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The Effects of Restoration Age and Prescribed Burns on Grassland Ant Community Structure

SEAN B. MENKE,[1] EMILEE GAULKE, ALLISON HAMEL, AND NICOLE VACHTER

Department of Biology, Lake Forest College, Lake Forest, IL 60045.

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ABSTRACT North American grassland environments are endangered as a result of degradation and conversion for agriculture and housing. Efforts to manage and restore grasslands have traditionally focused on monitoring plant communities to determine restoration success, but the incorporation of animal communities may provide important benchmarks of ecosystem function and restoration. Ants play many roles in maintaining ecosystem health in temperate grasslands, but relatively little is known about how ant communities respond to restoration. We studied the role that restoration age and prescribed burns have on ant communities in two types of Illinois grasslands, prairies and savannas, and identify indicator species of restoration success. Grassland environments included remnants and restorations that varied in age from newly restored sites, to sites that have been under restoration for >15 yr. We demonstrate that prairie and savanna ant communities are distinct, but respond to restoration in a similar manner. Three distinct prairie ant assemblages were identified based on the age of restoration of a site—sites <3 yr old, sites that have been under restoration >5 yr, and remnant prairies. Four distinct savanna ant assemblages were identified based on the age of restoration of a site—sites <3 yr old, sites 5–15 yr old, sites >15 yr old, and remnant savanna environments. After accounting for restoration age, time since last burn in both prairie and savannas does not explain community composition or species richness. Several ant species in both prairies and savannas have predictable changes in incidence that indicate their suitability for use as indicator species.

KEY WORDS prairie, savanna, indicator species, terrestrial invertebrate, Formicidae

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Temperate grassland ecosystems, including prairies and ecotonal savannas, are listed globally as endangered ecosystems (Noss et al. 1995, Hoekstra et al. 2005). They are some of the most threatened ecosystems in North America as a result of conversion for agriculture and housing, degradation, and fragmentation (Abrams 1992, Noss et al. 1995, Nemec 2014). In the central United States, >90% of grassland environments have been lost since the 19th century (Samson and Knopf 1994). Recent conservation efforts have focused on restoring degraded grasslands and reclaiming agricultural land to increase important ecosystem services such as habitat for native and endangered animals, soil retention and nutrient enrichment, and improved water quality (Samson and Knopf 1994, Whiles and Charlton 2005). Native grasslands and the ecotonal savannas were historically maintained by a combination of fire and grazing (Peterson and Reich 2001, Moranz et al. 2013). Modern restoration practices in degraded and remnant environments frequently begin with removal of brush and invasive plant species, followed by the implementation of a prescribed burn regime (Nielsen et al. 2003, Brudvig and Asbjornsen 2007), while restoration of reclaimed bare-field agriculture land begins with reseeding native vegetation, followed by a fire regime (Moranz et al. 2013). Prairie management and restoration have traditionally focused on monitoring plant communities as the metric for determining restoration success, but research is now demonstrating the utility of incorporating animal communities to provide tangible benchmarks of ecosystem function and restoration (Dufrene and Legendre 1997, Hodkinson and Jackson 2005, Nemec 2014).

Ants are excellent candidates for monitoring restoration success because they are one of the most common and abundant terrestrial animals on the planet, they are easy to collect, have high species richness, a good taxonomic base, and stationary nesting habits that allow them to be sampled over time (Hölldobler and Wilson 1990, Agosti et al. 2000). In addition, many species are successful colonizers of disturbed habitats and they respond to environmental change quickly, which often results in considerable economic impact as both agricultural and urban pests (Hölldobler and Wilson 1990). Ants are thought to play important roles in the functioning and health of grassland ecosystems due to their contributions to soil mixing, soil aeration, soil nutrient sequestration, and seed transport (Hölldobler and Wilson 1990, Trager 1990, Phipps 2006, Fagan et al. 2010). Ant communities or individual species have been successfully identified as indicators of environmental stress and disturbance in a variety of tropical environments (Hoffmann and Andersen 2003, Majer et al. 2007);

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[1] Corresponding author, e-mail: menke@lakeforest.edu.

however, their use as bioindicators in temperate grassland ecosystems is inconclusive (but see Kaspari and Majer 2000, Bestelmeyer and Wiens 2001, Fagan et al. 2010, Orlofske et al. 2010). Midwestern grasslands are home to ∼60 species of ants, with 25–35 species typically found in prairie remnants (Trager 1998). The utility of ants as bioindicators in temperate grasslands is currently inconclusive. Research by Orlofske et al. (2010) in NA tallgrass prairies asserts that ants are potential indicators, but this study was limited in taxonomic scope; Fagan et al. (2010) found that ant communities in English grasslands did not differ much with restoration age, but that one species was a good indicator, while New (2000), in SE Australia, found that variation in ant communities in grasslands was too great to use ants as an indicators.

Three interrelated processes play a role in patterns of species composition in restored grassland ecosystems—1) the starting point of restoration, 2) the age of the restoration, and 3) the restoration practices used, including burning, grazing, and herbicides (Whiles and Charlton 2005, Moranz et al. 2013, Nemec 2014). Most arthropod and, more specifically, ant community responses to grassland restoration have focused on a single type of grassland such as prairie or savanna or a single starting point of restoration such as a reclaimed agricultural field, pastureland, or savanna, undergoing succession to disturbed forest (but see Panzer 1995, Bestelmeyer and Wiens 2001). Relatively little work has been done on changes in ant communities with restoration age (Nemec 2014) and almost all of that work has compared a single set of restored grasslands to remnant grasslands (Panzer 1995, Kittelson et al. 2008, Orlofske et al. 2010, Nemec 2014). Phipps (2006) studied ant communities in a variety of different aged prairie restorations and found that ant species richness displayed a hump-shaped pattern with increasing restoration age, a peak in richness after 7–8 yr of restoration, and a decline in richness after 14–16 yr of restoration. When restored prairies are compared to virgin prairies, they are likely to have fewer or no mound-building Formica ants (Baxter and Hole 1967, Trager 1998, Foster and Kettle 1999). When mound-building ants are observed in restored prairies and disturbed grasslands, their colony densities are lower compared to virgin prairies, and colonization takes several years (Trager 1998, Foster and Kettle 1999, Foster 2004, Phipps 2006). The effect of prescribed burns on grassland ant communities is a growing area of study (Parr and Chown 2001, Moranz et al. 2013). Fire tends to have a stronger indirect than direct effect on ant communities (Nemec 2014). Direct effects are limited because most grassland ant colonies nest below ground (Underwood and Fisher 2006). Indirect effects of fire include altering nest availability for litter, twig and stem, and nut nesting species (Trager 1990); and alterations to the vegetation, thereby changing food resources (Underwood and Fisher 2006, Nemec 2014). Most studies have focused on only one or two of these factors and have rarely examined the interactions between the starting point of restoration, age of restoration, and the restoration practice used (Nemec 2014).

