

ECOLOGY OF BELOWGROUND INVERTEBRATES IN TALLGRASS PRAIRIE

by

MAC AARON CALLAHAM, JR.

B.A., University of Georgia, Athens, 1994

B.S., University of Georgia, Athens, 1994

M.S., University of Georgia, Athens, 1996

A DISSERTATION

submitted in partial fulfillment of the

requirements for the degree

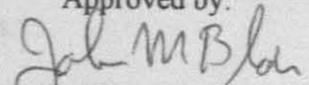
DOCTOR OF PHILOSOPHY

Division of Biology  
College of Arts and Sciences

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

2000

Approved by:



Major Professor  
John M. Blair

LD  
2668  
.D5  
BIOL  
2000  
L35  
C.2

## ABSTRACT

The aboveground responses of tallgrass prairie plant communities and ecosystem processes to fire and grazing are well characterized. However, responses of consumer groups to these disturbances are less well known. Invertebrates are an important component of soil ecosystems in tallgrass prairie, making up a diverse assemblage of organisms that perform a diverse set of functions. However, factors influencing their distribution and abundance are not well known.

We conducted studies examining the influence of different land management practices on the distribution of soil invertebrates at the Konza Prairie Biological Station (KPBS). We sampled the Belowground Plot Experiment at KPBS, a long-term experimental manipulation which addresses the influence of fire, mowing, and nutrient additions on belowground processes and organisms. Results indicated that native North American earthworms (*Diplocardia* spp.) were favored by the occurrence of fire and/or mowing, whereas an exotic European earthworm (*Aporrectodea trapezoides*) was more abundant in unburned, unmowed plots which had received fertilizer amendments. We suggest that changes in microclimate and/or belowground resource quality associated with absence of fire and mowing may be responsible for differences in native and exotic earthworm distributions. In another field study, we examined the differences between native and exotic earthworms with respect to their activity in the soil and their influence on plant nutrition. Native earthworms were more active during the growing season, but appeared to decrease availability of N to plants during the experiment.

We examined another group of soil invertebrates, the cicadas, at the landscape level and in the plot level study. We examined emergence densities of four annual cicada

species in upland grassland, lowland grassland, and lowland forest locations. Two species (*Tibicen aurifera* and *Cicadetta calliope*) were more abundant in upland landscape positions, and one species (*Tibicen pruinosus*) emerged only from forested areas. At all landscape positions, cicada emergence resulted in significant fluxes of N from belowground to aboveground ( $\sim 4 \text{ kg ha}^{-1}$ ). In the Belowground Plot Experiment, cicadas showed dramatic, species specific, responses to fire, mowing and fertilization treatments. *T. aurifera* emerged exclusively from unburned plots, whereas *C. calliope* was more abundant in burned plots which were not mowed, suggesting resource partitioning between species.

## TABLE OF CONTENTS

LIST OF TABLES.....	iv
LIST OF FIGURES.....	vi
ACKNOWLEDGEMENTS.....	x
INTRODUCTION.....	1
CHAPTER 1: INFLUENCE OF DIFFERING LAND MANAGEMENT ON THE INVASION OF NORTH AMERICAN TALLGRASS PRAIRIE SOILS BY EUROPEAN EARTHWORMS.....	14
SUMMARY.....	15
INTRODUCTION.....	16
MATERIALS AND METHODS.....	17
RESULTS.....	19
DISCUSSION.....	20
LITERATURE CITED.....	23
CHAPTER 2: NATIVE NORTH AMERICAN AND INTRODUCED EUROPEAN EARTHWORMS IN TALLGRASS PRAIRIE: BEHAVIORAL PATTERNS AND INFLUENCES ON PLANT GROWTH.....	29
ABSTRACT.....	30
INTRODUCTION.....	31
MATERIALS AND METHODS.....	33

RESULTS.....	37
DISCUSSION.....	40
CONCLUSIONS.....	44
ACKNOWLEDGEMENTS.....	45
LITERATURE CITED.....	45

### CHAPTER 3: FEEDING ECOLOGY AND EMERGENCE PRODUCTION OF ANNUAL CICADAS (HOMOPTERA: CICADIDAE) IN TALLGRASS PRAIRIE.....56

ABSTRACT.....	57
INTRODUCTION.....	58
MATERIALS AND METHODS.....	60
RESULTS.....	64
DISCUSSION.....	68
CONCLUSIONS.....	73
ACKNOWLEDGEMENTS.....	74
REFERENCES.....	74

### CHAPTER 4: INFLUENCE OF ANNUAL FIRE, MOWING, AND FERTILIZATION ON EMERGENCE DENSITY AND BIOMASS OF TWO ANNUAL CICADAS (HOMOPTERA: CICADIDAE) IN TALLGRASS PRAIRIE.....86

ABSTRACT.....	87
INTRODUCTION.....	88
MATERIALS AND METHODS.....	90

RESULTS.....	94
DISCUSSION.....	97
LITERATURE CITED.....	102
CHAPTER 5: INFLUENCE OF FIRE, MOWING, AND FERTILIZATION ON THE DENSITY AND BIOMASS OF BELOWGROUND MACROINVERTEBRATES IN TALLGRASS PRAIRIE.....	112
ABSTRACT.....	113
INTRODUCTION.....	114
MATERIALS AND METHODS.....	116
RESULTS.....	118
DISCUSSION.....	121
CONCLUSIONS.....	126
ACKNOWLEDGEMENTS.....	126
LITERATURE CITED.....	127
SUMMARY AND CONCLUSIONS.....	142

## LIST OF TABLES

### CHAPTER 2

Table 1. Mean proportions of earthworms collected from each depth during the study period.....	50
Table 2. Microbial biomass carbon, microbial biomass nitrogen, and microbial C/N from experimental cores with native, exotic, or no earthworms.....	51
Table 3. $\delta^{13}\text{C}$ of microbial biomass, total $\mu\text{g }^{13}\text{C}$ in microbial biomass standing stock per g soil, and $\delta^{13}\text{C}$ of $\text{CO}_2$ respired from non-fumigated soils from experimental cores with native, exotic, or no earthworms.....	52
Table 4. Mass, atom percent $^{15}\text{N}$ , standing stock nitrogen, and standing stock $^{15}\text{N}$ of aboveground tissues from plants grown in experimental cores.....	53

### CHAPTER 3

Table 1. Cicada emergence production and nitrogen flux from three habitats in the tallgrass prairie landscape.....	80
Table 2. Cicada emergence densities and percent similarity of plant communities.....	81
Table 3. Tissue chemistry for males, females, and exuviae of the three dominant species of grassland cicadas on KPRNA.....	82

### CHAPTER 4

Table 1. Body measurements of two cicada species from fertilized and unfertilized plots.....	106
--	-----

Table 2. Tissue chemistry of male and female *Cicadetta calliope* and *Tibicen aurifera*.....107

CHAPTER 5

Table 1. Regression equations for dry mass to ash-free dry mass conversions for various soil invertebrates.....131

## LIST OF FIGURES

### Chapter 1:

Figure 1. Abundances of native and exotic earthworms relative to land management treatments in A) October 1994 and B) April 1997. Note differences between Y-axes.....	26
Figure 2. Proportion of earthworm community composed of <i>Diplocardia</i> spp. relative to land management treatments..	27
Figure 3. Relationship between surface litter accumulation, and proportion of <i>Aporrectodea</i> spp. occurring in belowground plots during A) 1994 sampling and B) 1997 sampling. ....	28
<h3>Chapter 2:</h3>	
Figure 1. Rainfall (bars), temperature (solid line) and important dates during the experimental period. Climate data are from KPBS headquarters weather station. First dotted line indicates application of earthworm treatments, subsequent dotted lines indicate sampling dates.....	54
Figure 2. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of native and exotic earthworms during the study period.....	55

## Chapter 3:

Figure 1. Emergence phenology for dominant cicada species at KPRNA during the growing season of 1998. Note: emergence of a single specimen of *T. lyricen* is included with *T. pruinosus*. .....83

Figure 2. Sex-specific emergence densities through time of cicadas in grassland habitats at KPRNA. Note differences in scales for each plot.....84

Figure 3.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of cicadas collected from annually burned prairie at KPRNA during the growing season of 1998. Note: data for *M. cassini* are included as reference to a known (C<sub>3</sub>) tree feeding species.....85

## Chapter 4:

Figure 1. Densities of adult A) *Cicadetta calliope* and B) *Tibicen aurifera* emerging from selected treatments in the Belowground Plot Experiment, Summer 1999.....108

Figure 2. Energy and nitrogen fluxes associated with cicada emergence from the Belowground Plot Experiment.....109

Figure 3. Relationship between oviposition site availability and cicada density for both species (oviposition site availability is defined as the proportion of total plant shoots with either grass flowering culms or forb stems suitable for oviposition). Note: y-axis is log scale.....110

Figure 4. Conceptual model outlining possible interactions between cicadas and large ungulate grazers. Cicadas are hypothesized to partition resources spatially in the context of landscape level influences that grazers exert on aboveground vegetation structure...111

#### Chapter 5:

Figure 1. Density of earthworms in belowground plots October 1994. A) Native North American *Diplocardia* spp., and B) exotic European *Aporrectodea trapezoides*.....132

Figure 2. Biomass (ash-free dry mass) of earthworms in belowground plots October 1994. A) Native North American *Diplocardia* spp., and B) exotic European *Aporrectodea trapezoides*.....133

Figure 3. Proportion of total earthworm biomass in belowground plots that was from *Diplocardia* spp. in A) 1994 and B) 1999.....134

Figure 4. Density of earthworms in belowground plots in June 1999. A) Native North American *Diplocardia* spp., and B) exotic European *Aporrectodea trapezoides*, and C) *Octolasion cyaneum*.....135

Figure 5. Biomass (ash-free dry mass) of earthworms in belowground plots June 1999. A) Native North American *Diplocardia* spp., and B) exotic European *Aporrectodea trapezoides* and C) *Octolasion cyaneum*.....136

Figure 6. Density of cicada nymphs in belowground plots October 1994. A) <i>Tibicen aurifera</i> , B) <i>Cicadetta calliope</i> , and C) early instars.....	137
Figure 7. Biomass (ash-free dry mass) of cicada nymphs in belowground plots October 1994. A) <i>Tibicen aurifera</i> , B) <i>Cicadetta calliope</i> and C) early instars.....	138
Figure 8. Density of cicada nymphs in belowground plots June 1999. A) <i>Tibicen aurifera</i> , B) <i>Cicadetta calliope</i> and C) early instars.....	139
Figure 9. Biomass (ash-free dry mass) of cicada nymphs in belowground plots June 1999. A) <i>Tibicen aurifera</i> , B) <i>Cicadetta calliope</i> and C) early instars.....	140
Figure 10. Densities of A) Scarabaeidae larvae, B) Curculionidae larvae, and C) Elateridae larvae in the belowground plots June 1999.....	141

## ACKNOWLEDGEMENTS

This work is testimony to the fact that science is best done by committee. To say that this is “my” dissertation would not even approach the true nature of the work contained herein. This dissertation belongs to all the people who had input in one form or another, whether intellectual insight or dirt under the fingernails. First, John Blair deserves not only acknowledgement, but deep thanks as well for being an understanding and indulgent advisor. His willingness to allow deviations from the stated objectives of my program is responsible for the evolution of this dissertation into what it is, and I thank him for it. The members of my committee also deserve thanks. Loretta Johnson, Alan Knapp, Chuck Rice, and Tim Todd made this work possible, and all provided lab facilities, instruction, words of encouragement, and excellent advice during my time at K-state. A late addition to the committee was Matt Whiles who was responsible for getting the ball rolling on the entomological portion of the program, and there could have been no more appropriate person at K-state to fill in for a missing member on the day of the defense.

My lab mates Sara Baer, Duane Kitchen, Mark Norris, and later, Chris Harper deserve thanks for their patience and friendship during the long hours we spent together in the lab and in the annex. Duane deserves special attention and thanks for the many hundreds of hours that we spent together working on projects related to our dissertations, and the hundreds (thousands?) of hours beyond that we spent naturalizing, hunting, and fishing. Tina Kitchen also deserves thanks for her friendship and for her patience on those one or two occasions when we got home a little late. Many other friends helped

make the time spent at K-state more enjoyable, and foremost among them are the three horsemen of the apocalypse: Bill Rogers, Brett Rubenstein, and Mendy Smith. These three friends made my transition from Georgia to Kansas more fun than I thought it could be and I thank them for it. Other fellow grad students who have made the time here enjoyable are too numerous to mention and I won't try to list them, but they know who they are.

My family is one of the best things in my life. My parents have always supported me in every undertaking, and it is not possible to thank them for everything they have done to give me opportunities to be successful. My sisters and my brother (and their spouses) have all given unconditional love and support and they each continue to enrich my life with their families and their companionship.

Finally, Andrea Silletti deserves thanks for everything she has done for me. From sieving soil to cooking meals to growing tomatoes to making a home with me, she has reminded me of how to be happy, and for that I am eternally grateful.

-27 May 2000, Dwight, Kansas

## INTRODUCTION

### *Tallgrass prairie research at Konza Prairie Biological Station*

Tallgrass prairie, part of the grassland biome once covering vast areas of North America, is now represented by less than 5% of its original extent (Samson and Knopf 1994). The Flint Hills region of eastern Kansas and northeastern Oklahoma, having escaped wholesale conversion to rowcrop agriculture by virtue of its steep and stony soils, is the largest remaining expanse of tallgrass prairie. Grazing of livestock animals is the primary land use in the Flint Hills, and production of beef cattle is economically important in the region. However, changing land management practices in recent decades (such as exclusion of fire and overgrazing of rangeland), have resulted in increased cover of woody vegetation in the region, and in many cases conversion of grassland to forest-like vegetation is complete (Briggs et al. 1998, Hoch and Briggs 1999). For this reason (among others), an understanding of how land management practices can influence ecosystem products and processes in tallgrass prairie is as important as ever.

Periodic disturbances, such as fire and grazing by large ungulates are credited, in part, with the original development and subsequent maintenance of grassland ecosystems in vast areas of central North America (Knapp et al. 1998a, Axlerod 1985). Because precipitation levels near the eastern edge of the grassland biome are sufficient to support forest vegetation, a principal role of disturbances in these “tallgrass” prairie ecosystems is the maintenance of grassland vegetation by preventing the expansion of woody plants. Aside from this biome level influence of fire and grazing in tallgrass prairie, disturbances (or the absence thereof) affect virtually every other level of ecological organization in

these grasslands, ranging from the physiology of the individual grass leaf to plant community species composition to ecosystem primary production (Knapp 1985, Collins and Steinauer 1998, Collins et al. 1998, Knapp et al. 1998a, Knapp et al. 1999). Fire and grazing disturbances also affect ecosystem level processes such as nutrient retention and cycling (Tate 1990, Blair 1997). The profound effects of fire and grazing on ecosystem structure and function in tallgrass prairie have been a central area of study at the Konza Prairie Biological Station (KPBS) in eastern Kansas. These studies, over the last 20 years, have resulted in an excellent understanding of the mechanisms by which fire drives aboveground plant processes in tallgrass prairie, and provided a theoretical framework for questions about structure and function in other components of this ecosystem.

One area of particular interest to ecologists at KPBS has been that of structure and functioning of the belowground component of grassland ecosystems. Comparatively little is known about belowground processes in tallgrass prairie relative to aboveground processes. However, this situation is primarily a consequence of the difficulty of conducting studies in soil systems, and not a function of the perceived importance of belowground processes to overall ecosystem function (Blair et al. 1998, Rice et al. 1998). Indeed, a large proportion of annual primary production, and a majority of biomass, in tallgrass prairie is in the form of roots (Hayes and Seastedt 1987), and as such, answering questions concerned with processes belowground are critical to understanding the whole tallgrass prairie ecosystem.

Soil microbial communities are largely responsible for processing a majority of the primary production in tallgrass prairie either directly, as decomposers of plant detritus, or indirectly as decomposers of herbivorous consumers and their waste products.

Previous research conducted at KPBS has demonstrated that the characteristics of soil microbial communities in tallgrass prairie can vary with differing fire regimes. Although Garcia and Rice (1994) found no consistent differences in the total amount of microbial biomass in prairie that was burned compared to not burned, there are other lines of evidence, such as enhanced CO<sub>2</sub> flux from burned prairie (Knapp et al. 1998b), enhanced decomposition rates (O'Lear et al. 1996), and reduced net N mineralization (Blair 1997) in burned prairie, which suggest that microbial communities are indeed affected by fire.

Another area of interest in the belowground systems of tallgrass prairie is how macro- and micro-invertebrates are influenced by disturbances, and how they, in turn, through interactions with soil microbes, may affect soil ecosystem processes. Soil invertebrates have been demonstrated to be strong regulators of certain microbial processes (including decomposition, mineralization, etc.) in many temperate systems (Coleman and Hendrix 2000, Coleman and Crossley 1996), but invertebrate regulation of such processes in tallgrass prairie is poorly documented (Blair et al. 2000, Rice et al. 1998). The principal impediment to understanding the relationship between soil invertebrates and soil microbial processes, and their respective responses to fire, is the complex nature and multiplicity of fire and grazing effects on the soil system. For example, the removal of standing dead plant tissue and litter from the soil surface (a primary effect of fire or mowing/grazing) results in increased soil temperatures and decreased soil moisture status (Knapp and Seastedt 1986, Hulbert 1988); and these factors are often important determinants of soil invertebrate community composition and activity (Coleman and Crossley 1996, O'Lear and Blair 1999, Todd et al. 1999). In addition to effects on soil climate, removal of detritus results in an overall increase in

plant production compared to unburned prairie. This increase in primary production occurs in spite of the volatilization of nutrients (principally N) with fire, and the resulting low nutrient status of soils in annually burned prairie. The ultimate consequences of higher plant production in lower fertility soils are the production of plant tissue that has much higher C:N ratios in burned prairie, and increased dominance of plant species with higher nutrient use efficiencies (warm-season C<sub>4</sub> grasses). Clearly, the lower quality of plant tissue inputs, and decreased heterogeneity of those inputs, could have significant impacts on the composition and functioning of belowground systems. At the other extreme, exclusion of fire or grazing from tallgrass prairie systems results in the build up of large quantities of detritus. This detritus has the direct effect of shading and insulating soil such that it stays cooler and wetter for longer during the growing season (Knapp and Seastedt 1986). Another effect of fire exclusion is the increased diversity of plant communities in the tallgrass prairie landscape (Collins and Steinauer 1998). Thus, when examined simultaneously, the effects of fire and/or grazing (either frequent occurrence or absence) on soil climate, soil nutrient status, plant productivity, and plant tissue quality, make interpretation of potential interactions between soil processes and soil organisms very difficult. Following is a brief outline of studies conducted on various components of the soil ecosystem in tallgrass prairie at KPBS.

### ***Soil microbial ecology in tallgrass prairie***

Microbes (bacteria and fungi) are ubiquitous in soil, and are a crucial component of most terrestrial ecosystems. The soil microbial community is highly diverse, both taxonomically and functionally, and is composed of some organisms which are beneficial

to plant growth, but there are numerous detrimental organisms represented as well. Perhaps most importantly, soil microbes are responsible for processing and recycling of much of the organic matter in soil systems, and are the primary organisms involved in the processes of decomposition and mineralization. Additionally, these microbes form the base of the detrital food web, providing food resources for a wide variety of other organisms in soil, and it is often the interactions between soil microbes and organisms which feed upon them that determine rates of microbially mediated processes in soils.

In tallgrass prairie, studies on the microbial biomass in relation to fire are limited and have produced conflicting results. For example, Garcia and Rice (1994) found that five years of annual burning resulted in no consistent response from the microbial biomass, whereas Ojima et al. (1990) found that microbial biomass was slightly decreased in plots which were annually burned. Differences between these two studies have been attributed to the differences in the length of time annual burning had occurred, but differences in soil type between the two sites, and differences in site histories add to the difficulty in interpretation, and may also be responsible for equivocal results. Nonetheless, other work has suggested indirectly that the function and activity of soil microbes differs in soils under different burning regimes. For example, O'Lear et al. (1996) showed that the decomposition rate of a common substrate (wooden rods) was higher in annually burned prairie relative to unburned prairie, and Knapp et al. (1998b) showed that total soil CO<sub>2</sub> efflux was greater in soils under annually burned prairie. Finally, Groffman et al. (1993) found that although they could detect no differences in the size of microbial biomass, denitrification rates were higher in soils where fire had been excluded for long periods, relative to annually burned prairie. All of these studies

suggest that soil microbial communities behave differently under different fire regimes in tallgrass prairie.

Unfortunately, the interactions between soil microbes, and other soil organisms found in tallgrass prairie are not clearly understood. Although other soil organisms such as nematodes, microarthropods, and earthworms have all been shown to influence the size and/or turnover rates of microbial pools in other ecosystems, these relationships have not been verified in tallgrass prairie.

### ***Macroarthropods in tallgrass prairie***

Responses of macroarthropods to disturbances in tallgrass prairie are largely taxon specific, and have been little studied. Seastedt (1984) found that only herbivorous beetle larvae (primarily *Phyllophaga* spp.) exhibited a consistent positive response to burning. In that study, abundances of other groups of insect herbivores, detritivores, and predators were not affected by fire. However, in another study, Seastedt et al. (1986) found that densities of herbivorous cicada nymphs were higher in soils under burned prairie. One obvious potential mechanism for the observed increases in herbivorous macroarthropods is the increase in root production associated with annual burning (Rice et al. 1998, Johnson and Matchett *in press*), but other effects of fire such as changes in soil temperature and moisture conditions cannot be ruled out as possible contributors. In another study aimed at identifying belowground herbivore regulation of plant production, Todd et al. (1992) examined the influences of biocides on the density and biomass of several soil invertebrate groups and subsequent impacts on plant performance. Although biocide application resulted in general declines in abundance of herbivorous

macroarthropods, they were unable to detect differences in plant parameters after two years.

### ***Earthworms in tallgrass prairie***

Earthworms are the most abundant macro-invertebrate in tallgrass prairie soils, at least in terms of biomass (Blair et al. 2000, Todd et al. 1992). Furthermore, earthworms are recognized for their importance in several aspects of soil ecosystem function. They have the potential to affect virtually every soil process through their feeding, burrowing, and casting activities (Edwards and Bohlen 1996). In tallgrass prairie, fire affects both the abundance and distribution of earthworms, and may have implications for the species composition of earthworm communities (particularly with respect to the occurrence of non-native species).

Previous studies of earthworm distributions in tallgrass prairie with respect to fire treatments have shown the abundance of the native genus *Diplocardia* to be greater in annually burned prairie relative to prairie from which fire had been excluded (James 1982, James 1988). As with previously discussed taxa, the distribution and abundance of earthworms is thought to be, at least in part, a function of litter removal by fire. However it is difficult to separate the effects of litter accumulation on soil climate and the effects of burning on belowground plant production and plant tissue quality. In a study aimed at evaluating the independent effects of burning and soil moisture on earthworm communities, James (1988) manipulated soil moisture by irrigating annually burned and unburned prairie. He found that biomass of *Diplocardia smithii* increased in burned plots relative to those not burned, and was unaffected by irrigation, but that biomass of *D.*

*verrucosa* was greater in burned plots, and was also greater in irrigated plots. Biomass of the exotic genus *Aporrectodea* was highest in unburned plots, and was not influenced by irrigation in either burned or unburned plots. Thus, it appears that patterns observed for both native and exotic earthworms in relation to fire are not strongly influenced by moisture conditions, and may involve other factors such as plant tissue quality or changes in microbial activity.

### *Objectives*

In light of the challenges detailed above which face researchers interested in the belowground fauna of tallgrass prairie, the objective of the present work was to contribute to the general knowledge of invertebrates in tallgrass prairie soils by examination of the density and biomass of soil invertebrates both at the landscape level and in the context of a long-term plot level experimental manipulation of fire, mowing, and nutrient addition. In chapter one, I examined earthworms in the Belowground Plot Experiment at KPBS. Specific objectives for that study were to identify experimental treatment combinations that influenced the distributions of native and exotic earthworm taxa, and to identify possible mechanisms responsible for observed distributions. In chapter two, I experimentally manipulated earthworm populations in field-incubated microcosms. Specific objectives were to determine differences between native and exotic earthworms with respect to microbial biomass and nutrient uptake by plants. In chapter three, I examined emergence patterns of annual cicadas at the landscape scale. Objectives of this study were to enumerate cicada emergence density and nutrient flux (as cicada biomass) at three different topographic positions in the landscape. Chapter four

also examined cicada emergence, but in the Belowground Plot Experiment, and with the objective of examining effects of fire, mowing, and nutrient addition on emergence density. Finally, chapter five was a complete sampling of the Belowground Plot Experiment for all soil macroinvertebrates. The specific objective in chapter five was to examine the roles of fire, mowing, and N and P fertilization on the density and biomass of several groups of soil invertebrates.

### LITERATURE CITED

- Axlerod, D.I. 1985. Rise of the grassland biome, central North America. *Botanical Review* 51:163-201.
- Blair, J.M. 1997. Fire, N availability, and plant response in grasslands: a test of the transient maxima hypothesis. *Ecology* 78:2359-2368.
- Blair, J.M., T.C. Todd, M.A. Callaham, Jr. 2000. Responses of grassland soil invertebrates to natural and anthropogenic disturbances. In: Coleman D.C., P.F. Hendrix (eds). *Invertebrates as Webmasters in Ecosystems*. CAB International Press, pp 43-71.
- Blair, J.M., T.R. Seastedt, C.W. Rice, R.A. Ramundo. 1998. Terrestrial nutrient cycling in tallgrass prairie. In: Knapp, A.K., J.M. Briggs, D.C. Hartnett, and S.L. Collins (eds.). *Grassland Dynamics: Long-term Ecological Research in Tallgrass Prairie*. Oxford University Press, New York, pp. 222-243.
- Briggs, J.M., M.D. Nellis, C.L. Turner, G.M. Henebry, and H. Su. 1998. A landscape perspective of patterns and processes in tallgrass prairie. In: Knapp, A.K., J.M. Briggs, D.C. Hartnett, and S.L. Collins (eds.). *Grassland Dynamics: Long-term*

- Ecological Research in Tallgrass Prairie. Oxford University Press, New York, pp. 265-279.
- Coleman, D.C., and P.F. Hendrix (eds.). 2000. Invertebrates as Webmasters in Ecosystems. CAB International Press, 336 pages.
- Coleman, D.C., and D.A. Crossley, Jr. 1996. Fundamental of Soil Ecology. Academic Press, San Diego, 205 pages.
- Collins, S.L., A.K. Knapp, J.M. Briggs, J.M. Blair, and E.M. Steinauer. 1998. Modulation of diversity by grazing and mowing in native tallgrass prairie. *Science* 280:745-747.
- Collins, S.L., and E.M. Steinauer. 1998. Disturbance, diversity, and species interactions in tallgrass prairie. In: Knapp, A.K., J.M. Briggs, D.C. Hartnett, and S.L. Collins (eds.). *Grassland Dynamics: Long-term Ecological Research in Tallgrass Prairie*. Oxford University Press, New York, pp. 140-156.
- Edwards, C.A., and P.J. Bohlen. 1996. *Biology and Ecology of Earthworms*. (3rd ed.). Chapman and Hall, London, 426 pages.
- Garcia, F.O. and C.W. Rice. 1994. Microbial biomass dynamics in tallgrass prairie. *Soil Science Society of America Journal* 58:816-823.
- Groffman, P.M., C.W. Rice, and J.M. Tiedje. 1993. Denitrification in a tallgrass prairie landscape. *Ecology* 74:855-862.
- Hayes, D.C. and T.R. Seastedt. 1987. Root dynamics of tallgrass prairie in wet and dry years. *Canadian Journal of Botany* 65:787-791.
- Hoch, G.A and J.M. Briggs. 1999. Expansion of eastern red cedar in the northern Flint

- Hills, Kansas. Pages 9-15 In Proceedings of the Sixteenth North American Prairie Conference (J.T. Springer, ed.), University of Nebraska at Kearney, Kearney, NE.
- Hulbert, L.C. 1988. Causes of fire effects in tallgrass prairie. *Ecology* 69:46-58.
- James, S.W. 1982. Effects of fire and soil type on earthworm populations in a tallgrass prairie. *Pedobiologia* 24:37-40.
- James, S.W. 1988. The post-fire environment and earthworm populations in tallgrass prairie. *Ecology* 69:476-483.
- Johnson, L.C. and J.R. Matchett. Contrasting effects of grazing and burning on root productivity and root tissue quality in tallgrass prairie: feedbacks to prairie nitrogen cycling. *Ecology* (*In press*).
- Knapp, A.K. 1985. Effect of fire and drought on the ecophysiology of *Andropogon gerardii* and *Panicum virgatum* in a tallgrass prairie. *Ecology* 66:1309-1320.
- Knapp, A.K., J.M. Briggs, J.M. Blair, and C.L. Turner. 1998a. Patterns and control of aboveground net primary production in tallgrass prairie. In: Knapp, A.K., J.M. Briggs, D.C. Hartnett, and S.L. Collins (eds.). *Grassland Dynamics: Long-term Ecological Research in Tallgrass Prairie*. Oxford University Press, New York, pp. 193-221.
- Knapp, A.K., S.L. Conrad, and J.M. Blair. 1998b. Determinants of soil CO<sub>2</sub> flux from a sub-humid grassland: effect of fire and fire history. *Ecological Applications* 8:760-770.
- Knapp, A.K., and T.R. Seastedt. 1986. Detritus accumulation limits productivity in tallgrass prairie. *BioScience* 36:662-668.
- Ojima, D.S., W.J. Parton, D.S. Schimel, and C.E. Owensby. 1990. Simulated impacts of

- annual burning on prairie ecosystems. In: Collins, S.L., and L.L. Wallace (eds.).  
Fire in North American Tallgrass Prairies. University of Oklahoma Press,  
Norman, pp. 118-132.
- O'Lear, H.A., and J.M. Blair. 1999. Responses of soil microarthropods to changes in soil  
water availability in tallgrass prairie. *Biology and Fertility of Soils* 29:207-217.
- O'Lear, H.A., T.R. Seastedt, J.M. Briggs, J.M. Blair, and R.A. Ramundo. 1996. Fire and  
topographic effects on decomposition rates and nitrogen dynamics of buried wood  
in tallgrass prairie. *Soil Biology and Biochemistry* 28:323-329.
- Rice, C.W., T.C. Todd, J.M. Blair, T.R. Seastedt, R.A. Ramundo, and G.W.T. Wilson.  
1998. Belowground Biology and Processes. In: Knapp, A.K., J.M. Briggs, D.C.  
Hartnett, and S.L. Collins (eds.). *Grassland Dynamics: Long-term Ecological  
Research in Tallgrass Prairie*. Oxford University Press, New York, pp. 244-264.
- Samson, F. and F. Knopf. 1994. Prairie conservation in North America. *BioScience*  
44:418-421.
- Seastedt, T.R. 1984. Belowground macroarthropods of annually burned and unburned  
tallgrass prairie. *American Midland Naturalist* 111:405-408.
- Seastedt, T.R., D.C. Hayes, and N.J. Petersen. 1986. Effects of vegetation, burning, and  
mowing on soil macroarthropods of tallgrass prairie. In: Clambey, G.K., and  
R.H. Pemble (eds.), *Proceedings of the Ninth North American Prairie Conference*,  
Tri-College Press, Fargo, pp. 99-102.
- Tate, C.M. 1990. Patterns and controls of nitrogen in tallgrass prairie streams. *Ecology*  
71:2007-2018.
- Todd, T.C., J.M. Blair and G.A. Milliken. 1999. Effects of altered soil water availability

on a tallgrass prairie nematode community. *Applied Soil Ecology* 13:45-55.

Todd, T.C., S.W. James, and T.R. Seastedt. 1992. Soil invertebrate and plant responses to mowing and carbofuran application in a North American tallgrass prairie. *Plant Soil* 144:117-124.

**CHAPTER 1:**

**INFLUENCE OF DIFFERING LAND MANAGEMENT ON THE INVASION  
OF NORTH AMERICAN TALLGRASS PRAIRIE SOILS BY EUROPEAN  
EARTHWORMS**

Mac A. Callaham, Jr., and John M. Blair

Division of Biology, Kansas State University, Manhattan, KS, 66502

As published in: *Pedobiologia* 43:507-512 (1999)

## SUMMARY

European earthworms have come to dominate earthworm assemblages in most North American soils. The establishment of these invasive taxa is typically preceded by agricultural or other perturbation of the natural soil system. Grassland soils in the Flint Hills region of North America have escaped these perturbations because of their steep topography and stony soils; and as a consequence, still support native earthworms. Nevertheless, European taxa are invading these soils, and the conditions under which these invasions occur are unknown.

In fall of 1994 and spring of 1997 we sampled earthworms from experimental plots at Konza Prairie Research Natural Area in eastern Kansas, to assess the impacts of different land management practices on composition of the earthworm fauna in grassland soils. Since 1986, these plots have been subjected to all possible combinations of the following treatments: annually burned or unburned, annually mowed or unmowed, and annually fertilized (N and P) or unfertilized, totaling in eight treatments.

European taxa were significantly more abundant in unburned plots in samples collected in both 1994 and 1997 ( $p=0.03$  and  $p=0.07$  respectively). Furthermore, analysis of the 1997 data indicated a greater proportion of the earthworm community was composed of native taxa plots which were mowed ( $p=0.08$ ), and in plots which were not fertilized ( $p=0.09$ ). The accumulation of litter on the soil surface associated with absence of fire and/or grazing may be partly responsible for changes observed in the earthworm community.

