BRIONES, KIM M., M.S. Effects of Intercropping Switchgrass with Loblolly Pine on the Diet and Trophic Position of *Peromyscus leucopus.* (2011)
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Managed pine plantations have been recognized as potentially suitable land for producing biofuel feedstocks. Intercropping biofuel feedstocks, particularly switchgrass (*Panicum virgatum* L., a native C_4 grass), in existing loblolly pine (*Pinus taeda*) plantations is a potentially sustainable alternative to land conversion for feedstock production. However, little is known about how biofuel feedstock intercropping could affect biodiversity and ecosystem functioning in a managed forest system. I conducted a study of a common native omnivore, the white-footed mouse (*Peromyscus leucopus*), to examine if they would use planted switchgrass as a food source, or if they would use existing food resources associated with pine. This study was one of the first to examine ecological effects of forest management of biofuel feedstocks on a key forest consumer.

Rodents were live trapped in four replicates of three different treatments planted with: (1) pine only, (2) pine intercropped with switchgrass and (3) switchgrass-only. I assessed the diet sources and trophic position of mice using stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), respectively, in tissue samples collected in 2009 and 2010. I tested the hypothesis that switchgrass in intercropped landscapes would affect the dietary preference and trophic position of *P. leucopus*. In terms of intercropping, my main prediction was that diet and trophic position of mice in intercropped treatments would reflect a C_3/C_4 signal and trophic position would be lower than treatments with pine only, indicating a change in functional role.

In 2009 there was no effect of treatment, but there was an effect of season on diet and trophic position of *P. leucopus*. Across both seasons, mice consumed a combined C_3/C_4-based diet. However, in the summer, diet in all treatments was slightly more C_4-influenced, whereas in the fall diet was slightly more C_3-influenced. However, the influence was not dramatic in either season. Additionally, trophic position was higher in the fall and lower in the summer. In 2010, mouse diets in all treatments reflected a combined C_3/C_4-based diet. There was no effect of treatment or season on diet, but $\delta^{13}C$ of mouse tissue in switchgrass only treatments was pulled slightly toward a C_4 signal. In terms of trophic position, $\delta^{15}N$ values indicated that mice remained functionally omnivorous. While these findings did not suggest an influence of switchgrass on diet or trophic position of *P. leucopus*, results are based on only the first two years of the intercropping study. As treatments become more established, further study should be conducted to ensure that ecological roles of consumers remain intact.

EFFECTS OF INTERCROPPING SWITCHGRASS

WITH LOBLOLLY PINE ON THE DIET

AND TROPHIC POSITION OF

PEROMYSCUS LEUCOPUS

by

Kim M. Briones

A Thesis Submitted to
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CHAPTER I

INTRODUCTION

Energy flow through ecosystems is an important ecological phenomenon (Golley 1960; Lindeman 1991; Miller *et al.* 2008; Peterson and Fry 1987). In photosynthesis-based trophic systems, primary producers harness sunlight and inorganic compounds and convert these to organic matter (Chapin *et al.* 2002; Smith and Smith 2008). In turn, consumers derive their energy by directly by consuming plants or indirectly by consuming other animals. Depending on what a consumer eats defines that consumer's trophic level (Chapin *et al.* 2002; Thompson *et al.* 2007). Herbivores occupy the second trophic level; primary and secondary consumers occupy the third and fourth trophic levels, and so forth. Trophic omnivores differ in that they feed from multiple trophic levels (Thompson *et al.* 2007) and are capable of shifting their diet when necessary (Mcshea 2000; Polis and Strong 1996; Shaner and Macko 2011; Stapp *et al.* 1999; Vander Zanden *et al.* 1999; Wolff 1996). These multi-trophic interactions provide the structure for complex food webs (Chapin *et al.* 2002). Rodents are a group of animals that contribute to this ecological complexity, as many genera have a diverse diet spanning many trophic levels. Many rodents consume both plants and animals (Carey and Johnson 1995; Elkinton *et al.* 1996; Mittelbach and Gross 1984), and are also prey for higher order consumers (Carey and Johnson 1995; Hansson 2002, 1974; Roche *et al.* 1999). Thus, rodents provide an important energy link between producers and higher

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trophic consumers in terrestrial food webs (Hafner *et al.* 1998).

In addition to their role in energy transfer, rodents have other important functions in forest ecosystems. Rodents are dispersers of seeds and fungi, they aerate and mix soil via burrowing, they contribute to decomposition, and they also regulate invertebrate populations (Carey and Harrington 2001; Carey and Johnson 1995; Elkinton *et al.* 1996; Maser *et al.* 1978; Ostfeld *et al.* 1997).

Rodents are strongly influenced by changes in habitat structure (Davis *et al.* 2010; Loeb 1999; Mengak and Guynn 2003). Many rodents are associated with aspects of structural complexity such as snags, downed logs and stumps (coarse woody debris), as well as understory and overstory development and composition (Bowman *et al.* 2000; Carey and Harrington 2001; Carey and Johnson 1995; Loeb 1999; Mengak and Guynn 2003). These habitat elements provide protection from predators, are used as travel routes, and provide foraging and nesting habitat.

Rodents also show a strong response to resource availability, especially pulsed resource events (Clotfelter *et al.* 2007; Mccracken *et al.* 1999; Schmidt and Ostfeld 2008; Wolff *et al.* 1985). Oak masts which produce irregular synchronous eruptions of acorn crops (Kelly 1994; Silvertown 1980) are an important resource for many rodent species. Numerous studies have documented a positive correlation between acorn mast events and increased population densities in several species of rodents, especially in the genus *Peromyscus* (Clotfelter *et al.* 2007; Mccracken *et al.* 1999; Mcshea 2000; Schmidt and Ostfeld 2008; Wolff 1996). Annually, *Peromyscus* spp. are also influenced by seasonal availability of resources such as seeds, fruit and some arthropod larvae (Wolff *et al.*

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1985). In one study, *Peromyscus leucopus* (white-footed mouse) showed a strong response to an artificial pulse of seeds, but showed a weaker response to a natural cicada pulse (Shaner and Macko 2011). While a complete shift from one trophic level to another (e.g. omnivore shifts to herbivore) has not been documented in this generalist consumer, such a shift could alter food web interactions (Shaner and Macko 2011).

There has been increasing effort to assess the sustainability of forestry practices on biotic and abiotic components of managed forests (Kessler *et al.* 1992; Sharitz *et al.* 1992). Given their role in ecosystem functioning and their responsiveness to habitat structure and composition, rodent responses to forestry practices are often the focus of studies examining sustainability in managed forest landscapes (Carey and Harrington 2001; Pearce and Venier 2005). The effects of standard silvicultural (Whitaker 1963) practices on rodents including clear-cut harvesting, forest thinning, site preparation, and herbicide treatment (Constantine *et al.* 2004; Fuller *et al.* 2004; Iglay 2010; Kaminski *et al.* 2007; Lautenschlager 1993; Sullivan *et al.* 2009) have been examined, as have practices including incorporation of streamside management zones, retention of course woody debris, partial harvesting, variable tree retention, and retention of corridors (Constantine *et al.* 2004; Fuller *et al.* 2004; Gitzen *et al.* 2007; Le Blanc *et al.* 2010; Loeb 1999; Miller *et al.* 2004). General findings indicate a variety of responses to silviculture, depending on the level of disturbance to which a species is adapted. However, it is the changes in the understory that have been shown to most effect rodents (Fuller *et al.* 2004; Kaminski *et al.* 2007; Kirkland 1990).

Recently, there have been efforts to examine the feasibility of producing biofuel feedstocks in managed forests. North Carolina Senate Bill 2051 is a state bill that mandates that 10 percent of all liquid fuels shall be grown and produced in North Carolina by the year 2017. In order to produce biofuels at the scale necessary to meet this mandate, an abundance of land is required. Production of biofuel feedstocks has been controversial because it typically involves conversion of arable or otherwise undisturbed lands (Tilman *et al.* 2006). However, intercropping (growing two or more crops together simultaneously) biofuel feedstocks between rows of trees within existing forest plantations may be a sustainable option for biofuel feedstock production that takes advantage of unused land. In the southeastern United States, approximately 13 million hectares are covered by managed loblolly pine (*Pinus taeda*) plantations (Schultz 1999; Smith *et al.* 2009; Smith *et al.* 1997). Loblolly pine plantations are an abundant source of land where biofuel intercropping could be implemented.

Switchgrass (*Panicum virgatum* L.) is a promising bioenergy crop (Arnett 2000; Wright and Turhollow 2010) and a perennial C_4 grass native to the central and eastern U.S. It has a wide native range and grows in a variety of habitats and climatic conditions (Mclaughlin and Walsh 1998; Raghu *et al.* 2006). Once established, the deep root system provides high water and nutrient holding capabilities, and increases soil stabilization (Mclaughlin and Walsh 1998). As a perennial, switchgrass can be harvested for several years, reducing the need for annual seeding and disturbance to the soil. Overall, switchgrass can be grown and maintained with moderately low energy input, compared with other biofuel feedstocks (Schmer *et al.* 2008; Wright and Turhollow 2010).

In agricultural systems, monocropping leads to reduced biodiversity, whereas intercropping is more aligned with maintaining habitat heterogeneity (Mineau and Mclaughlin 1996), a key component of biodiversity (Carey 2003; Tews *et al.* 2004). Intercropping biofuel feedstock crops in managed forests is a potentially sustainable option for meeting state energy mandates. However, the ecological effects of biofuel feedstock intercropping are poorly understood. Although research on the ecological effects of forest management is prevalent, few studies have examined if or how biofuel feedstock intercropping could affect biodiversity in a managed forest system. Ecological effects of monocropping biofuel feedstock crops of switchgrass, miscanthus (*Miscanthus giganteus*) and reed canary-grass (*Phalaris arundinacea*) have been examined to some extent. For example, Robertson *et al.* (2011) did not detect a difference in avian species richness and abundance when comparing switchgrass monocrop and mixed-grass prairie biofuel feedstock patches. In another study, full (100%) and partial (60%) harvesting of switchgrass monocrops had greater avian species abundance when compared to corn and soybean monocrops, and un-harvested plots of switchgrass (Murray *et al.* 2003). Also, in a comparison of miscanthus and reed canary-grass biomass monocrops, small mammals were more abundant along plot edges (ranging from 1.3 to 7 ha.) versus the interior of these crops (Semere and Slater 2007).

In contrast, only one study that I am aware of has examined ecological effects of *intercropping* a biofuel feedstock crop in a managed forest. Marshall *et al.* (in Review) examined rodent responses (community diversity and abundance, and population demographics) to several pine and switchgrass intercropping treatments, and woody

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debris removal. Marshall *et al.* (in Review) found no difference in rodent community diversity among treatments. However, there were changes in species relative abundance associated with switchgrass intercropping. Invasive house mice (*Mus musculus*) were more abundant, whereas native white-footed mice (*Peromyscus leucopus*) were less abundant, in treatments that contained switchgrass.