Given the importance of ants in temperate grassland ecosystems and the relative paucity of studies focused on how ant communities change with restoration efforts, we aim to 1) compare the response of ant communities to habitat restoration in two grassland types, prairies and savannas, 2) determine the relative importance of time under restoration and time since last burn on ant species richness and incidence, and 3) identify indicator species of successful habitat restoration in prairies and savannas. By incorporating the ant community response to grassland restoration, we will acquire an important benchmark to measure changes in ecosystem function.

Materials and Methods

Study Design. Ant diversity was surveyed in two different environments; 47 prairie sites were sampled in June 2011 and 21 savanna sites in June 2012. The sites, located throughout McHenry County, Illinois, included remnant sites and sites that varied in age from just beginning the restoration process the year sampling occurred, to sites that have been under restoration for >15 yr (Supplementary Appendix 1 [online only], Fig. 1). Remnant prairie sites consisted of native tallgrass prairie that had never been tilled but may have been grazed at one point, while remnant savanna sites were oak savannas that had undergone succession to mixed hardwood forests. We classified the study sites into four different restoration categories: remnant, under restoration for ≤3 yr, under restoration for 7–15 yr, and under restoration for >15 yr.

Ants were sampled using 30 50-ml centrifuge tube (27 mm diameter) pitfall traps at each site. Pitfall traps were typically placed in a 5 by 6 grid with 15-m spacing between each trap. Each centrifuge tube was filled with 25 ml of propylene glycol with a drop of fragrance-free soap. Pitfalls were buried flush with the soil surface, and left capped, in place, at each site for at least a week to allow vegetation to recover from the disturbance. All pitfall traps were open for 120 h during a span of dry weather. Trap contents were collected, brought back to the lab and fixed in 95% ethanol.

Ants were sorted and identified to species using keys found in The Ants of Ohio (Coovert 2005), A Field Guide to the Ants of New England (Ellison et al. 2012), and Ants (Formicidae) of the Southeastern United States (MacGown 2014). Representative voucher specimens are deposited at the Chicago Field Museum of Natural History.

Data Analyses. All analyses are based on ant workers. Reproductives were not included because their origin can be difficult to track (Fisher 1998). Comparisons of ant species abundance are best accomplished by quantifying colony number rather than the number of individual worker ants (Ellison et al. 2007). Because pitfall traps are unable to distinguish the number of colonies in a site, our statistical comparisons of abundance use incidences rather than the number of workers. Incidences are the number of traps in which a species occurred, regardless of how many workers are in a given trap. Ant incidences in pitfall traps have

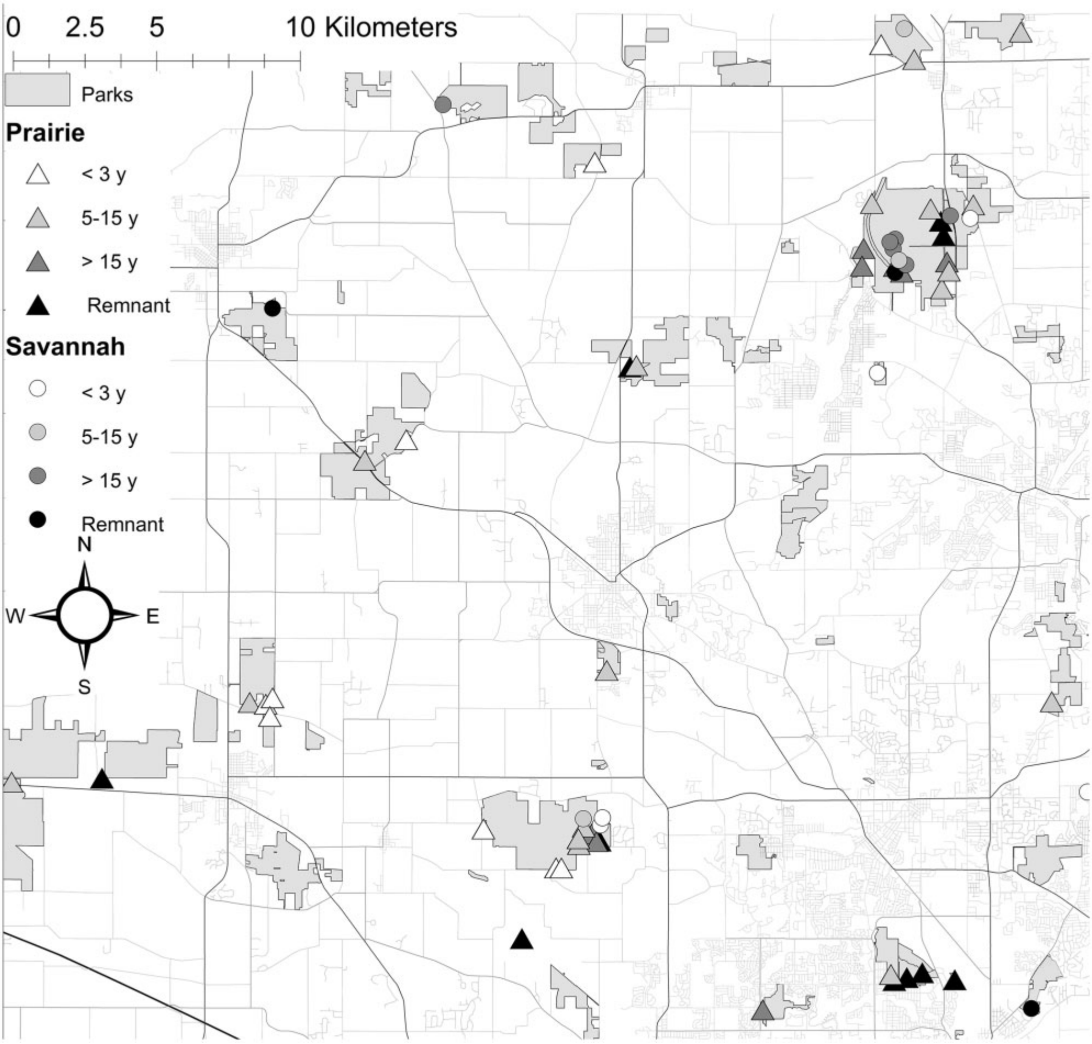

Fig. 1. Map showing the location of the 47 prairie (triangles) and 21 savanna (circles) study sites in McHenry County, IL. Gray areas represent land owned by the McHenry County Conservation District.