Our results suggest that land management practices may influence the suitability of these soils for European earthworms, and potential mechanisms for the differences observed in this study will be discussed.

**Key Words:** earthworms, Diplocardia, Aporrectodea, invasive species, fire

## INTRODUCTION

Understanding the ecology of invasive exotic organisms is crucial to understanding their potential impacts on ecosystems, both in terms of interactions with native organisms, and changes in ecosystem structure and function (e.g. Scheu & Parkinson 1994; Burtelow et al. 1998). Many North American soils are currently inhabited by an entirely European (and/or Asian) earthworm fauna (Reynolds et al. 1970; Reynolds 1994), and others are currently being invaded by these exotic species.

Early studies concerning the distribution and establishment of European earthworms (principally Lumbricidae) in North America suggested that these species were superior to native earthworms in their ability to compete for resources, and that native species were actively displaced by exotics in these competitive interactions (Smith 1928). However, more recent studies indicate that displacement of native earthworms is more likely the result of soil disturbances associated with human activities such as agriculture, logging, or land development (Stebbins 1969; Kalisz & Dotson 1989). Thus, the predominance of European earthworms in these soils is thought to be a function of their ability to tolerate soil disturbances, and not the direct result of competitive interactions with the native earthworm fauna.

Areas of North America once covered by tallgrass prairie have been subject to extensive soil disturbance, resulting from agricultural practices, and less than 5% of this area remains as native prairie (Samson & Knopf 1984). Much of the remaining tallgrass prairie exists in small fragmented patches which lack many of the factors historically important for the structure and function of these grasslands (e.g. fire and grazing). However, the shallow, stony soils in the Flint Hills region of North America have escaped extensive disturbance associated with agriculture or urban development, and large tracts of native tallgrass prairie remain there. As a consequence, these soils are still inhabited by native North American earthworm taxa, including Diplocardia spp. and Bimastos spp. (James 1992). However, these ecosystems are not entirely undisturbed, nor are they immune to invasion by European earthworm species. Tallgrass prairie is dependant on some periodic disturbances such as fire or grazing (Knapp et al. 1998), and the suppression of these natural disturbances may influence the establishment of European earthworms.

Our objectives were 1) to assess the distribution of native and exotic earthworms relative to several land management practices, and 2) to determine the possible mechanisms driving the distribution patterns of native and exotic earthworms in these grasslands.

## **MATERIALS AND METHODS**

Earthworms were sampled from the Belowground Experimental Plots at the Konza Prairie Research Natural Area (KPRNA) a tallgrass prairie research site near Manhattan,

Kansas, USA. The vegetation of KPRNA is characteristic of native tallgrass prairie and is dominated by the warm season tallgrasses Andropogon gerardii, Sorghastrum nutans, and Panicum virgatum, which co-occur along with many forb and shrub species (Gibson et al. 1993). The Belowground Experimental Plots were established in 1986 as part of the Long-Term Ecological Research program at KPRNA. A series of 64 12m x 12m plots were established on an Irwin silty clay loam (fine, mixed mesic, Pachic argiustoll) in a split strip-plot experimental design aimed at testing the effects of fire (annually burned vs. unburned), aboveground biomass removal (mowed vs. unmowed), and differing nutrient inputs (N fertilization, P fertilization, N and P fertilization, and no fertilization) on belowground processes and organisms (see Todd 1996, for details of the experimental design). Only plots receiving both nutrients (10.0g N m<sup>-2</sup> and 1.0 g P m<sup>-2</sup>) and plots receiving no nutrient additions were used in this study.

Earthworms were sampled in October 1994 as part of the ongoing data collection in the Belowground Experimental Plots (a 20cm x 50cm x 20cm deep soil monolith from each plot was passed through a 4 mm sieve in the field, and earthworms were collected and preserved in 70% ethanol). A second set of samples was collected in April 1997 to assess treatment effects on earthworm community structure during the spring when earthworms are more abundant (James 1992) (a 25cm x 25cm x 25cm deep soil monolith). Soils were stored at 4°C until sorted by hand in the laboratory. Earthworms in these soils were killed in 70% ethanol, and preserved in 5% formalin. Earthworms were identified to genus (James 1990; Schwert 1990), and abundance data, as well as proportion of total

community composed of native taxa, were analyzed using an ANOVA designed for the analysis of split strip-plot experiments (Todd 1996).

In order to assess potential relationships between earthworm community changes and vegetational changes associated with different management practices, plant data from KPRNA data archives for the Belowground Experimental Plots were used. These data included 1) grass biomass, 2) forb biomass, 3) previous years' surface litter, 4) current year's surface litter, and 5) total surface litter collected from two replicate 0.1 m<sup>2</sup> quadrats in each plot. Four year averages (1993-1996) for each of these vegetation parameters were regressed against the proportion of total earthworm community composed of exotic taxa. All data were log transformed to satisfy normality assumptions.

## RESULTS

Results of the 1994 sampling showed that the abundance (no. per m<sup>2</sup>) of Diplocardia spp. was not significantly affected by any land management treatment (Fig. 1a). However, the abundance of Aporrectodea spp. in 1994 was significantly ( $p=0.03$ ) higher in unburned plots than in burned plots (Fig. 1a).

In 1994, there was a significant interaction ( $p=0.07$ ) between burning and fertilizer treatments on the proportion of the total earthworm community consisting of Diplocardia spp. Inspection of the data revealed that in treatment combinations where fertilization seems to have had an effect, that effect was to decrease the proportion of Diplocardia spp. (Fig. 2).

Results of the 1997 sampling showed trends among treatments which were similar to those of the 1994 sampling (Fig. 1b), including a greater abundance of Aporrectodea spp. in unburned plots ( $p=0.07$ ).

In contrast to the 1994 sampling, the proportion of the earthworm community that consisted of Diplocardia spp. in 1997 was significantly influenced by mowing ( $p=0.08$ ) as well as fertilizer ( $p=0.09$ ), but with no significant interaction between burning and fertilization. The effect of mowing was to increase the mean proportion of Diplocardia spp. in the earthworm community from 0.45 in unmowed plots to 0.66 in mowed plots (Fig. 2), while the effect of fertilizer was to decrease the mean proportion of Diplocardia spp. from 0.67 in control plots to 0.44 in the fertilized plots (Fig. 2).

Regression analysis of relationships between proportion of earthworm community consisting of Aporrectodea spp. and vegetation parameters revealed that only total surface litter mass was significantly related to earthworm community composition. The four year average (1993-1996) of total litter mass in experimental plots was significantly positively related to the proportional abundance of Aporrectodea spp. in 1994 as well as in 1997 ( $p=0.07$  and  $p=0.02$ , respectively)(Fig. 3).

## DISCUSSION

The results of this study suggest that the distribution and abundance of invasive European earthworms in North American tallgrass prairie soils can be attributed, in part, to land management practices. Furthermore, in areas where exotic earthworms are most prevalent, it appears that displacement of native species may be occurring. Particularly

notable are the differences in earthworm community structure between what we consider to be the two extreme land management treatments -annually burned, mowed, without fertilizer, and unburned, unmowed, with supplemental nutrients (BMC vs. UNF on Fig. 1). In 1997, the earthworm communities were most dissimilar in these two extreme treatments, with native earthworm species dominant in the BMC plots and exotic earthworms most dominant in UNF plots (Fig. 1). The plots with the most intense “natural” disturbance regime (burned, mowed, with no added nutrients) also most closely approximate conditions prevalent prior to European settlement (frequently burned and grazed). These plots also most closely represent floristic and edaphic conditions of tallgrass prairie (Collins et al. 1998; Knapp & Seastedt 1986), and our study demonstrates that this is true of the earthworm community in these plots as well. Previous studies of earthworm distributions in tallgrass prairie with respect to fire treatments have shown the abundance of *Diplocardia* spp. to be greater in annually burned prairie relative to prairie from which fire had been excluded for eight years (James 1982). Results of the current study differ somewhat from James (1982), as we found no effect of burning on the absolute abundance of *Diplocardia* spp., although the relative abundance of *Diplocardia* spp. was favored by burning (Fig. 2). One potential explanation for the discrepancy between the two studies is the virtual absence of exotic taxa in the soils sampled by James (he reports only a single individual of *Aporrectodea* spp.), and therefore the absence of any effects on distributions arising from interactions between the two taxa.

One effect of suppressing fire and grazing disturbances in tallgrass prairie is the accumulation of plant litter on the soil surface, and the land management treatments

utilized in the belowground plot experiment at KPRNA result in continuum of surface litter accumulation. Indirect effects of this litter accumulation include increased soil moisture and decreased soil temperature, particularly in the early growing season. We suggest that microclimatic consequences of differing land management practices may contribute to changes in earthworm communities in tallgrass prairie.

Fire also influences the amount and quality of plant inputs belowground, which could affect the distribution of earthworms. Both the biomass and C:N ratio of live roots is greater in annually burned plots compared to burned plots (Blair 1997; Ojima et al. 1994). The lower quality plant tissue could be a factor limiting the expansion of exotic earthworms in tallgrass prairie. However, to date, no experiments have separated the effects of changes in soil climate, belowground plant production, and belowground resource quality associated with fire or mowing with regard to responses of the earthworm community in tallgrass prairie.

In spite of the correlative nature of this study, and the difficulty in identifying the specific mechanisms underlying invasion by exotic earthworms, our study demonstrates that relatively short term departures from natural disturbance regimes can result in detectable changes in the abundances of native and exotic earthworm taxa in these soils. An understanding of the relationship between land management and the distribution of native and exotic earthworms may help to predict changes in biogeochemistry of soils as changes in land cover and land use continue in the Flint Hills region.

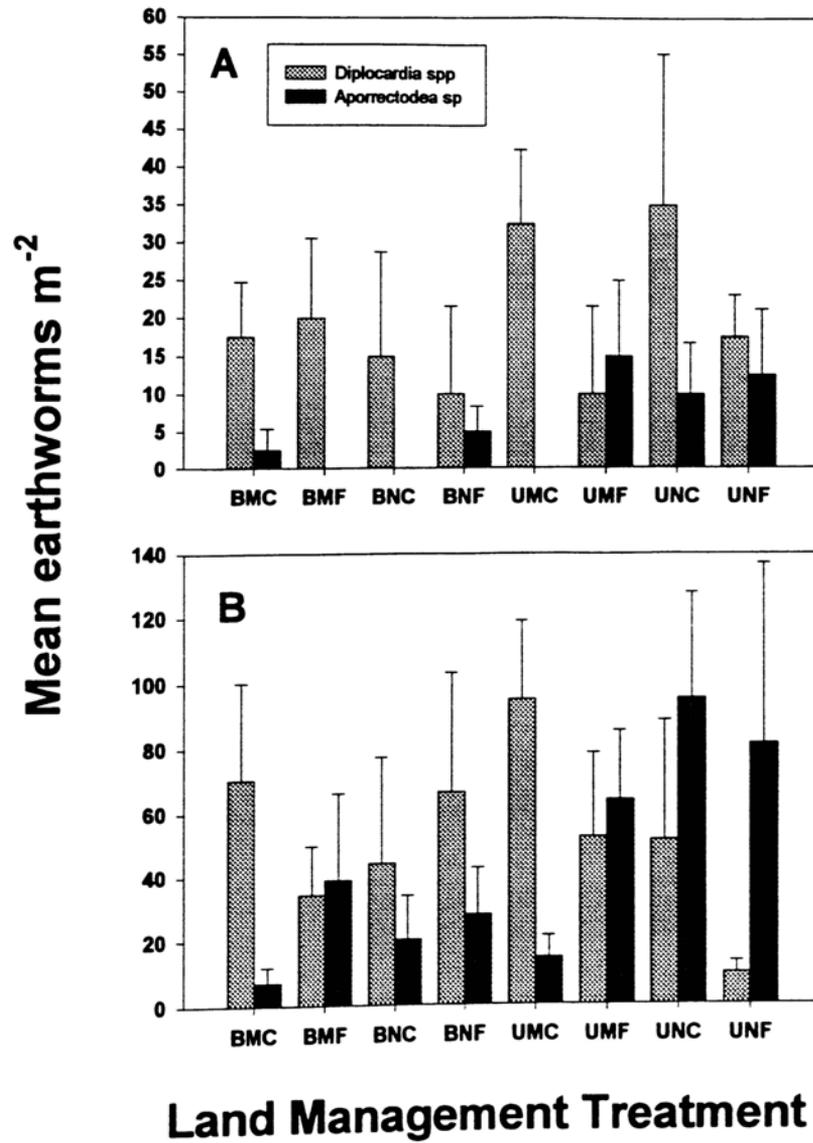
## LITERATURE CITED

- Burtelow, A.E., Bohlen, P.J., and Groffman, P.M. (1998) Influence of exotic earthworm invasion on soil organic matter, microbial biomass and denitrification potential in forest soils of the northeastern United States. *Applied Soil Ecology* 9:201-206.
- Blair, J.M. (1997) Fire, N availability, and plant response in grasslands: A test of the transient maxima hypothesis. *Ecology* 78:2359-2368.
- Collins, S.L., Knapp, A.K., Briggs, J.M., Blair, J.M., and Steinauer, E.M. (1998) Modulation of diversity by grazing and mowing in native tallgrass prairie. *Science* 280:745-747.
- Gibson, D.J., Seastedt, T.R., and Briggs, J.M. (1993) Management practices in tallgrass prairie: large- and small-scale experimental effects on species composition. *Journal of Applied Ecology* 30:247-255.
- James, S.W. (1982) Effects of fire and soil type on earthworm populations in a tallgrass prairie. *Pedobiologia* 24:37-40.
- James, S.W. (1990) Oligochaeta: Megascolecidae and other earthworms from Southern and Midwestern North America. In: Dindal, D.L. (ed) *Soil Biology Guide*. Wiley Interscience, New York.
- James, S.W. (1991) Soil, nitrogen, phosphorus, and organic matter processing by earthworms in tallgrass prairie. *Ecology* 72:2101-2109.
- James, S.W. (1992) Seasonal and experimental variation in population structure of earthworms in tallgrass prairie. *Soil Biology and Biochemistry* 24:1445-1449.
- Kalisz, P.J. and Dotson, D.B. (1989) Land-use history and the occurrence of exotic

- earthworms in the mountains of eastern Kentucky. *American Midland Naturalist* 122:288-297.
- Knapp, A.K., Briggs, J.M., Hartnett, D.C., and Collins, S.L. (1998) *Grassland Dynamics: Longterm Ecological Research in Tallgrass Prairie*. Oxford University Press, New York.
- Knapp, A.K. and Seastedt, T.R. (1986) Detritus accumulation limits productivity of tallgrass prairie. *BioScience* 36:662-668.
- Reynolds, J.W. (1994) The distribution of earthworms in Indiana: a case for the Post Quaternary Introduction Theory for megadrile migration in North America. *Megadrilogica* 5:13-32.
- Reynolds, J.W., Clebsch, E.E.C., and Reynolds, W.M. (1970) The earthworms of Tennessee (Oligochaeta). I. Lumbricidae. *Bulletin of the Tall Timbers Research Station* 17:1-133.
- Samson, F. and Knopf, F. (1994) Prairie conservation in North America. *BioScience* 44:418-421.
- Scheu, S. and Parkinson, D. (1994) Effects of invasion of an aspen forest (Canada) by *Dendrobaena octaedra* (Lumbricidae) on plant growth. *Ecology* 75:2348-2361.
- Schwert, D.P. (1990) Oligochaeta: Lumbricidae. In: Dindal, D.L. (ed) *Soil Biology Guide*. Wiley Interscience, New York.
- Smith, F. (1928) An account of changes in the earthworm fauna of Illinois and a description of one new species. *Bulletin of the Illinois Natural History Survey* 17:347-362.

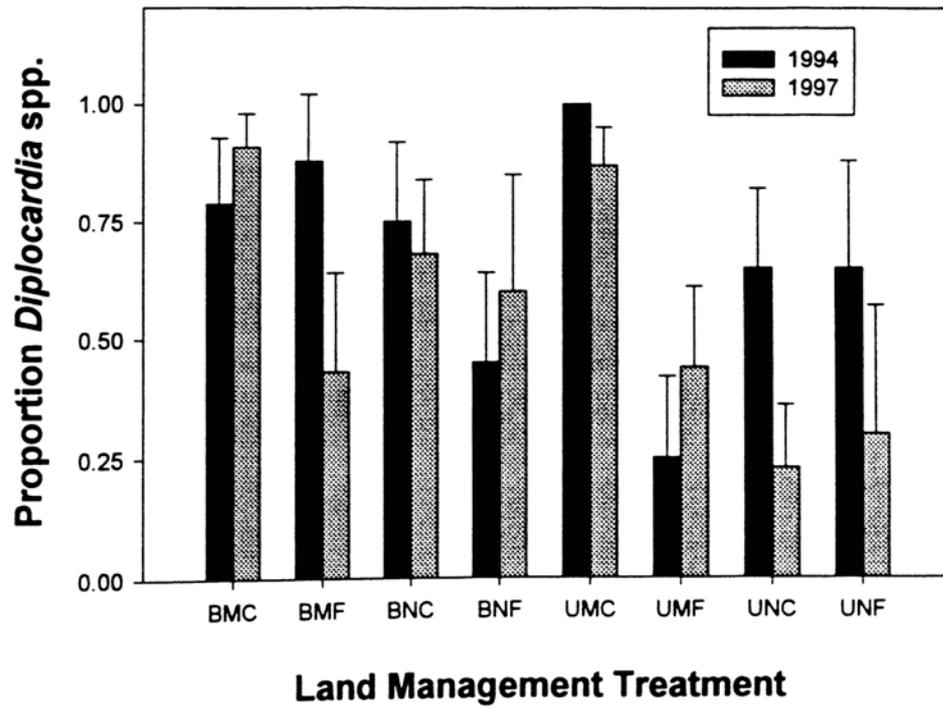
Stebbing, J.H. (1962) Endemic-exotic earthworm competition in the American midwest. *Nature* 196:905-906.

Todd, T.C. (1996) Effects of management practices on nematode community structure in tallgrass prairie. *Applied Soil Ecology* 3:235-246.



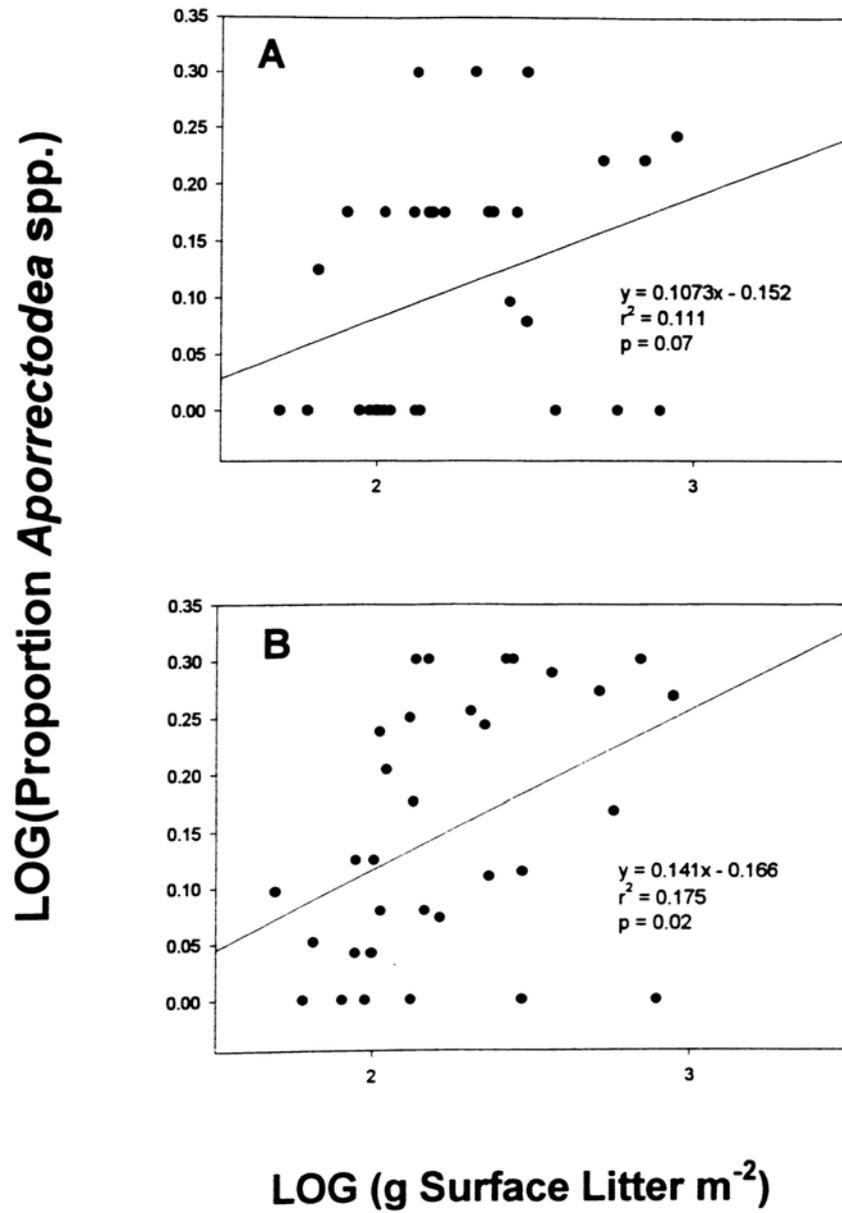
**Figure 1.** Abundances of native and exotic earthworms relative to land management treatments in A) October 1994 and B) April 1997. Note differences between Y-axes.

Note: B = burned annually, U = unburned, M = mowed annually, N = not mowed, C = unfertilized, F = nitrogen and phosphorus fertilizer added annually.



**Figure 2.** Proportion of earthworm community composed of *Diplocardia* spp. relative to land management treatments.

Note: X-axis labels as in Figure 2.



**Figure 3.** Relationship between surface litter accumulation, and proportion of *Aporrectodea* spp. occurring in belowground plots during A) 1994 sampling and B) 1997 sampling.

**CHAPTER 2:**

**NATIVE NORTH AMERICAN AND INTRODUCED EUROPEAN  
EARTHWORMS IN TALLGRASS PRAIRIE: BEHAVIORAL PATTERNS AND  
INFLUENCES ON PLANT GROWTH**

Mac A. Callaham, Jr.<sup>1</sup>, John M. Blair<sup>1</sup>, and Paul F. Hendrix<sup>2</sup>

<sup>1</sup>Division of Biology, Kansas State University, Manhattan KS 66502

<sup>2</sup>Institute of Ecology, University of Georgia, Athens GA 30605

## ABSTRACT

The Flint Hills region of eastern Kansas is the largest contiguous area of native tallgrass prairie in North America. A consequence of this area remaining in native vegetation has been the maintenance of a relatively undisturbed assemblage of soil fauna, including native earthworms. However, this area is subject to encroachment by European earthworm species, and differences between native and exotic earthworm species, with respect to soil function, are largely unknown. We conducted a field study to address questions regarding differences in behavior between *Diplocardia* spp. (native earthworms), and *Octolasion tyrtaeum* (an exotic species), as well as their relative influences on soil microbial biomass and plant uptake of nitrogen. The study was performed by manipulating the earthworm fauna inside PVC-encased soil cores (20 cm dia.) incubated under field conditions. Earthworm treatments included 1) a control with no earthworms present 2) native earthworms only, and 3) exotic earthworms only. Prior to the addition of native or exotic earthworms, seedlings of *Andropogon gerardii* (a native, dominant tallgrass) were established in each of the experimental cores, and a dilute solution containing  $^{13}\text{C}$  labeled glucose and  $^{15}\text{N}$  labeled ammonium sulfate was added to the soil as a tracer to facilitate examination of earthworm / microbe / plant interactions. Results indicated that during the study period (14 June - 27 July), native earthworms were significantly more active than exotic worms and quickly became labeled with  $^{13}\text{C}$  and  $^{15}\text{N}$ . Native earthworms also occurred at shallower depths than exotics throughout the study. Surprisingly, the increased activity by native earthworms did not result in increased plant productivity, but instead appeared to decrease the amount of N acquired by seedlings grown with native earthworms present. There were few

consistent effects of earthworms on microbial biomass C or N, but one significant result occurred on the final sample date (day 45) when exotic earthworms were found to be at shallower depths than previously, and there was a concomitant decrease in microbial biomass C in that soil depth. Microbial C/N also was affected by presence of native earthworms with a fairly consistent increase in this ratio in the presence of native worms. Taken together, the results of our study indicate that the activity of native earthworms may actually enhance competition for the limiting nutrient (N) between microbes and plants during the growing season in tallgrass prairie.

## INTRODUCTION

Earthworm assemblages across the North American continent are largely composed of introduced European species (Reynolds 1995). The establishment of these exotic species is usually preceded by some disturbance of the native soil ecosystem such as agricultural, or other development (e.g. Stebbings 1969, Kalisz and Dotson 1989, Dotson and Kalisz 1989). However, in some relatively undisturbed areas, native North American earthworms persist, and the ecology of these native earthworms has only recently begun to be studied (James 1990, 1995, Wood and James 1993, Callaham and Hendrix 1998, Winsome and McColl 1998). The Flint Hills physiographic region is a large (5 million ha) area of relatively undisturbed soils in eastern Kansas. Soils in this region have escaped agricultural disturbances because of their relatively steep topography and stoniness. One consequence of this lack of extensive soil disturbance in the Flint Hills is the persistence of native North American earthworm taxa (primarily a group of several species in the megascolecid genus *Diplocardia* Garman, and a lumbricid species

*Bimastos welchii* Smith). Nevertheless, several European lumbricid earthworm taxa are currently expanding their distribution in Flint Hills soils (e.g. *Lumbricus* spp., *Aporrectodea* spp. and *Octolasion* spp.), but the potential impacts of these introduced species on soil function and/or native earthworm populations is unknown.

Although there is a paucity of explicit evidence, it is unlikely that native and exotic earthworm taxa behave similarly with respect to nutrient cycling in tallgrass prairie soils (James and Cunningham 1989, James and Seastedt 1995). A great majority of the research performed in North America examining earthworm effects on nutrient cycling has been in agricultural systems (Blair et al. 1995, Parmelee et al. 1998, Hendrix 1998). A necessary consequence of the agricultural focus has been a coincidental focus on European earthworm species, and the influences of these exotic taxa on nutrient cycling and other soil processes are relatively well characterized (e.g. Doube and Brown 1998, Edwards and Bohlen 1996, Blair et al. 1997). Earthworm effects on nutrient cycling are usually manifested as changes in soil microbial pools and processes brought about by earthworm feeding and burrowing activities (e.g. Brown 1995, Lavelle et al. 1998, 1992). It is therefore reasonable to predict differences between native and exotic earthworm taxa and their respective influences on nutrient cycling processes because of observed (empirical and anecdotal) differences in their feeding, burrowing and casting activities as well as differences in the seasonality of their activities in tallgrass prairie soils (Callaham and Blair 1999, James 1992, James and Cunningham 1989). Thus, our objectives for this study were 1) to assess differences in the behavior and activity patterns of native and exotic earthworms; 2) to examine the influences of native and exotic earthworms on soil microbial biomass C and N; and 3) to examine the influences of native and exotic

earthworms on plant uptake of nitrogen and C and N transformations using stable isotopes.

## MATERIALS AND METHODS

### *Site description*

This study was conducted during early summer in 1998 at the Konza Prairie Biological Station (KPBS) approximately 15 km south of the Manhattan campus of Kansas State University (KSU). KPBS is a 3,487 ha tallgrass prairie preserve in the Flint Hills region of northeastern Kansas owned by the Nature Conservancy and operated by the Division of Biology at KSU. The climate at the site is continental with average annual precipitation of 835 mm, and approximate mean temperatures of 27°C in July and -3°C in January. This study was conducted on a footslope in the Kings Creek drainage basin, and soils at the site are characterized as Tully silty clay loams (fine, mixed, mesic Pachic Argiustolls) (Ransom 1998). Vegetation at the site was characteristic of lowland tallgrass prairie (Freeman 1998) and was dominated by the perennial warm-season grasses, Big Bluestem (*Andropogon gerardii*) and Indiangrass (*Sorghastrum nutans*). The study site had been burned annually or semi-annually for 12 years prior to 1998.

### *Field Methods*

Soil cores (20.3 cm in diameter and 25 cm deep) were taken from the field by pushing 30 cm sections of PVC pipe into the soil with a backhoe. These soil cores were removed from the site and frozen at -10°C for 15 d to kill any earthworms that were present in the soil at the time of coring. After freezing, the bottoms of the cores were covered with mesh (0.5 mm) to prevent immigration and emigration of earthworms from

experimental cores, and the cores were replaced in the field. The top 2 cm of soil in each core was shaved off to remove stolons and rhizomes of grasses present in the cores at the time of initial collection in order to remove competition for the experimental seedlings planted in the cores. We utilized new seedlings in this study because we expected plants already present in the cores to utilize stored N as a primary nutrient source during the growing season. Indeed, previous studies in tallgrass prairie have experienced difficulty in demonstrating plant N uptake from inorganic soil pools in the short term (Dell 1998).

Soil in cores was labeled with tracer amounts of  $^{13}\text{C}$  and  $^{15}\text{N}$ . Tracer solution was prepared by dissolving 6.2280 g of 99.9%  $^{13}\text{C}$  enriched glucose ( $\text{C}_6\text{H}_{12}\text{O}_6$ ) and 5.0636 g of 99.9%  $^{15}\text{N}$  enriched ammonium sulfate ( $[\text{NH}_4]_2\text{SO}_4$ ) into 30.0 L of deionized water. The tracer solution was applied to the cores by injection with a syringe. The syringe was inserted to a 10 cm depth into the soil, filled with 50 mL of solution, and injections were made at intervals as the needle of the syringe was incrementally removed from the soil, with 5.0 mL injections at 10, 8, and 6 cm depths and 10.0 mL injections at 4 and 2 cm depths and the remaining 15.0 mL sprayed directly onto the surface of soil in the core. Ten such injections (totaling 500mL of solution) were made per core according to a pattern on a template designed for uniform application of tracer solution throughout the core. Total quantities of  $^{15}\text{N}$  and  $^{13}\text{C}$  applied to cores were 43.46 mg  $^{13}\text{C}$  core $^{-1}$  and 18.9 mg  $^{15}\text{N}$  core $^{-1}$ . Following application of tracer solution, seedlings of *Andropogon gerardii* were planted in each core and earthworm treatments (native worms only, exotic worms only, and control) were applied on 14 June 1998. Earthworms were applied at densities (7 individuals of native or exotic species per core, equivalent to ~200 individuals m $^{-2}$ ) that approximated maximum field densities previously observed at KPBS (Callaham and

Blair 1999). All earthworms used in this study were collected during the two weeks prior to application by digging and hand-sorting from soils nearby (< 30 m) the location of experimental cores. Important dates and climate data for the study are shown in Figure 1.

### *Laboratory Methods*

Five cores of each treatment were randomly selected and destructively sampled on each of four sampling dates following earthworm application. Cores were removed after 7, 15, 30, and 45 days of earthworm treatment (see Fig. 1). Soil in each core was cut into three depths (0-5 cm, 5-10 cm and 10-20 cm). Soils were passed through a 4-mm sieve, and coarse organic matter (including live and dead roots) was removed prior to all other analyses. Soil microbial biomass C was determined for each depth of each core by a chloroform fumigation/incubation method (Brookes et al. 1985), and soil microbial biomass N was determined by chloroform fumigation-direct extraction followed by the alkaline persulfate digestion procedure of Cabrera and Beare (1993). Microbial biomass  $\delta^{13}\text{C}$  was determined by collection of  $\text{CO}_2$  from fumigated and non-fumigated incubations followed by direct injection into a continuous flow isotope ratio mass spectrometer (CFIRMS) (Europa Tracermass 20/20, Italy). The  $\delta^{13}\text{C}$  of microbial biomass was calculated as follows:

$$\delta^{13}\text{C}_{\text{MB}} = \frac{(\delta^{13}\text{C}_{\text{F}} * \text{C}_{\text{F}}) - (\delta^{13}\text{C}_{\text{NF}} * \text{C}_{\text{NF}})}{\text{C}_{\text{F}} - \text{C}_{\text{NF}}}$$

where  $\delta^{13}\text{C}_{\text{F}}$  and  $\delta^{13}\text{C}_{\text{NF}}$  were  $\delta^{13}\text{C}$  measurements of  $\text{CO}_2$  from fumigated and non-fumigated soils, and  $\text{C}_{\text{F}}$  and  $\text{C}_{\text{NF}}$  were concentrations of  $\text{CO}_2$  released from fumigated and non-fumigated soils, respectively.

Earthworms were removed from soils during sieving and kept refrigerated (4°C) until they could be processed. Earthworms were killed by immersion in boiling water for <1 s, and cut open lengthwise so that gut contents could be washed away from body tissues (as in Hendrix et al. 1999). Following gut washing, the earthworm tissues were freeze-dried for >72 h and ground with mortar and pestle. Tissues of all individual earthworms from a given core were pooled and thoroughly mixed, and sub-samples were analyzed for  $^{13}\text{C}$  and  $^{15}\text{N}$  content by CFIRMS (Europa 20/20 Tracermass).

Plants were sampled on the second and fourth sampling dates. The exclusion of the first date was due to inadequate quantities of plant tissue (i.e. the plants had not grown enough to warrant sampling). The lack of plant analysis from date three was a consequence of unanticipated grasshopper herbivory on experimental seedlings. Herbivory resulted in a total loss of seedlings from several cores following the second sample date – a situation that caused us to select plant-free cores for sampling on date three to allow for a full complement of plant sampling on date four. Thus, on the second and fourth sample dates, aboveground components of *A. gerardii* plants were collected from each core, dried at 65°C, weighed, and ground with mortar and pestle. Aboveground plant tissues were subsampled and analyzed for C and N content by combustion (Carlo Erba C/N analyzer), and for  $^{13}\text{C}$  and  $^{15}\text{N}$  content by CFIRMS.

