Testing effects of invasive fire ants and disturbance on ant communities of the longleaf pine ecosystem

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Abstract. 1. Invasive species and habitat disturbance are among the most important drivers of biodiversity loss and ecological change. Their individual effects, however, are difficult to disentangle because invasion and disturbance are often intimately linked. Here we test alternative hypotheses to determine if the invasive red imported fire ant, *Solenopsis invicta*, is a 'driver' or simply a 'passenger' of ecological change in a longleaf pine ecosystem.

2. We randomly assigned treatments of (1) unmanipulated, (2) soil disturbance, (3) fire ant removal and (4) soil disturbance + fire ant removal to experimental blocks and measured how ant communities changed over 2 years in thirty-six 15-m^2 plots.

3. Fire ant abundance in removal plots averaged 42% lower in pitfall traps and 95% lower on baits compared to unmanipulated, control plots. Species richness of co-occurring ants also decreased 42% in removal plots, with significant changes in community composition. Soil disturbance alone did not affect ant communities. Fire ant diet breadth–measured using carbon and nitrogen stable isotopes–increased up to 4.7-fold in soil disturbance + removal plots (i.e. 0.84‰^{2} to 3.94‰^{2}).

4. While non-target impacts of the fire ant removal treatment complicate interpretation, our results suggest fire ants follow an alternative 'interacting drivers' model in which partial recovery of some species occurs when populations of an invasive species are reduced. Further recovery of native ants may be limited by persisting, landscape-level effects of fire ants suppressing co-occurring ants below historical levels.

Key words. Amdro, disturbance, invasive species, social insect, stable isotope, *Solenopsis invicta*.

Introduction

Biological invasions are a serious threat to biodiversity with pervasive ecological, economic and evolutionary impacts (Elton, 1958; Vitousek *et al.*, 1996; Mack *et al.*, 2000; Mooney & Cleland, 2001; Wittman, 2014). The most pernicious invasive species are widely considered to be drivers of native species loss and ecological change, through disruption of community structure by competitive displacement and behavioural dominance (Porter & Savignano, 1990; O'Dowd *et al.*, 2003; Bertelsmeier

et al., 2015). However, disturbance [i.e. a relatively discrete event that disrupts the structure of an ecosystem, community or population (Turner, 2010)] often facilitates invasive species, confounding their impact and calling into question the mechanistic underpinning of the commonly-observed negative correlation between invasive and native species abundance or richness (Orians, 1986; Gurevitch & Padilla, 2004; Lockwood *et al.*, 2007). Thus, it is important but often difficult to tease apart the roles of disturbance and invasive species on changes in co-occurring species.

One approach to understanding determinants of ecological change in invaded communities is to consider whether an invasive species is a 'driver' or 'passenger' (MacDougall & Turkington, 2005). The 'driver' model posits that species

interact strongly and that native species are limited or excluded by competition with invasive species. Under this scenario, removal of an invasive species should increase native species richness and abundance. In contrast, the 'passenger' model posits that communities are primarily structured by factors other than interactions with invasive species (e.g. habitat disturbance, dispersal limitation) and that those factors are more beneficial to invasive species than native species. Under the 'passenger' model, removal of invasive species should have relatively little impact on native species. A third option–the 'interacting drivers' model–recognises a continuum between driver and passenger models, and posits additive or synergistic effects of habitat disturbance and invasive species that combine to reduce native species richness and abundance (Didham *et al.*, 2005). Under this model, removal or reduction in abundance of invasive species should result in partial recovery of some native species.

Here we test if the red imported fire ant, *Solenopsis invicta* Buren, follows the 'driver', 'passenger', or 'interacting driver' model in a multifactorial experiment within a longleaf pine ecosystem. Fire ants are considered among the most noxious invasive species in the United States and their distribution has expanded to many areas around the world (Buhs, 2004; Tschinkel, 2006; Ascunce *et al.*, 2011). Yet effects of non-native fire ants on co-occurring native ants, while extensively studied, remain contentious due to contrasting results from experimental and correlative studies (King & Tschinkel, 2006, 2008, 2013; LeBrun *et al.*, 2007; Resasco *et al.*, 2014; Roeder *et al.*, 2018). The role that habitat disturbance plays in the fire ant invasion story further complicates interpretation as evidence and criticism for 'passenger' and 'driver' models has arisen (King & Tschinkel, 2008; Stuble *et al.*, 2013). To test these alternative hypotheses, we randomly assigned treatments of (1) unmanipulated, (2) soil disturbance, (3) fire ant removal and (4) soil disturbance + fire ant removal to thirty-six 15-m^2 plots and measured how ant communities changed over 2 years. Specifically, we ask how these experimental treatments affected fire ant abundance and foraging activity, comparing those potential changes to corresponding shifts in species richness and community composition of co-occurring, mostly native, ant species. Also, to gain insight into observed patterns of response, we used stable isotope ratios to examine dietary shifts of fire ants in all treatments.

