NUTRIENT RESOURCES AND STOICHIOMETRY AFFECT THE ECOLOGY OF ABOVE- AND BELOWGROUND INVERTEBRATE CONSUMERS

by

JAYNE LOUISE JONAS

B.S., Wayne State College, 1998
M.S., Kansas State University, 2000

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Division of Biology
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Abstract

Aboveground and belowground food webs are linked by plants, but their reciprocal influences are seldom studied. Because phosphorus (P) is the primary nutrient associated with arbuscular mycorrhizal (AM) symbiosis, and evidence suggests it may be more limiting than nitrogen (N) for some insect herbivores, assessing carbon (C):N:P stoichiometry will enhance my ability to discern trophic interactions. The objective of this research was to investigate functional linkages between aboveground and belowground invertebrate populations and communities and to identify potential mechanisms regulating these interactions using a C:N:P stoichiometric framework. Specifically, I examine (1) long-term grasshopper community responses to three large-scale drivers of grassland ecosystem dynamics, (2) food selection by the mixed-feeding grasshopper *Melanoplus bivittatus*, (3) the mechanisms for nutrient regulation by *M. bivittatus*, (4) food selection by fungivorous Collembola, and (5) the effects of C:N:P on invertebrate community composition and aboveground-belowground food web linkages. In my analysis of grasshopper community responses to fire, bison grazing, and weather over 25 years, I found that all three drivers affected grasshopper community dynamics, most likely acting indirectly through effects on plant community structure, composition and nutritional quality. In a field study, the diet of *M. bivittatus* was dominated by forbs with grasses constituting only a minor fraction of their diet under ambient soil conditions, but grass consumption approximately doubled as a result of changes in grass C:N:P. *M. bivittatus* was found to rely primarily on selective consumption of foods with varying nutritional quality, rather than compensatory feeding or altering post-ingestive processes, to maintain C:N homeostasis in a laboratory experiment. In a soil-based mesocosm study, I show that Collembola feed on both saprophytic and AM fungi, in some cases exhibiting a slight preference for AM fungi. In the final study, although I did not find the expected indirect relationship between soil Collembola and aboveground herbivory as mediated through host plant quality, there were significant effects of root C:N and AM colonization on Collembola density and of plant C:N on aboveground herbivory. Overall, this research shows that host plant C:N:P stoichiometry can influence both above- and belowground invertebrate population, community, and food web dynamics.

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Aboveground and belowground food webs are linked by plants, but their reciprocal influences are seldom studied. Because phosphorus (P) is the primary nutrient associated with arbuscular mycorrhizal (AM) symbiosis, and evidence suggests it may be more limiting than nitrogen (N) for some insect herbivores, assessing carbon (C):N:P stoichiometry will enhance my ability to discern trophic interactions. The objective of this research was to investigate functional linkages between aboveground and belowground invertebrate populations and communities and to identify potential mechanisms regulating these interactions using a C:N:P stoichiometric framework. Specifically, I examine (1) long-term grasshopper community responses to three large-scale drivers of grassland ecosystem dynamics, (2) food selection by the mixed-feeding grasshopper *Melanoplus bivittatus*, (3) the mechanisms for nutrient regulation by *M. bivittatus*, (4) food selection by fungivorous Collembola, and (5) the effects of C:N:P on invertebrate community composition and aboveground-belowground food web linkages. In my analysis of grasshopper community responses to fire, bison grazing, and weather over 25 years, I found that all three drivers affected grasshopper community dynamics, most likely acting indirectly through effects on plant community structure, composition and nutritional quality. In a field study, the diet of *M. bivittatus* was dominated by forbs with grasses constituting only a minor fraction of their diet under ambient soil conditions, but grass consumption approximately doubled as a result of changes in grass C:N:P. *M. bivittatus* was found to rely primarily on selective consumption of foods with varying nutritional quality, rather than compensatory feeding or altering post-ingestive processes, to maintain C:N homeostasis in a laboratory experiment. In a soil-based mesocosm study, I show that Collembola feed on both saprophytic and AM fungi, in some cases exhibiting a slight preference for AM fungi. In the final study, although I did not find the expected indirect relationship between soil Collembola and aboveground herbivory as mediated through host plant quality, there were significant effects of root C:N and AM colonization on Collembola density and of plant C:N on aboveground herbivory. Overall, this research shows that host plant C:N:P stoichiometry can influence both above- and belowground invertebrate population, community, and food web dynamics.

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CHAPTER 1 - Introduction

Aboveground and belowground ecosystem components are intimately linked by plants, yet the dynamics that link above- and belowground components as a unified ecosystem are poorly understood and are legitimately considered an ecological frontier (Scheu and Setala 2002, Wardle 2002). Given the extensive network of interactions between above- and belowground subsystems, I selected a restricted subset of taxa located at strategic nodes that could had the potential to regulate above-belowground interactions (Fig. 1.1) because it is not feasible to adequately investigate all the above-belowground linkages. Plant shoots are the ultimate source of new carbon, while plant roots and the rhizosphere are key for the majority of belowground processes that drive carbon and nutrient cycling (Wardle 2002). Plants also form the basis of terrestrial invertebrate food webs, both above- and belowground. Much evidence suggests a potentially significant impact of soil-dwelling Collembola on arbuscular mycorrhizal fungi (AMF)-plant dynamics (Harris and Boerner 1990, Klironomos and Moutoglis 1999, Gange 2000), while it is also known that the nutritional composition of host plants can play an important role in the feeding ecology of insect herbivores (House 1969, Bernays and Chapman 1994, Simpson *et al.* 2004). Phosphorus (P) is the primary nutrient associated with mycorrhizal symbiosis that is formed between AMF and the roots of most grassland plant species (Smith and Read 2002), and recent hypotheses and some data suggest that it may be more limiting than nitrogen (N) for some aboveground insect herbivores (Elser *et al.* 2000, Schade *et al.* 2003, Perkins *et al.* 2004). Therefore, assessing carbon (C):N:P stoichiometry may greatly enhance my ability to discern trophic interactions in my model system (Coleman *et al.* 1983). Although the general focus of my research study takes a bottom-up perspective (i.e., arrorws in Fig. 1.1 are unidirectional), the interactions among components of the model are reciprocal and, therefore, influenced by top-down factors as well (Fig. 1.1). The goal of this research was to investigate interactions between aboveground and belowground invertebrates with plants and fungi as intermediaries, and to identify some of the mechanisms responsible for regulating interactions between various components of the food web. Using a stoichiometric approach, I investigated dynamic and mechanistic linkages regulating food webs including both above- and belowground

taxa. The central hypothesis was that over a range of soil nutrient conditions, fungivorous Collembola have a positive effect on mycorrhizal colonization of plant roots, increasing the nutritional quality of aboveground plant material for insect herbivores and thus influencing the degree of foliar herbivory. To address linkages in the conceptual model (Fig. 1.1), a series of experimental and observational studies were conducted.

In Chapter 2, using data from the Konza Prairie Biological Station (KPBS) Long-Term Ecological Research program that spans 25 years, I investigated the contributions of weather at annual and decadal scales, fire return interval, and grazing by bison to understand the dynamics of abundance and community composition in grasshopper assemblages from North American continental grassland. Each of these three primary large-scale drivers of grassland ecosystem dynamics are most likely to influence grasshopper population and community dynamics indirectly through changes in plant community structure, composition, or quality.

I examined the effects of fertilization and changes in host plant C:N:P on shifts in grass consumption by a mixed-feeding insect herbivore, *Melanoplus bivittatus* using natural abundance carbon isotopes ($^{12}C/^{13}C$) in a field experiment, in Chapter 3. The C isotope signatures of *M. bivittatus* collected from plots fertilized with or without nitrogen (+N), and with or without phosphorus (+P) are compared to the C isotope signatures of the plants in those plots to determine the proportion of assimilated C derived from C_4 grasses and C_3 forbs in each plot. I also examined the relationship between *M. bivittatus* diets and plant C:N:P stoichiometry.

As in most polyphagous herbivore species that perform best on diets containing plants from multiple families, mixed-feeding herbivores (those that feed on both forbs and grasses) also experience greatest performance when both forbs and grasses are consumed. Despite differences in elemental composition of forbs and grasses, mixed-feeding herbivores are able to maintain C:N:P homeostasis. Little is known about the mechanisms by which performance and homeostasis are maintained are in mixed-feeding herbivores. In Chapter 4, I assessed the roles of different regulatory mechanisms (food selection, compensatory feeding, or physiological adjustment) in *Melanoplus bivittatus*, a mixed-feeding insect herbivore, using natural and synthetic diet experiments.

Although soil-dwelling Collembola can influence plant growth and nutrient cycling, their specific role in soil food webs is poorly understood. Soil-free microcosm studies suggest that Collembola are primarily fungivores where they feed preferentially on saprophytic fungi (SF)

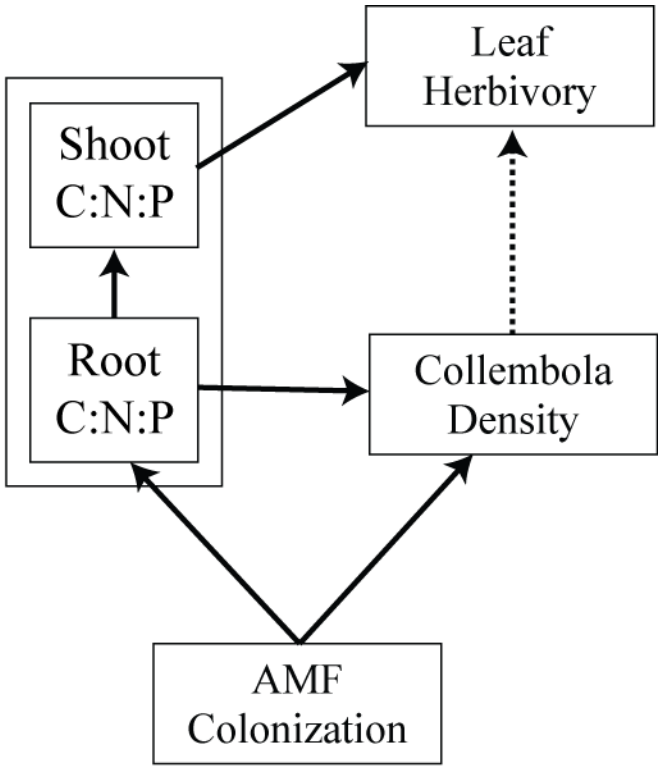
over other fungal types. In Chapter 5, I directly assessed collembolan consumption of arbuscular mycorrhizal fungi (AMF) and SF in a greenhouse experiment using plant-soil mesocosms and natural abundance stable carbon isotope techniques.

The objective of the research reported in Chapter 6 was to assess the linkages identified in the conceptual model using a field experiment. I examined the indirect linkages between soil-dwelling collembolans and aboveground herbivorous insects as they are influenced by shifts in plant root and shoot stoichiometry and arbuscular mycorrhzial fungi-plant symbiosis. Sampling each component of the model, including plant, Collembola, and grasshopper communities, in the long-term Belowground Plot Experiment at KPBS I employed structural equations modeling techniques to assess both the direct and indirect relationships hypothesized in the model.

In the final chapter of this dissertation, I briefly summarize and synthesize the results of these five studies as they relate to the conceptual aboveground-belowground invertebrate trophic model (Fig. 1.1). This synthesis suggests that fungivorous Collembola will positively affect the mycorrhizal symbiosis between AM fungi and plant roots, which would increase the quantity or nutritional quality of aboveground plant tissues for grasshoppers and thus increase the degree of foliar herbivory. These interactions between these above- and belowground invertebrate groups and plants should result in an indirect positive relationship between grasshoppers and Collembola. By using a stoichiometric approach, I also assessed the relative importance of N and P as limiting or co-limiting nutrients in the context of this tallgrass prairie invertebrate food web model.

Figures and Tables

Figure 1.1 Simplified conceptual aboveground-belowground food web model for tallgrass prairie systems guiding laboratory, greenhouse, and field experiments to address relationships between edaphic Collembola, arbuscular mycorrhizal fungi (AMF), plant carbon (C): nitrogen (N): phosphorus (P) stoichiometry, and aboveground herbivory by chewing insects.



CHAPTER 2 - Grasshopper (Orthoptera: Acrididae) communities respond to fire, bison grazing and weather in North American tallgrass prairie: A long-term study

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Abstract

Because both intrinsic and extrinsic factors influence insect population dynamics, operating at a range of temporal and spatial scales, it is difficult to assess their contributions. Long-term studies are ideal for assessing the relative contributions of multiple factors to abundance and community dynamics. Using data spanning 25 years, I investigate the contributions of weather at annual and decadal scales, fire return interval, and grazing by bison to understand the dynamics of abundance and community composition in grasshopper assemblages from North American continental grassland. Each of these three primary drivers of grassland ecosystem dynamics affects grasshopper population and community dynamics. Negative feedbacks in abundances, as expected for regulated populations, were observed for all feeding guilds of grasshoppers. Abundance of grasshoppers did not vary in response to frequency of prescribed burns at the site. Among watersheds that varied with respect to controlled spring burns and grazing by bison, species composition of grasshopper assemblages responded significantly to both after 25 years. However, after more than 20 years of fire and grazing treatments, the number of years since the last fire was more important than the managed long-term fire frequency *per se*. Yearly shifts in species composition (1983-2005), examined using nonmetric multidimensional scaling and fourth-corner analysis, were best explained by local weather events occurring early in grasshopper life cycles. Large-scale patterns represented by the Palmer Drought Severity Index and the North Atlantic Oscillation (NAO). NAO were significantly correlated with annual mean frequencies of grasshoppers, especially for forb- and

mixed-feeding species. Primary grassland drivers - fire, grazing and weather - contributing both intrinsic and extrinsic influences modulate long-term fluctuations in grasshopper abundances and community taxonomic composition.

Key Words: fire frequency, Konza Prairie, long-term ecological research (LTER), weather and insect populations

Introduction

Abundances and community structure of insects vary in response to both intrinsic and extrinsic factors, and understanding the relative roles of each to population and community processes is a long standing problem in ecology (Andrewartha and Birch 1954, Denno and McClure 1983, Barbosa and Schultz 1987, Royama 1992, Price 1995, Dempster and McLean 1998, Berryman 1999, Turchin 2003). Intrinsic influences from biotic interactions can act through multiple density- and frequency-dependent feedbacks that alter patterns of species abundances and coexistence (Dennis and Taper 1994, Brook and Bradshaw 2006). Extrinsic factors, as exemplified by weather conditions, act independently from the state of an insect population or community, but can affect insect population and community dynamics in a fashion reflecting their variable timing and extent (Dempster and McLean 1998). Effects of weather can sometimes act directly on insects. For example, body temperature influences metabolic processes, which in turn affects demographic responses and species interactions (Stamp 1993, Gutierrez 1996, Logan and Powell 2001, Logan *et al.* 2006). Weather can also act indirectly by altering the availability of resources (White 1993), affecting habitat structure, or influencing the strength of species interactions (Ovadia and Schmitz 2004). In grasslands, for example, differences in vegetation characteristics (biomass, structure, identity and diversity of plant species) and associated heterogeneity of these attributes strongly affect insect abundance, species richness and taxonomic composition (Evans 1988a, Rambo and Faeth 1999, Meyer *et al.* 2002, Joern 2004, Joern 2005). Variation in the action of both intrinsic and extrinsic factors, whatever the underlying mechanisms, influences observed patterns of insect population and community dynamics (Ovadia and Schmitz 2004). Because natural variability can have such effects on insect population and community dynamics, long-term studies greatly assist my ability to tease apart the nature of multiple combinations of key factors over time, facilitating the synthesis of the roles of external and internal drivers (Likens 1988, Magnuson 1990).

Grasshoppers are dominant insect herbivores in grasslands, naturally exhibiting much temporal variability in abundance and species composition (Joern and Gaines 1990, Lockwood 1993, 1997, Belovsky and Slade 2000, Meyer *et al.* 2002, Branson *et al.* 2006). Although multiple processes account for such responses (Joern and Gaines 1990), few studies assess the relative contribution of these effects over long periods of time. Both external factors driven largely by weather (Capinera 1987, Capinera and Horton 1989, Fielding and Brusven 1990) and density-dependent feedbacks from biotic interactions (Kemp and Dennis 1992, Chase and Belovsky 1994, Belovsky and Joern 1995, Belovsky and Slade 1995, Schmitz 1997, Schmitz and Sokol-Hessner 2002) may account for variation in grasshopper abundances and community composition. The boundaries of variability seen in grasshopper populations and communities over long periods remain to be fully delineated, and the mechanisms underlying the observed variability are largely unknown (Evans 1984, 1988a).

In this study, I analyze long-term grasshopper abundance data from Kansas Flint Hills tallgrass prairie to assess how fire frequency, bison grazing and weather – major drivers of tallgrass prairie structure and function (Knapp *et al.* 1998b) – modulate grasshopper abundances and species composition. Multiple studies at this site have shown the importance of these drivers to primary production, vegetation structure and plant community composition (Knapp *et al.* 1998b), but few studies have investigated the degree to which the same drivers affect consumers over the long term (Evans 1984, 1988a, 1988b, Kaufman *et al.* 1998).

Fire frequency and bison grazing clearly alter vegetation and plant communities with consequent influences on consumers in my study system (Collins *et al.* 1998, Knapp *et al.* 1999). I hypothesized that the effects of periodic fire on plant community dynamics would indirectly affect grasshoppers based on their feeding modes: grass-feeding species would be most abundant in annually burned sites, and forb-feeding species would be most common on sites with a 4-year fire return interval (FRI), a view supported by short term studies (Evans 1984, 1988b, Meyer *et al.* 2002, Joern 2004). Because they have a larger pool of potential host plants from which to select, I did not expect mixed-feeding species to be affected by FRI. I also expected that long-term grassland management practices would significantly shift the taxonomic composition of grasshopper communities as habitat characteristics changed (Joern 2004, Joern 2005). Species composition among watersheds, which was similar prior to the application of fire and grazing

treatments, was expected to diverge significantly in response to more than 20 years of alternate fire and grazing management activities.

As the third major grassland driver, weather has much potential to structure grasshopper communities at short-term and decadal time scales. Weather in North American continental grasslands is notoriously variable (Greenland *et al.* 2005) and may contribute to grasshopper population and community responses (Capinera 1987, Fielding and Brusven 1990). For example, grasshopper abundances and population growth rates from other North American sites have been significantly correlated with precipitation and temperature (*e.g.*, heating degree days) (Mukerji and Randell 1975, Gage *et al.* 1976, Mukerji and Gage 1978, Johnson and Worobec 1988, Capinera and Horton 1989, Fielding and Brusven 1990). At decadal scales, regional climate drivers such as the North Atlantic Oscillation (NAO), Pacific Decadal Oscillation (PDO), or El Niño-Southern Oscillation (ENSO) (Stenseth *et al.* 2002, Hurell *et al.* 2003, Mysterud *et al.* 2003, Hallett *et al.* 2004) may independently contribute to predicting temporal changes in species abundances and community structure. Although decadal weather patterns influence responses by other organisms (Lima *et al.* 1999, Merritt *et al.* 2001, Lima *et al.* 2002, Stenseth *et al.* 2002), the impact on continental North American grassland insects is unknown. Long-term trends suggest that decadal weather patterns in association with other factors could play a significant role in grasshopper population and community responses (Branson *et al.* 2006).

As grasshopper abundances and community species composition are expected to differ according to responses by key resources to fire frequency, bison grazing and weather (Evans 1984, 1988a, 1988b, Belovsky and Joern 1995, Belovsky and Slade 1995, Joern and Behmer 1998, Joern 2004, Branson 2005, Joern 2005), I address the following primary goals using long-term abundance data: (a) evaluate the effects of fire frequency on temporal changes in grasshopper abundance; (b) determine the likelihood of negative feedbacks characteristic of population regulation as contrasted with highly variable fluctuations driven by external factors; (c) investigate the importance of fire frequency and bison grazing on grasshopper community composition; and (d) contrast decadal-scale weather influences on grasshopper communities with those based on short-term annual weather events. Finally, conclusions from analyses of long-term data will be compared with those of short-term studies to get a sense of the proper scale for understanding grasshopper dynamics in this continental grassland. Population abundances of

three feeding guilds (grass-feeders, mixed-feeders and forb-feeders), which range from oligophagous to polyphagous accordingly, are treated separately in my analyses.

Methods and Approaches

Konza Prairie long-term grasshopper data

Data used in these analyses were collected at Konza Prairie Biological Station (KPBS), Manhattan, Kansas as part of the US National Science Foundation Long-Term Ecological Research (LTER) program and archived on the Konza LTER website, www.konza.ksu.edu (dataset cgr022). A replicated watershed-level experimental design at KPBS allows one to test the long-term effects of ungulate grazing and periodic fire on the tallgrass prairie ecosystem (Knapp and Seastedt 1998). Prescribed fire treatments were implemented between 1972 and 1977, and a bison (*Bos bison*) herd was introduced to a portion of KPBS in stages between 1987 and 1992.

Grasshoppers were sampled at all transects twice a year, during the mid-summer, from 1982-2005 on six to eight ungrazed watersheds and from 2002-2005 on six watersheds grazed by bison. Grasshoppers were sampled from two permanent upland transects in each watershed used. The abundance of each grasshopper species collected in each of 10 sets of sweep-net samples along each transect is reported; each dataset records abundances of 20 sweeps for a total of 200 sweeps per transect. Data from 1992-1995 were unavailable because samples were damaged prior to identification. Detailed information on the sampling protocol is described in the cgr022 metadata file available on the Konza Prairie LTER website.

Frequency of each grasshopper species was calculated from these data as the proportion of the 10 sets in which a species was collected at a given transect; all frequency values were arcsine-square root transformed prior to statistical analysis. Due to the sensitivity of sweep-net sample counts to sampling technique (Evans *et al.* 1983) and the inability to translate these count data directly to density (individuals/m^2), frequency was used as the most appropriate measure of abundance for comparisons among watersheds and years.

Statistical Analyses

An unbalanced hierarchical repeated measures analysis of variance was used to test the effects of fire, time and the interaction between fire and time on the mean frequency of species

within each grasshopper feeding guild. Grasshopper species were classified as belonging to one of three feeding guilds: grass-feeding, forb-feeding, or mixed-feeding (Mulkern *et al.* 1969, Campbell *et al.* 1974). Watersheds within treatments (1 year FRI: watersheds 1D and SpB, 4 year FRI: watersheds 4B and 4F, 20 year FRI: watershed 20B) and transects within watersheds were treated as random effects and time was a repeated measures factor. These watersheds range in size from 16.4 – 54.5 ha, with transects within each watershed located on upland fingers separated by 250 – 500 m. Grasshopper data from watersheds containing bison were not collected until 2002, therefore grazed treatments were not included in this analysis because the time series was too short.

Species composition was analyzed using non-metric multi-dimensional scaling (NMDS) and fourth corner statistics (Legendre *et al.* 1997, Dray and Legendre *in review*). NMDS ordination was based on Euclidean distance in PC-ORD (version 4) with each solution based on 40 runs of the data with the final solution compared against 50 Monte Carlo runs (McCune and Grace 2002). While I was able to non-parametrically and visually assess changes in species composition in relation to environmental variables with NMDS, fourth corner statistics allowed us to assess the effects of environmental variables on communities based on behavioral, morphological, or life history characteristics of the species. The fourthcorner package (http://biomserv.univ-lyon1.fr/~dray/) was used in the R program v. 2.4.1 (RDCT 2006) to conduct multivariate and univariate fourth corner analyses. Permutation models 2 and 4 were run for all datasets (Dray and Legendre *in review*). Permutation model 2 tests the hypothesis that species composition is linked to environmental variables (H_1), while model 4 tests the hypothesis that species composition is linked to species traits (H_2). To test the hypothesis that environmental variables influence composition based on species traits (H_3) at $\alpha_3=\alpha_1*\alpha_2=0.05$, I used $\alpha_1=\alpha_2=0.2236$ for both models 2 and 4 (Dray and Legendre *in review*). The species traits used were feeding guild, life history, flight ability, and size (S1). For the species at KPBS, species characteristics were independent of one another (chi-square analyses, $p>0.1$) except for feeding guild and flight ability which were marginally significant ($P=0.062$).

The mean frequency of species in each watershed was used to analyze community structure in 1982 and 2005 in response to watershed-scale management practices. The environmental matrix for these analyses included bison grazing and FRI as categorical variables

and the number of years since fire (YSF) as a quantitative variable. Fire-related data for KPBS were obtained from the "Burn History" dataset available on the Konza LTER website.

To assess changes in grasshopper communities over time as influenced by larger-scale weather patterns, the mean frequency of each species at KPBS was calculated for each year. The environmental matrix included 12 local precipitation and temperature factors chosen *a priori* as those most likely to affect grasshoppers during different developmental stages, a state-level index of drought conditions (Palmer Drought Severity Index), and three large-scale atmospheric phenomena which can affect interior continental North America: the Southern Oscillation Index (SOI), the Pacific Decadal Oscillation (PDO) index, and the North Atlantic Oscillation (NAO) index (Table 2.1). Local weather variables for KPBS were collected near the headquarters area at KPBS and assembled from the awe01 dataset available on the Konza LTER website. PDI values for Kansas were downloaded from the National Climate Data Center climate monitoring website (http://www.ncdc.noaa.gov/oa/climate/research/monitoring.html). SOI and NAO values were downloaded from the National Center for Atmospheric Research, Climate Analysis Section website (www.cgd.ucar.edu/cas/). PDO values were downloaded from the University of Washington Joint Institute for the Study of the Atmosphere and Oceans website (www.jisao.washington.edu/pdo/).

Results

Long-term trends in grasshopper abundance

The grass-feeding species, particularly *Phoetaliotes nebrascensis* and *Orphulella speciosa*, were the most abundant grasshoppers at KPBS; forb-feeding species, especially *Melanoplus keeleri*, *Mel. scudderi*, and *Hypochlora alba*, were not as common (Appendix A.1). Mixed-feeding species, including *Mel. femurrubrum* and *Mel. bivittatus*, were among least common grasshoppers at KPBS in these samples. Grass-feeding species frequencies displayed the greatest variability across years (coefficient of variation, CV = 4.5), while mixed-feeding species were the least variable (CV=1); the CV of frequency=3.3 for forb-feeding species. Despite the variability in abundances, plotting frequency_t versus frequency_{t+1} shows that all three feeding guilds vary through time around the line with a slope equal to one (no change in population size between years) (Fig. 2.1).

Effects of fire on grasshopper feeding guilds

Fire alone did not significantly impact the frequencies of any of the three feeding guilds, while all three did vary with time over the twenty-four years of this dataset (Table 2.2, Fig. 2.2). Only the mixed-feeding guild had a significant interaction between FRI and year (Fig. 2.2C), with abundance on 4- and 20- year FRI watersheds typically more similar to one another than on the annually burned watersheds, particularly from 1992 – 2005.

Effects of fire and grazing on grasshopper species composition

Grasshoppers were first sampled in 1982 after approximately ten years of fire management, but prior to the reintroduction of bison at KPBS. NMDS ordination did not detect structure in the species composition on the watersheds in 1982. Fourth-corner analysis showed a significant effect of YSF on composition based on the flight ability and size of the species (Table 2.3A). Poor fliers and medium-sized species were positively associated and strong fliers and large species were negatively associated with increased YSF (Appendix A.2).

NMDS analysis of 2005 grasshopper species composition resulted in an ordination with a final stress of 3.03 and instability of 0.00001 after 91 iterations. The 3-dimensional solution accounted for 98% of the variation in the data (axis 1: r^2=0.35, axis 2: r^2=0.24, axis 3: r^2=0.39). The number of years since the last fire (YSF) was associated with axes 1 and 2 of the ordination, while there was no detectable relationship with fire return interval (FRI) (Fig. 2.3A). There was also a significant effect of YSF on composition based on the flight ability of the species (Table 2.3B) in fourth-corner analysis. Poor fliers were positively associated with increased YSF, while strong fliers were negatively affected by YSF (Appendix A.2). NMDS ordination indicated that there was a large effect of bison grazing on grasshopper communities (Fig. 2.3), although fourth-corner results showed a strong effect of bison under model 2 only (Table 2.3B).

Influence of weather on grasshopper communities

Ordination of annual mean frequencies of grasshopper species at KPBS had a final stress of 4.73 and instability of 0.00001 after 61 iterations (Fig. 2.4). The 3-dimensional solution accounted for 96.6% of the variation in the data (axis 1: r^2=0.40, axis 2: r^2=0.28, axis 3: r^2=0.29). The ordination of annual mean frequencies revealed associations between grasshopper communities and growing season precipitation$_t$ (GSPPT$_t$) and PDI along axis 2 and NAO along axis 3 (Fig. 2.4). Nine weather variables were significant in fourth corner analysis (Table 2.3C);

of these, seven were local and two were larger-scale variables. Growing season precipitation at *t* and *t*-1, CV of growing season precipitation$_t$, and winter temperature were associated with shifts in species composition related to feeding guilds and flight ability, as was NAO. The positive phase of the NAO was associated with increased abundance of forb-feeders and poor fliers, while mixed-feeders and strong fliers were more common during the negative phase of the NAO cycle (Appendix A.3). Shifts in composition relating to size and phenology of the species were associated with growing season heating degree days$_{t-1}$, CV growing season precipitation at *t* and *t*-1, fall temperature, and PDI (Table 2.3C). Medium-bodied and late-hatching species were more common during years with high PDI values, while large-bodied and early-hatching species were more abundant during low PDI years (Appendix A.3).

Discussion

Long-term records indicate that grasshopper population and community dynamics at Konza Prairie are highly responsive to both extrinsic and intrinsic factors. Extrinsic effects of weather influence grasshopper abundances and community composition. Bison grazing also effects community composition, although it is not yet known if feedbacks exist between grasshopper and bison grazing. There is also strong evidence for population regulation in the form of negative-feedbacks for all feeding guilds (Fig. 2.1), an intrinsic response consistent with the action of density-dependence (Royama 1992, Turchin 2003, Brook and Bradshaw 2006). These feedback patterns were also found for most of the individual species (data not shown) such that the pattern seen at the feeding guild level generally reflected that of the species within each guild. My results extend the findings of Evans (1988a) and Collins (2000) who concluded that grasshopper communities at KPBS are regulated through biotic feedbacks. The general trend toward stability identified by Evans (1988a) using only 5 years of grasshopper data was supported by my analysis spanning 25 years of community data. The action of extrinsic drivers in this system interact with density-dependent processes, probably by altering resource availability or the ability of grasshopper consumers to utilize resources (Ovadia and Schmitz 2004). Understanding grasshopper responses in North American continental grasslands requires that I consider multiple influences that operate at a range of spatial and temporal scales.

I could not conduct a formal continuous time series analysis because of an unfortunate four-year gap in the data (1992-1995). I dealt with this break in the data by comparing pre- and

post-gap periods when assessing effects on community composition, or I relied on the time series segments that were available to assess regulation (Fig. 2.1). The gap in the record did prevent us from using some nonlinear analyses to evaluate population and community trends (Turchin 2003), but I do not feel that my general conclusions are compromised.

Effects of fire and bison on grasshopper feeding guilds

Fire had only a minor effect on fluctuations in grasshopper frequencies. After more than 25 years of fire management at KPBS, the mixed-feeding guild was the only one to show an interaction between fire frequency and year. Like fire management in most temperate North American grasslands, the majority of prescribed burning at KPBS, is currently conducted during the spring. Although I did not detect strong or consistent effects of fire on grasshopper abundance, it is possible that fire during other seasons may lead to more pronounced responses. For example, late summer fires are more likely to negatively effect grasshopper abundance because it is a time when many grasshopper species mate and lay eggs (Dempster 1963, Hewitt 1985).

Spring fires have significant effects on habitat characteristics. Although they may accelerate grasshopper phenology by removing litter and warming the upper soil layers (Evans 1984, Meyer *et al.* 2002), I expected that spring fires would have a stronger effect on grasshoppers due to changes in plant community composition and structure. Annual burning shifts plant communities toward warm-season C_4 grass dominance and decreases in cool-season C_3 grass and forb abundance (Hartnett and Fay 1998, Collins 2000). Although the warm-season grasses do not decrease, infrequent fires are typically associated with increased abundances of cool-season grasses, forbs and woody species than found on frequently burned sites (Hartnett and Fay 1998).

Grass-feeding species were somewhat but not significantly more common in annually burned than infrequently burned sites (Fig. 2.2A), as expected given the positive effects of annual spring fires on warm-season grasses. Two previous, short-term studies conducted at KPBS using different measures of abundance found grass-feeding species were most common on annually burned watersheds (Evans 1988b, Meyer *et al.* 2002); a third short term study showed a similar but not significant trend for increased densities on annually burned watersheds (Joern 2004). Four of the seven years in which the short-term studies were conducted are coincident with those used in the long-term dataset here and also showed grass-feeding species to be more abundant on

annually burned watersheds. However, grass-feeding species were found to be equally or more abundant on watersheds experiencing less frequent fire in several other years. Cool-season grasses, which are generally more common on infrequently burned watersheds, also tend to account for a large portion of the diets of many grass-feeding species (Mulkern *et al.* 1969), although most grass-feeders also readily consume warm-season grasses (Heidorn and Joern 1984, Pinder and Kroh 1987, Pinder and Jackson 1988, Barbehenn *et al.* 2004a). These unexpected patterns not only demonstrate the utility of long-term data for capturing nuances in temporal dynamics, but also my incomplete understanding of the mechanisms driving this ubiquitous group of consumers. This is of particular importance given that multiple short-term studies conducted at the same study site did not fully capture the relationship between fire and annual variability in grass-feeding species.

Although Evans (1984, 1988b) found that forb-feeding species were most common on watersheds with a 4-year FRI in short-term studies, my long-term results show that forb-feeder frequencies were not significantly affected by FRI. Forb-feeding species tended to be most abundant and diverse on the infrequently burned sites (Collins and Steinauer 1998), however, and it is possible that they were tracking changes in the composition or structure of plant communities. Since at least 1996, forb-feeder frequencies have shown an overall decline. Unfortunately, it appears that this decline began sometime between 1992 and 1995, the years for which grasshopper data are unavailable. Long-term shifts in plant communities (Collins 2000), especially in infrequently burned watersheds, may be negatively affecting forb-feeding species through decreased availability of suitable host plants or habitat structure.

Little is known about the ecology of mixed-feeding grasshopper species. Because they tend to occur in low abundances in native grasslands and typically prefer forbs (Mulkern *et al.* 1969, Campbell *et al.* 1974), mixed-feeding species have often been pooled with forb-feeding species (Evans 1984, 1988a, 1988b). I did not expect mixed-feeding species to be affected by spring fire given my view that spring fire would most likely affect grasshoppers indirectly by altering plant community composition and that mixed-feeders have a wider diet breadth (both grasses and forbs). The mixed-feeding guild, however, was the only one to show a significant interaction between FRI and year (Fig. 2.2c) although the pattern remains unclear.

The results of my analyses on the effects of both fire and bison grazing on species composition provide further support for my conclusion that spring fire alone is not a primary

driver of grasshopper dynamics. The first set of grasshopper samples at KPBS, collected in 1982, were taken five to ten years following initiation of fire treatments, but prior to the reintroduction of bison. Although NMDS ordination found no differences in communities at the sites sampled in 1982, fourth-corner analysis showed an effect of YSF associated with the flight ability of species in both 1982 and 2005. These results strongly suggest that species with well developed flight abilities responded to recently burned areas more quickly than those that are poor fliers.

After more than twenty-five years of fire and nearly fifteen years of bison grazing, however, the contributions and interactions of these ecosystem drivers on grasshopper community dynamics appear to be complex. The ordination identified bison grazing as having a strong influence on species assemblages in 2005 (Fig. 2.3A), with a general increase in the abundance of forb- and mixed-feeding species on grazed sites (Appendix A.2). Ungrazed sites were characterized by two dominant tallgrass prairie grasshoppers, *P. nebrascensis* and *O. speciosa*, both of which are grass-feeding species (Fig. 2.3B). Grazed watersheds were more likely to contain a diversity of species from all three feeding guilds, including several species which are generally uncommon (frequency <0.10), including *P. haldemanii*, *C. viridifasciata*, and *Mel. sanguinipe*s (Fig. 2.3B). In fourth-corner analyses, there were significant effects of bison grazing under model 2 only, which indicates that the relationship between grazing and grasshopper species composition is complex and I was not able to capture in my analysis the dynamics of the relationship with the actual species traits.

Grazed watersheds are characteristically more complex floristically than ungrazed sites with regard to plant communities (Towne *et al.* 2005, Collins and Smith 2006). Not only does grazing influence the identity of plant species present (including increased plant species diversity and increased cover of forbs) (Collins *et al.* 1998), but it also leads to greater variability in the horizontal and vertical structure of vegetation (Joern 2005, Towne *et al.* 2005). Areas of dense vegetation potentially provide refuge from predators (Schmitz 2006), while areas of bare ground facilitate thermoregulation and oviposition activities (Dempster 1963, Hewitt 1985, Joern 2005). In assessing the relationship between grasshopper species richness and several plant community characteristics, Joern (2005) found species richness to be most strongly associated with variability in plant canopy height, accounting for 45% of the variation in grasshopper species richness. Plant species richness showed a significant effect on grasshopper species richness (Joern 2005).

Both NMDS ordination and fourth-corner analysis also revealed a relationship between species composition and fire along axis 2. However, it was the number of years since the last fire, rather than overall fire frequency, that influenced species composition; communities on watersheds burned less than a year prior to sampling (YSF≤1) were different than those that had not been burned for many years (YSF≥9), regardless of grazing treatment. A similar result was reported by Joern (2004) with regard to overall grasshopper density. There were no significant effects of FRI on grasshopper density, while there was a significant correlation between density and the time since fire with the highest densities occurring in sites with YSF≤1. Bison tend to selectively graze recently burned areas (YSF<2) (Biondini *et al.* 1999). At KPBS the grazing treatment is not implemented on individually fenced watersheds; there is one large grazing enclosure encompassing 12 watersheds that receive the full suite of fire treatments. Therefore, long-term unburned watersheds in grazed areas may not be heavily influenced by bison during the growing season and essentially function as ungrazed sites from the perspective of grasshoppers.

These data also suggests that complex interactions between these two groups of consumers may operate. Based on consumption estimates and feeding characteristics (Pfadt 1994, Meyer *et al.* 2002), grasshoppers may potentially remove up to 20% annual net primary production (ANPP) in grasslands. Considering current stocking rates of bison at KPBS results in the removal of 20-25% ANPP, it is reasonable to expect that interactions between invertebrate and ungulate grazers are important. For instance, bison feed primarily on grasses and tend to select warm-season over cool-season grasses disproportionate to their abundance during the growing season (Plumb and Dodd 1993). Most of the grasshoppers strongly associated with ungrazed watersheds were the grass-feeding species that also tend to consume warm-season over cool-season grass species (Mulkern *et al.* 1969) (Fig. 2.3B).