Changes in rodent population abundance associated with switchgrass intercropping (Marshall *et al.* in Review) could affect food web dynamics. The observed decline of *P. leucopus* (white-footed mouse), one of the most common native rodents in the southeastern U.S. (Wolff 1996), in treatments with switchgrass may be associated with a decline in food resources. Although *P. leucopus* is an omnivore, consuming a variety of fruits, nuts, seeds, green foliage, fungi, and insects (Lackey *et al.* 1985; Shaner *et al.* 2007; Wolff *et al.* 1985), they may not have exploited the introduced basal food resource, switchgrass. Laboratory and field experiments have demonstrated that *P. leucopus* have specific energy and protein requirements, and make dietary choices based upon the nutritional content of foods (Lewis *et al.* 2001). If switchgrass does not fulfill these requirements, the pattern of declining abundances of *P. leucopus* may continue. In terms of energy flow, this could have cascading effects on higher trophic consumers that interact with *P. leucopus*, and have implications for forest food webs.

Dietary and trophic responses to switchgrass intercropping can be traced using stable isotopes. Stapp *et al.* (1999) used stable isotopes to show how the diet of an insular deer mouse (*Peromyscus maniculatus*) population shifted from a marine-derived diet in dry years, when terrestrial productivity was low to a terrestrial-based diet during wet El

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Niño years, when terrestrial productivity was high. This study revealed the importance of marine-derived resources in subsidizing insular *P. maniculatus* when terrestrial resources were reduced. Based on the observed changes in abundance of *P. leucopus* in response to switchgrass intercropping, their broad dietary preferences, and their widespread abundance in the southeast, *P. leucopus* is an excellent species to examine diet and trophic responses to switchgrass intercropping.

Physical properties of naturally-occurring stable isotopes make them useful tools in ecological studies. Different stable forms of chemical elements, referred to as stable isotopes, have a heavy and light mass such as the stable isotopes of carbon, ^{13}C and ^{12}C, respectively. Biological, chemical, and physical processes can change the ratios of the heavy to light isotopes (fractionation) in a very predictable manner (Crawford *et al.* 2008; Peterson and Fry 1987). These ratios can be measured in animal tissues relative to standards. Differences in abundances of heavy and light isotopes are typically expressed in delta notation (δ) as parts per thousand (‰) change from a standard, as follows: $\delta^H X = [(R_{sample}/R_{standard})-1] * 1000$, where X is the element, H is the heavy isotope, and R is the ratio of the heavy and light isotope. Pee dee belemnite limestone is the standard for $\delta^{13}C$ and atmospheric nitrogen is the standard for $\delta^{15}N$ (Peterson and Fry 1987).

Carbon and nitrogen stable isotopes (SI) are particularly useful in tracing a consumer's diet and trophic position because these isotopes exhibit unique patterns of enrichment relative to the diet of the consumer (Kelly 2000; Peterson and Fry 1987). Carbon SI ($\delta^{13}C$) signatures of consumers are enriched by about 1‰ (parts per thousand) relative to the diet. Thus, inferences can be made about what a consumer is eating based

on stable isotope estimates of carbon. Similarly, nitrogen SI (δ^{15}N) signatures of consumers are generally enriched 3–5 ‰ with each trophic level (Kelly 2000; Peterson and Fry 1987). Thus, if the δ^{15}N of the base of the food web is known, the trophic level of a consumer can be inferred.

Differential photosynthetic pathways of terrestrial C_3 plants versus C_4 plants increase the utility of SI analysis. C_3 and C_4 plants have distinct δ^{13}C signatures as a result of differential carbon fixation by these plant types (Ehleringer *et al.* 1992; Whelan *et al.* 1973). Switchgrass, like many other warm season grasses, use the C_4 photosynthetic pathway (Osborne and Freckleton 2009). However, most trees, shrubs, herbaceous plants and cool season grasses use the C_3 photosynthetic pathway (Cerling *et al.* 1993). C_3 plants typically have carbon SI (δ^{13}C) values that range from -35 to -21‰, whereas C_4 plants typically have higher δ^{13}C values that range from -14 to -10‰ (Boutton *et al.* 1991; Ehleringer 1991). Negative values of both plant types indicate they are depleted in δ^{13}C relative to the standard for δ^{13}C, Pee dee belemnite limestone. Given that stable isotopes are integrated into body tissues from the diet, SI analysis can be used to determine if an animal is subsisting on a C_3 (e.g., pine) or C_4 (e.g., switchgrass) based diet.

Metabolically inert tissues such as fur and skin are excellent tracers of long-term feeding patterns because the stable isotope signature of these tissues reflects the diet at the time of tissue growth (Crawford *et al.* 2008; Miller *et al.* 2008). Once tissue growth ceases, the stable isotope signature remains fixed until growth resumes (Baugh *et al.* 2004). In adult rodents, stable isotopes are incorporated in the fur at times of pelage

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formation, including when juveniles first form their sub-adult to adult pelage and when adults undergo a seasonal molt (Miller *et al.* 2008). In northern temperate climates adults typically undergo a winter molt in the fall (Wichman and Lynch 1991). However, molt may be less dramatic in southern temperate climates, including the North Carolina coastal plain. Few studies have examined isotope turnover in mammal skin. However, in nectar feeding bats, wing membrane isotope turnover ranged from three to four months (Voigt *et al.* 2003). Thus, skin has similar benefits for long-term diet analysis, as turnover of stable isotopes in skin occurs fairly slowly.

Using stable isotopes, I examined how the diet and trophic position of a native omnivore, *P. leucopus*, changed in response to management of biofuel feedstocks in a managed forest. Specifically, I focused on dietary and trophic shifts in response to several biofuel feedstock intercropping treatments. Changes in diet and trophic position have been described as an indicator of food web responses to ecosystem perturbations, including non-native species invasions, resource pulses, and seasonal climatic disturbances (Mcshea 2000; Polis and Strong 1996; Shaner and Macko 2011; Stapp *et al.* 1999; Vander Zanden *et al.* 1999; Wolff 1996). Assessing ecological implications of new management approaches is an important consideration for forest managers. Along with traditional timber management, forest managers must ensure sustainable management activities that conserve biodiversity and ecosystem functioning (Kaminski *et al.* 2007). This study is one of the first to evaluate effects of forest management of intercropped biofuel feedstocks on a key forest consumer.

Objectives, Hypothesis and Predictions

The objective of my study was to examine if diet and trophic position of a native omnivore (*P. leucopus*) changed in response to intercropping switchgrass in a managed loblolly pine forest using stable isotope analysis. I examined diet and trophic position changes in mice inhabiting three intercropping treatments including 1) pine with residual woody debris removed (pine biomass -; PB-), (2) pine and switchgrass intercropped with residual woody debris removed (pine x switchgrass, biomass -; P x SB-), and (3) switchgrass only with residual woody debris removed (S). Hereafter, I will refer to these treatments as "PB-", "P x SB-", and "S". The "PB-" treatment most closely represents typical forest management. The Removal of residual woody debris from these treatments simulated a biomass harvest. "S" treatments differed most from typical forest management.

Hypothesis 1. Switchgrass in intercropped landscapes affects dietary preference of *P. leucopus*.

Predictions

When switchgrass seeds are available (late summer/early fall), I predict the following, based on the opportunistic foraging behavior of *P. leucopus*: In "S" treatments, I predict that *P. leucopus* will consume switchgrass seeds, and will thus have enriched $\delta^{13}C$ tissue values that reflect a C_4-based diet (seed pulse response). In "PB-" treatments I predict that mice will primarily consume C_3 plant matter and invertebrates. Relative to mice in "S", mice in "PB-" treatments will have depleted $\delta^{13}C$ tissue values that reflect a C_3-based diet. In "P x SB-" treatments I predict that mice will consume C_3

and C_4 plant matter (planted switchgrass) and invertebrates. Because intercropped treatments will support a mixture of C_3 plants and planted switchgrass, I predict that mice will have $\delta^{13}C$ tissue values that reflect a combined C_3/C_4 signal that is intermediate to "S" and "PB-".

Hypothesis 2. Switchgrass in intercropped landscapes affects the trophic position of *P. leucopus.*

Predictions

When switchgrass seeds are available (late summer/early fall), I predict the following based on the opportunistic foraging behavior of *P. leucopus*: In the "S" treatment I predict that *P. leucopus'* trophic position will be low, compared to mice in "PB-", because they will also respond to the pulsed switchgrass seed resource, consuming more plant matter and less invertebrate prey. In the "PB-" treatment I predict that *P. leucopus* will occupy a higher trophic position, relative to "S", because they will consume a mixture of invertebrate prey and plant matter, thus they will have enriched $\delta^{15}N$ values. In the "P x SB-" treatment I predict that *P. leucopus'* trophic position will be low, compared to mice in "PB-", because they will also respond to the pulsed switchgrass seed resource, consuming more plant matter and less invertebrate prey.

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CHAPTER II

METHODS

The Lenoir 1 Sustainability Study Site is located in Lenoir County, North

Carolina, USA (Figure 1) on a loblolly pine plantation owned and managed by

Weyerhaeuser NR Company. The sustainability study is part of joint venture between

Weyerhaeuser Co. and Chevron (Catchlight Energy LLC), who established and

maintained the pine/switchgrass intercropping sites in eastern North Carolina. The 109 ha

study site was originally planted in 1974 as a loblolly pine plantation, with a site index of

70 (based on site potential, trees are approximately 70 feet in height after 25 years).

Water levels are maintained via linear ditches that run along forest edges and parallel

each other through forest interiors. The surrounding region is rural and consists of sandy

bottomland forest with a mosaic of agricultural and managed pine forest patches. Within

the study site, a 33.4 ha study area was designated for long-term multi-disciplinary

examination of the ecological effects of biofuels production on sustainability.

In 2008, the 33.4 ha study area was clear-cut harvested, and mechanically (V-

shearing and bedding) and chemically (herbicide treatment) site prepared. The study area

was divided into four blocks (Figure 1) with each block divided into 7 to 8 plots (2-ha,

referred to hereafter as treatment plot). Each plot was randomly assigned a different

treatment within a block. I examined four replicates of three treatments including (1)

"PB-", (2) "P x SB-", and (3) "S".

In December 2008 loblolly pine seedlings were planted in "PB-" and "P x SB-" treatment plots with approximately 6.1 m between rows. In early June 2009, switchgrass seeds were machine planted across the entirety of "S" plots and between rows of pine trees in the "P x SB-" treatment plots. With exception of the pine plantings and broadcast switchgrass seeds, all treatment plots were sparsely vegetated in early summer 2009. By the fall of 2009, switchgrass was established and set seed. In the summer of 2010 switchgrass seed broadcasting was not required, as switchgrass plants were established and are perennial. Therefore, in contrast with early summer 2009, switchgrass seeds were not present on the ground in summer 2010. All treatments continued to become more established and switchgrass set seed again in the fall of 2010 (Marshall *et al.* in Review).

Vegetation sampling was conducted bi-monthly between April and October 2010 (Marshall *et al.* in Review) to examine treatment plot vegetation composition. Treatments were effective in changing the composition of plants in each treatment plot. By October 2010 percent cover (%) of total area and height (m) was as follows: "S" plots were composed of switchgrass ($95.58 \pm 1.57\%$ and 1.45 ± 0.11 m) and forbs ($0.54 \pm 0.54\%$ and 0.28 ± 0.28 m); "P x SB-" were composed of loblolly pine ($9.25 \pm 1.55\%$ and 1.85 ± 0.11 m), grass ($75.17 \pm 2.73\%$ and 1.06 ± 0.11 m), forbs ($9.75 \pm 2.90\%$ and 1.14 ± 0.18 m), and woody debris ($2.63 \pm 1.0\%$ and 0.24 ± 0.08 m); and "PB-" plots were composed of loblolly pine ($13.67 \pm 7.06\%$ and 1.19 ± 0.23 m), grasses ($64.83 \pm 4.14\%$ and 0.90 ± 0.08 m), forbs ($14.0 \pm 4.3\%$ and 1.10 ± 0.18 m) and woody debris ($4.17 \pm 1.35\%$ and 0.15 ± 0.07 m) (Marshall *et al.* in Review).

