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Different behavioral patterns of the earthworms *Octolasion tyrtaeum* and *Diplocardia* spp. in tallgrass prairie soils: potential influences on plant growth

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Abstract This study addressed differences between *Diplocardia* spp. (a native North American earthworm) and *Octolasion tyrtaeum* (an introduced European species), with respect to behavior, influence on soil microbial biomass, and plant uptake of N in tallgrass prairie soils. We manipulated earthworms in PVC-encased soil cores (20 cm diameter) over a 45-day period under field conditions. Treatments included: (1) control with no earthworms, (2) *Diplocardia* spp. only, and (3) *O. tyrtaeum* only. Prior to addition of earthworms, seedlings of *Andropogon gerardii* (a dominant tallgrass) were established in each core, and a dilute solution of ^{13}C-labeled glucose and ^{15}N-labeled $(NH_4)_2SO_4$ was added to the soil to facilitate examination of earthworm/microbe/plant interactions. We found that *Diplocardia* spp. were significantly more active than *O. tyrtaeum*, and quickly assimilated ^{13}C and ^{15}N from the tracer. Individuals of *Diplocardia* spp. were present at shallower soil depths than *O. tyrtaeum* throughout the study. Contrary to expectation, this greater activity of *Diplocardia* spp. did not result in increased plant productivity. Rather, the activity of *Diplocardia* spp. was associated with less plant growth and smaller amounts of N acquired by *A. gerardii* seedlings compared to controls or *O. tyrtaeum* treatments. We observed few significant influences of earthworm treatments on microbial biomass C or N pool sizes, but the microbial C/N ratio was consistently greater in the presence of *Diplocardia* spp. relative to *O. tyrtaeum*. Results of this study indicate that activity of earthworms may enhance competition for N between microbes and plants during the growing season in tallgrass prairie.

Keywords Grassland · Microbial biomass · Soil invertebrates · Nitrogen · Stable isotopes

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Introduction

Earthworm assemblages across the North American continent are composed largely of introduced European species (Reynolds 1995). The establishment of these exotic species typically is preceded by disturbance of the native soil ecosystem through agricultural, or other development (e.g., Stebbings 1969; Dotson and Kalisz 1989; Kalisz and Dotson 1989). However, in relatively undisturbed soils, native North American earthworms persist (James 1990), and the ecology of these native earthworms has only recently begun to be studied (e.g., James and Cunningham 1989; James 1992; Callaham and Hendrix 1998; Winsome and McColl 1998). In contrast, there has been much research in North America examining earthworms in agricultural systems (e.g., Blair et al. 1995; Hendrix 1998; Parmelee et al. 1998). This agricultural focus has resulted in an excellent understanding of the influences of a relatively small number of European earthworm species on nutrient cycling and other soil processes in agricultural systems (e.g., Edwards and Bohlen 1996; Blair et al. 1997; Doube and Brown 1998). However, comparatively little is known about the influences of European earthworm taxa on processes in undisturbed native soil ecosystems of North America, and with a few exceptions (noted above) this is true of native taxa as well.

The Flint Hills physiographic region is a large (1.6 million ha) area of relatively undisturbed soils in eastern Kansas, USA. Soils in this region have escaped agricultural usage because of their steep topography and stoniness. One consequence of the lack of extensive soil disturbance in the Flint Hills is the presence of native North American earthworm taxa (primarily a group of species

in the megascolecid genus *Diplocardia* Garman, and a lumbricid species *Bimastos welchii* Smith) (James 1992). Nevertheless, several European lumbricid earthworm taxa are currently expanding their distributions in Flint Hills soils (e.g., *Lumbricus* spp., *Aporrectodea* spp. and *Octolasion* spp.). The influence of these introduced species on soil function and/or native earthworm populations is not well understood, but it is unlikely that native and introduced earthworm taxa behave similarly with respect to nutrient cycling in tallgrass prairie soils (James and Seastedt 1986; James and Cunningham 1989). Accordingly, objectives for this study were: (1) under field conditions, to assess differences in the behavior and activity patterns of *Diplocardia* spp. and *Octolasion tyrtaeum* Savigny (2) to examine the influences of the two earthworms on soil microbial biomass C (MBC) and N (MBN); and (3) to examine the influence of the two earthworms on plant uptake of N. We used stable isotopic tracers (^{15}N and ^{13}C) to facilitate the study of plant/microbe/earthworm interactions.