been shown to be correlated with colony number (Ellison et al. 2007). We calculated the asymptotic species richness for sample-based data using the Chao2 index. For calculation of the Chao2 index of asymptotic species richness, we pooled data from all of the sites for each restoration category. Chao2 calculations were conducted in EstimateS version 9.1 after being randomized 1,000 times (Colwell 2013).

Restoration and Fire Effects on Species Richness and Incidence. We used two-way ANOVAs to test how environment, restoration category, and how their interaction differed in species richness and incidence; all pair-wise comparisons were made with Tukey's multiple comparisons procedure. Estimated species richness within restoration categories is compared using the calculated 95% confidence intervals. Separate ANCOVAs were conducted for each environment to determine the importance of time since the last burn, while accounting for the age of the restoration, excluding remnant sites. Statistics were performed using JMP 8.0 (SAS Institute, Cary, NC).

Community Composition Changes. We performed permutational multivariate analysis of variance (PERMANOVA; McArdle and Anderson 2001) to test for differences in ant species square root transformed incidences between environment, restoration category, and their interaction. PERMANOVA is a permutational ANOVA, developed to test simultaneous responses of multiple variables to multiple factors. Analyses were based on Bray-Curtis dissimilarity with 99,999 permutations for each test and pair-wise comparisons. We then used principal coordinates analysis (PCoA) to visualize the distinctiveness of the ant assemblages in each environment across restoration categories. We also performed individual PCoAs for each environment. In order to analyze the effect of time since last burn while accounting for the age of restoration on ant communities, we conducted separate distance-based redundancy analyses (dbRDA; McArdle and Anderson 2001) for ant species square root transformed incidences in each environment. dbRDA is a permutational multiple linear regression based on Bray-Curtis dissimilarity with 99,999 permutations for each test. Statistics were performed in PRIMER v6.1 (Clarke and Gorley 2006).

Indicator Species. We evaluated which ant species were most responsible for differentiating communities in each restoration category using separate similarity percentage (SIMPER) analyses for prairies and savannas. SIMPER evaluates the contributions of each species to the Bray-Curtis dissimilarity of all pairs of samples between groups. Statistics were performed in PRIMER v6.1 (Clarke and Gorley 2006).

Results

Restoration and Fire Effects on Species Richness and Incidence. A total of 14,956 ants, comprising 48 species, were collected; 11,952 ants from 42 species representing 2,629 incidences in 1,385 pitfall traps were collected in prairies, while 3,004 ants from 37 species representing 1,298 incidences in 560 pitfall traps were collected in savannas (Supplementary Appendix 2 [online only]). Ant species richness differed by habitat (two-way ANOVA: $F_{1,60} = 11.22$, $P = 0.0014$) and restoration category (two-way ANOVA: $F_{3,60} = 21.08$, $P < 0.0001$), but there was a significant interaction (two-way ANOVA: $F_{3,60} = 4.97$, $P = 0.0038$). Species richness is lower in restored prairie sites compared to restored savanna sites, but was higher in prairie remnant sites than savanna remnant sites (Fig. 2A). Ant abundance, as measured by incidences in pitfall traps, was not different between habitats but did differ by restoration category (two-way ANOVA: $F_{3,60} = 20.94$, $P < 0.0001$), and there was a significant interaction (two-way ANOVA: $F_{3,60} = 10.30$, $P = 0.0024$). Ant incidence increases with age of restoration and is largest in remnant prairies, but smallest in remnant savannas (Fig. 2B). Estimated species richness is greater across all prairie sites and in remnant prairie sites compared to savannas, while both prairie and savanna restoration sites increase in estimated species richness with increasing time under restoration (Fig 3). Prairie sites that have been under restoration for at least 5 yr have as many estimated species as remnant sites, while savanna sites that have been under restoration for at least 5 yr have more species than remnant sites (Fig. 3).

For restored prairie sites, ANCOVAs to determine the importance of time since the last burn while accounting for the age of the restoration were nonsignificant predictors of ant species richness ($F_{2,25} = 2.05$, $P = 0.1495$, $r^2 = 0.14$) and incidence ($F_{2,25} = 1.55$, $P = 0.2312$, $r^2 = 0.11$). For savanna restoration sites, the overall ANCOVAs were significant in explaining ant species richness ($F_{2,14} = 10.93$, $P = 0.0014$, $r^2 = 0.61$) and incidence ($F_{2,14} = 3.97$, $P = 0.0432$, $r^2 = 0.36$), but time since last burn was nonsignificant (species richness: $F = 0.0023$, $P = 0.9622$, incidence: $F = 1.72$, $P = 0.2109$), after accounting for the effect of restoration age.

Community Composition Changes. Community composition differed between prairies and savannas (PERMANOVA: $P < 0.001$), by age of restoration (PERMANOVA: $P < 0.001$), with a significant interaction (PERMANOVA: $P < 0.001$). Prairies and savannas are separated on the x-axis of the PCoA (Fig. 4A), with prairie sites strongly correlated with presence of *Lasius neoniger* and savanna sites being strongly correlated with *Camponotus pennsylvanicus*, *Myrmica sp. AF-eva*, and *Lasius alienus.* Prairie sites are spaced along the x-axis from remnant sites on the left to the newest restoration sites on the right (Fig. 4B). Pairwise tests on prairie ant communities between restoration ages revealed three distinct communities—remnant prairies, prairies that had been under restoration for <3 yr, and prairies that had been under restoration for >5 yr (sites under restoration for 5–15 yr were indistinguishable from those that had been under restoration for >15 yr, $P = 0.1471$, Fig. 4B). Restored savanna sites are spaced along the diagonal, with the newest restorations in the upper left and the older restorations in the lower right, while the centroid from remnant sites occurs in the lower left corner (Fig. 4C). Pairwise tests on savanna ant communities demonstrated that ant communities in all four restoration classes were distinct (Fig. 4C).