Influence of weather on grasshopper communities

Of the three primary grassland ecosystem drivers, climate-associated weather patterns are the most unpredictable. Unlike fire and grazing treatments at KPBS, which are managed at the watershed level, weather patterns operate at a larger-spatial scale and are similar for all of the watersheds in any given year. Growing season precipitation was the only local weather factor correlated with species composition in NMDS ordination (axis 2) and also the only one in which

fourth corner analysis revealed shifts in composition to be associated with feeding guilds (Table 2.3C, Appendix A.3). Precipitation and temperature factors are the abiotic factors most often associated with grasshopper population dynamics (Dempster 1963, Gage and Mukerji 1977, Hewitt 1985, Capinera and Horton 1989). Sensitivity of grasshopper populations to precipitation is likely associated with tradeoffs between host plant quality, quantity, and susceptibility to predators, pathogens and parasites (Dempster 1963, Hewitt 1985, Joern and Gaines 1990, Belovsky and Slade 1995, Joern and Behmer 1998, Joern and Mole 2005). Temperature can affect insects both directly by influencing developmental rates (Mukerji and Randell 1975, Mukerji *et al.* 1977), and indirectly through changes in plant community structure and host quality (Richardson *et al.* 2002). I included PDI in my analysis as a measure of the combined effects of precipitation and temperature on grasshopper communities, although the index integrates these factors over a larger spatial extent than just KPBS. I found that PDI was important in both NMDS and fourth corner analyses. Fourth-corner analysis shows that PDI reflects the combined effects of the four local weather variables associated shifts in grasshopper species composition based on size and phenology (Table 2.3C). The weak positive relationship between NMDS axis 2 and both PDI and growing season precipitation, and the negative effect of PDI on early hatching species (Appendix A.3), suggests that interactions between temperature and precipitation may be most important in influencing development of grasshoppers during embryonic and early nymphal stages.

There was also a strong signal between the decadal level influence of NAO and grasshopper species composition (Fig. 2.4, Table 2.3C). To my knowledge, this is the first study to document a link between the NAO and terrestrial insects in North America, although it has been correlated with the population dynamics of spittlebugs in Finland (Halkka *et al.* 2006) and other taxa, including plants, amphibians, birds, and mammals, in both North America and Europe (Mysterud *et al.* 2003). A distinct shift in grasshopper communities between the positive and negative phases of the NAO is clear (Figs.2. 4 and 2.5). Although NAO may affect precipitation patterns, its primary effect on continental North America appears to be associated with increased wintertime temperatures (Hurell *et al.* 2003). The combined effects of three precipitation-related variables and winter temperatures on grasshopper species composition based on feeding guild and flight ability appear to be integrated by the NAO index (Table 2.3C). The NMDS ordination shows that following winters in which NAO was in its positive phase, many of the common

grasshopper species, including *H. alba*, *Hesperotettix* spp., *P. nebrascensis*, *Mel. scudderi*, *Mel. keeleri*, and *P. brachyptera*, were most abundant (Fig. 2.4B). Less common grasshopper species, including *C. olivacea*, *Mel. sanguinipes*, *Mer. picta*, *Mel. packardii*, and *B. gracile*, appeared to be more abundant during the negative phase of the NAO cycle. These shifts in species composition were associated with negative responses by mixed-feeding species and positive responses by species that are poor fliers (Appendix A.3). Detailed studies are required to uncover mechanisms for these responses.

The strong and consistent relationship between weather and shifts in composition related to flight ability of the species (Table 2.3C, Appendix A.3) was unexpected because most individuals are collected as nymphs with undeveloped wings. Previous growing season and winter weather, as well as NAO (which mainly affects winter weather) accounted for three of the five factors associated with grasshopper flight ability, which may indicate lagged effects of parental dispersal ability on grasshopper responses to weather.

Conclusions and Implications

Each of the three primary grassland ecosystem drivers influences grasshopper population and community dynamics. Despite significant effects of fire on plant communities, fire alone does not account for fluctuations of grasshopper abundances or species composition. However, the interaction between the length of time after fire and bison grazing significantly impacts grasshopper populations and communities at KPBS although the functional mechanisms underlying these responses are not well understood. Understanding the potential interactions between these two groups of grazers with otherwise vastly different habits will require future research. The relationship between NAO and grasshopper composition provides insights into a potential driver for periodic changes in overall grasshopper abundances at decadal time scales (Branson *et al.* 2006), and suggests ways in which the roles of annual precipitation and temperature patterns can be expanded to a longer time scale. Finally, comparing my results based on a 25-year LTER dataset to those of short-term studies illustrates the contributions of long-term studies to new insights as well as the reasonably high success of shorter studies to understanding mechanisms underlying grasshopper population dynamics. Although underlying mechanisms remain unclear, I revealed grasshopper community responses to both grassland management and weather patterns that were not captured by short-term studies.

My results indicate that the population and community dynamics of grasshoppers, a major group of grassland consumers, are driven by complex interactions including both intrinsic and extrinsic factors. Negative feedbacks among all three feeding guilds suggested that density-dependence is likely to play an important role in the regulation of these species. Factors extrinsic to grasshopper populations operating at different spatial and temporal scales also strongly influenced community-level dynamics. Grazing by bison had a much larger impact on these insect herbivores than did spring fire and large-scale weather patterns were also shown to be important drivers of community dynamics. Additionally, this is one of the first studies to document that NAO, operating at decadal scales, can influence terrestrial insect consumers. Given this result, it will be particularly interesting to explore the role of atmospheric oscillations (*e.g.*, NAO, ENSO, PDO) on long-term consumer dynamics in different ecosystems. As research on ecological responses to climate change progresses, understanding the influence of these large-scale oscillations on consumers may lead to important insights into ecosystem dynamics.

Acknowledgements

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Figures and Tables

Figure 2.1 Changes in annual mean frequencies of grass-feeding (black circle), forb-feeding (black inverted triangle), and mixed-feeding (black square) grasshopper species on ungrazed watersheds from 1982 to 2005 at Konza Prairie Biological Station (KPBS), Kansas with each year represented by one data point for each feeding guild. The dotted line represents no change in frequency from year (time *t*), to *t* + 1; points above the line indicate years of increasing, while those below the line represent years of decreasing frequency. For clarity, only the first (*t* = 1982) and most recent (*t* = 2004) data points for each feeding guild are labeled and appear as open symbols. There are no points for years *t* = 1991 up to and including *t* = 1995 because samples from 1992 to 1995 were damaged prior to identification.

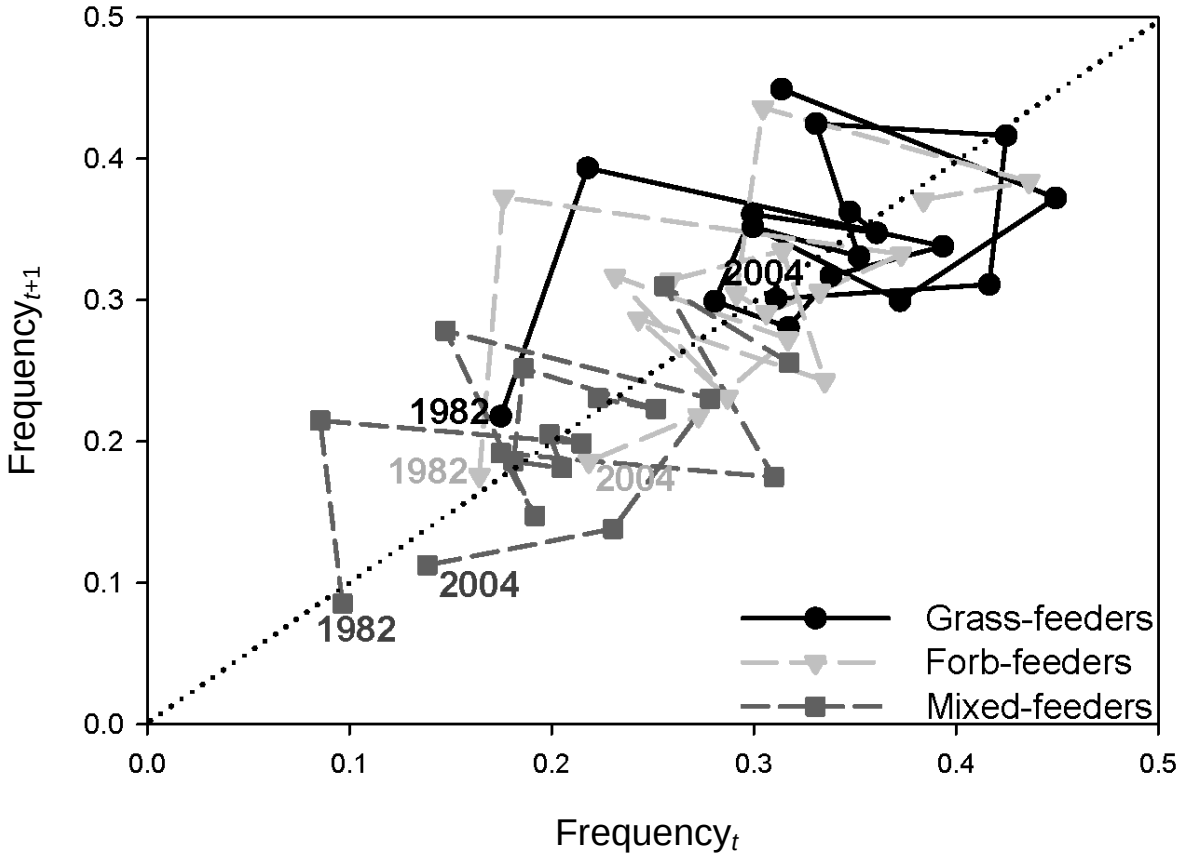

Figure 2.2 Annual mean frequencies of (A) grass-feeding, (B) forb-feeding, and (C) mixed-feeding grasshopper species in watersheds with prescribed burns scheduled annually (black circle), every 4 years (black inverted triangle), and every 20 years (black square) at KPBS. Four-year transects were burned in 1983, 1987, 1991, 1998, 2000, 2003, and 20-year transects were only burned in 1991. There are no data for 1992 up to and including 1995 because samples from those years were damaged prior to identification.

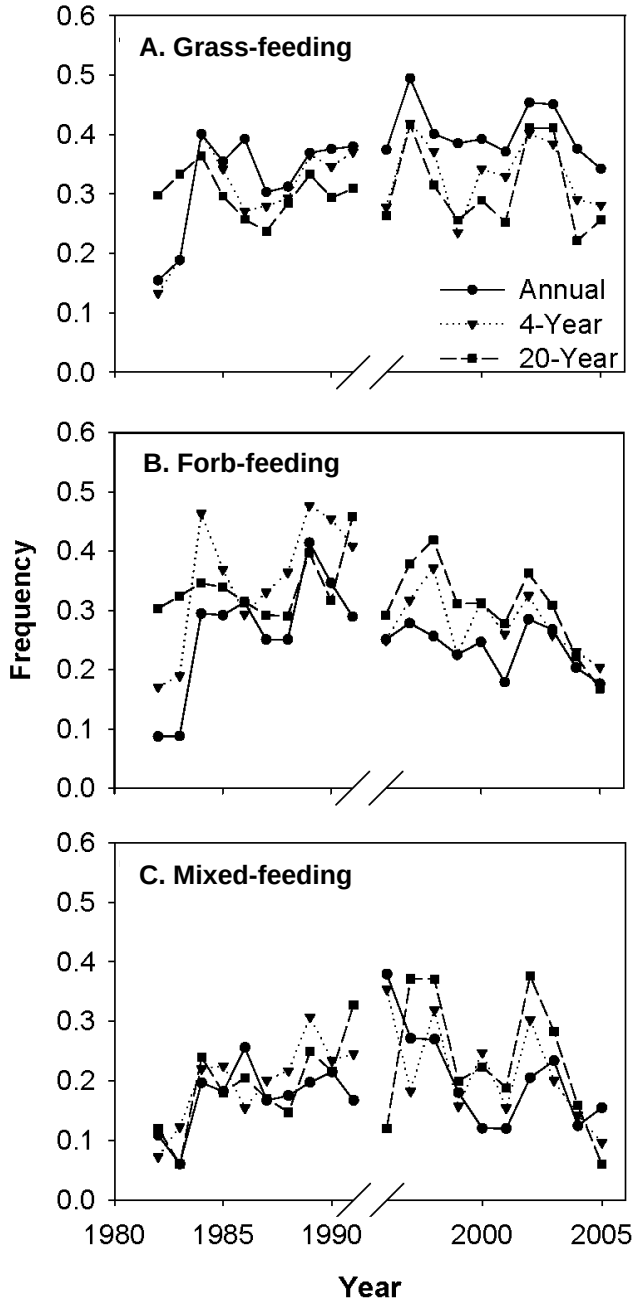

Figure 2.3 Non-metric multidimensional scaling ordination of grasshopper species composition of watersheds sampled in 2005; axes 1 and 3 account for 74% of the variation in the data. (A) Each point represents the species composition of the grasshopper community in either grazed (black circle) or ungrazed (black triangle) watersheds. The fire return interval of each watershed (1, 4, or 20 years) is indicated next to the point corresponding to that watershed, with the number of years since the last fire indicated in parentheses. (B) Each point represents a species with symbols denoting the feeding guild (grass-feeding black circle, forb-feeding black inverted triangle, and mixed-feeding black square) to which each species belongs. See Appendix A.1 for species' abbreviations.

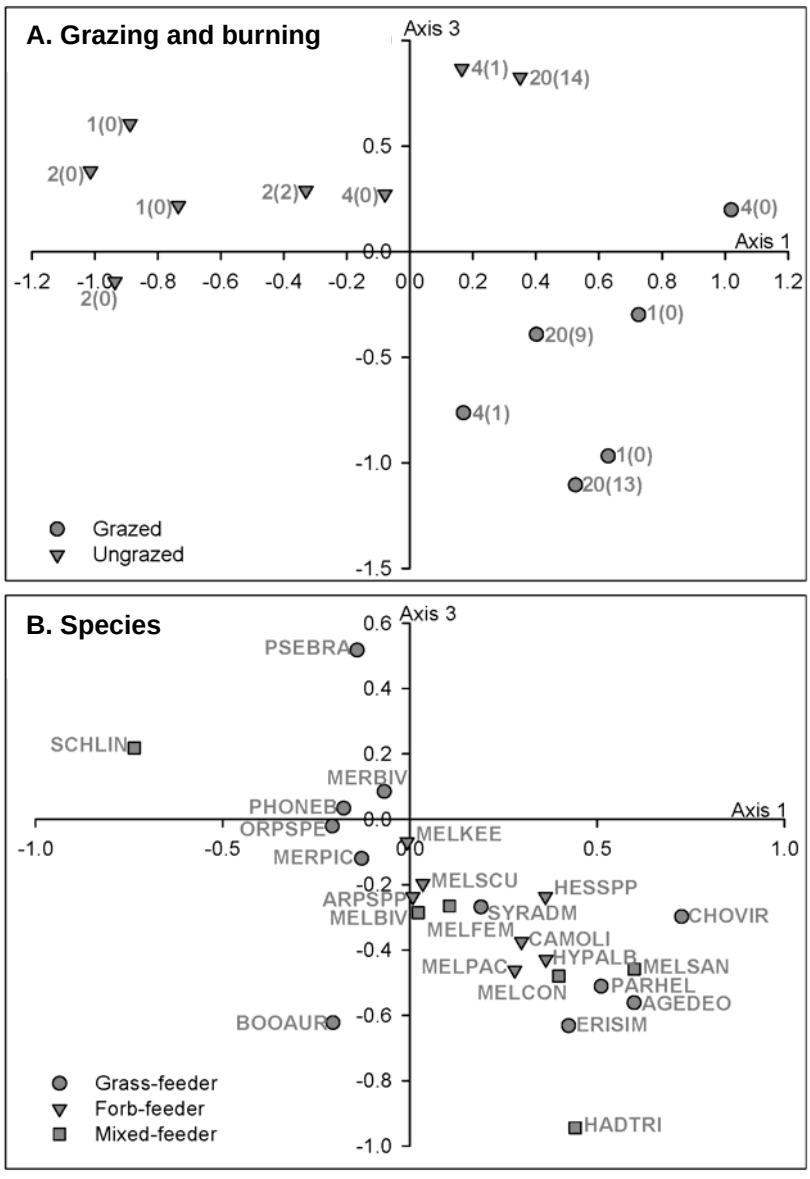

Figure 2.4 Non-metric multidimensional scaling ordination of annual mean grasshopper species composition at KPBS from 1982 to 1991 and 1996–2005; axes 1 and 3 account for 69% of the variation in the data. (A) Each point represents the species composition at KPBS in a given year with the last two digits of the year indicated next to each point. There are no data for 1992 up to and including 1995 because samples from those years were damaged prior to identification. (B) Each point represents a species. Symbols denote strong fliers (longer wings, black circle) or weak fliers (short wings, black inverted triangle). See Appendix A.1 for species' abbreviations. Community structure was correlated with the North Atlantic Oscillation index (NAO).

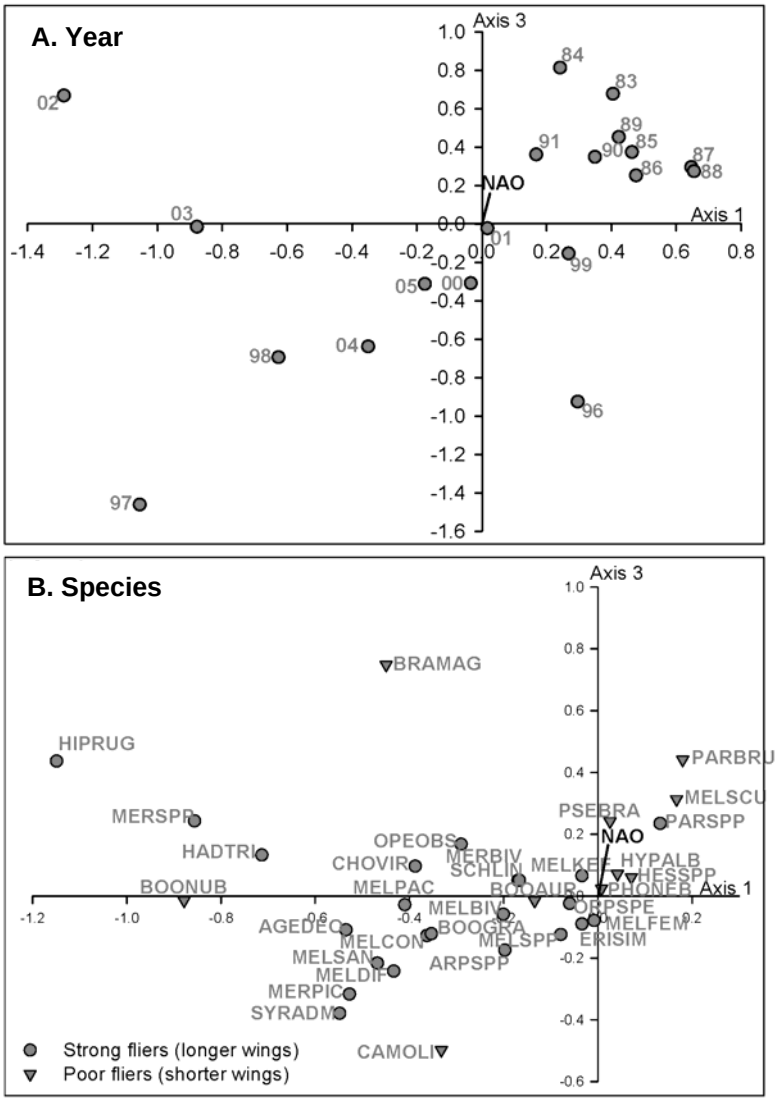

Figure 2.5 Annual mean frequencies of grass-feeding (black circle), forb-feeding (black inverted triangle), and mixed-feeding (black square) grasshopper species from 1982 to 2005 overlaid with the NAO (open diamond) index values for the same years. Points represent mean grasshopper frequencies on ungrazed watersheds during each year at KPBS.

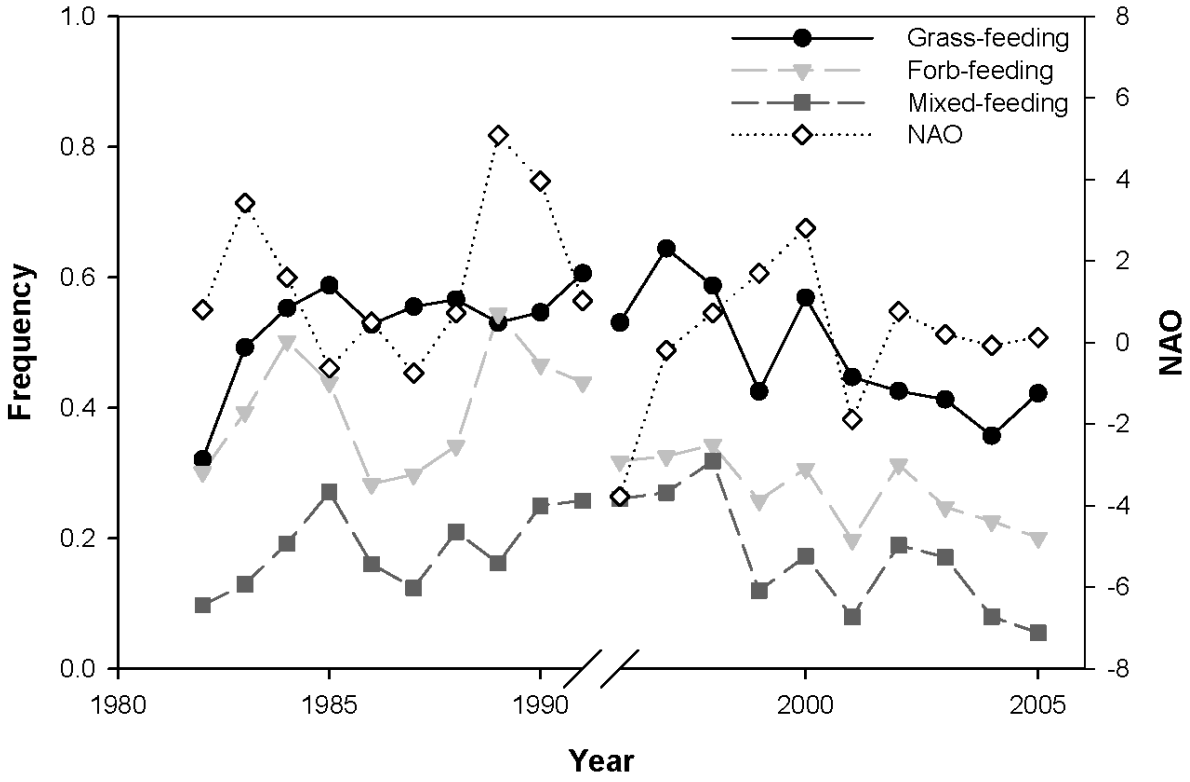

Table 2.1 Local, regional and large-scale variables used for analyses of weather effects on annual species composition of grasshopper communities at Konza Prairie Biological Station (KPBS).

a. Local weather events
Growing season (April-August)[a]
Total precipitation (mm)
Coefficient of variation of precipitation
Heating degree days (>20^{o}C)
Coefficient of variation of daily maximum temperature
Fall (September$_{t-1}$-November$_{t-1}$)
Average daily maximum temperature (^{o}C)
Total precipitation (mm)
Winter (December$_{t-1}$-March$_{t}$)
Average daily maximum temperature (^{o}C)
Total precipitation (mm)
b. Regional weather index
Palmer Drought Index
c. Decadal atmospheric phenomena
Southern Oscillation Index
North Atlantic Oscillation Index
Pacific Decadal Oscillation Index

[a]Analysis included growing season$_{t}$ and growing season$_{t-1}$ weather events

Table 2.2 Results of hierarchical repeated measures ANOVA tests on the effects of fire and time (year) on grass-feeding, forb-feeding, and mixed-feeding grasshopper species from 1982 to 2005 on watersheds at KPBS that were not grazed.

Factor	ndf	ddf	F	p-values
a. Grass-feeding species				
Fire	2	2	4.04	0.1985
Year	19	133	6.54	<0.0001
Fire*Year	38	133	1.06	0.3953
b. Forb-feeding species				
Fire	2	2	1.06	0.4865
Year	19	133	8.96	<0.0001
Fire*Year	38	133	1.37	0.0978
c. Mixed-feeding species				
Fire	2	2	0.49	0.6699
Year	19	133	6.15	<0.0001
Fire*Year	38	133	1.50	0.0495

Table 2.3 Multivariate fourth-corner analyses of the effects of land management in 1982 and 2005 and weather from 1982 to 2005 on grasshopper species composition based on species traits. Rejection of the null hypothesis (H_o: weather affects species composition based on the traits of the species) requires P < 0.2236 (results in bold) under both models 2 and 4 to maintain an overall $\alpha = 0.05$ (Dray and Legendre *in review*). GS Growing season, CV coefficient of variation.

Analysis	Environmental Variable	Feeding Guild			Flight Ability			Size			Phenology		
		Eta^2	Model 2	Model 4	Eta^2	Model 2	Model 4	Eta^2	Model 2	Model 4	Eta^2	Model 2	Model 4
a) 1982	Fire Return Interval	0.063	(0.760)	(0.359)	0.038	(0.742)	(0.429)	0.020	(0.846)	(0.725)	0.005	(0.903)	(0.941)
	Years Since Fire	0.125	(0.619)	(0.542)	**0.335**	**(0.191)**	**(0.184)**	**0.428**	**(0.012)**	**(0.082)**	0.044	(0.426)	(0.812)
b) 2005	Fire Return Interval	0.200	(0.264)	(0.432)	0.318	(0.203)	(0.256)	0.164	(0.246)	(0.511)	0.125	(0.253)	(0.613)
	Years Since Fire	0.106	(0.507)	(0.612)	**0.402**	**(0.150)**	**(0.167)**	0.132	(0.328)	(0.542)	0.115	(0.280)	(0.591)
	Bison grazing	2.412	(0.001)	(0.226)	0.495	(0.105)	(0.456)	0.163	(0.524)	(0.913)	0.832	(0.017)	(0.624)
c) Weather	GS precipitation$_t$	0.002	(0.205)	(0.503)	**0.004**	**(0.130)**	**(0.083)**	0.003	(0.130)	(0.393)	0.001	(0.649)	(0.835)
	CV GS precipitation$_t$	**0.003**	**(0.080)**	**(0.079)**	**0.005**	**(0.108)**	**(0.009)**	0.001	(0.349)	(0.342)	**0.004**	**(0.049)**	**(0.080)**
	GS heating degree days$_t$	0.000	(0.758)	(0.602)	0.001	(0.612)	(0.209)	0.001	(0.329)	(0.127)	0.001	(0.392)	(0.188)
	CV GS temperature$_t$	0.000	(0.868)	(0.668)	0.000	(0.959)	(0.871)	0.000	(0.935)	(0.851)	0.000	(0.738)	(0.428)
	GS precipitation$_{t-1}$	**0.005**	**(0.037)**	**(0.066)**	**0.004**	**(0.151)**	**(0.030)**	0.000	(0.903)	(0.935)	0.001	(0.426)	(0.515)
	CV GS precipitation$_{t-1}$	0.001	(0.567)	(0.604)	0.003	(0.260)	(0.049)	**0.003**	**(0.102)**	**(0.083)**	**0.002**	**(0.124)**	**(0.175)**
	GS heating degree days$_{t-1}$	0.001	(0.532)	(0.454)	0.003	(0.254)	(0.028)	**0.003**	**(0.088)**	**(0.042)**	**0.003**	**(0.062)**	**(0.058)**
	CV GS temperature$_{t-1}$	0.000	(0.955)	(0.934)	0.000	(0.933)	(0.852)	0.000	(0.933)	(0.911)	0.001	(0.673)	(0.560)
	Fall precipitation	0.001	(0.600)	(0.457)	0.002	(0.329)	(0.019)	0.000	(0.890)	(0.849)	0.002	(0.276)	(0.143)
	Fall temperature	0.000	(0.840)	(0.842)	0.000	(0.843)	(0.709)	0.000	(0.705)	(0.722)	**0.004**	**(0.025)**	**(0.023)**
	Winter precipitation	0.001	(0.361)	(0.094)	0.002	(0.336)	(0.010)	0.000	(0.663)	(0.398)	0.001	(0.489)	(0.174)
	Winter temperature	0.002	(0.325)	(0.405)	**0.005**	**(0.109)**	**(0.016)**	0.001	(0.644)	(0.732)	0.001	(0.352)	(0.456)
	Palmer Drought Index	0.001	(0.557)	(0.525)	0.001	(0.442)	(0.136)	**0.003**	**(0.149)**	**(0.089)**	**0.004**	**(0.018)**	**(0.013)**
	Southern Oscillation Index	0.000	(0.935)	(0.844)	0.000	(0.890)	(0.663)	0.000	(0.955)	(0.891)	0.000	(0.862)	(0.655)
	North Atlantic Oscillation Index	**0.006**	**(0.011)**	**(0.053)**	**0.004**	**(0.161)**	**(0.058)**	0.001	(0.302)	(0.515)	0.002	(0.263)	(0.500)
	Pacific Decadal Oscillation Index	0.001	(0.563)	(0.403)	0.000	(0.685)	(0.357)	0.000	(0.988)	(0.985)	0.000	(0.693)	(0.563)

Eta^2 statistics with *P*-values (9,999 permutations) from models 2 and 4 *in parentheses* are given for all tests.

CHAPTER 3 - Host plant quality alters grass:forb consumption by a mixed-feeding insect herbivore, *Melanoplus bivittatus* (Orthoptera: Acrididae)

Abstract

Factors affecting the nutritional ecology of mixed-feeding, polyphagous herbivores are poorly understood. Mixed-feeding herbivores do best when they consume both forb and grass species although they typically feed primarily on forbs, which are of relatively higher protein content than grasses. In a field experiment, I examined the effects of nitrogen and phosphorus fertilization and associated changes in host plant C:N:P on proportional grass consumption by a mixed-feeding insect herbivore, *Melanoplus bivittatus*, using natural abundance stable carbon isotope ($^{12}C/^{13}C$) methods. The C isotope signatures of *M. bivittatus* collected from plots fertilized with nitrogen (+N), phosphorus (+P), nitrogen and phosphorus (+N+P) and no-fertilizer were compared to the C isotope signatures of plants in those plots to determine the proportion of assimilated C derived from C_4 grasses and C_3 forbs in each plot. I also examined the relationship between *M. bivittatus* diets and plant C:N:P stoichiometry. The proportion of grass assimilated approximately doubled in N-fertilized treatments (39.1±0.05%) compared to non-fertilized treatments (19±0.004%), an increase associated with decreased C:N and increased N:P of grasses. These results indicate that mixed-feeding *M. bivittatus* can selectively feed to balance C:N:P intake even when choosing between two structurally and chemically different groups of plants. The strong relationship between diet selection and grass stoichiometry also suggests that plant nutrient composition may be more important than defensive chemistry in food choice.

Keywords: grasshopper, nitrogen (N), carbon (C), phosphorus (P), stoichiometry, tallgrass prairie, C_4 photosynthetic pathway, C_3 photosynthetic pathway, optimal foraging, *Phoetaliotes nebrascensis, Hesperotettix viridis.*

Introduction

Although the feeding ecology of polyphagous herbivores has been widely studied, little is known about the nutritional ecology of mixed-feeding herbivores. Polyphagous herbivores feed

on plants from a variety of plant families (Chapman 1990), where most polyphagous species feed only on plants in dicotyledonous families and fewer feed only on monocotyledonous plants. In an even smaller subset of polyphagous species, both dicots (*i.e.*, forbs) and monocots (*i.e.*, grasses) are consumed in a mixed-feeding strategy, presenting these herbivores with interesting problems because they must overcome the physical and chemical challenges unique to each plant group (grass vs. forb). Mixed feeding by insect herbivores is relatively uncommon (Mulkern *et al.* 1969, Joern 1983). Forbs usually make up the bulk of mixed-feeder diets with grasses contributing a variable but often minor component (Joern 1983, Bernays and Bright 1993). As seen in most polyphagous species which perform best on diets containing plants from multiple families (Rapport 1980, Hagele and Rowell-Rahier 1999), mixed-feeding herbivores also experience their greatest performance when both forbs and grasses are consumed (Bailey and Mukerji 1976, MacFarlane and Thorsteinson 1980, Randolph *et al.* 1995, Hagele and Rowell-Rahier 1999, Randolph and Cameron 2001, Miura and Ohsaki 2006). Increased performance on mixed diets indicates that diet selection may have evolved in these herbivores as a means to maximize fitness in complex nutrient and competition landscapes.

Forbs are generally considered a higher quality food for most herbivores than grasses from a primary nutrient standpoint because of higher nitrogen (N) (~protein), phosphorus (P) (~RNA) and sugar contents (Randolph *et al.* 1995). In grasses, not only are primary nutrient concentrations lower than in forbs, but protein and carbohydrate are often more concentrated in tough bundle sheath cells (C_4 photosynthetic pathway) than elsewhere in leaf tissues (Barbehenn *et al.* 2004b) with higher concentrations of non-digestible cellulose, lignin and silica in leaves (Massey *et al.* 2006). Secondary metabolites that can act as feeding deterrents or toxins are more abundant and diverse in forbs than in grasses (Bernays and Chapman 1994, Mole and Joern 1994).

Dietary nitrogen (N) is recognized as a key limiting nutrient for insect herbivores (Mattson 1980), although recent evidence suggests that P may also play an important role in regulating performance, especially growth rates (Sterner and Robinson 1994, Schade *et al.* 2003, Perkins *et al.* 2004). A large imbalance between the nutrient content of herbivores and that of their host plants generally exists (Mattson 1980, Dearing and Schall 1992, Logan *et al.* 2004). Despite this imbalance and the often extreme spatial and temporal variability of host plant quality in the field, herbivores are generally able to maintain stoichiometric homeostasis in their

tissues (Mattson 1980, Sterner 1997, Logan *et al.* 2004). Herbivores can maintain stoichiometric balance through compensatory feeding, physiological adjustment, or food selection mechanisms (Mattson 1980, Bernays and Chapman 1994, Yang and Joern 1994). Compensatory feeding for limiting nutrients usually leads to over-consumption of non-limiting or deterrent chemicals (Joern and Behmer 1997, Hagele and Rowell-Rahier 1999). Because it requires increased allocation of time and energy for feeding-related activities, compensatory feeding can also increase predation risk (Westoby 1978, Danner and Joern 2003, Berner *et al.* 2005). Changes in post-ingestive processes (gut passage rate, nutrient metabolism, etc.) to conserve limiting nutrients while excreting toxic compounds or nutrients consumed in excess can also influence feeding behavior in bird, mammal, and insect herbivores (Freeland and Janzen 1974, Heidorn and Joern 1987, Brugger 1991, Yang and Joern 1994, Barbehenn *et al.* 2004b). Polyphagous herbivores and mixed-feeders in particular may rely to a greater extent on food selection for maintaining homeostasis and performance (Dearing and Schall 1992, Pennings *et al.* 1993, Randolph *et al.* 1995, Raubenheimer and Simpson 2003b).

I used natural abundance stable C isotopes to assess the relative contribution of C from grass and forb host plants to body tissues of the mixed-feeding grasshopper, *Melanoplus bivittatus* (Say) (Orthoptera: Acrididae). The stable C-isotope technique has been widely used in food web and diet selection studies (Fry *et al.* 1978, Pinder and Jackson 1988, Briones *et al.* 1999, Fry 2006, Jonas *et al.* 2007) because characteristic stable C isotope signatures ($\delta^{13}C$) are produced during C-fixation in the C_3 ($\delta^{13}C \approx$ -27 to -30‰) and C_4 ($\delta^{13}C \approx$ -12 to -15‰) photosynthetic pathways, and the C isotope signature is conserved with transfer through trophic levels (Fry 2006). That is, the ^{13}C isotopic composition of an herbivore consumer reflects that of its host plant (or mixture of hosts) (Fry *et al.* 1978). In tallgrass prairie ecosystems, the natural abundance stable C isotope technique is particularly useful in field studies because the native vegetation consists primarily of C_4 grasses and C_3 forbs, which allows us to use an easily interpreted mixing model (Fry *et al.* 1978, Fry 2006) to calculate the relative abundance of grass- versus forb-derived C assimilated by *M. bivittatus*. Although C_3 grasses also occur at native prairie sites, they comprise a small proportion of total plant biomass and can be ignored here. I also analyzed $\delta^{13}C$ signatures of a grass-feeding grasshopper (*Phoetaliotes nebrascensis* (Thomas)) and a forb-feeding grasshopper (*Hesperotettix viridis* (Thomas)) for comparison and

validation of this technique to detect differences between grass- and forb-feeding habits, respectively.

My objective was to assess the impact of different nutrient environments on consumption of grasses and forbs by a mixed-feeding insect herbivore. Specifically, I examined the effects of long-term N and P fertilizer additions on (1) *M. bivittatus* tissue stoichiometry and (2) the proportion of grass consumed by *M. bivittatus*, and (3) the relationship between *M. bivittatus* grass consumption and host plant stoichiometry. I hypothesizedthat, despite changes in host plant C:N:P, *M. bivittatus* tissue C:N:P would be similar in all fertilizer treatments. Because it is likely that this mixed-feeding herbivore is able to achieve stoichiometric homeostasis through diet selection, the proportion of grass in the diet of *M. bivittatus* was expected to be higher in N fertilized treatments. Although recent evidence suggests that P plays a large role in the performance (*e.g.*, growth rates) of aquatic consumers (Elser and Hassett 1994, Sterner and Robinson 1994, Elser *et al.* 1996, Elser *et al.* 2000, Elser *et al.* 2003) and holometabolous terrestrial insects (Schade *et al.* 2003, Perkins *et al.* 2004), it may be of minor importance compared to N in hemimetabolous terrestrial insects (Loaiza *et al.,* unpublished data). Further, I predicted that as grass C:N decreased, the proportion of grass consumed by *M. bivittatus* would increase. Alternatively, an increase in grass consumption related to decreased forb C:N may suggest that the switch in diet is a means to avoid enhanced chemical or physical defenses of forbs under high nutrient resource conditions.

Methods

This study was conducted in the belowground plot experiment (BPE) established in 1986 at Konza Prairie Biological Station near Manhattan, Kansas. The annually burned, 12x12 m BPE plots sampled in this study are arranged in a randomized complete block design with 4 plots in each of 4 blocks. Plots within each block receive one of 4 randomly assigned fertilizer treatments: nitrogen (+N), phosphorus (+P), nitrogen and phosphorus (+N+P), or no-fertilizer control (0N0P). N is applied at a rate of 10 g $N/m^2/y$ as NH_4-NO_3 and P is applied at 1 g $P/m^2/y$ as superphosphate. All samples were collected between 30 June and 6 July 2004.

Aboveground plant community composition was estimated in 4 random 0.25-m^2 quadrats in each plot. Aboveground plant biomass was clipped at the soil surface in 0.05-m^2 sub-sections of each quadrat and sorted by functional group (C_4 grass or C_3 forb), dried at 60^{o}C for 48h, and

weighed. Cool season (C_3) grasses were rare (average cover 0-4%) in the plots and were not encountered in any of the clip samples.