### *Statistical Analyses*

All data were subjected to three way analyses of variance (PROC GLM, SAS Institute, Cary, NC) with date, soil depth and earthworm treatments as main effects variables. When necessary, data were log-transformed to satisfy normality assumptions.

The means separation procedure used to determine differences in treatment means was the Least Squares Means (LSMEANS/pdiff option, SAS Institute, Cary, NC).

## RESULTS

### *Earthworms*

Stable isotope analysis of earthworm tissues showed that native earthworms became rapidly enriched in  $^{15}\text{N}$  and  $^{13}\text{C}$  (Fig. 2). The enrichment of native earthworms was significantly greater ( $p < 0.01$ ) than that of exotic earthworms on all sample dates. Nevertheless, exotic worms did become enriched with the stable isotope tracers, and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of exotic worms on the fourth sampling date were significantly greater than for the first three dates (Fig. 2). Native earthworms became enriched with  $^{13}\text{C}$  and  $^{15}\text{N}$  at similar rates. For native earthworms, mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values on the first two dates did not differ significantly from one another, but by the third sample date they were significantly more enriched than on the first date. Values for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for native earthworms on the last two dates did not differ significantly from one another (Fig. 2).

Observations of earthworm distribution also indicated differences in the behavior of native and exotic earthworms. These data were collected by simply making note of the soil depth at which earthworms were collected on each sample date. Native earthworms were far more likely to be collected from the top 10 cm, and significantly ( $p < 0.05$ ) larger proportions of all native worms were collected from the top two depths (Table 1). In contrast, the likelihood of collecting exotic worms from the 10-20 cm soil depth was significantly ( $p < 0.001$ ) higher (approximately 8 times higher) than the likelihood of collecting these worms from the top two depths (Table 1).

### *Microbial Biomass*

There were few consistent differences in the sizes of microbial pools of C and N with respect to earthworm treatments. However, differences in microbial biomass C (MBC) did occur on the first date in the 0-5 cm depth, with significantly greater levels of MBC in earthworm cores than in the control cores (Table 2). On the final date, there were again differences in MBC at the 0-5 cm depth with significantly greater MBC in control cores than in exotic earthworm cores (Table 2). MBC values from native earthworm cores were intermediate (Table 2). Additionally, there were differences in MBC at the 5-10 cm depth on the fourth date. At this depth, control cores had significantly less MBC than did either native or exotic earthworm cores (Table 2).

There were also few differences in microbial biomass N (MBN) in experimental cores during the study. There were two exceptions to this general trend. On the second sampling date in the 10-20 cm soil depth, control cores had significantly higher levels of MBN than native earthworm cores, with exotic earthworm cores being intermediate (Table 2); and on the last sample date, control cores had significantly less MBN than exotic earthworm cores, with native earthworm cores intermediate (Table 2).

Differences in carbon to nitrogen ratios (C/N) of microbial biomass were typically reflective of significant differences in MBC or MBN. For example, C/N was significantly lower in control cores than in earthworm treatments for the 0-5 cm depth on the first date, and this difference is the result of lower MBC values on that date (Table 2). A single exception to this general relationship occurred in the 0-5 cm depth on the third sample date when there were significant differences detected in C/N but none for MBC or

MBN. In this instance, C/N was significantly higher in native earthworm cores than in exotic earthworm cores, and control cores were intermediate (Table 2).

Microbial biomass  $\delta^{13}\text{C}$  values showed very little response to earthworm treatments during the study. The single statistically significant difference in  $\delta^{13}\text{C}$  of the microbial biomass occurred on the second sample date at the 5-10 cm soil depth, when  $\delta^{13}\text{C}$  values for the microbial biomass were significantly ( $p < 0.1$ ) lower in exotic earthworm cores than in control cores (Table 3). However, there were several instances where the total standing stock of  $^{13}\text{C}$  in the microbial biomass was influenced by experimental treatments. Specifically, on the third date in the 0-5 cm soil depth, microbial biomass in native earthworm cores contained significantly ( $p < 0.07$ ) more total  $^{13}\text{C}$  than did exotic earthworm cores, and again on the fourth date (0-5 cm depth), the total standing stock of microbial  $^{13}\text{C}$  was significantly ( $p < 0.02$ ) lower in exotic cores than in controls (Table 3). Furthermore, at the 5-10 cm depth on the fourth date, total standing stock of microbial  $^{13}\text{C}$  was smaller ( $p < 0.02$ ) in control cores than in either native worm cores or exotic worm cores (Table 3).

### *Plants*

Several plant parameters showed statistically significant differences with respect to experimental treatments in this study. Perhaps most notable was the difference in total plant growth by the final date, with significantly less total aboveground biomass in plants grown in cores with native earthworms relative to the other two treatments (Table 4). Nitrogen content of plant tissues was also affected by earthworm treatments early in the study, with plants grown in cores containing exotic earthworms having significantly

higher concentrations of N than those in control cores ( $p < 0.10$ ) on the second sample date. This difference in plant tissue N concentration was not detected on the fourth date. However, although there were no differences in N concentration of plant tissue (%N), there were differences in total standing stock of N in aboveground plant tissues that were attributable to the above-mentioned decreased plant biomass production in native earthworm treatments on the final date (Table 4).

Results of stable isotope analysis on plant tissues revealed further effects of earthworm treatments. On the first date, the atom percent  $^{15}\text{N}$  (at%) of plant tissues was highest in control cores, lowest in native earthworm cores and intermediate in exotic earthworm cores, with significant ( $p < 0.05$ ) differences between means for native and control cores (Table 4). On the fourth date, the trend of higher at%  $^{15}\text{N}$  in control plants persisted but was no longer statistically significant. Standing stocks of  $^{15}\text{N}$  in aboveground plant tissues closely paralleled patterns observed for standing stocks of total N with significant differences only on the fourth date (Table 4). Again, these differences were largely the result of differences in total plant biomass.

## DISCUSSION

### *Earthworm Activities*

Stable isotope analyses revealed that the native earthworms assimilated more  $^{13}\text{C}$  and  $^{15}\text{N}$  than did the exotic worms over the course of the experiment (Fig. 2). This result suggests that the native earthworms may have been more active than exotic worms during the summer growing period examined in this experiment. The notion that exotic worms may have been largely dormant over the course of the experiment is corroborated by

observations made on sampling dates when exotic earthworms were frequently found at the very bottom of cores upon collection. Thus, one possible explanation for lack of label uptake by exotic worms is simply the fact that they spent most of the experiment in the lower 10 cm of cores (see Table 1) whereas only the top 10 cm of the cores was directly labeled. Nevertheless, it is also clear that the soil microbial biomass was labeled in the 10-20 cm depth (albeit to a lesser degree than shallower depths), and this label should have been incorporated into earthworm tissues over time had the exotic worms been actively feeding. In fact, enrichment of exotic earthworm tissues with  $^{13}\text{C}$  and  $^{15}\text{N}$  was unchanged over the first 30 d of the experiment (Fig. 2), and we considered this to be further evidence that these earthworms were inactive at this time. At any rate, whether exotic earthworms were dormant or simply feeding at deeper depths, these differences in activity patterns (relative to native earthworms) may be important with respect to their influences on plant nutrient uptake.

#### *Earthworm Effects on Microbial Biomass*

Responses of microbial biomass to earthworm activity are variable and are thought to be related to organic matter content of the soil examined, as well as to the species of earthworm studied (Blair et al. 1995, Wolters and Joergensen 1992). For example, soils with relatively high organic matter content consistently exhibit decreased amounts of microbial biomass in the fecal material of earthworms relative to bulk soil (e.g. Scheu 1987); whereas in soils with lower organic matter content the fecal material of earthworms typically has larger amounts of microbial biomass than bulk soil (Lavelle et al. 1992). Thus, it is likely that the overall effect of earthworms on bulk soil microbial

biomass is dependent upon the extent to which they feed, and the proportion of the total soil volume they process. In our study, effects of earthworms on microbial biomass were dependent upon the amount of time that earthworms had been in the soil and the depth at which they were active. On the first date, earthworms appeared to have a stimulatory effect on microbial biomass C in the 0-5 cm depth (Table 2), but this stimulation of MBC was transient and may have been the result of short term availability of labile carbon sources such as mucous or other metabolic products of earthworms. However, by the fourth date microbial biomass in the 0-5 depth was decreased in cores with exotic earthworms relative to control cores (Table 2). This decrease in MBC may be attributable to feeding of exotic worms at shallower soil depths on and/or near that date, and indeed exotic worms were encountered at this depth in larger proportion than on any other date (Table 1). We suggest that exotic earthworms broke dormancy at the end of the experiment as a result of cooler temperatures along with two large rainfall events (>50 mm) just before that date (Fig. 1), and associated high soil moisture at the end of the experiment. It is also interesting to note that on the date exotic earthworms broke dormancy, and were collected in significant numbers away from the 10-20 cm depth, they coincidentally became enriched in  $^{13}\text{C}$  from the added label (Fig. 2).

Although effects of earthworms on the size of microbial C and N pools were variable, another index of the microbial community (C/N ratio) was more consistent. On dates when microbial biomass C/N was affected by earthworms it was usually manifested as an increase in that ratio in the presence of native earthworms (Table 2). This may indicate that native earthworms were assimilating N that would otherwise be available to

microbes or plants, effectively increasing C/N of total microbial biomass and possibly decreasing total uptake of N by plants (see below).

### *Earthworm Influence on Plants*

Results from plant growth and plant tissue analyses were surprising in that native earthworms appeared to have a negative influence on the total uptake of nitrogen by *A. gerardii* plants, whereas exotic earthworms had no effect on N uptake compared to controls. This difference in N uptake was primarily due to differences in total plant growth, as plants in the native earthworm cores were significantly smaller than those in control or exotic earthworm cores. Thus, the activity of native earthworms appeared to result in an overall decrease in the availability of N to *A. gerardii*. This result is somewhat counter to previous findings with respect to native earthworm influence on native vegetation. In the only other study comparing native and exotic earthworms in tallgrass prairie, James and Seastedt (1986) found, in a microcosm study, that presence of *Diplocardia* spp. increased root growth of *A. gerardii* relative to plants grown in the presence of the exotic worm *Aporrectodea turgida*. However, that study utilized plants started from rhizomes and not seedlings as we used in our study, and this may explain the disparity of results. We were, in fact, unable to separate living roots from dead roots in the soil during sampling because the amount of living root material was small, and thus we could not evaluate the influence of native and exotic earthworms on root biomass.

The mechanism behind the decreased N availability observed in our study is unclear, but may be associated with immobilization of N in dead roots, as the procedure used to establish experimental cores (freezing of cores and removal of stolons and

rhizomes) certainly resulted in the input of unusually large amounts of dead root material. One potential explanation for the decreased availability of N in native earthworm cores is that native earthworm activity stimulated the immobilization of N in dead roots- an effect not seen in exotic earthworm cores or control cores. Nitrogen is among the most important limiting resources to plant growth in tallgrass prairie (Knapp et al. 1998, Blair 1997), and particularly so in annually burned prairie in years of abundant rainfall (such as the summer of 1998). In light of recent work demonstrating the existence of competition for N and other limiting nutrients between plants and microbes (e.g. Kaye and Hart 1997), it seems appropriate to list a potential influence on this interaction in tallgrass prairie: certain members of the soil fauna. In our study, native earthworms were active and assimilating N throughout the growing season, and this assimilation of N may have been at the expense of the plants grown in native earthworm cores.

## CONCLUSIONS

Earthworms had little impact on soil microbial biomass C or N, but when there were differences earthworms increased MBC early in the study, and had variable effects late in the study depending on soil depth (decreased MBC at shallow depths, increased MBC at deeper depths). Native earthworms had significantly more  $^{15}\text{N}$  and  $^{13}\text{C}$  label in their tissues than exotic worms, indicating greater feeding activity by natives during the experiment. Plants grown in cores with native earthworms were significantly smaller, and took up significantly less total N, than did control plants or plants grown in exotic earthworm cores. This decreased N availability to plants may have been the result of increased competition for N between plants and microbes that was stimulated by the activity of earthworms.

## ACKNOWLEDGEMENTS

This work was funded in part by a National Science Foundation Long-Term Ecological Research grant to Kansas State University and the Konza Prairie Biological Station. Lab and field assistance was provided by Duane Kitchen, Jim Larkins, Dennis Mossman, Jamie Nutt, Katie Page, and Andrea Silletti. Thanks are due to Mark Williams for stable isotope analysis.

## LITERATURE CITED

- Blair JM (1997) Fire, N availability, and plant responses in grasslands: a test of the transient maxima hypothesis. *Ecology* 78:2359-2368.
- Blair JM, Todd TC, Callaham MA Jr (2000) Responses of grassland soil invertebrates to natural and anthropogenic disturbances. In: Coleman DC, Hendrix PF (eds) *Invertebrates as Webmasters in Ecosystems*. CAB International Press, pp 43-71.
- Blair JM, Seastedt TR, Rice CW, Ramundo RA (1998) Terrestrial nutrient cycling in tallgrass prairie. In: Knapp AK, Briggs JM, Hartnett DC, Collins SC (eds) *Grassland Dynamics: Long-Term Ecological Research in Tallgrass Prairie*. Oxford University Press, New York, pp 222-243.
- Blair JM, Parmelee RW, Allen MF, McCartney DA, Stinner BR (1997) Changes in soil N pools in response to earthworm population manipulations in agroecosystems with different N sources. *Soil Biol Biochem* 29:361-367.
- Blair JM, Parmelee RW, Lavelle P (1995) Influences of earthworms on biogeochemistry. In: Hendrix, PF (ed) *Earthworm ecology and biogeography in North America*. Lewis Publishers, Boca Raton, pp 127-158.

- Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol Biochem* 17:837-842.
- Brown GG (1995). How do earthworms affect microfloral and faunal community diversity? *Plant Soil* 170:209-231.
- Cabrera ML, Beare MH (1993) Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. *Soil Sci Soc Am J* 57:1007-1012.
- Callaham MA Jr, Blair JM (1999) Influence of differing land management on the invasion of North American tallgrass prairie soils by European earthworms. *Pedobiologia* 43:507-512.
- Callaham MA Jr, Hendrix PF (1998) Impact of earthworms (Diplocardia: Megascolecidae) on cycling and uptake of nitrogen in coastal plain forest soils from northwest Florida, USA. *App Soil Ecol* 9:233-239.
- Dell CJ (1998) The impact of fire on nitrogen cycling in tallgrass prairie. PhD Thesis, Kansas State University, 152 pages.
- Dotson DB, Kalisz PJ (1989) Characteristics and ecological relationships of earthworm assemblages in undisturbed forest soils in the southern Appalachians of Kentucky, USA. *Pedobiologia* 33:211-220.
- Doube BM, Brown GG (1998) Life in a complex community: Functional interactions between earthworms, organic matter, microorganisms and plants. In: Edwards CA (ed) *Earthworm Ecology*. St. Lucie Press, Boca Raton, pp 179-211.
- Edwards CA, Bohlen PJ (1996) *Biology and Ecology of Earthworms* (3rd ed.). Chapman and Hall, London, 426 pages.

- Freeman CC (1998) The flora of Konza Prairie: A historical review and contemporary patterns. In: Knapp AK, Briggs JM, Hartnett DC, Collins SC (eds) *Grassland Dynamics: Long-Term Ecological Research in Tallgrass Prairie*. Oxford University Press, New York, pp 69-80.
- Hendrix PF (1998) Earthworms in agroecosystems: A summary of current research. . In: Edwards CA (ed) *Earthworm Ecology*. St. Lucie Press, Boca Raton, pp 103-122.
- Hendrix PF, Lachnicht SL, Callaham MA Jr, Zou X (1999) Stable isotopic studies of earthworm feeding ecology in tropical ecosystems of Puerto Rico. *Rapid Commun Mass Spectrom* 13:1295-1299.
- James SW (1995) Systematics, biogeography, and ecology of nearctic earthworms from Eastern, Central, Southern, and Southwestern USA. In: Hendrix, PF (ed) *Earthworm ecology and biogeography in North America*. Lewis Publishers, Boca Raton, pp 29-51.
- James SW (1992) Seasonal and experimental variation in population structure of earthworms in tallgrass prairie. *Soil Biol Biochem* 24:1445-1449.
- James SW (1990) Soil nitrogen, phosphorus and organic matter processing by earthworms in tallgrass prairie. *Ecology* 72:2101-2109.
- James SW, Seastedt TR (1986) Nitrogen mineralization by native and introduced earthworms: Effects on big bluestem growth. *Ecology*: 67:1094-1097.
- James SW, Cunningham MR (1989) Feeding ecology of some earthworms in Kansas tallgrass prairie. *Am Midl Nat* 121:78-83.
- Kalisz PJ, Dotson DB (1989) Land-use history and the occurrence of exotic earthworms in the mountains of eastern Kentucky. *Am Midl Nat* 122:288-297.

- Kaye JP, Hart SC (1997) Competition for nitrogen between plants and soil microorganisms. *Trends Ecol Evol* 12:139-143.
- Knapp AK, Briggs JM, Blair JM, Turner CL (1998) Patterns and controls of aboveground net primary productivity in tallgrass prairie. In: Knapp AK, Briggs JM, Hartnett DC, Collins SC (eds) *Grassland Dynamics: Long-Term Ecological Research in Tallgrass Prairie*. Oxford University Press, New York, pp 193-221.
- Lavelle P, Pahanasi B, Charpentier F, Gilot C, Rossi J, Derouard L, Andre J, Ponge J, Bernier N (1998) Large-scale effects of earthworms on soil organic matter and nutrient dynamics. In: Edwards CA (ed) *Earthworm Ecology*. St. Lucie Press, Boca Raton, pp 103-122.
- Lavelle P, Melendez G, Pashanasi B, Schaefer R (1992) Nitrogen mineralization and reorganization in casts of the geophagous tropical earthworm *Pontoscolex corethrurus* (Glossoscolecidae: Oligochaeta). *Biol Fertil Soils* 14:49-53.
- Martin A, Mariotti A, Balesdent J, Lavelle P (1992) Soil organic matter assimilation by a geophagous tropical earthworm based on  $\delta^{13}\text{C}$  measurements. *Ecology* 73:118-128.
- Parmelee RW, Bohlen PJ, Blair JM (1998) Earthworms and nutrient cycling processes: Integrating across the ecological hierarchy. In: Edwards CA (ed) *Earthworm Ecology*. St. Lucie Press, Boca Raton, pp 123-143.
- Ransom MD, Rice CW, Todd TC, Wehmueller WA (1998) Soils and soil biota. In: Knapp AK, Briggs JM, Hartnett DC, Collins SC (eds) *Grassland Dynamics: Long-Term Ecological Research in Tallgrass Prairie*. Oxford University Press, New York, pp 48-66.

- Reynolds JW (1995) Status of exotic earthworm systematics and biogeography in North America. In: Hendrix, PF (ed) Earthworm ecology and biogeography in North America. Lewis Publishers, Boca Raton, pp 1-28.
- Seastedt TR, James SW, Todd TC (1988) Interactions among soil invertebrates, microbes and plant growth in the tallgrass prairie. *Agric Ecosyst Environ* 24:219-228.
- Scheu S (1987) Microbial activity and nutrient dynamics in earthworm casts (Lumbricidae). *Biol Fertil Soils* 5:230-234.
- Stebbing JH (1969) Endemic-exotic earthworm competition in the American Midwest. *Nature* 196:905-906.
- Todd TC, James SW, Seastedt TR (1992) Soil invertebrate and plant responses to mowing and carbofuran application in a North American tallgrass prairie. *Plant Soil* 144:117-124.
- Wisome T, McColl JG (1998) Changes in chemistry and aggregation of a California forest soil worked by the earthworm *Argilophilus papillifer* Eisen (Megascolecidae). *Soil Biol Biochem* 30:1667-1677
- Wolters V, Joergensen RG (1992) Microbial turnover in beech forest soils worked by *Aporrectodea caliginosa* (Savigny)(Oligochaeta: Lumbricidae). *Soil Biol Biochem* 24:171-177.
- Wood HB, James SW (1993) Native and introduced earthworms from selected chaparral, woodland, and riparian zones in southern California. USDA General Technical Report PSW-GTR-142.

**Table 1.** Mean proportions ( $\pm$  standard error) of earthworms collected from each depth during the study period.

Depth	Treatment	Day 7	Day 15	Day 30	Day 45	All Dates
		Proportion	Proportion	Proportion	Proportion	Proportion
0-5 cm	Native	0.23 $\pm$ 0.15 a	0.00 $\pm$ 0.00 a	0.45 $\pm$ 0.07 a <sup>†</sup>	0.85 $\pm$ 0.07 a	0.43 $\pm$ 0.09 a
	Exotic	0.00 $\pm$ 0.00 y	0.00 $\pm$ 0.00 y	0.04 $\pm$ 0.04 y	0.39 $\pm$ 0.16 y	0.08 $\pm$ 0.05 y
5-10 cm	Native	0.52 $\pm$ 0.13 a	0.67 $\pm$ 0.26 b	0.40 $\pm$ 0.06 a	0.12 $\pm$ 0.06 b	0.40 $\pm$ 0.08 a
	Exotic	0.19 $\pm$ 0.11 y	0.00 $\pm$ 0.00 y	0.17 $\pm$ 0.11 y	0.11 $\pm$ 0.09 y	0.11 $\pm$ 0.05 y
10-20 cm	Native	0.25 $\pm$ 0.07 a	0.33 $\pm$ 0.26 b	0.15 $\pm$ 0.12 b	0.03 $\pm$ 0.03 b	0.17 $\pm$ 0.06 b
	Exotic	0.81 $\pm$ 0.11 z	1.00 $\pm$ 0.00 z	0.79 $\pm$ 0.10 z	0.50 $\pm$ 0.08 y	0.81 $\pm$ 0.06 z

Note: Within a given date and treatment, means followed by different letters are significantly different from one another ( $p < 0.05$  except where noted <sup>†</sup>  $p < 0.10$ )

**Table 2.** Microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and microbial C/N from experimental cores with native, exotic or no earthworms. Values for MBC and MBN are reported as  $\mu\text{g C or N g soil}^{-1}$ .

Depth	Treatment	Day 7			Day 15			Day 30			Day 45		
		MBC	MBN	C/N	MBC	MBN	C/N	MBC	MBN	C/N	MBC	MBN	C/N
0-5 cm	Control	978.3 a	76.0 a	13.3 a	1152.2 a	80.3 a	14.5 a	953.9 a	77.6 a	12.4 ab <sup>†</sup>	899.2 a <sup>†</sup>	78.0 a	11.5 a
	Native	1181.8 b	75.0 a	16.2 b	1063.1 a	82.3 a	13.0 a	1024.1 a	77.9 a	13.5 a	826.8 ab	80.8 a	10.4 ab
	Exotic	1178.3 b	71.2 a	16.8 b	1134.3 a	84.2 a	13.5 a	849.9 a	78.49 a	10.9 b	756.9 b	81.2 a	9.4 b
5-10 cm	Control	928.0 a	87.7 a	10.7 a	939.6 a	76.6 a	12.2 a	894.6 a	76.7 a	11.6 a	650.6 a	66.6 a	9.9 a
	Native	946.8 a	83.2 a	11.4 a	915.4 a	75.0 a	12.4 a	867.3 a	69.1 a	12.5 a	885.8 b	75.5 ab	11.8 b
	Exotic	987.5 a	85.7 a	11.6 a	970.0 a	73.2 a	13.3 a	945.0 a	77.9 a	12.0 a	949.8 b	81.8 b	11.6 ab
10-20 cm	Control	790.7 a	56.2 a	14.1 a	830.2 a	63.1 a	13.2 a	701.1 a	53.8 a	13.4 a	575.6 a	50.3 a	11.5 a
	Native	789.1 a	62.7 a	12.7 a	782.0 a	49.2 b	16.2 b	707.2 a	58.2 a	12.2 a	663.6 a	54.5 a	12.3 a
	Exotic	794.5 a	64.9 a	12.4 a	752.5 a	55.6 ab	13.5 a	586.5 a	53.2 a	11.0 a	684.5 a	58.2 a	13.4 a

Note: Within a given date and depth, means followed by different letters are significantly different from one another ( $p < 0.05$  except where noted <sup>†</sup>  $p < 0.10$ )

**Table 3.**  $\delta^{13}\text{C}$  of microbial biomass carbon ( $\text{MB}^{13}\text{C}$ ), total  $\mu\text{g }^{13}\text{C}$  in microbial biomass standing stock per g soil ( $^{13}\text{CSS}$ ), and  $\delta^{13}\text{C}$  of  $\text{CO}_2$  respired from non-fumigated soils ( $^{13}\text{CO}_2$ ) from experimental cores with native, exotic or no earthworms.

Depth	Treatment	Day 15			Day 30			Day 45		
		$\text{MB}^{13}\text{C}$	$^{13}\text{CSS}$	$^{13}\text{CO}_2$	$\text{MB}^{13}\text{C}$	$^{13}\text{CSS}$	$^{13}\text{CO}_2$	$\text{MB}^{13}\text{C}$	$^{13}\text{CSS}$	$^{13}\text{CO}_2$
0-5 cm	Control	365.55 a	1.738 a	54.96	298.87 a	1.368 ab	86.50	385.10 a	1.374 a	69.15
	Native	374.44 a	1.620 a	54.51	328.18 a	1.498 a	81.23	292.57 a	1.246 ab	59.51
	Exotic	361.91 a	1.708 a	60.07	318.58 a	1.238 b	57.08	363.98 a	1.100 b	63.43
5-10 cm	Control	256.59 a	1.302 a	52.95	215.56 a	1.198 a	74.23	241.26 a	0.893 b	85.36
	Native	241.34 ab	1.260 a	46.41	190.29 a	1.143 a	80.37	210.85 a	1.188 a	78.26
	Exotic	211.87 b	1.302 a	48.83	203.77 a	1.253 a	71.53	225.42 a	1.250 a	74.23
10-20 cm	Control	192.16 a	1.070 a	49.45	173.77 a	0.908 a	68.15	175.45 a	0.838 a	91.43
	Native	183.26 a	1.025 a	55.24	154.04 a	0.906 a	74.63	206.39 a	0.884 a	91.45
	Exotic	168.10 a	0.974 a	47.12	185.71 a	0.770 a	70.74	197.47 a	0.868 a	87.42

Note: Within a given date and depth, means followed by different letters are significantly different from one another ( $p < 0.05$  except where noted <sup>†</sup>  $p < 0.10$ ).

**Table 4.** Mass, atom percent  $^{15}\text{N}$  (At% $^{15}\text{N}$ ), standing stock nitrogen (SSN), and standing stock  $^{15}\text{N}$  (SS $^{15}\text{N}$ ) of aboveground tissues from plants grown in experimental cores.

Earthworm Treatment	Day 15					Day 45				
	Mass (g/ind.)	%N <sup>†</sup>	At% $^{15}\text{N}$	SSN (mg/ind.)	SS $^{15}\text{N}$ (mg/ind.)	Mass (g/ind.)	%N	At% $^{15}\text{N}$	SSN (mg/ind.)	SS $^{15}\text{N}$ (mg/ind.) <sup>†</sup>
Control (s.e.)	0.142 a 0.039	1.709 a 0.099	4.356 a 0.398	2.488 a 0.762	0.111 a 0.037	1.242 a 0.460	1.168 a 0.122	2.266 a 0.293	14.508 a 5.267	0.300 a 0.110
Native (s.e.)	0.158 a 0.051	1.840 ab 0.208	3.016 b 0.701	3.224 a 1.335	0.103 a 0.049	0.356 b 0.137	1.326 a 0.164	1.925 a 0.259	4.579 b 1.721	0.095 b 0.041
Exotic (s.e.)	0.088 a 0.017	2.137 b 0.170	3.922 ab 0.304	1.922 a 0.427	0.079 a 0.022	0.954 a 0.187	1.298 a 0.098	1.646 a 0.036	11.757 a 1.617	0.194 a 0.028

Note: Means in the same column followed by different letters are significantly different from one another ( $p < 0.05$ , except where noted <sup>†</sup>  $p < 0.10$ ).

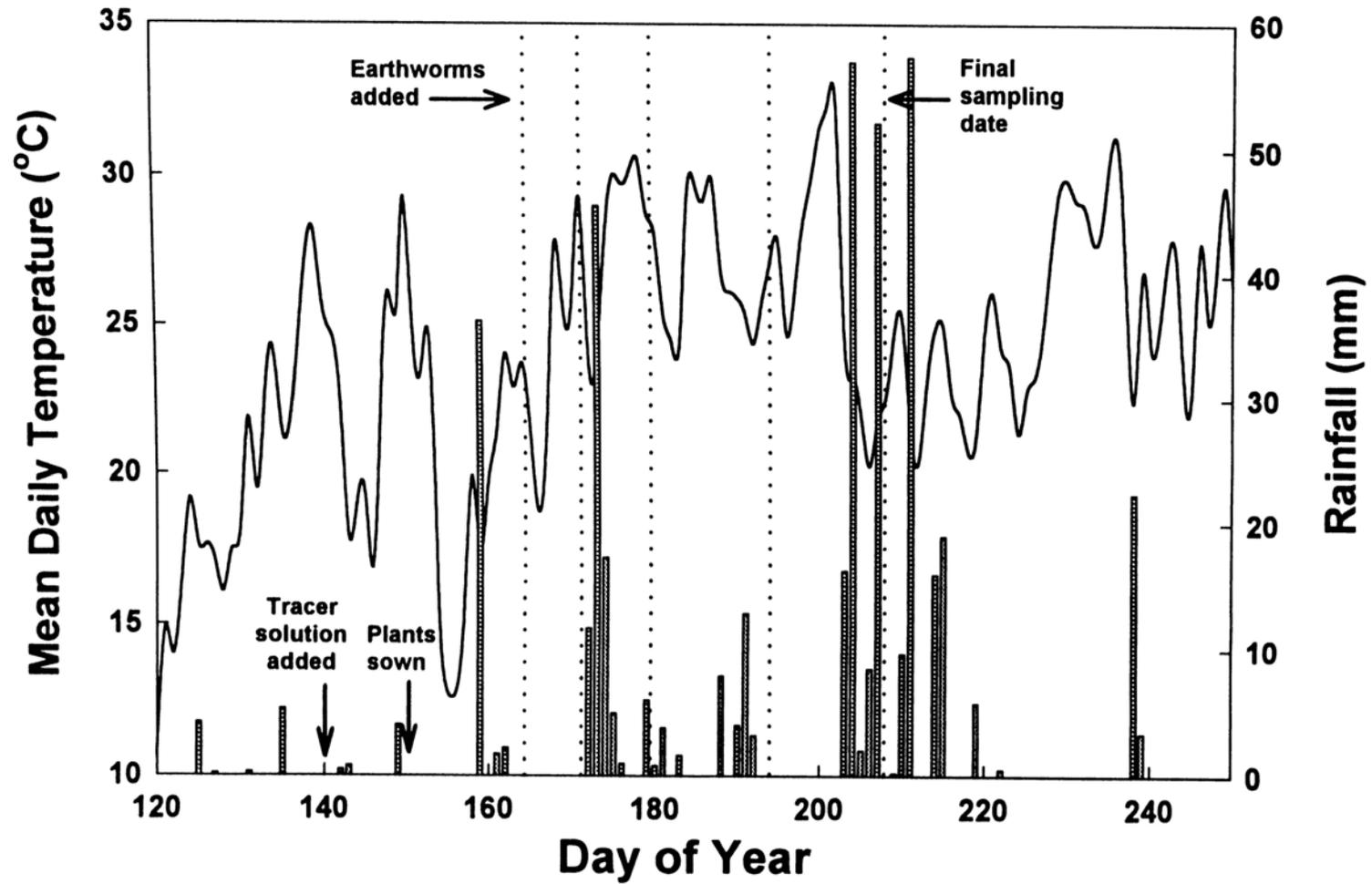
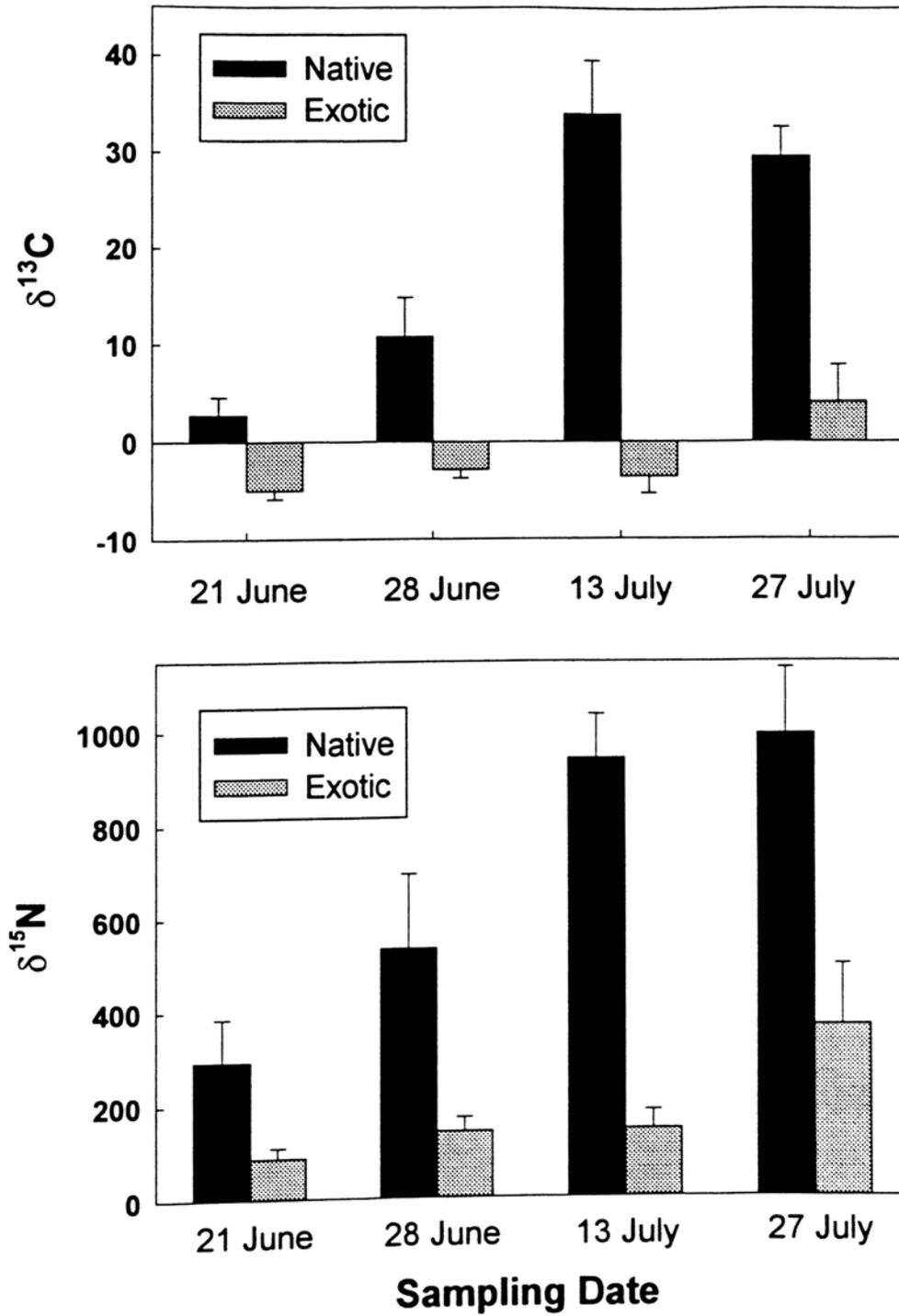


Figure 1. Rainfall (bars), temperature (solid line) and important dates during the experimental period. Climate data are from KPBS headquarters weather station. First dotted line indicates application of earthworm treatments, subsequent dotted lines indicate sampling dates.



