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Research

Reducing Native Ant Abundance Decreases Predation Rates in Midwestern Grasslands

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Abstract

Diverse and robust predator communities are important for effective prey suppression in natural and managed communities. Ants are ubiquitous components of terrestrial systems but their contributions to natural prey suppression is relatively understudied in temperate regions. Growing evidence suggests that ants can play a significant role in the removal of insect prey within grasslands, but their impact is difficult to separate from that of nonant predators. To test how ants may contribute to prey suppression in grasslands, we used poison baits (with physical exclosures) to selectively reduce the ant population in common garden settings, then tracked ant and nonant ground predator abundance and diversity, and removal of sentinel egg prey for 7 wk. We found that poison baits reduced ant abundance without a significant negative impact on abundance of nonant ground predators, and that a reduction in ant abundance decreased the proportion of sentinel prey eggs removed. Even a modest decrease (~20%) in abundance of several ant species, including the numerically dominant *Lasius neoniger* Emery (Hymenoptera: Formicidae), significantly reduced sentinel prey removal rates. Our results suggest that ants disproportionately contribute to ground-based predation of arthropod prey in grasslands. Changes in the amount of grasslands on the landscape and its management may have important implications for ant prevalence and natural prey suppression services in agricultural landscapes.

Key words: prey suppression, predation services, natural enemies, diversity, *Lasius neoniger*

Arthropods contribute to a variety of important ecosystem services in agricultural systems including pollination, decomposition, nutrient cycling, and natural pest control (Prather et al. 2013, Schowalter et al. 2018). The abundance and diversity of arthropods and the ecosystem services they provide are influenced by the abundance and distribution of crop and noncrop habitats within a landscape (Chaplin-Kramer et al. 2011, Shackelford et al. 2013, Veres et al. 2013). In particular, the occurrence of seminatural habitats (e.g., grasslands, hedgerows) has frequently been associated with increased abundances of beneficial arthropods (Bianchi et al. 2006), leading to increased provisioning of pollination (Blaauw and Isaacs 2014) and pest suppression services (Rusch et al. 2016). Perennial grasslands including natural and restored prairies, hayfields, pastures, and grasses grown as biomass crops, harbor diverse and abundant arthropod communities. These arthropod communities provide important ecosystem services within grasslands (Werling et al. 2011a, Werling et al. 2014) and also spillover into surrounding habitats (Liere et al. 2015). Perennial grasslands may also serve as reservoirs of biodiversity for recolonization into disturbed habitats (Tscharntke et al. 2005).

Ants are ubiquitous components of terrestrial ecosystems where they frequently play key roles as consumers and ecosystem engineers. As consumers, ants directly and indirectly affect the abundance, diversity, and behavior of other arthropods within an ecosystem (Wills and Landis 2018). Ants can directly reduce populations of other arthropods through predation or competition (Styrsky and Eubanks 2007, Sanders et al. 2011). They can also indirectly influence arthropod populations through nonconsumptive effects, where cues to the predator's presence (e.g., visual, chemical) cause changes in the development, growth, or feeding behavior of potential prey (Cembrowski et al. 2014, Mestre et al. 2014). Ants can also act as ecosystem engineers. They can concentrate resources within the nest, and increase soil nutrient content and its microbial activity (Dauber and Wolters 2000, Boulton and Amberman 2006). Ant nest

construction and maintenance activities redistribute organic matter and nutrients within the soil profile (Boulton and Amberman 2006, Halfen and Hasiotis 2010), creating conditions ideal for plant colonization. Therefore, changes to an ant community can potentially affect grassland biodiversity and above- and belowground community composition (Dean et al. 1997, Dostál 2007).

While ants are common in the upper Midwest U.S. grasslands (Beattie 1989, Ellison et al. 2012), with worker densities exceeding 1,800 per m^2 (Wodika et al. 2014), their contribution to prey suppression is often overlooked or underestimated (Grieshop et al. 2012, Nemec 2014). In general, ants search for a food as individuals and upon locating a resource recruit nest mates to the resource (Traniello 1989). Therefore, while individual foragers are dispersed (low densities) within a habitat and may only represent a small portion of total predators in a community, as a group (the colony) can efficiently remove prey items. Studies in European grasslands found densities of ants at 140–300 per m^2, and ants are collectively capable of collecting thousands of individual arthropods, consuming 200 times their body mass in a single season (Kajak et al. 1972). Establishing and maintaining the biomass necessary to reach densities of 140–1,800 ants per m^2 requires consuming a significant amount of arthropod biomass that likely includes arthropod plant pests.

Despite their importance in structuring ecosystems, relatively little is known about the ability of ants to provide prey suppression services in temperate perennial grasslands when compared with other systems (Nemec 2014). Most previous work examining ant prey suppression services examines their role in tropical or subtropical agricultural settings (Symondson et al. 2002). However, several studies suggest that ants may play a role in natural control of herbivore populations in temperate habitats. For example, ants have been identified as important predators of lepidopteran pests in turf (López and Potter 2000), coleopteran pests within meadows (Zhao et al. 2014) and agricultural fields. Ants have been identified as important predators in crops such as cotton (Styrsky and Eubanks 2007, Wickings and Ruberson 2016), corn (Kirk 1981, Perfecto 1991, Grieshop et al. 2012), blueberry, and biofuel grasses (Grieshop et al. 2012). In perennial systems like grasslands, ants have been observed foraging day or night, are quick to recruit to sentinel prey items, and are the most common predators removing sentinel pest prey items (López and Potter 2000, Grieshop et al. 2012). Therefore, ants are likely important predators of plant pests in temperate grasslands.

From the relatively few published works examining the effect of ants on ecosystem functions and services, researchers have relied upon physical or chemical means to selectively reduce ant populations. These methods could have negative impact on other nonant predators. Researchers have used ridged plastic sheets or halved PVC piping, coated with Fluon (a liquid Teflon) to exclude workers from entering plots (Sanders and Platner 2007, Wardle et al. 2011); these physical barriers could exclude other important insect predators. Because these physical suppression methods may simultaneously alter the occurrence of nonant predators, these methods may over-estimate the effects of ants because other predators are also reduced. More commonly, researchers have used chemical baits to reduce ant populations (López and Potter 2000, Parr et al. 2016) because chemical baits require less time and resources to initiate and can be used in larger scale experiments (Parr et al. 2016). Some commercially available ant baits are developed for low toxicity to nontarget organisms (Maxforce FC Fire Ant Bait 2015) and are designed to be specifically attractive to ants, further minimizing their nontarget effects. Nevertheless, nonant ground foraging predators can still be exposed to the chemical treatments from consumed baits directly or consuming exposed arthropods. Thus monitoring of nontarget arthropods is important in suppression approaches that utilize poison baits. Efforts to monitor nontarget arthropod predator communities in suppression studies is relatively rare (refer Parr et al. 2016).