Sweep net samples were collected along two 12-m transects separated by at least 5m in each plot. Samples were frozen following collection, then sorted and identified in the lab. Movement of grasshopper individuals between treatment plots was not expected given that grasshoppers tend remain within a locally small area (<4m) (Narisu *et al.* 1999). All individuals of the mixed-feeding acridid *M. bivittatus* were dried for 48h at 60°C prior to weighing. Individuals of the most abundant grass-feeding (*Phoetaliotes nebrascensis*) and forb-feeding (*Hesperotettix viridis*) species in the plots were also dried and weighed in the same manner. For some treatments, individuals did not provide enough biomass for elemental analysis which resulted in missing data for the analysis of diet composition data even though abundance data was presented.

All plant and grasshopper samples were coarsely ground in a 40 mesh Wiley mill and homogenized to a fine powder using a Wig'L Bug®. C and N elemental and isotopic composition of all samples was analyzed at the Stable Isotope Mass Spectrometry Lab (SIMSL) at Kansas State University on a Carlo Erba CE1100 (Carlo Erba Instrument, Milan, Italy) elemental analyzer connected to a ThermoFinnigan Delta Plus (Finnigan MAT, Bremen, Germany) mass spectrometer via a Conflo II EA/IRMS (Finnigan MAT, Bremen, Germany) open split interface. Isotopic values are presented in delta notation and expressed in units per mil (‰) (Fry 2006). Sample P content was analyzed at the Ecosystem Analysis Lab (University of Nebraska-Lincoln). Foliar phosphorus was converted to ortho-phosphate and measured colorimetrically at 660 nm following reaction with molybdate and ascorbic acid with antimony potassium tartrate as a catalyst.

Grasshopper diet composition was calculated using the simple, two-pool mixing model of Fry *et al.* (1978): $\delta^{13}C_{diet} = (\delta^{13}C_{C_4 plants})(\%C_4) + (\delta^{13}C_{C3\,plants})(\%C_3)$, where $\delta^{13}C_{animal}$ of individual grasshoppers was used as a proxy for $\delta^{13}C_{diet}$. Fry *et al.* (1978) found that the $\delta^{13}C_{animal}$ and $\delta^{13}C_{diet}$ had a nearly 1:1 relationship for coexisting grasshoppers in western Texas (USA).

The data were normally distributed with homogenous variances. Plant biomass and elemental composition was analyzed using analysis of variance for randomized complete block in SAS (SAS v.9.1.3, Cary, NC). Grasshopper biomass, proportion of grass assimilated, and C,

N, and P concentrations were analyzed using repeated measures analysis of variance for a randomized complete block design because multiple individuals of a species were often collected in a given plot. Stoichiometric ratios were analyzed using analysis of covariance with the numerator treated as the covariate and the denominator as the response variable (Raubenheimer and Simpson 1992, Horton and Redak 1993). Pearson's correlation coefficients were used to identify patterns between grass or forb C:N:P and the proportion of grass or forb, respectively, assimilated by *M. bivittatus*. Plot-level grass and forb C:N:P values were correlated with assimilation by individuals collected within the corresponding plot. The Dunn-Sidak adjustment was used to adjust p-values to maintain alpha across multiple correlations (Gotelli and Ellison 2004). Due to the low number of replicates available for sampling, an *a priori* α=0.10 was used.

Results

Warm-season grasses were dominant in all treatments. *Andropogon gerardii* Vitman was the most abundant plant species in both the 0N0P (43.9±15.6% cover) and +P (49.4±16.5% cover) treatments, while *Panicum virgatum* L. was the dominant species in the +N (57.4±4.3% cover) and +N+P (53±8.5% cover) treatments. In the 0N0P and +P treatments, *Solidago missouriensis* Nutt. exhibited the greatest forb cover although it was highly variable (12.5±12.5% and 13.1±8.9%, respectively). *Ambrosia psilostachya* DC. was the most common forb species in the +N+P (22.2±8.3% cover) and +N (15.6±5.6% cover) treatments; it was also consistently abundant in the 0N0P (10.3±1.8%) and +P (11.1±2.3% cover) plots.

Overall, plant biomass was significantly greater in the +N and +N+P treatments than in the 0N0P and +P treatments ($F_{3,21}$=34.63, p<0.0001) (Fig. 3.1A). Biomass of grasses was greater than that of forbs in all treatments ($F_{1,21}$=359.91, p<0.0001) (Fig.3.1A). However, the proportion of grass in the total plant biomass was not affected by fertilizer treatments ($F_{3,9}$=1.28, p=0.34).

There was no effect of fertilizer treatment on plant stable C isotopes ($F_{3,21}$=0.46, p=0.71) (Fig. 3.1B). As expected, the stable C isotope value of grasses was significantly higher than that of forbs ($F_{1,21}$=9030.24, p<0.0001) (Fig. 3.1B). Fertilizer treatments had no effect on plant C concentration, although tissue N was 63% higher in the +N and +N+P treatments, and P was 37% higher in +P and +N+P treatments (Table 3.1A). Forbs had higher N (1.37±0.11%) and P (0.15±0.01%) and lower C (42.2±0.47%) than grasses (N=0.77±0.04%, P=0.11±0.005%, C=43.8±0.15%). There was a significant interaction in N concentrations between fertilizer

treatment and plant functional group primarily because forb N concentrations were highest in +N+P, while grass N concentrations were similar in both the +N and +N+P treatments (Table 3.1A). Grass C:N in the 0N0P and +P treatments was the highest of all plants sampled, while plant C:N was lowest in forbs from +N and +N+P treatments (Fig. 3.2A, Table 3.1B). Overall, C:P was higher in grasses than forbs, and higher in plants from 0N0P and +N than +P and +N+P treatments (Fig. 3.2B, Table 3.1B). Grass N:P was significantly lower in +P than in 0N0P plots, while forb N:P was similar in those two treatments. This relationship accounts for the significant interaction between fertilizer and plant functional group on N:P (Table 3.1B). Plants in +N plots had significantly higher N:P than those from +P and +N+P treatments (Fig. 3.2C, Table 3.1B).

Although there was no difference in the abundance of *M. bivittatus* among +P, +N, or +N+P plots, only one individual was collected from 0N0P plots (Table 3.2); this treatment was removed from further analyses. There was no effect of fertilizer on *M. bivittatus* biomass ($F_{2,3}$=0.87, p=0.50). The abundance of *P. nebrascensis* was highest in +P treatments (Table 3.2); since this species was only collected in one each of the +N and +N+P plots the proportion of grass in *P. nebrascensis* diets could only be statistically analyzed for 0N0P and +P treatments. There was no effect of fertilizer treatment on the abundance of *H. viridis* (Table 3.2).

There was no effect of fertilizer on the proportion of C_4 grasses consumed by *P. nebrascensis* (Fig. 3.3A) or C_3 forbs consumed by *H. viridis* (Fig. 3.3B). The proportion of grass assimilated by *M. bivittatus* was significantly higher in the +N and +N+P treatments than in the +P treatments (Fig. 3.3C). There were no effects of fertilizer treatments on C, N, or P concentrations (Table 3.3A) or the C:N (Fig. 3.4A, Table 3.3B), C:P (Fig. 3.4B, Table 3.3B) or N:P (Fig. 3.4C, Table 3.3B) of *M. bivittatus*.

Forb C:N:P was not significantly correlated with the proportion of grass consumed by *M. bivittatus* (Table 3.4). A significant positive correlation was observed between the proportion of grass assimilated and grass C, N, and N:P (Fig. 3.5A), and a marginally significant negative relationship between grass C:N and grass assimilation resulted (Fig. 3.5B, Table 3.4).

Discussion

Mixed-feeding herbivores tend to feed primarily on forb species, but perform best when both forbs and grasses are included in their diet. My results for the mixed-feeding grasshopper *M. bivittatus* indicate that food selection at the plant functional group level is plastic and can

vary in response to changes in plant stoichiometry. An increase in the proportion of grass consumed by *M. bivittatus* was associated with decreased C:N and increased N:P of grasses. There was no effect of changes in grass or forb stoichiometry on diet selection by *P. nebrascensis* or *H. viridis* at the plant functional group level. A shift at this level was not expected for these two grasshopper feeding guilds, however, due to nutritional, behavioral and morphological characteristics of these grass- and forb-feeding species (Isley 1944, Bennack 1981, Patterson 1984, Meyer *et al.* 2002). It is possible that host-plant species on which the grass- and forb-feeders fed changed within the respective plant functional groups as a result of fertilizer application, but such dynamics were beyond the scope of this research.

The shift in *M. bivittatus* diets is most likely due to changes in host plant tissue chemistry rather than changes in plant community composition or structure. Long-term N-fertilization increased the biomass of grasses in this study, but it did not alter the relative availability of forbs and grasses within the treatments making it unlikely that increased grass biomass led to increased grass consumption by *M. bivittatus*. The dominant plant species in the N-fertilized treatments shifted from *A. gerardii* to *P. virgatum*, although both are native grasses and differ little in physiological and physical characteristics (Knapp 1985). The dominant forb was *A. psilostachya* in all treatments. The effects of N and P fertilizer on host plant stoichiometry were as expected: N fertilization led to a decrease in plant C:N and an increased N:P, while P fertilization was associated with decreased C:P and N:P.

Despite the effects of fertilization on plant C:N:P, *M. bivittatus* maintained C:N:P of body tissues within narrow limits in all treatments. Because of the time and energetic costs associated with compensatory feeding (discussed above) and the changes in post-ingestive processes needed to handle imbalances in nutrient intake (Simpson and Raubenheimer 2001, Simpson *et al.* 2004, Boersma and Elser 2006), I expected mixed-feeding herbivores to regulate food selection so nutrient intake would be as balanced as possible. For example, Raubenheimer and Simpson (1993) showed that herbivores balanced nutrient intake from artificial diets such that post-ingestive processing was most efficient. And, Pennings *et al.* (1993) found that a generalist herbivorous gastropod incurred a performance penalty rather than feeding in a compensatory manner when presented with single diets instead of mixed diets. Examining the relationship between the diet of *M. bivittatus* and host plant C:N:P, plants with intermediate N:P

(~9-12) and C:N (~40-50) were chosen to a greater extent than those at the extremes of the range (Fig. 3.5).

At a general level, the nutrient balance hypothesis (Pulliam 1975, Westoby 1978) and toxin dilution hypothesis (Freeland and Janzen 1974) are the two primary hypotheses under which the mechanisms for food selection by polyphagous herbivores have been studied (Bernays and Bright 1993). The toxin dilution hypothesis suggests that the fitness costs associated with excess consumption of plant secondary metabolites common in high quality foods, particularly those with high N, can be buffered by feeding on lower quality foods containing low concentrations of allelochemicals. Under the nutrient balance hypothesis, individuals are able to balance the intake of multiple nutrients by consuming hosts with complementary nutrient composition (Simpson and Raubenheimer 1993, Raubenheimer and Simpson 2003a, Simpson *et al.* 2004). It is likely, however, that plant allelochemical and nutrient content interact to affect food selection and performance of herbivores (Bucyanayandi and Bergeron 1990, Dearing and Schall 1992, Bernays and Bright 1993, Simpson and Raubenheimer 2000, 2001, Behmer *et al.* 2002) such that plant secondary metabolites are only detrimental when host nutrient content (*e.g.* protein:carbohydrate) is unbalanced.

Although I did not specifically test the mechanisms driving food selection (*i.e.*, toxin dilution hypothesis versus nutrient complementation hypothesis), my results suggest that host nutrient content was a more important determinant of food selection than toxin avoidance for *M. bivittatus*. Under the toxin dilution hypothesis, I would expect that the increase in grass consumption would be associated with an increase in N concentration or a decrease in the C:N of forbs. Concentrations of N-based allelochemicals were likely to be highest in forbs from the N fertilized treatments because forbs tend to increase allocation of resources to defensive compound synthesis when resources are not limiting (Coley *et al.* 1985, Ritchie 2000) although this is not always true (Mattson 1980, Riipi *et al.* 2002). However, because the magnitude of fertilizer effects on grass and forb C:N:P were not the same, and diet composition was most strongly associated with grass C:N:P, it is unlikely that the switch in *M. bivittatus* feeding was related to changes in forb defensive chemistry. Rather, my results show an increase in the consumption of grasses with increased grass quality (increased N:P and decreased C:N), which suggests nutrient complementation may be driving food selection. I did not analyze the allelochemistry of the plants, however, and therefore can not rule out the toxin dilution

hypothesis as a possible mechanism. It is interesting to note that C:N of the forbs in the 0N0P and 0N+P fertilizer treatments is similar to that of the grass in the +N and +N+P treatments. As expected, plant N seems to be more important in driving *M. bivittatus* food selection than P because increased grass consumption only occurred in plots receiving N fertilizer and was correlated with increasing grass N:P.

My results suggest that the mixed-feeding *M. bivittatus* feeds selectively on plants to balance C:N:P intake in the field. Although C:N stoichiometry is most commonly associated with host plant quality, I found that grass N:P showed the strongest relationship with the diet composition of my mixed-feeding herbivore. Although mixed-feeding grasshoppers typically prefer forbs, this study illustrates that this is not a static preference and that the nutritional ecology of mixed-feeders is separate and unique from that of either forb- or grass-feeding functional groups.

Acknowledgements

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Figures and Tables

Figure 3.1 (A) Biomass (g) and (B) stable carbon isotope values (δ^{13}C VPDB) of C_4 grasses and C_3 forbs from plots receiving no fertilizer (0N0P), phosphorus (+P), nitrogen (+N), or phosphorus and nitrogen (+N+P) fertilizer annually since 1986 at Konza Prairie Biological Station, Manhattan, KS. Bars with different letters are significantly different.

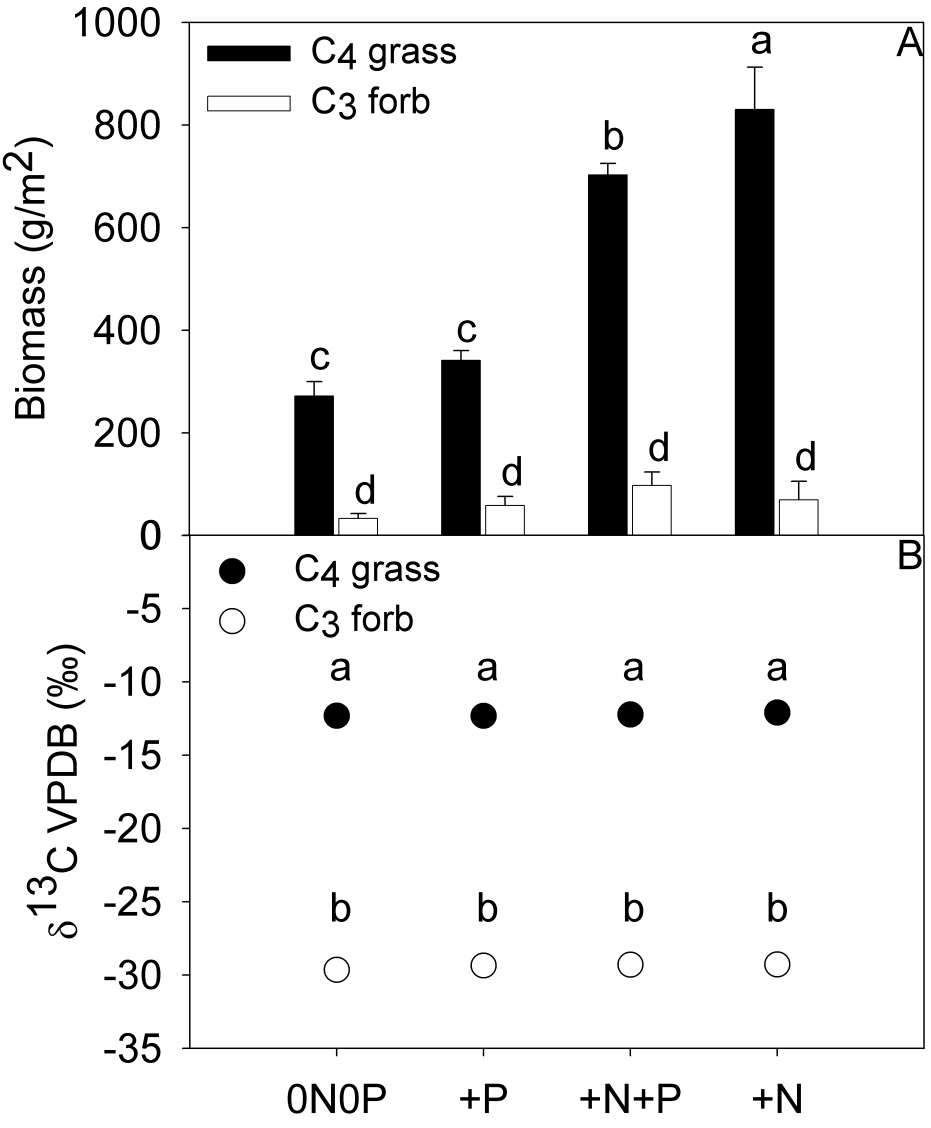


Figure 3.2 (A) Carbon:nitrogen, (B) carbon:phosphorus, and (C) nitrogen:phosphorus ratios of C_4 grass and C_3 forb tissue from plots receiving no fertilizer (0N0P), phosphorus (+P), nitrogen (+N), or phosphorus and nitrogen (+N+P) fertilizer annually since 1986. Data are presented as stoichiometric ratios; statistical analyses conducted using analysis of covariance with the numerator as the covariate and the denominator as the response variable.

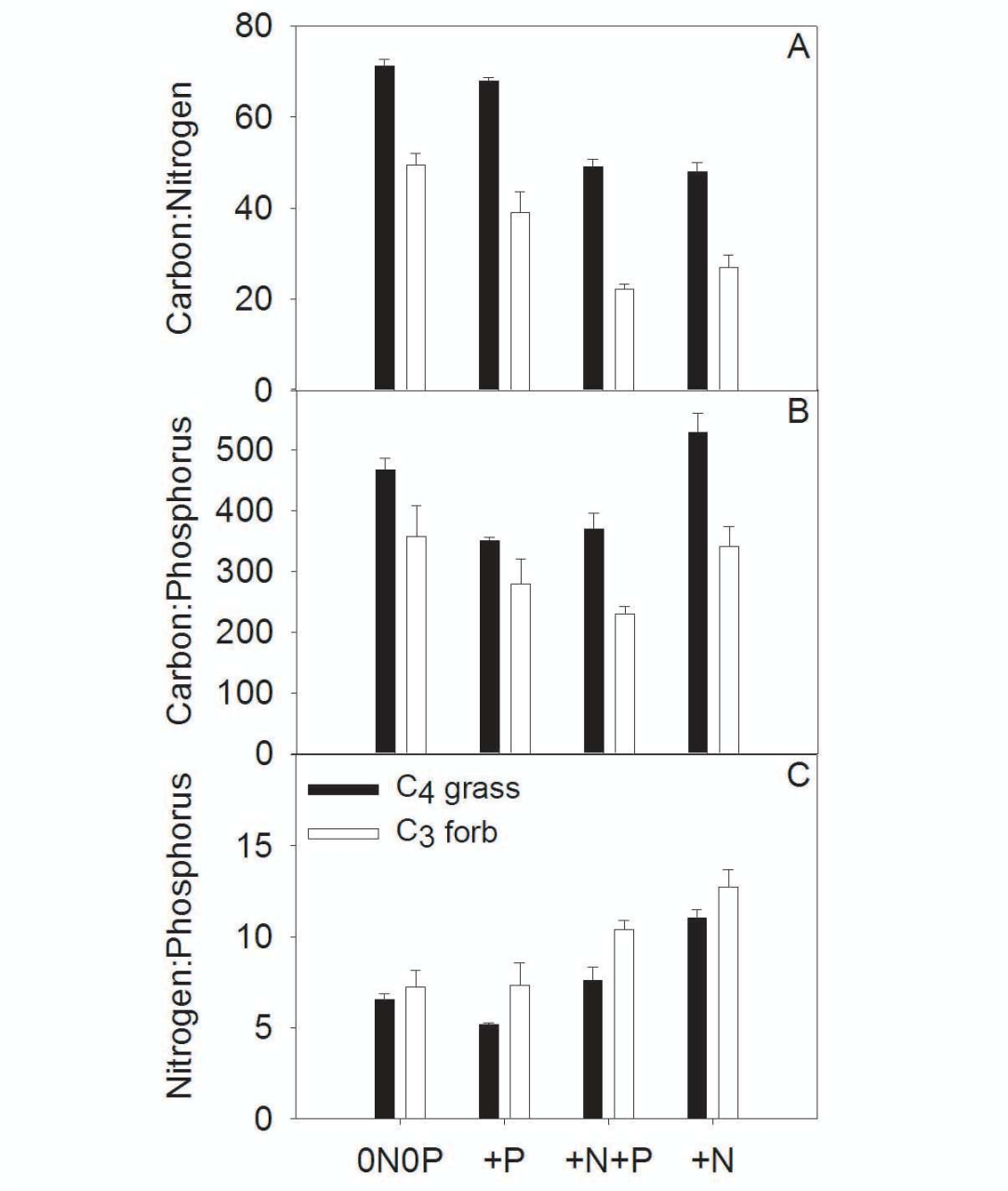


Figure 3.3 Proportion of C_4 grass assimilated into body tissues of (A) *Phoetaliotes nebrascensis* (grass-feeding grasshopper), (B) *Hesperotettix viridis* (forb-feed grasshopper), and (C) *Melanoplus bivittatus* (mixed-feeding grasshopper) from plots receiving no fertilizer (0N0P), phosphorus (+P), nitrogen (+N), or phosphorus and nitrogen (+N+P) fertilizer. *P. nebrascensis* occurred in only one of the +N and one of the +N+P plots, and *M. bivittatus* was collected from only one of the 0N0P plots. Since there was no replication for these species in the +N and +N+P or 0N0P treatments, respectively, they were excluded from analyses.

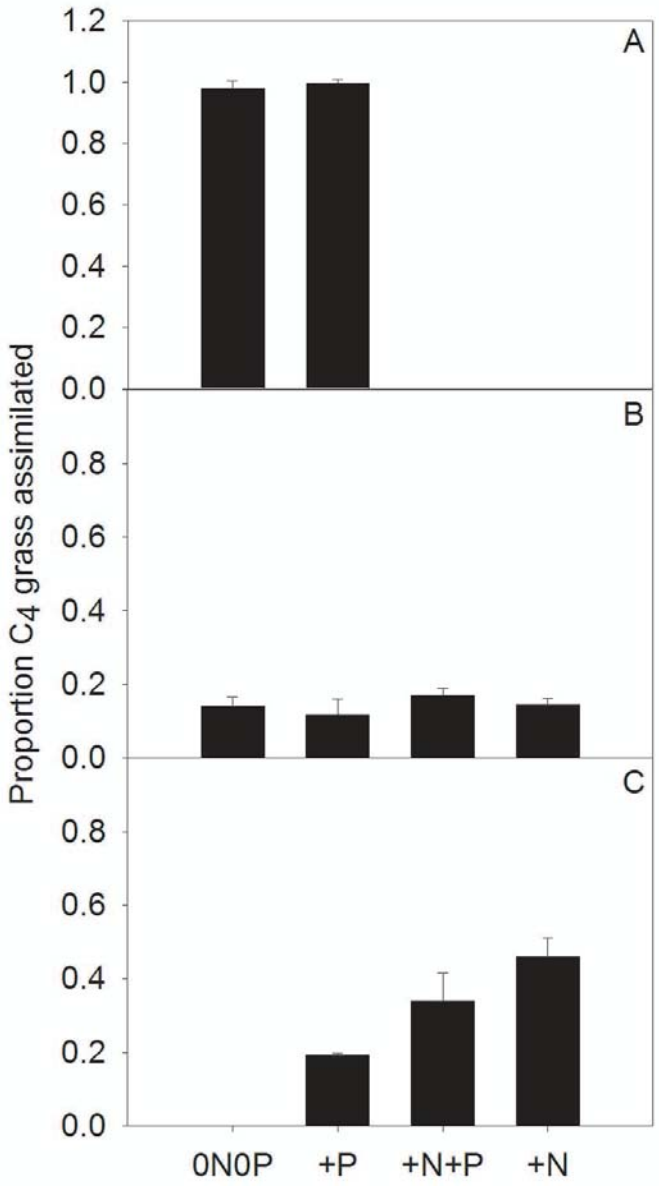


Figure 3.4 (A) Carbon:nitrogen, (B) carbon:phosphorus, and (C) nitrogen:phosporus ratios of *Melanoplus bivittatus* tissue from plots receiving no fertilizer (0N0P), phosphorus (+P), nitrogen (+N), or phosphorus and nitrogen (+N+P) fertilizer annually since 1986. Data are presented as stoichiometric ratios; statistical analyses conducted using analysis of covariance with the numerator as the covariate and the denominator as the response variable.

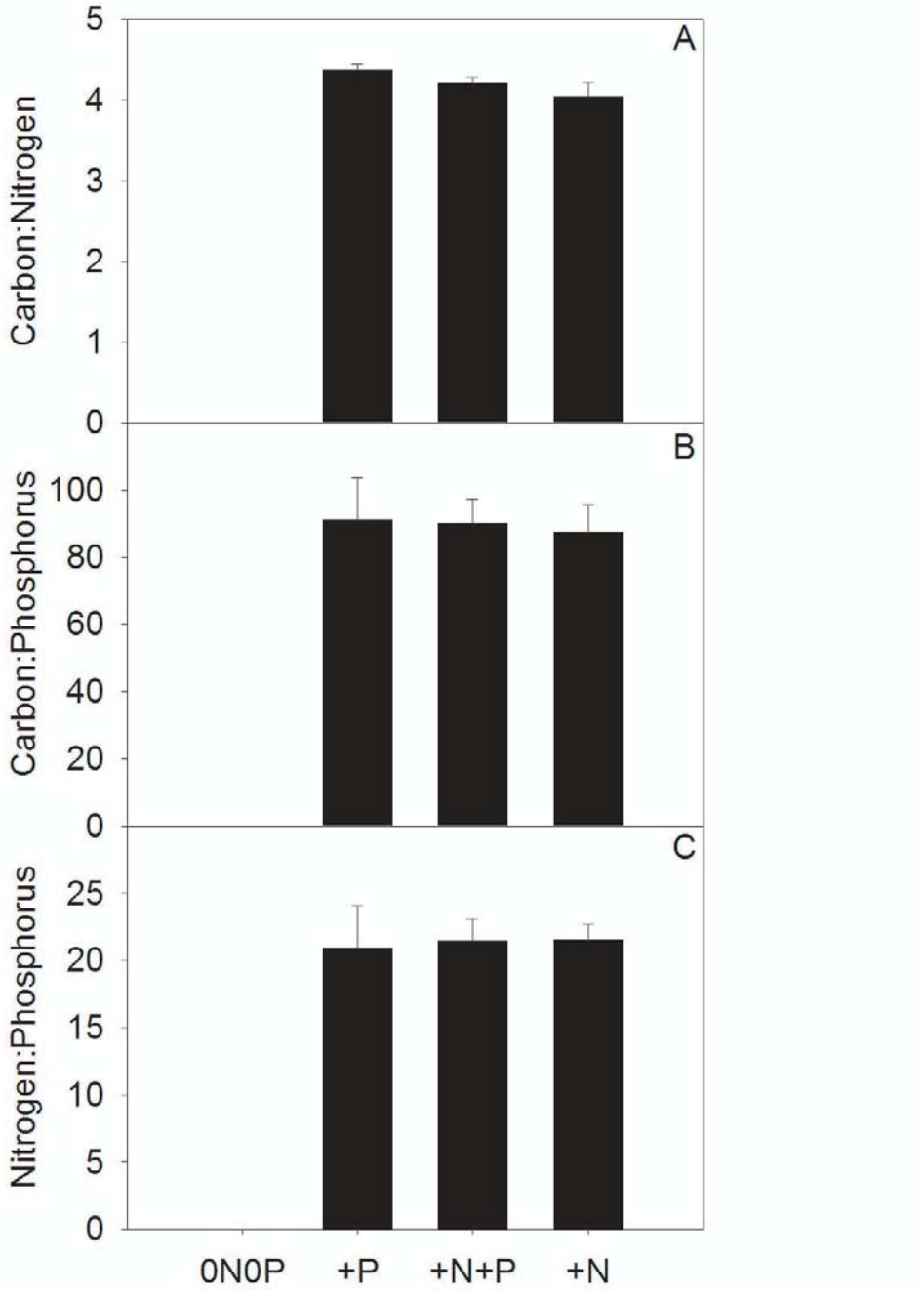


Figure 3.5 Relationship between the proportion of forb (open circle) and grass (closed circle) derived carbon assimilated by *Melanoplus bivittatus* and plot-level host plant (A) carbon:nitrogen and (B) nitrogen: phosphorus. Solid lines indicate significant correlations; correlations represented by broken lines were not significant.

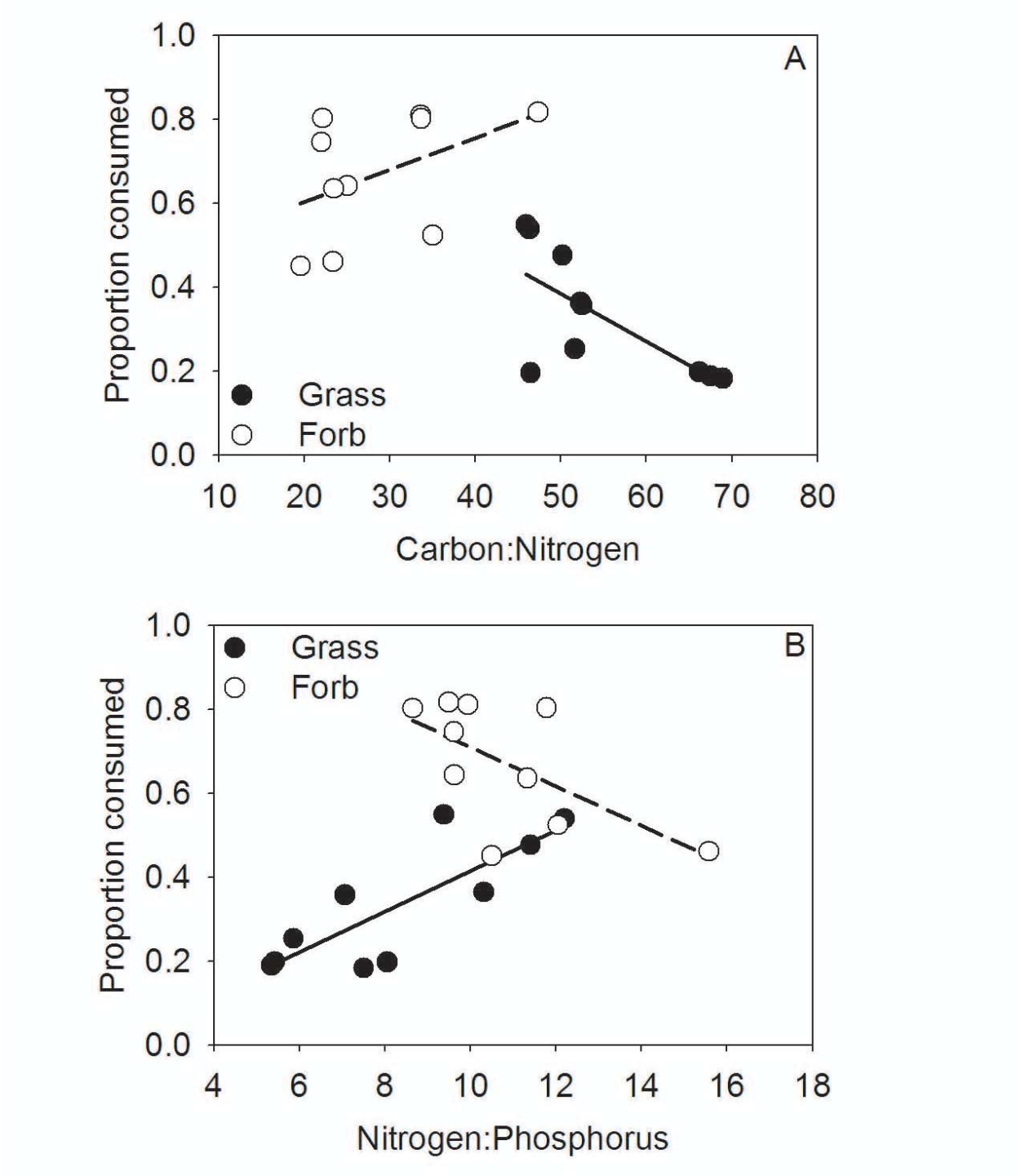


Table 3.1 (A) Analysis of variance (ANOVA) tables of fertilizer effects on grass and forb tissue carbon (C), nitrogen (N), and phosphorus (P) elemental concentrations and (B) analysis of covariance (ANCOVA) tables of fertilizer and plant functional group (FG) effects on plant stoichiometric ratios.

Response	Effect	Df	MS	F	p-value
A. ANOVA tables					
C concentration	Fertilizer	3	3.679	0.79	0.528
	FG	1	21.763	14.61	0.002
	Fertilizer*FG	3	8.977	2.01	0.166
	Block	3	13.214	2.85	0.098
	Fertilizer*Block	9	13.927	1.04	0.464
	Error	12	17.870		
N concentration	Fertilizer	3	2.197	52.82	<0.0001
	FG	1	2.848	175.21	<0.0001
	Fertilizer*FG	3	0.522	10.71	0.001
	Block	3	0.176	4.23	0.040
	Fertilizer*Block	9	0.195	0.85	0.586
	Error	12	0.195		
P concentration	Fertilizer	3	0.013	5.90	0.017
	FG	1	0.015	25.55	0.000
	Fertilizer*FG	3	0.001	0.39	0.765
	Block	3	0.003	1.33	0.324
	Fertilizer*Block	9	0.007	1.21	0.373
	Error	12	0.007		
B. ANCOVA tables					
C:N	Fertilizer	3	0.028	1.59	0.2366
	FG	1	0.021	4.47	0.058
	Fertilizer*FG	3	0.029	2.00	0.172
	C Concentraton	1	0.208	43.62	<0.0001
	Block	3	0.032	0.40	0.221
	Fertilizer*Block	9	0.056	0.97	0.338
	Error	11	0.053		
C:P	Fertilizer	3	0.006	11.13	0.0004
	FG	1	<0.0001	0.06	0.818
	Fertilizer*FG	3	0.002	3.16	0.068
	C Concentraton	1	0.002	10.41	0.008
	Block	3	0.000	0.40	0.759
	Fertilizer*Block	9	0.002	0.97	0.512
	Error	11	0.002		
N:P	Fertilizer	3	0.004	9.46	0.001
	FG	1	0.000	2.43	0.147
	Fertilizer*FG	3	0.003	5.98	0.011
	N Concentraton	1	0.002	13.68	0.004
	Block	3	0.001	1.63	0.243
	Fertilizer*Block	9	0.001	0.97	0.509
	Error	11	0.002		

Table 3.2 *Hesperotettix viridis*, *Melanoplus bivittatus*, and *Phoetaliotes nebrascensis* mean abundance per plot (±standard error) in the Belowground Plot Experiment at Konza Prairie Biological Station, Manhattan, Kansas. For each species, significant differences in abundance among treatments are indicated by different letters. 0N0P=non-fertilized control, +P=phosphorus fertilizer, +N=nitrogen fertilizer.

Treatment	*H. viridis*	*M. bivittatus*	*P. nebrascensis*
0N0P	1.25 (0.48)[a]	0.25 (0.25)[b]	3.75 (1.38)[b]
+P	1.00 (0.58)[a]	1.00 (0.58)[ab]	7.75 (1.6)[a]
+N+P	2.00 (1.68)[a]	1.75 (0.25)[a]	0.75 (0.75)[c]
+N	0.75 (0.48)[a]	1.25 (0.48)[a]	0.25 (0.25)[c]

Table 3.3 Repeated measures analysis of variance (ANOVA) results for *Melanoplus bivittatus* tissue carbon (C), nitrogen (N), and phosphorus (P) elemental concentrations and repeated measures analysis of covariance (ANCOVA) results for *M. bivittatus* tissue stoichiometric ratios. Df (n.d)=numerator, denominator degrees of freedom

Response	Effect	Df (n,d)	F	p-value
A. ANOVA tables				
C concentration	Fertilizer	2, 3	0.14	0.878
	Block	3, 3	0.37	0.781
N concentration	Fertilizer	2, 3	0.22	0.812
	Block	3, 3	0.34	0.802
P concentration	Fertilizer	2, 3	0.36	0.721
	Block	3, 3	4.40	0.128
B. ANCOVA tables				
C:N	Fertilizer	2, 3	0.52	0.642
	C Concentraton	1, 6	1.12	0.330
	Block	3, 3	0.54	0.686
C:P	Fertilizer	2, 3	2.78	0.208
	C Concentraton	1, 6	7.22	0.036
	Block	3, 3	15.22	0.026
N:P	Fertilizer	2, 3	0.22	0.812
	N Concentraton	1, 6	13.18	0.011
	Block	3, 3	25.47	0.012

Table 3.4 Correlation between the proportion of C_4 grass assimilated by *Melanoplus bivittatus* and (A) concentrations of carbon (C), nitrogen (N), and phosphorus (P) and (B) C:N, C:P, and N:P stoichiometric ratios of C_3 forb and C_4 grass tissues. Significant correlations at $p<0.02$ with Dunn-Sidak adjustment for multiple comparisons at $\alpha=0.10$.

	C_3 forb	C_4 grass
	Pearson's *r* (p-value)	Pearson's *r* (p-value)
A. Elemental concentrations		
C	-0.34(0.340)	0.83(0.003)
N	0.47(0.173)	0.74(0.014)
P	0.07(0.841)	-0.53(0.116)
B. Stoichiometric ratios		
C:N	-0.45(0.194)	-0.70(0.024)
C:P	-0.05(0.884)	0.56(0.092)
N:P	0.63(0.053)	0.81(0.005)

CHAPTER 4 - Host plant selection is a primary means of nutrient regulation in a mixed-feeding insect herbivore

Abstract

Some polyphagous insect herbivores are also mixed-feeders under natural conditions, eating both forbs and grasses. Despite differences in elemental composition of forbs and grasses, mixed-feeding herbivores are able to maintain C:N:P homeostasis, often performing best when both forbs and grasses are consumed. However, the mechanisms by which performance and elemental homeostasis are maintained in mixed-feeding herbivores are poorly studied. I assessed the roles of different regulatory mechanisms (food selection, compensatory feeding, or physiological adjustment) in the mixed-feeding grasshopper, *Melanoplus bivittatus*, with natural and synthetic diets. Because *M. bivittatus* is a relatively mobile mixed-feeding grasshopper, I hypothesize that stoichiometric homeostasis is achieved primarily through food selection. In a natural diet experiment, leaves of nitrogen fertilized (+) and unfertilized (-) grass (G) and forb (F) collected at Konza Prairie Biological Station were provided and individual growth and development was measured. In a second experiment, high (H) or low (L) quality diets (with respect to C:N) with (+) or without (-) plant extracts were provided. *M. bivittatus* use of diets differing in C:N and macronutrient (protein and carbohydrate) content was examined in a third experiment. All experiments had a paired-choice design with all possible combinations of diets provided to 5th instar *M. bivittatus* nymphs in individual cages for the duration of the stadium. Individuals provided with F+ as at least one of the two diets offered had the highest survival, while those given only grass material (G+/G+, G+/G-, G-/G-) experienced 97% mortality. Although the total amount of food consumed did not differ among treatments, significantly more F+ diet was consumed in F+/G+ and F+/G- treatments. In the artificial diet experiment, survival was 90% on the H+/H+ and lowest on the L/L combinations. Total consumption did not differ among treatments, though H- was preferred over H+ and H+ was preferred over L+. Patterns of performance and utilization of diets were similar in terms of both macronutrient and C:N content. For surviving individuals, my results suggest that *M. bivittatus* nymphs have the ability

to employ all three regulating mechanisms although they are likely to rely primarily on food selection to maintain performance and internal elemental homeostasis when faced with variable resources as would be encountered in a field environment.