**Figure 2.**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of native and exotic earthworms during the study period.

**CHAPTER 3:**

**FEEDING ECOLOGY AND EMERGENCE PRODUCTION OF ANNUAL  
CICADAS (HOMOPTERA: CICADIDAE) IN TALLGRASS PRAIRIE**

Mac A. Callaham, Jr.<sup>1</sup>, Matt R. Whiles<sup>2†</sup>, Clinton K. Meyer<sup>2</sup>, Brent L. Brock<sup>1</sup>, and  
Ralph E. Charlton<sup>2</sup>

<sup>1</sup>Division of Biology, Kansas State University, Manhattan, KS 66506-4901

<sup>2</sup>Department of Entomology, Kansas State University, Manhattan, KS 66506-4004

<sup>†</sup>Present Address: Department of Zoology, Life Sciences II, 351  
Southern Illinois University, Carbondale, IL 62901-6501, USA

**As accepted for publication in *Oecologia* (2000)**

## ABSTRACT

The emergence phenology and feeding ecology of annual cicadas in tallgrass prairie is poorly documented. However, these large insects are abundant, and their annual emergence represents a potentially important flux of energy and nutrients from belowground to aboveground. We conducted a study at Konza Prairie Research Natural Area in eastern Kansas to characterize and quantify cicada emergence and associated energy and nutrient fluxes. We established emergence trap transects in three habitat types (upland prairie, lowland prairie, and riparian forest), and collected cicadas every three days from May to September. A subset of trapped cicadas was used for species- and sex-specific mass, nutrient and stable isotopic analyses. Five species were trapped during the study, of which three were dominant. Cicadetta calliope and Tibicen aurifera exhibited significantly higher emergence production in upland prairie than in lowland prairie, and were not captured in forested sites at all. Tibicen dorsata emerged from all three habitat types, and though not significant, showed a trend of greater abundance in lowland grasslands. Two less abundant species, Tibicen pruinosus and Tibicen lyricen, emerged exclusively from forested habitats. Nitrogen fluxes associated with total cicada emergence were estimated to be  $\sim 4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  in both grassland habitats, and  $1.01 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  in forested sites. Results of stable isotope analyses showed clear patterns of resource partitioning among dominant cicada species emerging from grassland sites. T. aurifera and C. calliope had  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures indicative of feeding on shallowly rooted  $\text{C}_4$  plants such as the warm-season grasses dominant in tallgrass prairie ecosystems, whereas T. dorsata signatures suggested preferential feeding on more deeply rooted  $\text{C}_3$  plants.

**Key Words:** insect emergence, grassland, nutrient flux, resource partitioning, stable isotopes

## INTRODUCTION

The basic ecology of annually emerging cicada species in North American grasslands is poorly known. This is primarily because cicadas are not considered to be agricultural pests, with the exception of occasional damage to fruit trees by the oviposition activity of periodical cicadas (Miller and Crowley 1998). Indeed, there is little evidence that belowground feeding by cicadas on the roots of their host plants has any negative effect on plant performance (e.g. White and Sedcole 1993).

Most research on cicadas in tallgrass prairie to date has focused on nymphal stages in the life cycle. Several studies have documented nymphal responses to various disturbances such as fire, mowing (grazing), or nutrient addition (Seastedt 1984; Seastedt et al. 1988; Todd et al. 1992). However, these studies focused on responses of the whole soil invertebrate community, and not on cicadas specifically, and none examined responses at levels of taxonomic resolution below that of family. Nevertheless, several cicada species emerge annually from tallgrass prairie soils, and emergence patterns and feeding ecology of different species are poorly understood.

Annual cicadas are abundant in tallgrass prairie, and their emergence represents a movement of C-stored energy and nutrients (such as N) from belowground to aboveground pools. In fact, the emergence of soil dwelling insects such as cicadas is one of the few non-gaseous fluxes of N from belowground to aboveground pools. This movement of N is of particular interest in tallgrass prairie ecosystems, because N

availability is a principal determinant of aboveground net primary production (Blair 1997; Knapp et al. 1998).

Examination of stable isotopic signatures (specifically  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) is a well established method for studying the feeding ecology of organisms, but has only recently been used to study the feeding ecology of belowground organisms (Martin et al. 1992; Neilson et al. 1998; Schmidt et al. 1997, 1999; Hendrix et al. 1999). This technique is made possible by virtue of naturally occurring variation in the isotopic composition of organic material arising from biological, chemical, and physical processes. A classic example of isotopic discrimination is the difference in  $\delta^{13}\text{C}$  signatures of plants utilizing  $\text{C}_3$  vs.  $\text{C}_4$  photosynthetic pathways.  $\text{C}_4$  plants discriminate less against  $^{13}\text{C}$  atoms during photosynthesis and therefore have less depleted signatures than do  $\text{C}_3$  plants ( $\delta^{13}\text{C} \approx -12\text{‰}$  vs.  $\delta^{13}\text{C} \approx -26\text{‰}$ , respectively). Thus, by examining the  $\delta^{13}\text{C}$  of the consumer of interest it is often possible to identify sources of organic C (e.g. DeNiro and Epstein 1978, Gearing 1991). In the case of  $\delta^{15}\text{N}$ , it is sometimes possible to determine the trophic status of an organism in a given food web because of increasing  $^{15}\text{N}$  enrichment of tissues with each increase in trophic level (Deniro and Epstein 1981).

Among soil invertebrates, cicadas present a unique opportunity to utilize stable isotopic techniques because they feed exclusively on the xylem sap of their perennial host plants (Cheung and Marshall 1973), sometimes feeding on a single root for their entire life-span belowground (Beamer 1928). We hypothesized that xylem sap would contain C derived only from the host plant, and N derived exclusively from soil pools (for non-legumes) and from plant-storage pools early in the growing season. Therefore, it should be possible to detect what types of plants (e.g. those utilizing  $\text{C}_3$  vs.  $\text{C}_4$  photosynthetic

pathways) different cicada species are feeding upon. Because cicadas have relatively long life-cycles (the shortest known cicada life-cycle is four years), their tissues may be considered a composite sample of the sometimes variable isotopic composition of xylem fluid components, although rates of turnover (and fractionations/discriminations associated with metabolism) of C and N in body tissues are unknown for these insects.

We used emergence trapping of cicadas and analysis of mass, nutrient content, and stable isotopic composition of cicada tissues to address the objectives of this study, which were 1) to characterize and quantify emergence patterns for all species of annually emerging cicadas in upland prairie, lowland prairie, and riparian forest habitats in a tallgrass prairie ecosystem; 2) to quantify energy and nutrient (specifically N) fluxes associated with the emergence of cicadas in each habitat type; and 3) to examine the feeding ecology of cicadas in this ecosystem.

## **MATERIALS AND METHODS**

### *Site Description*

This study was conducted at the Konza Prairie Research Natural Area (KPRNA), a research site operated by the Division of Biology at Kansas State University (KSU). KPRNA is located in the Flint Hills physiographic region, a narrow (50–200 km) band of tallgrass prairie extending from extreme southeast Nebraska to northeast Oklahoma. Principle research foci at this Long-Term Ecological Research site include population, community, and ecosystem responses to various disturbances such as fire and grazing (Knapp et al. 1998). For this study, we used a KPRNA management unit in the lower Kings Creek drainage that was burned annually for 14 years (except 1987). Grassland

vegetation at the site is characteristic of tallgrass prairie and is dominated by the warm-season C<sub>4</sub> tallgrasses big bluestem (Andropogon gerardii Vitman), little bluestem (A. scoparius Michx.), and indian grass (Sorghastrum nutans (L.) Nash). Additionally, numerous species of sub-dominant C<sub>3</sub> grasses, sedges, and forbs occur within the matrix of warm-season grasses (Freeman 1998). Woodland vegetation in the tallgrass prairie landscape occurs as thin riparian strips in lowland areas, dominated by bur oak (Quercus macrocarpa Michx.), chinkapin oak (Q. muhlenbergii Engelm.), American elm (Ulmus americana L.), and hackberry (Celtis occidentalis L.). For a full description of vegetation at KPRNA, see Freeman (1998).

### *Field Methods*

Cicadas were sampled by emergence trapping throughout the growing season of 1998. Emergence traps consisted of metal mesh (3.2 mm aperture) cylinders with a basal area of 2500 cm<sup>2</sup>. Trap transects were established in mid-May 1998 on each of three topographic positions at the study site: upland prairie (elevation ≈ 390 m, relatively shallow soil, relatively low primary production); lowland prairie (elevation ≈ 340 m, deeper soils, higher primary production); and riparian forest (elevation ≈ 330 m, deepest soil, forest vegetation). The management unit utilized in this study consisted of three separate watersheds, and three transects were randomly located (in upland, lowland, and forested habitats) within each of these watersheds for a total of 9 independent transects. Transects consisted of 8 or 10 traps in a line spaced 5-10 m apart at randomly selected locations within each topographic position. Emergent adult cicadas and nymphal exuviae were collected from traps every 2-4 days during the entire 1998 growing season (May-

Sept.). Voucher specimens of all cicada species collected during the study were deposited in the KSU Museum of Entomological and Prairie Arthropod Research.

To examine potential relationships between vegetation and cicada emergence patterns, plant species composition was determined inside each trap on a single date at the end of the growing season (late Sept.). Additionally, aerial cover was estimated for each plant species occurring in the traps using a cover class method modified from Daubenmire (1968).

### *Laboratory Methods*

All cicadas were identified to species and sexed. Sex-specific dry mass (DM) and ash-free dry mass (AFDM) were determined from a subset of male and female individuals ( $n = 5-12$  for each sex) of each species. Dry mass was determined by drying individual cicadas at 55°C for four days, and AFDM was determined by ashing individuals at 500°C for 4 hours. Sex-specific AFDM values were applied to all emerging individuals to estimate emergence production ( $\text{g AFDM m}^{-2}$ ) by topographic position. When possible, exuviae of emerged individuals were collected from traps and DM and AFDM were determined. Species-specific AFDM values for exuviae were included in estimates of emergence production.

Another subset of individuals from each sex of each species ( $n = 5$ ) was analyzed for N and C content. Individual cicadas were freeze-dried under high vacuum for >72 hours prior to grinding with mortar and pestle. Subsamples of ground cicada tissue were then weighed into tin capsules, and tissue N and C content were determined by combustion (Carlo-Erba C/N NA 1500). Average sex- and species-specific dry mass

values were multiplied by average %N to estimate N flux ( $\text{kg N ha}^{-1}$ ) for each species by topographic position. As with emergence production estimates, exuviae were also collected and analyzed for %N and %C, and these were included in total flux estimates.

Stable isotopic content of cicada tissues was determined on additional subsamples of freeze-dried individuals ( $n = 5$  for each sex of each species) by continuous flow isotope ratio mass spectrometry (Finnegan delta-C), after sample gas preparation by combustion. Reference materials for isotopic analysis were acetanilide ( $\delta^{13}\text{C} = -30.30 \text{ ‰}$  relative to PeeDee belemnite (PDB) and  $\delta^{15}\text{N} = -2.80 \text{ ‰}$  relative to atmospheric N) and bovine liver ( $\delta^{13}\text{C} = -21.55 \text{ ‰}$  relative to PDB and  $\delta^{15}\text{N} = 7.44 \text{ ‰}$  relative to atmospheric N).

### *Statistical Analyses*

All emergence data were analyzed with analysis of variance (ANOVA) procedures (SAS institute, Cary NC). For these analyses, emergence values for individual traps were averaged over an entire trap array and that average was used in ANOVA analyses (thus,  $n = 3$  for each of the 3 landscape positions). We used Duncan's Least Significant Difference means separation procedure to identify statistically significant differences in mean emergence values for each species from each of the landscape positions. Because of the low number of replications and the high variability inherent in this type of field sampling, we tested for significance at  $\alpha = 0.10$ .

To assess potential relationships between plant species composition and cicada emergence, cover values for each individual plant species were regressed against cicada emergence values for each trap. Additionally, relationships between plant community

composition and cicada emergence patterns were examined by comparing plant communities at different sites (both within and across landscape positions) and relating average cicada emergence at each site to plant community at each site. We analyzed the influence of plant community on cicada emergence by selecting the sites with the highest cicada emergence densities for each species (i.e. upland site 2 for T. aurifera and C. calliope, lowland site 1 for T. dorsata, and forest site 1 for T. pruinosa), and calculating percent similarity of vegetation at all other transect sites relative to the sites with highest cicada density. Percent similarity was calculated as:  $PS = 1 - (\sum |p_i - q_i| / 2)$  where  $p_i$  is the proportional average abundance (as percent cover) of species  $i$  at site 1, and  $q_i$  is the proportional average abundance (as percent cover) of species  $i$  at site 2 (Brower et al. 1998). Average cicada emergence density for each site was then regressed against the range of similarity values relating all other sites to those with the highest cicada densities.

Tissue N data were analyzed with ANOVA (SAS Institute, Cary NC) procedures and Duncan's least significant difference means separation procedure ( $\alpha = 0.05$ ) was used for comparisons between species. Similarly,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  data were analyzed for differences among species with ANOVA procedures ( $\alpha = 0.05$ ) and Duncan's Least Significant Difference means separation method.

## RESULTS

### *Cicada Species Composition and Emergence Patterns*

Five species of annual cicada were trapped in 1998 (Fig. 1). Cicadetta calliope and Tibicen aurifera were trapped only in grassland habitats, whereas T. pruinosa (along

with a single specimen of T. lyricen) were trapped only in forested sites (Table 1). Tibicen dorsata was trapped in both grassland and forested sites (Table 1). Cicadetta calliope was first to emerge (23 June) and had significantly ( $p < 0.05$ ) higher emergence production in upland sites than in lowland sites (Table 1). The C. calliope emergence lasted only 12 days, with ~45% of individuals emerging on the first day (Fig. 2). Males and females of C. calliope emerged in roughly equal numbers throughout the emergence period (Fig. 2). Tibicen pruinosa began emerging on 18 July, and T. aurifera and T. dorsata began emerging on 2 August (Fig. 1). Tibicen aurifera had significantly higher emergence production in upland sites than in lowland sites ( $p < 0.10$ ), while T. dorsata exhibited a trend of higher emergence production in lowland prairie than in the other two landscape positions (Table 1). The emergence of T. aurifera lasted for 41 days and was slightly protandric as only males emerged (in relatively low numbers) for the first 20 days, followed by a peak of male emergence on the 30th day (Fig. 2). Females of T. aurifera began to emerge on the 20th day of the overall emergence with peak emergence ~10 days later than the peak of male emergence (Fig. 2). Emergence of T. dorsata spanned 38 days with a large proportion (>60%) of the total number of females emerging during the first 12 days (Fig. 2). Male emergence was more protracted for T. dorsata with relatively high numbers (compared to female T. dorsata) emerging over the first 25 days (Fig. 2).

#### *Influence of Plant Community on Cicada Emergence*

Regressions between cicada emergence and percent cover of individual plant species revealed no significant relationships between the cover of any single plant species

and emergence for any species of cicada, suggesting that within a habitat type (grassland or forest), cicadas are generalist feeders. However, plant community analyses revealed significant relationships between plant communities and emergence of cicadas (Table 2). Cicadetta calliope emergence was significantly related to similarity values comparing the plant community at the second upland prairie site to all other sites (column UP2 in Table 2). Tibicen aurifera emergence was also significantly related to plant community at UP2 (Table 2). There were no significant relationships between plant community and T. dorsata emergence. Tibicen pruinosa emergence patterns were related to plant community similarity values of other sampling sites to the vegetation at the first forested site (column RF1 in Table 2).

#### *Mass and Nutrient Analyses*

Mass and tissue chemistry analyses for the three dominant species are summarized in Table 3. There were large differences in the size of cicada species collected over the course of the study with dry mass for individuals (including exuviae) ranging from > 1.1 g for females of T. dorsata to < 0.03 g for males of C. calliope (Table 3). Dry mass values for T. pruinosa and T. lyricen were intermediate to T. dorsata and T. aurifera (data not shown). Average %N for all male and female cicadas, regardless of species, revealed a significantly ( $p < 0.05$ ) higher %N for males (Table 3). However, total mass of N per individual was always greater for females, because of their greater individual mass. Between species, average %N was greatest for T. aurifera and least for T. dorsata. Average %C was significantly higher for T. dorsata than the other two species. Within species, females always had significantly higher %C than males (Table

3). T. pruinosa and T. lyricen were not included in tissue chemistry analyses, because not enough individuals were collected to perform both AFDM and nutrient analyses.

Estimates of landscape level fluxes of N showed patterns closely related to AFDM for the different landscape positions (Table 1). Total fluxes were not different between upland and lowland prairie sites because of approximately equal contributions of different species in each landscape position. Fluxes of N from grassland sites were significantly larger than from forested sites (Table 1).

#### *Stable isotope analyses*

Stable isotope analyses indicated that two species of cicada (C. calliope and T. aurifera) fed on C<sub>4</sub> plants, with  $\delta^{13}\text{C}$  signatures averaging -11.25‰ and -10.15‰, respectively (Fig. 3). In contrast, individuals of T. dorsata had average  $\delta^{13}\text{C}$  signatures of -25.17‰, indicative of feeding on C<sub>3</sub> plants (Fig. 3). We also analyzed tissues from individuals of Magicicada cassini, a species of 17-year periodical cicada, which also emerged from the forested sites in 1998 (Whiles et al., in review). We included periodical cicada tissues as a reference for other cicada species, because M. cassini is known to be a tree feeding species (White and Strehl 1978) and should therefore (at our site) invariably have a C<sub>3</sub> type  $\delta^{13}\text{C}$  signature. Magicicada cassini tissues did reflect C<sub>3</sub> feeding with values averaging -25.09‰ (Fig. 3). Tibicen pruinosa and T. lyricen were not included in this analysis because an insufficient number of specimens was collected in emergence traps.

Nitrogen isotope analysis revealed similar patterns of separation to the  $\delta^{13}\text{C}$  analysis with respect to cicada species. The two C<sub>4</sub> feeding species had significantly less

enriched  $\delta^{15}\text{N}$  signatures than did the two  $\text{C}_3$  feeding species (Fig. 3) (average  $\delta^{15}\text{N}$  of -0.06‰ and -0.63‰ for *C. calliope* and *T. aurifera*, respectively and +4.07‰ and +3.75‰ for *T. dorsata* and *M. cassini*, respectively).

## DISCUSSION

### *Cicada Emergence Patterns*

The cicada emergence patterns observed in this study provide evidence that various factors influence the distribution and abundance of different cicada species in the tallgrass prairie landscape. The most obvious difference between species collected during the study were differences in timing of emergence (Fig. 1). *Cicadetta calliope* emerged first, in late June, followed closely by *T. pruinosa*, while *T. aurifera* and *T. dorsata* emerged simultaneously in early August (Fig. 1). There were differences in emergence patterns with respect to topographic position as well. For example, two species, *C. calliope* and *T. aurifera*, were more likely to emerge from upland prairie than from lowland prairie, and were not observed to emerge from forested soils at all (Table 1). In contrast, *T. pruinosa* and *T. lyricen* emerged exclusively from forested habitats (Table 1). Thus, to some degree, the composition of the belowground invertebrate community in the tallgrass prairie landscape appears to be determined by the type of vegetation present. However, plant community composition is highly dependent upon factors such as topographic position, water availability, and disturbance regime (Collins and Steinauer 1998; Hartnett and Fay 1998), all of which can also influence the composition of belowground invertebrate assemblages (Seastedt et al. 1988; Todd 1996; Blair et al. *in press*; Callaham and Blair *in press*). Therefore, the total makeup of the

belowground invertebrate community is likely the result of complex interactions between biotic and abiotic forces acting simultaneously.

Various factors may be responsible for the higher emergence production observed for *C. calliope* and *T. aurifera* in upland sites (Table 1). We originally hypothesized that cicada abundance would be correlated with some single plant species that cicadas preferred for a host, but found no such correlation for any individual plant species. We then examined relationships between plant community compositions and cicada emergence patterns, and found some relationships that were statistically significant (Table 2), but it was difficult, with similarity index values, to identify what characteristics of the plant community were responsible for the observed relationships. For the purposes of this study, similarity values (Table 2) were viewed as a continuum of decreasing vegetation similarity of other sites relative to the site where cicada emergence was highest (for each species of cicada collected). This approach assumed that the plant community at a site with high cicada densities was in some way responsible for the high cicada densities. We interpreted decreased similarity of vegetation at other sites to be indicative of a departure from the floristic conditions that maximized cicada density (table 2). However, percent similarity values of 0.50 or greater are sometimes interpreted as indicative of general similarity between communities (e.g. Odum 1950). In this case, five of the six grassland sites in our study could be considered floristically similar to one another (Table 2), and as such, differences in cicada emergence between sites would be best explained by variables other than plant community composition. Therefore, as differences in plant community parameters may explain some of the differences in cicada emergence, it is important to note that there are many other differences between upland

and lowland grassland habitats that may be responsible (in whole or in part) for the patterns (both for cicadas and plants) that we observed. For example, certain cicada species may have a preference for the soil type most frequently found in upland sites at KPRNA, which has considerably lower clay content in topsoil (~28% clay in uplands vs. ~42% clay in lowlands, for 0-20 cm depth) (Ransom et al. 1998). Other topography-related factors that could potentially influence cicada emergence patterns include differences in soil depth (shallower in uplands), soil moisture (drier in uplands), and aboveground plant productivity (lower in uplands, although differences in belowground plant productivity and/or biomass with topographic position has not been measured at KPRNA) (Knapp et al. 1998). Again, we suspect that given significant differences in emergence production of cicadas with topography, and significant relationships between plant community and cicada emergence, the real mechanisms which dictate cicada distributions in this landscape are likely an admixture of biotic and abiotic factors.

### *Nutrient fluxes*

Nitrogen fluxes associated with cicada emergence represent a significant flux of this limiting nutrient in tallgrass prairie. Annual inputs of inorganic N via bulk precipitation for KPRNA vary between 6 and 12 kg N ha<sup>-1</sup>yr<sup>-1</sup> and total inputs of N (including N<sub>2</sub> fixation and organic N in precipitation) have been estimated to range from 11 to 25 kg N ha<sup>-1</sup>yr<sup>-1</sup> (Blair et al. 1998). Thus, cicada emergence (~4 kg N ha<sup>-1</sup>yr<sup>-1</sup>, Table 1) represents a redistribution of a significant fraction of that total in grassland sites. It is likely that N leaving soil pools by way of cicada emergence returns fairly quickly to soil pools via decomposers or detritivores. However, an unknown portion of cicada

tissue N passes through at least one additional aboveground trophic level, as they are frequently preyed upon by vertebrates (Krohne et al. 1991; Steward et al. 1988) as well as invertebrates such as spiders, sphecid wasps, and ants (personal observations).

Estimated nutrient fluxes from forested habitats as annual cicada tissue were about 25% of those estimated for grassland habitats (Table 1). However, the estimate for N flux from forested sites is probably conservative, as observations in the field suggested that our sampling strategy was inappropriate for tree feeding species. Our traps were placed randomly along the forest floor, but we suspect that T. pruinosa and T. lyricen emergence patterns are non-random, perhaps concentrating near the bases of large trees. Further (and more intensive) sampling may provide better estimates of actual energy and nutrient fluxes associated with these woodland species. Another important flux from forested habitats in cicada tissues occurs during mass emergence of the periodical cicada Magicicada cassini. This species also emerged from forested sites at KPRNA during the summer of 1998, and (when projected over 17 years) flux of N and C associated with periodical cicadas adds  $\sim 0.37 \text{ kg N ha}^{-1}\text{yr}^{-1}$  and  $\sim 0.37 \text{ g C m}^{-2}\text{yr}^{-1}$  (AFDM) to our estimates for forested sites (Whiles et al. in press).

### *Feeding Ecology*

Stable isotopic signatures of cicada tissues provide further evidence for resource partitioning among cicada species in tallgrass prairie. Cicadas with  $\delta^{13}\text{C}$  signatures similar to those of  $\text{C}_4$  plants are likely feeding on the roots of the warm-season  $\text{C}_4$  grasses dominant in this ecosystem. Interestingly, the two cicada species with  $\text{C}_4$  type  $\delta^{13}\text{C}$  signatures (C. calliope and

T. aurifera) emerge at different times during the growing season (Fig. 1), suggesting resource partitioning in time. One resource for which these two grass-feeding cicadas may compete is suitable oviposition sites. This type of resource partitioning has been observed in periodical cicadas by White (1980) as different species of 17-year cicadas utilize different tree species for oviposition. In the case of annual grassland cicadas, C. calliope emerges early in the growing season (Fig. 1), and may oviposit in the flowering stalks of early developing grasses such as side oats grama (Bouteloua curtipendula (Michx.)), blue grama (B. gracilis (H.B.K.)) or switch grass (Panicum virgatum L.), whereas T. aurifera may utilize the flowering stalks of grasses such as Indian grass (Sorghastrum nutans) or big bluestem (Andropogon gerardii) which are abundant during the time of T. aurifera emergence. Thus, the separation of the two species in time may be related to differences in the phenology of their host plants, although further research is needed to confirm the existence of such a phenomenon. On the other hand, T. dorsata had signatures which suggest that it feeds on deeply rooted C<sub>3</sub> forbs or woody vegetation (Fig. 3). Interestingly, T. dorsata maintained its C<sub>3</sub> type signature even when collected in plots with high cover values for warm season grasses, suggesting that feeding on C<sub>3</sub> forb roots may be an obligate condition for T. dorsata. Thus, as two species with the same food resource (C. calliope and T. aurifera) emerge at different times during the growing season, it is notable that the two species that emerge simultaneously (T. aurifera and T. dorsata) utilize different food resources.

Results of the  $\delta^{15}\text{N}$  analysis provide further insight into the feeding ecology of grassland cicadas. The relatively enriched signatures of T. dorsata (Fig. 3) may suggest feeding on more deeply rooted plants. Several studies have reported  $\delta^{15}\text{N}$  enrichment of bulk soil

with depth, particularly in undisturbed natural ecosystems (e.g. Nadelhoffer and Fry 1988, Gebauer and Schulze 1991, Hendrix et al. 1999). Previous investigations at KPRNA have found that  $\delta^{15}\text{N}$  signatures of the soil profile at several locations (including annually burned prairie and riparian forest) exhibit this trend of increasing enrichment with depth (P.F. Hendrix, unpublished data). Still, interpretation of  $\delta^{15}\text{N}$  signatures in cicada tissues requires caution, because of potential variability in soil  $\delta^{15}\text{N}$  patterns at the landscape level (Sutherland et al. 1993), and indeed within the tissues of a single plant (Handley and Raven 1997; Handley and Scrimgeour 1997). Because of complex nutrient transformations within the plant, and associated fractionations and discriminations, the  $\delta^{15}\text{N}$  values of organic N in the xylem sap of plants may not simply be a reflection of the  $\delta^{15}\text{N}$  values of soil pools. As such, several alternative explanations exist for the differences observed in  $\delta^{15}\text{N}$  of cicada tissues in this study. These include differences in the degree of mycorrhizal dependence (cf. Handley et al. 1993) of  $\text{C}_3$  and  $\text{C}_4$  plants in tallgrass prairie, differences in N storage patterns of  $\text{C}_3$  and  $\text{C}_4$  plants, or (completely independent of plants) differences in the metabolisms of  $\text{C}_3$  and  $\text{C}_4$  feeding cicadas. Regardless of the mechanism responsible for differences in  $\delta^{15}\text{N}$  values of cicada species, it is important to note that these differences are not indicative of the trophic level at which cicadas feed.

## CONCLUSIONS

Five species of annual cicadas emerged from an annually burned landscape at KPRNA during the growing season of 1998. Cicada emergence resulted in the redistribution of approximately  $4 \text{ kg N ha}^{-1}$  in grassland habitats, and represents a

significant flux of N from belowground to aboveground pools. Cicadas appear to be partitioning resources in time and in space, and stable isotopic evidence suggests that cicadas partition food resources with some species specializing on C<sub>4</sub> grasses while others are C<sub>3</sub> forb or tree feeders. Continued emergence trap sampling, coupled with thorough sampling of soils and plants, is likely to provide further information on the basic biology and feeding ecology of cicadas in the future.

### ACKNOWLEDGEMENTS

Funding for this study was provided in part by an NSF Long-Term Ecological Research grant awarded to the Division of Biology at Kansas State University. Stable isotope analyses were carried out by T. Maddox at the Institute of Ecology, University of Georgia. Thanks to J. Blair for valuable comments on an earlier version of the manuscript and to P. Hendrix for use of soil data and many stimulating discussions. Thanks are due to S. Baer, J. Jonas, D. Kitchen, A. Silletti, and M. Smith for logistical support throughout the study.