An analysis of the rank order of change based on calculated similarities from the PERMANOVA revealed that the pattern in community similarity as age of restoration increases differs between prairies and savannas. In general, there is greater similarity between restoration ages in savanna sites (47.69–58.30%) than prairie sites (23.97–51.26%; Tables 1 and 2). In prairies, the ant communities become more similar to those found in remnant prairies with increasing age of restoration (Table 1). In prairies, ant communities were least similar (17.91%) between remnant sites and sites that had been under restoration for <3 yr, while the greatest similarity (43.70%) occurred between remnant sites and sites that had been under restoration for >15 yr (Table 1). In savannas, there appears to be no correlation between age of restoration and similarity to remnant sites (Table 2). But, as age of restoration increases in savannas, the ant communities become more similar to older restoration sites (Table 2). In savannas, ant communities differ more within remnant sites (33.48% similarity) than between any restoration age categories (Table 2).

DbRDA on the importance of time since last burn treatment after accounting for the age of restoration in determining community composition in restored prairie sites, revealed that the time since restoration began was significant ($P = 0.0256$, $r^2 = 0.08$), while adding time since last burn did not significantly improve the model ($P = 0.3407$, $r^2 = 0.04$). The dbRDA for restored savanna sites showed a similar pattern with time since restoration as significant ($P = 0.0001$, $r^2 = 0.26$), and that adding time since last burn did not significantly improve the model ($P = 0.7343$, $r^2 = 0.04$).

Indicator Species. In prairies, the most common ant was *Lasius neoniger*, which occurred in 27% of the

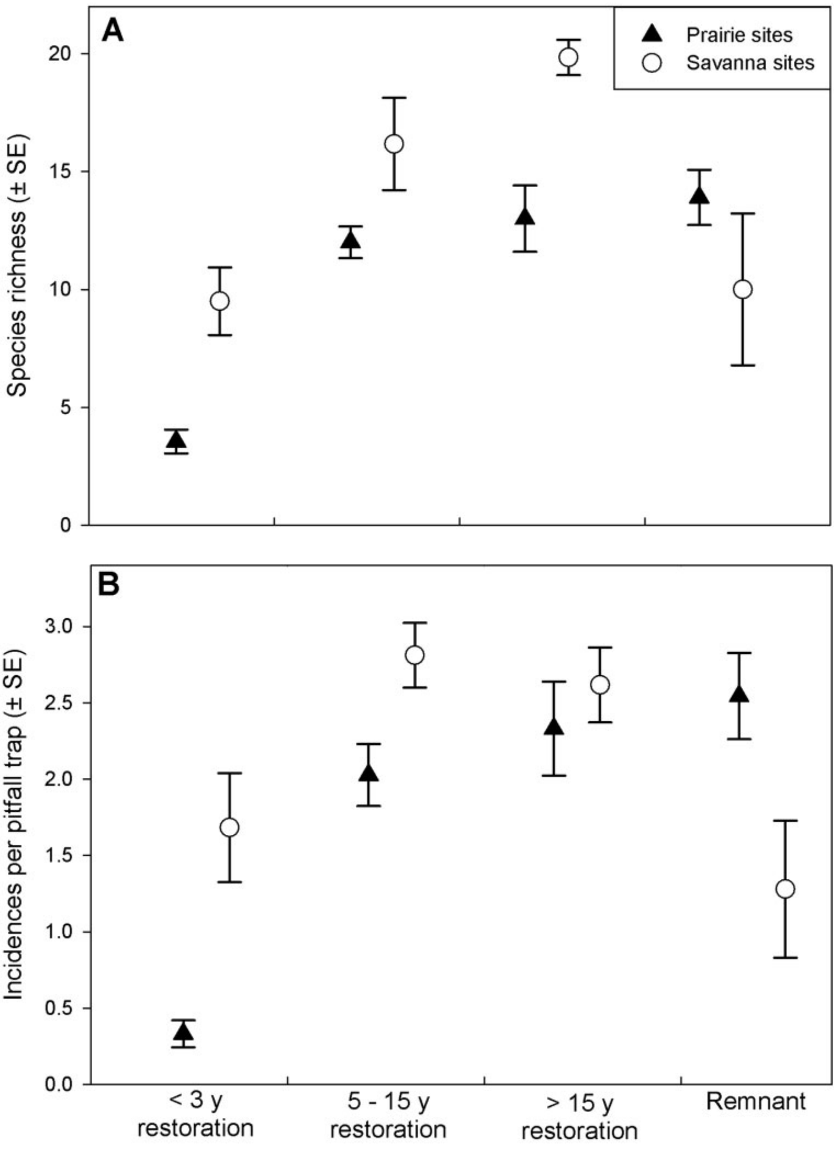

Fig. 2. (A) Average ant species richness and (B) worker abundance as measured by incidence in pitfall traps for both prairies (triangles) and savannas (circles) across four habitat restoration categories. Error bars represent one standard error.

pitfall traps, and no other species occurred in >20% of the pitfall traps (Supplementary Appendix 2 [online only]). Three species occurred in >25% of the traps in remnant prairies—*Solenopsis molesta*, *Aphaenogaster picea*, and *L. alienus*, and all three of the species became more common with increasing age of restoration (Fig. 5A). Three species were most common at intermediate ages of restoration, occurring in >20% of traps: *L. neoniger*, *Myrmica fracticornis*, and *Formica montana* (Fig. 5A). Based on the PCoA of prairie sites, three species were strongly indicative (Spearman correlation > 0.7) of increasing age under restoration—*A. picea*, *L. alienus*, and *S. molesta.* The SIMPER analysis revealed that initially, the transition from restoration sites <3 yr old to sites 5–15 yr old was due to a large increase in six species (Table 3). Sites that had been under restoration for >15 yr had a continued increase in *F. montana* and *S. molesta*, a decrease in *L. neoniger* and *Tetramorium caespitum*, the replacement of *M. detritinodis* by *M. fracticornis*, and the increased importance of *A. picea* (Table 3). The differences between sites with >15 yr of restoration and remnant prairies was driven, in part, by the continued increase in *S. molesta* and *A. picea*, the replacement of *L. neoniger* by *L. alienus* and *M. fracticornis* by *M. sp. AF-eva*, and the decrease in *F. montana* (Table 3).