Keywords: *Melanoplus bivittatus*, mixed-feeding herbivore, toxin-dilution hypothesis, nutrient-regulation hypothesis, artificial diet, natural diet, host plant quality, homeostasis

Introduction

Mixed-feeding herbivores eat both forbs and grasses, potentially providing a larger range of available resources than that encountered by other polyphagous species that feed only on forbs or grasses. In return, mixed-feeding species must also be able to cope efficiently with the unique physical and chemical properties of both forbs and grasses in order to maintain nutritional homeostasis. Because forbs tend to have higher total N concentrations, and a larger proportion of highly digestible leaf tissue C than grasses (Mole and Joern 1994, Randolph *et al.* 1995, Anderson *et al.* 2004), they are often considered to be of higher nutritional quality than grasses. Despite higher primary nutrient content, forbs also contain higher concentrations of secondary metabolites that act as feeding deterrents, compared to grasses (Harborne 1988, Mole and Joern 1994), reducing their suitability as food. Like generalist herbivores that usually perform best when more than one type of food is available (Bernays and Bright 1993, Hagele and Rowell-Rahier 1999, Miura and Ohsaki 2006), mixed-feeding herbivores usually perform best when feeding on both forbs and grasses (Bailey and Mukerji 1976, MacFarlane and Thorsteinson 1980, Bernays *et al.* 1994, Randolph and Cameron 2001). Although mixed-feeding herbivores occur across taxa, including rodents (Lacher *et al.* 1982, Bucyanayandi and Bergeron 1990, Randolph and Cameron 2001), lizards (Dearing and Schall 1992) and grasshoppers (Joern 1983), relatively little is known about the specific and unique nutritional processes and feeding ecology of mixed-feeding herbivores as opposed to polyphagous herbivores in general.

The ability of herbivores to maintain relatively constant elemental homeostasis despite a large imbalance between plant and herbivore nutrient needs has been widely reported (Slansky and Feeny 1977, Bernays 1998, Fagan *et al.* 2002, Sterner and Elser 2002, Fink and Von Elert 2006). Three general pathways by which homeostasis can be achieved in the face of variable

resources are generally recognized: compensatory feeding, altered post-ingestive processes, and food selection (Simpson and Abisgold 1985). Compensatory feeding, or adjusting consumption to meet the intake needs of the most limiting nutrients, has been reported in many herbivorous species (Heidorn and Joern 1987, Wratten *et al.* 1988, Yang and Joern 1994, Berner *et al.* 2005). Although compensatory feeding may be the most common and efficient means of maintaining nutrient intake by herbivores, especially for specialists or taxa with limited mobility (Bernays 1998, Huberty and Denno 2006), there are several potential costs. Costs include increased predation risk associated with exposure to predators from increased time spent and activity during feeding (Werner and Anholt 1993, Hagele and Rowell-Rahier 1999), increased processing costs associated with over-consumption of non-limiting nutrients (Simpson *et al.* 2004), detoxification costs if foods containing defensive chemicals are ingested in large quantities (Slansky and Wheeler 1992, Hagele and Rowell-Rahier 1999, Simpson and Raubenheimer 2001), and under-consumption of carbohydrates when herbivores adapted to low nutrient hosts limit total intake when fed high nutrient foods (*i.e.* Bertrand's Rule) (Raubenheimer and Simpson 2003b, Raubenheimer *et al.* 2005, Boersma and Elser 2006).

While compensatory feeding may be relatively more common among specialist or immobile species, generalist and vagile herbivores are more likely to rely on food selection to meet nutritional demands (Raubenheimer and Simpson 2003b, Huberty and Denno 2006). In attempting to understand the mechanisms driving food selection by herbivores, two dominant, historically-significant hypotheses are the toxin-dilution hypothesis (Freeland and Janzen 1974) and the nutrient-balance hypothesis (Westoby 1978). Although often framed as opposing hypotheses, interactions between plant nutrient and toxin composition are likely to drive food selection, especially among generalist herbivores (Dearing and Schall 1992, Hagele and Rowell-Rahier 1999, Simpson and Raubenheimer 2000, 2001). For example, Simpson and Raubenheimer (2000, 2001) and Behmer *et al.* (2002) found that deleterious effects of tannic acid on consumption and performance of two grasshopper species occurred only when their diet was imbalanced with respect to protein and carbohydrate.

Herbivores can also alter post-ingestive processing rates to maintain constant body nutritional composition. For insect herbivores, changes in gut size and gut passage rates affect the efficiency with which nutrients are selectively extracted from food and assimilated into body tissue (Yang and Joern 1994, Barbehenn *et al.* 2004b). Although herbivores may exhibit a

tendency toward relying on one of these pathways more than the others, both food selection and compensatory feeding are likely to also impact food processing. When fed high-N plants, Slansky and Feeny (1977) found that *Pieris rapae* larvae had lower consumption and higher efficiency of conversion of ingested food to body mass compared to those fed low-N plants. Fink and Von Elert (2006) reported that an herbivorous gastropod exhibited both compensatory feeding and differential excretion of nutrients in order to maintain C:N:P homeostasis.

Using a common mixed-feeding grasshopper species, *M. bivittatus* (Say), and both natural and synthetic diets in laboratory experiments, I (1) examined the potential methods by which a mixed-feeding herbivore maintains elemental homeostasis (*i.e.*, compensatory feeding, food selection, and post-ingestive processes), and (2) assessed the roles of secondary chemistry and nutrient balance in food selection. In an associated field study, I observed a broad shift in the diet of *M. bivittatus* from primarily forb-based (~80%) to one that contained a nearly equal proportion of grass and forb in response to N fertilization (Chapter 3). Based on these results, I expected *M. bivittatus* to rely primarily on food selection rather than either compensatory feeding or post-ingestive processing to balance elemental composition. Because I expected that food selection would play an important role in the feeding ecology of this species, I also examined the relative importance of toxin dilution and nutrient balance for feeding decisions and performance of *M. bivittatus*. If feeding decisions are based on limiting the intake of N-based plant defensive compounds, I hypothesized that preference would be shown for non-fertilized plants or artificial diets free of plant extracts compared to fertilized plants or artificial diets containing plant extracts in choice tests. If food selection is based on nutrient composition, however, individuals should select a combination of food plants to maintain balanced nutrient intake.

The relationships between herbivores and the relative concentrations of macronutrients in dietary resources are important for understanding the physiological basis for and consequences of feeding decisions and have been widely studied (Bernays and Simpson 1982, Simpson and Abisgold 1985, Abisgold and Simpson 1987, Simpson and Raubenheimer 1993, Bernays and Chapman 1994, Simpson and Raubenheimer 2000, Raubenheimer and Simpson 2003b). Relating these dynamics to larger ecological processes, which are often measured in terms of carbon (C) and nitrogen (N), however, is facilitated by an ecological stoichiometric approach (Sterner and Elser 2002). Therefore, I examine patterns of intake in terms of both protein: carbohydrate and C:N ratios to provide a more direct link for understanding the nutritional

dynamics of this mixed-feeding herbivore in an ecological context. Although I expected the magnitude of responses to change depending on whether protein:carbohydrate or C:N was being measured, the patterns observed for these different nutritional attributes should track one another closely.

Methods

Collection and rearing of M. bivittatus

Adult *Melanoplus bivittatus* (Say) (two-striped grasshopper) were collected at Konza Prairie Biological Station, Manhattan, Kansas during late summer-early fall 2005 and reared to obtain eggs. Grasshoppers were fed organic wheat, organic romaine lettuce, and a mixture of organic wheat bran and brewers yeast. Females oviposited in moist, autoclaved sand. Once removed from the cages, eggs were kept at 30^{o}C for approximately six weeks, and sand was kept moist with distilled water; 0.5% methyl paraben was added to prevent fungal infections. After a six-week incubation, containers with eggs were kept at 4^{o}C for ~12 weeks to break egg diapause, and then maintained at room temperature (~30^{o}C) until hatching. After hatching, nymphs were fed *ad libitum* on the diet described above through the fourth instar (~30-45 days).

Basic experimental set-up

Experiments were performed in 18.5x13x9cm ventilated, clear-plastic containers with two 4cm diameter food dishes, a perch and a 30ml water dish (Fig. 4.1). Within 6-12h after molting to the fifth instar, each nymph was weighed and placed in an experimental cage containing a randomly assigned diet treatment. Because the water content of food (Slansky and Feeny 1977, Chapman 1990, Joern and Mole 2005) and leaf mechanical defenses (Miura and Ohsaki 2004) including cell walls (Clissold *et al.* 2006) can affect feeding of insect herbivores, all diets used in this study were dried and ground (see below). Nymphs were maintained for the duration of the stadium under a 14:10h photoperiod and constant temperature within each phase (35^{o}C light, 30^{o}C dark). During the experiment, each cage was isolated from all other cages by dividers so that activity of individuals in adjacent cages did not influence the feeding behaviors of one another. Initial wet weights for newly hatched fifth instar nymphs were converted to dry weights using a regression equation based on the wet and dry weights of twenty-five additional individuals (dry weight = (0.24505*wet weight)+0.0084, adjusted r^2=0.66, $p<0.0001$).

Natural diet experiment

Plant material for the natural diet experiment was collected from the Belowground Plot Experiment (BPE) at Konza Prairie Biological Station, Manhattan, Kansas. Warm-season grass (G) and forb (F) leaf material was collected in mid-June and early October 2005 from plots fertilized with N since 1986 (G+, F+), and from those that had not been fertilized with N (G-, F-). *Andropogon gerardii* Vitman was the most abundant grass in the unfertilized plots, while *Panicum virgatum* L. was the dominant species in N fertilized plots. *Solidago missouriensis* Nutt. showed the greatest average forb cover in unfertilized plots, but was highly variable. *Ambrosia psilostachya* DC. was the most common forb species in the fertilized plots, and was also consistently abundant in the unfertilized plots. Given the wide diet breadth of *M.bivittatus*, natural variation in plant species composition of the natural diets (rather than using only a single grass and single forb species) so individuals had access to the full suite of host plants available to them in the field. All samples were dried at 60^{o}C for 24h and ground to coarse powder in a 40 mesh Wiley mill. Replicate subsamples of each diet were analyzed for C and N content using a CarloErba 1500 Elemental Analyzer (Thermo Electron, Milan, Italy); the C:N of each diet differed significantly from one another (Table 4.1A). The four diets (F+, F-, G+, G-) were presented to individual nymphs as paired-choice tests with treatments consisting of all possible pair-wise combinations of diets (total 10 treatments); there were 10 replicates of each treatment.

Artificial diet experiment

Standard artificial diets containing macro- and micronutrient concentrations known to support grasshopper growth (Simpson and Raubenheimer 1993) were modified to create high quality and low quality base diets (Appendix B.1A, B.1B). The high quality diet (H-) had a C:N similar to that of forbs (C:N=28.13±1.63), while the low quality diet (L-) was in the range of warm-season grasses (C:N=54.10±2.95). To assess the potential influence of plant allelochemicals on feeding behavior, forbs from the N fertilized plots used in the natural diet experiment were extracted with methanol, water, and chloroform following the protocol of Bernays and Chapman (1975). Extracts from 1g plant material were added per 1g dry diet to a portion of each of the base diets to create high quality and low quality diets containing secondary metabolites (H+ and L+, respectively). Diets were stored at 35^{o}C until dry. Although the extracts likely contained free amino acids which could increase nutritional quality, Bernays and Chapman

(1975) found that these extracts had overall deterrent effects on grasshopper feeding. Addition of the extracts had no effect on the C:N of the diets (H+ vs. H- $t_{1,16}$=1.00, p=0.33, L+ vs. L- $t_{1,16}$=1.17, p=0.26). As in the natural diet experiment, pair-wise combinations of the four artificial diets (H+, H-, L+, L-) were randomly assigned to nymphs in a paired-choice design with treatments consisting of all possible combinations of diets (total 10 treatment combinations). There were 10 replicates of each treatment.

Macronutrient diet experiment

Five artificial diets differing in protein and carbohydrate concentration were prepared to compare the effects of C:N and protein:carbohydrate on feeding by *M. bivittatus* (Appendix B.1A, B.1C). Four of the five diets had a combined protein (p) and carbohydrate (c) content of 42% (p7:c35, p14:c28, p21:c21, p28:c14, p35:c7), while the fifth diet was extremely low quality with only 7% protein and 7% carbohydrate (Simpson and Raubenheimer 1993). Seven treatments consisting of paired diets were presented to nymphs (p7:c7 v. p21:c21, p7:c7 v p7:c35, p7:c7 v p35:c7, p21:c21 v. p7:c35, p21:c21 v. p14:c28, p21:c21 v. p28:c14, p21:c21 v. p35:c7); there were eight replicates of each treatment.

Grasshopper elemental composition

Either upon molting to adulthood or death, grasshoppers were dried for 1 week at 35^{o}C and weighed. All twenty-five control individuals and experimental individuals that reached adulthood were ground to fine powder using liquid N and a mortar and pestle and analyzed for C and N using a CarloErba 1500 Elemental Analyzer (Thermo Electron, Milan, Italy).

Statistical analyses

Final dry weights, days to molt/death, amount consumed of each diet, and the amount of frass produced were recorded at the end of the experiment for each individual. We then calculated amount of weight gained/lost, relative growth rates ((ln(final dry weight)-ln(initial dry weight))/total number of days alive), amount of diet assimilated (consumption-frass), amount of C and N consumed, and N-use efficiencies (NUE) (Slansky and Feeny 1977) for all three experiments, and the amount of protein and carbohydrate consumed in the artificial and macronutrient experiments.

For each experiment, we examined three measures of performance (survival, development, relative growth rate), total consumption, food selection, and five post-ingestive processing metrics (assimilation, approximate digestibility, efficiency of conversion of ingested food, efficiency of conversion of digested food, and NUE) of individuals that survived to the adult stage. All response variables were transformed as necessary to achieve normality. Homogeneity of variances was tested using the Brown-Forsythe test (Milliken and Johnson 1992) and the Kenward-Rogers denominator degrees of freedom adjustment was used for response variables with heterogeneous variances. A square-root transformation was used for RGR in the artificial diet experiment and development time data were log+1 transformed for all three experiments. Total consumption and total carbon consumption in the natural and artificial diet experiments were log+1 transformed; total nitrogen consumption was log(log+1) transformed in both the natural and artificial diet experiments.

Failure time analysis was used to test the effects of the treatments on the time to death or molting (Proc LIFEREG, SAS v.9.1, Cary, NC). Results of this analysis are presented as the estimated cumulative density function (CDF) of either mortality or survival through time. The estimated CDF indicates the probability that an individual will experience mortality at a given time if still alive at the beginning of that time interval (Fox 2001).

Paired t-tests were used to examine differences in consumption of the two diets within each treatment and analysis of variance (ANOVA) on those differences was used to compare selectivity between treatments. Analysis of covariance (ANCOVA) with initial weight as the covariate was used to analyze RGR and assimilation in the natural diet experiment, and total consumption in both the natural and artificial diet experiments. ANOVA was used to analyze days to molt, RGR, and assimilation for the artificial and macronutrient diet experiments; initial weight was not used as a covariate in these analyses because it was not correlated with the responses. Given the statistical issues associated with conducting analyses on ratio-based metrics (Horton and Redak 1993, Raubenheimer 1995), we also used ANCOVA to test approximate digestibility (main effect: assimilation, covariate: consumption), efficiency of conversion of ingested food (ECI; main effect: weight gain, covariate: consumption), and efficiency of conversion of digested food (ECD; main effect: weight gain, covariate: assimilation) and NUE (main effect: biomass N gained, covariate: N ingested). One-way ANOVA and ANCOVA tests

were performed with Fisher's LSD used for post-hoc pairwise comparisons of the treatments when the main effect was significant (Proc mixed, SAS v.9.1, Cary, NC).

RESULTS

Natural diet experiment

The C:N of all four natural diets differed from one another. The F-diets had significantly lower C:N than the G-diets and diets from N fertilized (+) plots had significantly lower C:N than those from unfertilized (-) plots ($F_{3,16}$=583.08, p<0.0001) (Table 4.1A).

Overall survival of 5th instar nymphs was highest when fed diet combinations that included F+ as at least one of the two diets offered (F+/F+: 60%, F+/F-: 30%, F+/G+: 70%, F+/G-: 60%), while those that received G+/G+ (0%), G+/G- (10%), or G-/G- (0%) had very low survival. Failure-time analysis indicated that individuals on the mixed diet treatments (F/G) generally took longer to complete the experiment (whether died or molted) than those fed forbs only or grasses only (Wald χ^2_9=63.4, p<0.0001)(Fig. 4.2A). Relative concentrations of body tissue C:N of individuals were similar in all treatments (Fig. 4.3A), as all treatments aligned along the same C:N trajectory. For individuals that survived to the adult molt, diet combination treatments did not influence either RGR or the length of the 5th instar (Table 4.2B).

The total amount of diet consumed, when adjusted for initial weight, did not differ among the diet combination treatments (Fig. 4.4A). Within the F+/F+ and F-/F- diet combination treatments, individuals consumed the same amount of diet from each dish (Fig. 4.5A, Appendix B.2A). In the F/G treatments, individuals consumed significantly more of the F than G diet, regardless of whether or not the plant material had been fertilized with N. In the F+/F- treatment, significantly more of the F+ diet was consumed than the F- diet. Individuals within each treatment maintained consistent C:N intake (Fig. 4.6). Intake of C:N among treatments was similar in the F+/F-, F+/G+, F+/G- treatments, with it being slightly higher in the F+/F+ and significantly lower in the F-/F- and F-/G+ treatments (Fig. 4.6).

There were no effects of the diet combination treatments on measures of post-ingestive processing. Assimilation, AD, ECD, and ECI were maintained at similar levels by all nymphs (Table 4.2A).

Artificial diet experiment

High quality (H) diets had significantly lower C:N ratios than the low quality (L) diets. The addition of plant extracts did not significantly alter the C:N of either H or L diets ($F_{3,16}$=23.16, p<0.0001) (Table 4.1B).

Overall survival of nymphs was significantly affected by the diet combinations with survival highest among nymphs given H+/H+ (90%) or H+/H- (60%) diet combinations, and lowest on the L/L combinations irrespective of plant extracts (L+/L+: 0%, L-/L-: 0%, L+/L-: 10%). According to failure-time analysis, most individuals completed the experiment (died or molted) within approximately 30 days except those given the H+/H- or L+/L+ treatments which took significant longer (Wald χ^2_9=41.4, p<0.0001) (Fig. 4.2B). At the time of molting, all individuals had similar body tissue C:N (Fig. 4.3B). Neither RGR nor the number of days in the 5th instar was significant affected by the artificial diet combinations (Table 4.2B).

There were no differences in the total amount of diet consumed among the diet combination treatments (Fig. 4.4B). In the H+/L+ treatments, significantly more H+ diet was consumed than L+ diet, there were no significant differences in consumption between the diets within the other treatments in which more than one individual survived (Fig. 4.5B, Appendix B.2B). The C:N of the diet consumed was highest in all treatments containing H+ as at least one of the two diets offered (Fig. 4.6), while those with H- (except H+/H-) had a lower intake C:N.

Post-ingestive processing was similar in all nymphs in the artificial diet experiment. Assimilation, AD, ECD, and ECI did not vary with diet combination treatments (Table 4.2B).

Macronutrient diet experiment

No differences in the C:N ratios of the p7:c7 and p7:c35 diets were observed, and the p28:c14 diet did not differ from either the p21:c21 or p35:c7 diets. All other macronutrient diets differed significantly from one another ($F_{5,24}$=77.06, p<0.0001) (Table 4.1C).

Overall survival was 75% or 87.5% on all treatments (*i.e.*, only 1 or 2 of 8 individuals died). Failure-time analysis indicated that individuals completed the experiment in 10 to 20 days with individuals given the diet combination with the lowest overall concentration of protein (p7:c7/p7:c35) taking the longest to complete the experiment (Wald χ^2_6=37.5, p<0.0001) (Fig. 4.2C). Individuals fed the p7:c7 and p7:c35 diet had significantly higher body tissue C:N than other individuals (Fig. 4.3C). There was no significant response of RGR to diet combinations in

the macronutrient diet experiment, while individuals fed the p7:c7/p7:c35 diets took significantly more time to complete the 5th instar than did those fed any of the other diet combinations (Table 4.2C).

The diet combination treatments did not significantly influence total consumption of individuals (Fig. 4.4C). In all treatments with p21:c21, more of the p21:c21 diet was consumed than the other diet with which it was paired except when in the p21:c21/p28:c14 treatment (Fig. 4.5C, Appendix B.2C). There was no difference in the amount of either p21:c21 or p28:c14 consumed. In the treatments containing one of the 42% total macronutrient content diets and the p7:c7 (14% macronutrient), the 42% macronutrient diet was always consumed to a greater degree than the p7:c7 diet (Fig. 4.5C). Individuals with the low macronutrient diets consumed significantly different C:N, with those in the p7:c7/p35:c7 treatment achieving lower intake C:N than those on the p7:c7/p7:c35 treatment (Fig. 4.7A). All other diet combination treatments had intake C:N ratios between the two treatments containing the low macronutrient diets. Similar patterns were observed when the amount of protein:carbohydrate consumed was analyzed (Fig. 4.7B).

Diet combination treatments did not affect assimilation, AD, ECI, ECD, or NUE (Table 4.2C).

Discussion

My results show that among individuals surviving to adulthood, resource C:N and protein:carbohydrate ratios strongly influence diet selection and performance by *M. bivittatus*. All four diets used in the natural diet experiment differed in C:N, while the high quality artificial diets exhibited lower C:N than the low quality diets (Table 4.1). Despite these differences in food C:N, *M. bivittatus* individuals achieved relatively constant body tissue C:N across all treatments in the natural and artificial diet experiments (Fig. 4.3A, B). Although I expected C:N homeostasis in all experiments (Slansky and Feeny 1977, Fagan *et al.* 2002, Fink and Von Elert 2006), the C:N of individuals in the macronutrient experiment was not regulated as tightly as in individuals in the natural and artificial diet experiments. As protein:carbohydrate of the available diets increased, body tissues from individuals showed progressively lower C:N ratios (Fig. 4.3C).

Performance, measured by RGR and development time, was similar for surviving individuals on all diets within each experiment. This was unexpected as changes in diet quality are usually associated with changes in herbivore growth in other acridid species (MacFarlane and Thorsteinson 1980, Raubenheimer and Simpson 1993, Joern and Behmer 1997, Hagele and Rowell-Rahier 1999, Barbehenn *et al.* 2004b, Berner *et al.* 2005, Fink and Von Elert 2006, Ode 2006). Post-ingestive processes (AD, ECI, ECD) of surviving individuals were not influenced by diet combination treatments, nor was the total amount of food consumed in any of the experiments. In the natural diet experiment, there was a trend toward slightly higher consumption in the F-/F- treatment, especially compared to F+/F+, indicating that the amount eaten can be adjusted as necessary to meet nutrient intake needs in no-choice situations.

Although there was only weak evidence of compensatory feeding within any given experiment, total consumption was about twice as high on the natural diets than on the artificial and macronutrient diets (Fig 4.4). Cellulose was the only non-digestible carbohydrate in the synthetic (artificial and macronutrient) foods and digestible carbohydrate concentrations were higher than in naturally occurring plants (Raguse and Smith 1966, Menke and Trlica 1982, Taylor *et al.* 1989, Read and Morgan 1996, Oedekoven and Joern 2000). Not only were the synthetic foods free of lignin and other non-digestible structural carbohydrates, but nutrients were not packaged within cell walls which have been shown to inhibit the ability of grasshoppers to extract nutrients from plants (Clissold *et al.* 2006). This shows that *M. bivittatus* can employ compensatory feeding in response to broad changes in nutrient packaging and concentration. Given the drastic differences between the natural and synthetic diets and that compensatory feeding was not employed within the context of any of the individual experiments, I do not expect the mixed-feeding *M. bivittatus* to widely exhibit compensatory feeding to regulate nutrient intake under natural conditions.

The N-containing compounds in plants account for a large proportion of foliar digestible C (Anderson *et al.* 2004). Similarly, the protein sources used in the synthetic foods (artificial and macronutrient experiments) had slightly higher C content (peptone~46% C and albumen~57% C) than the digestible carbohydrates (sucrose=42% C, dextrin=40% C). In the synthetic foods, the digestible C fraction increased (from 44 to 50%) with increasing protein concentration even though the concentration of carbohydrate decreased. So even though Oedekoven and Joern (2000) found no general relationship between %N and % non-structural carbohydrates in 7

dominant grasses, the low C:N synthetic foods used in this study had higher protein content as well as a higher proportion of labile C than high C:N foods.

There was evidence for selective feeding by *M. bivitattus* in all three experiments, especially in the natural diet experiment. Forbs were preferred over grasses, and fertilized forbs were generally selected over those that were not fertilized. Likewise, high quality artificial diets were generally favored over those of low quality, although this was only significant in the H+/L+ treatment. As a result of this selective feeding, an intake C:N ratio of approximately 24 was maintained across most treatments even though the absolute amount of C consumed varied between 30-70mg in the artificial diet experiment and 60-230mg in the natural diet experiment (Fig. 4.6). The exceptions to this were those feeding on the lower quality diets where intake C:N was as low as possible given the C:N of the foods in those treatments. But again, an intake C:N of about 38 was seen in individuals on the lower trajectory in both natural and artificial diet experiments.

Unlike the other two experiments, intake C:N was not regulated as tightly in the macronutrient experiment (Fig. 4.7A). Individuals within each treatment tended to consume C and N in constant proportions, but there was variation between treatments. Individuals in the p7:c7/p7:c35 and p7:c7/p35:c7 treatments had the highest (~50) and lowest (~10) intake C:N ratios, respectively. Intake protein:carbohydrate (Fig. 4.7B) roughly mirrors that of intake C:N (Fig. 4.6A) with treatments containing p21:c21 intermediate between the p7:c7/p7:c35 and p7:c7/p35:c7 treatments In all treatments containing p21:c21, it was always the preferred food except when paired with p28:c14 in which case both were chosen equally. There was also always selection against p7:c7 foods, even when paired with the food in which protein was most dilute (p7:c35). *Schistocera gregaria*, another mixed-feeding grasshopper species, was found to regulate consumption such that there appeared to be a combined protein plus carbohydrate optimum even if the two were unbalanced with regard to one another (Simpson and Raubenheimer 2000). Although nutrients are imbalanced in p7:c35 compared to p7:c7 the total nutrient content is higher (combined protein plus carbohydrate 42% versus 14%, respectively) and thus more optimal.

Despite selection for higher quality (low C:N) foods, lower quality foods were never completely rejected. For example, grass accounted for about 10% of the material ingested in most of the mixed-diet combinations and about 40% in F-/G+ of the natural diet experiment.

That the proportion of grass consumed increased three-fold on F-/G+ provides support for my results from a field study where the contribution of grass to the diet of *M. bivittatus* doubled from control (~20% grass) to N-fertilized (~40% grass) plots and the increase was associated with decreased C:N of grasses and unrelated to forb C:N (Chapter 3).

Although *M. bivittatus* showed strong selection for foods based on C:N (or carbohydrate: protein), my results suggest that plant defensive chemistry was not as important in food selection by *M bivittatus*. However, there were non-significant increases in consumption of extract-free food in the H+/H- (n=6) and H+/L- (n=1) treatments. N-containing alkaloids are more deterrent to grasshoppers than tannins or other phenols (Bernays and Chapman 1977, Bernays *et al.* 1994, Mole and Joern 1994), including *M. bivittatus* (Harley and Thorsteinson 1967), and forbs generally produce more N-based (*e.g.*, alkaloids, non-protein amino acids, etc.) compounds than either grasses or woody plants (Feeny 1976, Bernays and Chapman 1977, Mole and Joern 1994, Throop *et al.* 2004). When plants are fertilized with N, production of N-based secondary metabolites tends to increase (McKey 1979, Rhoades 1979) while investment in C-based secondary compounds decreases (Bernays and Chapman 1994, Ritchie 2000). The dominant species in the forb diets and plant extracts was *A. psilostachya*. Hazlett and Sawyer (1998) found this species to have high alkaloid (N-based deterrents) content. Therefore, I reasonably expect the presence of N-based secondary metabolites in the extracts (Bernays and Chapman 1975), even though I did not directly test for them. Bernays and Chapman (1975, 1977) found that the extraction procedure removed only 65-80% of secondary compounds from plant material, but at least one of the extracts from each forb tested had deterrent effects on feeding of two grass-feeding grasshopper species. Because Bernays *et al.* (1994) found that alkaloids only had deterrent effects on a mixed-feeding grasshopper at high concentration, I used N-fertilized forb material so the concentration of natively produced N-based secondary chemicals would be as high as possible in my extracts.

The diet combinations used in these experiments had a large effect on survival. Individuals forced to feed on low quality diets (G/G or L/L) experienced 97% mortality which occurred relatively early (except L+/L+) in both experiments. In the natural diet experiment, those fed the low quality diets had a higher probability of dying more quickly (<20 days) than those in the artificial diet experiment (20-70 days) (Fig. 4.2A,B). Average mortality for those given high quality diets (F/F or H/H) was 43-63% and those on mixed diets (F/G or H/L) was 57-

65%. Individuals who died prior to reaching the adult stage had significantly lower total consumption, ECI and ECD and higher intake C:N than those that survived in the natural and artificial diet experiments (data not shown). In the macronutrient experiment, both consumption and ECI were lower in grasshoppers that died. And although there was no difference in intake C:N based on survival, intake protein: carbohydrate ratios were significantly lower in those that died (data not shown). This result also suggests that host nutritional quality exerts a stronger influence on *M. bivittatus* performance than defensive chemistry.

Conclusions

Despite the significant variation in resource C:N stoichiometry, *M. bivittatus* a mixed-feeding insect herbivore regulated intake C:N to maintain body tissue homeostasis. As expected, food selection based upon dietary nutrition was the primary mechanism used to regulate elemental homeostasis and maintain performance, though my results also suggest the ability to use compensatory feeding in the face of extreme imbalances in diet quality. Although physiologically less meaningful than protein and carbohydrate dynamics, studying herbivore feeding ecology in terms of elemental stoichiometry yielded similar responses as macronutrients and is likely to enhance my ability to relate herbivory to ecosystem-level processes such as nutrient cycling.

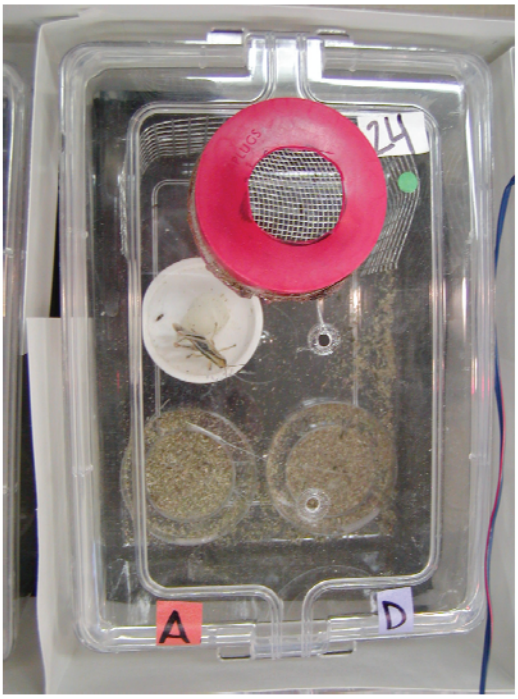
Acknowledgements

I thank S. Behmer and J. Apple for insightful discussions in designing this study and A. Kula, J. Hill and S. Parsons for laboratory assistance. J.M. Blair, D.C. Hartnett, and T.C. Todd provided helpful comments on previous drafts of this manuscript. This work was funded by NSF DEB0456522 and NSF LTER.

Figures and Tables

Figure 4.1 . Experimental setup of (A) food dishes, water dish, and perch inside individual 18.5 x 13 x 9 cm cages and (B) layout of cages in laboratory during the experiments.

A. Individual cage



B. Experimental design

Figure 4.2 . Estimated cumulative density functions (CDF) of mortality among *Melanoplus bivittatus* 5th instar nymphs in the (A) natural and (B) artificial diet experiments. Because only 1 or 2 individuals died prior to molting in the macronutrient diet experiment, estimated CDF of survival to the adult stage of *M. bivittatus* nymphs is presented for the (C) macronutrient diet experiment. Each line represents the probability of an individual experiencing mortality (panels A and B) or molting to the adult stage (panel C) if still alive at a given time (day) as estimated by failure-time analyses (Fox 2001). In panel B, only one individual in the H+/H+ treatment died prior to molting, so that treatment is represented by a single point.

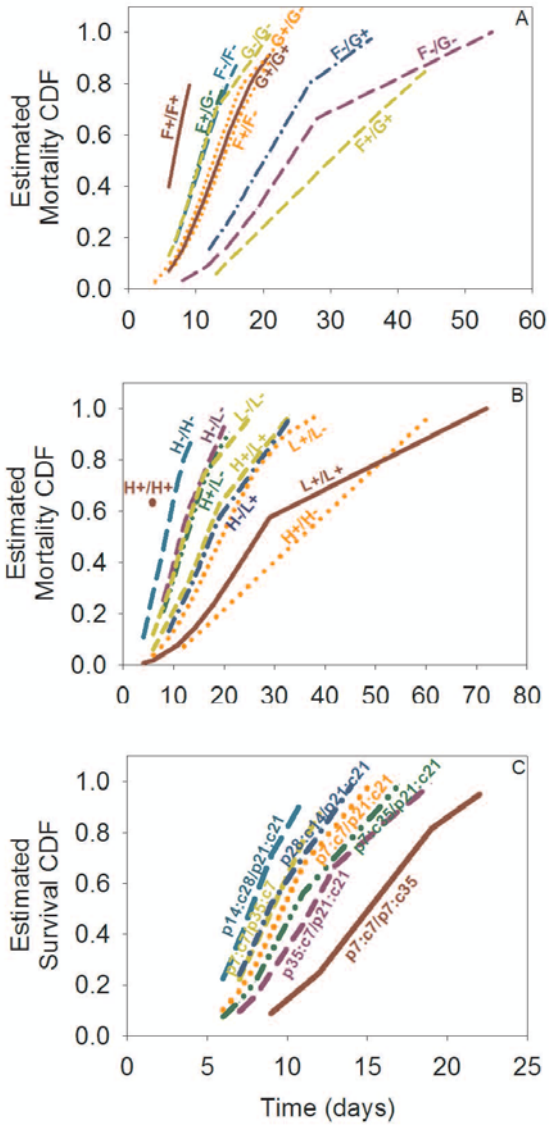


Figure 4.3 Tissue carbon (C) and nitrogen (N) content of *Melanoplus bivittatus* newly hatched adults from the (A) natural, (B) artificial, and (C) macronutrient diet experiments. Gray symbols represent treatments in which only one individual survived. (A) ANCOVA: diet treatment $F_{5,20}$=1.50, P=0.23, C (covariate) $F_{1,20}$=0.70, P=0.41, (B) ANCOVA: diet treatment $F_{5,23}$=1.90, P=0.13, C (covariate) $F_{1,23}$=50.86, P<0.0001, (C) ANCOVA: diet treatment $F_{6,39}$=4.98, P=0.001, C (covariate) $F_{1,39}$=141.81, P<0.0001.

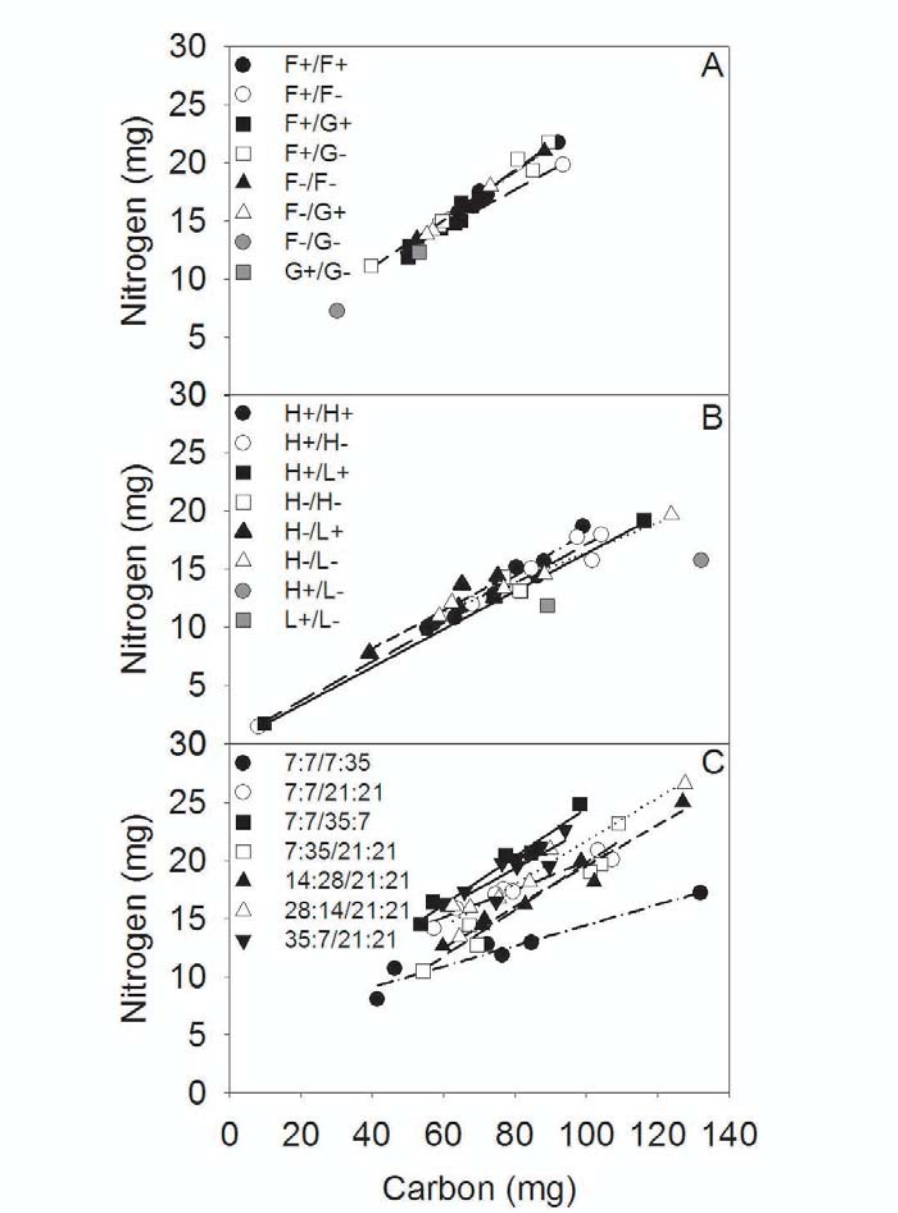


Figure 4.4 Total amount of food consumed (g) by *Melanoplus bivittatus* nymphs during the 5th nymphal stadium in the (A) natural, (B) artificial, and (C) macronutrient diet experiments. All data graphed are untransformed; statistical analyses were conducted on log+1 transformed values for (A) and (B). Only one individual survived treatments F-/G- and G+/G- in (A) and L+/L- and H+/L- in (B). (A) ANCOVA: diet treatment $F_{5,20}$=1.14, P=0.37, initial weight (covariate) $F_{1,20}$=4.80, P=0.04, (B) ANCOVA: diet treatment $F_{5,23}$=2.14, P=0.10, initial weight (covariate) $F_{1,23}$=11.87, P=0.002, (C) ANOVA (initial weight not significant): diet treatments $F_{6,40}$=2.02, P=0.09. Error bars represent ± 1 standard error.