### REFERENCES

- Beamer RH (1928) Studies on the biology of Kansas Cicadidae. *Kan U Sci Bull* 20:155-263.
- Blair JM (1997) Fire, N availability, and plant responses in grasslands: a test of the transient maxima hypothesis. *Ecology* 78:2359-2368.
- Blair JM, Todd TC, Callaham MA Jr (In press) Responses of grassland soil invertebrates

- to natural and anthropogenic disturbances. In: Coleman DC, Hendrix PF (eds) *Arthropods as Webmasters in Ecosystems*. CAB International Press, pp xxx-xxx.
- Blair JM, Seastedt TR, Rice CW, Ramundo RA (1998) Terrestrial nutrient cycling in tallgrass prairie. In: Knapp AK, Briggs JM, Hartnett DC, Collins SC (eds). *Grassland Dynamics: Long-Term Ecological Research in Tallgrass Prairie*. Oxford University Press, New York, pp 222-243.
- Brower JE, Zar JH, von Ende CN (1998) *Field and Laboratory Methods For General Ecology*. WCB/McGraw Hill, Boston, 273 pp.
- Callaham MA Jr, Blair JM (In press) Influence of differing land management on the invasion of North American tallgrass prairie soils by European earthworms. *Pedobiologia* .
- Cheung WWK, Marshall AT (1973) Water and ion regulation in cicadas in relation to xylem feeding. *J Insect Ph* 19:1801-1816.
- Collins SC, Steinauer EM (1998) Disturbance, diversity, and species interactions in tallgrass prairie. In: Knapp AK, Briggs JM, Hartnett DC, Collins SC (eds) *Grassland Dynamics: Long-Term Ecological Research in Tallgrass Prairie*. Oxford University Press, New York, pp 140-156.
- Daubenmire R (1968) Ecology of fire in grasslands. *Adv Ecol Res* 5:209-266.
- DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochim Cosm Acta* 42:495-506.
- DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim Cosm Acta* 45:341-351.
- Freeman CC (1998) *The flora of Konza Prairie: A historical review and contemporary*

- patterns. In: Knapp AK, Briggs JM, Hartnett DC, Collins SC (eds) *Grassland Dynamics: Long-Term Ecological Research in Tallgrass Prairie*. Oxford University Press, New York, pp 69-80.
- Gearing JN (1991) The study of diet and trophic relationships through natural abundance  $^{13}\text{C}$ . In: Coleman DC, Fry B (eds). *Carbon Isotope Techniques*. Academic Press, San Diego, pp 201-218.
- Gebauer G, Schulze ED (1991) Carbon and nitrogen isotope ratios in different compartments of a healthy and a declining *Picea abies* forest in the Fichtelgebirge, NE Bavaria. *Oecologia* 87:198-207.
- Handley LL, Daft MJ, Wilson J, Scrimgeour CM, Ingleby K, Sattar MA (1993) Effects of the ecto- and VA-mycorrhizal fungi *Hydnagium carneum* and *Glomus clarum* on the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of *Eucalyptus globulus* and *Ricinis communis*. *Plant Cell Environ* 16:375-382.
- Handley LL, Raven JA (1997) The use of natural abundance of nitrogen isotopes in plant physiology and ecology. *Plant Cell Environ* 15:965-985.
- Handley LL, Scrimgeour CM (1997) Terrestrial plant ecology and  $^{15}\text{N}$  natural abundance: The present limits to interpretation for uncultivated systems with original data from a Scottish oldfield. *Adv Ecol Res* 27:133-212
- Hartnett DC, Fay PA (1998) Plant populations: Patterns and processes. In: Knapp AK, Briggs JM, Hartnett DC, Collins SC (eds) *Grassland Dynamics: Long-Term Ecological Research in Tallgrass Prairie*. Oxford University Press, New York, pp 81-100.
- Hendrix PF, Lachnicht SL, Callaham MA Jr, Zou X (1999) Stable isotopic studies of

- earthworm feeding ecology in tropical ecosystems of Puerto Rico. *Rapid Commun Mass Spectrom* 13:1295-1299.
- Knapp AK, Briggs JM, Blair JM, Turner CL (1998) Patterns and controls of aboveground net primary productivity in tallgrass prairie. In: Knapp AK, Briggs JM, Hartnett DC, Collins SC (eds) *Grassland Dynamics: Long-Term Ecological Research in Tallgrass Prairie*. Oxford University Press, New York, pp 193-221.
- Krohne DT, Couillard TJ, Riddle JC (1991) Population responses of Peromyscus leucopus and Blarina brevicauda to emergence of periodical cicadas. *Am Midl Nat* 126:317-321.
- Martin A, Mariotti A, Balesdent J, Lavelle P (1992) Soil organic matter assimilation by a geophagous tropical earthworm based on  $\delta^{13}\text{C}$  measurements. *Ecology* 73:118-128.
- Miller F, Crowley W (1998) Effects of periodical cicada ovipositional injury on woody plants. *J Arboric* 24:248-253.
- Nadelhoffer KJ, Fry B (1988) Controls on natural nitrogen-15 and carbon-13 abundances in forest soil organic matter. *Soil Sci Soc Am J* 52:1633-1640.
- Neilson R, Hamilton D, Wishart J, Marriott CA, Boag B, Handley LL, Scrimgeour CM, McNicol JW, Robinson D (1998) Stable isotope natural abundances of soil, plants and soil invertebrates in an upland pasture. *Soil Biol Biochem* 30:1773-1782.
- Odum EP (1950) Bird populations of the Highlands (North Carolina) Plateau in relation to plant succession and avian invasion. *Ecology* 31:587-605.
- Ransom MD, Rice CW, Todd TC, Wehmueller WA (1998) Soils and soil biota. In:

- Knapp AK, Briggs JM, Hartnett DC, Collins SC (eds) *Grassland Dynamics: Long-Term Ecological Research in Tallgrass Prairie*. Oxford University Press, New York, pp 48-66.
- Schmidt O, Scrimgeour CM, Handley LL (1997) Natural abundance of  $^{15}\text{N}$  and  $^{13}\text{C}$  in earthworms from a wheat and a wheat-clover field. *Soil Biol Biochem* 29:1301-1308.
- Schmidt O, Scrimgeour CM, Curry JP (1999) Carbon and nitrogen stable isotope ratios in body tissue and mucus of feeding and fasting earthworms (*Lumbricus festivus*). *Oecologia* 118:9-15.
- Seastedt TR (1984) Belowground macroarthropods of annually burned and unburned tallgrass prairie. *Am Midl Nat* 111:405-408.
- Seastedt TR, James SW, Todd TC (1988) Interactions among soil invertebrates, microbes and plant growth in the tallgrass prairie. *Agric Ecosyst Environ* 24:219-228.
- Steward VB, Smith KG, Stephen FM (1988) Red-winged blackbird predation on periodical cicadas (Cicadidae: *Magicicada* spp.): bird behavior and cicada responses. *Oecologia* 76:348-352.
- Sutherland RA, van Kessel C, Farrell RE, Pennock DJ (1993) Landscape-scale variations in plant and soil nitrogen-15 natural abundance. *Soil Sci Soc Am J* 57:169-178.
- Todd TC (1996) Effects of management practices on nematode community structure in tallgrass prairie. *App Soil Ecol* 3:235-246.
- Todd TC, James SW, Seastedt TR (1992) Soil invertebrate and plant responses to mowing and carbofuran application in a North American tallgrass prairie. *Plant Soil* 144:117-124.

- Whiles MR, Callaham MA Jr, Meyer CK, Brock BL, Charlton RE (in press) Emergence of periodical cicadas from a Kansas riparian forest: densities, biomass, and nitrogen flux. *Am Midl Nat*
- White EG, Sedcole JR (1993) A study of the abundance and patchiness of cicada nymphs (Homoptera: Tibicinidae) in a New Zealand subalpine shrub-grassland. *NZ J Zoo* 20:38-51.
- White J, Strehl CE (1978) Xylem feeding by periodical cicada nymphs on tree roots. *Ecol Entom* 3:323-327.
- White J (1980) Resource partitioning by ovipositing cicadas. *Am Nat* 115:1-28.

**Table 1.** Cicada emergence production (g AFDM m<sup>-2</sup>yr<sup>-1</sup>) and nitrogen flux (kg N ha<sup>-1</sup>yr<sup>-1</sup>) from three habitats in the tallgrass prairie landscape.

	Upland Prairie	Lowland Prairie	Riparian Forest
<b>AFDM</b>			
<i>C. calliope</i>	0.25 a	0.06 b	0.00 b
<i>T. pruinosa</i> <sup>†</sup>	0.00 b	0.00 b	0.57 a
<i>T. dorsata</i>	1.27 a	2.25 a	0.06 a
<i>T. aurifera</i>	2.17 a	1.01 b	0.00 b
Total	3.69	3.32	0.63
<b>N flux</b>			
<i>C. calliope</i> <sup>†</sup>	0.26 a	0.07 b	0.00 c
<i>T. pruinosa</i> <sup>†</sup>	0.00 b	0.00 b	0.70 a
<i>T. dorsata</i>	1.38 ab	2.77 a	0.31 b
<i>T. aurifera</i>	2.62 a	1.23 b	0.00 b
Total	4.26	4.07	1.01

Note: Values in a row followed by different letters are significantly different from one another (Duncan's LSD  $\alpha = 0.10$ , except rows where indicated, <sup>†</sup>  $\alpha = 0.05$ ). Nitrogen flux for *T. pruinosa* was estimated by using average %N of other species along with DM measurements for *T. pruinosa*.

**Table 2.** Cicada emergence densities and percent similarity of plant communities. Each column in the plant community similarity portion of the table has similarity values relating plant communities from all other sites to a site with maximum cicada density for one species (e.g. UP2 for *T. aurifera* and *C. calliope*). Also shown are adjusted  $r^2$  and p-values for regressions of cicada emergence and each column of similarity values. Regressions that were significant at  $p < 0.01$  are shown in bold print.

Site	Cicada emergence (ind m <sup>-2</sup> )				Plant Community Similarity (Percent Similarity)		
	Cc	Ta	Td	Tp	UP2	LP1	RF1
UP 1	3.0	4.4	1.1	0.0	0.689	0.666	0.052
UP 2	11.5	15	1.0	0.0	1.000	0.678	0.032
UP 3	5.0	11.5	1.5	0.0	0.649	0.492	0.023
LP 1	3.5	5.0	4.5	0.0	0.678	1.000	0.026
LP 2	1.5	6.5	1.5	0.0	0.747	0.700	0.050
LP 3	0.5	6.0	2.5	0.0	0.233	0.118	0.014
RF 1	0.0	0.0	0.4	1.6	0.032	0.026	1.000
RF 2	0.0	0.0	0.5	0.5	0.073	0.050	0.440
RF 3	0.0	0.0	0.0	1.0	0.052	0.026	0.490
<i>C. calliope</i>				$r^2$	<b>0.620</b>	-	-
				p	<b>0.007</b>	-	-
<i>T. aurifera</i>				$r^2$	<b>0.662</b>	-	-
				p	<b>0.005</b>	-	-
<i>T. dorsata</i>				$r^2$	-	0.338	-
				p	-	0.059	-
<i>T. pruinosa</i>				$r^2$	-	-	<b>0.962</b>
				p	-	-	<b>0.000</b>

Note: For site, UP = upland prairie sites, LP = lowland prairie sites, RF = riparian forest sites. For cicada emergence, Cc = *Cicadetta calliope*, Ta = *Tibicen aurifera*, Td = *Tibicen dorsata*, Tp = *Tibicen pruinosa*

**Table 3.** Tissue chemistry for males, females, and exuviae of the three dominant species of grassland cicadas on KPRNA. Dry mass and AFDM values are means per individual. Tissue chemistry values are means (n = 10 for species n = 5 for sexes).

Species	dry mass (g)	AFDM (g)	%N	%C	C/N
<i>C. calliope</i>	0.0355	0.0337	10.73 ab	52.90 b	5.01 ab
male	0.0255	0.0238	11.60	51.59	4.50
female	0.0455	0.0435	9.87	54.21	5.52
exuviae	0.0055	0.0038	5.96	29.32	4.92
<i>T. aurifera</i>	0.1904	0.1829	11.26 a	53.04 b	4.78 b
male	0.1750	0.1679	12.03	51.83	4.34
female	0.2058	0.1979	10.48	54.24	5.23
exuviae	0.0595	0.0296	7.88	38.38	4.87
<i>T. dorsata</i>	0.9751	0.9488	10.15 b	55.50 a	5.57 a
male	0.8606	0.8366	10.36	54.18	5.26
female	1.0896	1.0610	9.93	56.82	5.88
exuviae	0.1954	0.1046	n/d	n/d	n/d
<b>All males</b>	0.3537	0.3428	11.33 a	52.53 b	4.70 b
<b>All females</b>	0.4470	0.4341	10.10 b	55.09 a	5.55 a

Note: Means followed by different letters within a column are significantly different from one another (Duncan's LSD,  $\alpha = 0.05$ ). n/d = not determined.

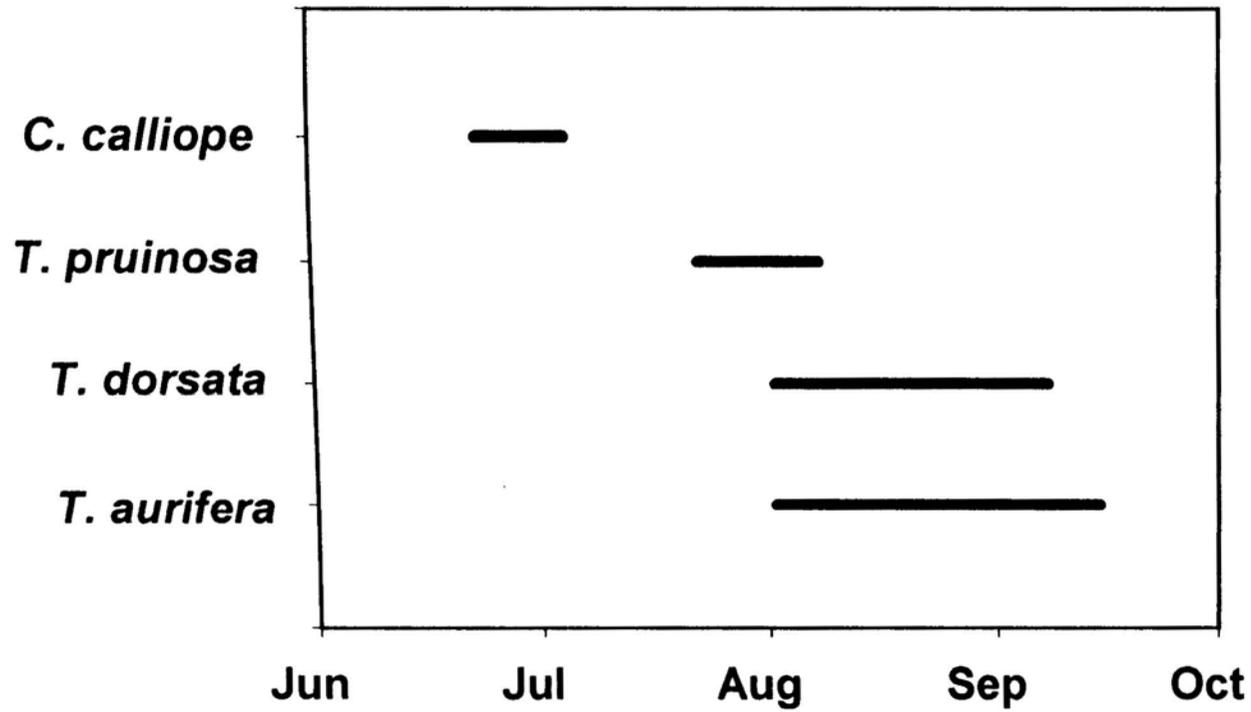


Figure 1. Emergence phenology for dominant cicada species at KPRNA during the growing season of 1998. Note: emergence of a single specimen of *T. lyricen* is included with *T. pruinosa*.

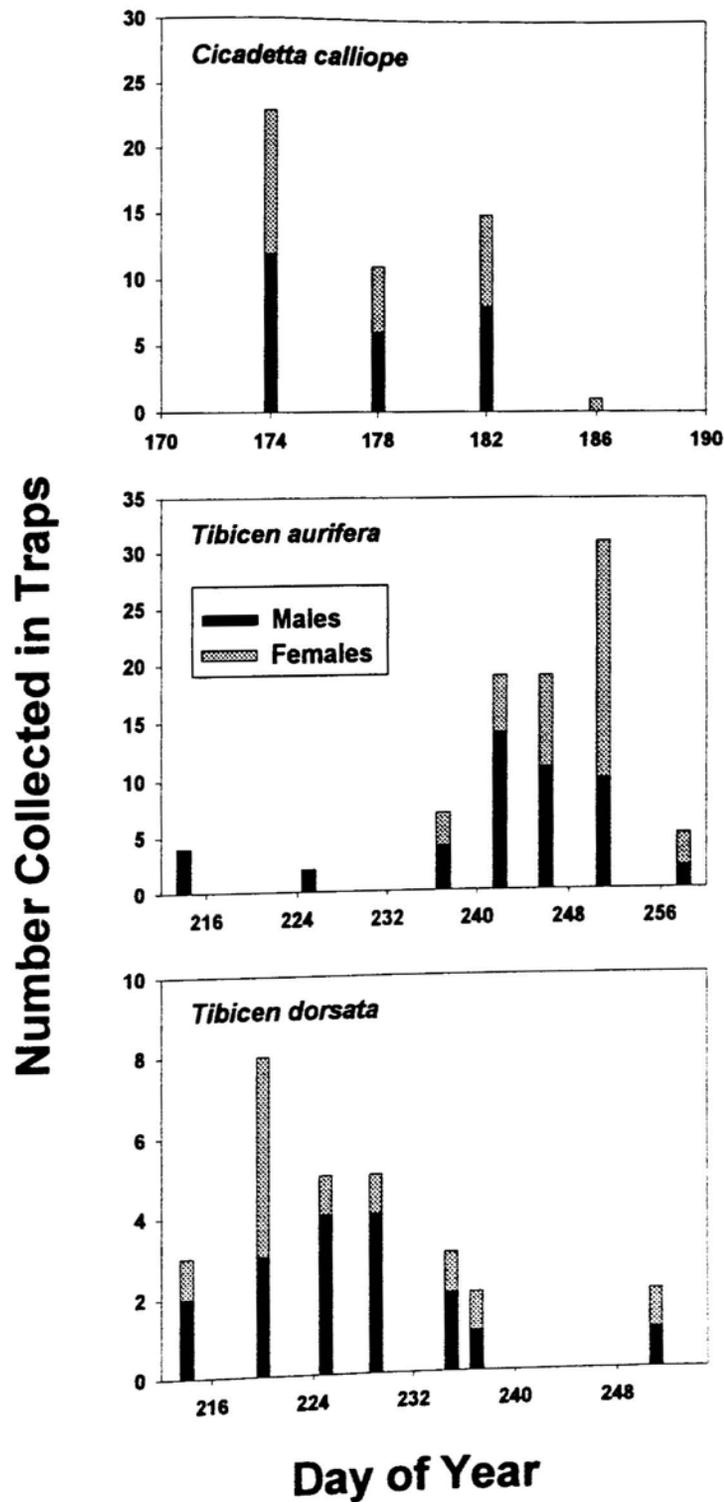


Figure 2. Sex-specific emergence densities through time of cicadas in grassland habitats at KPRNA. Note differences in scales for each plot.



**CHAPTER 4:**

**INFLUENCE OF ANNUAL FIRE, MOWING AND FERTILIZATION ON  
EMERGENCE DENSITY AND BIOMASS OF TWO ANNUAL CICADAS  
(HOMOPTERA: CICADIDAE) IN TALLGRASS PRAIRIE**

Mac A. Callaham, Jr.<sup>1</sup>, Matt R. Whiles<sup>2</sup>, and John M. Blair<sup>1</sup>

<sup>1</sup>Division of Biology, Kansas State University, Manhattan KS 66506-4901

<sup>2</sup>Department of Zoology, Life Sciences II, 251

University of Southern Illinois, Carbondale, IL 62901-6501

## ABSTRACT

The ecology of annual cicadas in tallgrass prairie is poorly documented. These large insects are abundant, and their annual emergence represents an important flux of energy and nutrients. However, factors influencing their distribution and abundance are virtually unknown. Two important land management practices commonly employed in the Flint Hills region of Kansas are annual burning of rangeland and mowing grassland vegetation for hay during the growing season. In 1999, we trapped emerging cicadas in plots from a long-term experimental manipulation aimed at examining effects of these land management practices on belowground organisms and processes. The study utilized plots managed in a factorial experimental design which incorporates annual burning, mowing, and fertilization (100 kg N ha<sup>-1</sup> and 10 kg P ha<sup>-1</sup>). Results showed that *Cicadetta calliope* responded positively to fire, but negatively to mowing, and individuals were most abundant in plots that were burned, unmowed, and fertilized. We suggest that this increased abundance of cicadas is a direct response to increased availability of oviposition sites aboveground. Furthermore, females of *C. calliope* showed a statistically significant increase in overall body size in response to fertilization, with average increases in body size of ~9% relative to individuals from unfertilized plots. Another species, *Tibicen aurifera*, responded negatively to fire, and was most abundant in unburned plots. The mechanism behind this negative response to fire is unclear, but may be related to differences in plant tissue quality belowground in unburned plots. In contrast to *C. calliope*, density of *T. aurifera* was unaffected by mowing or fertilization, but individual females of *T. aurifera* were significantly larger in fertilized plots relative to unfertilized. Cicada emergence resulted in N fluxes ranging from 0.05-0.16 g N m<sup>-2</sup> in

unburned plots, but there were negligible N fluxes from annually burned plots with the exception of burned, unmowed, and fertilized plots where high density of *C. calliope* resulted in a flux of 0.16 g N m<sup>-2</sup>. Observed differences in cicada abundances (and subsequent N fluxes) were clearly related to land management practices and attributable in part to the resultant impacts of such management upon plant community composition and structure.

**Key Words:** Cicadas, Macroarthropods, Fire, Insect emergence, Land management, Nitrogen flux, Grassland

## INTRODUCTION

The basic biology and ecology of annual cicadas in North American grasslands are poorly understood, and have only recently begun to receive serious attention after a hiatus of more than 70 years in cicada research in tallgrass prairie (Beamer 1928, Callaham et al. *In press*). However, these large insects are abundant in tallgrass prairie ecosystems, and their annual emergence represents a significant flux of energy and nutrients from belowground to aboveground (Callaham et al. *In press*). Previous investigations have shown that topographic position in the tallgrass prairie landscape can influence the distribution and abundance of different cicada species, but the influences of commonly employed land management practices, such as annual fire or haying, on these insects have yet to be examined.

The Flint Hills region of eastern Kansas represents the largest contiguous tract of relatively undisturbed tallgrass prairie vegetation in North America. The great majority (>95%) of land area once covered in tallgrass prairie has, in the last 150 years, been

converted to agricultural or urban use (Sampson and Knopf 1994). The Flint Hills region escaped large scale conversion to row-crop agriculture because of its relatively steep topography and rocky soils, and consequently the principal land use in this region is grazing of livestock on native prairie vegetation. A common management practice used for maintenance of this rangeland is annual burning of detritus in the spring. Land managers use fire as a tool because removal of grass litter from the soil surface results in increased productivity of warm-season grasses and thus increased amounts of forage for livestock (Knapp et al. 1998a, Knapp and Seastedt 1986). In addition to increased grass production, annual or semi-annual burning limits the encroachment of woody vegetation which is not suitable for grazing (Briggs et al. 1998). Another important and widespread land use in areas not directly grazed by livestock is mowing of large areas of grass vegetation for hay in mid-summer. Although the effects of these different land management practices on plant community composition and aboveground primary production are well documented (Gibson et al. 1993, Collins et al. 1998, Knapp et al. 1998a, Knapp and Seastedt 1986), the effects that such practices have on various consumer groups, particularly those living belowground, are not well understood (Rice et al. 1998).

Cicadas are long-lived belowground herbivores that are abundant in soils of tallgrass prairie. Previous investigations of the effects of disturbances (burning, mowing, etc.) on belowground invertebrates have yielded variable results. For example, Seastedt (1984) found no differences in cicada density in plots that had been either burned annually or left unburned for five years. However, in a later study, Seastedt et al. (1986) found that densities of cicada nymphs, along with other belowground herbivores, were

higher in burned plots relative to plots that were mowed, but that there was no statistical difference between burned and unburned plots. It is notable that these previous studies of cicada responses to disturbances in tallgrass prairie addressed changes in the entire belowground invertebrate community and did not determine cicada responses at levels of taxonomic resolution below that of family. Additionally, these studies were all conducted with short term experimental manipulations (relative to the typical cicada life cycle), and as a result, cicada responses to experimental treatments may not have had time to be fully manifested. For example, fire induced changes in plant community composition, a factor which may underlie belowground herbivore responses, result from indirect effects of fire and accrue over long periods (Knapp et al. 1998b, Collins and Steinauer 1998) The shortest known cicada life cycle is four years (Borror et al. 1989), and it is possible that idiosyncratic, species-specific, responses of cicadas may have been outside the purview of these studies.

Our objectives for this study were to examine the responses of cicadas (at the species level) to long-term (10+ years) burning, mowing, fertilizer treatments. We hypothesized that in the context of a long-term experimental manipulation, we would be able to detect differences in the density and biomass of two common cicada species, *Tibicen aurifera* and *Cicadetta calliope*, with respect to different land management practices.

## MATERIALS AND METHODS

### *Site Description and Experimental Design*

This study was conducted at the Konza Prairie Biological Station (KPBS) in the Flint Hills of eastern Kansas. This Long-Term Ecological Research site is owned by the

Nature Conservancy and operated by the Division of Biology at Kansas State University. The climate at the site is continental with average annual precipitation of 835 mm (75% of which falls during the growing season), and mean monthly temperatures are  $-4^{\circ}\text{C}$  in January and  $27^{\circ}\text{C}$  in July (Knapp et al. 1998a). For this study we utilized selected plots from the Belowground Plots Experiment, a long-term experimental manipulation that has been underway at KPBS since 1986. The long-term objective of the Belowground Plot Experiment has been to determine the influences of different land management practices on belowground communities and processes (Rice et al. 1998). Specifically, the treatment structure of the experiment is a three-way factorial arrangement of two burning treatments (annual Spring burning or not burned), two mowing treatments (mowed and raked annually in early July or not mowed), and four different nutrient addition treatments (plots with no nutrients added, plots with  $10\text{ g N m}^{-2}$  added, plots with  $1\text{ g P m}^{-2}$  added, and plots with both N and P added). Thus in the 64 total plots, there are 4 replicates for each of 16 treatment combinations arranged in a split-strip plot experimental design structure with burning treatments applied to whole plots, mowing applied to half of each sub-plot, and fertilization treatments applied in opposing strips within the plots (Todd 1996). For our study, we utilized a subset of half of all possible treatment combinations by using plots that were burned or unburned, mowed or unmowed, and fertilized with both N and P or unfertilized.

### *Field and Lab Methods*

We determined densities of emerging cicadas from the experimental plots by trapping them in their adult form as they emerged from belowground (see Callaham et al.

*In press* and Whiles et al. *In press*). Traps were constructed of wire mesh (~3 mm aperture) cylinders with a basal area of 0.25 m<sup>2</sup>. Three traps were placed in each plot and traps were checked for cicadas every 2 or 3 days for the duration of the growing season. We also collected the shed exuviae from emerged cicadas, and used these to verify the total number of adults trapped during the growing season. At the end of the growing season (mid October) when traps were disassembled, we carefully searched the vegetation and soil surface inside the traps for any dead individuals or exuviae that may have been missed during the summer.

Twice during the growing season, we assessed the vegetation structure in plots where trapping occurred. Sampling dates were chosen to coincide with periods of oviposition for the different cicada species (06 July for *C. calliope* and 06 October for *T. aurifera*). This sampling consisted of counting shoots of grasses and forbs in small quadrats (10 x 20 cm) randomly located within the experimental plots. We counted shoots in 3 quadrats for each plot on both dates. In addition to total plant shoots, we also counted the number of grass flowering culms and forb stems in the quadrats that were suitable sites for cicada oviposition to estimate the number of available oviposition sites under different management conditions. Cicadas use any vegetation that fits certain size requirements for oviposition (personal observations) and thus, for our estimates, oviposition sites were defined as any grass flowering stalk (culm) or forb stem within the sampling quadrat.

In order to assess the effects of fertilizer application on body size of cicadas, we measured individual cicadas from fertilized and control plots on a dissecting scope fitted with an ocular micrometer (for *C. calliope*) or manual calipers (for *T. aurifera*). We used

length of body parts as a conservative measure of size instead of individual body mass because of concerns that females may have lost body mass (through oviposition) by the time they were collected. We made 3 different measurements for each individual including head width at the eyes, total body length, and forewing length. For *C. calliope*, we measured 10 females from fertilized plots and compared them to 10 females collected from similar grasslands >1 km off-site because too few individuals were collected from the unfertilized experimental plots. For male *C. calliope* we measured 7 individuals from fertilized plots and 4 individuals from unfertilized plots. For *T. aurifera*, N=7 for males and females from both fertilized and unfertilized plots. Additionally, we selected a subset (N=5 for each sex of *C. calliope*, and N=7 for each sex of *T. aurifera*) of male and female individuals from fertilized and unfertilized plots and performed tissue nitrogen analysis. Individual cicadas were freeze-dried and ground with mortar and pestle and tissue N content was determined by coupled combustion and gas chromatography (Carlo-Erba NA1500).

### *Statistical Analyses*

Estimates of density (number of individuals per square meter) were calculated by averaging emergence over the three traps in a given plot and using the plot averages in statistical analyses (i.e. N=4 for a given experimental treatment). We tested for differences in means by subjecting the data to mixed model analysis of variance (PROC MIXED, SAS Institute, Cary NC) suitable for analyzing split-strip plot experimental data. Energy and nutrient fluxes (g biomass m<sup>-2</sup> and g N m<sup>-2</sup>, respectively) were estimated by multiplying average mass of final instar nymphs collected from fertilized and unfertilized

plots (Callaham, unpubl. data) by density and tissue N concentration of emergent individuals of each species from fertilized and unfertilized plots.

We used linear regression analysis to explore relationships between plant community structure and cicada emergence patterns. We performed regressions relating cicada density to all of the parameters collected during the sampling described above (grass stems, forb stems, oviposition sites, and proportion of total stems that were oviposition sites), as well as regressions relating cicada emergence to the percent cover of selected plant species (data from KPBS Belowground Plot Experiment data archive) including the dominant warm-season grasses, *Andropogon gerardii*, *Sorghastrum nutans*, *Andropogon scoparius*, and *Panicum virgatum*.

## RESULTS

### *Cicada Densities*

There were clear differences in the densities of cicadas with respect to land management practices (Fig. 1). Furthermore, different cicada species had differential responses to land management practices. *Cicadetta calliope* emerged first and was strongly influenced by fire with higher densities in burned plots than in unburned plots ( $p < 0.01$ ). However, there was also a strong interaction between fire and mowing for this species, such that *C. calliope* was captured in higher numbers in plots that were burned but not mowed relative to burned plots that were mowed ( $p < 0.0001$ ). Finally, the land management practice that maximized *C. calliope* density was burning, absence of mowing and nutrient addition (Figure 1a). This effect was remarkably strong with densities of *C. calliope* ~ 4x higher in burned, unmowed and fertilized plots than the

treatment combination with the next highest densities (burned, unmowed, unfertilized plots).

The response of *T. aurifera* to different land management treatments was less complex than that of *C. calliope*, but was no less pronounced (Figure 1b). *T. aurifera* had significantly higher densities in plots that had not been burned relative to annually burned plots, with no individuals of *T. aurifera* collected in traps in burned plots (Figure 1b). *T. aurifera* was not significantly impacted by any other land management practice in this experiment.

#### *Cicada Body Size and Chemistry*

Because of strong effects of fire and mowing on distributions of cicadas, we were unable to test for effects of fire and mowing on body size or tissue chemistry (i.e. there were too few individuals collected from some treatments to perform measurements). However, we were able to test for differences in body size and tissue chemistry in terms of fertilizer effects. Fertilizer effects on cicada body size were most evident in females of both species (Table 1). For *C. calliope*, females were significantly ( $p < 0.0001$ ) larger than males and females collected from plots receiving nutrient additions were significantly larger than those from unfertilized plots. This increase in size was seen in all of the body measurements. Male *C. calliope* were less responsive to fertilizer additions, but still showed indications of larger size with significantly larger wings ( $p < 0.01$ ) and body length ( $p < 0.08$ ). *T. aurifera* also responded to fertilization (Table 1). Differences were statistically significant only for females of *T. aurifera* and were only marginally significant ( $p = 0.06$ ) for head width (Table 1). Results of tissue chemistry analysis for

cicadas collected from different fertilizer treatments revealed that individuals (both male and female) collected from fertilized plots had lower percent N than their counterparts in unfertilized plots (Table 2).

### *Energy and Nitrogen Flux*

Cicada emergence from belowground to above ground resulted in biomass and N fluxes ranging from ~0.5 to 1.3 g biomass m<sup>-2</sup> and 0.05 to 0.16 g N m<sup>-2</sup> respectively, in unburned plots (Fig. 2). Biomass of *T. aurifera* was responsible for >99% of the total N flux from burned plots. Nitrogen fluxes from unburned plots were negligible with the exception of burned, unmowed and fertilized plots where *C. calliope* density was highest. From these plots, the N flux from belowground to aboveground was similar to the largest fluxes from unburned plots (~0.16 g N m<sup>-2</sup>).

### *Cicada distribution and plant parameters*

Analyses examining the relationships between cicada abundance and various plant parameters showed that, of the many plant species examined, the percent cover of the warm-season grass species *Panicum virgatum* was best correlated with abundance of *C. calliope* in the belowground plots (data not shown). However, another parameter that was significantly related to *C. calliope* density was oviposition site density. Oviposition site density, measured as the relative density of grass flowering culms and forbs in these plots, was significantly correlated with *C. calliope* density (Fig. 3). There were no significant relationships between the density of *T. aurifera* and any of the plant parameters examined in this study.

## DISCUSSION

### *Land Management and Cicada Density*

There were profound effects of land management on the density of both cicada species examined in this study (Fig. 1). Although both species have been demonstrated to feed on C<sub>4</sub> grasses (Callaham et al. 2000), the responses of the two species with respect to land management (which differentially affect grass cover) were divergent. The response of *C. calliope* to land management treatments was marked by a strong interaction between burning, mowing, and fertilization (Fig. 1a). Fortunately, there were other biological parameters in this experiment that showed similar interactive responses to treatment combinations, allowing a potential explanation for the *C. calliope* response. Specifically, the density of grass shoots and the proportion of these shoots that were suitable sites for oviposition (i.e. flowering stems) were greatly increased by the same combination of treatments that maximized *C. calliope* density (Fig. 3). Simply stated, our observations suggest that plots with low availability of oviposition sites (plots where potential oviposition sites were mown to <5 cm) will consequently have low densities of *C. calliope*. Therefore, it is not surprising that another experimental manipulation in this study that affects flowering stem density is also an important determinant of *C. calliope* density. In burned, unmowed plots that received fertilizer, the density of grass flowering stalks was twice that of unmowed and unfertilized plots, and this increase was accompanied by a three-fold increase in *C. calliope* density. However, it is important to note that increased cicada abundance with increased oviposition site availability could simply be the result of coincidentally increased cicada survivorship under soil conditions which promote high oviposition site availability in the plant community.