In savannas, the four most common species were *L. alienus* (39%), *A. picea* (25%), *Camponotus pennsylvanicus* (25%), and *M. sp. AF-smi* (24%; Supplementary Appendix 2 [online only]). Three species were more

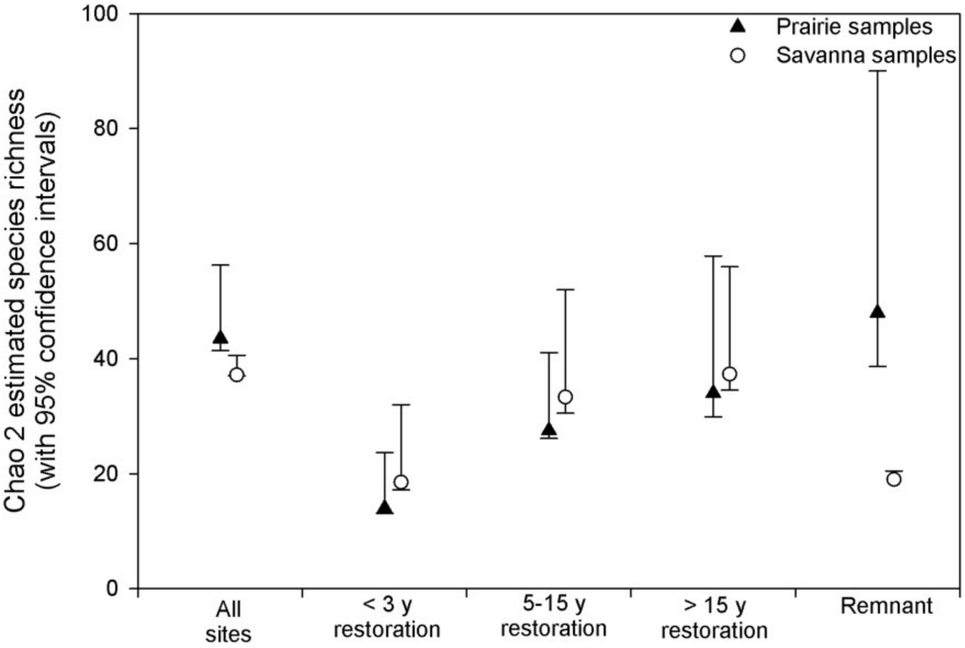

Fig. 3. Chao 2 asympototic estimates of species richness comparing prairies (triangles) and savannas (circles) by restoration categories. Error bars represent 95% confidence intervals.

common in remnant sites than any other stage of restoration—*F. incerta* (16%), *L. flavus* (10%), and *M. fracticornis* (10%; Fig. 4B). Five relatively common species increased to greater than 10% occurrence in pitfall traps with increasing restoration age—*Temnothorax curvispinosus*, *Brachymyrmex depilis*, *M. pinetorium*, *M. punctiventris*, and *M. detritinodis* (Fig. 5B). Based on the PCoA of savanna sites, three species were strongly indicative (Spearman correlation > 0.7) of increasing age under restoration—*Formica incerta*, *M. pinetorum*, and *Tapinoma sessile*, while one species was strongly indicative decreasing age under restoration—*M. sp. AF-smi*. The SIMPER analysis revealed that as sites underwent the restoration process from remnant forests to sites <3 yr old, *Camponotus pennsylvanicus*, *M. sp. AF-smi*, and *L. alienus* increased in incidence, while *T. sessile*, *F. subsericea*, and *M. sp. AF-eva* decreased in incidence. As restoration age increased to 5–15 yr old, *L. alienus* and *M. sp. AF-smi* continued to increase in incidence, *A. picea*, *F. pallidefulva*, *T. curvispinosus*, and *M. pinetorum* all increased in incidence. As sites continued to greater than 15 yr of restoration, *M. sp. AF-smi* was replaced by *M. pinetorum* and *M. punctiventris*, *Tapinoma sessile* became common again, and *F. pallidefulva* and *L. alienus* became less common.

Discussion

Summary. In this study, we address three topics related to how grassland ant communities respond to habitat restoration. We determine 1) how ant communities respond to time since restoration began and the use of fire in restoration, 2) how ant communities found in prairies and savannas differ in their response to restoration, and 3) indicator species for use in documenting successful habitat restoration. We demonstrated that ant species richness and incidence, as an indicator of colony number (Ellison et al. 2007), in both prairies and savannas increase with age of restoration (Figs. 2 and 3) and that the time since a site was last burned does not explain patterns in species richness or incidence, after accounting for how long the site had been under active management. Prairie ant communities in restored environments form two groups—one found in newly restored sites <3 yr old and one found in sites that have been under restoration for >5 yr, with communities in older restorations distinct from but more similar to those found in remnant prairies (Fig. 4B). Savanna ant communities in restored environments are distinct in each of the three restoration age categories and all three communities are distinct from those found in remnant savanna environments (Fig. 4C). After accounting for restoration age, the time since a site was last burned in both prairie and savanna restorations does not explain patterns of community composition. Several ant species in both prairie and savanna restorations have predictable changes in incidence that indicate their suitability for use as indicator species.

While ants are acknowledged to play important roles in grassland ecosystems, especially in terms of restoration of ecosystem services such as their direct impacts on soil quality (Baxter and Hole 1967, Trager 1998, Nemec 2014, Wodika et al. 2014) and mutualisms with plants and other invertebrates (Trager 1990, Nemec