Here we present results of a study aimed at isolating the role of ants as predators in perennial grassland systems. We selectively suppressed ant populations in perennial grasslands in two common garden experiments using poison baits designed to depress ant populations while minimizing the impacts on nonant predators. The selective suppression of ants was accomplished by using both chemical (a low dosage poison baits) and physical (mesh hardware cloth covering baits) means to isolate the effect of ants as predators in grasslands. We then measured the diversity and abundance of ant and nonant predators and their impact on removal of sentinel pest eggs. If ants play a significant role in predation of prey in perennial grasslands, we predicted that a reduction in ant abundance would result in decreased prey removal rates in grasslands.

Materials and Methods

Study Sites

We adopted a common garden approach because we wanted to minimize environmental variation (e.g., grassland management, fire history, vegetation type, and slope) that can naturally occur across sites and that could alter the effectiveness of ants to locate and carry bait to nests. However, we wanted to determine whether different ant communities would respond in similar ways to our treatments. Therefore, this study was repeated in two geographically separate locations in Michigan and Wisconsin. We selected the locations within each state based on similarities in ant abundances and community composition from previous sampling (Kim et al. 2017). The Michigan study location was located within the Edger Waterfowl Protection Area in Barry County, MI, (42°38′49.44″N, 85°23′1.08″W) and the Wisconsin location within the Brooklyn Natural Wildlife Area in Dane County (42°52′3.08″N, 89°29′18.22″W). Both locations were former hayfields converted to native warm-season grasslands and managed primarily by fire (both locations last burned in 2012) for migratory birds and other wildlife. Agricultural fields, wooded areas, and perennial grasslands surround both locations. The Michigan grassland was established in 2008 and consisted of grasses (e.g., *Schizachyrium scoparium*, *Panicum virgatum*, and *Elymus canadensis*) and wildflowers (e.g., *Rudbeckia hirta*, *Solidago rigida*, and *Chamaecrista fasciculata*). The Wisconsin location was established in 2004 and included grasses (e.g., *S. scoparium*, *P. virgatum*, and *E. canadensis*) and wildflowers (e.g., *R. hirta*, *Solidago altissima*, and *Trifolium pratense*). Sampling during the experiment occurred in 2015 from early June to July for a total of 7 wk.

Baiting and Sampling

To test how reducing ant abundance affects pest predation rates, we created four pairs of 8 × 8 m plots within each location. We randomly selected one plot within each pair to serve as the 'control' and the other as the 'poison' treatment. Plots within each pair were separated by 20 m, with each pair separated by 30 m. The plots within location were considered independent because for the ants in our region foraging is generally limited to only several meters (0–5 m) from the nest (Ness et al. 2004, 2016) and preliminary experiments (2014) indicated that poison baits did not impact ant abundance >5 m from baits. Each plot was further subdivided into four 4 × 4 m quadrants with a sampling station established in the center of each quadrant (i.e., 2 m from any edge and 4 m from any other sampling

station). At each station, we deployed baits (control or poison), an ant pitfall trap, and sentinel egg cards (details below), each separated by 0.5 m. Given published foraging distances of all temperate grassland ant species ranges from <0.5 to 10 m this density of baits provided us enough coverage within the experimental plots while minimizing the effect of poison baits in adjacent plots (Pudlo et al. 1980, Traniello and Levings 1986, Ness et al. 2016).

To suppress ant populations, we created two types of baits by mixing fipronil (Termidor SC, BASF Corporation, NC) into a honey bait and peanut butter bait at a concentration of 0.0095% (w/v). We chose fipronil because it is both slow acting (allowing for transfer between individuals) and effective at reducing survival of both queens and workers at low dosages (Hooper-Bui and Rust 2000). The poison baits were deployed as single point sources at the center of a sampling station (two baits, one honey and one peanut butter bait per 4 × 4 m area). Control plots received a honey (8 g) and peanut butter (9 g) baits of equal volume containing no poison. Baits were deployed in 20 ml scintillation vials fitted with 0.5 cm^2 mesh hardware cloth covers to exclude most other predaceous arthropods larger than ants, e.g., ground beetles. We replaced all baits on a weekly basis.

Ground foraging arthropod predators were surveyed using pitfall traps (100 ml specimen cups, 5 cm diameter) filled with 75 ml of a 50–50 mixture of propylene glycol and water to preserve the fallen insects. Pitfalls were placed out for 48 h and we avoided sampling during periods of heavy rainfall to avoid over-flowing pitfalls. We surveyed ground foraging predators for each of the 7 wk because ant abundance and diversity are known to shift throughout the growing season in temperate climates (Fellers 1989, Caut et al. 2013). All ants (Family: Formicidae) collected in the pitfalls were identified to the species level (Ellison et al. 2012). Nonant ground predators were also counted and identified to the following taxonomic levels: beetles (Family: Carabidae, Staphylinidae); spiders (Family: Lycosidae, Linyphiidae, Salticidae, and Thomisidae); harvestmen (Order: Opiliones); earwigs (Order: Dermaptera); and crickets (Family: Gryllidae). In addition, slugs (Class: Gastropoda) and millipedes (Class: Diplopoda) were also included as predators because they have previously been observed consuming sentinel egg prey (Grieshop et al. 2012).

Prey suppression services were measured as the removal rates of corn earworm (*Helicoverpa zea* Boddie (Lepidoptera: Noctuidae)) eggs from sentinel egg cards (Werling et al. 2011b). At each sampling station, index cards with approximately 75 earworm eggs (freeze-killed) were placed in petri dishes and covered with a petri dish lid, with a 6 cm diameter hole covered by 0.5 cm^2 hardware cloth glued to the lid. The hardware cloth helped to exclude ground foraging predators that were larger than ants from entering the petri dishes thus isolating the role of ants in prey removal. Egg cards were placed out for 48 h during the same period as each pitfall sampling and the remaining eggs on each card were compared with the initial number of eggs to calculate the mean percent removal. Previous work using video monitoring and direct observations has shown ants to be the primary consumer of sentinel eggs (Grieshop et al. 2012, López and Potter 2000).