To determine if diet and trophic position of *P. leucopus* were influenced by treatment, I collected fur and skin samples from mice. I conducted live trapping between July 15 – December 9, 2009 and July 19 – November 14, 2010 in all treatment plots. Trapping grids (10-m arrays) measuring 30m x 60m were established within each treatment plot, approximately 20 m from the edge of each plot. Each trap line was located parallel to each pine row and was composed of one randomly placed Longworth and six Sherman live traps. Traps were baited with oat seeds in the summer and a combination of oats and sunflower seeds in the fall. Traps were set at sunset (1700-2030 pm) and checked the following morning at sunrise (0600-0830).

Standard data collected for each capture included trap location, species, sex, reproductive condition, and mass. Each individual was marked with a unique number ear-tag (Monel Numeric, size 1005-1). Upon capture, juveniles were distinguished from adults based on pelage color. Juveniles have overall grey pelage, whereas adults have dorsal rufous brown pelage and ventral white pelage. When molting into adult pelage, rufous fur on adults initially grows laterally, progressing up the dorsum and is visibly distinct from the juvenile pelage (Golley *et al.* 1966; Miller *et al.* 2008).

To interpret stable isotope signatures of *P. leucopus* tissues, I collected invertebrates from three functional groups: herbivores, omnivores, and predators. I did this to obtain representative stable isotope values with which to compare diet and trophic position with *P. leucopus*, a functional omnivore. Therefore, collection of invertebrates was not meant to establish exactly what *P. leucopus* was consuming but rather to establish how *P. leucopus* compared with other consumers in the treatment plots. I also collected samples

of loblolly pine and switchgrass from each of the three treatment plots to provide a basal stable isotope source of comparison.

Tissue Collection and Processing

Mouse Tissue Collection

Tissue samples were collected from adult male and female *P. leucopus* in all treatment plots. Skin samples were collected from the ear using a biopsy punch only during 2009 and fur samples were trimmed from the dorsum during the second year of sampling. Most skin samples were too small to meet minimum weight requirements for isotope analysis, so that I changed methods to collect fur samples from individuals. Collection of fur is less invasive and larger samples can be collected to meet minimum weight requirements for analyses. To determine if fractionation between the diet and skin or fur were similar, I also collected skin samples from some of the mice that I sampled for fur samples in 2010. Fur samples were stored dry in micro-centrifuge tubes. Skin samples were stored in 95% ethanol in micro-centrifuge tubes. All samples were stored at -20° C within three hours of collection. All animal handling and tissue collection were conducted according to Sikes *et al.*(2011), NCWRC collecting protocol (09SC00162, 10SC00162), and IACUC (10-04, 09-09).

Terrestrial Invertebrate Tissue Collection

To compare SI values of *P. leucopus* with that of potential invertebrate prey items, I conducted invertebrate sampling within each treatment plot during the 2010 field

season. I collected invertebrates using a combination of branch beating and hand picking.

Invertebrate sampling was conducted twice per month between June 22, 2010 and

November 20, 2010, from approximately 1600-1930. The time that sampling was

conducted was based on other field scheduling needs. Each sampling session consisted of

sampling at four randomly selected mouse live-trap stations in each treatment plot. At

each of the four mouse live-trap stations within each treatment plot, four plants were

sampled for invertebrates by branch beating for one minute each. Ground-dwelling

invertebrates were also collected by hand picking for an additional minute at each of the

four mouse live trap stations. Thus, each treatment plot was sampled for a total of sixteen

minutes for branch beating and four minutes for hand picking for each sampling event.

Invertebrates were placed in vials with 95 percent ethanol and stored at room temperature

until identification. I identified most invertebrates to family level and classified each

family by general feeding guild (e.g., herbivore, omnivore, and predator) using Marshall

(2006) and Arnett (2000). A few taxonomic groups were classified as decomposers.

Unidentified spiders (Araneae) and butterfly/moth larvae (Lepidoptera) were treated as

predator and herbivore functional groups, respectively. Millipedes (Diplopoda) were only

identified to class.

Vegetation Tissue Collection

To compare SI values of *P. leucopus* with that of potential plant food items, I

collected vegetation samples within each treatment plot during the 2010 field season.

Because I was interested in determining if *P. leucopus* shifted from a primarily C_3-based

diet to a C_4-based diet (switchgrass), and because pine and switchgrass were the

dominant plant forms, I only collected pine (C_3) and switchgrass (C_4). I conducted

vegetation sampling once in the summer and once in the fall for all treatment plots

between June 2010 and November 2010. Samples of switchgrass seeds and loblolly pine

leaves were randomly collected within each treatment plot. Pine needles were collected in

lieu of pine seeds, because seeds were not available when sampling was conducted.

While I did not anticipate *P. leucopus* would consume pine needles, I collected needles to

obtain $\delta^{13}C$ isotopic signals from the dominant C_4 plant in the study area. Plant material

collected from an individual plant was defined as one sample. All plant material was

placed in clean freezer bags, and stored at - 20°C.

Tissue Processing for Isotope Analysis

In preparation for SI analysis, I rinsed fur and skin tissue samples in 2:1

chloroform:methanol solution to remove surface oils. I air-dried fur and skin samples for

48 hours under a fume hood. I rinsed invertebrate and plant samples with deionized water

and dried each sample at 60°C for 48 hours. Once dry, I ground invertebrate and plant

samples to a fine powder with a mortar and pestle. All samples were weighed (mice and

invertebrates: 0.20 – 1.0 mg and plants: 1.0 – 6.0 mg) in tin foil capsules on a

microbalance. Weighed samples were crushed into a small ball and placed in a 96-well

plate. Skin samples collected via biopsy punch were often too small for minimum weight

requirements (0.20 mg) for isotope analysis. When skin samples were too small they

were pooled with a second skin sample, by treatment and proximity of date collected

(average = 3.5 days). Stable isotope analysis for 2009 samples was performed in a single batch at the Woods Hole Stable Isotope Laboratory. Stable isotope analysis for 2010 samples was performed in four separate batches at the U.C. Santa Cruz Stable Isotope Laboratory. Both $\delta^{13}C$ and $\delta^{15}N$ were analyzed from each sample simultaneously.

Statistical Analysis

I evaluated all data for normality and homogeneity of variances using Shapiro-Wilk and Levene's tests, respectively. When Shapiro-Wilk and Levene's tests were not significant (P>0.05), I conducted parametric statistical analyses. When normality and/or homogeneity of variance assumptions were violated, I removed outliers and re-checked assumptions. If assumptions were still violated, I then transformed the data using log10, square-root-transformations. However, in most cases removal of outliers and transformations often did not alleviate assumption violations. Therefore, I conducted non-parametric analyses on rank-transformed data (Conover and Iman 1981). All data are shown as mean ± 1 SE. Statistical significance was accepted at p ≤ 0.05. All statistical analyses were performed using SPSS Version 16.0.

To capture isotope signals before and after fall molt of *P. leucopus* and before and after switchgrass seeds were available on the ground, I grouped samples by season: summer (July- September) and fall (October - November). I made statistical comparisons of stable isotope values of mouse tissue ($\delta^{13}C$ and $\delta^{15}N$) between the two seasons (summer and fall) and among the three different treatments (PB -, P x SB-, and S). I also examined the interaction of season x treatment.

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Because I had skin samples for 2009, and skin and fur samples for 2010, I treated these data sets differently. For 2009 data, I conducted a two-factor Analysis of Variance (ANOVA) to determine if there was an effect of season and treatment on $\delta^{13}C$ and $\delta^{15}N$ values of skin. Because there was an effect of season on $\delta^{13}C$ and $\delta^{15}N$ values, I conducted a post-hoc one-way ANOVA to examine where the differences lie. For 2010, I conducted a paired t-test for the subset of mice for which I had skin and fur samples to determine if both tissues incorporate $\delta^{13}C$ and $\delta^{15}N$ values in a similar manner. I did this to ensure that there were not major discrepancies between conclusions in 2009 and 2010. However, since I was not comparing 2009 and 2010, nor was I comparing skin and fur from individuals in 2010, small differences would not affect interpretation of results. I then conducted a two-factor ANOVA to examine effects of treatment and season (independent variables) on $\delta^{13}C$ and $\delta^{15}N$ values (dependent variables) with *P. leucopus* fur from 2010.

Additionally, I conducted a separate two-factor ANOVA, using treatment as the only independent variable and $\delta^{13}C$ and $\delta^{15}N$ values of source tissues (*P. leucopus* fur, and herbivorous, omnivorous and predatory invertebrates) as the dependent variables. I did not include season as a factor because there was no effect of season on stable isotope values of *P. leucopus* fur in 2010 (see results). I conducted this separate analysis to determine if *P. leucopus*' diet and trophic position changed similarly or differently to invertebrates, as further support for any treatment effects. When the interaction term of source x treatment was significant, I conducted a test of simple main effects. I conducted

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all post hoc analysis using Least Significant Difference (LSD) and adjusted α to 0.02, as more conservative adjustments (*e.g.* Bonferroni) (Perneger 1998) failed to detect obvious significant mean differences.

To examine potential invertebrate prey availability in 2010, I statistically analyzed mean abundances of invertebrates by family and functional group (herbivore, omnivore, and predator) collected among the three treatments using one-way ANOVAs. For family analyses, I pooled the data from the two methods of collection (branch beating and hand picking) because there was no difference in statistical findings when the data were not pooled. I grouped Coleoptera that could not be identified to family as "other", but only included Coleoptera in the family statistical analysis because functional groups could not be assigned. I excluded invertebrate families or functional groups whose total abundances were less than 10 individuals and that were not collected in all treatments. I also excluded decomposers from statistical analyses by functional group because decomposers were not collected in all treatments. Therefore, I statistically compared the mean abundance of ten families and three orders (Table 4), and three invertebrate functional groups (Table 5), among each of the three treatments. For analysis of mean abundances of invertebrates by family among treatments, I adjusted the level of significance with a Bonferroni correction of 0.005.

I did not statistically compare potential plant food items (loblolly pine and planted switchgrass) among treatments, as my primary interest was in obtaining $\delta^{13}C$ and $\delta^{15}N$ basal reference values. This was required to infer the source of carbon in the diet and trophic position of *P. leucopus*. Thus, I only made qualitative comparisons of $\delta^{13}C$ and

$\delta^{15}N$ values of *P. leucopus* and basal resources in the treatment plots. I grouped means ± 1 SE of $\delta^{13}C$ and $\delta^{15}N$ values for plants only by treatment.