In contrast to *C. calliope*, the responses of *Tibicen aurifera* to land management were dominated by a strong negative response to burning. *T. aurifera* was collected exclusively from unburned plots, but the mechanism behind this response is unknown. Possible explanations for *T. aurifera* responses to burning center around belowground resource quality or aboveground vegetation structure. In a recent sampling of the belowground plots, root standing stock biomass was observed to be lower in unburned plots relative to burned plots. However, the tissue quality of roots (inferred from C/N) was higher in unburned plots as C/N ratios were lower than in burned plots (D. Kitchen, unpublished data). This increased root tissue quality in unburned plots may be related to changes in plant community composition (Gibson et al. 1994, Collins et al. 1998), or increased nitrogen availability in unburned plots (Blair 1997). Another potential explanation for the increased density of *T. aurifera* in unburned plots is that they are attracted to standing dead vegetation for oviposition. Exclusion of fire from tallgrass prairie results in the accumulation of a layer of detritus which can be up to 30 cm deep (Knapp and Seastedt 1986). This detrital layer may provide protection for *T. aurifera*, or may be selected by *T. aurifera* because of a low probability that it will be eaten by grazers (as senescent vegetation is of lower nutritional quality than fresh forage). Finally, exclusion of fire also results in a greater abundance of forb vegetation, and *T. aurifera* has been observed to oviposit into the stems of forbs as well as grass flowering stalks (Whiles and Callaham, personal observations). Thus, *T. aurifera*'s preference for unburned plots may be explained in part by availability of forb stems.

### *Body size and tissue chemistry*

In addition to the increase in density of *C. calliope* in fertilized plots, there was also a significant difference in the body size of individuals collected from these plots (Table 1). Furthermore, even though there was no influence of fertilization on density of *T. aurifera*, this species also responded to fertilization with larger females emerging from fertilized plots. Presumably, this increase in body size is directly related to the increased quality (and/or quantity) of plant resources available for cicada consumption in fertilized plots. Whatever the direct cause of larger body size in cicadas from fertilized plots, the likely result is increased fecundity of these larger individuals relative to their counterparts from unfertilized plots. Increased fecundity with larger body sized has been demonstrated for several arthropod taxa (e.g. Hogue and Hawkins 1991, Slansky and Scriber 1985, Sweeny and Vannote 1981).

Results from cicada tissue analysis revealed the interesting pattern of higher N content (as percent of dry mass) in cicadas from unfertilized plots. The mechanism behind this phenomenon is unclear, but may be related to several factors. One potential explanation is that cicadas grown in plots with relatively high N availability were able to put more effort into energy storage, and because these carbon rich storage molecules made up more of their total body weight, the C/N ratio of their tissues was increased. Fat content has been shown to increase with body size in digger wasps (Strohm 2000), and it is likely that this relationship exists in cicadas also. Another possible explanation for higher relative N content in cicadas from unfertilized plots is that plant species composition in fertilized plots was responsive to such a degree that differences in plant

tissue composition (and limitation of cicada feeding options) were responsible for differences in cicada tissue chemistry.

### *Nitrogen flux*

Results of this study show that in unburned, unfertilized plots the emergence of cicadas represents a significant flux of N from belowground to aboveground (Fig. 2). This is primarily due to the emergence of *T. aurifera* from unburned, unfertilized plots. *T. aurifera* is a large bodied cicada (~4x the size of *C. calliope*), and as such has a much larger per capita impact on nutrient flux. Cicada emergence from unburned plots resulted in fluxes of 0.05-0.16 g N m<sup>-2</sup> which is equivalent to ~10% of the total annual input of N via precipitation in these systems (Blair et al. 1998). The magnitude of N flux associated with cicada emergence is comparable to other system-level N fluxes, including hydrologic export and gaseous N fluxes (Blair et al. 1998), though the fate of this cicada biomass N is uncertain. The only burned plot that exhibited significant flux of N in cicada biomass was the burned, unmowed and fertilized plots. The fact that these plots received fertilizer at a rate of 10 g N m<sup>-2</sup> makes the flux in cicada biomass negligible (~1.5%). The cicada mediated fluxes of N from the burned plots in this experiment were similar to those observed for *T. aurifera* and *C. calliope* in lowland prairie by Callaham et al. (2000) in a landscape level study.

### *A Conceptual Model*

The results of this plot level study led us to consider possible manifestations of the observed cicada density and distribution phenomena at the landscape level. For

example, what is the adaptive significance of preferential use of unburned vegetation in a landscape characterized by periodic occurrence of fire (as is the case with *T. aurifera*)? However, fire is not the only important force shaping tallgrass prairie vegetation. Grazing activity has been demonstrated to be instrumental in the mediation of plant species diversity (Collins et al. 1999), and bison have been dubbed a keystone species because of their importance in this process (Knapp et al. 1999). Grazing activity of large ungulates (by American bison) is considered to have been important in the historical development and maintenance of the tallgrass prairie biome, and large ungulate grazing (domestic cattle) continues to have significant impact on this ecosystem in the present (Briggs et al. 1998). In fact, grazers and fire interact with one another to generate a vegetation mosaic of burned and unburned patches across the landscape at multiple scales (ranging from 10-1000 m), and this mosaic may be influential in the distribution of cicadas over such scales (for illustration see Fig. 1 of Knapp et al. 1998). Here, we propose a conceptual model that takes into account the interactive effects of fire and grazing on vegetation characteristics in tallgrass prairie, and superimposes these characteristics on the empirical observations for cicada distributions from this study (Fig. 4). This conceptual model suggests that cicada oviposition behavior is determined by the vegetation mosaic imposed by fire and grazing. Potential mechanisms behind *T. aurifera* selecting standing dead vegetation include the decreased likelihood of egg mortality resulting from consumption by ungulates, as grazers frequently avoid unburned areas in favor of recently burned forage. If *T. aurifera* does exhibit some preference for oviposition in standing dead vegetation (as observed in this study, Fig. 1b), then oviposition would likely occur in unburned patches in the landscape. In subsequent

years, however, unburned/ungrazed patches from previous years will have higher fuel loads and will have a higher probability of burning in the next fire event (G. Hoch, unpublished data). It is therefore likely that grazers will preferentially switch from burned patch to unburned patch from year to year. In contrast to *T. aurifera*, *C. calliope* preferentially oviposits in areas that have been recently burned (Fig 1a), and in the context of the landscape mosaic of burned and unburned areas will, in any given year, oviposit in areas not utilized by *T. aurifera*. This differential use of burned and unburned areas in the landscape amounts to spatial and temporal resource partitioning between the two species, and our model predicts that the likelihood is low that first instar nymphs of both species will hatch into the same soil volume in the same year. Thus, our model predicts that the interaction between cicadas, grazers and fire is a shifting mosaic that results in cicada oviposition in different locations in the landscape from year to year (Fig. 4). This conceptual framework provides many testable hypotheses, and more work is needed to confirm the operation of mechanisms proposed.

#### LITERATURE CITED

- Beamer, R.H. 1928. Studies on the biology of Kansas Cicadidae. Kansas University Science Bulletin 20:155-263.
- Blair, J.M. 1997. Fire, N availability, and plant response in grasslands: a test of the transient maxima hypothesis. Ecology 78:2359-2368.
- Blair, J.M., T.R. Seastedt, C.W. Rice, R.A. Ramundo. 1998. Terrestrial nutrient cycling in tallgrass prairie. In: Knapp, A.K., J.M. Briggs, D.C. Hartnett, and S.L. Collins (eds.). Grassland Dynamics: Long-term Ecological Research in Tallgrass

- Prairie. Oxford University Press, New York, pp. 222-243.
- Briggs, J.M., M.D. Nellis, C.L. Turner, G.M. Henebry, and H. Su. 1998. A landscape perspective of patterns and processes in tallgrass prairie. In: Knapp, A.K., J.M. Briggs, D.C. Hartnett, and S.L. Collins (eds.). *Grassland Dynamics: Long-term Ecological Research in Tallgrass Prairie*. Oxford University Press, New York, pp. 265-279.
- Callaham, M.A. Jr., M.R. Whiles, C.K. Meyer, B.L. Brock, R.E. Charlton.  
Feeding ecology and emergence production of annual cicadas (Homoptera: Cicadidae) in tallgrass prairie. *Oecologia*, In press.
- Collins, S.L., A.K. Knapp, J.M. Briggs, J.M. Blair, and E.M. Steinauer. 1998.  
Modulation of diversity by grazing and mowing in native tallgrass prairie. *Science* 280:745-747.
- Collins, S.L., and E.M. Steinauer. 1998. Disturbance, diversity, and species interactions in tallgrass prairie. In: Knapp, A.K., J.M. Briggs, D.C. Hartnett, and S.L. Collins (eds.). *Grassland Dynamics: Long-term Ecological Research in Tallgrass Prairie*. Oxford University Press, New York, pp. 140-156.
- Knapp, A.K., J.M. Blair, J.M. Briggs, S.L. Collins, D.C. Hartnett, L.C. Johnson, and E.G. Towne. 1999. The keystone role of bison in North American tallgrass prairie. *BioScience* 49:39-50.
- Knapp, A.K., J.M. Briggs, D.C. Hartnett, and S.L. Collins (eds.). 1998a. *Grassland Dynamics: Long-term Ecological Research in Tallgrass Prairie*. Oxford University Press, New York, 364 pages.
- Knapp, A.K., J.M. Blair and J.M. Briggs. 1998. Long-term ecological consequences of

- varying fire frequency in a humid grassland in T.L. Pruden and L.A. Brennan, editors. *Fire in Ecosystem Management: Shifting The Paradigm from Suppression to Prescription*. Tall Timbers Fire Ecology Conference Proceedings No. 20., Tall Timbers Research Station, Tallahassee, FL. pp. 173-178.
- Knapp, A.K., and T.R. Seastedt. 1986. Detritus accumulation limits productivity in tallgrass prairie. *BioScience* 36:662-668.
- Rice, C.W., T.C. Todd, J.M. Blair, T.R. Seastedt, R.A. Ramundo, and G.W.T. Wilson. 1998. Belowground Biology and Processes. In: Knapp, A.K., J.M. Briggs, D.C. Hartnett, and S.L. Collins (eds.). *Grassland Dynamics: Long-term Ecological Research in Tallgrass Prairie*. Oxford University Press, New York, pp. 244-264.
- Samson, F. and F. Knopf. 1994. Prairie conservation in North America. *BioScience* 44:418-421.
- Seastedt, T.R. 1984 b. Belowground macroarthropods of annually burned and unburned tallgrass prairie. *American Midland Naturalist* 111:405-408.
- Seastedt, T.R., D.C. Hayes, and N.J. Petersen. 1986. Effects of vegetation, burning, and mowing on soil macroarthropods of tallgrass prairie. In: Clambey, G.K., and R.H. Pemble (eds.), *Proceedings of the Ninth North American Prairie Conference*, Tri-College Press, Fargo, pp. 99-102.
- Strohm E. 2000. Factors affecting body size and fat content in a digger wasp. *Oecologia* 123:184-191.
- Slansky F., and J.M. Scriber. 1985. Food consumption and utilization. In: Kerkut G. and L. Gilbert (eds.), *Comprehensive insect physiology, biochemistry, and pharmacology*. Pergamon, Oxford, pp. 87-161.

Todd, T.C. 1996. Effects of management practices on nematode community structure in tallgrass prairie. *Applied Soil Ecology* 3:235-246.

Table 1. Body measurements of two cicada species from fertilized and unfertilized plots. All measurements are in millimeters (mm). Significance of differences between means are indicated (p-value) (Least Squares means separation procedure).

		<u><i>Cicadetta calliope</i></u>		<u><i>Tibicen aurifera</i></u>	
		<u>Male</u>	<u>Female</u>	<u>Male</u>	<u>Female</u>
Head Width	+Nutrients	4.24	4.64	10.91	10.71
	-Nutrients	4.18	4.45	10.62	10.36
	p-value	0.505	0.011	0.133	0.064
Body Length	+Nutrients	13.26	15.90	24.93	24.20
	-Nutrients	12.43	14.27	25.47	22.94
	p-value	0.071	0.0001	0.354	0.039
Wing Length	+Nutrients	16.30	17.30	31.09	30.67
	-Nutrients	15.16	15.76	30.12	29.06
	p-value	0.006	0.0001	0.115	0.010

Table 2. Tissue chemistry of male and female *Cicadetta calliope* and *Tibicen aurifera*. Values are means (standard error in parentheses).

Species	%N		%C		C/N	
	+Nutrients	-Nutrients	+Nutrients	-Nutrients	+Nutrients	-Nutrients
<i>Cicadetta calliope</i>						
Male	10.90 (0.53)	11.74 (0.99)	54.42 (0.89)	53.23 (1.44)	5.09 (0.40)	4.62 (0.52)
Female	9.00 (0.14)*	10.97 (0.48)	56.68 (0.72) <sup>†</sup>	54.29 (0.94)	6.30 (0.11)	5.00 (0.31)
<i>Tibicen aurifera</i>						
Male	11.57 (0.27)	11.75 (0.40)	53.97 (0.43)	53.82 (0.48)	4.68 (0.12)	4.61 (0.18)
Female	11.85 (0.12) <sup>†</sup>	11.39 (0.17)	56.33 (0.35)*	55.02 (0.23)	5.20 (0.09)	4.84 (0.09)

Note: Within species, sex, and element, means followed by \* are significantly different from one another ( $p < 0.05$ ), and <sup>†</sup> ( $p < 0.10$ ) (Least Squares means separation procedure).

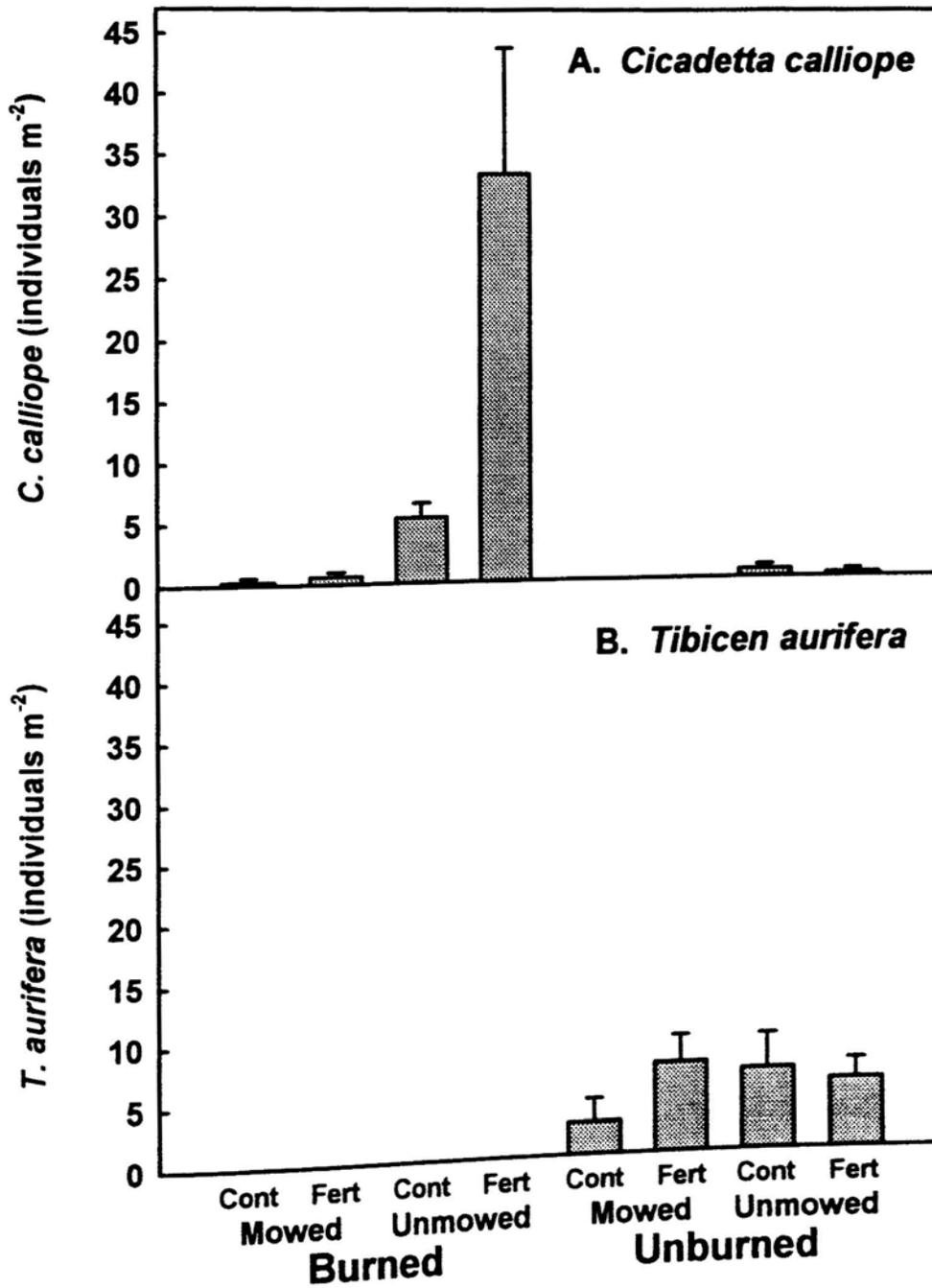


Figure 1. Densities of adult A) *Cicadetta calliope* and B) *Tibicen aurifera* emerging from selected treatments in the Belowground Plot Experiment, Summer 1999.

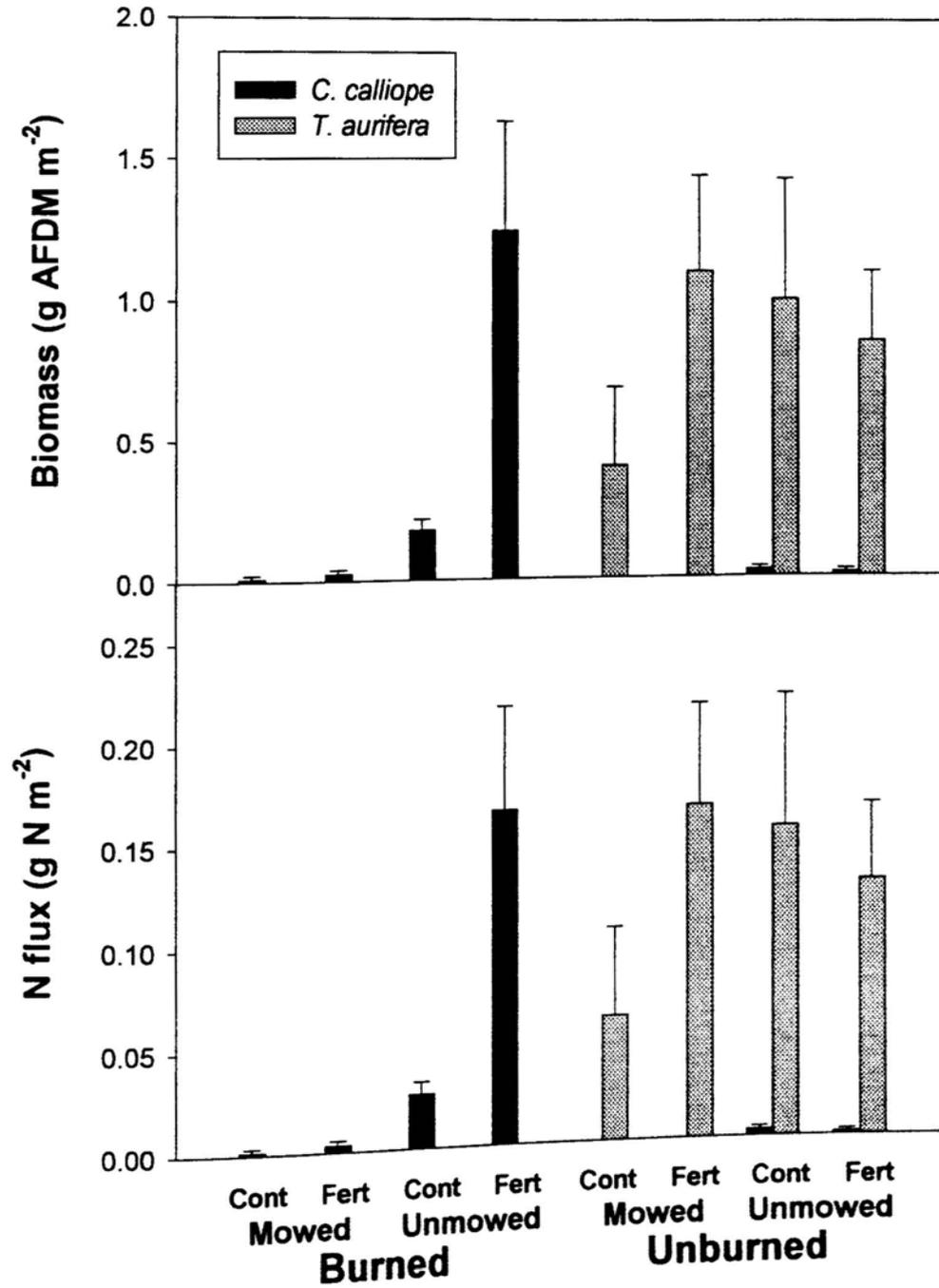


Figure 2. Energy as ash-free dry mass (AFDM) and nitrogen fluxes associated with cicada emergence from the Belowground Plot Experiment.

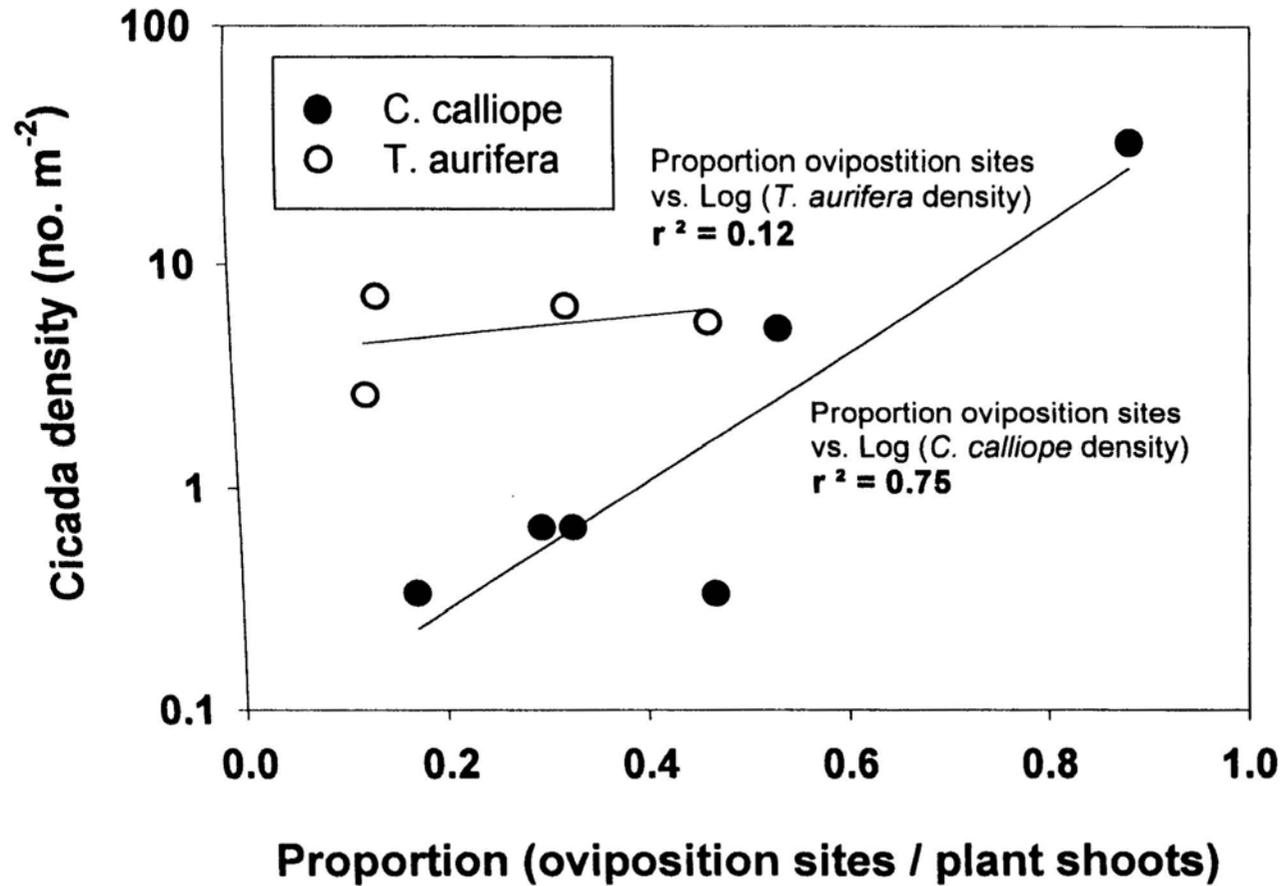


Figure 3. Relationship between oviposition site availability and cicada density for both species (oviposition site availability is defined as the proportion of total plant shoots with either grass flowering culms or forb stems suitable for oviposition).

Note: y-axis is log scale.

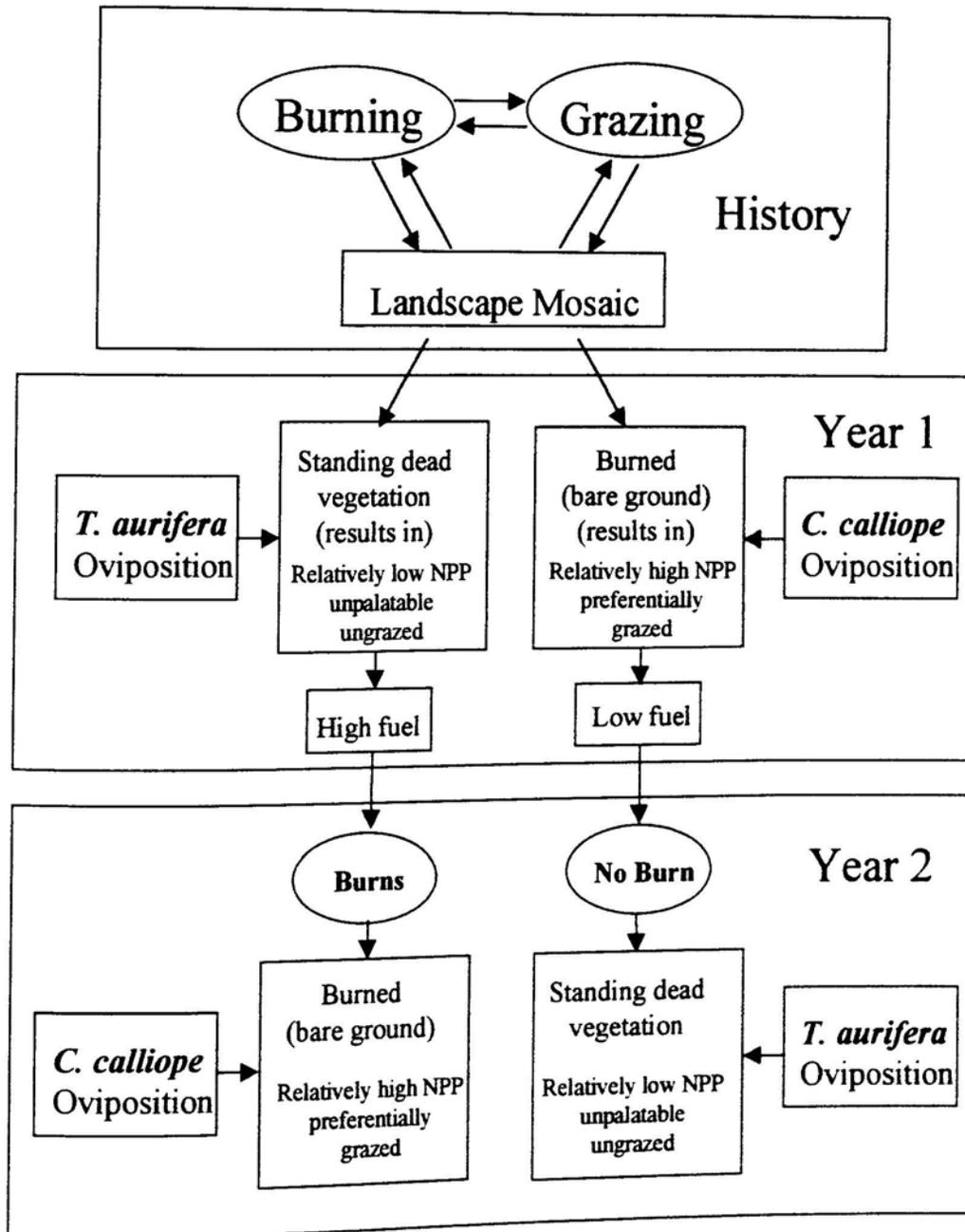


Figure 4. Conceptual model outlining possible interactions between cicadas and large ungulate grazers. Cicadas are hypothesized to partition resources spatially in the context of landscape level influences that grazers exert on aboveground vegetation structure.

**CHAPTER 5:**

**INFLUENCE OF FIRE, MOWING, AND FERTILIZATION ON THE DENSITY  
AND BIOMASS OF BELOWGROUND MACROINVERTEBRATES IN  
TALLGRASS PRAIRIE**

Mac A. Callaham, Jr., and John M. Blair

Division of Biology, Kansas State University, Manhattan, KS 66506-4901

## ABSTRACT

The responses of tallgrass prairie plant communities and various ecosystem processes (such as primary production and nutrient cycling) to fire and grazing are well characterized. However, responses of consumer groups, and particularly organisms living belowground, to these disturbances are less well known. In 1999, we sampled belowground macroinvertebrates from plots in a long-term experiment at Konza Prairie Biological Station which is designed to examine the effects and interactions of annual fire, annual mowing, and fertilization on belowground communities and processes. We also analyzed samples from a similar sampling in 1994. Results indicated that several groups were influenced singly or interactively by fire, mowing, and fertilization. In general, European earthworms were more abundant in plots from which fire had been excluded, and the proportion of the total community made up by native earthworms decreased in unburned, unmowed, and fertilized plots. Nymphs of two cicada species were collected (*Cicadetta calliope* and *Tibicen aurifera*) with each responding differently to the treatments. *Cicadetta calliope* nymphs were more abundant in burned plots, but were also influenced by mowing, with reduced numbers in plots that had been mowed. *Tibicen aurifera* nymphs were collected almost exclusively from unburned plots and their density was positively affected by phosphorus fertilization. Responses of other invertebrate taxa to the treatments were inconsistent, and some differed compared to observations made in previous shorter-term studies. Our results suggest that departures from natural disturbance regimes (i.e. fire and grazing) may render soils more susceptible to encroachment by European earthworm taxa, and that interactions between fire and

grazers (and the resultant vegetation mosaic) may be important in the structuring of insect communities, particularly cicadas.

**Key words:** earthworms, cicadas, scarabs, herbivores, disturbance, tallgrass prairie, grassland invertebrates, fire, soil invertebrates

## INTRODUCTION

Tallgrass prairie ecosystems are maintained by periodic disturbances such as fire and grazing (Knapp et al. 1998). These disturbances serve to limit the encroachment of woody vegetation into areas that would otherwise support forest vegetation. Annual spring fire is a common land management practice in the Flint Hills region of eastern Kansas used by livestock ranchers to maintain rangeland and maximize production of warm-season forage grasses. Additionally, large land areas are mowed annually for hay (as supplemental winter feed for livestock). Responses of plant species composition to fire, mowing, and grazing disturbances are well documented (Gibson et al. 1993, Collins and Steinauer 1998, Collins et al. 1998, Knapp et al. 1999), and ecosystem process level responses to such disturbances are also well known (Knapp et al. 1998, Blair 1997, Knapp and Seastedt 1986). However, responses of belowground invertebrates to these important disturbances in tallgrass prairie are less well known (Blair et al. 2000). These organisms are important regulators of soil processes in some soil systems (*cf.* Coleman and Hendrix 2000, Coleman and Crossley 1996), and their influences on processes are often driven by their densities.

Earthworms are influential organisms in soil systems where they are abundant. Earthworms have multiple impacts on soil ecosystems through their burrowing and feeding behavior, and their effects include improved aeration, water infiltration, and incorporation of organic matter (Edwards and Bohlen 1996). Indeed, earthworms have been dubbed ecosystem engineers because of their profound physical effects on soils (Lawton et al. 1994). However, earthworms are also important in terms of soil biological processes such as nutrient cycling and microbial turnover (Brown 1995), with increased soil microbial activity and nutrient mineralization in soils where earthworms are present (e.g. Zhang and Hendrix 1995, Scheu 1987). Although earthworm effects on soil systems are relatively well known, most ecological information for earthworms comes from studies conducted with European taxa. Nevertheless, there are numerous species of native North American earthworms extant in relatively undisturbed soils, and less is known about these taxa. Large assemblages of earthworms composed of native North American species are found in the Flint Hills region of eastern Kansas (James 1990, 1995), but European taxa have been introduced to the area and are expanding into soils occupied by native earthworms (Callaham and Blair 1999). Changes in land management such as exclusion of fire and/or expansion of forest vegetation in the Flint Hills (Briggs et al. 1998), may be related to the establishment of exotic European earthworms, but the interactions between native and exotic earthworm populations are poorly understood.

Herbivorous insects make up the majority of individuals and biomass of belowground insects in tallgrass prairie soils (Seastedt 1984, Seastedt et al. 1986). Previous investigations have shown these insects to vary in their responses to burning and

mowing, but these studies have all been performed over relatively short term experimental periods. Herbivorous insects are important regulators of plant productivity in some systems, and are frequently responsive to changes in resource quantity or quality (Schowalter 2000), and their responses to long-term changes in plant community composition, and plant derived resource quality are poorly understood in tallgrass prairie.