Analysis

Pitfall counts within a plot (n = 4) were pooled as a single measurement, and egg removal was calculated as the proportion of total eggs removed from the four sentinel egg stations. We excluded four samples collected in Michigan from all analyses (a total of 147 ants). These samples were from a single pitfall station, where *Solenopsis molesta* abundance exceeded four times the abundance of any other collection from that week and likely represented a trap placed within or immediately next to their subterranean nest. We used Simpson's 1-D to estimate ant diversity within each plot. We analyzed the effects of the poison treatment on ant abundance and diversity, nonant predator abundance, and egg removal rates using a repeated measures ANOVA, using SAS (PROC MIXED, SAS 9.4). State (Michigan or Wisconsin), treatment (poison or control), week (1–7), and all two- and three-way interactions were treated as fixed effects and plot within state as a random effect using an auto-regressive covariance matrix. Ant abundance was $\log_{10}(x + 1)$ transformed and nonant ground predator abundance was $\log_{10}$ transformed to normalize the data. We used LSMEANS to examine any differences between treatments for each state. To examine if ant community composition differed between treatments, we performed a permutational ANOVA (PERMANOVA) where we summed the abundance of each taxa for each plot and by week, and constructed a Bray–Curtis distance matrix. As states differed significantly in ant and nonant predator abundance, we performed PERMANOVAs for each state separately in the vegan package of R (v3.4.0, Oksanen et al. 2018). Similarly, we also ran PERMANOVAs for nonant predators to determine whether the poison treatment affected nonant predator community composition.

To determine the relative contributions of individual species (ant and nonant predator communities) to treatment differences in community composition, we used species-specific coefficients with 95% confidence intervals generated from PERMANOVA based on the fit of individual species abundance to the linear model. Taxa with less than three observations were excluded because these values disproportionally affect the analysis (Kindt and Coe 2005). The abundance of remaining taxa was $\log_{10}(x + 1)$ transformed. Finally, we tested for relationships between egg removal and the abundance of ants, crickets, or all nonant predators collected in poison and control treatments using regression analysis in R (v3.4.0). Poison treatment and the abundance of ants, crickets, and nonant predators were included as explanatory variables. State was not included because there was no effect of state in the repeated-measures analysis for egg removal ($F_{1, 15.9}$ = 0.46, P = 0.510). For this analysis, we included the collection data used in repeated measures analyses (discussed earlier). The mean proportion of eggs removed was bound between 0 and 1 and, therefore, was arcsine transformed.

Results

We collected 1,202 ants in Michigan and 2,643 in Wisconsin throughout the duration of the experiment. We identified 19 species in Michigan and 12 species in the Wisconsin grassland with seven species in common (Table 1). The most commonly collected species in both states was *Lasius neoniger*, which on average comprised approximately 28% of the ants collected in Michigan and 80% in Wisconsin. In Michigan, the next most common ants in descending order included *Solenopsis molesta* (22%), *Aphaenogaster rudis* (14%), and *Myrmica AF-smi* (12%). In Wisconsin, the other most common ants we collected were *Formica montana* (12%), *S. molesta* (3%), and *Myrmica nearctica* (2%).

Ant Abundance, Diversity, and Community Composition

Overall, poison baiting significantly suppressed ant abundance in poison plots across the entire study ($F_{1,16}$ = 9.57, P = 0.007) with up to an 80% reduction in ants per trap. However, the poison baiting effect was most evident in Michigan, where ant abundance was significantly suppressed in plots with poison bait treatment (t = 2.98,

Table 1. Ant species collected and their relative percentage from 7 wk of sampling in summer 2015

Species	Michigan		Species	Wisconsin	
	Control	Poison		Control	Poison
Lasius neoniger	39.53%	11.31%	*Lasius neoniger**	88.81%	65.68%
Solenopsis molesta	7.31%	25.30%	*Solenopsis molesta**	1.91%	3.74%
Aphaenogaster rudis	17.22%	12.20%	*Aphaenogaster rudis**	0.80%	0.39%
Stenamma brevicorne	3.47%	2.08%	*Stenamma brevicorne**	0.80%	1.08%
Temnothorax ambiguus	0.12%	0.30%	*Temnothorax ambiguous**	0.12%	0.49%
Ponera pennsylvanica	1.24%	4.76%	*Ponera pennsylvanica**	0.00%	0.29%
Prenolepis imparis	1.61%	3.87%	*Prenolepis impairs**	0.00%	0.10%
Myrmica AF-smi	14.25%	11.61%	*Formica montana*	6.27%	22.22%
Myrmica detrinoitis	5.95%	7.44%	*Myrmica nearctica*	0.68%	4.52%
Nylanderia parvula	3.47%	2.08%	*Formica argenta*	0.43%	0.79%
Myrmica brevispinosa	1.49%	3.57%	*Crematogaster cerasi*	0.18%	0.10%
Tetramorium immigrans	0.62%	10.71%	*Lasius alienus*	0.00%	0.59%
Brachymyrmex depilis	1.49%	1.79%	**Total Ants Collected**	**1,626**	**1017**
Myrmica incompleta	1.24%	0.60%			
Formica pergandei	0.00%	1.49%			
Tapinoma sessile	0.62%	0.00%			
Myrmica fracticornis	0.37%	0.00%			
Formica pallidefulva	0.00%	0.60%			
Formica incerta	0.00%	0.30%			
Total ants collected	807	336			

The totals are separated by treatment (total collected in poison vs control treatment) and state. Overall, *L. neoniger* is the most common ant species collected. Shared species are denoted with asterisks.

d.f. = 16, P = 0.009). The number of ants collected was reduced by 50–80% and generally, the percent reduction in ants collected in poison plots increased, relative to control plots, through the duration of the sampling. Although the number of ants collected in Wisconsin was consistently ~50% lower in poison plots than control plots the difference was not statistically significant (t = 1.39, d.f. = 16, P = 0.182). Ant abundance differed significantly between states ($F_{1, 16}$ = 16.98, P < 0.001) and over time ($F_{6, 74.4}$ = 22.36, P < 0.001) (Fig. 1A).