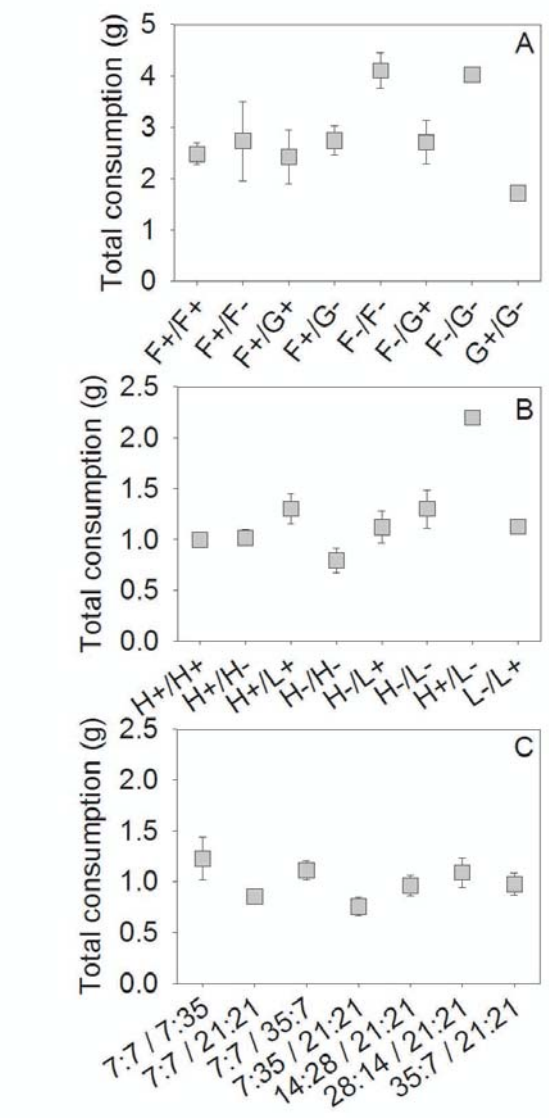


Figure 4.5 Average amount of each diet consumed (g) in all treatments in which at least one *Melanoplus bivittatus* individual survived to molt in the (A) natural, (B) artificial, and (C) macronutrient diet experiments. Data for each treatment were analyzed separately using paired t-tests. Only one individual survived treatments F-/G- and G+/G- in (a) and L+/L- and H+/L- in (b). Error bars represent ± 1 standard error. *p<0.10 **p<0.05

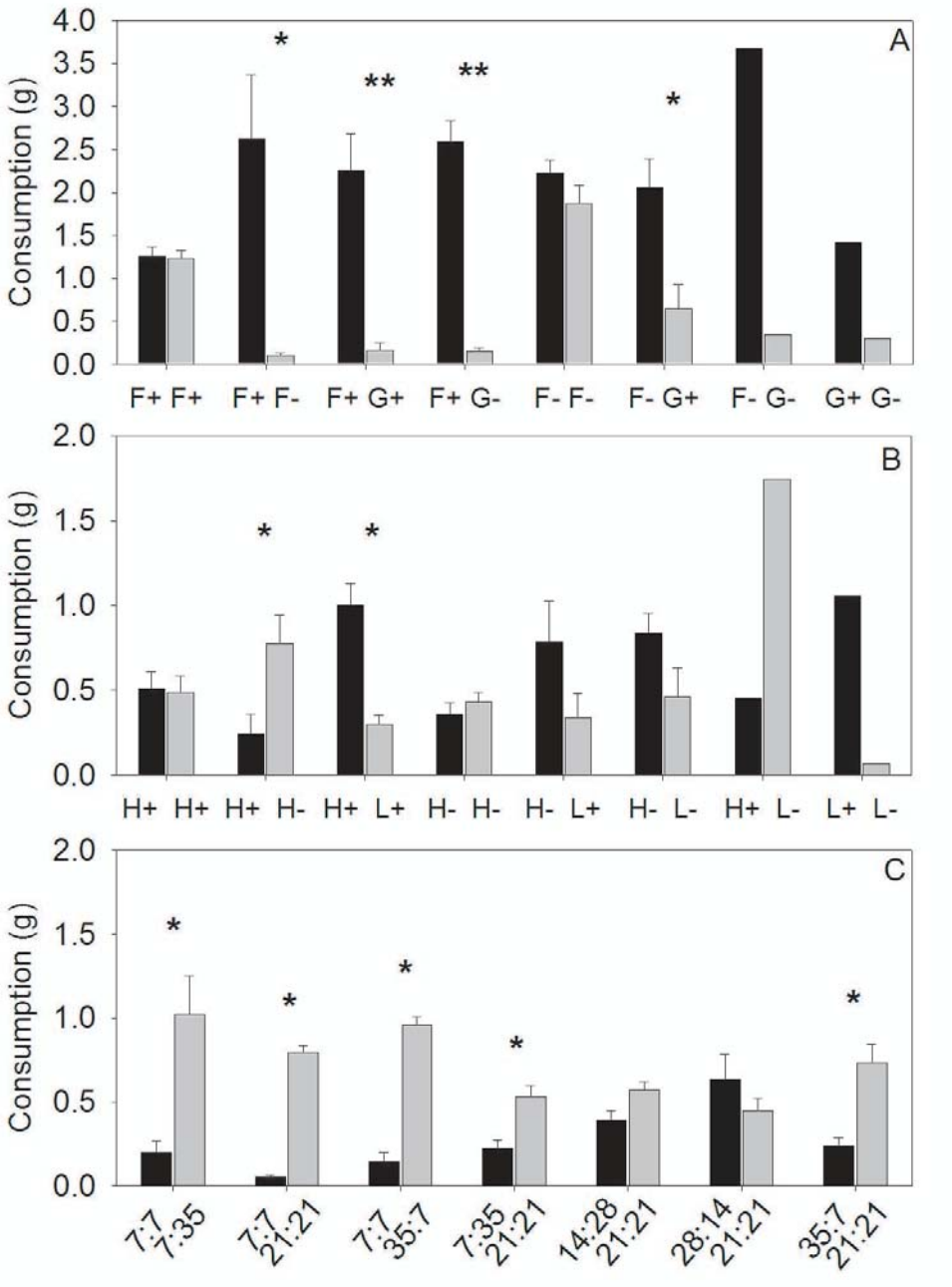


Figure 4.6 The effects of diet combination treatments on total carbon and nitrogen consumption (g) of *Melanoplus bivittatus* 5th instar nymphs surviving to adulthood in the natural (upper right) and artificial (lower left) diet experiments. Natural diets ANCOVA: diet treatment $F_{5,20}$=141.90, P<0.0001, C (covariate) $F_{1,20}$=2772.22, P<0.0001, Artificial diets ANCOVA: diet treatment $F_{5,23}$=8.86, P<0.0001, C (covariate) $F_{1,23}$=144.27, P<0.0001.

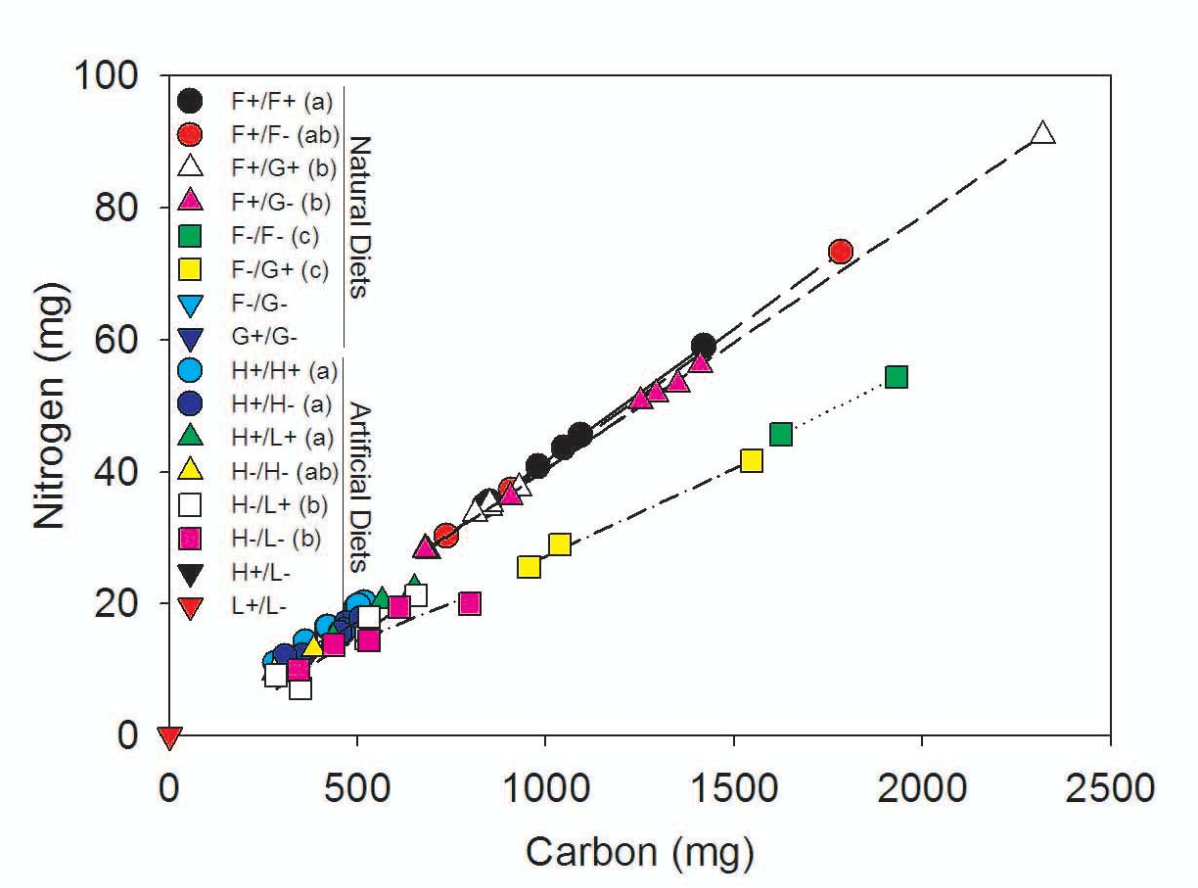


Figure 4.7 The relationship between (A) carbon and nitrogen and (B) protein and carbohydrate consumption (g) by *Melanoplus bivittatus* nymphs fed different macronutrient diet combinations. (A) ANCOVA: diet treatment $F_{6,39}$=10.00, P<0.0001, C (covariate) $F_{1,39}$=60.86, P<0.0001, (B) Unequal slopes ANCOVA at mean carbohydrate: diet treatment $F_{6,39}$=15.06, P<0.0001, C (covariate) $F_{1,39}$=26.48, P<0.0001.

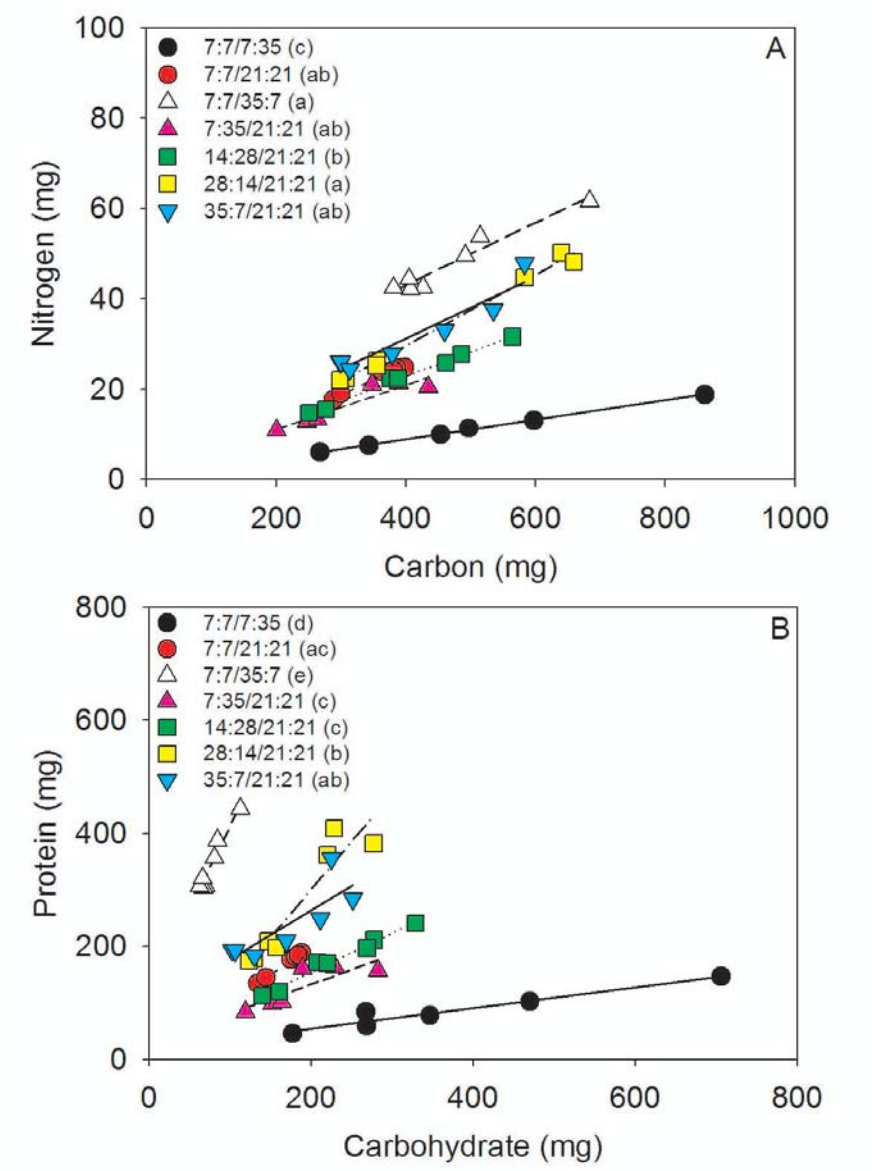


Table 4.1 Carbon:Nitrogen (C:N) ratios of food material used in the (A) natural diet, (B) artificial diet, and (C) macronutrient diet experiments. Different letters denote significant differences ($p<0.05$) between C:N of diets.

Diet	%Carbon	%Nitrogen	Carbon:Nitrogen (C:N)
A) Natural Diets			
Fertilized forb (F+)	41.84 (0.09) [d]	1.74 (0.03) [a]	24.05 (0.46) [a]
Non-fertilized forb (F-)	43.31 (0.17) [b]	1.22 (0.02) [b]	35.60 (0.58) [b]
Fertilized grass (G+)	44.40 (0.07) [a]	1.07 (0.03) [c]	41.50 (0.95) [c]
Non-fertilized grass (G-)	42.91 (0.11) [c]	0.64 (0.01) [d]	66.62 (0.46) [d]
B) Artificial Diets			
High quality +plant extracts (H+)	42.47 (0.11) [a]	1.66 (0.14) [a]	26.26 (2.17) [a]
High quality (H-)	41.83 (0.19) [b]	1.43 (0.12) [a]	30.01 (2.36) [a]
Low quality +plant extracts (L+)	41.79 (0.04) [b]	0.83 (0.05) [b]	50.94 (3.05) [b]
Low quality (L-)	41.93 (0.08) [b]	0.76 (0.07) [b]	57.25 (5.00) [b]
C) Macronutrient Diets			
7% protein: 7% carbohydrate (p7:c7)	41.47 (0.05) [d]	1.00 (0.08) [d]	42.32 (3.09) [a]
7% protein: 35% carbohydrate (p7:c35)	40.98 (0.05) [e]	0.89 (0.05) [d]	46.77 (2.82) [a]
14% protein: 28% carbohydrate (p14:c28)	41.44 (0.09) [d]	1.83 (0.13) [c]	23.07 (1.43) [b]
21% protein: 21% carbohydrate (p21:c21)	41.82 (0.12) [c]	2.76 (0.11) [b]	15.27 (0.54) [c]
28% protein: 14% carbohydrate (p28:c14)	42.22 (0.15) [b]	3.41 (0.09) [b]	12.42 (0.32) [c]
35% protein: 7% carbohydrate (p35:c7)	42.82 (0.11) [a]	4.85 (0.21) [a]	8.89 (0.38) [c]

Table 4.2 Means (±SE) and analysis of variance/covariance results of performance and post-ingestive processing measures by 5th instar *Melanoplus bivittatus* nymphs in the (A) natural, (B) artificial, and (C) macronutrient diet experiments. Relative growth rates (RGR) and developmental time were used as performance metrics, while assimilation, approximate digestibility (AD), efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD), and nitrogen use efficiency (NUE) were used to assess post-ingestive processing.

Diet Treatment	Performance		Post-ingestive processing				
	RGR Mean (SE)	Development Mean (SE)	Assimilation Mean (SE)	AD Mean (SE)	ECI Mean (SE)	ECD Mean (SE)	NUE Mean (SE)
A. Natural Diets							
F+/F+	0.038 (0.005)	8.33 (0.99)	0.65 (0.14)	0.25 (0.04)	0.02 (0.00)	0.08 (0.02)	18.18 (1.06)
F+/F-	0.025 (0.004)	12.67 (2.73)	0.54 (0.15)	0.20 (0.04)	0.01 (0.00)	0.08 (0.02)	12.50 (3.22)
F+/G+	0.041 (0.009)	11.71 (3.06)	0.47 (0.12)	0.19 (0.01)	0.02 (0.00)	0.11 (0.03)	13.29 (2.27)
F+/G-	0.029 (0.011)	9.33 (0.61)	0.75 (0.27)	0.26 (0.08)	0.01 (0.01)	0.08 (0.03)	15.24 (2.99)
F-/F-	0.022 (0.017)	18.00 (6.00)	0.86 (0.00)	0.21 (0.02)	0.01 (0.01)	0.05 (0.03)	15.56 (8.90)
F-/G+	0.026 (0.015)	13.33 (0.88)	0.88 (0.33)	0.31 (0.07)	0.01 (0.01)	0.06 (0.05)	18.03 (5.25)
Response variable	RGR	Day to Molt	Assimilation	Assimilation	Weight gain	Weight gain	N gained
F	0.66	2.12	0.66	0.76	0.03	0.14	0.80
P-value	0.66	0.1	0.655	0.586	1.000	0.979	0.561
Covariate	Initial weight			Consumption	Consumption	Assimilation	N ingested
F	4.39			19.32	0.11	3.31	1.40
P-value	0.05			0.000	0.743	0.084	0.250
B. Artificial Diets							
H+/H+	0.052 (0.009)	12.22 (2.88)	0.40 (0.04)	0.41 (0.03)	0.06 (0.01)	0.16 (0.02)	20.21 (4.76)
H+/H-	0.026 (0.033)	10.83 (0.40)	0.44 (0.05)	0.42 (0.03)	0.06 (0.02)	0.15 (0.06)	24.35 (15.49)
H+/L+	-0.006 (0.074)	11.33 (0.33)	0.53 (0.07)	0.40 (0.01)	0.03 (0.05)	0.08 (0.12)	10.57 (24.45)
H-/H-	0.049 (0.003)	10.00 (3.00)	0.29 (0.10)	0.36 (0.07)	0.07 (0.01)	0.20 (0.01)	36.13 (10.70)
H-/L+	0.030 (0.012)	16.20 (3.87)	0.63 (0.11)	0.55 (0.03)	0.03 (0.01)	0.06 (0.02)	13.45 (9.19)
H-/L-	0.044 (0.008)	14.20 (2.13)	0.68 (0.20)	0.49 (0.07)	0.05 (0.01)	0.12 (0.03)	26.75 (7.34)
Response variable	RGR	Day to Molt	Assimilation	Assimilation	Weight gain	Weight gain	N gained
F	0.62	0.81	1.77	1.85	2.09	2.04	0.34
P-value	0.69	0.56	0.158	0.143	0.103	0.111	0.883
Covariate				Consumption	Consumption	Assimilation	N ingested
F				67.13	1.32	0.02	1.38
P-value				<0.0001	0.262	0.886	0.252
P-value							

Table 4.2 (Continued)

Diet Treatment	Performance		Post-ingestive processing				
	RGR Mean (SE)	Development Mean (SE)	Assimilation Mean (SE)	AD Mean (SE)	ECI Mean (SE)	ECD Mean (SE)	NUE Mean (SE)
C. Macronutrient Diets							
7:7/7:35	0.028 (0.014)	14.83 (1.99)	0.67 (0.18)	0.52 (0.05)	0.03 (0.02)	0.07 (0.04)	19.14 (10.76)
7:7/21:21	0.065 (0.012)	9.57 (1.13)	0.35 (0.01)	0.41 (0.02)	0.08 (0.01)	0.20 (0.03)	34.31 (3.59)
7:7/35:7	0.048 (0.006)	9.29 (0.89)	0.34 (0.06)	0.30 (0.03)	0.05 (0.01)	0.17 (0.02)	19.36 (2.57)
7:35/21:21	0.072 (0.016)	9.50 (1.65)	0.32 (0.11)	0.37 (0.12)	0.11 (0.03)	-0.09 (0.26)	43.92 (16.92)
14:28/21:21	0.056 (0.012)	8.14 (0.59)	0.46 (0.09)	0.46 (0.04)	0.07 (0.01)	0.17 (0.04)	32.69 (5.43)
28:14/21:21	0.069 (0.008)	8.86 (0.91)	0.53 (0.14)	0.44 (0.07)	0.07 (0.01)	0.19 (0.03)	25.72 (4.10)
35:7/21:21	0.055 (0.009)	10.29 (1.70)	0.38 (0.06)	0.37 (0.02)	0.07 (0.01)	0.19 (0.03)	31.53 (3.70)
Response variable	RGR	Day to Molt	Assimilation	Assimilation	Weight gain	Weight gain	N gained
F	1.8	2.35	1.42	1.02	0.69	0.51	1.12
P-value	0.12	0.05	0.231	0.428	0.661	0.796	0.367
Covariate				Consumption	Consumption	Assimilation	N ingested
F				13.68	2.65	0.22	3.95
P-value				0.001	0.112	0.640	0.054

CHAPTER 5 - Consumption of mycorrhizal and saprophytic fungi by Collembola in grassland soils

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Abstract

Although soil-dwelling Collembola can influence plant growth and nutrient cycling, their specific role in soil food webs is poorly understood. Soil-free microcosm studies suggest that Collembola are primarily fungivores where they feed preferentially on saprophytic fungi (SF) over other fungal types. I directly assessed collembolan consumption of arbuscular mycorrhizal fungi (AMF) and SF using plant-soil mesocosms and natural abundance stable carbon isotope techniques. Mycorrhizal *Andropogon gerardii* (C_4 grass) seedlings were placed in pots containing Collembola and soil from a C_3 plant dominated site, while mycorrhizal *Pascopyrum smithii* (C_3 grass) seedlings were placed in pots with Collembola and soil collected at a C_4 plant dominated site. After six weeks, collembolans assimilated carbon derived from C_3 and C_4 sources in both *A. gerardii* and *P. smithii* treatments. Comparing Collembola isotope values in AMF versus AMF-suppressed treatments, my data show that both AMF and SF were consumed in these experimental soil environments.

Keywords: arbuscular mycorrhizal fungi, saprophytic fungi, Collembola, grassland, natural abundance stable isotopes, $\delta^{13}C$, *Andropogon gerardii*, *Pascopyrum smithii*

Introduction

Soil microarthropods can play important roles in ecosystem processes, such as nutrient cycling (Scheu and Setala 2002, Rooney *et al.* 2006). Edaphic Collembola have been shown to affect nitrogen (N) and phosphorous (P) content of plants (Harris and Boerner 1990), as well as rates of carbon (C) cycling in soils (Johnson *et al.* 2005). The heterogeneous and opaque nature of soil environments, however, limits my ability to assess the mechanisms by which soil microarthropods affect ecosystem dynamics. Understanding the feeding habits of soil

microarthropods, such as Collembola, in the soil environment is an important step in identifying such mechanisms. I directly examine collembolan consumption of plant symbiotic arbuscular mycorrhizal fungi (AMF) and decomposer saprophytic fungi (SF) in a reciprocal mesocosm experiment using natural abundance stable C isotope techniques.

Saprophytic fungi are ubiquitous in soils and are the primary component of the fungal pathway of soil food webs (Coleman *et al.* 1983, Rooney *et al.* 2006). They tend to decompose more recalcitrant forms of plant residue and soil organic matter than bacteria, and by balancing the faster nutrient turnover rates of labile organic matter through bacterial pathways, SF are important in ecosystem N and P dynamics (Coleman *et al.* 1983, Wall and Moore 1999). An increased abundance of SF-feeding soil fauna has been associated with decreased rates of decomposition (Petersen and Luxton 1982, Hedlund and Sjogren Ohrn 2000).

The symbiosis formed between AMF and plant roots is important to the productivity of many terrestrial ecosystems (Smith and Read 2002). This mycorrhizal symbiosis is characterized by fungal uptake of soil P and N, largely unattainable by plants which are exchanged for plant-derived C (Smith and Read 2002). In grassland ecosystems AMF colonize the roots of most plant species (Wilson and Hartnett 1997, 1998, Smith and Read 2002). While productivity of the dominant warm-season grass species responds positively to AMF (Wilson and Hartnett 1998), other species, including some forbs and sub-dominant grasses, may be negatively affected by the symbiosis (Wilson and Hartnett 1997). With these varied relationships between different plant species and AMF colonization, feeding on AMF by soil Collembola may result in complex effects on plant performance and community structure (Harris and Boerner 1990, Gange 2000, Partsch *et al.* 2006).

Collembola are a widely distributed and abundant group of soil microarthropods with densities of up to 57,000 individuals/m^2 in grassland soils (Gange and Bower 1997). Although they can employ diverse feeding strategies, edaphic species are considered to be primarily fungivorous (Bardgett *et al.* 1993, Hopkin 1997, Rusek 1998). The presence of Collembola has been associated with increased growth and respiration of AMF (Thimm and Larink 1995), as well as increased plant growth and nutrient concentrations (Harris and Boerner 1990, Larsen and Jakobsen 1996). Although detailed mechanisms for these responses are unknown, moderate collembolan grazing may stimulate growth of AMF (Lussenhop 1996, Bakonyi *et al.* 2002, Lussenhop and BassiriRad 2005) or enhance plant available N and P through fecal deposition or

by stimulating SF-mediated decomposition (McGonigle 1994, Rusek 1998, Gange 2000). Additionally, collembolan grazing has also been found to decrease rates of soil C cycling by severing AMF hyphal networks (Johnson *et al.* 2005).

Elucidating collembolan feeding habits in soil habitats will provide a more mechanistic understanding of their role in soil food webs and nutrient dynamics (Scheu *et al.* 1999, Partsch *et al.* 2006). It is unclear whether Collembola preferentially consume AMF or SF (Finlay 1985, Moore *et al.* 1985, Harris and Boerner 1990, Kaiser and Lussenhop 1991, Thimm and Larink 1995, Klironomos and Ursic 1998, Klironomos *et al.* 1999, Klironomos and Moutoglis 1999, Jorgensen *et al.* 2003, Scheu and Folger 2004), although soil-free laboratory microcosm experiments conclude they have a general preference for SF (Verhoef *et al.* 1988, Klironomos and Kendrick 1996, Klironomos *et al.* 1999, Jorgensen *et al.* 2003, Schreiner and Bethlenfalvay 2003, Tiunov and Scheu 2005). Given the complexity of the soil environment, however, actual consumption of fungal resources may not reflect collembolan preferences *per se*. For example, the ability of most soil microarthropods, including Collembola, to disburse to resources and forage throughout the soil matrix is constrained by structural and architectural characteristics of the soil, including pore spaces (Schrader and Lingnau 1997, Larsen *et al.* 2004), which may result in more opportunistic rather than selective feeding patterns.

The primary objective of my study is to evaluate Collembola feeding habits on AMF and SF in complex plant-soil systems. I conducted an experiment with Collembola in mesocosms containing (1) non-sterile C_4 soils and mycorrhizal C_3 plants and (2) non-sterile C_3 soils and mycorrhizal C_4 plants. Because natural variation in stable C isotope concentrations ($\delta^{13}C$) of organic matter derived from C_3 versus C_4 photosynthetic pathways is generally maintained through trophic levels, analysis of Collembola body tissue $\delta^{13}C$ allows us to assess C assimilated from AMF ($\delta^{13}C$ similar to plants) and SF ($\delta^{13}C$ similar to soil organic matter) (Fry 2006). By including reciprocal plant-soil treatments, I am able to assess feeding habits in the presence of obligate (*Andropogon gerardii*) and facultative (*Pascopyrum smithii*) mycotrophic grasses. I expect Collembola will consume AMF to some extent in both treatments, although consumption of AMF is likely to be greater in mesocosms with the obligate mycotroph since AMF are likely to be most abundant in that treatment. I also assess the population response of Collembola to the presence of AMF in these systems. Because of increased resource diversity, I hypothesize that

Collembola populations will be greatest when both fungal types are present. This study is the first to assess Collembolan feeding habits in soil-based mesocosms.

Methods and materials

An experiment with reciprocal treatments (Fig. 5.1) that manipulated the $\delta^{13}C$ signatures of plants, Collembola, soil, and both fungal types was conducted during the summer 2005 at Kansas State University, Manhattan, Kansas, USA using soils and Collembola collected at Konza Prairie Biological Station (KPBS). In each trial, the $\delta^{13}C$ signature of SF and AMF were different from one another (representative of C_3 vs. C_4 substrates used by the fungi) so that I could determine relative consumption by Collembola using their $\delta^{13}C$ values (Fry 2006).

Pascopyrum smithii trial

P. smithii (Rydb.) (western wheatgrass), a facultative mycotroph, is a native C_3 grass. *P. smithii* seeds were sown in C_3 soil collected at KPBS and grown for four weeks prior to initiation of the experiment to allow AMF colonization of roots. Three replicate plants were harvested before the experiment began to ascertain the degree of AMF colonization. Roots were stained with Trypan blue following the method of Koske and Gemma (1989) and scored for percent AMF colonization using the magnified gridline intersect method developed by McGonigle *et al.* (1990). In addition to indicating the strength of the relationship between AMF and the plant, percent AMF root colonization is also positively correlated with the biomass of hyphae in soil (Hart and Reader 2002). Average *P. smithii* colonization was 16% in four week old seedlings.

Soil was collected at KPBS from beneath a native C_4 plant community dominated by *A. gerardii*. The soil contained 20 $\mu g\ g^{-1}$ available P (Bray I), 32 $\mu g\ g^{-1}$ NH_4-N, and 3$\mu g\ g^{-1}$ NO_3-N as determined by the Kansas State University Soil Testing Laboratory. Collembola (Onychiuridae and Isotomidae) were elutriated from a portion of the C_4 soil so their C isotope signature would initially be similar to that of the C_4 soil. One *P. smithii* seedling was transplanted into each of 16 plastic pots (6 cm diameter x 25 cm deep) containing 530 g (dry weight) non-sterile C_4 soil. At the same time, six to ten of the C_4-labelled Collembola (equivalent to 2123-3539 individuals/m^2) were placed in each C_4-labelled soil pot approximately 2 cm below the soil surface. Every attempt was made to add ten individuals per pot, however individuals were occasionally lost during the transfer process. Benomyl (methyl 1-

(butylcarbamoyl)-2-benzimidazole carbamate) was applied to half (8) of the pots at a rate of 8.3g/m^3 every 2 weeks to suppress AMF root colonization. This is a fungicide previously used to successfully suppress AMF in greenhouse mesocosms with no phytotoxic effects on either of the grass species used in my current study (Wilson and Hartnett 1997).

Four pots each of the mycorrhizal and fungicide treatments were destructively harvested approximately three weeks and six weeks after initiation of the experiment, sufficient time for collembolan reproduction and turnover of body tissue C (Briones *et al.* 1999, Chamberlain *et al.* 2004). Aboveground plant biomass was clipped, dried at 60^{o}C for 48-h, and weighed. Roots were removed from the soil, stained, and scored for AMF colonization using the methods described above. The soil was left in the pots which were wrapped in foil and stored at 4^{o}C for less than 48-h prior to elutriation of live Collembola from the soil. Soil, plant, and Collembola were analyzed for ^{13}C signature using a Sercon GSL prep unit fitted to a Europa 20-20 continuous flow mass spectrometer (Sercon, Crewe, United Kingdom). Values for ^{13}C are reported in $\delta^{13}C$ notation and referenced against the Vienna Pee Dee Belemnite (VPDB) standard (0‰ or 1.12372% ^{13}C). Because the soil in each pot was elutriated, I analyzed eight replicate subsamples of bulk soil to estimate the soil $\delta^{13}C$ value.

Andropogon gerardii *trial*

A. gerardii Vitman (big bluestem) is a native C_4 grass and an obligate mycotroph (Wilson and Hartnett 1998). *A. gerardii* seeds were sown in native C_4 grassland soil and allowed to grow for four weeks to allow AMF colonization of *A. gerardii* roots. AMF colonization was scored using the methods described above on three replicate plants prior to initiation of the experiment. Average *A. gerardii* colonization was 24% in four week old seedlings.

Soil was collected at KPBS from an area that had supported predominantly C_3 vegetation for at least twelve of the past fifteen years. The soil contained 21 μg g^{-1} available P (Bray I), 20 μg g^{-1} NH_4-N, and 0.6 μg g^{-1} NO_3-N as determined by the Kansas State University Soil Testing Laboratory. Collembola (Onychiurdiae and Isotomidae) were elutriated from a portion of the C_3 soil so their C isotope signature would initially be similar to that of the C_3 soil.

One *A. gerardii* seedling, six to ten C_3-labelled Collembola (2123-3539 individuals/m^2), and C_3 soil were placed in each of 16 pots as in the *P. smithii* trial. Benomyl was applied as described above for the AMF-suppressed treatment. Half of the pots from each treatment were

harvested after approximately three weeks. The remaining pots were harvested after six weeks. All pots were sampled as described for the *P. smithii* trial. There was insufficient root material to assess AMF colonization in one *A. gerardii* pot treated with fungicide at each harvest time.

Data analysis

The experiment was a complete factorial with 2 time intervals (3 and 6 weeks) and 2 fungicide levels (+ and -) with four replications. Two-way analysis of variance (SAS v.9.1, Cary NC) was used to assess plant biomass, mycorrhizal root colonization, and Collembola $\delta^{13}C$ and density responses. Data for each plant-soil trial were analyzed separately because I did not have a factorial design of plant and soil C isotope combinations (*i.e.* C_3 plant in C_3 soil, C_4 plant in C_4 soil). Collembola C isotope analysis was performed only on individuals from the families Onychiuridae and Isotomidae which are the same groups added to the pots at the beginning of the experiment. Individuals from these two families elutriated from the same pots were pooled to ensure adequate biomass for mass spectroscopy analysis. Individuals from other families (Sminthuridae, Hypogasturidae, and Entomobryidae) were rare.

Results

P. smithii *trial*

There was no difference in *P. smithii* biomass between mycorrhizal and AMF-suppressed pots after three weeks, but mycorrhizal plants had significantly higher biomass than plants from AMF-suppressed pots after six weeks (Table 5.1A, Fig. 5.2A). Plants treated with fungicide had significantly lower mycorrhizal root colonization than those that did not receive fungicide (Table 5.1A, Fig. 5.2C). The mean $\delta^{13}C$ signature of *P. smithii* was -30.80±0.36‰; there was no difference in the C isotope values between mycorrhizal and AMF-suppressed pots (Table 5.1A). The C isotope value of the C_4 soil was -13.17± 0.14‰.

In the *P. smithii* trial, mycorrhizal treatment did not have a significant influence on the density of Collembola, although density did increase significantly from three to six weeks (Table 5.1A, Fig. 5.3A). There was no difference in Collembola $\delta^{13}C$ between AMF-suppressed and mycorrhizal pots after three weeks; after six weeks Collembola in the mycorrhizal pots had significantly lower $\delta^{13}C$ values than the six week AMF-suppressed or either treatment after three

weeks (Table 5.1A, Fig. 5.4A). One of the six week AMF-suppressed pots contained only one live collembolan and did not provide enough biomass for C isotope analysis.

A. gerardii *trial*

One mycorrhzial *A. gerardii* plant at three weeks was more than 25 times larger than any of the other replicates, and was removed as an outlier from further analysis. Aboveground biomass of *A. gerardii* showed a significant increase from three to six weeks, and mycorrhizal plants were significantly larger than those in which mycorrhizae had been suppressed after six weeks (Table 5.1B, Fig. 5.2B). Fungicide significantly reduced AMF root colonization of plants (Table 5.1B, Fig. 5.2D). Overall, the *A. gerardii* $\delta^{13}C$ signature was -11.38±0.18‰, although it was significantly less negative in the mycorrhizal (-10.94 ±0.23‰) than AMF-suppressed pots (-11.82 ±0.20‰) (Table 5.1B). The C_3 signature of the bulk soil used in this experiment was -20.12± 0.29‰.

In addition to being more abundant in the mycorrhizal *A. gerardii* pots than the AMF-suppressed pots, the density of collembolans was also significantly higher after six weeks than three weeks (Table 5.1B, Fig. 5.3B). Because three of the four experimental pots that had been treated with fungicide did not contain live Collembola (density = 0 individuals/m^2) after three weeks, I could not test for an interaction between time and fungicide treatment on Collembola $\delta^{13}C$ values. There were no significant differences in the $\delta^{13}C$ signature of Collembola based on either time or mycorrhizal treatment (Table 5.1B, Fig. 5.4B).