CHAPTER III

RESULTS

In 2009, trapping was conducted between 15 July and 9 December on 61 nights

(8,064 trap nights). In 2009, 153 unique individual *P. leucopus* were captured. In 2010,

trapping was conducted between 19 July and 14 November on 45 nights (6,048 trap

nights). In 2010, 160 unique individual *P. leucopus* were captured. In 2009, out of 153

individuals sampled for tissue, 55 samples were selected for SI analysis based on age,

plot and season. Out of the 55 samples, 31 samples met the minimum weight

requirements (0.20mg) for SI analysis, but 24 samples did not. Out of the 24 samples that

did not meet minimum weight requirements, twelve pairs of samples were pooled based

on age, plot and season. Therefore, a total of 43 skin samples ("PB-" = 17, "P x SB" =

12, "S" = 14) were submitted for SI analysis in 2009. In 2010, out of 112 individuals

sampled for tissue, 75 fur samples were submitted for SI analysis based on age, plot and

season. Of these 75 individuals, 28 paired skin samples were also selected for SI analysis

to compare differences in skin and fur. Following analysis of isotope data for 2010, three

pairs of extreme outliers were removed from the data set and were not used for any

further analyses. Therefore, 72 fur ("PB-" = 36, "P x SB" = 26, "S" = 10) and 25 skin

samples ("PB-" = 13, "P x SB" = 11, "S" = 1) were analyzed for 2010.

In 2010, I conducted invertebrate sampling between June 22 and November 20 (30 sampling occasions). During branch beating I collected a total of 752 individuals from "PB-", 864 individuals from "P x SB-", and 767 individuals from "S", representing eight orders and 36 families (Table 1). During hand picking, I collected 32 individuals from PB-", 48 individuals from P x SB-", and 41 individuals from "S", representing one class, seven orders, and 14 families (Table 2). Of the total number of invertebrates that were collected by branch beating and hand picking combined, I collected representatives from four functional groups including herbivores, omnivores, predators, and decomposers (Table 3). Out of the total number of invertebrates collected by branch beating and hand picking, 146 individuals were selected based on functional group, plot, and season, and submitted for stable isotope analyses. In terms of invertebrate abundance, there was no difference between treatments in the mean abundance of invertebrates by family (Table 4). Of these invertebrates, Acrididae (grasshoppers), Cicadellidae (leafhoppers), Pentatomidae (stink bugs), Araneae (spiders), and Tettigoniidae (katydids) were among the most abundant invertebrates found overall, at least by branch beating (Table 1). Similarly, when compared by functional group, there was no difference in mean abundance of herbivores, omnivores, or predators among each of the treatments (Table 5).In 2010, I conducted plant sampling between Sept. 2 - 14 and November 18 – 20 (8 sampling days). I collected a total of 84 plant samples including 50 loblolly pine and 34 switchgrass samples. A total of 44 loblolly pine and 34 switchgrass samples were selected based on plot and season, and submitted for stable isotope analyses.

2009 Stable Isotopes

In 2009, there was no effect of treatment ($F_{2,37} = 2.40$, $p = 0.11$) on rank-transformed mean $\delta^{13}C$ values of *P. leucopus*. There was an effect of season ($F_{1,37} = 14.43$, $p = 0.001$) on rank-transformed mean $\delta^{13}C$ values of *P. leucopus* (Figure 2 and 3a). Treatment did not vary by season, in terms of rank-transformed mean $\delta^{13}C$ values of *P. leucopus* ($F_{2,37} = 0.26$, $p = 0.77$). The effect of season was significant in "PB-" treatments ($F_{1,15} = 5.54$, $p = 0.03$) and in "P x SB-" treatments ($F_{1,10} = 8.41$, $p = 0.02$), whereby mean $\delta^{13}C$ values of *P. leucopus* were 1.6‰ and 2.3‰ more enriched in the summer than in the fall, respectively (Figure 3a). There was no effect of season in "S" treatments ($F_{1,12} = 2.36$, $p = 0.15$; Figure 3a). Regardless of seasonal differences within treatments, mean $\delta^{13}C$ values among treatments were intermediate between $\delta^{13}C$ of loblolly pine and switchgrass (Figure2).

In 2009, there was no effect of treatment ($F_{2,37} = 0.46$, $p = 0.63$) on mean $\delta^{15}N$ values of *P. leucopus* (Figure 2 and 3b). There was an effect of season on mean $\delta^{15}N$ values of *P. leucopus* ($F_{1,37} = 17.03$, $p < 0.001$) (Figure 3b). The effect of season on mean $\delta^{15}N$ values was significant in "PB-" treatments ($F_{1,15} = 12.99$, $p = 0.003$; Figure 3b) and marginally significant in "S" ($F_{1,12} = 4.19$, $p = 0.06$), whereby mean $\delta^{15}N$ values were 1.66‰ (PB-) and 1.35‰ (S) more enriched in the fall than in the summer (Figure 3b). The effect of season on mean $\delta^{15}N$ values of *P. leucopus* was not significant in "P x SB-" ($F_{1,10} = 3.34$, $p = 0.10$; Figure 3b). The interaction of treatment x season on mean $\delta^{15}N$ values of *P. leucopus* was not significant ($F_{2,37} = 0.47$, $p = 0.63$).

2010 Stable Isotopes

In 2010, I tested if stable isotope values differed depending on tissue type (skin and fur) used. Carbon isotope values of skin and fur differed ($t = -3.83$, df = 24 p = 0.001) (Figure 4a). On average, skin was 0.28‰ more enriched in mean $\delta^{13}C$ than fur. Nitrogen isotope values of skin and fur did not differ ($t = 1.00$, 24 df = 24, p = 0.33) (Figure 4b). Because I was not comparing results across years, this small difference did not affect my conclusions. Furthermore, I only utilized stable isotope data that was derived from hair samples for 2010. Had I made yearly comparisons or utilized a combination of tissue types from different individuals in 2010, the difference between skin and fur would have required a correction factor to account for the discrepancy. However, since these were not the kind of comparisons I made, 2010 analysis hereafter is based only on fur.

In 2010, I tested if treatment or season affected stable isotope values of *P. leucopus* fur. There was no effect of season on rank-transformed mean $\delta^{13}C$ values of *P. leucopus* fur ($F_{1, 66} = 1.37$, p = 0.25). There was an effect of treatment on rank-transformed mean $\delta^{13}C$ values of *P. leucopus* ($F_{2, 66} = 5.31$, p = 0.01; Figure 5 and 6a). Switchgrass only treatments differed from "PB-" (p = 0.004) and "P x SB-" (p = 0.04), whereby mean $\delta^{13}C$ values of *P. leucopus* in "S" plots were 0.77‰ more enriched than "P x SB-" and 0.87‰ more enriched than "PB-" treatments (Figure 6a). The interaction of treatment x season on mean $\delta^{13}C$ values of *P. leucopus* was not significant ($F_{2, 66} = 1.41$, p = 0.25). In general, $\delta^{13}C$ values across treatments were intermediate between $\delta^{13}C$ for loblolly pine and switchgrass (Figure 5). In 2010 there was no effect of season

($F_{1, 66} = 1.88$, p = 0.18) or treatment ($F_{2, 66} = 0.08$, p = 0.93) on rank-transformed mean $\delta^{15}N$ values of *P. leucopus* (Figure 6b). The interaction of treatment x season on mean $\delta^{15}N$ values of *P. leucopus* was not significant ($F_{2, 66} = 0.25$, p = 0.78).

As further support for treatment effects on *P. leucopus*, I followed the two-factor ANOVA with a separate two-factor ANOVA to determine how *P. leucopus'* diet and trophic position compared with the diet and trophic position of herbivorous, omnivorous, and predatory invertebrates (i.e., sources). There was an effect of treatment ($F_{2,203} = 10.07$, p < 0.001), source ($F_{3,203} = 5.70$ p = 0.001), and an interaction of treatment x source ($F_{6,203} = 2.21$, p = 0.04) on rank-transformed mean $\delta^{13}C$ values (Figure 6 and 7a). Within treatments, sources differed in "PB-" ($F_{3,203} = 4.89$, p = 0.003) and "P x SB-" treatments ($F_{3,203} = 6.96$, p < 0.001), but did not differ in "S" treatments ($F_{3,203} = 0.94$ p = 0.42). In "PB-" treatments, rank-transformed mean $\delta^{13}C$ values for *P. leucopus* did not differ from omnivorous or predatory invertebrates, but did differ from herbivorous invertebrates, whereby mean $\delta^{13}C$ values of *P. leucopus* were 1.24‰ more enriched than herbivores (Figure 7a). In "P x SB-" treatments rank-transformed mean $\delta^{13}C$ values of *P. leucopus* did not statistically differ from herbivores, omnivores, or predators (Figure 7a). However, in "P x SB-", p-values for pairwise comparisons between *P. leucopus* and herbivores (p = 0.023) and predators (p = 0.025), barely exceeded the rejection criteria (α=0.02). Although sources did not differ statistically in "S", *P. leucopus* and omnivorous invertebrates had lower rank-transformed mean $\delta^{13}C$ values than herbivores and predators. Within treatments, the most consistent pattern was that *P. leucopus* and

omnivorous invertebrates did not differ from one another. Among treatments, rank-transformed mean $\delta^{13}C$ values of *P. leucopus*, as well as omnivorous invertebrates, did not differ. However, *P. leucopus* did have a tendency for gradual increase in rank-transformed values from "PB-" to "P x SB-" to "S". In comparison, rank-transformed mean $\delta^{13}C$ values of herbivorous and predatory invertebrates did differ among treatments. In general, $\delta^{13}C$ values of *P. leucopus* and invertebrates were intermediate between $\delta^{13}C$ for loblolly pine and switchgrass across all treatments (Figure 5). However, herbivorous and predatory invertebrate $\delta^{13}C$ values showed a marked shift toward a C_4 signal in "S".

There was no difference in rank-transformed mean $\delta^{15}N$ values of sources among treatments ($F_{2,203} = 1.68$, $p = 0.19$; Figure 5 and 7b). There was a difference of sources within treatments ($F_{3,203} = 37.73$, $p < 0.001$) (Figure 5 and 7b). In "PB-" treatments rank-transformed mean $\delta^{15}N$ values of *P. leucopus* did not differ from predators, but did differ from herbivores and omnivores. Although statistically similar to predators, *P. leucopus* had the highest rank-transformed mean $\delta^{15}N$ values in "PB-" treatments (Figure 7b). In "P x SB-" treatments rank-transformed mean $\delta^{15}N$ values of *P. leucopus* differed from all sources, whereby *P. leucopus* had the highest values (Figure 7b). In "S" treatments, rank-transformed mean $\delta^{15}N$ values of *P. leucopus* did not differ from omnivores and predators, but did differ from herbivores (Figure 7b). Although statistically similar to omnivores and predators, *P. leucopus* had the highest rank-transformed mean $\delta^{15}N$ values in "S" treatments (Figure 7b). The interaction of treatment

x source on rank-transformed mean $\delta^{15}N$ values was not significant ($F_{6,203} = 1.63$, p = 0.14).

CHAPTER IV

DISCUSSION

While intercropping biofuels within a managed pine forest is a potentially

sustainable option for meeting energy demands, ecological effects are poorly understood.

My study was one of the first to examine how the functional role of a native omnivore

changes in response to intercropping switchgrass in a loblolly pine plantation. I examined

changes in the functional role of *P. leucopus* with stable isotope analysis of mouse tissue

and potential food sources during the first two years of a biofuel feedstock intercropping

experiment.