In both Michigan and Wisconsin, ant diversity was not different between the poison and control treatments ($F_{1, 16}$ = 0.71, P = 0.411, Fig.1B). There was a significant effect of week ($F_{6, 77.5}$ = 1.61, P = 0.155) and state ($F_{1, 16}$ = 27, P < 0.001), but no significant interaction of state and poison treatment on ant diversity ($F_{1, 16}$ = 2.68, P = 0.121). In Wisconsin, ant diversity was generally higher in poison plots but not statistically different (t = –1.75, d.f. = 16, P = 0.099). The composition of the ant community was significantly different by poison treatment in Michigan ($F_{1, 51}$ = 5.25, P = 0.016) (Fig. 2A) and Wisconsin ($F_{1, 53}$ = 4.96, P = 0.026) (Fig. 2B). In Michigan, the abundance of *L. neoniger*, *Aphenogaster rudis*, *Myrmica AF-smi*, *Stennama brevicorne*, *Nylanderia parvula* was more common in control plots than in treatment plots and *Tetramorium immigrans* (formerly *T. caespitum*) was more commonly found in treatment plots than control plots (Fig. 2A). In Wisconsin, *L. neoniger* was more commonly collected in control plots and *Lasius alienus* and *Myrmica nearctica* were more commonly collected in treatment plots than control plots (Fig. 2B).

Nonant Predator Abundance and Community Composition

There was no overall effect of poison treatment ($F_{1, 16}$ = 2.56, P = 0.130) on nonant ground predator abundance, but a significant effect of week ($F_{6, 77.3}$ = 40.42, P < 0.001) and state ($F_{1, 16}$ = 99.00, P < 0.001) (Fig. 3). Nonant predator abundance was approximately two times greater in the Michigan site than in Wisconsin (Fig. 3).

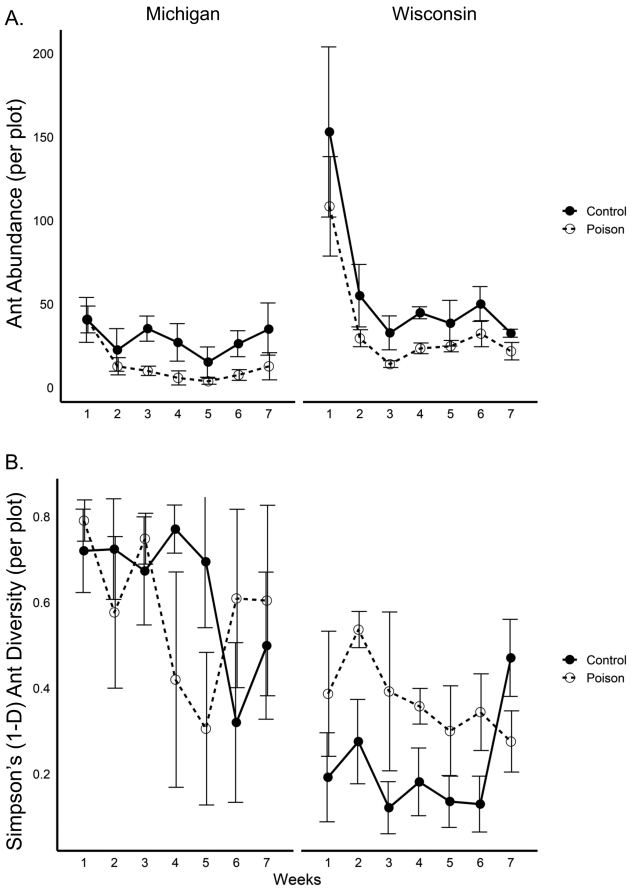

Fig. 1. (A) Ant abundance and (B) Simpson's 1-D diversity by week in Michigan and Wisconsin over the course of the experiment. Control treatments are represented by black circles and solid lines and poison treatments are represented by open circles and dashed lines.

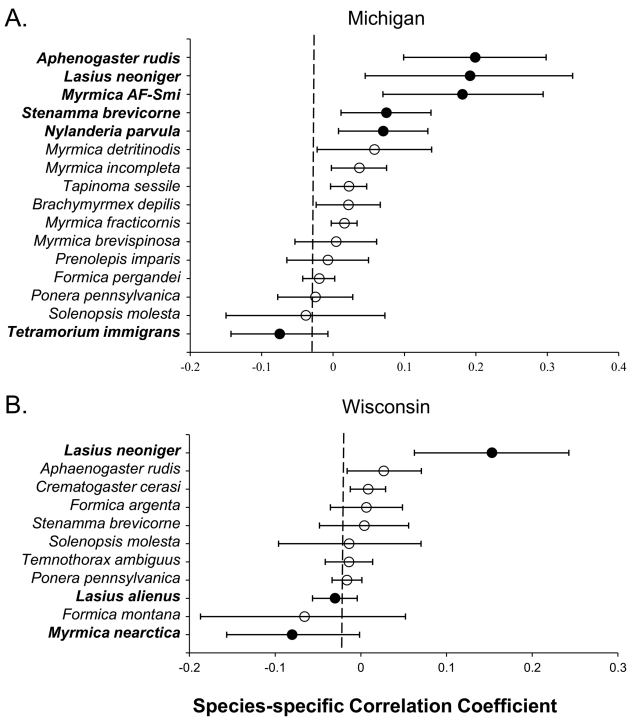

Fig. 2. The species-specific coefficients with 95% confidence intervals based on PERMANOVA results for the poison treatment effect on ant abundances in (A) Michigan and (B) Wisconsin. Coefficients where 95% CI do not overlap zero (dashed lined) are filled and species names bolded. These represent taxa impacted by treatment. Positive values indicate a taxon was more commonly collected in control compared to the poison treatment and negative values indicate a taxon was more commonly collected in poison treatment compared to control.