Discussion

My approach differs from previous Collembola feeding studies which have relied on indirect methods such as fecal counts (Klironomos and Kendrick 1996, Klironomos and Ursic 1998, Klironomos *et al.* 1999), the location of individuals relative to different food sources (Verhoef *et al.* 1988, Hedlund and Sjogren Ohrn 2000, Jorgensen *et al.* 2003) or the presence of dark hyphae in the guts of collembolans (Schreiner and Bethlenfalvay 2003, Bardgett *et al.* 2005). By assessing the stable C isotope signatures of collembolan body tissues, I found that they assimilated C from both AMF and SF; AMF was assimilated to a greater degree in the *P. smithii* trial than in the *A. gerardii* trial.

Because the C isotope signatures are generally maintained through trophic levels (Fry 2006), the C isotope values of the plants were used as a surrogate for those of the AMF. Likewise, the isotope values of the bulk soil were used as a proxy for non-mycorrhizal fungi because SF were expected to be processing soil organic matter, which should have the same signature as the soil. Given the differences in the natural abundance stable isotope values of the soil and plants in my treatments, there were four potential outcomes in my experiment. First, if collembolans were feeding exclusively on root material their isotope values would reflect that of the plant and not differ between the AMF-suppressed and mycorrhizal pots. Secondly, if they fed only on AMF their isotope values would be similar to that of the plant in the mycorrhizal treatment. Thirdly, if they were feeding only on SF their $\delta^{13}C$ signature would reflect that of the SF in the bulk soil. Finally, collembolan isotope values intermediate between the soil and plant values would indicate mixed-feeding on both AMF and SF.

In the *P. smithii* trial, my results provide strong evidence that Collembola consumed AMF in addition to SF. Using the two-pool isotope mixing model of Fry (2006), AMF constituted up to 57% of their diet, while SF ranged from 43 to 82% of their diet. It is important to note that although AMF was suppressed by the fungicide, it was not eliminated. Therefore, the C_4 signal in the AMF-suppressed pots may be due to either AMF grazing or root herbivory. The C_4 portion of collembolan tissues most likely resulted from AMF grazing however, since the Collembola isotope value was significantly more depleted (closer to the plant isotope value) in the mycorrhizal pots compared to the AMF-suppressed pots after six weeks (Fig. 5.4B).

In the *A. gerardii* trial, collembolan C isotope values were most similar to the bulk soil which should indicate feeding on SF only. However, the collembolan values were more depleted than those of the bulk soil and fractionation of C isotopes between trophic levels generally results in enrichment, not depletion, of the ^{13}C isotope (Fry 2006). Additionally, the C isotope value of the soil was higher than anticipated; it was -20‰, whereas a value more similar to C_3 vegetation (-27‰ to -30‰ was expected). The field from which the C_3 soil was collected had been planted with C_3 vegetation for only 12 of the last 15 years. Portions of older C_4-C in the stable C pool mixed with more recent C_3-C could have resulted in the higher than expected soil $\delta^{13}C$ signature. Additionally, carbonates (~+8‰ $\delta^{13}C$) are present in KPBS soils and would also lead to a less negative $\delta^{13}C$ signature than expected. Accounting for these issues, the isotope value of SF was likely closer to -25 ‰ based on the C signature of C_3 vegetation (*e.g. P. smithii*

$\delta^{13}C$ = -30.8 ‰) and studies of C isotope fractionation in decomposer fungi (Kohzu *et al.* 1999, Trudell *et al.* 2004) (dashed line, Fig. 5.4A). Given this assumption and using the two-member isotope mixing model of Fry (2006), the isotope values of Collembola in the *A. gerardii* treatment indicate that approximately 50-71% of their diet consisted of SF. Because Benomyl did not completely eliminate AMF in the fungicide treatment, I am unable to discern unambiguously whether the 29-50% of the plant-derived C assimilated by collembolans was the result of root herbivory or AMF grazing.

Although Gange and Bower (1997) reported a negative response of the collembolan *Folsomia candida* to Benomyl, I found no evidence of persistent negative effects of the fungicide in my experiments. In the *P. smithii* trial, there were no differences in collembolan densities between the AMF-suppressed and AMF pots. Although only one of the four replicate AMF-suppressed *A. gerardii* mesocosms harvested after three weeks contained live Collembola, they were abundant in all replicate AMF-suppressed pots (3200-6700 individuals/m^2/pot) harvested after six weeks. Tomlin (1981) found that onychiurid and podurid collembolans, in addition to prostigmatid and cryptostigmatid mites, responded positively to Benomyl although both mesostigmatid mites and earthworms were negatively affected. In a study by Martikainen *et al.* (1998), Benomyl did not affect enchytraied or nematods, nor did it alter microbial respiration or inorganic soil nitrogen.

Collembolan densities in both the *P. smithii* and *A. gerardii* trials reached the 2.9–13.1x10^3 individuals/m^2 range reported for Kansas tallgrass prairie (Seastedt 1984) by week 6. The significant increase in collembolan densities in both trials from week 3 to week 6 suggests that they were successfully reproducing and resource availability was not limiting population growth during that period. Further, the mycorrhizal *A. gerardii* treatment supported significantly larger collembolan densities than the AMF-suppressed pots. Kaiser and Lussenhop (1991) found a similar response to mycorrhizal versus non-mycorrhizal treatments when Collembola were introduced to pots containing seedling *Glycine max*. The lack of a density response to the mycorrhizal treatment in the *P. smithii* trial may indicate Collembola-fungus-plant species-specific responses similar to those reported by Salamon *et al.* (2004) and Milcu *et al.* (2006).

Collembolan patterns of feeding on AMF and SF differed between the *P. smithii* and *A. gerardii* treatments. Their diet consisted of less AMF when it was associated with the obligate mycotroph (*A. gerardii*), and more AMF when it was associated with the facultative mycotroph

(*P. smithii*). This discrepancy may be due to nutritional or chemical differences between C_3 and C_4-derived C compounds. For instance, Chamberlain *et al.* (2004) found that when fed diets of the same yeast species grown on different C sources (C_3 versus C_4), collembolan tissues had different fatty acid profiles. Or, because Collembola, AMF, and SF species were not standardized between the two experiments, the different outcomes may be due to interactions at the species-level (Jorgensen *et al.* 2003, Salamon *et al.* 2004) which I am not able to address further with this study.

Because I was primarily interested in the role of AMF relative to SF in collembolan diets and SF were found in greater abundance than AMF in an adjacent tallgrass prairie restoration experiment (P.M. White, *unpublished data*), I did not specifically analyze the fungal communities of the soils used in this experiment. Soil-dwelling Collembola may consume other fungal types (*i.e.* pathogenic fungi) that occur in soils, which may account for a portion of consumption I attribute to SF. However, given that Collembola fed SF or ectomycorrhizal fungi (Scheu and Folger 2004) began reproducing about a week earlier than those fed pathogenic fungi (Sabatini and Innocenti 2000) and that pathogenic fungi species can lead to 100% mortality of some collembolan species (Sabatini and Innocenti 2000), I would not expect pathogenic fungi to contribute significantly to their diets when alternative resources are available.

The mycorrhizal treatment was associated with increased aboveground biomass of both *A. gerardii* and *P. smithii* only at the six week harvest (when seedlings were ten weeks old). At that time, AMF colonization of both *A. gerardii* (38±3.1%) and *P. smithii* (15±1.1%) was lower than the levels reported by Wilson and Hartnett (1998) (50.2% and 19.3%, respectively). Because my experiment was terminated when plants were still seedlings, the plants may not have had ample time for AMF colonization to reach levels consistent with those of perennial plants. This illustrates the importance of accounting for stage- or age-based interactions and organism reproduction rates when designing such multi-trophic level experiments or attempting to apply results at the field scale.

At the ecosystem scale, AM fungi are important in regulating C flux from plants to the soil (Zhu and Miller 2003, Rillig 2004, Olsson and Johnson 2005). They can consume up to 20% of plant C (Jakobsen and Rosedahl 1990, Watkins *et al.* 1996) and are often the largest contributor to soil microbial biomass (Miller *et al.* 1995). The importance of AM mycelial networks for belowground C flow highlights the need to investigate the extent of impact these

abundant fungal-feeding invertebrates impose on these systems (Johnson *et al.* 2005). My results reported here indicate collembolans should be viewed as selective polyphagous feeders that depend, at least in part, on fungi that are readily available to them. Selective feeding by fungal-feeding collembolans may affect fungal community composition and therefore the relative dominance of AM versus SF taxa. Whether the effects of these shifts will be observed at the plant community level remain to be investigated. However, mycorrhizal fungal diversity and/or composition may impact ecosystem productivity at the species level (van der Heijden *et al.* 1998, Jonsson *et al.* 2001, van der Heijden 2002) and even at the genetic level (Koch *et al.* 2006). Reductions in SF abundance due to fungivorous Collembola have resulted in increased longevity of seeds in soil seed banks, potentially influencing the dynamics of plant populations and the assembly of plant communities (Mitschunas *et al.* 2006). Further comprehensive multifactorial interaction studies that examine both above- and belowground community dynamics are needed before more specific conclusions can be drawn.

Conclusions

Using stable isotope techniques, this is the first study to demonstrate consumption of both saprophytic and mycorrhizal fungi by Collembola in soil-based mesocosms. My results indicate that (1) grassland Collembola will consume both AMF and SF, and (2) collembolan densities can respond positively to the presence of AMF in the soil environment. Collembolan grazing on both saprophytic and mycorrhizal fungi may alter fungal-fungal (Tiunov and Scheu 2005) and plant-fungal (Larsen and Jakobsen 1996) interactions with consequent implications for plant performance and nutrient cycling (Harris and Boerner 1990).

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Figures and Tables

Figure 5.1 Experimental design of *Andropogon gerardii* and *Pascopyrum smithii* trials in relation to C_3- and C_4-labelled Collembola and soil organic matter (SOM) components, as well as application of the fungicide Benomyl (AMF-suppressed pots). Arbuscular mycorrhizal fungi (AMF) colonize and receive carbon from plant roots, while saprophytic fungi (SF) use SOM as a carbon source.

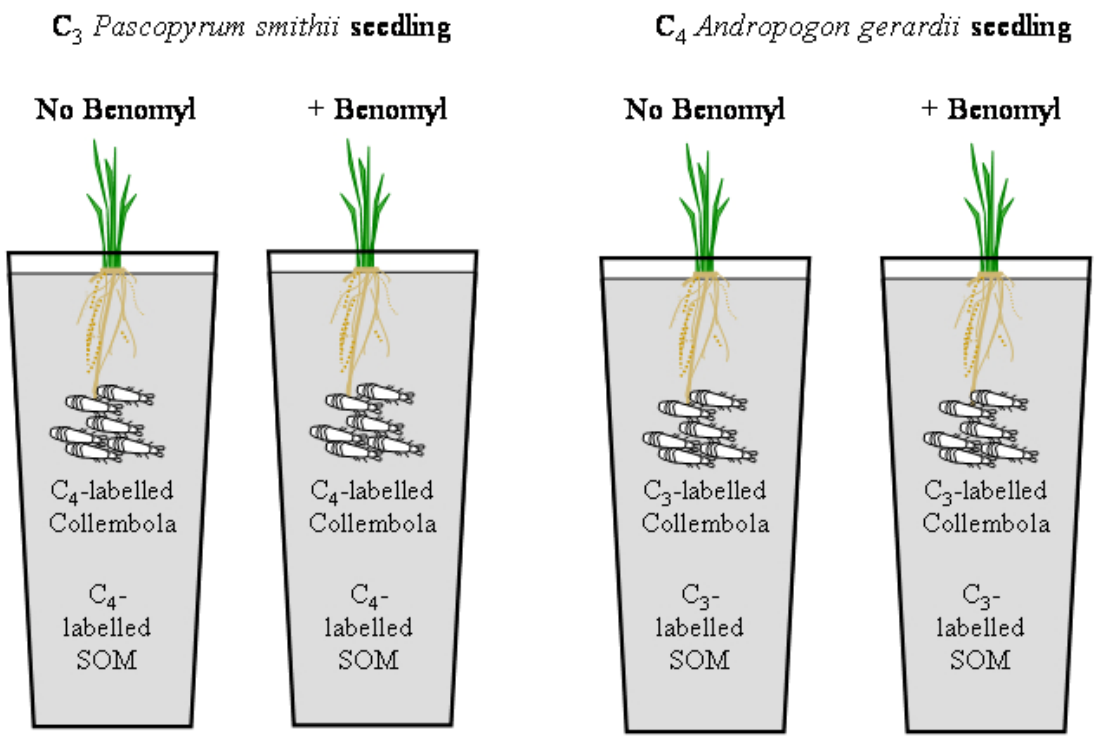

Figure 5.2 Biomass (g dry weight ± 1 SE) of (A) *Pascopyrum smithii* and (B) *Andropogon gerardii* and percent root colonization by mycorrhizal fungi of (C) *P. smithii* and (D) *A. gerardii* three and six weeks after initiation of the experiments. Plants were either treated with a fungicide (AMF-suppressed) or allowed to maintain a mycorrhizal association. Error bars represent ± 1 standard error. Horizontal bars with different letters indicate significant differences (p<0.10). Only time was significant in panel (B) *A. gerardii* biomass as indicated by the solid lines and letters above the 3 week and 6 week bars.

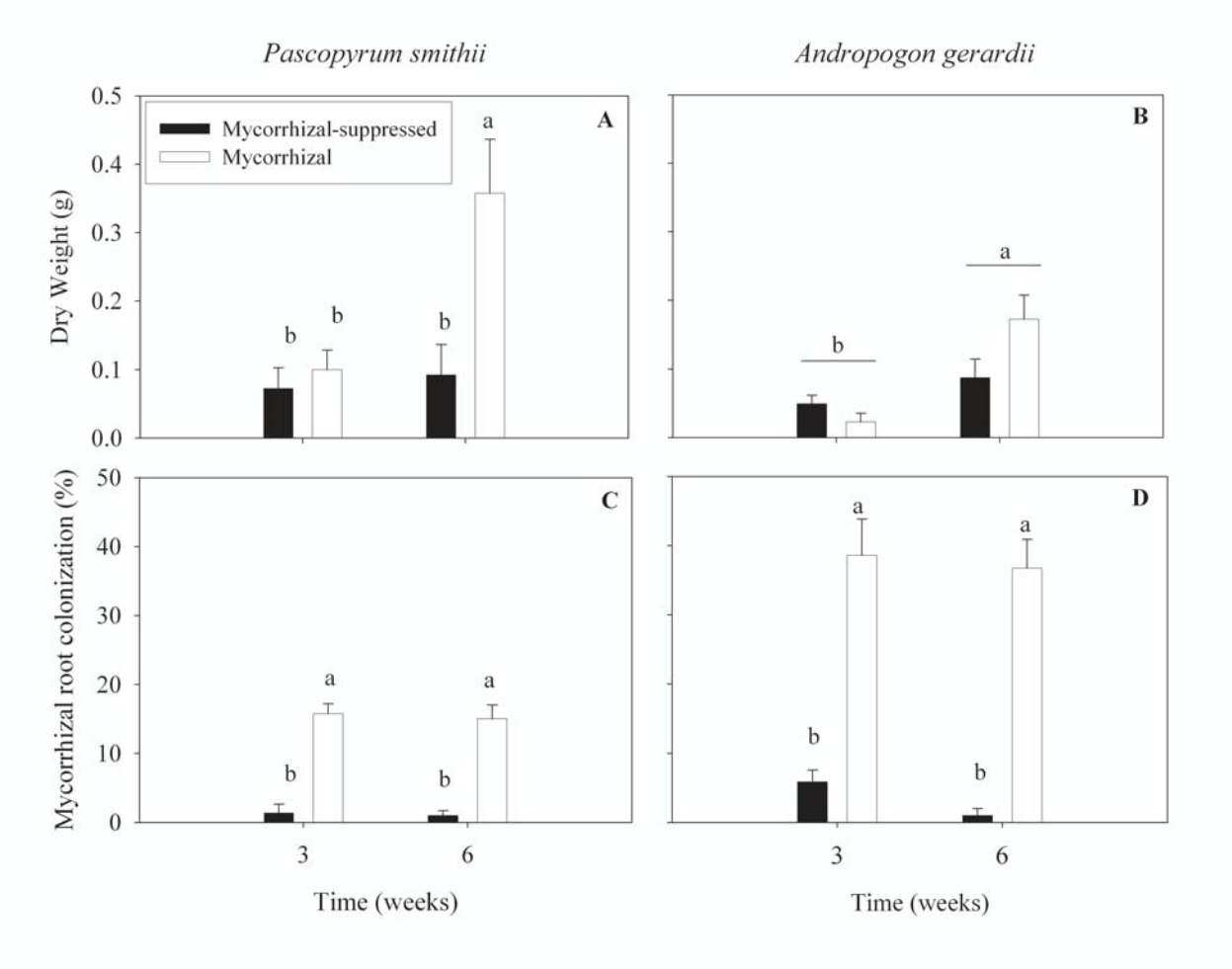

Figure 5.3 Density (individuals/m^2 ±SE) of Collembolans after three or six weeks in mesocosms with (A) *Pascopyrum smithii* in C_4 soil or (B) *Andropogon gerardii* in C_3 soil. Plants were either treated with a fungicide (AMF-suppressed) or allowed to maintain a mycorrhizal association. Error bars represent ± 1 standard error. Bars with different letters indicate significant differences (p<0.10).

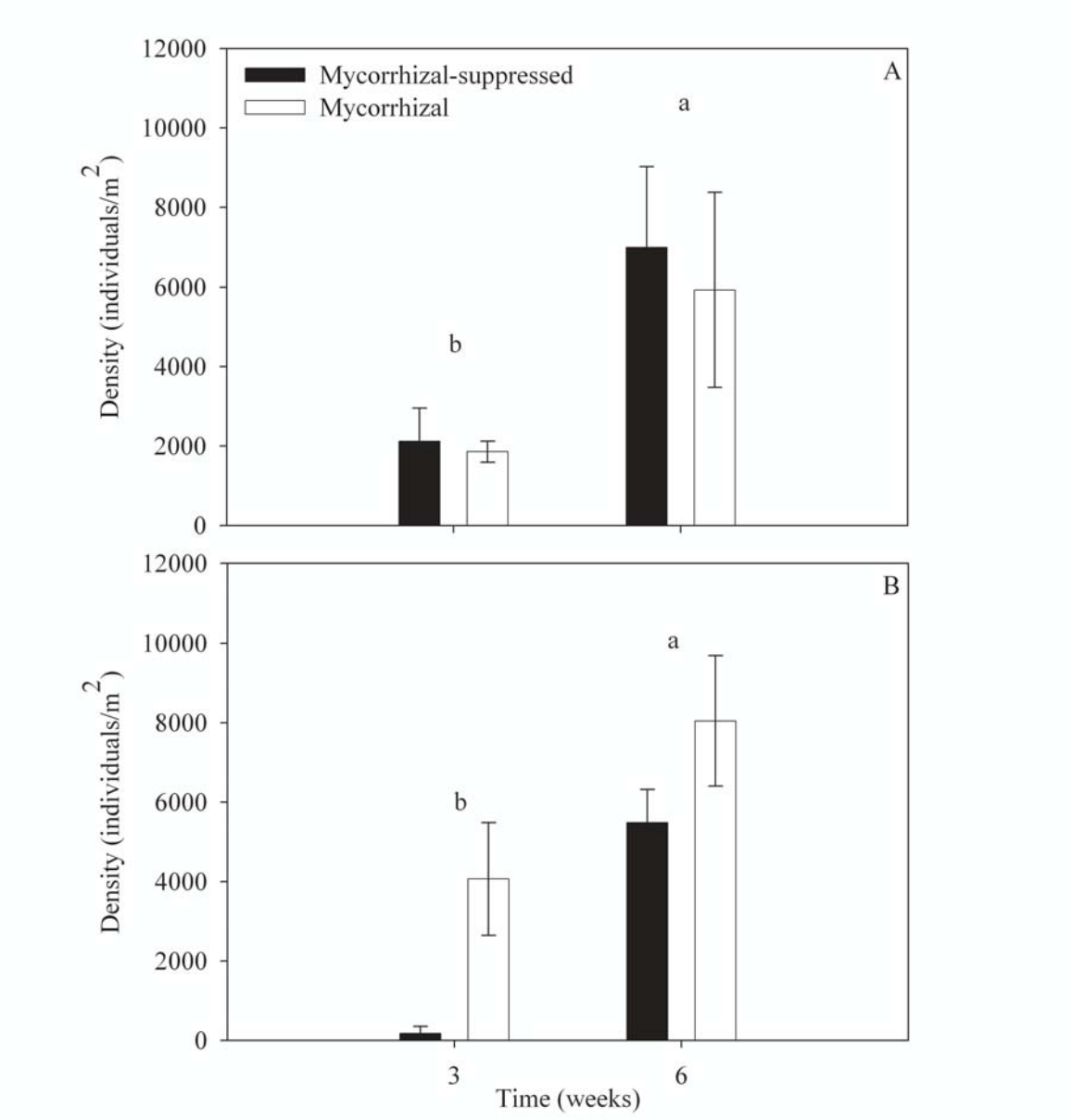

Figure 5.4 Stable carbon isotope signature (δ^{13}C VPDB ‰ ± 1 SE) of edaphic Collembola grown in mesocosms with (A) *Pascopyrum smithii* in C_4 soil or (B) *Andropogon gerardii* in C_3 soil for three or six weeks. Mesocosms were either treated with a fungicide (AMF-suppressed) or allowed to maintain a mycorrhizal association. Shaded areas represent 95% confidence intervals of plant and soil carbon isotope signatures δ^{13}C ‰). The dashed line in panel B represents the estimated signature of saprophytic fungi in the C_3 soil (Kohzu *et al.* 1999, Trudell *et al.* 2004). Error bars represent ± 1 standard error. Bars with different letters indicate significant differences ($p<0.10$).

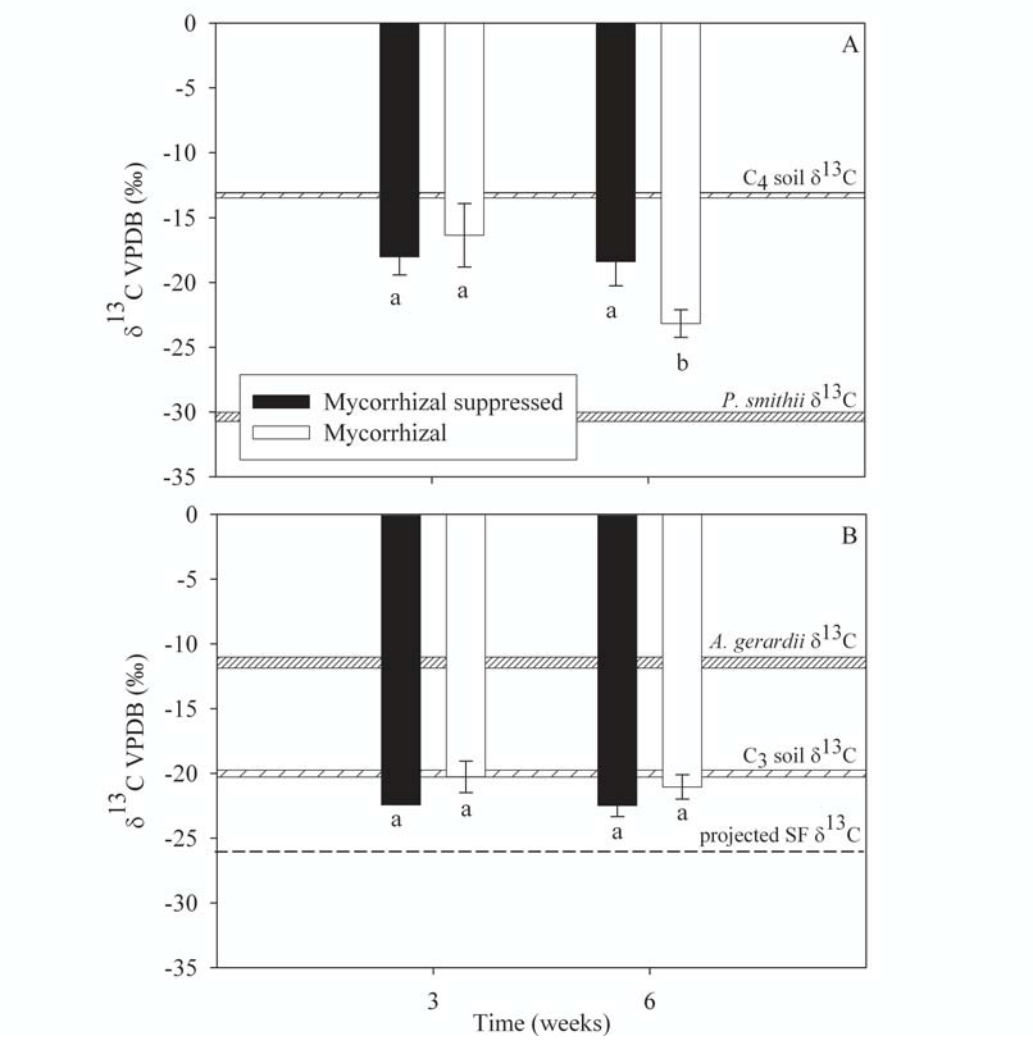

Table 5.1 Results of analysis of variance tests for significant effects of time (T), fungicide application (F), and the interaction between time and fungicide (T*F) in (A) *Pascoyrum smithii* and (B) *Andropogon geradii* treatments. F-statistics are reported with all significant statistics (p<0.10) in bold. There was no replication in stable carbon isotope ($\delta^{13}C$) data for Collembola at 3 weeks in the *A. gerardii* treatment, therefore the interaction was not determined (ND). df (n, d)=degrees of freedom (numerator, denominator).

		Factors		
Response variable	df (n, d)	Time (T)	Fungicide (F)	T*F
A. *Pascopyrum smithii*				
Plant biomass	1, 12	**7.78****	**8.61****	**5.68****
Root colonization	1, 10	0.15	**105.00*****	0.02
Plant $\delta^{13}C$	1, 10	3.13	0.11	0.01
Collembola density	1, 12	0.00	1.24	0.06
Collembola $\delta^{13}C$	1, 11	**4.02***	0.76	**3.25***
B. *Andropogon gerardii*				
Plant biomass	1, 12	1.28	2.23	0.10
Root colonization	1, 10	0.69	**72.35*****	0.13
Plant $\delta^{13}C$	1, 12	0.35	**8.00****	0.92
Collembola density	1, 12	**46.69*****	**13.22*****	0.96
Collembola $\delta^{13}C$	1, 10	0.25	2.03	ND

CHAPTER 6 - Long-term nitrogen and phosphorus fertilization affects above- and belowground invertebrate communities and food web linkages

Abstract

Aboveground and belowground consumers are intimately linked by processes mediated through primary producers. The population and community dynamics of aboveground consumers are much more broadly understood than those of belowground consumers in terrestrial systems, but both interact with plants and potentially each other through either direct or indirect means. This study assessed indirect linkages between soil-dwelling collembolans and aboveground herbivorous insects in a tallgrass prairie ecosystem as they are influenced by shifts in plant root and shoot stoichiometry and an arbuscular mycorrhizal fungi-plant symbiosis. I sampled plant attributes, Collembola, and grasshopper communities in long-term (20 year) experimental plots receiving N and P fertilizer in a factorial design. Species composition of all three taxonomic groups showed significant shifts in response to fertilizer treatments, primarily N. Univariate analysis also suggested that N is a much more important driver of observed food web dynamics than P in this system. Anticipated indirect relationships between collembolans and aboveground herbivores mediated through plants was not identified by structural equations modeling for the variables I considered, although each invertebrate group did show significant direct relationships with plants in their respective sub-system.

Keywords: Carbon, nitrogen, phosphorus, Collembola, herbivory, grasshoppers, structural equation modeling, fourth-corner analysis, arbuscular mycorrhizal fungi

Introduction

The study of food webs is an important tool for understanding interactions that contribute to the organization of ecological communities (Morin 1999, Elser *et al.* 2000). Despite interdependence between above- and belowground processes for the flow of nutrients through

terrestrial ecosystems, much remains to be known about the functional interactions that link the two subsystems (Wardle 2002). Producers are the primary source of new organic carbon to most terrestrial ecosystems, while belowground organisms typically drive nutrient cycling processes. Rather than studying food webs within each component as independent units, there is a compelling need to understand aboveground and belowground components as a single, networked ecosystem (Scheu and Setala 2002, Wardle 2002).

Tallgrass prairies are relatively productive mesic grasslands with the largest proportion of biomass and diversity of the ecosystem often occurring belowground (Licht 1998, Ransom *et al.* 1998, Rice *et al.* 1998). As such, they provide an ideal system in which to study interactions between belowground and aboveground subsystems. At Konza Prairie Biological Station (KPBS), for example, mean aboveground plant biomass varies between 185-600 $g/m^2/yr$, while belowground biomass ranges from about 860-1100 $g/m^2/yr$ (Knapp *et al.* 1998a). Seastedt (1984) reported that belowground arthropod biomass averages 4.2 g/m^2 while that of aboveground arthropods is generally between 1 and 2 g/m^2 (Evans 1989). Missing from these analyses is an understanding of the dynamic interactions among participants.

A key factor leading to the productivity of grassland ecosystems is the arbuscular mycorrhizal (AM) symbiosis formed between AM fungi and the roots of many grassland plant species (Wilson and Hartnett 1997, Hartnett and Fay 1998, Wilson and Hartnett 1998, Smith and Read 2002). This symbiosis forms with over 90% of grassland plant species and is characterized by fungal uptake of largely immobile and/or unavailable soil nutrients, primarily phosphorus (P), which are exchanged for plant-derived C (Smith and Read 2002). Mycorrhizal symbiosis plays an important role in plant nutrient acquisition in grasslands (Hartnett and Fay 1998, Rice *et al.* 1998), typically leading to increased foliar nutrient concentrations (primarily P) and plant productivity (Schlesinger 1997, Rice *et al.* 1998, Kula *et al.* 2005). In addition to the role of the symbiosis in plant nutirition, AM fungi can also aid plants with water regulation and protection against pathogens (Schreiner *et al.* 1997, Al-Karaki 1998, Smith and Read 2002) and increase soil aggregate formation (Schreiner *et al.* 1997, Wilson 2003).

Collembola are a ubiquitous and abundant group of soil microarthropods (Hopkin 1997, Schrader and Lingnau 1997) with densities of up to 57,000 individuals/m^2 reported in grassland systems (Petersen and Luxton 1982, Hopkin 1997, Schrader and Lingnau 1997). Although Collembola taxa employ diverse feeding strategies, edaphic species are generally fungivorous

(Bardgett *et al.* 1993, Hopkin 1997, Rusek 1998), feeding on both saprophytic fungi and AMF (Moore *et al.* 1985, Thimm and Larink 1995, Jonas *et al.* 2007). Studies have shown that Collembola can influence AMF and plant N and P dynamics (Harris and Boerner 1990, McGonigle 1994, Lussenhop 1996, Klironomos and Moutoglis 1999, Scheu *et al.* 1999, Bakonyi *et al.* 2002, Lussenhop and BassiriRad 2005), as well as ecosystem C flow (Johnson *et al.* 2005).

Changes in host plant foliar quality can affect feeding and performance of aboveground herbivores (House 1969, Wratten *et al.* 1988, Bernays and Simpson 1990, Joern and Gaines 1990, Raubenheimer 1992, Joern and Behmer 1997, Richardson *et al.* 2002). In grasslands, insects are often dominant herbivores (Tscharntke and Greiler 1995) capable of reducing plant biomass and altering plant community dynamics (Brown and Gange 1989). In general, N is often the limiting nutrient for insect herbivores (Mattson and Haack 1987, White 1993, Bernays and Chapman 1994) because carbon-rich compounds (*e.g.*, carbohydrates) are much more abundant in plant tissues than N-containing compounds such as protein (Raubenheimer 1992, Bernays and Chapman 1994, Bernays and Minkenberg 1997, Whittaker 2001). Although little is known about the role of P in determining plant quality for terrestrial insect herbivores, recent studies suggest P may be more limiting than N for some terrestrial herbivore species, such as caterpillars (Elser *et al.* 2000, Schade *et al.* 2003, Perkins *et al.* 2004).

The overall goal of this research was to assess trophic relationships between aboveground and belowground invertebrates as they are potentially mediated by plant C:N:P and AM symbiosis (Fig. 6.1). By conducting this research in the context of a long-term N and P fertilizer experiment, I was also able to examine the relative role of two potentially limiting or co-limiting nutrients (Elser *et al.* 1996) likely to influence aboveground-belowground food web interactions in the tallgrass prairie ecosystem. I had three primary objectives: (1) to assess the effects of N and P fertilization on trait-based responses of plant, collembolan, and grasshopper communities, (2) to examine the effects of N and P fertilization on plant biomass and stoichiometry, AM fungal colonization of plant roots, and Collembola and grasshopper abundances, and (3) to simultaneously analyze the relationships between fungivorous Collembola densities, AM fungi, plant C:N:P, and aboveground herbivory using structural equations modeling (SEM).

SEM is a statistical method for uncovering putative causal pathways, both direct and indirect, in a multivariate framework using a model hypothesized *a priori* and a series of structural equations (Shipley 2000, Grace 2006). According to Grace (2006), SEM when used in

conjunction with manipulative experiments strengthens the ability of this technique to identify cause-and-effect relationships between paths. An SEM model based on my conceptual food web (Fig. 6.1) was be used to assess potential causal relationships between the hypothesized linkages.

I expected that long-term shifts in C:N:P dynamics caused by N and P fertilization would lead to changes in species composition related to functional and life history traits. For instance, few leguminous forb species are expected to occur in areas of chronic N addition (Huenneke *et al.* 1990), while annual plant species will likely be more common under these conditions (Huenneke *et al.* 1990, Brooks 2003). Grasshopper communities are expected to track fertilizer-induced changes in host plant nutrient quality, including those resulting from shifts in plant species composition (as reflected in plant C:N:P) (Chapter 3). The occurrence of mixed-feeding species is expected to be highest in plots fertilized by both N and P. AM fungal colonization of plant roots should be higher in N-fertilized plots to increase plant P uptake as these systems are potentially driven toward P-limitation. In plots receiving additional P, AM colonization has been shown previously to decrease (Johnson *et al.* 2003, Wilson 2003). Although Collembola will consume both saprophytic and mycorrhizal fungi (Gange 2000, Scheu and Folger 2004, Jonas *et al.* 2007), I expected soil-dwelling collembolan densities to be greatest in plots with the highest AM colonization if AM fungi is an important component of their diets. Reflecting shifts in grasshopper communities, I expected the abundance of grasshoppers to be highest in plots receiving both N and P fertilizer due to increased resource quality of aboveground foliage. Given these predicted relationships between Collembola and belowground resources and between grasshoppers and aboveground resources, I expected SEM analyses to identify an indirect, positive interaction between Collembola and foliar herbivory.

Methods

Field sampling

Samples were collected from the Belowground Plot Experiment (BPE) at Konza Prairie Biological Station, Manhattan, KS. The BPE plots used in this study were arranged as a randomized complete block experiment with a 2x2 factorial treatment structure replicated in 4 blocks. Established in 1986, these 12m x 12m plots have been fertilized annually with N (10g N/m^2 as NH_4NO_3), P (1 g P/m^2 as superphosphate), both N and P, or neither N nor P (control plots). Plots were sampled during the summer (mid-June through early July) and fall (mid-

August through early October) in 2004, 2005, and 2006. All plots sampled for this study have been burned each spring since 1986.

Plant species composition was estimated using visual estimates of canopy cover and Daubenmire cover classes (Daubenmire 1959) in four random 0.25m^2 quadrats, and aboveground plant biomass was clipped in four randomly located 0.05-m^2 quadrats in each plot. Aboveground biomass was separated by warm-season grass and forb functional groups, dried at 60^oC for 48h and weighed. Plant roots were removed from four 5cm x 5cm soil cores per plot per sampling time and dried for 48h at 60^oC prior to weighing. Approximately half of the root biomass from each sample was stained with Trypan blue following the method of Koske and Gemma (1989) and scored for percent AMF colonization using the magnified gridline intersect method developed by McGonigle *et al.* (1990). The other half of the root sample and all aboveground plant biomass (grass and forb separately) were ground to a fine powder in liquid N with a mortar and pestle. Vegetation height was recorded at sixteen points per plot at each sampling in 2005 and 2006.

Four 5cm x 5cm soil cores were randomly removed from each plot. Immediately after collection, cores were placed in modified Berlese funnels in a refrigerator (Crossley and Blair 1991) for six days to collect soil invertebrates. Collembola were identified to genus following Christiansen and Bellinger (1998).

Herbivory by chewing insects was estimated by classifying the proportion of leaf area removed (0=no damage, 1=1-20%, 2=21-40%, 3=41-60%, 4=61-80%, 5=81-100%) on at least sixty shoots (30 grasses, 30 forbs) in each plot. Average whole plant herbivory was calculated as the sum of the number of damaged leaves weighted by the midpoint of the damage class divided by the total number of leaves on the plant (Kula *et al.* 2005). At least fifteen of the thirty grasses scored were *Andropogon gerardii* and fifteen of the forbs were *Ambrosia psilostachya* to control for differences in plant species composition between plots. As the dominant insect herbivore in tallgrass prairies, I collected grasshoppers from two 12m sweep net transects from each plot during each sampling period. All grasshoppers were counted and identified to species.

Laboratory analysis

All biomass samples for which adequate material was available were analyzed for C, N, and P content. A Carlo Erba 1500 Elemental Analyzer (Thermo Electron, Milan, Italy) was used

for C and N analysis (Blair Nutrient Dynamics Lab, Kansas State University). Foliar phosphorus was converted to ortho-phosphate and measured colorimetrically (660nm) following reaction with molybdate and ascorbic acid with antimony potassium tartrate as a catalyst at the Ecosystem Analysis Lab at the University of Nebraska (Lincoln, NE).

Statistical analysis

Fourth-corner analyses were conducted to assess shifts in plant, Collembola, and grasshopper communities. This analysis takes into account shifts in the identity of species within plots as well as their abundance. Multi- and univariate fourth-corner analyses (Legendre *et al.* 1997, Jonas and Joern 2007, Dray and Legendre *in review*) were conducted to test the hypothesis that shifts in community composition resulted from trait-based responses to environmental variables using the fourthcorner package (http://www.biomserv.univ-lyon1.fr/~dray/) in R v. 2.5.1 (R Development Core Team, Vienna, Austria). For each dataset, permutations 2 (tests link between species composition and environment) and 4 (tests link between species composition and species traits) were run with $P<0.2236$ in both models 2 and 4 to maintain an overall $\alpha=0.05$ for effects of environment on trait-based species composition (Dray and Legendre *in review*). For analysis of plant communities, an environmental matrix was based on N and P fertilizer treatments and the trait matrix included functional group (forb, grass), photosynthetic pathway (C3, C4), life history (annual, perennial), whether or not the species is native to tallgrass prairies, and family characteristics of each species. In addition to N and P treatments, the environmental matrix used for Collembola community analysis contained AMF colonization data. The trait matrix for Collembola included the presence/absence of pigmentation, presence/absence of eyes, and family. The absence of both pigmentation and eyes are characteristic of species that spend their life cycle belowground and are not associated with the litter or surface layers (Hopkin 1997, Verhoef *et al.* 2000). The environmental matrix used for grasshopper community analysis included N and P fertilization, as well as vertical structure of the plant community (calculated as the CV of plant height), forb biomass (g/m^2) and grass biomass (g/m^2). The grasshopper trait matrix included feeding guild (forb-feeding, grass-feeding, mixed-feeding), phenology (early season, mid-season, late season), size (small, medium, large), and flight ability (poor flier, strong flier) of the species. Two-factor analysis of variance testing the main effects of N and P and the

interaction between them was conducted on plant biomass, invertebrate abundance, AMF root colonization, and leaf herbivory.