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; soils at the site are characterized as Tully silty clay loams (fine, mixed, mesic Pachic Argiustolls) (Ransom et al. 1998). Vegetation at the site 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.

Study organisms

The two earthworms used in this study were *Diplocardia* spp., a member of a genus native to North America; and *O. tyrtaeum*, a common introduced European species in North American soils. These taxa were selected because both earthworms have the endogeic ecological strategy (sensu Bouché 1977), and utilize similar food resources (natural abundance stable isotope data suggest that both species feed on material with isotopic content similar to bulk soil organic matter in shallow soil layers; P.F. Hendrix, unpublished data). The representatives of *Diplocardia* at KPBS cannot be identified reliably at the species level without dissection (except *Diplocardia kansensis* which is identified by virtue of its pigmentation), but all species that have been examined (again, except *D. kansensis*) have similar feeding and burrowing behaviors (James and Cunningham 1989). *O. tyrtaeum* is one of four exotic earthworm taxa currently found at KPBS, and has expanded its distribution at the site in recent years (personal observations; S. James, personal communication; P. F. Hendrix, unpublished data). All earthworms used in this study were collected during 2 weeks prior to the experiment by digging pits and hand-sorting soils within 30 m of the location of the experimental cores.

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 cores were removed and frozen at –10°C for 15 days 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, 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 collection. This was done to remove competition for experimental seedlings planted in the cores. We used seedlings in this study because we expected mature plants in the cores to utilize stored N as a primary nutrient source during the growing season. Previous studies in tallgrass prairie have had difficulty demonstrating short-term N uptake by mature plants because of this use of stored N (Dell 1998).

Soil in cores was labeled with tracer amounts of ^{13}C and ^{15}N. The tracer solution was prepared by dissolving exactly 6.2280 g of oven-dried (50°C) 99.9% ^{13}C-enriched glucose ($C_6H_{12}O_6$) and 5.0636 g of oven-dried (50°C) 99.9% ^{15}N-enriched $(NH_4)_2SO_4$ into 30.0 l deionized water. The solution was applied to cores on 20 and 21 May 1998, by injection with a syringe. The needle of the syringe was inserted to a 10 cm depth into the soil, and the syringe was filled with 50 ml solution. Each injection was made at intervals as the needle of the syringe was incrementally removed from the soil, with 5-ml injections at 10, 8, and 6 cm depths, and 10 ml injections at 4 and 2 cm depths. The remaining 15 ml was sprayed directly onto the soil core surface. Ten injections were made per core according to a template designed for uniform application of solution throughout the core. Total quantities of ^{15}N and ^{13}C applied to cores were 43.46 mg ^{13}C $core^{-1}$ and 18.9 mg ^{15}N $core^{-1}$. After application of tracer solution, a seedling of *A. gerardii* was planted in each core. Earthworm treatments (*Diplocardia* spp. only, *O. tyrtaeum* only, or control) were applied on 14 June 1998. Although there is a substantial difference in body size between the two species (*O. tyrtaeum* has 3–4 times more biomass per adult individual) we elected to apply the earthworm treatments on the basis of density because our research focus was a comparison of the two species in terms of per capita influences (rather than per unit biomass) on soil processes. Accordingly, earthworms were applied to cores at approximate densities observed for both species in previous field studies (7 individuals $core^{-1}$ or ~200 individuals m^{-2}) (Callaham and Blair 1999).