The difference between nonant predator abundance between states was in part driven by the abundance of slugs, as they represented 87% of the nonant predators collected in Michigan (Table 2). The nonant predator community composition in Michigan was significantly different in response poison treatment ($F_{1, 48}$ = 4.93, P < 0.001) (Fig. 4A). Similarly, in Wisconsin the nonant predator community composition was significantly different in poison treatment ($F_{1, 48}$ = 4.34, P < 0.001) (Fig. 4B). In both Michigan and Wisconsin, crickets contributed significantly to differences in nonant predator community composition but in an opposing pattern. In Michigan, crickets were more abundant in poison than control treatments while the opposite was true of Wisconsin (Fig. 4A and B). A repeated-measures analysis for crickets found no significant effect of poison treatment ($F_{1, 16}$ = 2.36, P = 0.140) on cricket abundance. We found a significant week ($F_{6, 75.8}$ = 70.09, P < 0.001) and state by treatment ($F_{1, 16}$ = 16.78, P < 0.001) effect on cricket abundance. In Wisconsin, the poison plots had significantly reduced abundance of crickets (t = 3.98, d.f. = 16, P = 0.001), while in Michigan we observed a slight increase in cricket abundance in poison versus control plots (t = –1.81, d.f. = 16, P = 0.090) (Table 2).

Sentinel Egg Removal

We found a significant effect of poison treatment ($F_{1, 15.9}$ = 29.58, P < 0.001) and week ($F_{6, 71.2}$ = 19.34, P < 0.001) on egg removal. There was no effect of state on egg removal ($F_{1, 15.9}$ = 0.46, P = 0.510) (Fig. 5) or interaction between state and treatment ($F_{6, 71.2}$ = 25.97, P = 0.120). Poison treatment generally reduced egg removal by 50% in both states. The poison treatment significantly reduced egg removal in Michigan (t = 2.67, d.f. =15.9, P = 0.017) and Wisconsin (t = 5.02, d.f. = 15.9, P < 0.001) (Fig. 5). There was a significant relationship of four explanatory variables on proportion of eggs removed in our grasslands (r^2 = 0.235, $F_{4, 107}$ = 8.210, P < 0.001). In line with our previous analysis, poison treatment has negative relationship to proportion of eggs removed (β coefficient = - 0.142, P = 0.012). There was a positive relationship ant abundance and proportion of eggs removed (β coefficient = 0.231, P < 0.001) (Fig. 6A), but no significant relationship between cricket (β coefficient = 0.011, P = 0.815) (Fig. 6B), or nonant predator (β coefficient, P = 0.125) (Fig. 6C) abundance and proportion of eggs removed. In a separate analysis, examining the linear regression of each taxa and egg removal, we calculated each taxa's Pearson correlation coefficient. We found a similar pattern in that ants (r = 0.372) had the strongest relationship with egg removal relative to crickets (r = 0.081) or nonant predators (r = 0.011) (Fig. 6).

Discussion

Overall we found that a poison-baiting treatment caused a reduction in ant abundance and this in turn was associated with a reduction in sentinel egg removal rates. The poison treatment had no significant effect on the overall ant diversity but we did observe changes in the ant community composition. For example, of the seven shared species only one (*L. neoniger*) was negatively affected by the poison treatment, suggesting that in addition to a decrease in abundance, the proportional reduction of this dominant species may have also been associated with lower predation rates. We did not find evidence that the poison treatment had a significant effect on the overall nonant arthropod predator abundance lending more evidence to the conclusion that it was ant reductions and shifts in community composition that were responsible for a decrease in predation rates. We did find an inconsistent effect of poison treatment on crickets when examining shifts in community composition but observed no other shifts in the other nonant predators considered. Unlike previous studies

we were able to tease apart the impact of ants and nonant predators on predation rates and our study suggests that ants play a role in pest egg predation and that they contribute to the natural biological control in grasslands.

We found a significant reduction in ants collected in response to poison treatment. The efficacy of fipronil at low doses is well established from previous studies (Collins and Callcott 1998, Hooper-Bui and Rust 2000). Fipronil is effective because it can quickly reduce ant populations and when diluted, the effects are delayed allowing workers to share bait with other workers or reproductives via trophallaxis (Hooper-Bui and Rust 2000). The poison baiting was so effective we were able to reduce ant population in both states, despite dramatic differences in the initial abundances of *L. neoniger* between states. In Wisconsin *L. neoniger*, comprised 66–96% of the total ant community in control plots throughout the experiment. In contrast, *L. neoniger* never comprised greater than 57% of the total ant community in control plots in Michigan (Supp Table 1 [online only]). Initial differences in a numerically dominant ant species, like *L. neoniger*, may be an important factor influencing the efficacy of suppression, and observed patterns. For example, the numerical dominance of *L. neoniger* may have limited our ability to reduce ant abundance to zero in the poison treatment plot. The baits were often empty when we replaced them and *Lasius* spp. were commonly observed feeding at the baits or found within empty vials. Future efforts to suppress ant populations that include *L. neoniger* populations, may need to consider increasing baiting effort to reduce ant populations to zero.

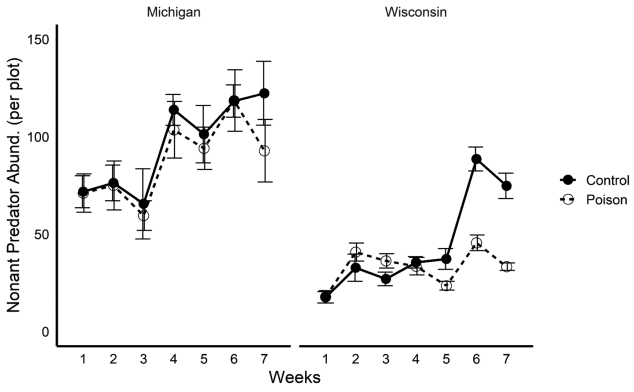

Fig. 3. Nonant ground predator abundance per plot by week in Michigan and Wisconsin. The control treatments are represented by black circles and black lines and the poison treatments are represented by open circles and dashed lines.