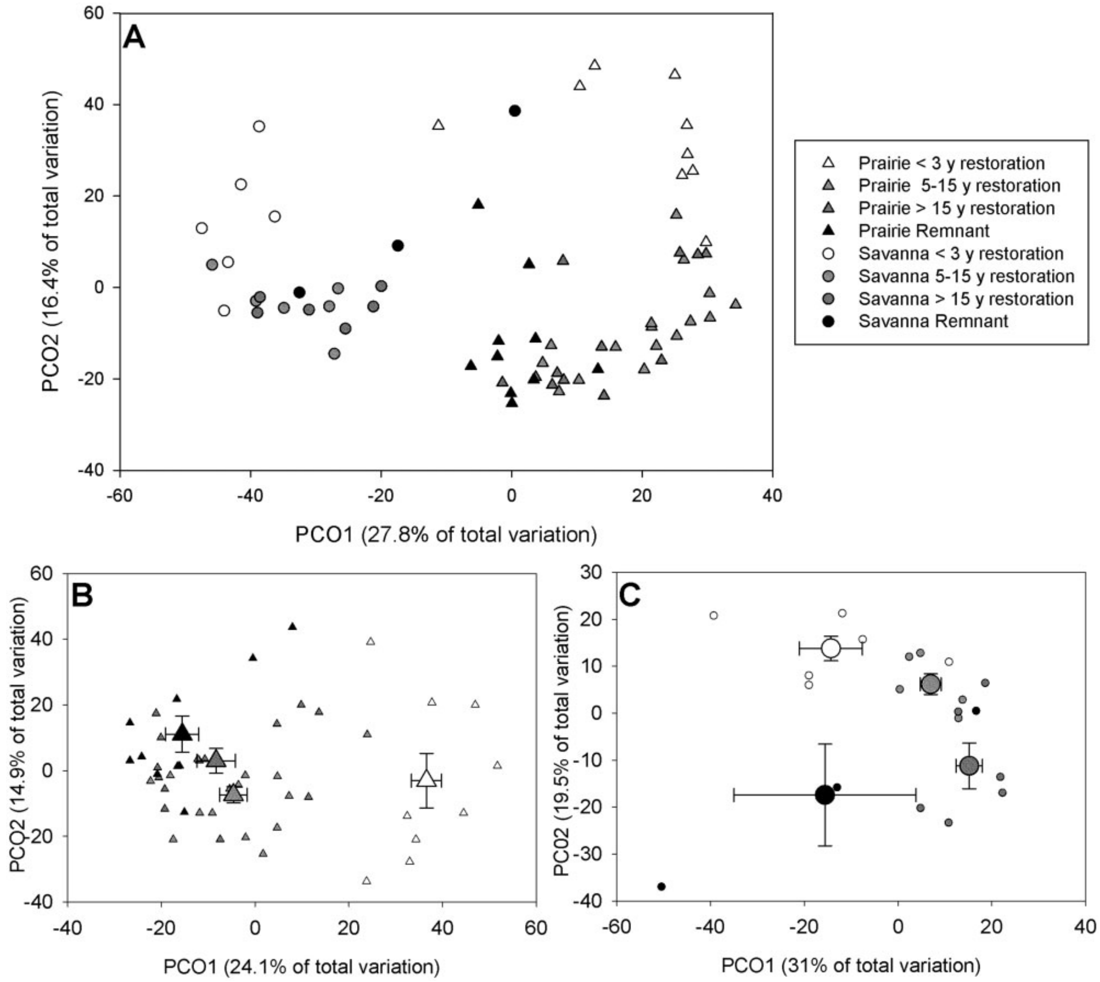

Fig. 4. Two-dimensional PCoA plots based on square root transformed abundance (as measured by incidences) of ant community composition. (A) All plots sampled, (B) all prairie plots sampled, and (C) all savanna sites sampled. Triangles represent prairie sites, circles represent savanna sites, and darker colors represent older restoration sites. Large symbols represent the average community composition for each restoration category.

Table 1. Average percent similarity between and within prairie sites calculated in PERMANOVA

	Percentage similarity			
	<3 years	5–15 years	>15 years	Remnant
<3 years	31.6			
5–15 years	28.13	55.23		
>15 years	23.97	51.26	49.87	
Remnant	17.91	42.15	43.00	43.70

Table 2. Average percent similarity between and within savanna sites calculated in PERMANOVA

	Percentage similarity			
	<3 years	5–15 years	>15 years	Remnant
<3 years	61.02			
5–15 years	56.41	64.18		
>15 years	47.69	58.30	62.26	
Remnant	40.24	41.30	40.36	33.48

2014), little is known about how restored communities change through time. This is, in part, due to the lack of long term data sets documenting changes in a single site (but see Phipps 2006). We used 68 grassland sites in a space-for-time substitution to expand on our existing knowledge of how ant communities change to a consistently applied restoration practice of prescribed burning. Remnant grasslands are reported to have greater species richness than restored sites (Nemec 2014). Our results expand on these findings by demonstrating that species richness in restored prairies can be similar to remnant sites after only 5 yr of restoration practices on an abandoned farm field, while in savannas species, richness in sites restored for only 5 yr may exceed that found in remnant sites (Fig. 2). But, ant community structure in remnant grasslands is distinct