Laboratory methods

Five cores of each treatment were randomly selected and destructively sampled 7, 15, 30, and 45 days after earthworm treatments were applied (Fig. 1). Sampling dates were selected to be close enough together to detect short-term earthworm effects on microbial parameters, but wide enough apart to cover much of the early growing season in tallgrass prairie, as this is the period when a majority of plant production and nutrient uptake occurs. Upon sampling, soil in each core was cut into three layers (0–5, 5–10, and 10–20 cm); each layer was passed through a 4-mm sieve, and coarse organic matter (including live and dead roots) was removed. Soil MBC was determined for each depth of each core by a chloroform fumigation/incubation method (Brookes et al. 1985). Soil MBN was determined by chloroform fumigation/direct extraction with alkaline persulfate digestion (Cabrera and Beare 1993). Microbial biomass $\delta^{13}C$ was determined by collection of CO_2 from fumigated and non-fumigated incubations and direct injection into a continuous-flow isotope ratio mass spectrometer (CFIRMS) (Europa Tracermass 20/20, Italy). The $\delta^{13}C$ of microbial biomass was calculated as:

$$\delta^{13}C_{MB} = \frac{(\delta^{13}C_F \times C_F) - (\delta^{13}C_{NF} \times C_{NF})}{C_F - C_{NF}} \quad (1)$$

where $\delta^{13}C_F$ and $\delta^{13}C_{NF}$ were $\delta^{13}C$ measurements of CO_2 from fumigated and non-fumigated soils, and C_F and C_{NF} were concentra-

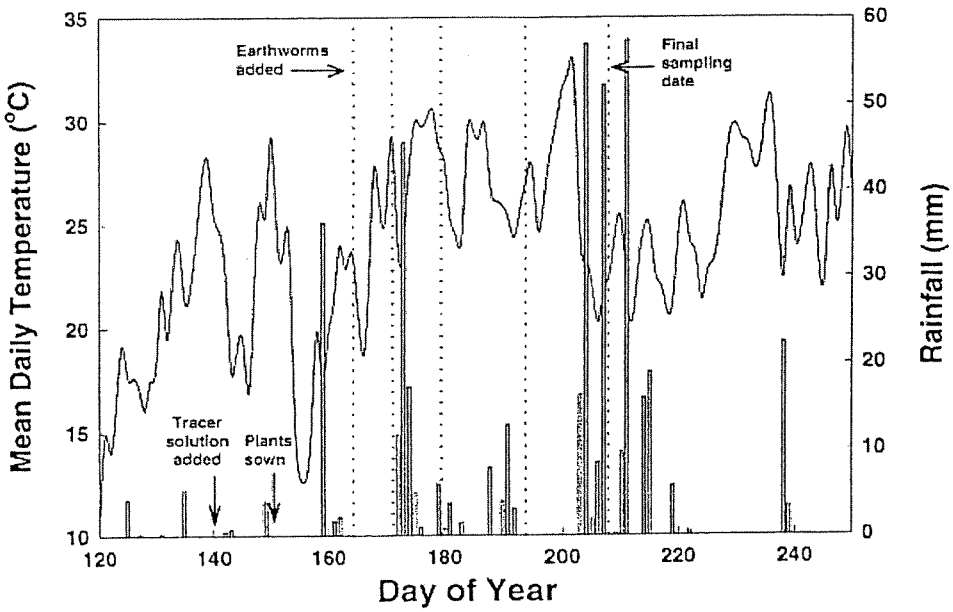

Fig. 1 Rainfall (*bars*), temperature (*solid line*) and important dates during the experiment. Climate data are from KPBS headquarters weather station. *First vertical dotted line* indicates application of earthworm treatments; *subsequent vertical dotted lines* indicate sampling dates

Table 1 Mean proportions (±SE) of *Diplocardia* spp. and *Octolasion tyrtaeum* collected from each depth during the study period. Within a given date and treatment, means followed by *different letters* are significantly different from one another ($P<0.05$ except where noted)