Objectives for the present study were to examine influences of annual fire, mowing, and nutrient addition on belowground invertebrate density and biomass (including native and exotic earthworms and herbivorous insects). We addressed these objectives in the context of a long-term plot level experiment by analyzing invertebrate samples from 1994 (after 9 years of experimental treatment) and 1999 (after 14 years of experimental treatment).

## **MATERIALS AND METHODS**

### *Site Description and Experimental Design*

This study was conducted at the Konza Prairie Biological Station (KPBS) in the Flint Hills of eastern Kansas. This Long-Term Ecological Research site is owned by the Nature Conservancy and operated by the Division of Biology at Kansas State University. The climate at the site is continental with average annual precipitation of 835 mm (75% of which falls during the growing season), and mean monthly temperatures are  $-4^{\circ}\text{C}$  in January and  $27^{\circ}\text{C}$  in July (Knapp et al. 1998). For this study we sampled plots in the Belowground Plot Experiment, a long-term experimental manipulation that has been underway at KPBS since 1986. The long-term objective of the Belowground Plot Experiment has been to determine the influences of different land management practices

on belowground communities and processes (Rice et al. 1998). Specifically, the treatment structure of the experiment is a three-way factorial arrangement of two burning treatments (annual Spring burning or not burned), two mowing treatments (mowed and raked annually in early July or not mowed), and four different nutrient addition treatments (plots with no nutrients added, plots with 10.0 g N m<sup>-2</sup> added, plots with 1.0 g P m<sup>-2</sup> added, and plots with both N and P added). Thus in the 64 total plots, there are 4 replicates for each of 16 treatment combinations arranged in a split-strip plot experimental design structure with burning treatments applied to whole plots and mowing treatments applied to half of the sub-plots and fertilization treatments applied in opposing strips within mowing treatments (Todd 1996).

#### *Field and Laboratory Methods*

To estimate invertebrate densities, we sampled all 64 plots in a one week period in early June 1999 by collecting soil monoliths (25 x 25 cm, and 25 cm depth), and sealing them in plastic bags where they were stored at 4°C until they could be carefully hand sorted. Sorting consisted of breaking all soil aggregates into pieces (<5 mm) and collecting all organisms encountered. Specimens were preserved in 70% EtOH. All native North American earthworm species were identified to genus, and European earthworms were identified to species. Arthropods were identified to order and to family when possible (and to species in the case of final instar cicadas). All identifications were made using keys from Dindal (1990). The 1994 invertebrate sampling took place over a 3 week period in October (at the end of growing season). Sampling consisted of digging

monoliths (20 x 50 cm, 40 cm deep) and passing all soil in the monolith through a 6 mm screen in the field. Specimens were preserved and identified as with the 1999 sampling.

We estimated invertebrate biomass as ash-free dry mass (AFDM), by ashing individuals of each taxonomic group from the 1999 sampling at >450°C for >6 h, and subtracting remaining ash mass from dry (50°C) mass. We used biomass data from 1999 sampling to generate linear regression equations to estimate AFDM from dry mass values for the 1994 specimens. Equations used for dry mass to AFDM conversions are shown in Table 1.

### *Statistical Analyses*

All data were subjected to mixed model analysis of variance suitable for the analysis of split-strip plot experimental designs (PROC Mixed, SAS Institute, Cary NC). Means separation analysis was Least Squared Means (LSMEANS/pdiff option, SAS Institute).

## RESULTS

### *Earthworms*

Earthworm densities in the 1994 sampling indicated no response of native earthworms (*Diplocardia* spp.) to any single land management treatment (Fig. 1a), but there was a significant three way interaction ( $p=0.02$ ) between the three treatments. In general, within a set of burning and mowing treatments, the effect of N fertilization (N and F treatments on Fig. 1) was a decrease in the abundance of *Diplocardia* spp. There were no significant (at  $\alpha = 0.1$ ) differences in the abundance of *Aporrectodea trapezoides* (a European exotic) in response to experimental treatments in 1994 (Fig. 1b). Earthworm

biomass in 1994 showed similar trends to earthworm abundance (Fig 2.) except that individuals of European earthworms were larger than native earthworms in all treatments. This larger per capita biomass of European earthworms affected the proportion of the total earthworm community biomass that consisted of native species in 1994 (Fig. 3a). This proportion was significantly affected by burning treatments, and there was a significant ( $p=0.01$ ) interaction between burning, mowing and nitrogen addition (Fig. 3a). Specifically, the proportion of the community made up of *Diplocardia* was above 75% in all burned plots except those that were unmowed and had N fertilizer added (+N and +N+P plots). In unburned plots, proportion of earthworm biomass exhibited high variability ranging from 100% (in unburned, mowed, unfertilized plots) to <5% (in unburned, mowed, fertilized +N+P)(Fig. 3a).

In 1999, earthworms exhibited different trends than those in the 1994 sampling. There was a general decline in the abundance of *Diplocardia* spp. in unburned plots, but the effect of fire exclusion was not statistically significant ( $p=0.11$ ). There was, however, a statistically significant ( $p=0.01$ ) effect of mowing on the abundance of *Diplocardia* spp. in 1999, with greater abundances in mowed plots (Fig. 4a). Also in 1999, *A. trapezoides* showed a trend of increased abundance in unburned plots ( $p=0.08$ ) (Fig 4b). Also of note from the 1999 sampling was the occurrence of another European earthworm species, *Octolasion cyaneum*, which was not collected in the 1994 sampling (Fig. 3c). The distribution of *O. cyaneum* was not significantly related to any of the experimental treatments, but was significantly related to the block in which sampling occurred. In other words, *O. cyaneum* was (with the exception of a single individual) collected exclusively from the two blocks on the eastern half of the experimental area. Earthworm

biomass in 1999 was again similar in distribution across experimental treatments, but European earthworms had higher per capita biomass (Fig. 5). The proportion of total earthworm biomass that consisted of native earthworms again illustrates this larger per capita impact of European earthworms (Fig 3b), with a steady and significant decline in this proportion in burned plots.

### *Cicadas*

In 1994, cicada nymphs showed clear responses to land management practices, with the exception of *Cicadetta calliope* nymphs. The only statistically significant response of *C. calliope* nymphs in 1994 was an interactive response to the three experimental treatments ( $p=0.04$ ), with the highest densities being collected from plots that were unmowed and received P fertilizer (Fig. 6b). In contrast to *C. calliope*, in 1994 nymphs of *T. aurifera* were collected almost exclusively from unburned plots (Fig 6a), and this difference was statistically significant ( $p=0.005$ ). Early instar cicadas also were significantly more abundant in unburned plots in 1994 ( $p=0.01$ ), and they were also significantly more abundant in mowed plots ( $p=0.04$ ) (Fig. 6c). Cicada biomass followed patterns of distribution very closely, but there were differences between species with *T. aurifera* having much higher per capita effect on biomass abundance, and overall cicada biomass was significantly higher in unburned plots (Fig 7).

In 1999, Cicada nymphs exhibited somewhat different patterns of distribution and biomass than in 1994. *Tibicen aurifera* continued to be significantly ( $p=0.02$ ) affected by fire with greater abundance in unburned plots, but there was also a significant ( $p=0.02$ ) interaction between mowing and fertilizer addition. This interaction between

experimental treatments yielded the largest numbers of *T. aurifera* nymphs in plots that were unmowed and which received P amendments ( $p=0.02$ ) (Fig. 8a). In 1999, *C. calliope* was dramatically more abundant in burned plots than in unburned plots (Fig. 8b), and there was a strong interaction between burning and mowing treatments ( $p=0.01$ ), as very few individuals of *C. calliope* were collected from plots that had been mowed in burned or unburned plots. In a similar pattern 1994, the distribution of early instar cicada nymphs in 1999 was influenced by fire ( $p=0.005$ ) and mowing ( $p=0.0005$ ) with greater abundances in unburned and unmowed plots (Fig. 8c). Cicada biomass in 1999 was similar to density patterns but with *T. aurifera* having a much larger per capita influence on total biomass flux than *C. calliope* or early instars (Fig. 9).

Other major macroinvertebrate groups collected over the course of the study included the herbivorous beetle families Scarabaeidae, Curculionidae, and Elateridae (Fig. 10). None of these taxa were significantly (at  $\alpha = 0.10$ ) affected by the experimental treatments employed in the study.

## DISCUSSION

### *Land Management Influence on Earthworm Community*

The results of this study suggest that the distribution and abundance of invasive European earthworms in North American tallgrass prairie soils are, at least in part, attributable to land management practices. Furthermore, in areas where these exotic earthworms are most prevalent, it appears that displacement of native species may be occurring. Particularly notable are the differences in earthworm community structure between the two extreme land management treatments -annually burned, mowed, without

fertilizer, and unburned, unmowed, with supplemental nutrients. For both sampling dates, the proportion of earthworm biomass composed of native taxa was 75% or higher in burned, mowed plots, but this proportion either became variable and erratic (as in 1994) or declined steadily (1999) as treatment combinations became decreasingly "prairie" like (Fig 3). The plots with the most intense natural disturbance regime (burned, mowed, with no added nutrients) most closely approximate conditions prevalent prior to European settlement (frequently burned and with aboveground biomass removal). These plots are also the most typical with respect to floristic and edaphic characteristics of tallgrass prairie (Collins et al. 1998, Knapp and Seastedt 1986), and our study demonstrates that this is true of the earthworm community in these plots as well. Previous studies of earthworm distributions in tallgrass prairie with respect to fire treatments have shown the abundance of *Diplocardia* spp. to be greater in annually burned prairie relative to prairie from which fire had been excluded for eight years (James 1982). Results of the current study are not exactly in accordance with James (1982), as we found no effect of burning on the absolute abundance of *Diplocardia* spp. in (Fig 1a), but the relative abundance of *Diplocardia* spp. was favored by burning in our study (Fig. 3). One potential explanation for the discrepancy between our study and James (1982) is the virtual absence of exotic taxa in the soils sampled by James (he reports only a single individual of *Aporrectodea* spp.), and therefore the absence of any effects on distributions arising from interactions between the two taxa.

One effect of suppressing fire and grazing disturbances in tallgrass prairie is the accumulation of plant litter on the soil surface, and the land management treatments utilized in this experiment result in what is essentially a gradient of surface litter

accumulation. Indirect effects of this litter accumulation include increased soil moisture and decreased soil temperature, particularly in the early growing season. We suggest that microclimatic consequences of differing land management practices may contribute to changes in earthworms community in tallgrass prairie. However, it is difficult to decouple the effects of litter accumulation on soil climate and the effects of burning and mowing on belowground plant production, and plant tissue quality. For example, Todd et al. (1992) demonstrated that mowing treatments resulted in significantly less live root biomass, and an increase in the abundance of *Diplocardia* spp. relative to unmowed plots. The results of the 1999 sampling from our study are similar to the findings of Todd et al. (1992), as we found increased abundance of *Diplocardia* spp. related to mowing treatments (Fig. 4), and mowing also favored the relative abundance of *Diplocardia* spp. in the earthworm community (Fig. 3).

Fire also influences the quality and amount of plant tissue inputs belowground, and this could be another factor involved in the distribution of earthworms in tallgrass prairie soil. The amount of live root tissue in annually burned plots has been shown to be significantly greater than in unburned plots, and live roots in annually burned plots can have C:N up to nearly double that of unburned plots (Blair 1997, Ojima et al. 1994). Clearly, the lower quality plant tissue could potentially be a factor limiting the expansion of exotic earthworms in tallgrass prairie.

In spite of the correlative nature of this study, and the difficulty in identifying the specific mechanisms underlying invasion by exotic earthworms, our study demonstrates that departures from natural disturbance regimes can result in detectable changes in the abundances of native and exotic earthworm taxa in these soils. The changes in

earthworm community structure may have functional consequences due to differences in the feeding ecology and behavior of these earthworm taxa (James 1991, James and Cunningham 1989, James and Seastedt 1986).

#### *Land Management Influence on Cicadas*

There were profound effects of land management on the density of each cicada species examined in this study (Figs. 6 and 8). Although both species have been demonstrated to feed on C<sub>4</sub> grasses (Callaham et al. 2000), the responses of the two species with respect to land management (which differentially affect grass cover [Gibson et al. 1993]) were divergent. The response of *C. calliope* to land management treatments was marked by a strong interaction between burning, mowing, and fertilization in 1994 (Fig. 6a), and then by an interaction between burning and mowing in 1999. The pattern from 1999 is very similar to the findings of another study on cicada responses to land management where Callaham et al. (Chapter 4) found that adults of *C. calliope* emerged exclusively from burned and unmowed plots. They hypothesized that the distributional pattern observed for *C. calliope* was primarily the result of aboveground vegetation structure and that oviposition site availability may be a determinant of *C. calliope* density.

In contrast to *C. calliope*, the responses of *Tibicen aurifera* to land management were dominated by a strong negative response to burning. *T. aurifera* was collected almost exclusively from unburned plots with only a single individual collected from an annually burned plot in the two sampling years. The mechanism behind this response is unknown. One potential explanation for *T. aurifera* responses to burning is increased

belowground resource quality in unburned prairie. In a recent sampling of the belowground plots, root standing stock biomass was observed to be lower in unburned plots relative to burned plots. However, the tissue quality of roots (inferred from C/N) was higher in unburned plots as C/N ratios were lower than in burned plots (D. Kitchen, unpublished data). This increased root tissue quality in unburned plots may be related to changes in plant community composition (Gibson et al. 1994, Collins et al. 1998), or increased nitrogen availability in unburned plots (Blair 1997). Another potential explanation for the increased density of *T. aurifera* in unburned plots is that they are attracted to standing dead vegetation for oviposition. Exclusion of fire from tallgrass prairie results in the accumulation of a layer of detritus which can be up to 30 cm deep (Knapp and Seastedt 1986). This detrital layer may provide protection for *T. aurifera*, or may be selected by *T. aurifera* because of a low probability that it will be eaten by grazers (as senescent vegetation is of lower nutritional quality than fresh forage). Finally, exclusion of fire also results in a greater abundance of forb vegetation, and *T. aurifera* has been observed to oviposit into the stems of forbs as well as grass flowering stalks (M. Whiles and M. Callaham, personal observations). Thus, *T. aurifera's* preference for unburned plots may be explained in part by availability of forb stems.

Interestingly, the differential influence of fire and mowing on the distributions of different species of cicadas (with *C. calliope* more abundant in burned plots and *T. aurifera* in unburned plots) suggests that these two species may be partitioning resources between one another spatially by utilizing different parts of the landscape in different years. Interactions between fire and grazers result in a mosaic of burned and unburned

vegetation in the landscape, and different cicada species appear to have preferences for each component of this mosaic (see Chapter 4 for further discussion).

## CONCLUSIONS

Several soil invertebrate taxa responded significantly to the experimental treatments utilized in the Belowground Plot Experiment. Native North American earthworm species (*Diplocardia* spp.) were favored by annual burning, and European earthworms (*Aporrectodea trapezoides*) were more abundant in plots where fire had been excluded since 1986. The proportion of total earthworm biomass made up of native taxa decreased steadily as the gradient of treatment combinations became more distant from the natural disturbance regime of tallgrass prairie. Cicadas responded differently to treatments depending on species. *Cicadetta calliope* was more abundant in burned plots that had not been mowed, and *Tibicen aurifera* was more abundant in unburned plots. This pattern of cicada distribution suggests spatial resource partitioning between the two species. We did not detect significant responses to the experimental treatments in other arthropod taxa examined in the study.

## ACKNOWLEDGEMENTS

This work was supported by a Long-Term Ecological Research grant awarded to Kansas State University, Division of Biology by the National Science Foundation. We thank Rosmary Ramundo for collection of the 1994 samples and for maintenance of the plots. We gratefully acknowledge Duane Kitchen, Jamie Nutt, Katie Page, and Andrea Silletti for assistance with sorting samples from 1999.

## LITERATURE CITED

- Blair, J.M. 1997. Fire, N availability, and plant response in grasslands: a test of the transient maxima hypothesis. *Ecology* 78:2359-2368.
- Blair, J.M., T.C. Todd, M.A. Callaham, Jr. 2000. Responses of grassland soil invertebrates to natural and anthropogenic disturbances. In: Coleman DC, Hendrix PF (eds) *Invertebrates as Webmasters in Ecosystems*. CAB International Press, pp 43-71.
- Briggs, J.M., M.D. Nellis, C.L. Turner, G.M. Henebry, and H. Su. 1998. A landscape perspective of patterns and processes in tallgrass prairie. In: Knapp, A.K., J.M. Briggs, D.C. Hartnett, and S.L. Collins (eds.). *Grassland Dynamics: Long-term Ecological Research in Tallgrass Prairie*. Oxford University Press, New York, pp. 265-279.
- Brown, G.G. 1995. How do earthworms affect microfloral and faunal community diversity? *Plant Soil* 170:209-231.
- Callaham, M.A. Jr., M.R. Whiles, C.K. Meyer, B.L. Brock, R.E. Charlton. Feeding ecology and emergence production of annual cicadas (Homoptera: Cicadidae) in tallgrass prairie. *Oecologia* (*In press*).
- Callaham, M.A., Jr. and J.M. Blair. 1999. Influence of differing land management on the invasion of North American tallgrass prairie soils by European earthworms. *Pedobiologia* 43:507-512.
- Coleman, D.C., and P.F. Hendrix (eds.). 2000. *Invertebrates as Webmasters in Ecosystems*. CAB International Press, 336 pages.
- Coleman, D.C., and D.A. Crossley, Jr. 1996. *Fundamentals of Soil Ecology*. Academic

- Press, San Diego, 205 pages.
- Collins, S.L., A.K. Knapp, J.M. Briggs, J.M. Blair, and E.M. Steinauer. 1998.  
Modulation of diversity by grazing and mowing in native tallgrass prairie.  
*Science* 280:745-747.
- Collins, S.L., and E.M. Steinauer. 1998. Disturbance, diversity, and species interactions  
in tallgrass prairie. In: Knapp, A.K., J.M. Briggs, D.C. Hartnett, and S.L. Collins  
(eds.). *Grassland Dynamics: Long-term Ecological Research in Tallgrass Prairie*.  
Oxford University Press, New York, pp. 140-156.
- Dindal, D.L. 1990. *Soil Biology Guide*. John Wiley and Sons, New York, 1349 pages.
- Edwards, C.A., and P.J. Bohlen. 1996. *Biology and Ecology of Earthworms*. (3rd ed.).  
Chapman and Hall, London, 426 pages.
- Gibson, D.J., T.R. Seastedt, and J.M. Briggs. 1993. Management practices in tallgrass  
prairie: large- and small-scale experimental effects on species composition. *J.*  
*Appl. Ecol.* 30:247-255
- James, S.W. 1995. Systematics, biogeography, and ecology of nearctic earthworms from  
Eastern, Central, Southern, and Southwestern USA. In: Hendrix, PF (ed)  
*Earthworm ecology and biogeography in North America*. Lewis Publishers, Boca  
Raton, pp 29-51.
- James, S.W. 1990. Soil nitrogen, phosphorus and organic matter processing by  
earthworms in tallgrass prairie. *Ecology* 72:2101-2109.
- James, S.W. 1982. Effects of fire and soil type on earthworm populations in a tallgrass  
prairie. *Pedobiologia* 24:37-40.
- James, S.W., and T.R. Seastedt. 1986. Nitrogen mineralization by native and introduced

- earthworms: Effects on big bluestem growth. *Ecology*: 67:1094-1097.
- James, S.W. and M.R. Cunningham. 1989. Feeding ecology of some earthworms in Kansas tallgrass prairie. *Am Midl Nat* 121:78-83.
- Knapp, A.K., J.M. Blair, J.M. Briggs, S.L. Collins, D.C. Hartnett, L.C. Johnson, and E.G. Towne. 1999. The keystone role of bison in North American tallgrass prairie. *BioScience* 49:39-50.
- Knapp, A.K., J.M. Briggs, D.C. Hartnett, and S.L. Collins (eds.). 1998. *Grassland Dynamics: Long-term Ecological Research in Tallgrass Prairie*. Oxford University Press, New York, 364 pages.
- Knapp, A.K., and T.R. Seastedt. 1986. Detritus accumulation limits productivity in tallgrass prairie. *BioScience* 36:662-668.
- Ojima, D.S., D.S. Schimel, W.J. Parton and C.E. Owensby. 1994. Long and short-term effects of fire on nitrogen cycling in tallgrass prairie. *Biogeochemistry* 24: 67-84.
- Rice, C.W., T.C. Todd, J.M. Blair, T.R. Seastedt, R.A. Ramundo, and G.W.T. Wilson. 1998. Belowground Biology and Processes. In: Knapp, A.K., J.M. Briggs, D.C. Hartnett, and S.L. Collins (eds.). *Grassland Dynamics: Long-term Ecological Research in Tallgrass Prairie*. Oxford University Press, New York, pp. 244-264.
- Scheu, S. 1987. Microbial activity and nutrient dynamics in earthworm casts (*Lumbricidae*). *Biol Fertil Soils* 5:230-234.
- Schowalter, T.D., 2000. Insects as regulators of ecosystem development. In D.C. Coleman and P.F. Hendrix (eds.), *Invertebrates as webmasters in ecosystems*. CABI Publishing, New York, pp 99-114.
- Seastedt, T.R. 1984. Belowground macroarthropods of annually burned and unburned

tallgrass prairie. *American Midland Naturalist* 111:405-408.

Seastedt, T.R., D.C. Hayes, and N.J. Petersen. 1986. Effects of vegetation, burning, and mowing on soil macroarthropods of tallgrass prairie. In: Clambey, G.K., and R.H. Pemble (eds.), *Proceedings of the Ninth North American Prairie Conference*, Tri-College Press, Fargo, pp. 99-102.

Todd, T.C., S.W. James, and T.R. Seastedt. 1992. Soil invertebrate and plant responses to mowing and carbofuran application in a North American tallgrass prairie. *Plant Soil* 144:117-124.

Zhang, Q., and P.F. Hendrix. 1995. Earthworm (*Lumbricus rubellus* and *Aporrectodea caliginosa*) effects on carbon flux in soil. *Soil Sci. Soc. Am J.* 59:816-823.

**Table 1.** Regression equations for dry mass to ash-free dry mass conversions for various soil invertebrates. Equations were derived from specimens collected and ashed in 1999.

<b>Organism</b>	<b>N</b>	<b>Equation</b>	<b>r<sup>2</sup></b>
<b>Earthworms</b>			
<i>Diplocardia</i> spp.	41	$y = 0.526x + 0.00136$	0.853
<i>Aporrectodea trapezoides</i>	60	$y = 0.583x + 0.00494$	0.896
<i>Octolasion cyaneum</i>	21	$y = 0.396x + 0.00207$	0.991
<b>Cicadas</b>			
<i>Cicadetta calliope</i>	46	$y = 0.978x - 0.00265$	0.977
<i>Tibicen aurifera</i>	26	$y = 0.907x + 0.00086$	0.941
Early instar cicadas	20	$y = 0.871x + 0.00111$	0.996
<b>Beetles</b>			
Scarabaeidae	13	$y = 0.993x - 0.00206$	0.998
Curculionidae	18	$y = 0.998x - 0.00002$	0.999
Elateridae	18	$y = 0.978x - 0.00019$	0.999

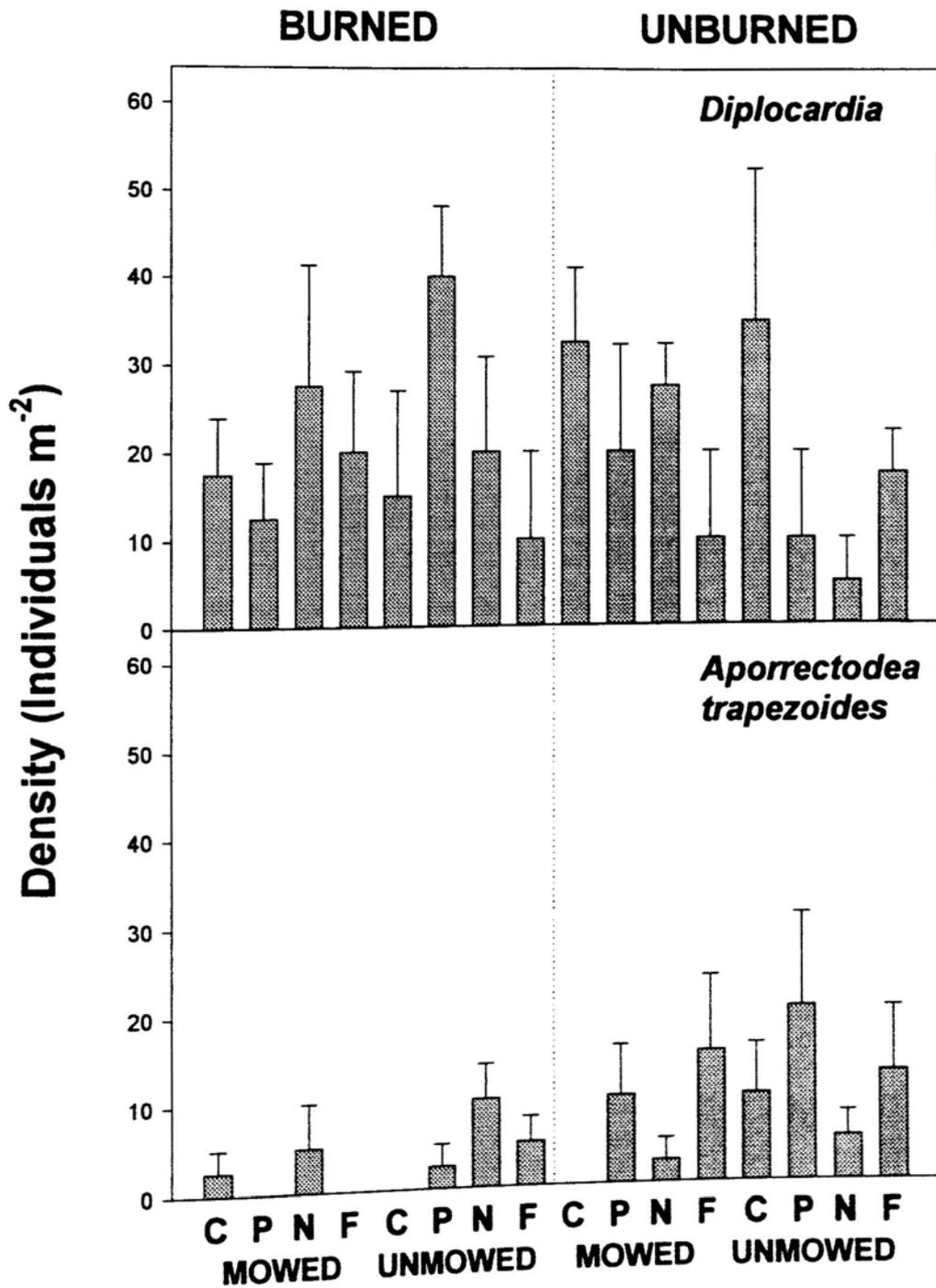


Figure 1. Density of earthworms in Belowground Plots October 1994. A) Native North American *Diplocardia* spp., and B) European exotic *Aporrectodea trapezoides*. Note: x-axis labels C = no nutrient amendments, P = phosphorus amendment only, N = nitrogen amendment only, and F = both nitrogen and phosphorus amendments.

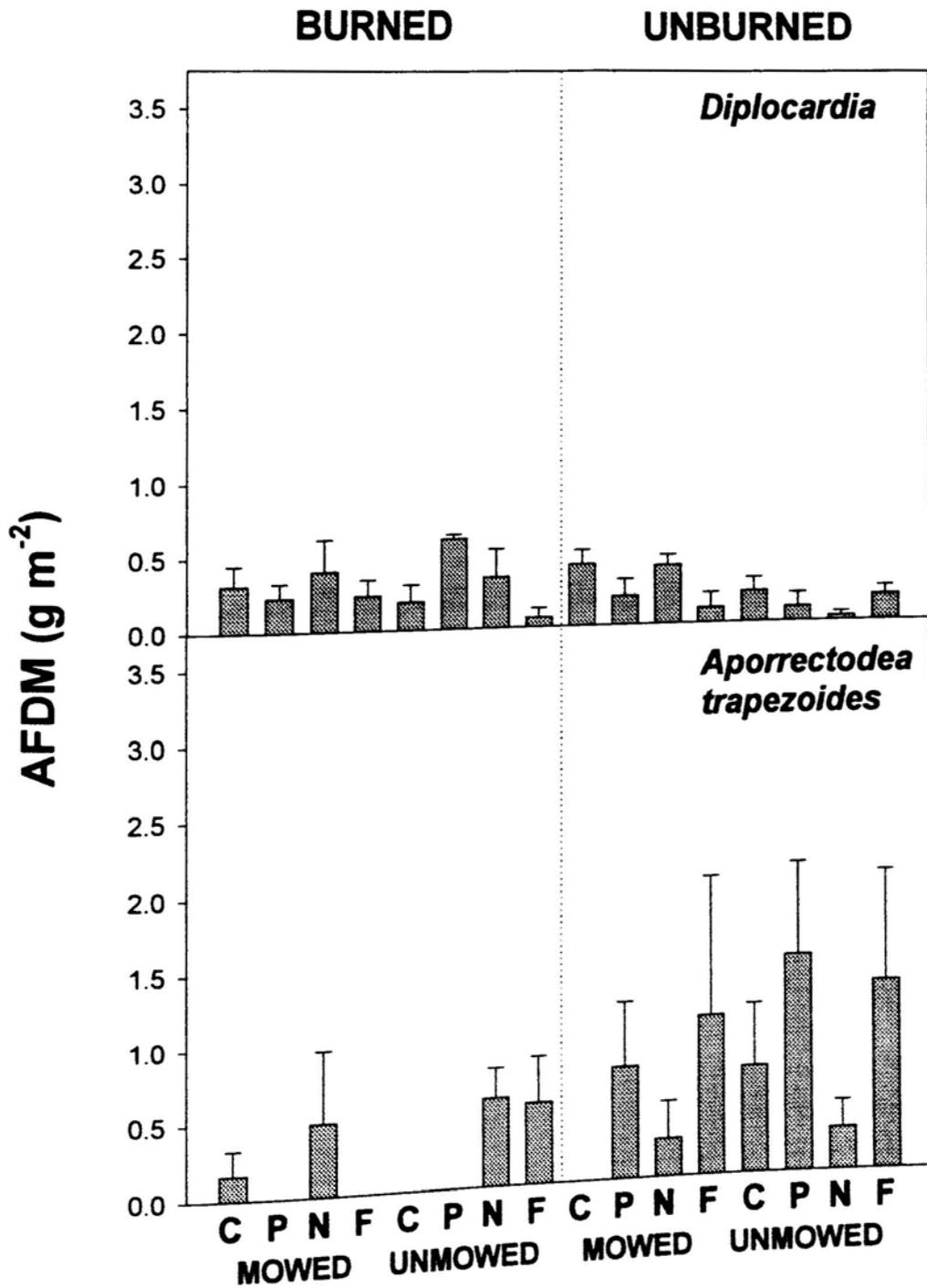


Figure 2. Biomass (ash-free dry mass = AFDM) of earthworms in belowground plots October 1994. A) Native North American *Diplocardia* spp., and B) European exotic *Aporrectodea trapezoides*. Note: x-axis labels as in Figure 1.

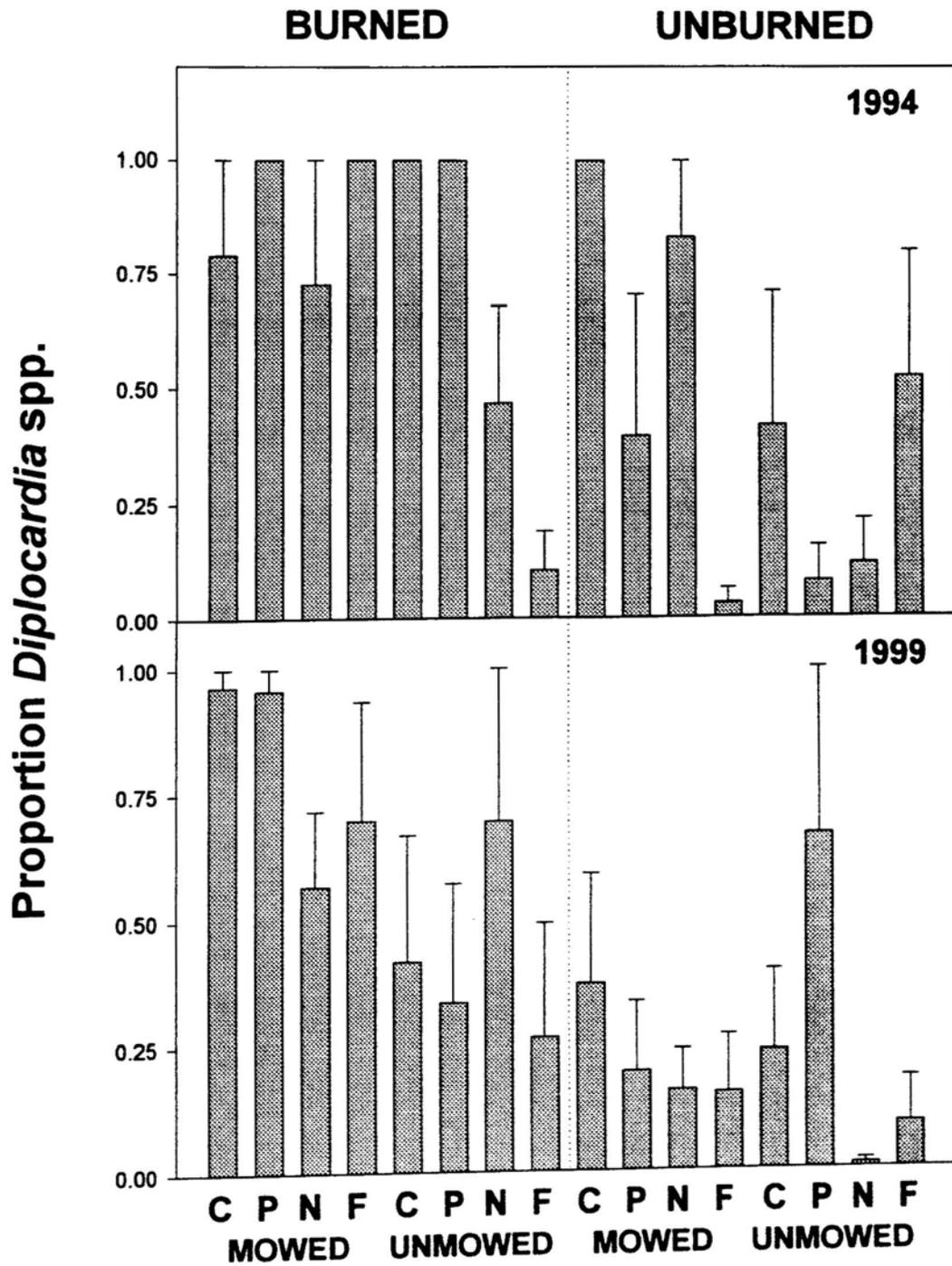


Figure 3. Proportion of total earthworm biomass (as ash-free dry mass) in belowground plots that was from *Diplocardia* spp. in A)1994, and B)1999. Note: x-axis labels as in Figure 1.