In year 1 of my study, there was not a treatment response on the diet or trophic

position of *P. leucopus* but there was a seasonal response. This seasonal response was

likely an artifact of mechanical site preparation, rather than a response to switchgrass

intercropping. With the exception of pine seedlings and/or switchgrass seeds that were

broadcast on the ground, all treatments in year 1 had sparse vegetation and associated

food resources at the beginning of the study in the summer because site preparation

occurred in the summer of year 1. Thus, enriched ^{13}C values and lowered trophic

response of *P. leucopus* in the summer was probably a result of availability of

switchgrass seed that was broadcast on the ground. Since there were few plant resources,

and potentially few invertebrate resources in all treatment plots at the beginning of the

study, it is plausible that *P. leucopus* consumed switchgrass seeds that were present on the ground. In the summer mice in "P x SB-" and "S" treatments likely foraged on the broadcast seeds that were available in these respective treatments. In the summer, mice in "PB-" treatments, were likely subsidized by switchgrass seed in neighboring switchgrass-associated treatments. A similar dietary shift to an abundant resource was observed in a congener, *Peromyscus maniculatus* (deer mouse), whom shifted from a terrestrial diet to a marine diet during a year of lowered terrestrial productivity (Stapp *et al.* 1999). *Peromyscus leucopus'* trophic response in the summer is consistent with this interpretation since lower $\delta^{15}N$ values indicate more plant matter in the diet and depleted $\delta^{13}C$ values suggest some influence of switchgrass. By the fall of year 1, loblolly pine and switchgrass had become established, and early seral plant species likely colonized the treatment plots. The shift toward depleted $\delta^{13}C$ values combined with enriched $\delta^{15}N$ values in the fall is consistent with the re-colonization of plant resources, as more established plots likely provided more food resources, including invertebrate prey. Despite this shift, $\delta^{13}C$ values still reflected a combination of C_3 and C_4 resources in the diet, but the C_4 influence was weaker than the summer. Ultimately, results from year 1 did not specifically address my research question. However, these results provide evidence that changes in functional role of *P. leucopus* can be detected using stable isotopes. The remainder of my discussion focuses on my results from year 2 (2010), as these results relate directly to my hypotheses. That $\delta^{13}C$ values overall were intermediate between loblolly pine and switchgrass, indicates there was a combined influence of both C_3 and C_4 resources over both seasons, but the C4 influence was strongest in the summer.

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In year 2, skin and fur were statistically different in $\delta^{13}C$. This difference, although small, may be the result of different stable isotope turnover rates and/or different fractionation effects of skin and fur. As with other tissues, skin and fur incorporate stable isotopes when the tissue is being generated (Crawford *et al.* 2008; Miller *et al.* 2008). In wild *Peromyscus* spp., fur is generated only at a few discrete times throughout the year (i.e., seasonal molt or development of adult pelage) (Miller *et al.* 2008; Wichman and Lynch 1991), and so provides an indication of diet before and during hair growth (long-term diet). However, in lab mice, skin cell production occurred continually, up to 60% at any one time (Potten 1974). Thus, the stable isotope signal of skin should reflect a mixture of both long term and recent diet. While I am unaware of any comparisons of fractionation rates in skin and fur in rodents, bat tissues have shown minimal fractionation differentiation. For example, Sullivan *et al.* (2006) found variation (approx. 1‰ for $\delta^{13}C$ and $\delta^{15}N$) in skin and fur in bats among different sampling sites, but skin was generally more enriched than fur. Voigt and Kelm (2006) found similar differentiation in $\delta^{13}C$ and $\delta^{15}N$, except fur was more enriched. Regardless of the difference that I found in skin and fur, I only used fur in my 2010 analysis. Thus, any tissue differences have no impact on my conclusions.

In year 2 of my study, while a treatment effect on the diet of *P. leucopus* was detected in "S" treatments, this effect was not detected when invertebrate sources were included in the model. Nonetheless, the trend toward enrichment of $\delta^{13}C$ values of *P. leucopus* in "S" compared with "PB-" and "P x SB-" was unchanging, regardless of the

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ANOVA model used. Despite this trend, mean $\delta^{13}C$ values of *P. leucopus* in all treatments were not strongly C_3 or C_4 biased, even in "S" treatments. This is consistent with my prediction for "P x SB-", but inconsistent with my predictions for "PB-" and "S" treatments. What this suggests is that mice consumed a variety of resources, both C_3 and C_4-based in nature, even in "S". The composition of vegetation within each of these treatments may explain the intermediate signal. Vegetation sampling conducted in October in year 2, showed that "PB-" and "P x SB-" treatments were largely composed of grass, pine, and forbs (Marshall *et al.* in Review). However, grasses accounted for over half the total cover of these treatments. Even with switchgrass as the dominant grass in intercropped treatments, both "P x SB-" and "PB-" also likely supported a combination of native C_3 and C_4 plants, which could account for the intermediate $\delta^{13}C$ signal. Alternatively, vegetation sampling showed that "S" treatments were largely composed (>90%) of grasses and these grasses were predominantly switchgrass (Marshall *et al.* in Review). This abundance of C_4 grasses likely explains the shift of $\delta^{13}C$ toward a C_4 signal. However, given the shift was not as dramatic as I had expected suggests that mice subsidized their diets from other resources, perhaps along the perimeter of the "S" treatments.

It should be mentioned that loblolly pine needles and switchgrass seed are not analogous food resources. Thus, my interpretation could be slightly inaccurate. However, $\delta^{13}C$ reported for slash pine (*Pinus elliottii*) seeds, another southeastern pine species, ranged from are -29.02‰ to -27.98‰ and switchgrass leaves were -12.8‰, (Jahren 2004; Still *et al.* 2003). Using these values to compare pine needle to switchgrass leaf or pine

seed to switchgrass seed, I still maintain that mice incorporated a combination of C_3 and C_4 resources into their diet.

While $\delta^{13}C$ values indicated a combined C_3/C_4 carbon source in the diet, mean $\delta^{15}N$ values of *P. leucopus* indicated they likely consumed a variety of plant and animal matter (omnivory). In "PB-", mice were < 3‰ more enriched in ^{15}N than predatory and herbivorous invertebrates and in "S" mice were < 3‰ more enriched in ^{15}N than omnivorous invertebrates. In contrast, ^{15}N values for mice in "P x SB-" were ≥ 3‰ more enriched than herbivorous invertebrates. While this might indicate "P x SB-" mice were more predatory, I conclude that these mice are also maintaining omnivory, as their trophic position did not differ from mice in "PB-" and "S". What this indicates is that both plants and lower-level consumers (invertebrates) had similar influence over *P. leucopus'* isotopic signature across treatments. This is consistent with the work of Shaner *et al.* (2007), who found that *P. leucopus* preferred habitat patches with a mixture of foods (seeds and mealworms) in contrast to patches with fewer options (seeds only or mealworms only). Other studies have also showed that *P. leucopus* consume a variety of invertebrate and plant resources (Wolff *et al.* 1985). Given that switchgrass was the dominant plant in the "S" treatment, it was not surprising that mean $\delta^{13}C$ of these mice was slightly more enriched. However, since the $\delta^{15}N$ values of mice in "S" treatments were not statistically different than mice in "PB-" and "P x SB-" indicates there was not a dramatic change in functional role in "S", as I had expected. Again, $\delta^{13}C$ and $\delta^{15}N$ or *P. leucopus* in "PB-" and "P x SB-" were nearly identical, indicating that mice in the intercropped treatments function no differently that they do in the monocropped pine

treatments. This is important because the monocropped pine treatment most closely represents typical forest management. Thus, regardless of treatment, *P. leucopus* maintained their role as functional omnivores.

In contrast to *P. leucopus*, there was a strong response to treatment for herbivorous and predatory invertebrates at lower trophic levels. This response was most notable in the "S" treatment. Specifically, enriched ^{13}C values (Figure 7a) combined with the lower trophic position (Figure 7b) of herbivorous invertebrates in "S" treatments reflected a response to the switchgrass in the "S" treatment. Similarly, the shift of predatory invertebrates' δ^{13}C values toward a C_4 signal in "S" treatments suggests that the response of the primary consumer progressed up the invertebrate food web (Figure 7a and 7b). Interestingly, in "S" treatments, omnivorous invertebrates were depleted in ^{13}C compared with predatory invertebrates. This indicates that omnivorous invertebrates did not interact with herbivorous or predatory invertebrates. Instead, omnivorous invertebrates were likely subsidized by other resources within "S", or along the perimeter of the plots.

Among the different families of invertebrates, those families and functional groups that were represented within each of the treatments did not differ in total abundance among the treatments in 2010 (Table 4 and 5). Given that potential prey was equally available among treatments, it was not surprising that *P. leucopus*' diet did not change among treatments in 2010.

There are several possible explanations as to why a stronger C_4 signal in monocropped switchgrass or intercropped treatments, was not detected in *P. leucopus*'

tissue. One possible explanation is that *P. leucopus* consumed fewer switchgrass seeds, than I expected, because the seeds were too small. Optimal foraging theory predicts that a consumer should maximize the amount of energy gained per unit of handling time of a prey item (Emlen 1966; Macarthur and Pianka 1966). Several studies have shown that when offered seeds of differing sizes, *Peromyscus* spp. tend to prefer large seeds over smaller seeds (Kantak 1983; Mittelbach and Gross 1984). Given the relatively small size of switchgrass seed (~0.57 mg (Howe and Brown 1999), it may have been more profitable for *P. leucopus* to consume other resources that were available in this system.

Likewise, *P. leucopus* may have consumed fewer switchgrass seeds, than I expected, because they did not provide adequate energy or nutrient content to meet *P. leucopus'* metabolic requirements. Studies have shown that *P. leucopus* and *P. maniculatus* choose energy-rich food over protein-rich food, or high-energy food that is low in protein (Lewis *et al.* 2001; Vickery *et al.* 1994). This preference has also been documented in other small mammals (Kerley and Erasmus 1991). However, to my knowledge energy and nutrient content in switchgrass seeds has not been examined. There have been studies that examine how birds metabolize and assimilate energy from seeds including switchgrass. In these studies, switchgrass seed rated low in potential energy for greater prairie chicken and scaled quail (Heffron and Parrish Jr. 2005; Saunders and Parrish 1987). Feeding trials, in the field, could elucidate how the physical and chemical properties of switchgrass seed compare with other potential seed resources at my study site, and whether this could explain the lack treatment response on the diet and trophic position of *P. leucopus*.

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Marshall *et al.* (in Review) found that more *P. leucopus* were found in plots without switchgrass, whereas more *M. musculus* were found in monoculture switchgrass plots. Based on these findings, Marshall *et al.* (in Review) suggested that either *M. musculus* outcompete *P. leucopus* in monoculture switchgrass plots, or that switchgrass does not provide suitable resources for *P. leucopus*. If the resource suitability hypothesis is correct, this could also explain why *P. leucopus* did not have a stronger response to switchgrass in "S" treatments. Marshall *et al.* (in Review) suggested that switchgrass-dominated understories in plots with switchgrass might not have provided sufficient resources (food and cover) to support *P. leucopus*. Based on vegetation sampling conducted in October 2010, "S" treatments were less diverse in vegetation structure and composition, compared with "PB-" and "P x SB-" (Marshall *et al.* in Review). Based on invertebrate prey sampling, prey availability was the same across all treatments (Table 4 and 5). That *P. leucopus* responded marginally, but not as strongly as anticipated in "S", indicated that vegetation composition and structure, not prey availability, likely was the main reason for lack a stronger response. This is consistent with studies have demonstrated that *P. leucopus* prefer structurally diverse microhabitat features (*i.e.*, course woody debris, understory complexity) versus less structural diversity for nesting and protection from predators (Greenberg 2002; Kaufman *et al.* 1983; Kirkland 1990; Menzel *et al.* 1999).