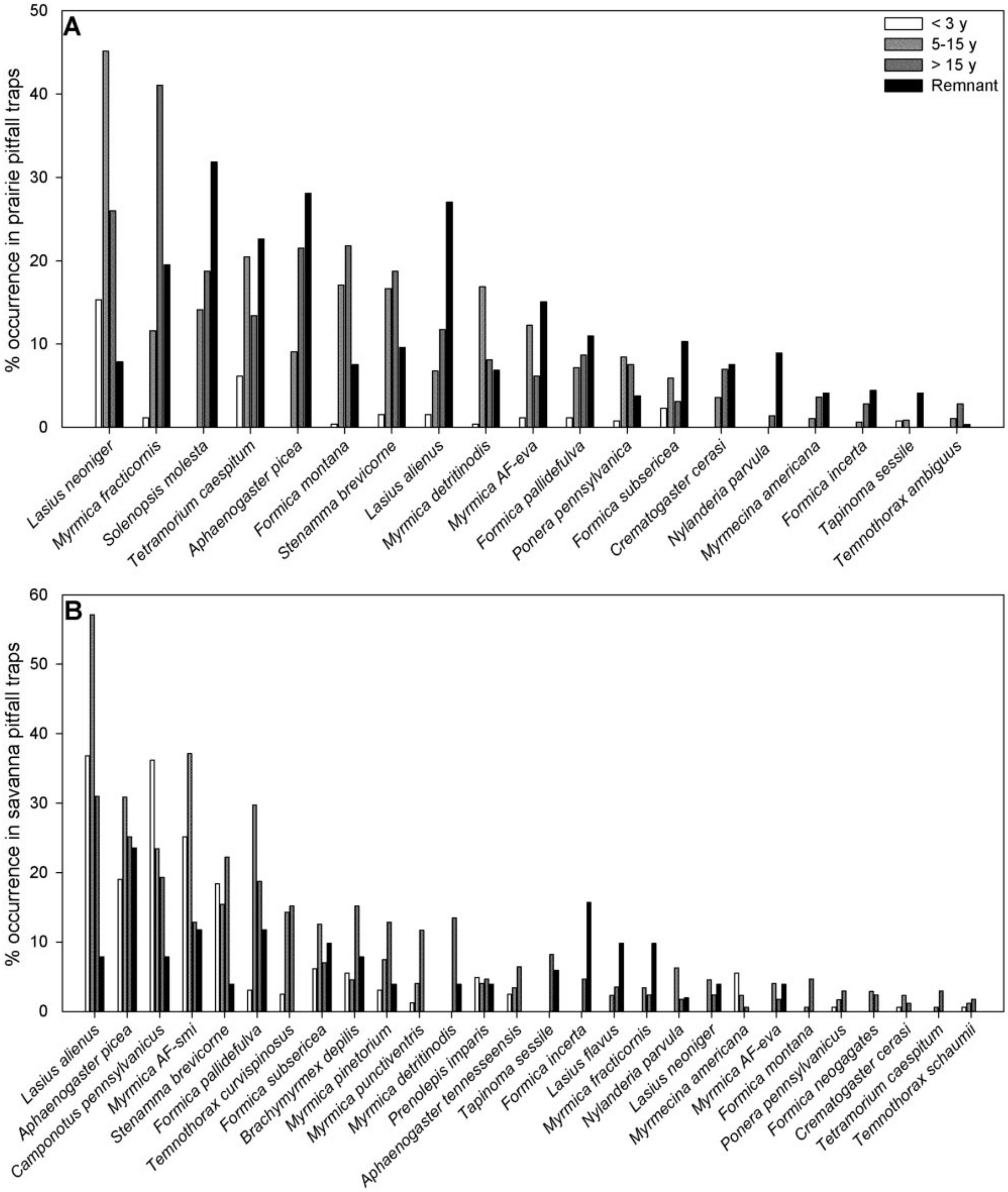

Fig. 5. Abundance of ant species, as measured by percent occurrence in pitfall traps, in each restoration category for (A) prairie and (B) savanna sites. The species are ordered by their total incidence in each environment.

from sites that have been under restoration for >20 yr (Fig. 3). The change in community structure with restoration age takes different trajectories in prairies and savannas. In prairies, as time under restoration increases, ant communities become increasingly similar to those found in remnant sites, while in restored savannas, ant communities appear to be on a trajectory that diverges from those found in remnant sites (Tables 1 and 2; Fig. 4). Ant communities in early prairie restorations are distinct from those in early savanna restorations, but with increasing time under restoration, ant communities in prairies and savannas become increasingly similar (Fig. 4A).

Changing vegetation structure may be one possible explanation for the observation that distinct ant communities become more similar with time in newly restored prairies and savannas. Prairie restorations in this study began as reclaimed farmland, consisting of

Table 3. Ant species responsible for differentiation in prairie sites (SIMPER analysis: species explaining 50% of the variation)

Ant species	Percentage similarity		Avg. dissimilarity	Contribution (%)
	Restoration 1	Restoration 2		
<3 years	**<3 years**	**5–15 years**		
Lasius neoniger	2.86	6.37	8.34	11.16
Myrmica detritinodis	0.21	3.69	6.50	8.68
Formica montana	0.20	3.18	5.97	7.98
Solenopsis molesta	0.00	3.22	5.73	7.67
Tetramorium caespitum	1.81	3.79	5.54	7.41
Stenamma brevicorne	0.70	3.46	5.32	7.12
5–15 years	**5–15 years**	**>15 years**		
Myrmica fracticornis	2.39	5.37	5.08	10.18
Formica montana	3.18	3.54	3.77	7.55
Lasius neoniger	6.37	4.42	3.76	7.53
Aphaenogaster picea	2.15	3.80	3.24	6.49
Tetramorium caespitum	3.79	2.94	3.13	6.27
Solenopsis molesta	3.22	3.51	3.02	6.04
Myrmica detritinodis	3.69	2.21	2.74	5.49
>15 years	**>15 years**	**Remnant**		
Myrmica fracticornis	5.37	3.20	4.48	7.69
Lasius neoniger	4.42	1.37	3.86	6.61
Lasius alienus	2.24	4.66	3.69	6.34
Solenopsis molesta	3.51	4.77	3.51	6.03
Formica montana	3.54	1.90	3.34	5.74
Aphaenogaster picea	3.80	4.59	3.31	5.69
Tetramorium caespitum	2.94	4.07	2.89	4.96
Myrmica sp. AF-eva	2.06	2.33	2.72	4.67
Stenamma brevicorne	3.94	2.44	2.41	4.14

Table 4. Ant species responsible for differentiation in savanna sites (SIMPER analysis: species explaining 50% of the variation).

Ant species	Percentage similarity		Avg. dissimilarity	Contribution (%)
	Restoration 1	Restoration 2		
Remnant	**Remnant**	**<3 years**		
Camponotus pennsylvanicus	2.04	5.86	6.87	11.13
Myrmica sp. AF-smi	2.36	4.59	4.63	7.50
Aphaenogaster picea	3.76	3.26	4.62	7.49
Lasius alienus	2.84	5.70	4.09	6.63
Tapinoma sessile	2.31	0.00	4.03	6.52
Formica subsericea	2.42	1.55	3.23	5.24
Myrmica sp. AF-eva	1.42	0.00	3.09	5.01
<3 years	**<3 years**	**5–15 years**		
Formica pallidefulva	1.04	5.32	4.77	10.71
Aphaenogaster picea	3.28	5.36	3.33	7.48
Temnothorax curvispinosus	0.86	3.12	2.93	6.59
Formica subsericea	1.55	3.16	2.71	6.09
Myrmica sp. AF-smi	4.59	5.83	2.55	5.73
Lasius alienus	5.70	7.46	2.52	5.56
Myrmica pinetorum	1.02	2.12	2.06	4.62
Camponotus pennsylvanicus	5.86	4.65	1.96	4.41
5–15 years	**5–15 years**	**>15 years**		
Myrmica sp. AF-smi	5.83	2.04	3.56	8.41
Myrmica detritinodis	0.00	2.78	2.19	5.17
Brachymyrmex depilis	1.72	3.50	1.78	4.19
Tapinoma sessile	0.00	2.15	1.76	4.16
Formica pallidefulva	5.32	3.98	1.70	4.02
Temnothorax curvispinosus	3.12	3.70	1.67	3.95
Myrmica punctiventris	1.40	3.36	1.61	3.79
Lasius alienus	7.46	5.51	1.58	3.73
Myrmica pinetorum	2.12	3.48	1.50	3.53
Stenamma brevicorne	3.83	4.29	1.46	3.44
Camponotus pennsylvanicus	4.65	4.12	1.41	3.32
Formica subsericea	3.16	2.37	1.37	3.23