Depth	Treatment	Proportion				
		Day 7	Day 15	Day 30	Day 45	All dates
0–5 cm	*Diplocardia*	0.23±0.15 a	0.00±0.00 a	0.45±0.07 a*	0.85±0.07 a	0.43±0.09 a
	O. tyrtaeum	0.00±0.00 y	0.00±0.00 y	0.04±0.04 y	0.39±0.16 y	0.08±0.05 y
5–10 cm	*Diplocardia*	0.52±0.13 a	0.67±0.26 b	0.40±0.06 a	0.12±0.06 b	0.40±0.08 a
	O. tyrtaeum	0.19±0.11 y	0.00±0.00 y	0.17±0.11 y	0.11±0.09 y	0.11±0.05 y
10–20 cm	*Diplocardia*	0.25±0.07 a	0.33±0.26 b	0.15±0.12 b	0.03±0.03 b	0.17±0.06 b
	O. tyrtaeum	0.81±0.11 z	1.00±0.00 z	0.79±0.10 z	0.50±0.08 y	0.81±0.06 z

*$P<0.10$

tions of CO_2 released from fumigated and non-fumigated soils, respectively.

Earthworms were removed during sieving and kept cool (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). The earthworm tissues then were freeze-dried for >72 h and ground with a mortar and pestle. Tissues of all individual earthworms from a given core were pooled and thoroughly mixed. Tissue sub-samples were analyzed for ^{13}C and ^{15}N content by CFIRMS.

Plants were sampled on the second and fourth sampling dates. Plants were not sampled on the first date due to inadequate quantities of plant tissue (i.e., the plants had not grown enough to warrant sampling). The lack of plant analysis data from date three was due to unanticipated herbivory by grasshoppers on the seedlings. This herbivory eliminated seedlings from several cores after the second sampling 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 sampling dates, aboveground components of *A. gerardii* plants were collected from each core, dried at 65°C, weighed, and ground with a mortar and pestle. These plant tissues were subsampled and analyzed for C and N content by combustion (Carlo Erba C/N analyzer), and for ^{13}C and ^{15}N content by CFIRMS.

Statistical analyses

All data were subjected to three-way ANOVA (PROC GLM; SAS Institute, Cary, N.C.) with date, soil depth and earthworm treatment as main effects variables. When necessary, data were log-transformed to satisfy normality assumptions. The SAS least squares means (LSMEANS/pdiff option; SAS Institute) procedure was used to identify significant differences in treatment means.

Results

Earthworms

Stable isotope analysis of earthworm tissues revealed that *Diplocardia* spp. rapidly became enriched with ^{15}N and ^{13}C (Fig. 2). The enrichment of *Diplocardia* spp. was significantly greater ($P<0.01$) than that of *O. tyrtaeum* on all sampling dates. However, *O. tyrtaeum* did become enriched with the stable isotope tracers, and their tissue $\delta^{13}C$ and $\delta^{15}N$ values on the fourth sampling date were significantly greater than for the first three dates (Fig. 2). For *Diplocardia*, mean $\delta^{13}C$ values for the first two dates