Regardless of how *P. leucopus* responded in "S", mice in intercropped treatments did respond as I had expected. Thus, the important result was that in "P x SB-" *P. leucopus* maintained their functional role, as it did not differ from mice in "PB-"

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treatments. This suggests that, at least in this early stage of research, food web interactions and ecosystem services linked to *P. leucopus* would not be affected by intercropping switchgrass in a loblolly pine plantation.

There are two caveats to my conclusions. First, *P. leucopus'* weak response to switchgrass may be related to the timing of the experiment. My study took place during the first two years following site preparation. Although switchgrass was well established by the second year of the study, it was a novel resource. *Peromyscus leucopus* may not have responded as strongly to switchgrass, as expected, because they did not yet learn of its' availability. Because *P. leucopus* is not known as a strong climber of grasses, the window of time that they could access seeds was only when seeds fell to the ground at the end of growing season. Once established, seed was available when switchgrass plants dropped seeds in the late summer/early fall of 2009 and 2010. In mammals, finding food may occur by trial and error, but more often it is the result of social learning from the parents (Rymer *et al.* 2008). In African striped mice (*Rhabdomys pumilio*), it is often the mother who demonstrates palatability of novel foods to her offspring, through olfactory or other sensory cues (Rymer et al. 2008). It has also been suggested that rodent fathers play a role in transmitting information about food and foraging to their offspring (Rymer et al. 2008; Schug *et al.* 1992). Thus, long-lived adults (up to 2 yrs. (Schug *et al.* 1991) who have lived through a few seed events have a better chance of learning about switchgrass and passing that information to their offspring. Therefore, the probability of finding mice that have learned about this pulsed resource should increase after several years of establishment. Revisiting this research question several years into the experiment

could provide more insight on *P. leucopus'* response when the novelty of this resource diminishes.

Second, the scale of this study was small. Trapping grids measured approximately 1,800 m^2, which is smaller than the documented home ranges of some male *P. leucopus* (Schug *et al.* 1991; Wolff *et al.* 1985). Thus, some individuals were not necessarily confined to one treatment plot. However, during my study, very few individuals were captured in more than one treatment plot and these individuals were not used in my analysis. Replicating this study on larger spatial scale would account for intraspecific variation in home range sizes of *P. leucopus*.

This intercropping experiment was one of the first of its kind to address land conversion and food security concerns associated with biofuel feedstock production. My study was one of the first to assess potential ecological effects of intercropping biofuels in a managed southeastern pine forest. My results suggest that this potentially new forest management approach would not impact food web interactions and ecosystem services associated with *P. leucopus*. However, future work should be considered to assess effects of biofuel intercropping on other consumers in this system.

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TABLES

Table 1. Total Number of Invertebrates by Family Collected by Branch Beating at The Lenoir 1 Sustainability Study Site, Lenoir Co., NC.

Invertebrates were collected in pine with residual woody debris removed (PB-), pine intercropped with switchgrass and residual woody debris removed (P x SB-), and switchgrass only (S) treatments in 2010.

Order	Family	Functional Group	PB-	PxSB-	S	Total
Orthoptera	Acrididae	herbivore	145	161	115	421
Hemiptera	Cicadellidae	herbivore	89	80	183	352
Hemiptera	Pentatomidae	herbivore	63	91	127	281
Araneae	unidentified	predator	101	104	68	273
Orthoptera	Tettigoniidae	herbivore	21	58	64	143
Lepidoptera	Geometridae	herbivore	62	79	0	141
Hemiptera	Coreidae	herbivore	70	37	3	110
Lepidoptera	unidentified larvae	herbivore	22	34	23	79
Hemiptera	Reduviidae	predator	14	24	40	78
Hemiptera	Nabidae	predator	13	18	46	77
Hymenoptera	Formicidae	omnivore	26	35	6	67
Orthoptera	Gryllidae	omnivore	16	34	15	65
Hemiptera	Cercopidae	herbivore	6	25	26	57
Coleoptera	unidentified	NA	25	12	11	48
Coleoptera	Chrysomelidae	herbivore	15	20	5	40
Coleoptera	Coccinellidae	predator	8	6	2	16
Coleoptera	Elateridae	omnivore	4	11	1	16
Coleoptera	Curculionidae	herbivore	7	5	1	13
Coleoptera	Phalacridae	herbivore	11	0	2	13
Coleoptera	Melolothinae	herbivore	11	0	0	11
Coleoptera	Staphylinidae	predator	0	0	11	11
Coleoptera	Carabidae	omnivore	1	2	7	10
Coleoptera	Cicinidelidae	predator	2	2	4	8
Coleoptera	Anthicidae	omnivore	0	1	5	6
Orthoptera	Tetrigidae	herbivore	2	4	0	6
Coleoptera	Lycidae	herbivore	0	5	0	5
Coleoptera	Cetoniinae	herbivore	1	3	0	4
Mantodea	Mantidae	predator	1	3	0	4
Coleoptera	Aphodiinae	decomposer	3	0	0	3
Coleoptera	Cantharidae	omnivore	1	1	1	3
Hemiptera	Cicadidae	herbivore	2	1	0	3
Hemiptera	Largidae	herbivore	3	0	0	3
Hemiptera	Lygaeidae	herbivore	2	1	0	3
Hemiptera	Miridae	omnivore	2	1	0	3
Coleoptera	Mordellidae	herbivore	0	2	0	2
Hemiptera	Berytidae	omnivore	1	0	1	2
Hemiptera	Membracidae	herbivore	1	1	0	2
Blattodea	Blattellidae	omnivore	0	1	0	1
Coleoptera	Lampyridae	omnivore	0	1	0	1
Hemiptera	Issidae	herbivore	1	0	0	1
Hemiptera	Phymatidae	predator	0	1	0	1
		Total	752	864	767	2383

Table 2. Total Number of Invertebrates by Family Collected in 2010 by Hand Picking at The Lenoir 1 Sustainability Study Site, Lenoir Co., NC.

Invertebrates were collected in pine with residual woody debris removed (PB-), pine intercropped with switchgrass and residual woody debris removed (P x SB-), and switchgrass only (S) treatments.

Order/class	Family	Group	PB-	PxSB-	S	Total
Coleoptera	Carabidae	omnivore	9	8	19	36
Orthoptera	Gryllidae	omnivore	6	9	10	25
Araneae	unidentified	predator	3	7	6	16
Hymenoptera	Formicidae	omnivore	0	9	0	9
Diplopoda (class)	unidentified	decomposer	4	0	3	7
Orthoptera	Acrididae	herbivore	2	5	0	7
Coleoptera	Tenebrionidae	omnivore	1	2	0	3
Hemiptera	Cercopidae	herbivore	0	2	1	3
Lepidoptera larvae	unidentified	herbivore	2	0	1	3
Coleoptera	Silphidae	decomposer	2	0	0	2
Hemiptera	Cicadellidae	herbivore	1	1	0	2
Hemiptera	Reduviidae	predator	0	2	0	2
Lepidoptera	Geometridae	herbivore	0	2	0	2
Blattodea	Blattellidae	omnivore	1	0	0	1
Coleoptera	Coccinellidae	predator	1	0	0	1
Hemiptera	Pentatomidae	herbivore	0	0	1	1
Orthoptera	Tettigoniidae	herbivore	0	1	0	1
		Total	32	48	41	121

Table 3. Total Number of Invertebrates by Functional Group Collected by Branch Beating and Hand Picking in 2010 at The Lenoir 1 Sustainability Study Site, Lenoir Co., NC.

Invertebrates were collected in pine with residual woody debris removed (PB-), pine intercropped with switchgrass and residual woody debris removed (P x SB-), and switchgrass only (S) treatments.

Functional Group	PB-	P x SB-	S
Herbivore	539	619	552
Omnivore	68	114	65
Predator	143	167	177
Decomposer	9	0	3
Total	759	900	797

Table 4. Mean Total Abundance (± 1SE)/Treatment of Invertebrates by Family Collected by Branch Beating and Hand Picking in 2010 at The Lenoir 1 Sustainability Study Site, Lenoir Co., NC.

Data are from invertebrates collected in pine with residual woody debris removed (PB-), pine intercropped with switchgrass and residual woody debris removed (P x SB-), and switchgrass only (S) treatments in 2010. Invertebrate families whose total abundance was less than 10 individuals and that were not present in all three treatments were excluded from this analysis. *P* values are from one-way ANOVA. The level of significance was adjusted with a Bonferroni correction, *P* value = 0.005. *Only keyed to order.

Family	PB-	*n*	PxSB-	*n*	S	*n*	*P* value
Acrididae	36.75 ± 8.20	147	41.50 ± 8.50	163	28.75 ± 3.0	115	0.50
Araneae (unidentified)*	26.0 ± 6.80	104	27.80 ± 6.14	111	18.50 ± 1.5	74	0.50
Carabidae	2.50 ± 0.87	10	3.33 ± 0.67	10	6.50 ± 1.70	26	0.11
Coleoptera (unidentified)*	6.25 ± 2.40	25	4.33 ± 1.20	12	3.67 ± 1.80	11	0.65
Cercopidae	3.0 ± 2.0	6	6.75 ± 1.44	27	6.75 ± 2.43	27	0.50
Chrysomelidae	3.75 ± 1.03	15	6.67 ± 1.20	20	1.67 ± 0.33	5	0.03
Cicadellidae	22.50 ± 3.23	90	20.25 ± 3.0	81	45.75 ± 18.41	183	0.24
Gryllidae	5.50 ± 2.60	22	10.75 ± 4.33	43	6.25 ± 2.02	25	0.47
Lepidoptera larvae (unidentified)*	6.0 ± 1.23	24	8.50 ± 1.20	34	6.0 ± 2.30	24	0.50
Nabidae	3.25 ± 1.03	13	4.50 ± 1.20	18	11.50 ± 2.22	46	0.01
Pentatomidae	15.75 ± 4.33	63	22.75 ± 1.40	91	32.0 ± 4.42	128	0.04
Reduviidae	4.67 ±1.33	14	6.50 ± 1.32	26	10.0 ± 1.80	40	0.11
Tettigoniidae	5.25 ± 1.32	21	14.75 ± 3.15	59	16.0 ± 3.0	64	0.03

Table 5. Mean Total Abundance (± 1SE) of Invertebrates by Functional Group Collected by Branch Beating and Hand Picking in 2010 at The Lenoir 1 Sustainability Study Site, Lenoir Co., NC.

Data are from invertebrates collected in pine with residual woody debris removed (PB-), pine intercropped with switchgrass and residual woody debris removed (P x SB-), and switchgrass only (S) treatments. Decomposers were excluded from this analysis because they were not present in all three treatments. *P* values are from one-way ANOVA.

Functional Group	PB-	*n*	PxSB-	*n*	S	*n*	*P* value
Herbivore	77.0 ± 27.76	534	103.17 ± 31.23	607	92.0 ± 31.22	549	0.82
Omnivore	8.50 ± 2.75	51	16.30 ± 4.82	87	8.13 ± 1.42	36	0.15
Predator	28.60 ± 7.15	139	23.86 ± 7.82	158	29.50 ± 9.01	171	0.86

Figure 1. Map of Weyerhaeuser NR Company's Lenoir 1 Sustainability Study Located in Lenoir County, NC.