Two-factor analysis of covariance was used to test for N and P main effects and interactions on plant C:N, C:P, and N:P ratios with the ratio denominator as the response variable and the numerator treated as a covariate. In all ANOVA/ANCOVA analyses, repeated measures analysis was used to account for sampling within (summer and fall) and between (2004, 2005, 2006) years. Because of the nested effect of time and to control for heterogeneous variances, the Kenward-Roger denominator degrees of freedom adjustment was used and accounts for inconsistencies in denominator degrees of freedom within and among tests. All ANOVA/ ANCOVA tests were conducted in SAS v9.1 (SAS Inst., Cary, NC).

Structural equations modeling (SEM) (Joreskog and Sorbom 1995, Mueller 1996, Shipley 2000, Grace 2006) was conducted in LISREL 8.8 Student (SSI, Int., Lincolnwood, IL) to assess relationships among the ecosystem components outlined in my aboveground-belowground food web model (Fig. 6.1). Collembola densities used for SEM analysis were composed only of the onychiurids and isotomids as these are the primarily fungivorous families. Three models (based on plant C:N, C:P, and N:P) were run based on asymptotic covariance matrices of the data due to the presence of ordinal variables in my dataset; fit statistics and model AICc values were used to choose the best-fit model.

Results

Plants

Fourth-corner analysis of plant communities indicated that N-fertilizer was associated with a shift away from grass and leguminous species, as well as perennials (Tables 6.1, 6.2). P-fertilizer was associated with an increase in forb and legume species, which was also reflected by the positive relationship between C_3 plants and P-fertilization.

The biomass of both forb (Fig. 6.2A, Appendix C.1A) and grass (Fig. 6.2B, Appendix C.1A) shoots was approximately 100% higher in N-fertilized plots than those in which N was not added. There was no effect of P-fertilizer on forb or grass shoot biomass. Although there was no effect of either N or P fertilizer on root biomass (1.09±0.06 g/m^2) (Appendix C.1A), the pattern of total plant biomass (shoots plus roots) reflected that of the forbs and grasses with

biomass in the N-fertilized plots being more than double the biomass of the plots that were not fertilized with N (Fig. 6.2C, Appendix C.1A).

Plant C:N:P stoichiometry was also influenced by N and P fertilization. Application of N fertilizer significantly decreased the C:N of roots and forb and grass shoots and increased the N:P of both roots and grass shoots (Fig. 6.3, Appendix C.1B). P fertilizer significantly decreased grass shoot C:P and decreased root, grass shoot, and forb shoot N:P, but had no effect on the C:P of roots or forb shoots (Fig. 6.3, Appendix C.1B).

Plants roots had significantly higher AMF colonization in N-fertilized plots ($F_{1,65.5}$=27.73, p<0.0001) (Fig. 6.4A), while colonization was not influenced by P-fertilization ($F_{1,68.9}$=0.10, p=0.75) nor by an interaction between N and P ($F_{1,65.7}$=0.42, p=0.52).

Invertebrates

There were no effects of N or P fertilizer on Collembola densities at the family level (Table 6.3, Appendix C.1C), except among the entomobryid collembolans. Entomobryid densities were significantly lower in the control plots (neither N nor P fertilizer) than in any of the three other treatments (Table 6.3). Collembolans with pigmented exoskeletons and eyes were positively associated with N-fertilizer; pigmented species were also negatively influenced by P-fertilizer (Tables 6.1, 6.2).

The abundances of grasshopper feeding guilds were not affected by N or P fertilization (Table 6.3, Appendix C.1C). Average overall percent leaf area removed by chewing insects was greatest in N-fertilized plots (Fig. 6.4B). This pattern of increased herbivory in plots receiving N-fertilizer was also seen in both forbs and grasses when analyzed separately (Appendix C.1A).

Fourth-corner analysis showed that changes in grasshopper species composition were related to feeding guilds and size classes. Grass-feeding grasshopper species showed a negative response to N-fertilizer and forb biomass, while the forb- and mixed-feeding guilds were positively associated with increased N and forb biomass. Increased variability in the vertical structure of plant communities had a positive effect on small-bodied species (Tables 6.1, 6.2).

Food web analysis

Structural equations models using field data led to a good fit with my simplified aboveground-belowground food web based on plant C:N (Fig. 6.5A) and plant N:P (Fig. 6.5B),

while the model based on plant C:P (not shown) was inadequate (Table 6.4). The model based on plant C:N was a slightly better fit to the data with a lower model AICc.

As described in the above analysis, N had a significant positive effect on AMF colonization (Fig. 6.4A). The density of fungivorous Collembola was positively associated with both AMF colonization and root C:N, while herbivory decreased significantly with increasing shoot and decreasing root C:N. All other hypothesized paths were non-significant. This analysis also detected significant indirect effects of N on both collembolan densities (through AMF and root C:N) and aboveground herbivory (through root C:N), although no indirect links between collembolans and aboveground herbivory were evident.

In the plant N:P based SEM, the effects of N on AMF colonization and AMF colonization on collembolan densities were similar to those in the plant C:N based SEM (Fig. 6.5B). In this model, Collembola densities had a negative response to increasing root N:P and herbivory was negatively associated with increasing shoot and root N:P.

Discussion

The use of C:N:P stoichiometry to study trophic interactions is particularly useful for examining the flow of nutrients between above- and belowground food webs because it integrates large-scale ecosystem (*i.e.* nutrient cycling) and organismal biomolecular dynamics (Sterner and Elser 2002, Raubenheimer and Simpson 2004, Verde *et al.* 2004). My analysis of a simple model connecting aboveground and belowground invertebrate consumers shows that variation in host plant C:N:P associated with long-term N and P fertilization influenced both aboveground herbivory and the density of fungivorous soil collembolans, but did not reveal an expected indirect relationship between the two consumer groups as I had hypothesized. Because shifts were seen in response to both C:N and N:P, but not C:P, N appears to be the primary driver of these relationships.

The extent of AM symbiosis is influenced by plant-available soil nutrients such that plant allocation of C to AMF increases as P becomes more unavailable (Johnson *et al.* 2003). At KPBS, N fertilizer was found to increase both AMF root colonization and extradical hyphal growth (Rice *et al.* 1998, Wilson 2003), while P fertilizer had a negative effect on hyphal growth (Rice *et al.* 1998, Johnson *et al.* 2003). The AM symbiosis can improve water relations, provide protection against pathogens, increase plant growth, and increase nutrient concentrations of host

plants (Al-Karaki 1998, Smith and Read 2002). For instance, Kula *et al.* (2005) reported decreased foliar N:P in seven tallgrass prairie plant species in response to AM symbiosis. These results suggest that P may be limiting and provide an explanation for the ubiquity of mycorrhizal symbiosis among tallgrass prairie plant species. Despite this and other such evidence for terrestrial P- limitation (Elser *et al.* 2000, Johnson *et al.* 2003), both uni- and multivariate analyses suggest that N-limitation is more important in structuring food web dynamics in this tallgrass prairie ecosystem as has been widely reported based on other approaches (Tilman 1984, Knapp *et al.* 1998a, Ritchie 2000). It is important to note, however, that P fertilization did not significantly affect all above- and belowground stoichiometric relationships (*i.e.*, forb and root C:P were not influenced by P fertilization). Although P fertilizer significantly increased the %P of grass tissues approximately 0.03% compared to the treatments that did not receive P, there was no significant effect of P fertilizer on the concentration of P in forb tissues (data not shown). It is likely that increased P availability resulted in increased forb growth only and not increased tissue P concenctrations, particularly in treatments receiving N fertilizer (Fig. 6.2A).

Increased N was associated with increased occurrence of non-leguminous forbs, forb biomass and herbivory, as well as the occurrence of forb-feeding grasshopper species. Evans (1988b) reported a similar pattern in which there was a positive correlation between forb biomass and the abundance of forb-feeding grasshopper individuals. Likewise, in fourth corner analyses both grass and grass-feeding grasshopper species composition declined in response to N-fertilization. Because the composition of forb- and grass-feeding grasshopper species appears to be tracking shifts in host plant composition according to fourth corner analyses, the occurrence of specific host plant(s) may be of relatively greater importance to these groups than general foliar quality of plants in a given plot, although more specific analysis of host plant occurrence, stoichiometry, and secondary chemistry and grasshopper gut contents would be needed to adequately assess this possibility. Although there was an increase in grass herbivory in N-fertilized plots, despite no change in overall grasshopper abundance, it is likely due to the increased presence of mixed-feeding species which can adjust resource consumption to meet nutritional needs (Simpson and Raubenheimer 2000). In a related field study (Chapter 3), the mixed-feeding grasshopper *Melanoplus bivittatus* shifted relative consumption of forbs and grasses as grass C:N:P varied. Given that burning can alter plant tissue N concentrations (Ojima *et al.* 1994, Turner and Knapp 1996, Reich *et al.* 2001), the observed increase in consumption of

grasses in sites that had not been burned for at least two years by *Melanoplus sanguinipes* (Porter and Redak 1997) is also likely a response to altered host plant quality.

Shifts in the collembolan community were associated with pigmentation and eye number. Species with pigmented exoskeletons were more common in N-fertilized plots, while those with increased number of eyes were more common in P-fertilized sites. Because the autecology of most collembolan species is poorly known, it is unclear why these particular traits would respond to altered resource environments (Hopkin 1997, Winkler and Kampichler 2000). However, highly pigmented species as well as those with well-developed eye patches tend to be epigeic to hemiedaphic rather than edaphic (Hopkin 1997, Chagnon *et al.* 2000) and employ different feeding strategies. Soil-dwelling species tend to be fungal-feeders while those more closely associated with the soil surface and litter layers tend to feed to a larger degree on plant matter (Hopkin 1997, Rusek 1998). The increase in Collembolan densities associated with increased root C:N identified in SEM analysis may be due to exudation of C compounds by the roots increasing fungal growth, both AM and saprotrophic, in the rhizosphere which would increase total resource quantity for Collembolans (Sun and Fries 1992, Kuzyakov et al. 2007). That the truly soil-dwelling species, primarily the Onychiuridae and Isotomidae (Chagnon *et al.* 2000), were negatively associated with N-fertilization was unexpected given that AMF colonization was greatest in the N fertilized plots. It is likely that these are indirect responses to fertilizer-induced changes in microhabitat selection related to soil chemical and physical properties (Rusek 1998, Chagnon *et al.* 2000) because the members of these collembolan families tend to be highly sensitive to soil pH (Chagnon *et al.* 2000), compaction (Dittmer and Schrader 2000, Larsen *et al.* 2004), and soil aggregate formation (Wiggins *et al.* 1979, Kanal 2004). Although most onychiurid and isotomid species collected in the study by Chagnon *et al.* (2000) were positively associated with acidic soils, several species were negatively affected by soil acidity. Long-term N fertilization not only alters the quality and quantity of the resource environment, but also tends to decrease soil pH (Bardgett *et al.* 1999) and alter soil aggregate distribution (Wilson 2003) which may be more responsible for the observed shifts in the collembolan community associated with N-fertilization than changes in fungal resource availability. Further study on these relationships is warranted.

Although fourth-corner analysis documented shifts in invertebrate species composition associated with N or P fertilization, the abundance of collembolans and grasshoppers at the

family or feeding guild levels, respectively, did not respond to fertilizer treatments. Entomobryid collembolans were the only group to show increased abundance due to N and a marginally significant response to the interaction between N and P with densities in the control treatments (no N or P fertilizer) lower than in any of the other treatments. Because the species in this family are typically associated with the soil surface and litter layers (Hopkin 1997, Chagnon *et al.* 2000), this group maybe responding to changes in the quality of plant litter and soil organic matter in the N fertilized plots.

Because of the ability of invertebrate organisms to maintain elemental homeostasis across a range of resource environments (Mattson 1980, Sterner 1997), I did not use invertebrate C:N:P in my model. Rather, I used collembolan density and leaf herbivory as measures of invertebrate activity and to track their responses to changes in ecosystem C:N:P. Both aboveground and belowground invertebrate groups showed significant relationships to plant C:N:P as I expected, although the indirect relationship I expected between collembolans and aboveground herbivory was not identified in my SEM analysis. Although aboveground plant responses to changes in soil invertebrates was not found in the first five years of the BGP study (Rice *et al.* 1998), such interactions have been reported in greenhouse studies. For example, Scheu *et al.* (1999) found that the presence of Collembola in experimental pots had a significant impact on aphid reproduction by altering host plant nutrient status.

Although my model was analyzed in a bottom-up framework, top-down factors are also likely important in affecting my observed patterns. For instance, insect herbivory can lead to increased C-exudation from roots (Holland and Detling 1990) and increased rates of P-cycling (Seastedt and Crossley 1984), each of which would also be expected to affect mycorrhizal dynamics. Collembola can also exert top-down effects on plants, such as influencing host plant N and P concentrations (Boerner and Harris 1991). These responses were not measured in this study.

Conclusions

In this field experiment that employed long-term N and P fertilization, I did not identify indirect interactions between above- and belowground invertebrate food webs mediated through host plants as predicted. Long-term N and P fertilization significantly affected both producer and consumer communities and food-web linkages, though not always in the direction I

expected. The most widespread ecosystem changes were associated with N-fertilization, although my results also suggest that P-fertilization played a minor role in structuring plant and collembolan communities. Comparison of three food web models, based on plant C:N, C:P or N:P, indicated that plant C:N was the most important driver of collembolan and aboveground herbivory dynamics. Although detected previously in the greenhouse experiment of Scheu *et al.* (1999), an indirect linkage between fungivorous collembolans and aboveground herbivores was not identified in my field study despite strong relationships between the abundance or activity of each invertebrate group and plant nutrient characteristics. Because this simplified model was examined in the context of a complex field environment, including additional ecosystem components (*e.g.*, saprophytic fungi, belowground herbivores), future research should clarify many of these relationships and allow for a more thorough analysis of both top-down and bottom-up forces.

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Figure 6.1 Hypothesized model of aboveground-belowground invertebrate trophic relationships mediated by arbuscular mycorrhizae and plant carbon (C): nitrogen (N): phosphorus (P) ratios. Solid arrows indicate direct and broken arrows represent indirect interactions.

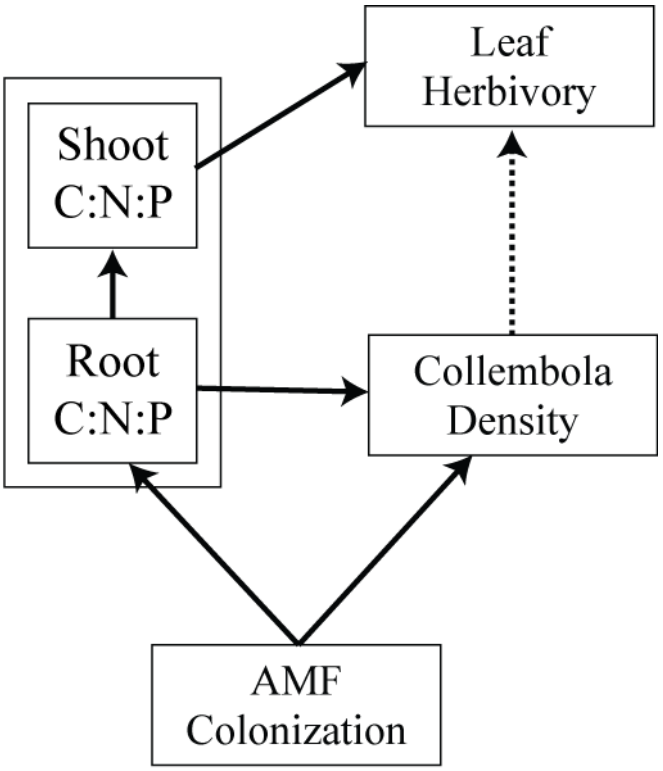


Figure 6.2 Average biomass (g/m^2) across all sampling periods of (A) aboveground forbs, (B) aboveground grasses, (C) roots, and (D) total shoots plus roots in plots receiving an additional 0 or 10g nitrogen (N)/m^2/yr and 0 or 1 g phosphorus (P)/m^2/yr at the Belowground Plot Experiment at Konza Prairie Biological Station, Manhattan, KS. Error bars represent 1 standard error. See Appendix C.1A for ANOVA statistics.

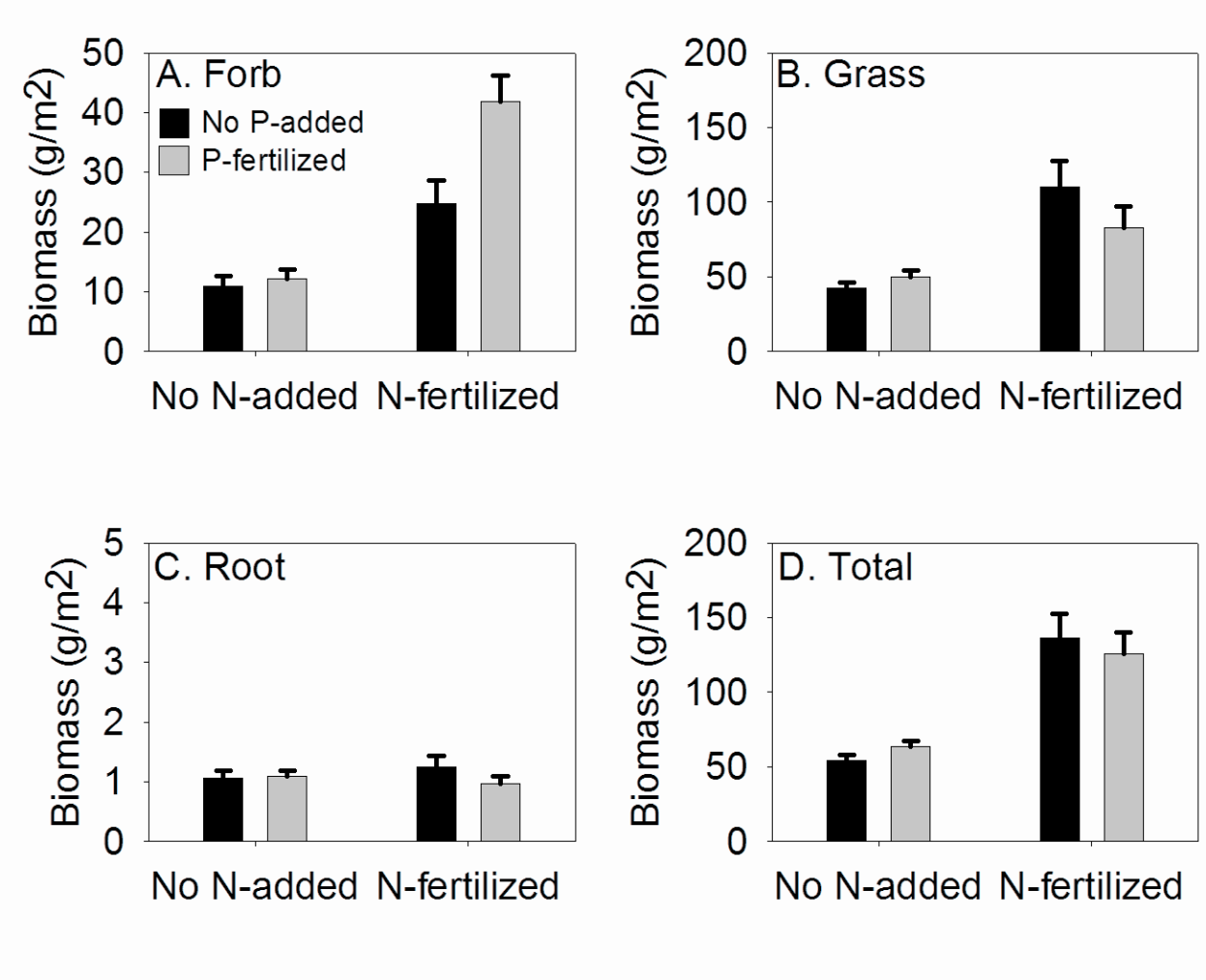


Figure 6.3 Average C:N, C:P, and N:P ratios across all sampling periods of plant roots and shoots in plots receiving an additional 0 or 10g nitrogen (N)/m^2/yr and 0 or 1 g phosphorus (P)/m^2/yr. (A) Root C:N, (B) Forb C:N, (C) Grass C:N, (D) Root C:P, (E) Forb C:P, (F) Grass C:P, (G) Root N:P, (H) Forb N:P, (I) Grass N:P. Error bars represent 1 standard error. See Appendix C.1B for ANCOVA results.

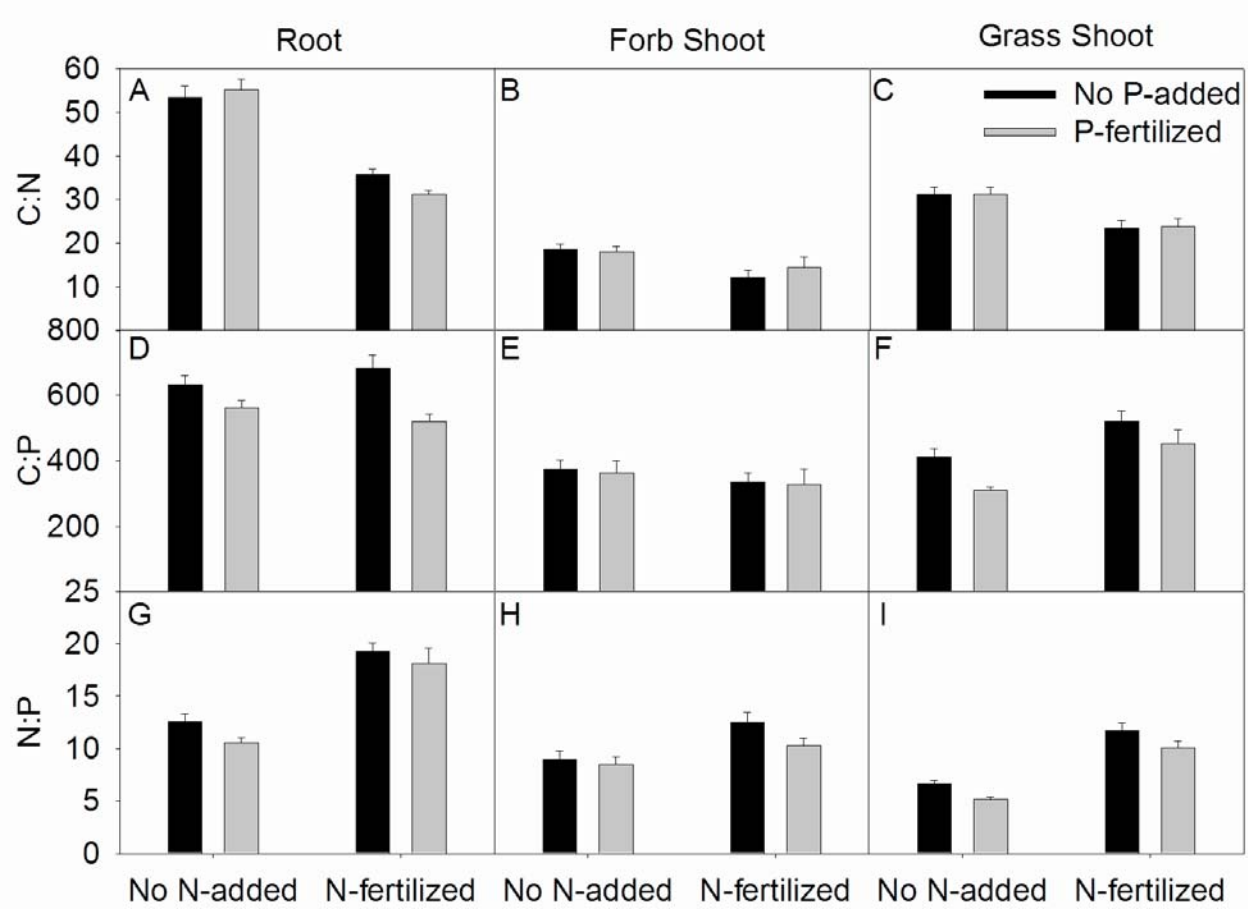


Figure 6.4 The effects of nitrogen (N) fertilization on average (A) arbuscular mycorrhizal fungi (% root colonization) and (B) insect chewing damage (% leaf area removed). Error bars represent 1 standard error. See Appendix C.1A for ANOVA results.

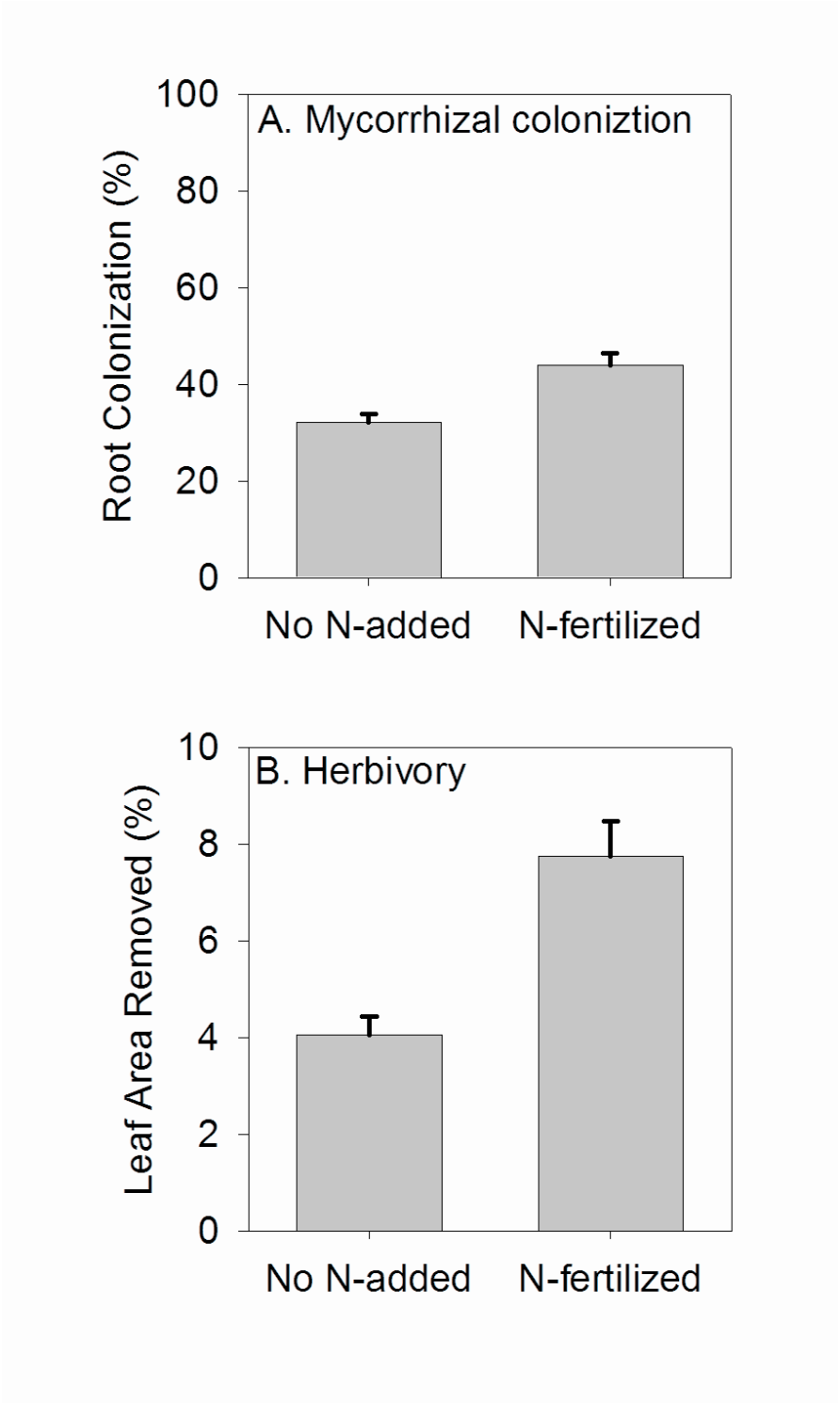
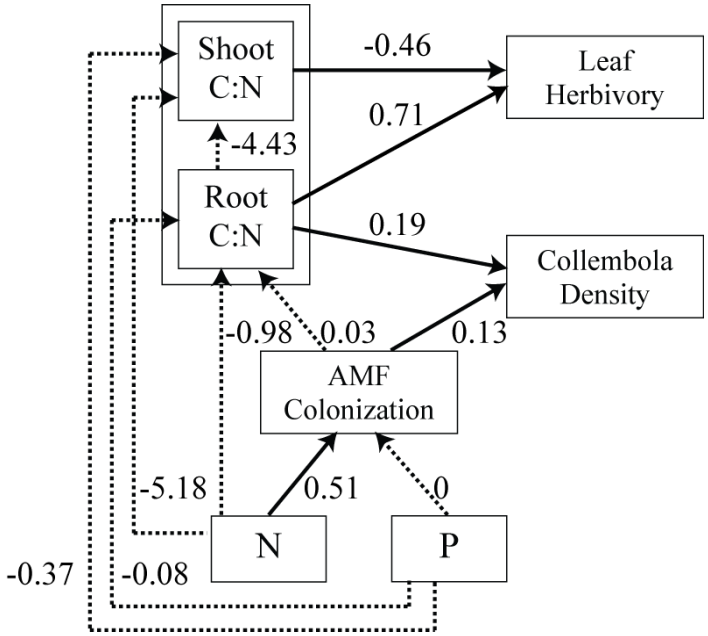


Figure 6.5 Structural equation models based on (A) plant C:N or (B) plant N:P of the hypothesized relationships between invertebrates, arbuscular mycorrhizal fungi (AMF) and host plant stoichiometry (Fig. 6.1). Fit statistics (Table 6.4) indicate that this model is a good fit for the data and is a better fit than the models based on either plant N:P or C:P. Covariance parameters are provided next to each arrow. Solid arrows indicate significant paths, broken arrows indicate those that are insignificant.

A.



B.

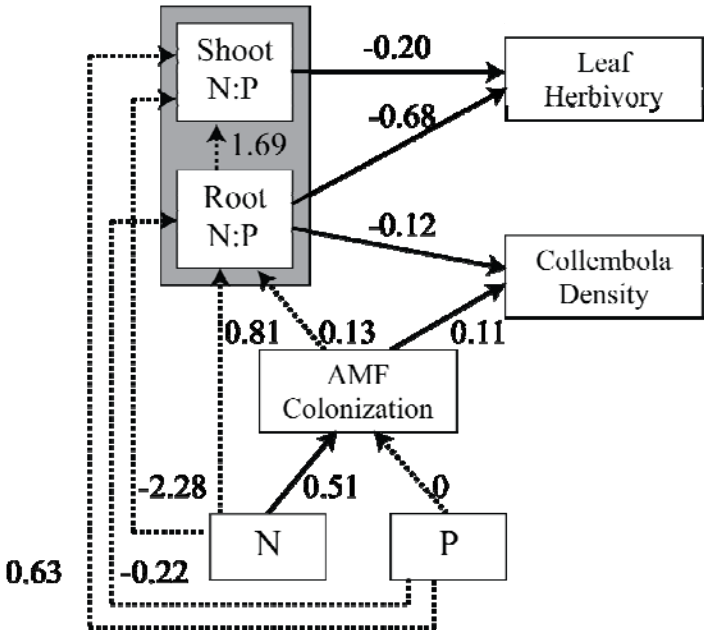


Table 6.1 Statistical results of fourth-corner analysis on the response of plant species composition to nitrogen (N) and phosphorus (P) fertilizer, grasshopper communities to N, P and variability in the vertical structure of the plant community, and collembolan species/morphospecies to N, P and arbuscular mycorrhizal root colonization. Rejection of the null hypothesis (Ho: environmental conditions have no effects on species composition based on species traits) requires P<0.2236 (results in bold) under both models 2 and 4 to maintain an overall α=0.05 (Dray and Legendre, in review). Eta^2 statistics with P-values (9,999 permutations) from models 2 and 4 given in parentheses are given for all tests. The list of species and species traits are given in Appendix C.2.

		Nitrogen			Phosphorus			Forb Biomass			Grass Biomass			Variablity in Vertical Structure[a]			Mycorrhizal Root Colonization[b]		
		Eta^2	Model 2	Model 4	Eta^2	Model 2	Model 4	Eta^2	Model 2	Model 4	Eta^2	Model 2	Model 4	Eta^2	Model 2	Model 4	Eta^2	Model 2	Model 4
Plants	Functional Group	**0.048**	**(0.003)**	**(0.170)**	**0.012**	**(0.210)**	**(0.076)**												
	Photosynthetic Pathway	0.001	(0.506)	(0.818)	**0.007**	**(0.097)**	**(0.094)**												
	Life History	**0.027**	**(0.011)**	**(0.066)**	0.002	(0.486)	(0.320)												
	Nativity	0.007	(0.234)	(0.459)	0.006	(0.263)	(0.118)												
Grasshoppers	Feeding Guild	**0.111**	**(0.002)**	**(0.184)**	0.004	(0.775)	(0.218)	**0.134**	**(0.000)**	**(0.037)**	0.036	(0.191)	(0.512)	0.018	(0.358)	(0.132)			
	Life History	0.000	(0.994)	(1.000)	0.002	(0.710)	(0.511)	0.001	(0.916)	(0.996)	0.001	(0.888)	(0.985)	0.009	(0.226)	(0.438)			
	Size	0.040	(0.003)	(0.592)	0.003	(0.673)	(0.376)	0.019	(0.081)	(0.773)	0.047	(0.001)	(0.440)	**0.017**	**(0.105)**	**(0.158)**			
	Flight Ability	0.017	(0.049)	(0.511)	0.002	(0.466)	(0.193)	0.005	(0.311)	(0.755)	0.029	(0.003)	(0.332)	0.004	(0.362)	(0.384)			
Collembola	Pigment	**0.010**	**(0.093)**	**(0.115)**	0.004	(0.274)	(0.244)										0.005	(0.267)	(0.279)
	Number of Eyes	0.001	(0.551)	(0.612)	**0.006**	**(0.149)**	**(0.167)**										0.001	(0.556)	(0.608)
	Presence of Furcula	0.001	(0.544)	(0.727)	0.000	(0.605)	(0.733)										0.001	(0.414)	(0.630)
	Size	0.000	(0.968)	(0.973)	0.002	(0.521)	(0.469)										0.002	(0.523)	(0.536)
	Post-Antennal Organ	0.001	(0.320)	(0.573)	0.003	(0.101)	(0.306)										0.018	(0.245)	(0.497)

[a]Vertical structure=coefficient of variation of vegetation heights; included in environmental matrix for grasshopper analysis only.
[b]Mycorrhizal colonization included in environmental matrix for Collembola analysis only

Table 6.2 Univariate fourth-corner analysis results for environmental variable-species trait combinations that were significant in multivariate fourth-corner analysis (Table 6.1). Rejection of the null hypothesis (H_o: environment does not affect species composition through species traits) requires P<0.2236 under both models 2 and 4 (Dray and Legendre, *in review*). G-statistics and the sign of the relationships (+ positive, -negative) are reported for analysis of 2 categorical variables; Homogeneity and F-statistics are reported for analyses of 1 quantitative variable versus 1 categorical variable. P-values from each model are in parentheses and are based on 9,999 permutations of the data.

			Nitrogen (N)						Phosphorus (P)					
			No added N			N-fertilizer			No added P			P-fertilizer		
Taxa	Trait	State	Statistics	G	Model 2	Model 4	G	Model 2	Model 4	G	Model 2	Model 4	G	Model 2	Model 4
Plants															
	Functional Group		Global test	217.05	(0.002)	(0.145)				50.1629	(0.219)	(0.076)			
		Forb	G-statistic	884.53	(0.464)	(0.495)	1099.67	(0.464)	(0.495)	934.618	(0.546)	(0.636)	1049.58	(0.546)	(0.636)
			Sign	-			+			-			+		
		Grass	G-statistic	973.61	(0.464)	(0.135)	844.32	(0.464)	(0.406)	992.603	(0.421)	(0.083)	825.323	(0.421)	(0.275)
			Sign	+			-			+			-		
		Legume	G-statistic	333.27	(0.004)	(0.495)	67.50	(0.004)	(0.014)	147	(0.492)	(0.083)	253.765	(0.492)	(0.275)
			Sign	+			-			-			+		
	Photosynthetic Pathway		Global test							28.3625	(0.102)	(0.091)			
		C3	G-statistic							1153.05	(0.353)	(0.0004)	1354.81	(0.353)	(0.002)
			Sign							-			+		
		C4	G-statistic							921.174	(0.353)	(0.0004)	773.864	(0.353)	(0.002)
			Sign							+			-		
	Life History		Global test	119.01	-0.012	(0.067)									
		Annual	G-statistic	58.333	(0.029)	(0.706)	217.158	(0.029)	(0.269)						
			Sign	-			+								
		Perennial	G-statistic	2133.1	(0.124)	(0.706)	1794.32	(0.124)	(0.269)						
			Sign	+			-								

Table 6.2 (Continued)

Taxa	Trait	State	Statistics	Nitrogen (N)						Phosphorus (P)						Forb Biomass			Variability in Vertical Structure[a]			Mycorrhizal Root Colonization[b]		
				No added N			N-fertilizer			No added P			P-fertilizer											
				G	Model 2	Model 4	G	Model 2	Model 4	G	Model 2	Model 4	G	Model 2	Model 4	F	Model 2	Model 4	F	Model 2	Model 4	F	Model 2	Model 4
Grasshoppers																								
	Feeding Guild		Global test	112.14	(0.002)	(0.193)										76.352	(0.000)	(0.038)						
		Forb-feeding	G-statistic	178.00	(0.447)	(0.952)	284.00	(0.447)	(1)							0.484	(1.000)	(1.000)						
			Sign	-			+									0.259	(0.004)	(0.170)						
		Grass-feeding	G-statistic	281.00	(0.020)	(0.451)	118.00	(0.020)	(1)							0.249	(0.010)	(1.000)						
			Sign	+			-									-0.365	(0.001)	(0.028)						
		Mixed-feeding	G-statistic	40.00	(0.078)	(1)	89.00	(0.078)	(1)							0.133	(1.000)	(1.000)						
			Sign	-			+									0.147	(0.005)	(0.170)						
	Size		Global test																8.46	(0.106)	(0.161)			
		Small	Homogeneity																0.28	(0.928)	(1.000)			
			F-statistic																0.11	(0.119)	(0.212)			
		Medium	Homogeneity																0.60	(0.081)	(1.000)			
			F-statistic																-0.04	(0.266)	(0.306)			
		Large	Homogeneity																0.10	(0.747)	(0.186)			
			F-statistic																-0.09	(0.091)	(0.212)			
Collembola																								
	Pigment		Global test	4575.1	(0.099)	(0.112)																		
		Absent	G-statistic	98167	(0.645)	(1)	91750	-0.645	(1)															
			Sign	+			-																	
		Present	G-statistic	111583	(0.213)	(1)	156750	-0.213	(1)															
			Sign	-			+																	
	Number of eyes		Global test							2737.68	(0.150)	(0.169)												
			Homogeneity							0.50	(0.843)	(0.659)	0.49	(0.843)	(0.521)									
			F-statistic							-0.08	(0.154)	(0.175)	0.08	(0.154)	(0.175)									

Table 6.3 Abundance (±SE) of invertebrates from annually burned plots in the Belowground Plot Experiment at Konza Prairie Biological Station, Manhattan, KS with and without nitrogen (N) and phosphorus (P) fertilizer additions. Densities (#/m^2) are given for each collembolan family collected and abundances (#/sweep transect) of grasshoppers categorized by feeding guilds. Entomobryid collembolans were the only group to differ significantly; means followed by the same letter do not differ at α=0.05. ANOVA statistics are given in Appendix C.1.