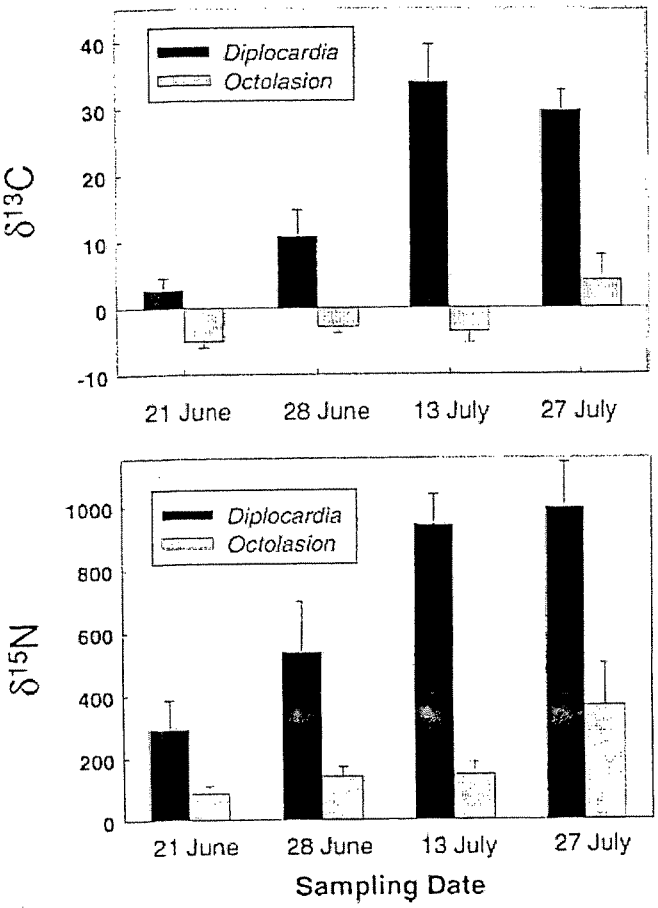

Fig. 2 $\delta^{13}C$ and $\delta^{15}N$ signatures of *Diplocardia* spp. and *Octolasion tyrtaeum* during the study period

did not differ significantly, but by the third sampling date, were significantly more enriched than on the first date. There was no difference between $\delta^{13}C$ values of *Diplocardia* spp. for dates three and four (Fig. 2).

Observations of earthworm depth distribution also revealed differences in the behavior of *Diplocardia* spp. and *O. tyrtaeum*. These data were obtained by making note of the soil depth at which earthworms were collected on each sampling date. *Diplocardia* spp. were far more likely to be collected from the top 10 cm, and significantly ($P<0.05$) larger proportions of all *Diplocardia* spp. were collected from the top two depths (Table 1). In contrast, the likelihood of collecting *O. tyrtaeum* from the 10–20 cm soil depth was about 8 times greater ($P<0.0001$) than the likelihood of collecting these worms from the 0–10 cm depths (Table 1).

Microbial biomass

Differences in the sizes of microbial pools of C and N, in response to earthworm treatments, were inconsistent. Differences in MBC were evident on the first date (0–5 cm), with significantly greater levels of MBC in earthworm cores than in the control cores (Table 2). On

Table 2 Microbial biomass C (*MBC*; μg C g soil^{-1}), microbial biomass N (*MBN*; μg N g soil^{-1}), and microbial C/N from experimental cores containing no earthworms (*Control*), Diplocardia spp., or *O. tyrtaeum*. Within a given date and depth, means followed by *different letters* are significantly different from one another ($P<0.05$ except where noted)

Depth	Treatment	Day 7			Day 15			Day 30			Day 45		
		MBC (μg C g soil^{-1})	MBN (μg N g soil^{-1})	C/N	MBC (μg C g soil^{-1})	MBN (μg N g soil^{-1})	C/N	MBC (μg C g soil^{-1})	MBN (μg N g soil^{-1})	C/N	MBC (μg C g soil^{-1})	MBN (μg N g soil^{-1})	C/N
0–5 cm	Control	978.3 a	76.0 a	13.3 a	1,152.2 a	80.3 a	14.5 a	953.9 a	77.6 a	12.4 ab*	899.2 a*	78.0 a	11.5 a
	Diplocardia	1,181.8 b	75.0 a	16.2 b	1063.1 a	82.3 a	13.0 a	1,024.1 a	77.9 a	13.5 a	826.8 ab	80.8 a	10.4 ab
	O. tyrtaeum	1,178.3 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
	Diplocardia	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
	O. tyrtaeum	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
	Diplocardia	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
	O. tyrtaeum	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