Treatments examined include: (1) pine with residual woody debris removed (PB -), (2) pine and switchgrass intercropped with residual woody debris removed (P x SB -), and (3) switchgrass only (S). Additional treatments existed within each of the four blocks. However, these additional treatments were not part of my study and are not shown. Map created by J. Homyack.
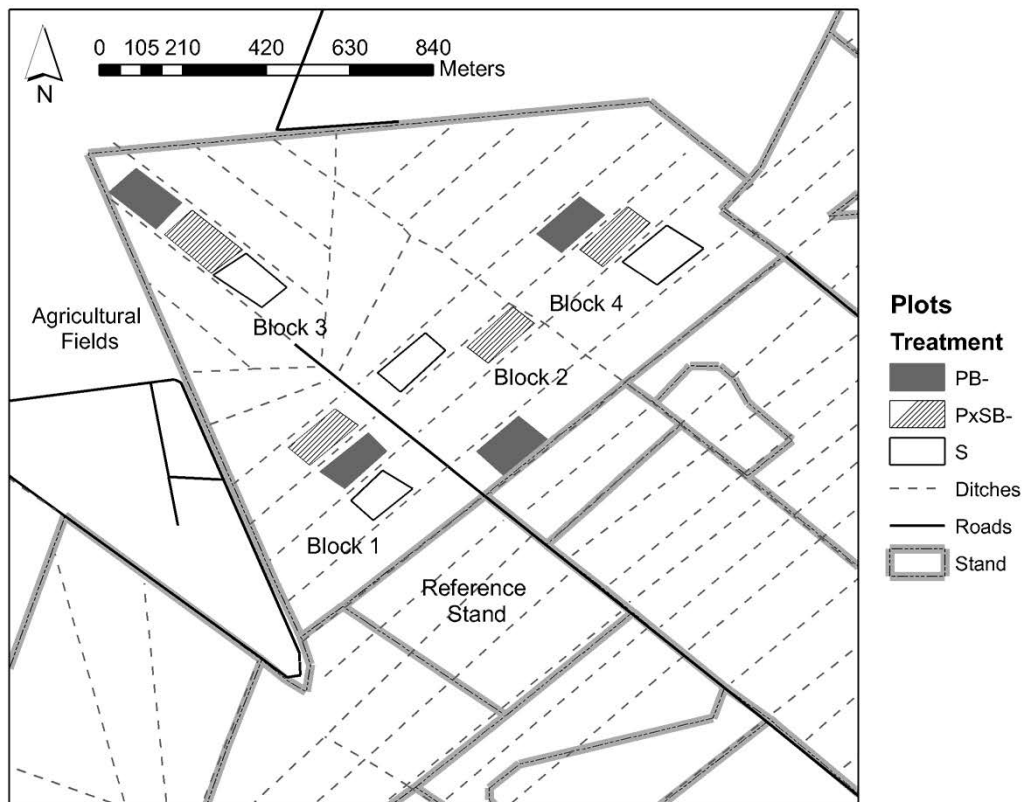
Figure 2. Bi-Plot of Mean ± 1 SE δ^{13}C and δ^{15}N Values for *P. Leucopus* Skin Samples in Summer and Fall 2009.

Skin was collected from *P. leucopus* in (1) pine with residual woody debris removed (PB-), (2) pine and switchgrass intercropped with residual woody debris removed (P x SB-), and (3) switchgrass only (S). Treatments are represented as follows: grey (PB-), stripes (P x SB-), and white (S). The δ^{13}C of mice in "PB-" and "P x SB-" in the summer (squares) was statistically different from mice in these treatments in fall (circles). Mean ± 1 SE δ^{13}C and δ^{15}N values for loblolly pine and switchgrass (triangles) collected during the 2010 field season are shown for comparison. The δ^{15}N of mice in "PB-" and "S" in the summer was statistically different from mice in these treatments in the fall. Data are from sampling conducted at the Weyerhaeuser NR Company Lenoir 1 Sustainability study site, Lenoir Co., NC.
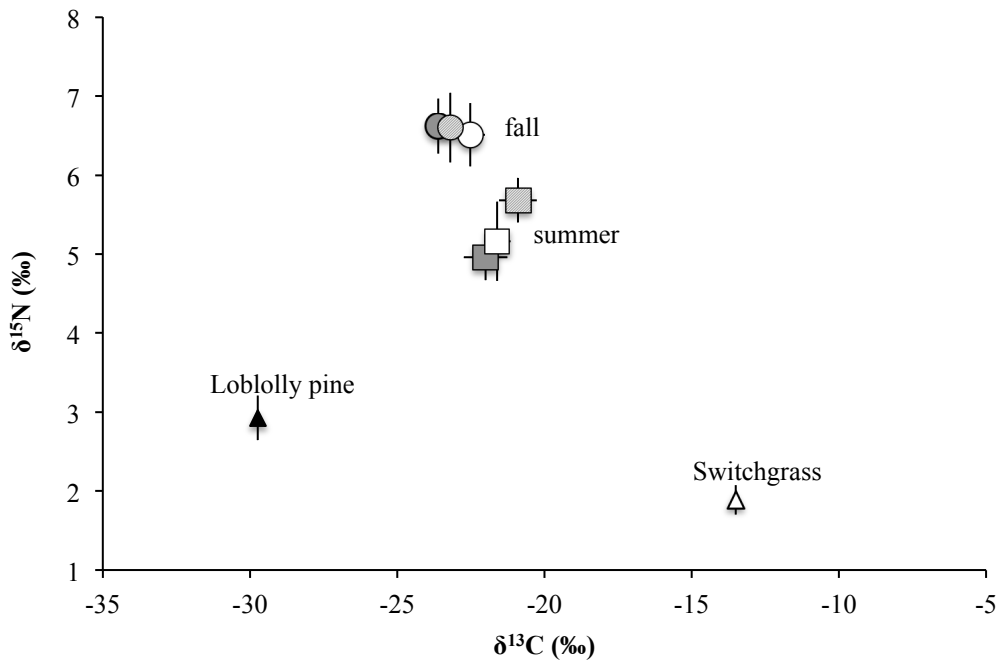
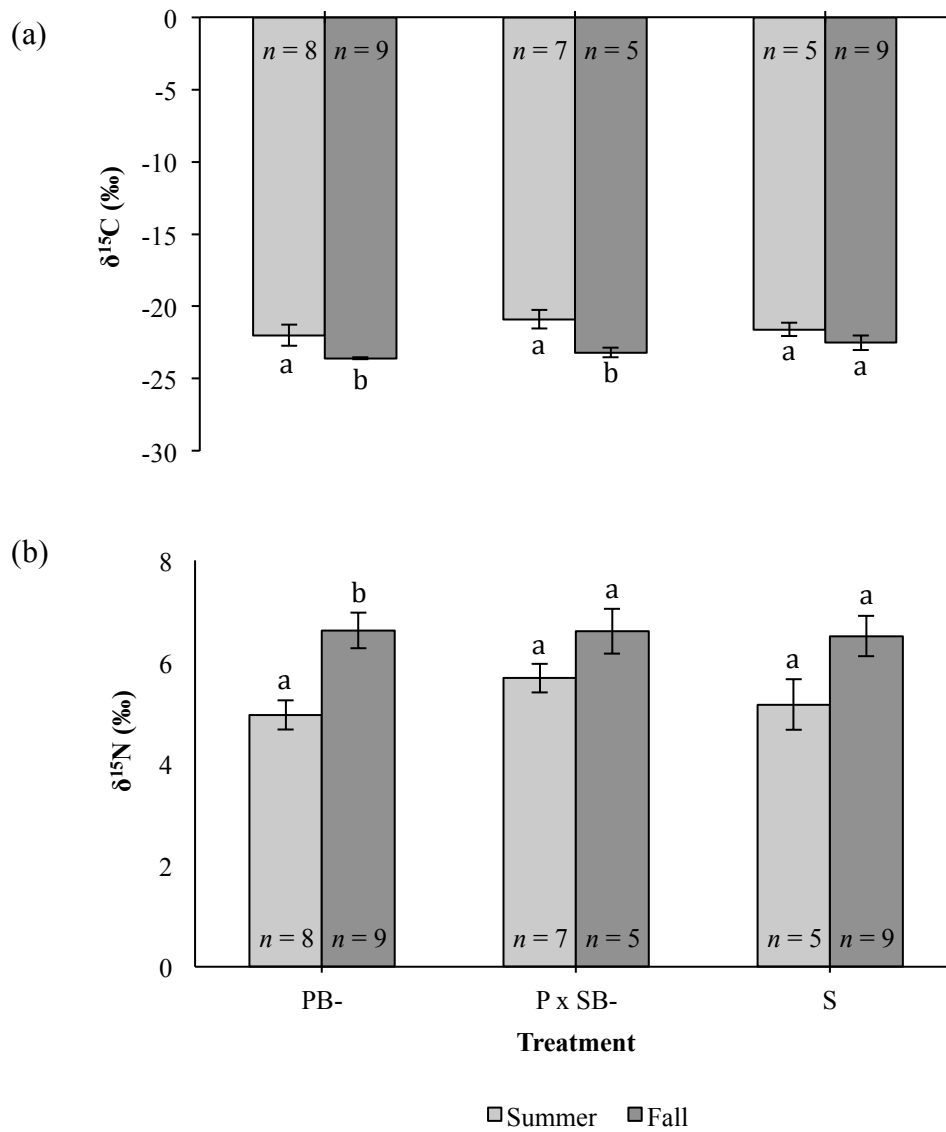**Figure 3.** Mean ± 1 SE in (a) $\delta^{13}C$ and (b) $\delta^{15}N$ Values for *P. Leucopus* Skin Collected in Summer and Fall 2009.

Skin was collected from *P. leucopus* in (1) pine with residual woody debris removed (PB-), (2) pine and switchgrass intercropped with residual woody debris removed (P x SB-), and (3) switchgrass only (S). Different letters indicate statistically significant differences. Data are from live trapping conducted in summer and fall 2009 at the Weyerhaeuser NR Company Lenoir 1 Sustainability study site, Lenoir Co., NC.

Figure 4. Mean ± 1 SE in (a) $\delta^{13}C$ and (b) $\delta^{15}N$ Values Between Skin and Fur Collected From *P. leucopus* in 2010.