Ant species varied between states, with more species collected in Michigan (n = 19) than Wisconsin (n = 12) and we observed no effect of poison treatment on ant diversity in response to poison treatment. The ant diversity in poison treatment plots in Wisconsin tended to be higher but we found no clear pattern in Michigan. However, we found that the ant community composition in both states shifted in response to poison treatment. Of the seven-shared species between the states (Table 1), only one species, *L. neoniger*, was associated with a decline in response to the poison treatment in both states (Fig. 2). This was the only species to decline in response to poison treatment in Wisconsin, while in Michigan four other species declined in response to poison treatment. In an exclusion study that also examined the ant community composition, Parr et al. (2016), found no evidence of a shift in ant community composition in response to poison bait treatment. In reducing *L. neoniger* abundance, we may have also reduced their impact on less dominant species, and subsequently affected the ant community composition. In Wisconsin, for example, the reduction of *L. neoniger* within poison treatments corresponded to an increase in relative abundance of several ant species, notably *L. alienus* and *M. nearctica* (Fig. 3). We did not observe a shift in diversity because *L. alienus* and *M. nearctica* make up a relatively small portion (~5%) of the total ant species collected (Table 1).

The nonant predator abundance was twofold to threefold higher in Michigan than in Wisconsin. In Michigan slugs (83–90%) dominated the nonant predator collections, while in Wisconsin slugs only account for 8–10% of the nonant predators collected (Table 2). In Wisconsin, the most abundant predators collected were crickets (11–44%) and 'other spiders' (33–59%) which only constituted 1–2% of collections in Michigan (Table 2). In spite of these differences, we observed no significant difference in nonant predator abundance in response to poison treatments. We did observe that during the last 2 wk of the experiment, crickets became very abundant at the Wisconsin site. Further analysis of changes in cricket abundance showed evidence that crickets may have been negatively affected by poison treatments in Wisconsin (Fig. 4). More specifically, we found fewer crickets were collected in the Wisconsin poison treatments relative to control treatments. However, the impact of poison treatments on cricket abundance is inconsistent. In Michigan, we collected more crickets in poison treatment relative to control treatments (Fig. 4) (Table 2). The inconsistency in cricket abundance

Table 2. Nonant ground predators collected and the relative percentage of each group from 7 wk of sampling in summer 2015

	Michigan (%)		Wisconsin (%)	
	Control	Poison	Control	Poison
Slugs	89.54	83.80	8.34	10.33
Other spiders	1.16	1.75	32.65	59.14
Crickets	0.19	1.51	44.37	10.72
Staphylinids	5.16	8.14	2.91	4.21
Linphiidae	1.08	1.99	5.19	9.19
Millipedes	2.17	2.20	0.39	0.57
Crab spiders	0.04	0.00	2.68	2.30
Carabids	0.34	0.37	1.49	1.05
Harvestmen	0.34	0.12	0.94	1.34
Jumping spiders	0.00	0.12	1.02	1.15
Earwigs	0.00	0.00	0.00	0.00
Totals predators collected	2,676	2,457	1,271	1,045

The totals are separated by treatment (total collected in poison treatment versus control treatment) and state.

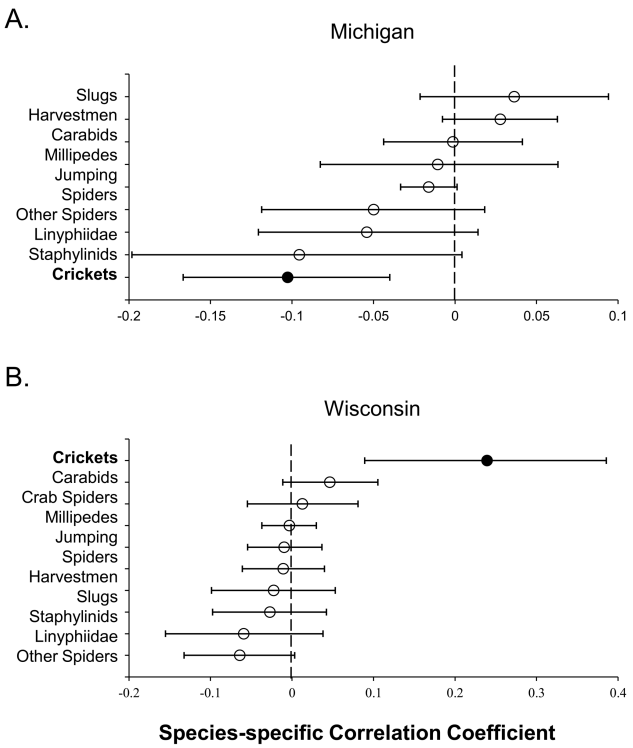

Fig. 4. The species-specific coefficients and 95% confidence interval for the poison treatment effect on nonant ground predator abundances in (A) Michigan and (B) Wisconsin. Coefficients where 95% CI do not overlap zero (dashed lined) are filled and species names bolded. These represent taxa impacted by treatment. Positive values indicate a taxon was more commonly collected in control compared with the poison treatment and for negative values, a taxon was more commonly collected in poison treatment compared to control.

between states is likely why we do not observe an associated effect on the overall nonant predator abundance in response to poison treatment (Fig. 3). Based on our results, it is difficult to determine whether poison treatment negatively impacts crickets. However, we did observe an overall decrease in ant abundance and consistent shifts in community composition (with *L. neoniger*). This suggests that our poison baiting methods effectively reduced ant abundance, while minimizing the poison's effects on nontarget predators.

Across all collections we found that an increase in ant abundance was positively associated with a higher pest egg removal. We found no evidence of a relationship between egg removal and cricket and nonant predator abundance. This suggests that ants likely contributed significantly to egg removal relative to nonant arthropod predators. We do acknowledge that throughout the experiment nonant predators may have contributed to some egg removal, e.g., immature crickets may have been small enough to pass through the hardware cloth and consume eggs. Moreover, we considered a wide breadth of predators including crickets, slugs, and spiders that have been previously identified as predators visiting sentinel prey in grasslands (Grieshop et al. 2012). However, while we observed an increase in total nonant predator abundance through the duration of our experiment (Fig. 3), we did not observe an associated increase in egg removal (Fig. 5). Furthermore, we found a relationship between nonant predator abundance and egg removal (Fig. 6C). Given that were able to consistently reduce ant abundance by poison treatment, with minimal impact on nonant predators, and observed a positive relationship between ant abundance and egg removal, suggests that ants are a major contributor to prey removal in grasslands.