*$P<0.10$

Table 3 $\delta^{13}C$ of microbial biomass C (*MB^{13}C*), and total micrograms ^{13}C standing stock per gram soil in microbial biomass (*^{13}CSS*), from experimental cores containing *Diplocardia* spp., *O. tyrtaeum*, or no earthworms (*Control*). Within a given date and depth, means followed by *different letters* are significantly different from one another ($P<0.05$ except where noted)

Depth	Treatment	Day 15		Day 30		Day 45	
		MB^{13}C	^{13}CSS	MB^{13}C	^{13}CSS	MB^{13}C	^{13}CSS
0–5 cm	Control	365.55 a	1.738 a	298.87 a	1.368 ab	385.10 a	1.374 a
	Diplocardia	374.44 a	1.620 a	328.18 a	1.498 a	292.57 a	1.246 ab
	O. tyrtaeum	361.91 a	1.708 a	318.58 a	1.238 b	363.98 a	1.100 b
5–10 cm	Control	256.59 a	1.302 a	215.56 a	1.198 a	241.26 a	0.893 b
	Diplocardia	241.34 ab	1.260 a	190.29 a	1.143 a	210.85 a	1.188 a
	O. tyrtaeum	211.87 b	1.302 a	203.77 a	1.253 a	225.42 a	1.250 a
10–20 cm	Control	192.16 a	1.070 a	173.77 a	0.908 a	175.45 a	0.838 a
	Diplocardia	183.26 a	1.025 a	154.04 a	0.906 a	206.39 a	0.884 a
	O. tyrtaeum	168.10 a	0.974 a	185.71 a	0.770 a	197.47 a	0.868 a

Table 4 Mass, atom percent ^{15}N (*at%^{15}N*), standing stock of N (*SSN*), and standing stock of ^{15}N (*SS^{15}N*) of aboveground tissues from plants grown in experimental soil cores. Means in the same column followed by *different letters* are significantly different from one another ($P<0.05$, except where noted). *ind.* Individual

Earthworm	Day 15					Day 45				
	Mass (g ind.$^{-1}$)	%N*	at%^{15}N	SSN (mg ind.$^{-1}$)	SS^{15}N (mg ind.$^{-1}$)	Mass (g ind.$^{-1}$)	% N	at%^{15}N	SSN (mg ind.$^{-1}$)	SS^{15}N (mg ind.$^{-1}$)*
Control	0.142 a	1.709 a	4.356 a	2.488 a	0.111 a	1.242 a	1.168 a	2.266 a	14.508 a	0.300 a
SE	0.039	0.099	0.398	0.762	0.037	0.460	0.122	0.293	5.267	0.110
Diplocardia	0.158 a	1.840 ab	3.016 b	3.224 a	0.103 a	0.356 b	1.326 a	1.925 a	4.579 b	0.095 b
SE	0.051	0.208	0.701	1.335	0.049	0.137	0.164	0.259	1.721	0.041
Octolasion	0.088 a	2.137 b	3.922 ab	1.922 a	0.079 a	0.954 a	1.298 a	1.646 a	11.757 a	0.194 a
SE	0.017	0.170	0.304	0.427	0.022	0.187	0.098	0.036	1.617	0.028

*$P<0.10$

the final date, we again measured differences in MBC at the 0–5 cm depth, with significantly greater MBC in control cores than in *O. tyrtaeum* cores (Table 2). There were contrasting differences in MBC at the 5–10 cm depth on the fourth date when control cores had significantly less MBC than did cores containing either earthworm (Table 2).

Differences in MBN were also minimal. Two exceptions to this general trend were observed. On the second sampling date (10–20 cm), control cores had significantly greater levels of MBN than *Diplocardia* cores, with *O. tyrtaeum* cores being intermediate (Table 2); on the last sample date (5–10 cm), control cores had significantly less MBN than cores containing *O. tyrtaeum*, with cores containing *Diplocardia* spp. being intermediate (Table 2).