Samples were pooled across all treatments including (1) pine with residual woody debris removed, (2) pine and switchgrass intercropped with residual woody debris removed, and (3) switchgrass only. Different letters indicate statistically significant differences. Data are from live trapping conducted in 2010 at The Weyerhaeuser NR Company Lenoir 1 Sustainability Study Site, Lenoir Co., NC.
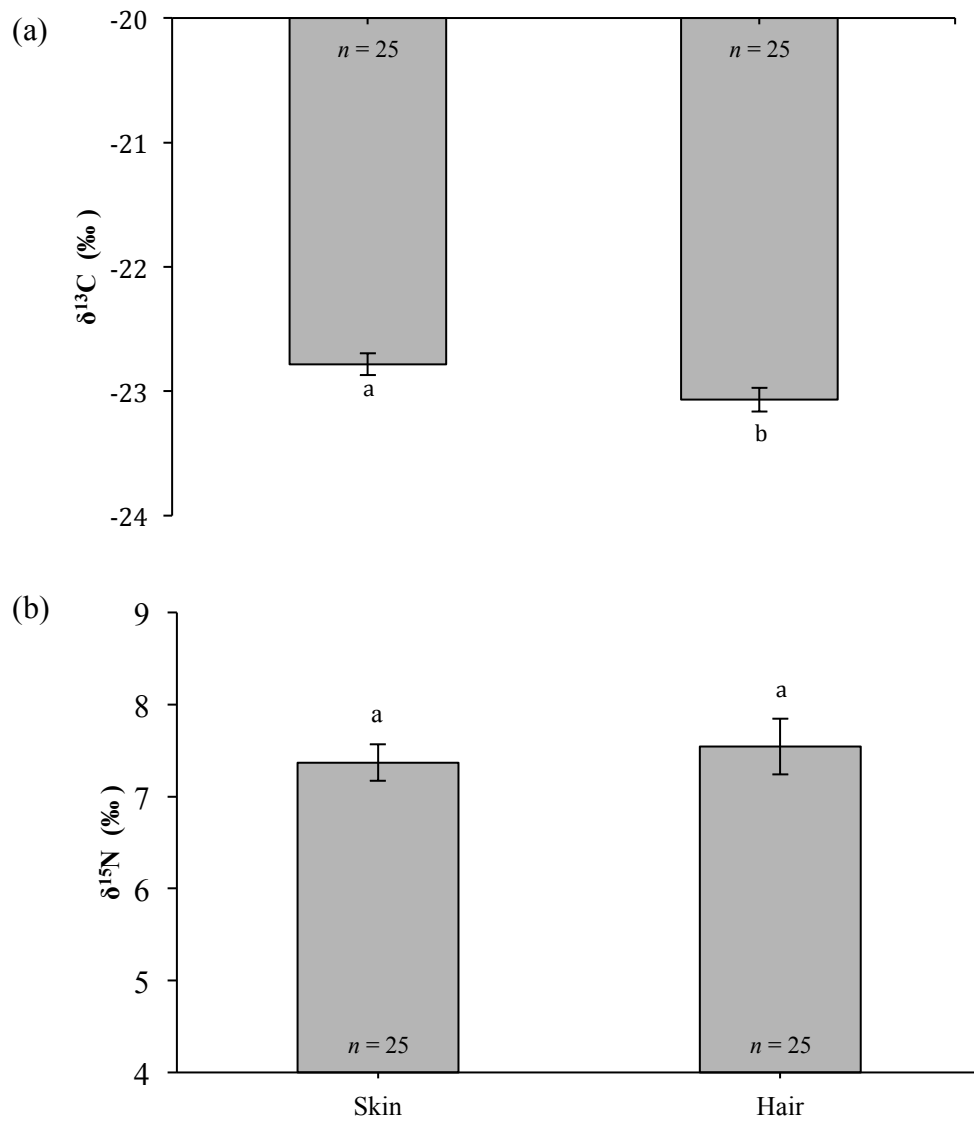
Figure 5. Mean ± 1 SE δ^{13}C and δ^{15}N Values for *P. leucopus* Fur Samples in 2010.

Peromyscus leucopus fur (○), whole invertebrates (□), loblolly pine needles (▲), and switchgrass seed (Δ) were collected in (1) pine with residual woody debris removed (PB-), (2) pine and switchgrass intercropped with residual woody debris removed (P x SB-), and (3) switchgrass only (S). Treatments are represented as follows: PB- (gray), P x SB- (stripes), and S (white). Invertebrates are grouped by trophic feeding guild (Herb: herbivores, Omni: omnivores, and Pred: predators) for each treatment. Data are from sampling conducted at the Weyerhaeuser NR Company Lenoir 1 Sustainability study site, Lenoir Co., NC. Sample sizes in PB- are PELE (n = 38), Herb (*n* = 31), Omni (*n* = 5), Pred (*n* = 16); P x SB-: PELE (*n* = 25), Herb (*n* = 34), Omni (*n* = 9), Pred (*n* = 20), and S: PELE (*n* = 10), Herb (*n* = 17), Omni (*n* = 3), and Pred (*n* = 10).
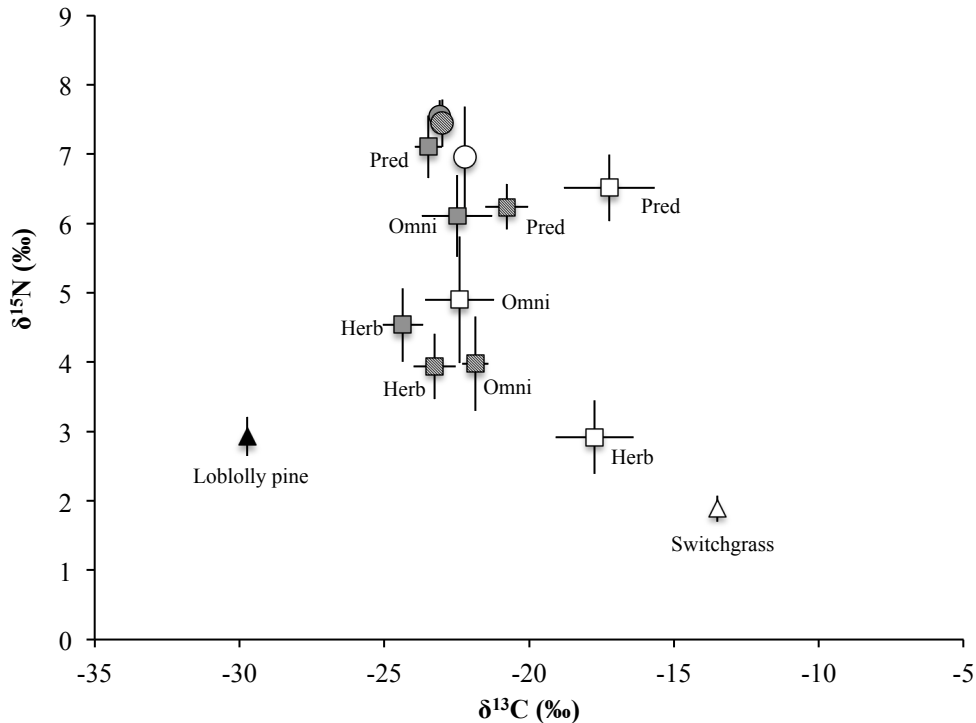
Figure 6. Mean ± 1 SE in (a) δ¹³C and (b) δ¹⁵N Values of *P. leucopus* Fur Samples Collected in 2010.

Tissue was collected from *P. leucopus* in (1) pine with residual woody debris removed (PB-), (2) pine and switchgrass intercropped with residual woody debris removed (P x SB-), and (3) switchgrass only (S) treatments. Different letters indicate statistically significant differences. Data are from live trapping conducted in 2010 at the Weyerhaeuser NR Company Lenoir 1 Sustainability study site, Lenoir Co., NC.
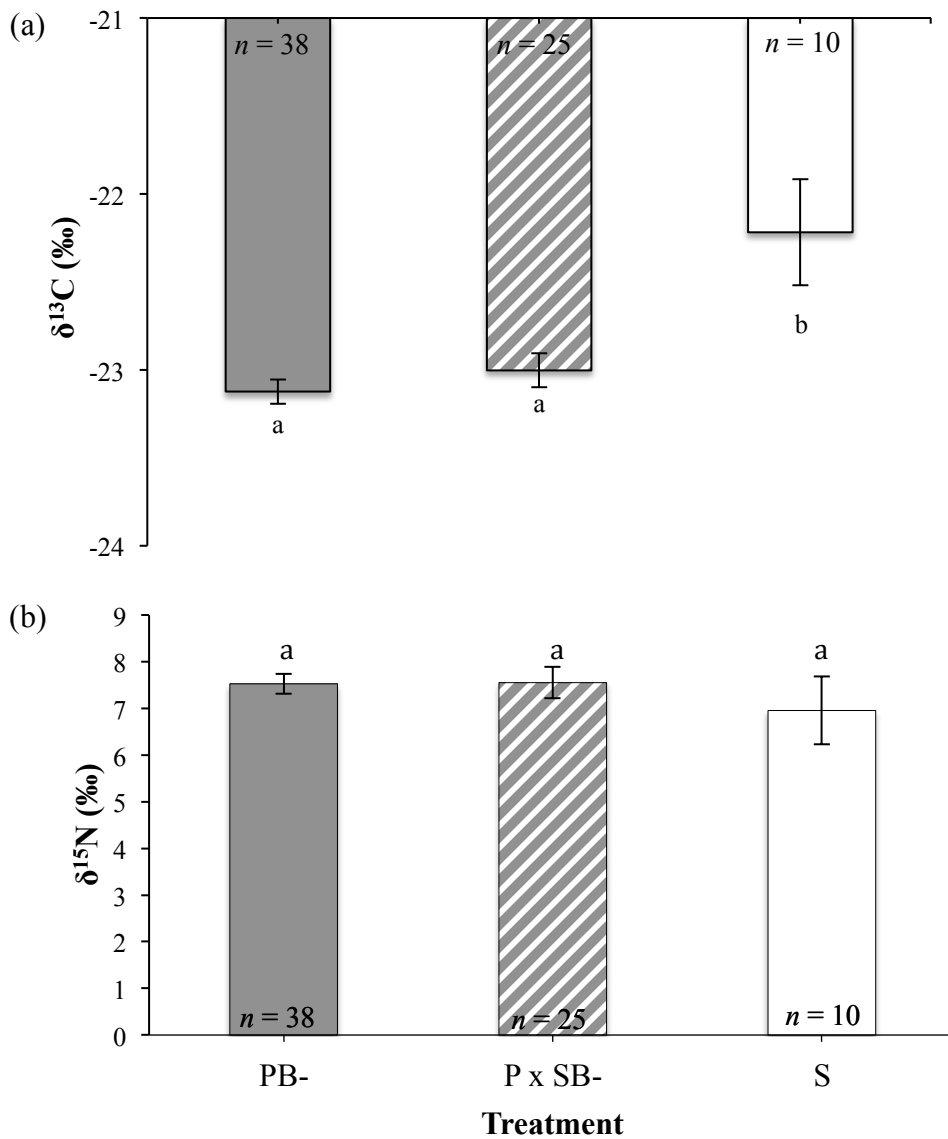
Figure 7. Mean ± 1 SE $\delta^{13}C$ and $\delta^{15}N$ Values for *P. leucopus* Skin and Fur Tissue Samples in 2010.

Peromyscus leucopus fur (circles), herbivore (diamonds), omnivore (squares), and predator (triangles) invertebrate tissue samples were collected from (1) pine with residual woody debris removed (PB-), (2) pine and switchgrass intercropped with residual woody debris removed (P x SB-), and (3) switchgrass only (S) treatments in 2010. Sample sizes in PB- are Pele (n = 36), Herb (n = 29), Omni (n = 5), Pred (n = 16); P x SB-: Pele (n = 26), Herb (n = 28), Omni (n = 15), Pred (n = 20), and S: Pele (n = 10), Herb (n = 15), Omni (n = 5), and Pred (n = 10). Data are from sampling conducted at the Weyerhaeuser NR Company Lenoir 1 Sustainability study site, Lenoir Co., NC.