Despite not completely reducing ant populations to zero (Table 1, Fig. 1), poison treatments still had a significant effect on proportion eggs removed. This suggests that completely reducing ant populations is not necessary to observe an effect on prey removal rates and provides a guide for future work to explore the potential of ants in providing prey suppression services. Previous work has shown that ants are effective lepidopteran egg predators in other perennial systems (López and Potter 2000, Grieshop et al. 2012) and within agricultural settings (Kirk 1981, Perfecto 1991, Nuessly and Sterling 1994, Yusa 2001), thus it is likely ants are a significant ground predators, and are contributing to prey suppression within grasslands. It also appears that *L. neoniger* is one of the key ant species contributing to prey suppression based on previous work (Kirk 1981, López and Potter 2000) and the ant communities presented here. This species may also be excluding other ants species, particularly in Wisconsin. Finally, the contribution of *L. neoniger* to pest prey suppression may not be limited to grasslands, as they are one of most common ants in Midwestern corn fields (Ballard and Mayo 1979).

As components within grasslands, ants are known to play important roles as consumers and ecosystem engineers (Wills and Landis 2018) but relatively little work explores the consequences of these interactions (Nemec et al. 2014). Our study, to our knowledge, is the first aimed at isolating the role of ants as predators in temperate perennial grassland systems that also thoroughly explores responses in nonant predator abundance and community composition. By selectively suppressing ant populations while also minimizing the impacts of poison baiting on nonant predator abundance and community composition, we found that reducing ant abundance was associated with reduced prey suppression, an effect potentially cascading to other ecosystem services. Our results are supported by Parr et al. (2016), that found reductions in ant populations altered rates of herbivory and decomposition in a South African savanna because ant predation on the insect herbivores and decomposers were

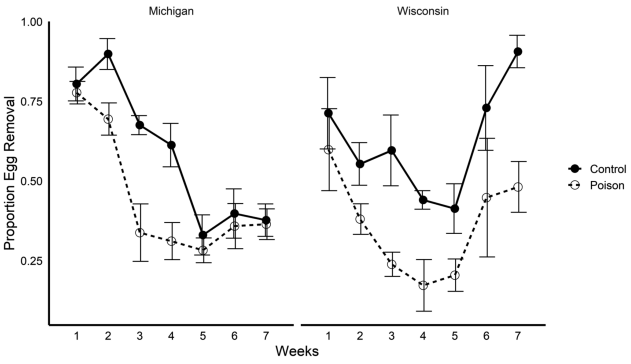

Fig. 5. Mean proportion eggs removed per plot by week in Michigan and Wisconsin. The control treatments are represented by black circles and black lines and the poison treatments are represented by open circles and dashed lines.

reduced. Additionally, our work also outlines a possible method for isolating the role of ants as consumers from other nonant ground foraging predators to explore the role of ants as predators in grassland systems. It also helps build a foundation for additional work exploring if grasslands are potentially pest sinks because of ant predation or if grassland ant species disperse into the neighboring areas and subsequently affect prey suppression in these habitats.

Grasslands support diverse invertebrate communities that can provide valuable ecosystem services within agricultural landscapes but are threatened by changes in land-use, fragmentation, and climate change (Hoekstra et al. 2005). In contrast, interest in using perennial grasses in bioenergy cropping systems (Landis et al. 2018, Robertson et al. 2017) could increase their occurrence in Midwestern landscapes with important implications for ants (Kim et al. 2017) and other beneficial insects (Werling et al. 2011a, Werling et al. 2014). The addition of even relatively small patches of grasslands can harbor generalist predatory ant species like *L. neoniger* (Campbell and Crist 2017), and increasing the number of patches within a landscape may improve ant species diversity (Dauber et al. 2005) and functional diversity (Crist 2009). To improve our ability to maximize ecosystem services in human-managed landscapes, future work exploring the role of land use change on ecological processes should consider ants as numerically dominant and effective predators in grasslands.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

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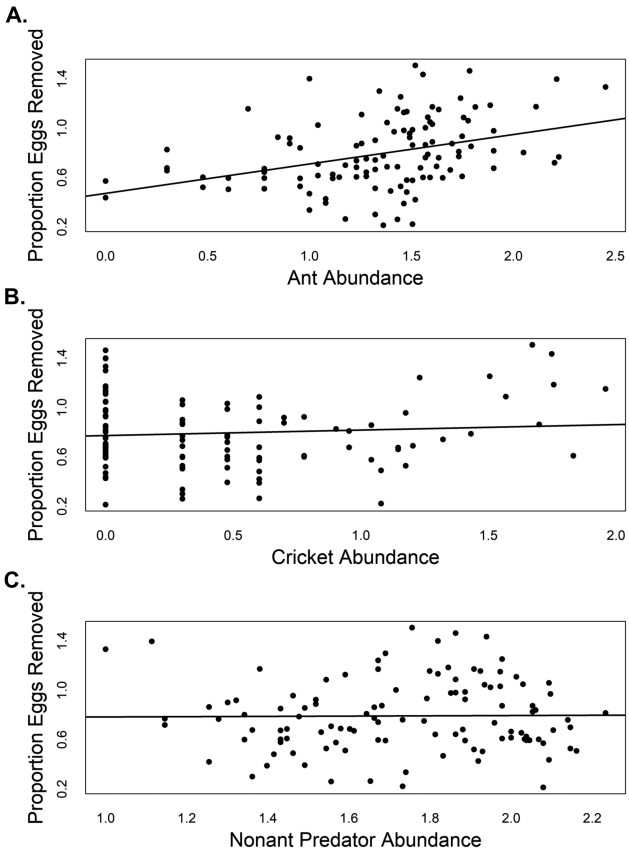

Fig. 6. The relationships between the log-transformed abundance of (A) ants, (B) crickets, and (C) nonant predators to the mean proportion eggs removed (arcsine-transformed) from both states and treatment.

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Supplementary Table 1. List and proportion of ant species collected in Michigan per week. The total is based on the number of ants collected from each in field for all seven weeks of the experiment.