Differences in C to N ratios (C/N) of microbial biomass typically were associated with significant differences in MBC or MBN. For example, C/N was lower in control cores than in earthworm treatments for the 0–5 cm depth on the first date; this difference was 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 sampling date. For this sample we found differences in C/N but none for MBC or MBN. In this instance, C/N was significantly higher in cores with *Diplocardia* spp. than in cores containing *O. tyrtaeum*.

Microbial biomass $\delta^{13}C$ values showed little response to earthworm treatments during the study. The single statistically significant difference in $\delta^{13}C$ of the microbial biomass occurred on the second sample date (5–10 cm depth) when $\delta^{13}C$ values for the microbial biomass were significantly ($P<0.1$) lower in cores containing *O. tyrtaeum* compared to control cores (Table 3). However, there were several instances where the total standing stock of ^{13}C in the microbial biomass was influenced by experimental treatments. Specifically, on the third date (0–5 cm), microbial biomass in *Diplocardia* cores contained more total ^{13}C than did the cores with *O. tyrtaeum* ($P<0.07$), and on the fourth date (0–5 cm), the standing stock of microbial ^{13}C was lower in *O. tyrtaeum* cores than in controls ($P<0.02$) (Table 3). On the fourth date (5–10 cm), the standing stock of microbial ^{13}C was smaller ($P<0.02$) in control cores than in cores containing either *Diplocardia* spp. or *O. tyrtaeum* (Table 3).

Plants

Several plant parameters varied in response to earthworm treatments. Most prominent was the difference in

total plant growth on the final date, with significantly less total aboveground biomass for plants that had grown in *Diplocardia* cores (Table 4). The N content of plant tissues also was affected by earthworm treatment early in the study. Plants grown in *O. tyrtaeum* cores had significantly higher N concentrations compared to those in control cores ($P<0.10$, day 15), but plant N concentrations did not differ significantly on the fourth date (day 45). Although we found no differences in the N concentration of plant tissue (% N), we did observe differences in total standing stock of N in aboveground plant tissues. These differences were attributable to the above-mentioned decreased plant biomass production in *Diplocardia* treatments on the final date (Table 4).

Results of stable isotope analyses of plant tissues revealed additional effects of the earthworm treatments. On the first date, the atom percent ^{15}N (at%^{15}N) of plant tissues was highest in control cores, lowest in *Diplocardia* cores and intermediate in *O. tyrtaeum* cores (Table 4). On the fourth date, the trend of higher at%^{15}N in control plants persisted, but was no longer statistically significant. Standing stocks of ^{15}N in aboveground plant tissues closely paralleled those observed for standing stocks of total N, with significant differences being detected only on the fourth date (Table 4). Again, these differences were largely the result of differences in total plant biomass.

Discussion

Earthworm activity

Stable isotope analyses revealed that, over the course of the experiment, *Diplocardia* spp. assimilated more ^{13}C and ^{15}N than did *O. tyrtaeum* (Fig. 2). This result suggests that *Diplocardia* spp. may have been more active than *O. tyrtaeum* during the summer growing period, when the experiment was conducted. The notion that *O. tyrtaeum* was dormant over much of the course of the experiment is corroborated by observations made on early sampling dates when they were found at the very bottom of cores upon collection. Thus, one possible explanation for the lack of label uptake by *O. tyrtaeum* is simply 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, microbial biomass was labeled in the 10–20 cm depth (albeit to a lesser degree than shallower depths), and this label would have been detected in tissues earlier, had *O. tyrtaeum* been actively feeding. Because enrichment of *O. tyrtaeum* tissues with ^{13}C and ^{15}N was unchanged over the first 30 days of the experiment (Fig. 2), we conclude that these earthworms were inactive at this time. We suggest that this pattern of activity denotes a fundamental difference in the biology of the two earthworms examined in this study: that native *Diplocardia* spp. are better adapted to life in warmer drier soils than their European counterparts, *O. tyrtaeum*. At any rate, whether *O. tyrtaeum* was dormant or active at deeper depths, the contrast to *Diplocardia* activity patterns may have implications for nutrient cycling processes as these introduced earthworms expand their distribution in tallgrass prairie soils.