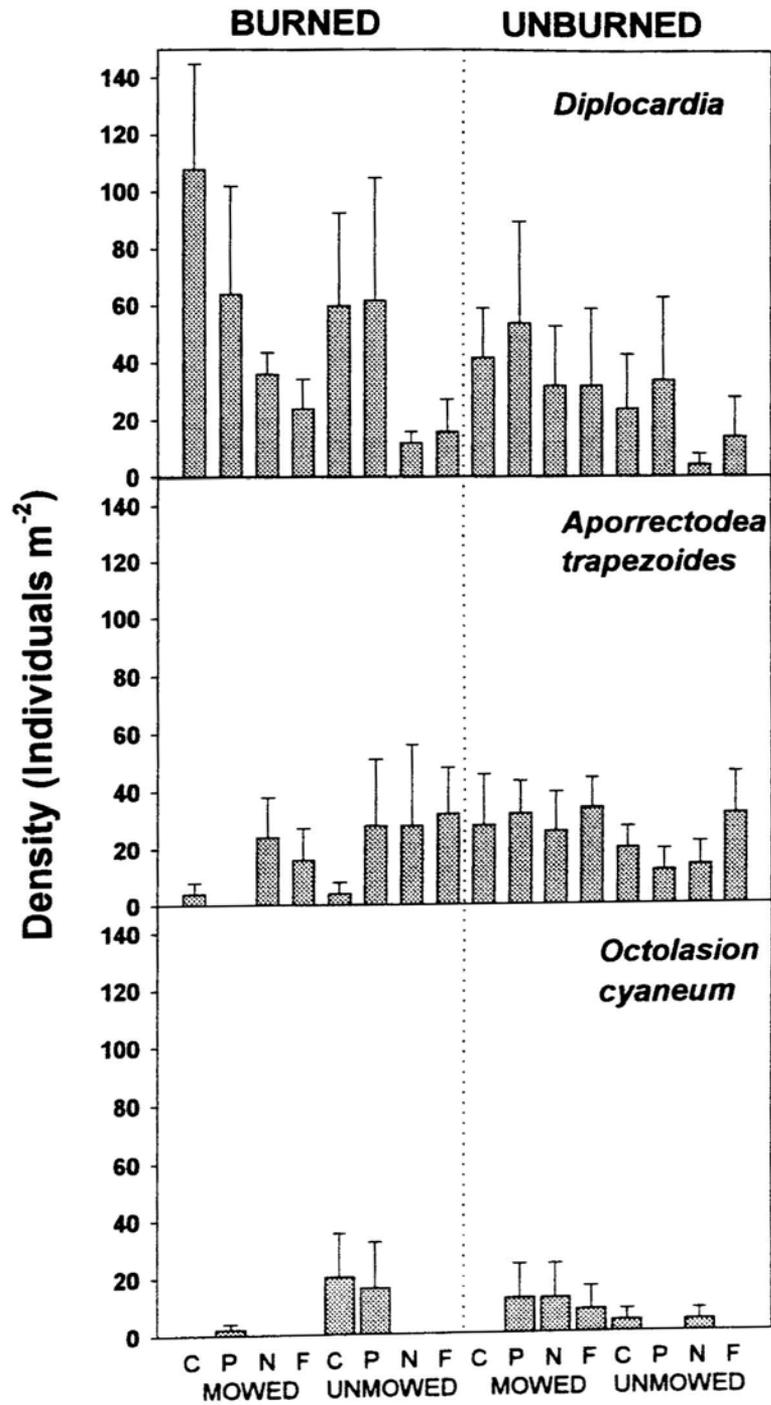


Figure 4. Density of earthworms in belowground plots in June 1999. A) Native North American *Diplocardia* spp., B) European exotic *Apporectodea trapezoides*, and C) *Octolasion cyaneum*. Note: x-axis labels as in Figure 1.

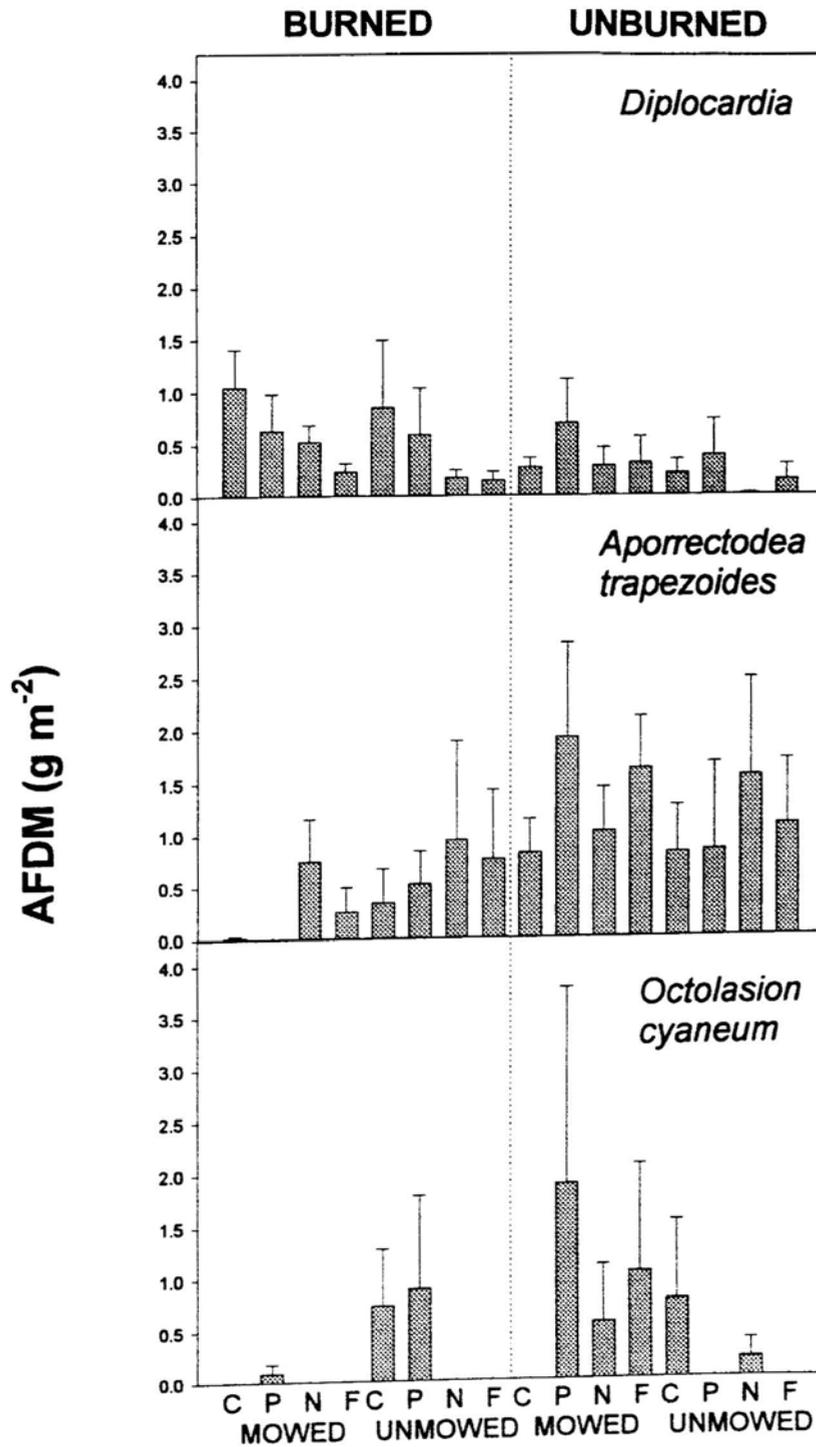


Figure 5. Biomass (ash-free dry mass = AFDM) of earthworms in belowground plots June 1999. A) Native North American *Diplocardia* spp., B) European exotic *Aporectodea trapezoides*, and C) *Octolasion cyaneum*. Note: x-axis labels as in Figure 1.

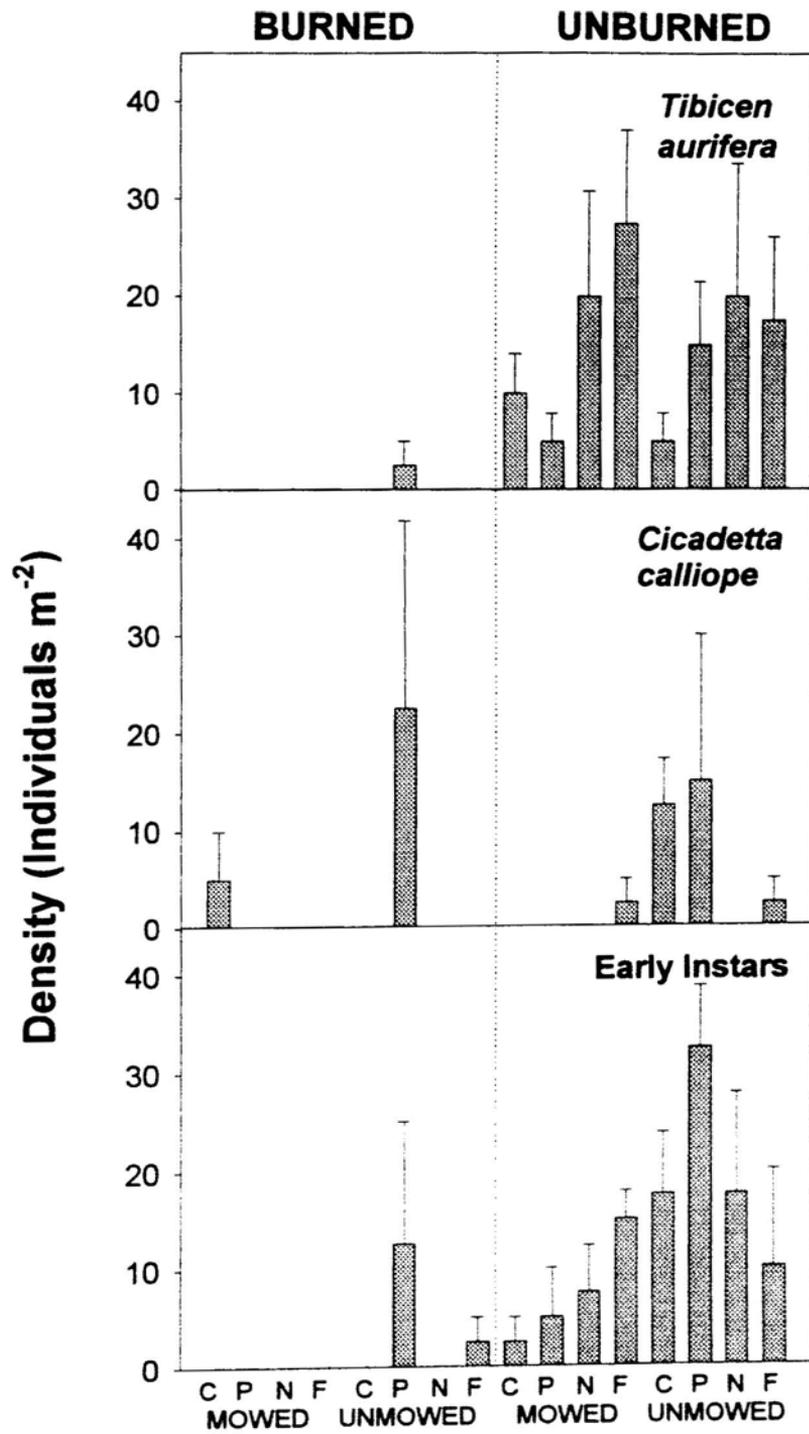


Figure 6. Density of cicada nymphs in belowground plots October 1994. A) *Tibicen aurifera*, and B) *Cicadetta calliope* C) early instars. Note: x-axis labels as in Figure 1.

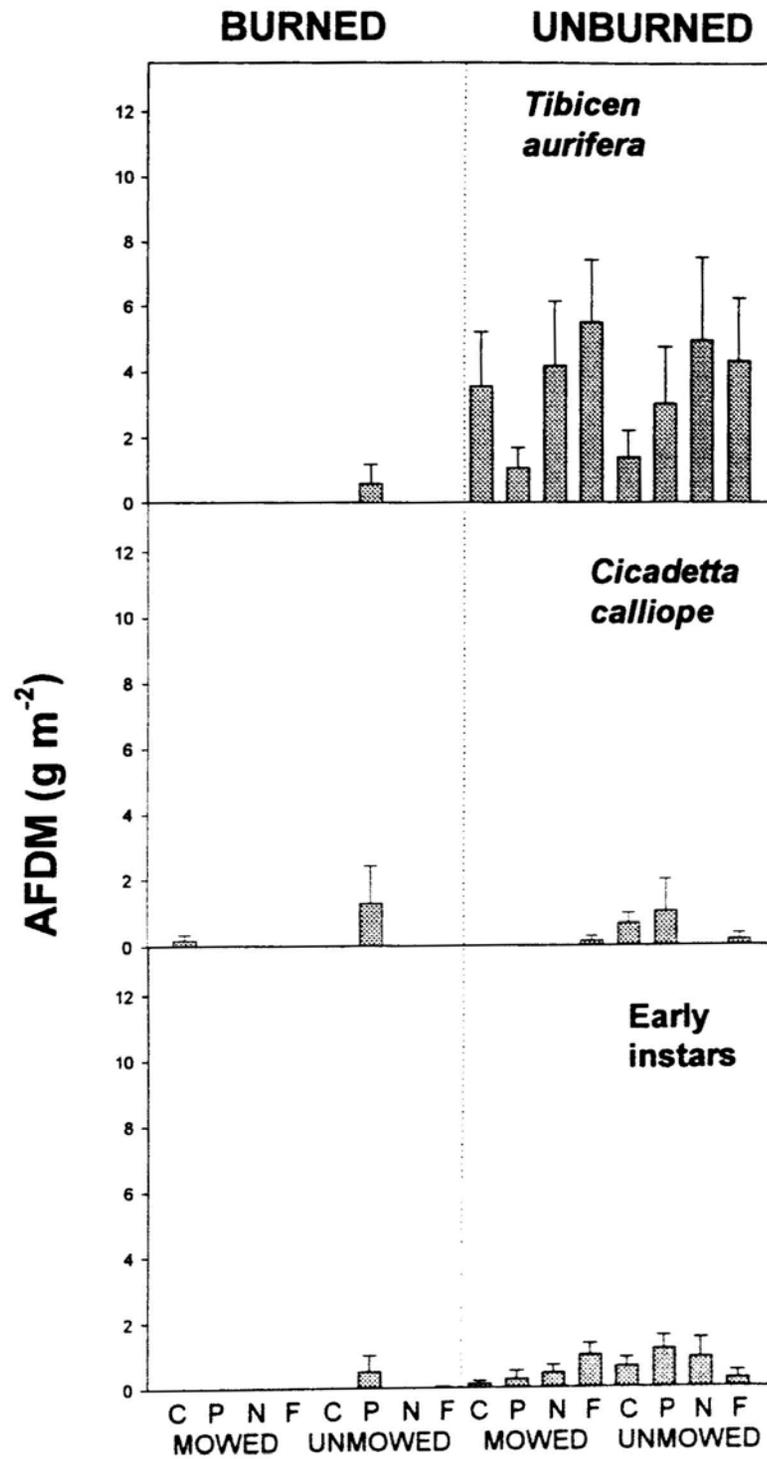


Figure 7. Biomass (ash-free dry mass = AFDM) of cicada nymphs in belowground plots October 1994. A) *Tibicen aurifera*, B) *Cicadetta calliope*, and C) early instars. Note: x-axis labels as in Figure 1.

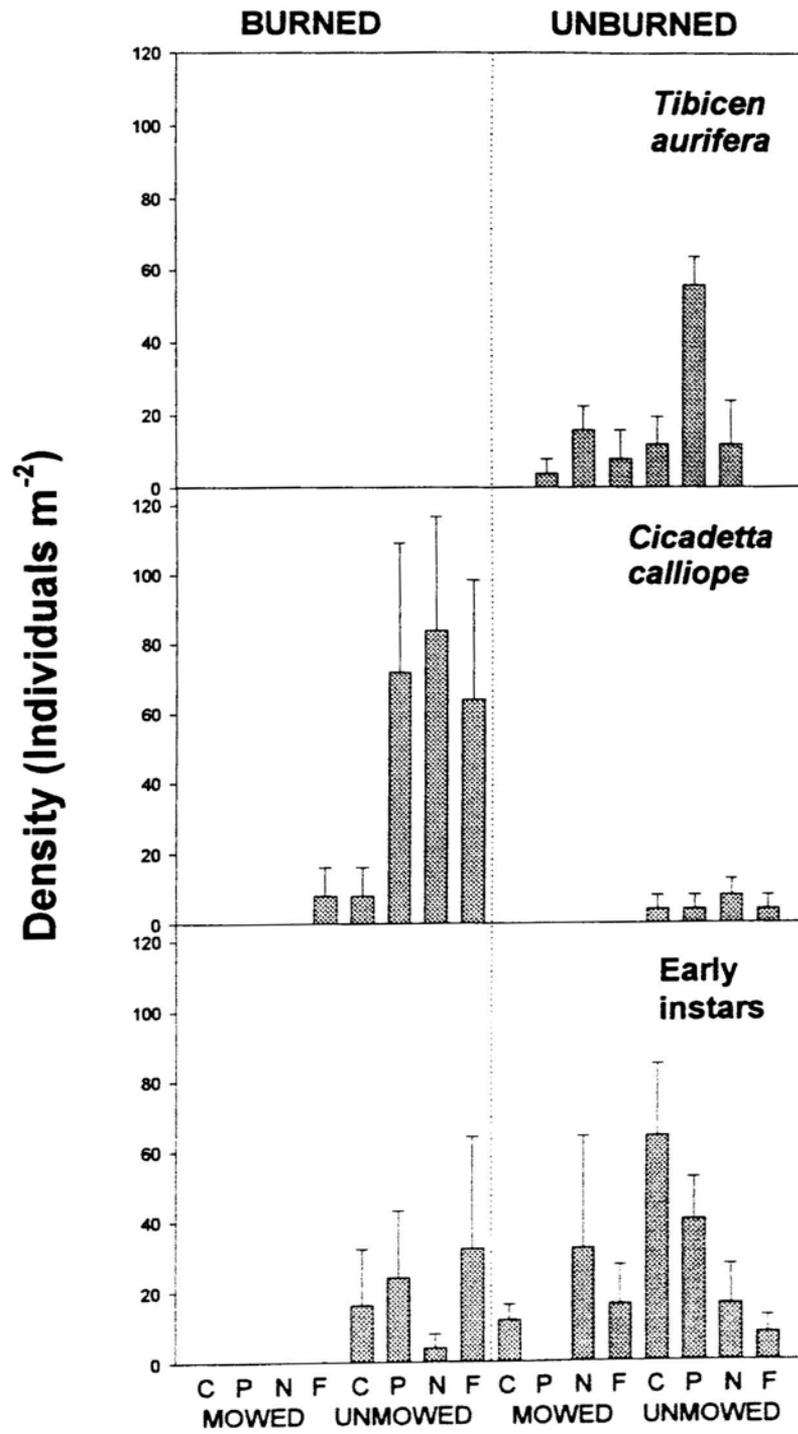


Figure 8. Density of cicada nymphs in belowground plots June 1999. A) *Tibicen aurifera*, B) *Cicadetta calliope*, and C) early instars. Note: x-axis labels as in Figure 1.

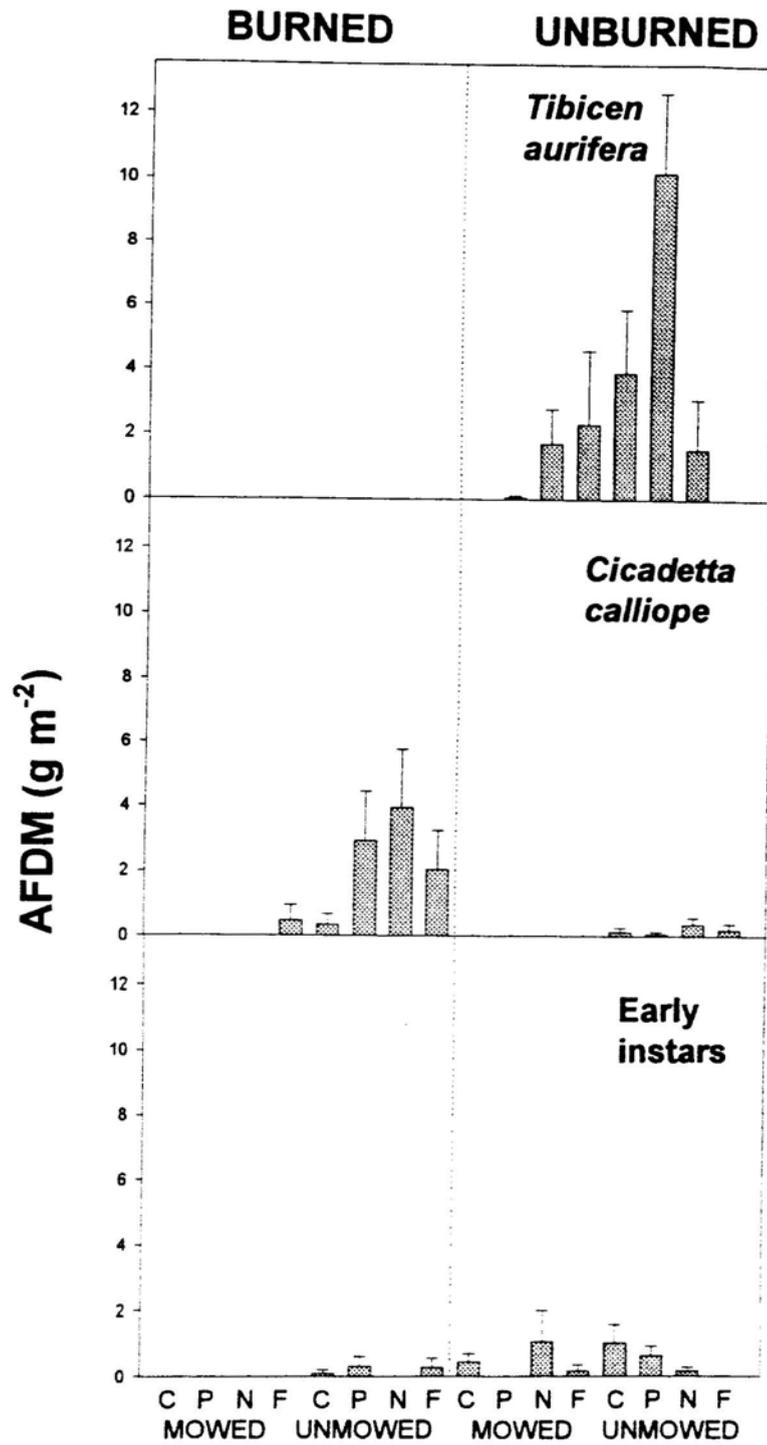


Figure 9. Biomass (ash-free dry mass = AFDM) of cicada nymphs in belowground plots June 1999. A) *Tibicen aurifera*, B) *Cicadetta calliope*, and C) early instars. Note: x-axis labels as in Figure 1.

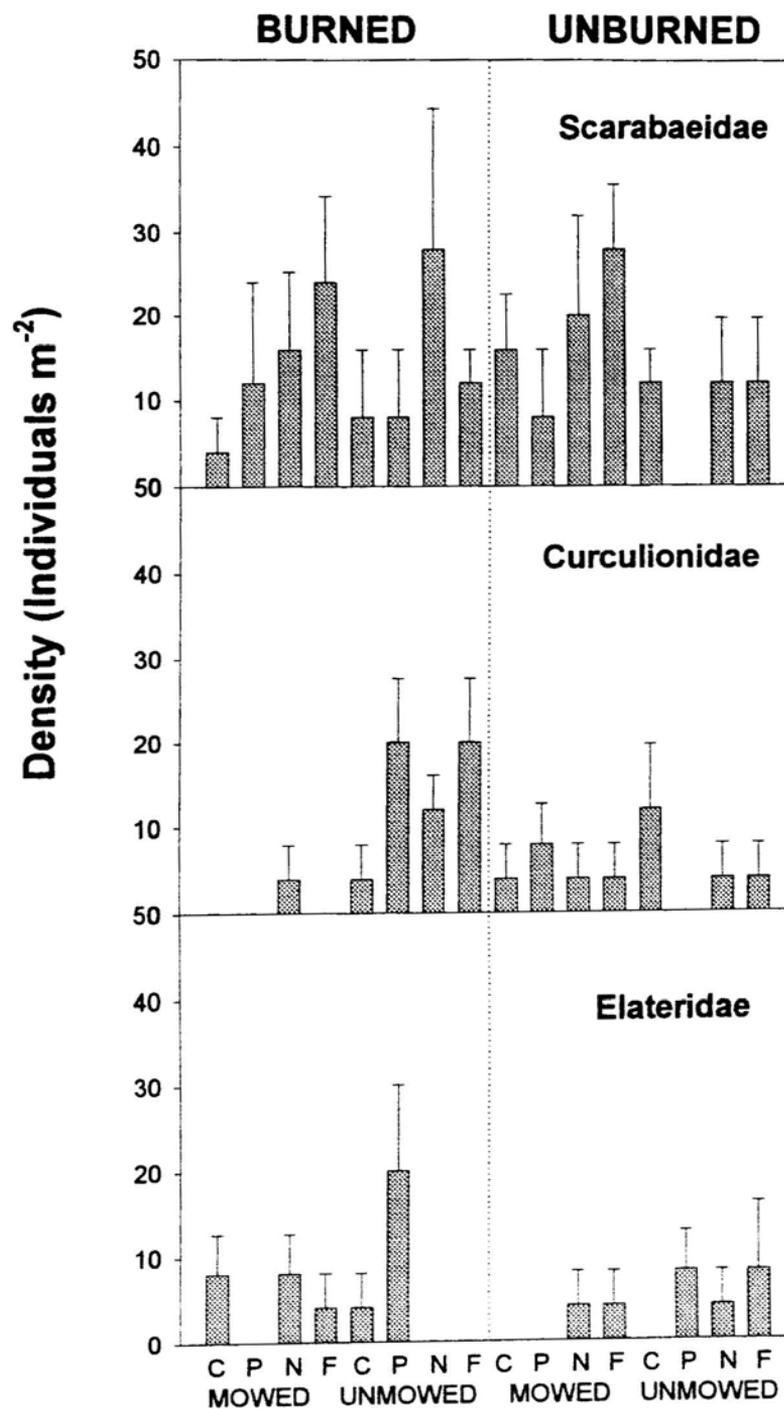


Figure 10. Densities of A) Scarabaeidae larvae, B) Curculionidae larvae, and C) Elateridae larvae in belowground plots June 1999. Note x-axis labels as in Figure 1.

## SUMMARY AND CONCLUSIONS

The results of this work showed that several soil invertebrate groups responded (either positively or negatively) to land management practices commonly employed in the Flint Hills region of eastern Kansas. Furthermore, there were genus and/or species specific responses to certain experimental treatment combinations that lend insight to the potential mechanisms which drive the overall structure of soil faunal assemblages in tallgrass prairie. Among these there were responses of earthworms to the treatment combinations utilized in the Belowground Plot Experiment at Konza Prairie Biological Station (KPBS). Results from chapters 1 and 5 showed that native North American earthworms (*Diplocardia* spp.) were favored, in terms of their relative abundance, by treatment combinations that simulated common disturbances (fire and grazing) and that resulted in conditions thought to have been most similar to pre-European settlement prairie ecosystems (i.e. they were the most dominant component of the earthworm community in plots that were annually burned, annually mowed, and/or received no fertilizer amendments). On the other hand, European earthworms (specifically *Aporrectodea trapezoides*) were most abundant in plots that were subjected to treatments that excluded fire and mowing. The distributional patterns observed for native and exotic earthworms appear to be, at least in part, related to edaphic conditions associated with these different management treatments. Exclusion of fire and/or mowing results in build up of a detrital layer, which leads to increased soil moisture and decreased soil temperature, and these differences may be responsible for the replacement of native earthworms with exotic taxa in plots where fire and mowing had been excluded.

Chapter 2 was an experimental assessment of how different earthworm communities (such as those identified in Chapters 1 and 5) affect nutrient cycling processes in tallgrass prairie soils. The results of this experiment demonstrated that native earthworms and exotic earthworms behave differently during the growing season, with the natives maintaining a higher level of activity during warmer and drier conditions than the exotics (as evidenced by their uptake of stable isotope tracers and their depth distribution in the soil). This increased activity resulted in differences in plant performance between treatments. Plants grown in cores with native earthworms were substantially smaller and took up less total nitrogen than plants grown with no earthworms, or plants grown in the presence of exotic earthworms. Although artificial experimental conditions may have contributed to these surprising results, this study clearly demonstrated that native earthworms were more active in (and had more influence on) the growing season nitrogen cycle in experimental cores than their exotic counterparts.

Another major group of grassland soil invertebrates, the cicadas, were examined in chapters 3, 4, and 5. The annual cicadas examined in these studies were differentially abundant with respect to landscape position and were responsive to different land management practices in the Belowground Plot Experiment at the species level. Two species (*Tibicen aurifera* and *Cicadetta calliope*) of three species emerging from grassland habitats were more abundant in upland sites. These two species were demonstrated (by examination of their  $\delta^{13}\text{C}$  signatures) to feed primarily on warm season  $\text{C}_4$  grasses, whereas the third species (*Tibicen dorsata*) found to emerge from all landscape positions including forested sites had  $\delta^{13}\text{C}$  signatures indicative of feeding

upon C<sub>3</sub> plants (probably trees and shrubs). One other relatively abundant species (*Tibicen pruinosus*) was found to emerge exclusively from forested sites.

Another aspect of cicada biology that was of interest in these studies was the total flux of energy and nutrients (particularly N) from soil as emergence biomass. It was estimated that annual cicada emergence resulted in redistribution of ~4 kg N ha<sup>-1</sup> from belowground to aboveground in upland and lowland grassland habitats, and ~1 kg N ha<sup>-1</sup> from forested sites.

In the Belowground Plot Experiment, cicadas exhibited strong, species specific, responses to burning, mowing, and fertilizer treatments. *Tibicen auriferus* emerged exclusively from plots that had not been burned (chapter 4) and nymphs of *T. auriferus* were collected (with the exception of a single individual) only from unburned plots (chapter 5). In contrast to the pattern observed for *T. auriferus*, *Cicadetta calliope* was found to emerge in greater abundance from burned plots, and was more abundant in fertilized plots. Again, the pattern for *C. calliope* emergence was similar to the pattern of distribution of *C. calliope* nymphs in the Belowground Plot Experiment. The differential emergence of *T. auriferus* and *C. calliope* is hypothesized to be the result of resource partitioning between the two species, and is further hypothesized to be related to heterogeneity imposed upon vegetation at the landscape level by large ungulate grazers.

The combined results of this work show that belowground invertebrates, and the factors influencing their distribution and abundance, can potentially have impacts on ecosystem level processes such as nutrient uptake and nutrient flux. Both of the major groups examined, earthworms and cicadas, were implicated as being potentially important in N cycling in this system. Native earthworms influenced plant uptake of N

during the growing season, whereas soil with exotic earthworms was no different from earthworm-free soil with respect to plant N uptake. This pattern clearly has ecosystem level consequences in terms of ongoing changes in land management practices in the Flint Hills region, and the potential resultant changes in earthworm communities. Further monitoring of earthworm distributions with respect to land management practices, and more intensive examination of differences between native and exotic taxa with respect to N cycling should prove interesting in the future. Cicada emergence represents a significant redistribution of N from belowground to aboveground in tallgrass prairie, and the fate of cicada biomass N is essentially unknown (or at best, not quantified). Whether, or in what proportion, cicada biomass N flows to higher trophic levels or returns to soil pools is an intriguing problem for future work. Additionally, the impact of cicada feeding on plants (i.e. how plants respond to cicada herbivory) is unknown. Hopefully, this dissertation provides information that may serve as a starting point for new studies on the influences of soil invertebrates on ecosystem process in tallgrass prairie.