	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7	
Michigan	Control	Poison	Control	Poison	Control	Poison	Control	Poison	Control	Poison	Control	Poison	Control	Poison
Lasius neoniger	0.43	0.06	0.57	0.04	0.27	0.15	0.32	0.05	0.61	0.07	0.25	0.23	0.45	0.04
Solenopsis molesta	0.03	0.50	0.04	0.57	0.09	0.45	0.10	0.45	0.03	0.40	0.08	0.33	0.12	0.47
Aphenogaster rudis	0.12	0.11	0.06	0.04	0.28	0.03	0.18	0.05	0.11	0.00	0.27	0.20	0.15	0.06
Myrmica Af-smi	0.17	0.13	0.08	0.04	0.13	0.00	0.14	0.00	0.10	0.00	0.17	0.03	0.16	0.08
Myrmica detritinodis	0.06	0.01	0.11	0.20	0.10	0.14	0.06	0.05	0.03	0.13	0.05	0.00	0.00	0.00
Tetramorium immigrans	0.01	0.07	0.00	0.00	0.01	0.06	0.00	0.14	0.02	0.13	0.00	0.03	0.01	0.16
Stenamma brevicorne	0.01	0.00	0.04	0.04	0.06	0.05	0.05	0.00	0.03	0.13	0.07	0.00	0.00	0.00
Nylanderia parvula	0.02	0.00	0.02	0.02	0.02	0.03	0.06	0.00	0.03	0.00	0.05	0.03	0.04	0.04
Ponera pennsylvanica	0.02	0.02	0.01	0.06	0.00	0.03	0.01	0.18	0.00	0.00	0.03	0.03	0.01	0.04
Prenolepis imparis	0.02	0.04	0.00	0.00	0.00	0.00	0.05	0.05	0.03	0.00	0.02	0.10	0.00	0.00
Myrmica brevispinosa	0.05	0.04	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.01	0.00	0.02	0.00
Brachymyrmex depilis	0.03	0.01	0.01	0.00	0.02	0.02	0.00	0.05	0.00	0.13	0.01	0.00	0.01	0.00
Myrmica incompleta	0.01	0.01	0.04	0.00	0.02	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Formica pergandei	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10
Tapinoma sessile	0.00	0.00	0.00	0.00	0.01	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.01	0.00
Myrmica fracticornis	0.01	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Temnothorax ambiguus	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
Formica pallidefulva	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Formica incerta	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total Ants Collected	**162**	**163**	**90**	**33**	**141**	**41**	**108**	**22**	**61**	**15**	**105**	**30**	**140**	**33**

Supplementary Table 2. List and proportion of ant species collected in Wisconsin per week. The total is based on the number of ants collected from each in field for all seven weeks of the experiment.

	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7	
Wisconsin	Control	Poison	Control	Poison	Control	Poison	Control	Poison	Control	Poison	Control	Poison	Control	Poison
Lasius neoniger	0.88	0.51	0.88	0.58	0.96	0.73	0.90	0.79	0.97	0.84	0.94	0.81	0.66	0.87
Formica montana	0.08	0.40	0.05	0.09	0.02	0.13	0.03	0.05	0.03	0.09	0.02	0.10	0.19	0.07
Solenopsis molesta	0.01	0.03	0.03	0.05	0.01	0.00	0.00	0.10	0.00	0.00	0.01	0.04	0.11	0.03
Myrmica nearctica	0.00	0.03	0.02	0.19	0.00	0.05	0.02	0.03	0.00	0.00	0.00	0.03	0.01	0.02
Stenamma brevicorne	0.00	0.00	0.01	0.07	0.00	0.00	0.03	0.01	0.01	0.01	0.01	0.01	0.00	0.00
Aphaenogaster rudis	0.01	0.00	0.00	0.00	0.02	0.04	0.00	0.00	0.00	0.01	0.00	0.00	0.02	0.00
Formica argenta	0.00	0.01	0.00	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Temnothorax ambiguus	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00
Lasius alienus	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.02	0.00	0.02	0.00	0.01	0.00	0.00
Crematogaster cerasi	0.00	0.00	0.01	0.00	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ponera pennsylvanica	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00
Prenolepis imparis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total Ants Collected	**612**	**434**	**220**	**118**	**131**	**56**	**179**	**94**	**152**	**99**	**202**	**129**	**130**	**87**

Supplementary Table 3. The complete summary table of the repeated measures ANOVA for all metrics.

Ant Abundance (per plot)

Effect	F value	p - value
State	$F_{1, 16} =$ 16.98	0.0008
Poison Treatment (Treatment)	$F_{1, 16} =$ 9.57	0.007
State*Treatment	$F_{1, 16} =$ 1.26	0.2785
Week	$F_{6, 74.4} =$ 22.36	<0.0001
State*Week	$F_{6, 74.4} =$ 5.14	0.0002
Week*Treatment	$F_{6, 74.4} =$ 3.52	0.004
State*Treatment*Week	$F_{6, 74.4} =$ 2.00	0.0765

Simpson's (1-D) Ant Diversity (per plot)

Effect	F value	p - value
State	$F_{1, 16} =$ 24.71	0.0001
Poison Treatment (Treatment)	$F_{1, 16} =$ 0.71	0.4111
State*Treatment	$F_{1, 16} =$ 2.68	0.1212
Week	$F_{6, 77.5} =$ 1.61	0.1549
State*Week	$F_{6, 77.5} =$ 1.17	0.3309
Week*Treatment	$F_{6, 77.5} =$ 2.00	0.0752
State*Treatment*Week	$F_{6, 77.5} =$ 2.16	0.0559

Non-Ant Predator Abundance (per plot)

Effect	F value	p - value
State	$F_{1, 16} =$ 99.06	<0.0001
Poison Treatment (Treatment)	$F_{1, 16} =$ 2.56	0.1294
State*Treatment	$F_{1, 16} =$ 0.57	0.4606
Week	$F_{6, 74.3} =$ 40.42	<0.0001
State*Week	$F_{6, 74.3} =$ 10.95	<0.0001
Week*Treatment	$F_{6, 74.3} =$ 6.41	<0.0001
State*Treatment*Week	$F_{6, 74.3} =$ 3.36	0.0054

Proportion of Egg Removal (per plot)

Effect	F value	p - value
State	$F_{1, 15.9} =$ 0.46	0.5088
Poison Treatment (Treatment)	$F_{1, 15.9} =$ 29.58	<0.0001
State*Treatment	$F_{1, 15.9} =$ 2.77	0.1155
Week	$F_{6, 71.2} =$ 19.34	<0.0001
State*Week	$F_{6, 71.2} =$ 12.30	<0.0001
Week*Treatment	$F_{6, 71.2} =$ 2.14	0.0594
State*Treatment*Week	$F_{6, 71.2} =$ 1.62	0.1543