Effects of earthworms on microbial biomass

Effects of earthworms on soil MBC are difficult to generalize, and can be variable depending on the organic matter content of the soil, as well as the species of earthworm studied (Shaw and Pawluk 1986; Wolters and Joergensen 1992; Blair et al. 1995). However, the net effect of earthworms on bulk soil MBC seems to depend on the amount of labile C mobilized/assimilated during gut transit, and the proportion of the total soil volume that is processed. In our study, effects of earthworms on MBC were dependent upon the amount of time that earthworms had been in the soil, and the depth at which the earthworms were active. On the first date, earthworms appeared to have a stimulatory effect on MBC in the 0–5 cm depth (Table 2), but this effect was transient and may have been the result of short-term availability of labile C sources such as mucous or other metabolic products of earthworms. By the fourth date, MBC in the 0–5 cm depth was lower in cores containing *O. tyrtaeum*, relative to control cores (Table 2), and this decrease may have been due to feeding by *O. tyrtaeum* at shallower soil depths on or near that date. Indeed, *O. tyrtaeum* was encountered at this depth on the fourth date in a larger proportion than on any other date (Table 1). We suggest that *O. tyrtaeum* broke dormancy and moved into shallower soil depths in response to cooler temperatures and two large rainfall events (>50 mm) just before the experiment ended (Fig. 1). Associated with the break in *O. tyrtaeum* dormancy was ^{13}C enrichment of their tissues (Fig. 2), indicating not only movement into shallow soil layers, but also active feeding on labeled soil and MBC.

The effects of earthworms on the size of microbial C and N pools were variable, but another index of the microbial community – the 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 the C/N ratio in the presence of *Diplocardia* spp. (Table 2). This response may indicate that *Diplocardia* were assimilating N that otherwise would have been available to microbes or plants, thereby effectively elevating the C/N of the microbial biomass and possibly decreasing the uptake of N by plants.

Earthworm influence on plants

Plant growth and plant tissue analyses unexpectedly indicated that *Diplocardia* spp. may have had a negative influence on the total uptake of N by *A. gerardii*; in contrast, *O. tyrtaeum* had no effect on the uptake of N by *A. gerardii*, relative to controls. The earthworm-mediated

differences in total N uptake were due primarily to differences in total plant growth: plants in cores containing *Diplocardia* spp. were significantly smaller than those in control cores or cores containing *O. tyrtaeum*. Thus, the activity of *Diplocardia* spp. appeared to decrease the availability of N to *A. gerardii*. This result does not agree with previous findings, which indicate that *Diplocardia* spp. can positively influence native vegetation (James and Seastedt 1986). The greenhouse study by James and Seastedt (1986) used plants started from rhizomes, rather than seedlings, which may account for the different outcomes. It is likely that the outcome of their study was influenced by the fact that the rhizome-started plants had access to stored N reserves, and may have had a better opportunity to respond to earthworm treatments.

The mechanism behind the decreased availability of N to plants in cores containing *Diplocardia* spp. is unclear, but may be associated with immobilization of N in dead roots. The procedure we used to establish experimental cores (freezing of cores and subsequent removal of stolons and rhizomes) certainly resulted in a large pulse of dead root material relative to natural annual inputs. One possible explanation for the decreased availability of N in *Diplocardia* spp. cores is that stimulation of microbial processes by *Diplocardia* activity caused N to become immobilized in dead roots – an effect not seen in *O. tyrtaeum* cores, or control cores. N is known to be a limiting resource in tallgrass prairie (Blair 1997; Knapp et al. 1998), and this is particularly the case in annually burned prairie during 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 consider earthworms as a potential influence on this interaction in tallgrass prairie. In our study, *Diplocardia* spp. were active and assimilating N throughout the growing season, and this assimilation of N may have been at the expense of plants grown in the presence of *Diplocardia* spp.

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