bare dirt fields with almost no ant species, while savanna restorations originated as secondary forest sites with intact ant faunas. As the physical environment in both prairies and savannas began to respond to the restoration practices of invasive plant and brush removal, intentionally seeding native plants, and prescribed burns followed by natural colonization of plants, the two environments became structurally more similar. While we did not conduct vegetation surveys as part of this study, all restoration sites were seeded with a similar profile of native grassland species (S. M., unpublished data) and differ mostly in the amount of tree and shrub canopy allowed to persist. One might also expect ant communities to converge on similar outcomes, given the lack of any true prairie specialist ants in North America (Trager 1998). The savanna ant fauna will always be somewhat distinct from prairie sites because of the increased number of woodland cavity nesting habitats and associated forest specialist ant species such as *Temnothorax* and *Myrmica* species (Talbot 1934, Gregg 1944, Houdeshell et al. 2011, Menke and Vachter 2014).

Contrary to previous studies suggesting that ants are not useful indicators of grassland restoration (Panzer 1995, New 2000, Fagan et al. 2010, Wodika et al. 2014), we demonstrate that the lack of specialist grassland ant species in North America (Trager 1998) does not preclude their use as bioindicators of restoration status. While Orlofske et al. (2010) found ants to be good indicators of remnant prairie status when they were taxonomically lumped together, and Moranz et al. (2013) demonstrated that different ant functional groups responded to restoration practices in a predictable manner, we identified several good indicator species that are relatively common (Tables 3 and 4; Fig. 5), easy to identify (Coovert 2005, Ellison et al. 2012), and easy to monitor with classic baiting and trapping techniques (Menke and Vachter 2014). The three most common species in prairie remnants (*A. picea*, *L. alienus*, and *S. molesta*) all increase in incidence with increasing restoration age. Both *Aphaenogaster picea* and *L. alienus* are known myrmecochorous species which collect seeds from numerous plant species (Ellison et al. 2012), while *A. picea* colony growth has been demonstrated to benefit from plant elaisomes (Clark and King 2012) while *L. alienus* tends to feed at a lower trophic level by frequently tending honeydew-secreting insects (Tillberg et al. 2006). The thief ant, *S. molesta*, is often found in a parasitic association with nests of other ant species and they are also known to tend below ground honeydew-producing insects (Coovert 2005, Ellison et al. 2012). In savanna restorations, the pattern is a little less clear, with multiple common species at all stages of restoration. Excluding the remnant savannas, which are second growth forests, three of the common species at the oldest restoration sites (*M. pinetorum*, *M. punctiventris*, and *T. curvispinosus*) were uncommon in early restoration ages. Species of *Myrmica* are known to be important seed dispersers of forest plants, while *T. curvispinosus* is a common acorn nesting ant which feeds on honeydew and plant nectar (Ellison et al. 2012). Some of the disparity in the apparent utility of ants as indicator species between our findings and previous studies, especially in North American grasslands, can be attributed to differences in study designs. Sampling techniques vary from those that are not designed for adequately sampling ants (Panzer 1995, Wodika et al. 2014), to lack of species-level taxonomy (Panzer 1995, Orlofske et al. 2010, Moranz et al. 2013), to comparisons limited between remnant and restored sites with no gradations in the level or type of restoration (Nemec 2014). Our study was unique because we specifically and thoroughly sampled the ant fauna, identified all of our samples to the species level, and sampled ants across a gradient of replicated restoration ages in two different types of grasslands.

We found no significant effect of prescribed burns on ant species richness, incidence, or community structure, after accounting for the age of restoration in our grassland sites. These results support other studies in North American grasslands (Nemec 2014). While Moranz et al. (2013) found that relatively frequent fires favored *F. montana*, the overall effect on the ant community was small. While fire frequency has been documented to play a large role in structuring grassland vegetation (Samson and Knopf 1994, Peterson and Reich 2008, Cavender-Bares and Reich 2012) and leaf litter arthropod communities (Siemann et al. 1997, Coleman and Rieske 2006, Houdeshell et al. 2011, Moranz et al. 2013), the relatively small role of fire on ant communities in grassland restoration is due, in part, to three interrelated factors. First, most controlled burns by McHenry County Conservation District occur in early spring, when ants are inactive due to cold temperatures (Panzer 1995, Moranz et al. 2013). Second, there are relative few aboveground cavity nesting species and most ant nests occur at least partially underground and are therefore not susceptible to the direct effects of fire (Trager 1998, Underwood and Fisher 2006, Houdeshell et al. 2011, Nemec 2014). Third, grassland plants are fire adapted and they quickly regrow to provide habitat and food resources (Siemann et al. 1997, Cavender-Bares and Reich 2012, Moranz et al. 2013).

In conclusion, in temperate grassland ecosystems, ant community structure shifts along predictable pathways so that ant communities can be used to assess the trajectory of habitat restoration. Our research expands on previous efforts to assess the effect of grassland restoration practices on ant communities by systematically sampling changes in ant community structure using species-level taxonomy across multiple stages of grassland restoration in both prairie and savanna grasslands. Our results corroborate previous studies by demonstrating that prescribed burning, when used in early spring, plays a relatively minor role in ant community composition (Houdeshell et al. 2011, Moranz et al. 2013). We also corroborate previous work, which shows that ant communities rapidly recolonize restored grasslands (Phipps 2006, Nemec 2014, Wodika et al. 2014), while expanding on previous studies to highlight indicator species for both prairie and savanna restorations. Ants play many important ecological roles in temperate grassland ecosystems (Hölldobler and Wilson 1990, Nemec 2014) and the relative ease of sampling and commonness in these systems (Agosti et al. 2000, Lach et al. 2010) makes them an ideal tool for monitoring the success of habitat restoration.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

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