		No P-added	P-fertilized
Collembola (Individuals/m^2)			
Entomobryidae	No N-added	2250.0 ± 611.6 [a]	2875.0 ± 471.3 [b]
	N-fertilized	3791.7 ± 838.3 [b]	3708.3 ± 935.4 [b]
Hypogasturidae	No N-added	1916.7 ± 454.1	1416.7 ± 506.9
	N-fertilized	2291.7 ± 652.4	1916.7 ± 564.4
Isotomidae	No N-added	1041.7 ± 272.4	791.7 ± 240.6
	N-fertilized	666.7 ± 311.2	875.0 ± 367.7
Onychiuridae	No N-added	2041.7 ± 440.2	2750.0 ± 889.2
	N-fertilized	1963.0 ± 415.1	3190.5 ± 906.6
Sminthuridae	No N-added	722.2 ± 277.8	400.0 ± 235.0
	N-fertilized	866.7 ± 273.7	666.7 ± 198.0
Grasshoppers (Individuals/transect)			
Forb-feeding	No N-added	0.9 ± 0.2	0.9 ± 0.2
	N-fertilized	1.0 ± 0.3	2.0 ± 0.6
Grass-feeding	No N-added	1.1 ± 0.2	1.8 ± 0.3
	N-fertilized	0.7 ± 0.2	0.6 ± 0.2
Mixed-feeding	No N-added	0.2 ± 0.0	0.3 ± 0.1
	N-fertilized	0.3 ± 0.1	0.6 ± 0.2

Table 6.4 Fit statistics from structural equations models used to compare data collected in the Belowground Plot Experiment at KPBS to my model of trophic relationships between aboveground and belowground invertebrates (Figs. 6.1, 6.5). The best-fit model (based on plant C:N) is that with a non-significant Satorra-Bentler scaled χ^2, root mean square error of approximation (RMSEA) at or near 0, comparative fit index (CFI) at or near 1, and with the smallest model AICc values compared to the other models tested.

Model	χ^2 (P-value)[a]	RMSEA	CFI	Model AIC_c
C:N	6.320 (0.502)	0.000	1.000	123.176
N:P	6.600 (0.472)	0.000	1.000	123.451
C:P	55.116 (0.000)	0.272	0.599	171.967

[a]Satorra-Bentler scaled χ^2

CHAPTER 7 - Conclusions

In these studies, I broadly examined interactions between aboveground and belowground invertebrates, focusing on plants and fungi as intermediaries by using a stoichiometric approach. I also investigated some linkages more specifically to identify potential mechanisms regulating such interactions. Given the extensive network of interactions, it is not possible to investigate all interactions, so I selected a range of taxa located at strategic nodes that could possibly regulate above-belowground interactions. My central hypothesis was that over a range of soil nutrient conditions, fungivorous Collembola would have a positive effect on mycorrhizal colonization of plant roots, increasing the nutritional quality of aboveground plant material for insect herbivores and thus influencing the degree of foliar herbivory. Through a series of laboratory, greenhouse, and field experiments, I examined the effects of host plant stoichiometry on community and individual responses by grasshoppers and the relationships between the plant-mycorrhizal symbiosis and fungivorous soil-dwelling Collembola.

Using data spanning 25 years from the Konza Prairie Biological Station (KPBS) Long-Term Ecological Research (LTER) program, I investigated (Chapter 2) the contributions of weather at annual and decadal scales, fire return interval, and grazing by bison to the dynamics of grasshopper assemblages in North American continental grassland. Each of the three primary drivers of grassland ecosystem dynamics was found to affect grasshopper population and community dynamics. Negative feedbacks in abundances as expected for regulated populations were observed for all feeding guilds of grasshoppers. Among watersheds that varied with respect to controlled spring burns and grazing by bison, species composition of grasshopper assemblages responded significantly to both after 25 years. However, the number of years since the last fire was more important than the managed long-term fire frequency *per se*. Yearly shifts in species composition (1983-2005), examined using nonmetric multidimensional scaling and fourth-corner analysis, were best explained by local weather events occurring early in grasshopper life cycles. Large-scale patterns represented by the Palmer Drought Severity Index and the North Atlantic Oscillation (NAO) were significantly correlated with annual mean frequencies of grasshoppers, especially forb- and mixed-feeding species. Primary grassland drivers - fire, grazing and weather - through both intrinsic and extrinsic influences modulate long-term fluctuations in grasshopper

abundances and community taxonomic composition. Given the patterns of responses, it is likely that these drivers primarily act indirectly on most grasshoppers through changes in the quality, quantity, and structure of the plant community.

I examined the effects of host plant quality associated with nitrogen and phosphorus fertilization on proportional grass consumption by a mixed-feeding insect herbivore, *Melanoplus bivittatus* using natural abundance stable carbon (^{13}C) isotope methods (Chapter 3). The proportion of grass assimilated approximately doubled in N-fertilized treatments (39.1±0.05%) compared to non-fertilized treatments (19±0.004%), an increase associated with decreased C:N and increased N:P of grasses. There were no effects of P fertilizer on consumption patterns. These results indicate that mixed-feeding *M. bivittatus* can selectively feed to balance C:N:P intake even when choosing between two structurally and chemically different groups of plants.

Given the results of the field study, I then conducted a series of laboratory experiments to assess the roles of different regulatory mechanisms (food selection, compensatory feeding, or physiological adjustment) in *M. bivittatus*, with natural and synthetic diets (Chapter 4). Individuals provided with high quality diets as at least one of two diet choices had the highest survival, while those given only low quality foods experienced 97% mortality. In all experiments, the high quality diets were consumed preferentially over the low quality diets, although the low quality food was never completely avoided. I found no evidence of compensatory feeding within the natural or synthetic diet experiments alone, but individuals fed natural diets tended to consume approximately twice as much food as those fed synthetic diets. This probably reflects the generally higher availability of nutrients in controlled artificial diets (*e.g.*, no structural impediments such as cell walls). Five measures of post-ingestive food processing (assimilation, approximate digestibility, efficiency of conversion of ingested food, efficiency of conversion of digested food, or nitrogen use efficiency) were not affected by diet choice treatments in any of the experiments. Patterns of performance and utilization of diets were similar regardless of whether they were based on macronutrient or C:N content. For surviving individuals, my results suggest that *M. bivittatus* nymphs rely primarily on host plant selection to maintain internal elemental homeostasis and performance, although they have the ability to employ a compensatory feeding strategy when faced with variable resources as would be encountered in a field environment.

In the belowground portion of the hypothesized food web, I expected an interaction between fungivorous Collembola and arbuscular mycorrhizal (AM) fungi (Chapter 5). Most soil-free microcosm feeding experiments indicate that collembolans have a strong preference for saprophytic fungi over AM fungi. To better assess the feeding habits of Collembola in the complexity of the soil environment, I examined consumption of AM and saprophytic fungi by Collembola in plant-soil mesocosms using natural abundance stable carbon isotope techniques. After six weeks, Collembola isotope values indicated that both saprophytic and AM fungi were consumed, with a slight preference for AM fungi detected when a cool-season grass was the mycorrhizal host.

I sampled field plots that have been subjected to long-term N and P fertilization to assess indirect linkages between soil-dwelling collembolan and aboveground herbivorous insects as they are mediated by plant root and shoot C:N:P stoichiometry and AM fungi-plant symbiosis (Chapter 6). Composition of plant, Collembola, and grasshopper communities shifted significantly in response to fertilizer application (primarily N). Univariate analyses also suggested that N is a much more important driver of plant and invertebrate dynamics than P in this system. The expected indirect relationship between collembolans and aboveground herbivores was not identified by structural equations modeling, although each invertebrate group did show significant direct relationships with the C:N of plant roots and shoots, respectively. The lack of an aboveground response to changes in soil invertebrates, however, was also reported by Rice *et al.* (1998).

Overall, this research indicates that above- and belowground invertebrates may interact indirectly with one another through interactions with host plants. In field and laboratory experiments, I found that grasshopper population and community dynamics can be influenced by host plant stoichiometry (Chapters 3, 4, 6). The feeding ecology of soil-dwelling Collembola was shown to be influenced by AM fungi which form a symbiosis with plant roots (Chapters 5 and 6). Although the indirect linkage between Collembola and grasshoppers was not detected in the N and P fertilizer field experiment (Chapter 6), the above- and belowground systems are much broader and more complex than captured in the hypothesized food web. Finally, my results based on N and P fertilizer studies (Chapters 3 and 6) and host plant stoichiometry (Chapters 3, 4, 6) indicate that N is likely more limiting and may affect consumer dynamics more than P in this tallgrass prairie ecosystem.

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Appendix A - Chapter 2 Appendices

Table A.1 Annual mean number of individuals (±SE) and mean frequency (±SE) for all grasshopper species collected at Konza Prairie Biological Station from 1982 to 2005. Frequency was calculated as the proportion of samples in which a species occurred along each transect.

	Abbreviation	Life History	Size	Flight ability	Individuals	Frequency
Grass-feeding species						
Phoetaliotes nebrascensis (Thomas)	PHONEB	Mid-season	Medium	Poor	1421.9 ±219.9	0.756 ±0.033
Eritettix simplex (Scudder)	ERISIM	Early season	Small	Strong	8.0 ±2.6	0.101 ±0.021
Syrbula admirabilis (Uhler)	SYRADM	Mid-season	Medium	Strong	62.2 ±43.1	0.182 ±0.035
Orphulella speciosa (Scudder)	ORPSPE	Late season	Small	Strong	937.0 ±135.4	0.607 ±0.029
Mermiria picta (Walker)	MERPIC	Mid-season	Large	Strong	18.6 ±7.7	0.087 ±0.020
Mermiria bivittata (Serville)	MERBIV	Early season	Large	Strong	46.4 ±13.5	0.169 ±0.014
Mermiria spp.	MERSPP	Mid-season	Large	Strong	1.2 ±1.0	0.017 ±0.009
Pseudopomala brachyptera (Scudder)	PSEBRA	Early season	Medium	Poor	14.7 ±3.5	0.137 ±0.013
Boopedon auriventris McNeill	BOOAUR	Mid-season	Large	Poor	7.5 ±2.2	0.096 ±0.010
Boopedon nubilum (Say)	BOONUB	Mid-season	Large	Poor	0.1 ±0.1	0.005 ±0.005
Boopedon gracile (Rehn)	BOOGRA	Mid-season	Large	Strong	1.2 ±0.5	0.034 ±0.012
Ageneotettix deorum (Scudder)	AGEDEO	Mid-season	Small	Strong	25.7 ±10.9	0.104 ±0.032
Opeia obscura (Thomas)	OPEOBS	Mid-season	Small	Strong	2.1 ±1.0	0.041 ±0.013
Chortophaga viridifasciata (DeGeer)	CHOVIR	Early season	Medium	Strong	4.1 ±2.3	0.058 ±0.016
Pardalophora apiculata (Harris)	PARAPI	Early season	Large	Strong	0.2 ±0.1	0.015 ±0.008
Pardalophora haldemanii (Scudder)	PARHEL	Early season	Large	Strong	2.1 ±1.2	0.029 ±0.012
Pardalophora spp.	PARSPP	Early season	Large	Strong	3.9 ±1.8	0.058 ±0.018
Forb-feeding species						
Brachystola magna (Girard)	BRAMAG	Early season	Large	Poor	0.2 ±0.1	0.015 ±0.008
Hypochlora alba (Dodge)	HYPALB	Late season	Small	Poor	206.4 ±42.5	0.304 ±0.023
Campylacantha olivaceae (Scudder)	CAMOLI	Late season	Medium	Poor	72.5 ±23.4	0.170 ±0.038
Hesperotettix spp.[a]	HESSPP	Mid-season	Medium	Poor	173.7 ±54.1	0.129 ±0.086
Melanoplus scudderi (Uhler)	MELSCU	Late season	Small	Poor	249.0 ±67.2	0.314 ±0.039
Melanoplus packardii Scudder	MELPAC	Mid-season	Medium	Strong	3.5 ±1.6	0.057 ±0.012
Melanoplus differentialis (Thomas)	MELDIF	Mid-season	Large	Strong	0.5 ±0.2	0.035 ±0.011
Melanoplus keeleri (Thomas)	MELKEE	Late season	Medium	Strong	321.9 ±65.6	0.394 ±0.024
Melanoplus spp.	MELSPP	Mid-season	Medium	Strong	10.4 ±4.1	0.103 ±0.025
Arphia spp.[b]	ARPSPP	Early season	Large	Strong	14.4 ±4.8	0.060 ±0.062
Mixed-feeding species						
Schistocerca lineata Scudder	SCHLIN	Mid-season	Large	Strong	1.5 ±0.5	0.059 ±0.013
Paratylotropidia brunneri Scudder	PARBRU	Late season	Large	Poor	0.7 ±0.2	0.045 ±0.011
Melanoplus sanguinipes (Fabricius)	MELSAN	Late season	Medium	Strong	8.9 ±4.6	0.079 ±0.018
Melanoplus femurrubrum (DeGeer)	MELFEM	Mid-season	Small	Strong	149.8 ±24.3	0.279 ±0.022
Melanoplus bivittatus (Say)	MELBIV	Mid-season	Large	Strong	55.0 ±12.2	0.172 ±0.014
Melanoplus confusus Scudder	MELCON	Early season	Small	Strong	36.7 ±28.0	0.130 ±0.025
Hadrotettix trifasciatus (Say)	HADTRI	Early season	Large	Strong	2.6 ±1.7	0.036 ±0.013
Hippiscus rugosus (Scudder)	HIPRUG	Mid-season	Large	Strong	1.0 ±0.8	0.011 ±0.007

[a] *Hesperotettix viridis* (Thomas) and *H. speciosus* (Scudder) pooled

[b] *Arphia xanthoptera* (Burmeister), *A. simplex* Scudder, and *A. conspersa* (Scudder) pooled

Table A.2 Results of univariate fourth-corner analyses of grasshopper species composition in (A) 1982 and (B) 2005 using permutation models 2 and 4 to analyze relationships that were significant in multivariate analysis (Table 2.3). Rejection of the null hypothesis (H_o: management affects species composition through species traits) requires P<0.2236 under both models 2 and 4 (Dray and Legendre in review). G-statistics and the sign of the relationships (+ positive, -negative) are reported for analysis of 2 categorical variables; F-statistics are reported for analyses of 1 quantitative variable versus 1 categorical variable. P-values from each model are in parentheses and are based on 9999 permutations of the data.

Characteristic/ Trait	Statistic	a) 1982			b) 2005								
		Years Since Fire			Years Since Fire			Grazed			Ungrazed		
		F	Model 2	Model 4	F	Model 2	Model 4	G	Model 2	Model 4	G	Model 2	Model 4
Feeding Guild	Global test							2.453	(0.0007)	(0.2246)			
Forb-feeder	G-statistic							17.862	(0.0072)	(0.5520)	13.961	(0.0072)	(0.8488)
	Sign							+			-		
Grass-feeder	G-statistic							20.249	(0.2460)	(0.8488)	23.543	(0.2460)	(0.8488)
	Sign							+			-		
Mixed-feeder	G-statistic							10.486	(0.0007)	(0.8488)	4.807	(0.0007)	(0.5184)
	Sign							+			-		
Flight Ability	Global test	0.335	(0.1906)	(0.1842)	0.402	(0.1496)	(0.1670)						
Poor fliers	Homogeneity	0.486	(0.1626)	(0.7600)	0.386	(0.8604)	(0.9096)						
	Correlation (r)	**0.107**	**(0.2104)**	**(0.1830)**	**0.066**	**(0.1696)**	**(0.1678)**						
Strong fliers	Homogeneity	0.502	(0.9153)	(0.7600)	0.609	(0.2558)	(0.1700)						
	Correlation (r)	**-0.107**	**(0.2104)**	**(0.1830)**	**-0.066**	**(0.1696)**	**(0.1678)**						
Size	Global test	0.428	(0.0117)	(0.0820)									
Small	Homogeneity	0.405	(0.9356)	(0.9942)									
	Correlation (r)	-0.060	(0.0817)	(0.2377)									
Medium	Homogeneity	0.395	(0.1863)	(0.9942)									
	Correlation (r)	**0.158**	**(0.0298)**	**(0.0540)**									
Large	Homogeneity	0.169	(0.8468)	(0.4239)									
	Correlation (r)	**-0.129**	**(0.0267)**	**(0.1206)**									

Table A.3 Univariate fourth-corner analyses of grasshopper species composition in response to weather variables using permutation models 2 and 4 to analyze significant multivariate relationships (Table 2.3). Rejection of the null hypothesis (H_o: weather affects species composition based on the traits of the species) requires P<0.2236 under both models 2 and 4 (Dray and Legendre in review). F-statistics are reported with P-values (9999 permutations) in parentheses.

Trait	State	Statistic	GS PPT_t			CV GS PPT_t			GS PPT_{t-1}			WINT TEMP			GS HDD_{t-1}			CV GS PPT_{t-1}			FALL TEMP			PDI			NAO		
			F	Model 2	Model 4	F	Model 2	Model 4	F	Model 2	Model 4	F	Model 2	Model 4	F	Model 2	Model 4	F	Model 2	Model 4	F	Model 2	Model 4	F	Model 2	Model 4	F	Model 2	Model 4
Feeding Guild		Global test				F=0.219	(0.0807)	(0.0786)	F=0.297	(0.0339)	(0.0660)																F=0.372	(0.0109)	(0.0525)
	Forb-feeder	Homogeneity				0.380	(1.0000)	(1.0000)	0.379	(0.8322)	(1.0000)																0.400	(1.0000)	(0.9660)
		Correlation (r)				**0.041**	**(0.1416)**	**(0.0996)**	**-0.044**	**(0.1108)**	**(0.1002)**																**0.045**	**(0.0998)**	**(0.1628)**
	Grass-feeder	Homogeneity				0.469	(1.0000)	(1.0000)	0.461	(0.5937)	(1.0000)																0.438	(0.0558)	(0.9660)
		Correlation (r)				-0.002	(0.4632)	(0.4715)	-0.002	(0.4503)	(0.4697)																0.008	(0.3389)	(0.3959)
	Mixed-feeder	Homogeneity				0.148	(1.0000)	(0.8157)	0.156	(0.8322)	(0.9189)																0.157	(1.0000)	(0.9660)
		Correlation (r)				**-0.053**	**(0.0840)**	**(0.0996)**	**0.063**	**(0.0282)**	**(0.1002)**																**-0.073**	**(0.0057)**	**(0.0414)**
Flight Ability		Global test	F=0.575	(0.1274)	(0.0843)	F=0.636	(0.1033)	(0.0089)	F=0.521	(0.1455)	(0.0280)	F=0.639	(0.1007)	(0.0139)													F=0.479	(0.1664)	(0.0584)
	Poor fliers	Homogeneity	0.430	(0.7742)	(0.8861)	0.429	(0.7768)	(0.8885)	0.429	(0.7238)	(0.8884)	0.454	(0.6710)	(0.9155)													0.045	(0.6557)	(0.9103)
		Correlation (r)	**-0.067**	**(0.1308)**	**(0.0562)**	**0.070**	**(0.1040)**	**(0.0004)**	**-0.063**	**(0.1448)**	**(0.0094)**	**-0.070**	**(0.0998)**	**(0.0046)**													**0.061**	**(0.1718)**	**(0.0552)**
	Strong fliers	Homogeneity	0.566	(0.7742)	(0.2186)	0.566	(0.7768)	(0.2094)	0.567	(0.7238)	(0.2154)	0.541	(0.5996)	(0.1558)													0.545	(0.6480)	(0.1734)
		Correlation (r)	**0.067**	**(0.1308)**	**(0.0562)**	**-0.070**	**(0.1040)**	**(0.0004)**	**0.064**	**(0.1448)**	**(0.0094)**	**0.070**	**(0.0499)**	**(0.0046)**													**-0.061**	**(0.1718)**	**(0.0552)**
Size		Global test							F=0.219	(0.0884)	(0.0443)	F=0.207	(0.0971)	(0.0888)											F=0.161	(0.1555)	(0.0903)			
	Small	Homogeneity							0.385	(1.0000)	(1.0000)	0.400	(0.8454)	(1.0000)											0.392	(1.0000)	(1.0000)			
		Correlation (r)							**-0.029**	**(0.2058)**	**(0.1404)**	**0.045**	**(0.0543)**	**(0.0732)**											0.009	(0.3502)	(0.3150)			
	Medium	Homogeneity							0.433	(0.2055)	(1.0000)	0.437	(0.8454)	(1.0000)											0.434	(0.4272)	(1.0000)			
		Correlation (r)							**-0.016**	**(0.2058)**	**(0.2217)**	-0.006	(0.3410)	(0.4180)											**0.029**	**(0.0774)**	**(0.1998)**			
	Large	Homogeneity							0.179	(1.0000)	(0.0018)	0.159	(0.8454)	(0.0084)											0.172	(1.0000)	(0.0048)			
		Correlation (r)							**0.058**	**(0.1053)**	**(0.0177)**	**-0.049**	**(0.1164)**	**(0.0732)**											**-0.049**	**(0.1354)**	**(0.0387)**			
Phenology		Global test				F=0.223	(0.0474)	(0.0815)	F=0.204	(0.0648)	(0.0585)	F=0.155	(0.1275)	(0.1685)	F=0.261	(0.0258)	(0.0221)	F=0.278	(0.0199)	(0.0105)									
	Early season	Homogeneity				0.145	(1.0000)	(0.2718)	0.145	(0.8694)	(0.2487)	0.139	(1.0000)	(0.2922)	0.128	(0.6378)	(0.1548)	0.138	(1.0000)	(0.2412)									
		Correlation (r)				**-0.050**	**(0.0819)**	**(0.0854)**	**0.054**	**(0.0525)**	**(0.0522)**	-0.025	(0.1751)	(0.2358)	**0.047**	**(0.0738)**	**(0.0486)**	**-0.062**	**(0.0201)**	**(0.0087)**									
	Mid-season	Homogeneity				0.439	(0.3438)	(0.5148)	0.448	(0.8304)	(0.5580)	0.429	(0.2118)	(0.4872)	0.463	(0.9400)	(0.6578)	0.457	(1.0000)	(0.6212)									
		Correlation (r)				-0.010	(0.3239)	(0.3365)	-0.005	(0.3963)	(0.4091)	-0.030	(0.1434)	(0.2358)	**-0.057**	**(0.0045)**	**(0.0138)**	0.004	(0.4328)	(0.4389)									
	Late season	Homogeneity				0.413	(1.0000)	(0.9802)	0.403	(0.8694)	(0.9781)	0.429	(1.0000)	(0.9824)	0.404	(0.9400)	(0.9766)	0.400	(1.0000)	(0.9731)									
		Correlation (r)				**0.046**	**(0.0819)**	**(0.0297)**	**-0.033**	**(0.1866)**	**(0.0924)**	**0.048**	**(0.0579)**	**(0.0651)**	**0.025**	**(0.1698)**	**(0.1555)**	**0.040**	**(0.0998)**	**(0.0730)**									

Appendix B - Chapter 4 Appendices

Table B.1 Proportion of each ingredient used to construct synthetic diets, including the vitamin mix (A) for the artificial (B) and macronutrient (C) diet experiments.

A. Vitamin Mix

INGREDIENT	%
Thiamine	1.41
Riboflavin	1.41
Nicotinic Acid	5.63
Pyridoxine	1.41
Folic Acid	1.41
Myo-Inositol	14.08
Calcium Pantothenate	2.82
p-Aminobenzoic Acid	1.41
Choline Chloride	70.38
Biotin	0.06

B. Artificial Diets

	Quality	
	High	Low
INGREDIENT	%	%
a-Cellulose	53.97	54.00
Caesin[a]	5.97	2.65
Peptone[a]	2.16	1.00
Albumen[a]	2.16	1.00
Sucrose[b]	15.32	18.65
Dextrin[b]	16.37	18.65
Linoleic Acid	0.55	0.55
Cholesterol	0.55	0.55
Wesson's Salts	2.50	2.50
l-Ascorbic Acid	0.27	0.27
Vitamin Mix	0.18	0.18

C. Macronutrient Diets

	p7:c7	p7:c35	p14:c28	p21:c21	p28:c14	p35:c7
INGREDIENT	%	%	%	%	%	%
a-Cellulose	81.95	53.97	53.97	53.97	53.97	53.97
Caesin[a]	4.20	4.20	8.40	12.59	16.79	20.99
Peptone[a]	1.40	1.40	2.80	4.20	5.60	7.00
Albumen[a]	1.40	1.40	2.80	4.20	5.60	7.00
Sucrose[b]	3.50	17.49	13.99	10.49	7.00	3.50
Dextrin[b]	3.50	17.49	13.99	10.49	7.00	3.50
Linoleic Acid	0.55	0.55	0.55	0.55	0.55	0.55
Cholesterol	0.55	0.55	0.55	0.55	0.55	0.55
Wesson's Salts	2.50	2.50	2.50	2.50	2.50	2.50
l-Ascorbic Acid	0.27	0.27	0.27	0.27	0.27	0.27
Vitamin Mix	0.18	0.18	0.18	0.18	0.18	0.18

[a]=primary protein sources

[b]=primary digestible carbohydrate sources

Table B.2 Results of paired t-tests used to analyze the difference in consumption of each diet presented to individuals in the diet combination treatments of the (A) natural, (B) artificial, and (C) macronutrient diet experiments. Values in bold indicate significant differences in consumption of the two foods at α=0.05.

Diet Treatment	t-value	P-value
A. Natural Diet Experiment		
F+/F+	0.1	0.919
F+/F-	6.16	**<.0001**
F+/G+	7.83	**<.0001**
F+/G-	8.38	**<.0001**
F-/F-	0.72	0.4781
F-/G+	-3.43	**0.0025**
B. Artificial Diet Experiment		
H+/H+	0.14	0.8866
H+/H-	-2.21	**0.037**
H+/L+	-2.04	**0.0522**
H-/H-	-0.18	0.8574
H-/L+	-1.69	0.1045
H-/L-	-1.41	0.1719
C. Macronutrient Diet Experiment		
p7:c7/p7:c35	-5.95	**<.0001**
p7:c7/p21:c21	-5.75	**<.0001**
p7:c7/p35:c7	-6.35	**<.0001**
p7:c35/p21:c21	-2.23	**0.0312**
p14:c28/p21:c32	-1.39	0.1713
p28:c14/p21:c21	-1.46	0.1512
p35:c7/p21:c32	3.85	**0.0004**

Appendix C - Chapter 6 Appendices

Table C.1 Analysis of variance/covariance results for analyses of (A) plant biomass, herbivory, and mycorrhizal root colonization, (B) plant carbon (C):nitrogen (N), C:phosphorus (P), and N:P of forb and grass aboveground tissues and plant roots, and (C.) Collembola abundance and grasshopper density.

A.		Biomass			Herbivory			Mycorrhizal Root Colonization		
Response	Factors	df	F	P-value	df	F	P-value	df	F	P-value
Forb	N	1,6	44.21	0.001	1,6	11.39	0.015			
	P	1,6	8.08	0.030	1,6	0.13	0.728			
	N*P	1,6	3.65	0.105	1,6	0.25	0.638			
Grass	N	1,6	8.98	0.024	1,6.11	32.52	0.001			
	P	1,6	0.43	0.535	1,6.11	0	0.950			
	N*P	1,6	4.99	0.067	1,6.11	0.12	0.740			
Root	N	1,87	0.04	0.835	Root Herbivory Not sampled			1,65.7	27.73	<0.0001
	P	1,87	0.45	0.503				1,68.9	0.10	0.749
	N*P	1,87	1.73	0.192				1,65.7	0.42	0.517
Total	N	1,6	78.71	0.0001	1,86	76.77	<0.0001			
	P	1,6	0.93	0.373	1,86	0.15	0.704			
	N*P	1,6	3.19	0.124	1,86	0.81	0.370			

B.		Forb			Grass			Root		
Ra	Response	Factors	df	F	P-value	df	F	P-value	df	F	P-value
C:I	%N	N	1,6.13	17.06	0.006	1,38	97.16	<0.0001	1,75.2	10.04	0.002
		P	1,6.1	0.08	0.786	1,5.84	0.16	0.706	1,75.5	0.03	0.855
		N*P	1,6.01	0.31	0.598	1,6.33	0.94	0.367	1,75.8	2.18	0.144
		%C	1,88.4	9.44	0.003	1,82.9	7.41	0.008	1,70.8	5.01	0.028
C:I	%P	N	1,6.75	2.42	0.166	1,25.2	0.18	0.673	1,83.2	0.34	0.559
		P	1,6.67	4.89	0.065	1,5.8	11.88	0.014	1,83.5	0.48	0.492
		N*P	1,6.44	0.16	0.706	1,6.07	1.89	0.218	1,83.6	2.3	0.133
		%C	1,85	1.98	0.163	1,89.5	9.39	0.003	1,82	2.55	0.114
N:I	%P	N	1,86.6	0.38	0.537	1,33.9	31.04	<0.0001	1,81.3	78.45	<0.0001
		P	1,82	5.86	0.018	1,83.9	22.09	<0.0001	1,80.2	4.55	0.036
		N*P	1,82.1	0.86	0.356	1,83.9	1.19	0.279	1,80.3	0.32	0.575
		%N	1,83	15.09	0.0002	1,8	5.41	0.048	1,88.9	573.38	<0.0001

Table C.1 (Continued)

C.	Collembola Response	Factors	df	F	P-value	Grasshoppers Response	Factors	d	F	P-value
	Total	N	1,78	0.87	0.355	Total	N	1	1.42	0.277
		P	1,78	0.01	0.913		P	1	0.01	0.933
		N*P	1,78	0.03	0.859		N*P	1	0.11	0.752
	Entomobryids	N	1,77.9	4.06	0.047	Forb-feeding	N	1	3.91	0.091
		P	1,77.9	3.15	0.080		P	1	0.37	0.566
		N*P	1,77.9	3.92	0.051		N*P	1	0.54	0.486
	Hypogasturids	N	1,78	0.05	0.828	Grass-feeding	N	1	1.11	0.325
		P	1,78	2.5	0.118		P	1	0.01	0.918
		N*P	1,78	0.01	0.936		N*P	1	0.40	0.545
	Isotomids	N	1,6.33	4.18	0.084	Mixed-feeding	N	1	2.98	0.133
		P	1,6.33	0.12	0.738		P	1	2.25	0.182
		N*P	1,6.33	2.65	0.152		N*P	1	0.64	0.452
	Onychiurids	N	1,78	0.46	0.499					
		P	1,78	0.44	0.508					
		N*P	1,78	0.51	0.477					
	Sminthurids	N	1,78	2.88	0.094					
		P	1,78	0.67	0.415					
		N*P	1,78	1.51	0.222					

Table C.2 Characteristics of (A) plant, (B) grasshopper, and (C) Collembola species collected in the Belowground Plot Experiment at Konza Prairie Biological Station, Manhattan, KS. (A) Plant functional groups: F=Forb, G=Grass, L=Legume. (B) Grasshopper feeding guilds: FF=Forb-feeding, GF=Grass-feeding, MF=Mixed-feeding, Phenology: portion (early, mid, or late) of the growing season during which most individuals of a given species are adults.

A. Plant species	Functional Group	Photosynthetic Pathway	Family	Life History	Nativity
Achillea millefolium L.	F	C3	Asteraceae	Perennial	Native
Ambrosia psilostachya DC.	F	C3	Asteraceae	Perennial	Native
Andropogon gerardii Vitman	G	C4	Poaceae	Perennial	Native
Antennaria neglecta Greene	F	C3	Asteraceae	Perennial	Native
Asclepias speciosa Torr.	F	C3	Asclepiadaceae	Perennial	Native
Asclepias spp.	F	C3	Asclepiadaceae	Perennial	Native
Asclepias syriaca L.	F	C3	Asclepiadaceae	Perennial	Native
Asclepias tuberosa L.	F	C3	Asclepiadaceae	Perennial	Native
Asclepias verticillata L.	F	C3	Asclepiadaceae	Perennial	Native
Asclepias viridiflora Raf.	F	C3	Asclepiadaceae	Perennial	Native
Symphyotrichum ericoides (L.) Nesom	F	C3	Asteraceae	Perennial	Native
Baptisia spp.	L	C3	Fabaceae	Perennial	Native
Bothriochloa bladhii (Retz.) S.T. Blake	G	C4	Poaceae	Perennial	Introduced
Bouteloua curtipendula (Michx.) Torr.	G	C4	Poaceae	Perennial	Introduced
Brickellia eupatorioides (L.) Shinners	F	C3	Asteraceae	Perennial	Native
Carex spp.	G	C3	Cyperaceae	Perennial	Native
Cyperus spp.	G	C3	Cyperaceae	Perennial	Native
Dalea candida Michx. ex. Willd.	L	C3	Fabaceae	Perennial	Native
Dalea pupurea Vent.	L	C3	Fabaceae	Perennial	Native
Desmodium spp.	L	C3	Fabaceae	Perennial	Native
Dichanthelium oligosanthes (J.A. Schultes) Gould	G	C3	Poaceae	Perennial	Native
Helianthus annuus L.	F	C3	Asteraceae	Annual	Native
Koeleria macrantha (Ledeb.) J.A. Schultes	G	C3	Poaceae	Perennial	Native
Lespedeza capitata Michx.	L	C3	Fabaceae	Perennial	Native
Lespedeza spp.	L	C3	Fabaceae	Perennial	Native
Mimosa nuttallii (DC.) B.L. Turner	L	C3	Fabaceae	Perennial	Native
Orbexilum pedunculatum (P. Mill.) Rydb.	L	C3	Fabaceae	Perennial	Native
Oxalis stricta L.	F	C3	Oxalidaceae	Perennial	Native
Panicum virgatum L.	G	C4	Poaceae	Perennial	Native
Physalis spp.	F	C3	Solanaceae	Perennial	Native
Poa pratensis L.	G	C3	Poaceae	Perennial	Native
Psoralidium tenuiflorum (Pursh) Rydb.	L	C3	Fabaceae	Perennial	Native
Rumex spp.	F	C3	Polygonaceae	Perennial	Native
Schizachyrium scoparium (Michx.) Nash	G	C4	Poaceae	Perennial	Native
Solidago canadensis L.	F	C3	Asteraceae	Perennial	Native
Solidago gigantea Ait.	F	C3	Asteraceae	Perennial	Native
Solidago missouriensis Nutt.	F	C3	Asteraceae	Perennial	Native
Solidago rigida L.	F	C3	Asteraceae	Perennial	Native
Solidago spp.	F	C3	Asteraceae	Perennial	Native
Sorghastrum nutans (L.) Nash	G	C4	Poaceae	Perennial	Native
Sporobolus heterolepsis (Gray) Gray	G	C3	Poaceae	Perennial	Native
Symphoricarpos occidentalis Hook.	F	C3	Caprifoliaceae	Perennial	Native
Trifolium spp.	L	C3	Fabaceae	Perennial	Introduced
Vernonia baldwinii Torr.	F	C3	Asteraceae	Perennial	Native

Table C.2 (Continued)

B. Grasshopper species	Feeding Guild	Phenology	Size	Flight Ability
Ageneotettix deorum (Scudder)	GF	Mid	Small	Strong
Arphia simplex Scudder	FF	Early	Large	Strong
Arphia spp.[b]	FF	Early	Large	Strong
Boopedon auriventris McNeill	GF	Mid	Large	Poor
Campylacantha olivaceae (Scudder)	FF	Late	Medium	Poor
Chortophaga viridifasciata (DeGeer)	GF	Early	Medium	Strong
Hesperotettix speciosus (Scudder)	FF	Mid	Medium	Poor
Hesperotettix spp.[a]	FF	Mid	Medium	Poor
Hesperotettix viridis (Thomas)	FF	Mid	Medium	Strong
Hypochlora alba (Dodge)	FF	Late	Small	Poor
Melanoplus bivittatus (Say)	MF	Mid	Large	Strong
Melanoplus confusus Scudder	MF	Early	Small	Strong
Melanoplus differentialis (Thomas)	FF	Mid	Large	Strong
Melanoplus femurrubrum (DeGeer)	MF	Mid	Small	Strong
Melanoplus keeleri (Thomas)	FF	Late	Medium	Strong
Melanoplus packardii Scudder	FF	Mid	Medium	Strong
Mermiria bivittata (Serville)	GF	Early	Large	Strong
Melanoplus sanguinipes (Fabricius)	MF	Late	Medium	Strong
Melanoplus scudderi (Uhler)	FF	Late	Small	Poor
Melanoplus spp.	FF	Mid	Medium	Strong
Orphulella speciosa (Scudder)	GF	Late	Small	Strong
Pardalophora haldemanii (Scudder)	GF	Early	Large	Strong
Phoetaliotes nebrascensis (Thomas)	GF	Mid	Medium	Poor
Syrbula admirabilis (Uhler)	GF	Mid	Medium	Strong

C. Collembola genus	Family	Pigmentation	Eyes
Anurophorus	Isotomidae	Absent	Present
Entomobrya	Entomobryida	Present	Present
Folsomia	Isotomidae	Absent	Absent
Hypogastura	Hypogasturida	Present	Present
Onychiurus	Onychiuridae	Absent	Absent
Orchesella	Entomobryida	Present	Present
Priostoma	Isotomidae	Absent	Present
Pseudosinella	Entomobryida	Absent	Present
Sensiphorura	Onychiuridae	Absent	Absent
Sinella	Entomobryida	Absent	Absent
Sminthurus	Sminthuridae	Present	Present
Tullbergia	Onychiuridae	Absent	Absent
Unknown 1	Entomobryida	Present	Present
Unknown 2	Hypogasturida	Present	Present
Unknown 3	Isotomidae	Absent	Present
Unknown 4	Onychiuridae	Absent	Absent
Unknown 5	Sminthuridae	Present	Present
Xenylla	Hypogasturida	Present	Present