Materials and methods

Study site and experimental treatments

All experiments were run from May 2011 to August 2012 at the Savannah River Site, a National Environmental Research Park, in South Carolina, U.S.A. (33.20°N, 81.40°W). We established nine experimental blocks within longleaf pine forest stands that were thinned and burned on regular intervals as part of management for red-cockaded woodpeckers (*Picoides borealis*). Each experimental block consisted of four 15 m × 15 m plots in a row that were spaced 15 m apart, a distance that exceeds the average foraging range of many genera of ants (Parr *et al.*, 2007). Each plot within a block was randomly assigned a treatment of unmanipulated (hereafter 'Control'), soil disturbance (hereafter 'Dist'), fire ant removal (hereafter

'Amdro') or a combination of 'Dist' and 'Amdro' (hereafter 'Both').

'Dist' treatments were applied in June 2011 using a disc harrow attachment on a bulldozer by disking an 'X' pattern through the middle of the plot such that the majority of the area was disturbed. 'Amdro' treatments were applied repeatedly, using a granular fire ant pesticide, Amdro® (Active ingredient: Hydramethylnon: [CAS No. 67485–29-4] 0.73%). Because Amdro® is non-specific, we were careful to minimise poisoning native ant species. In July 2011, we placed bait stations of oil-packed tuna on index cards interspersed throughout the plot and a surrounding 5-m buffer to recruit fire ant workers. Once large numbers of fire ants had recruited to the tuna baits, we removed the tuna and replaced it with ∼20 g of Amdro®. We carefully monitored the Amdro® bait stations until foraging stopped or the Amdro® had been removed. We quickly removed any Amdro® bait stations visited by native ant species. Amdro® baiting in 2011 was repeated twice per plot, approximately 8 ± 2 days apart. In 2012, we modified this technique because we found that some fire ants persisted in the Amdro® plots. The new technique involved placing Sandies® pecan shortbread cookie crumbs at 1-m intervals in a grid across the plot and the 5-m buffer area. Once fire ants had discovered and recruited to the cookie baits, we followed them back to their nest. We then placed ∼20 g of Amdro® near the nest entrance and covered the Amdro® and nest entrance with an inverted 5-gallon (∼19 L) plastic bucket, pushed into the sandy soil. This technique allowed fire ants continual access to baits while preventing access from non-target species. After 2 days, we collected the buckets and any remaining Amdro®. We repeated this technique every 2 weeks throughout the summer from May to August. 'Both' treatments received manipulations that were a combination of 'Dist' and 'Amdro'.

Ant sampling with pitfall traps and baits

We sampled ants using pitfall traps and bait surveys before treatments in May 2011 and after treatments in August of 2011 and 2012. Pitfall traps were 5-dram vials (inner diameter: 28.6 mm) filled one-third full with propylene glycol-based Peak® RV & Marine anti-freeze. During each sampling period and within each plot, we set out 16 pitfall traps flush with the soil surface, 5 m apart in a 4 × 4 grid for 48 h. To reduce 'digging-in effects', we inserted capped vials into pitfall trap holes for 48 h prior to sampling (Greenslade, 1973). All ants were identified using taxonomic keys (MacGown, 2003) and stored in 95% EtOH. As another metric of fire ant abundance, we estimated foraging activity with bait surveys in the same month as pitfall trapping. We placed tuna baits (oil-packed; ∼15 cm^3) on 5-cm^2 index cards in the centre and four corners of each plot. We visited the baits after 10 min and every subsequent 15 min for a total of 70 min, estimating the number of fire ants at each bait at each time.

Stable isotope analyses

We used carbon (^{13}C/^{12}C) and nitrogen (^{15}N/^{14}N) stable isotope analyses to estimate trophic relationships and similarities

in diet breadth among fire ants across treatments. To do this, we pooled and homogenised workers from pitfall traps within each plot from the final round of sampling. In addition to fire ants, which persisted in all plots (see Results section), we also analysed 16 other ant species that were found in at least three plots to determine the number of species that are potentially competing for resources. We dried pooled samples for each species to constant mass and weighed them to the nearest 0.001 mg. Delta values (δ) were calculated as:

$$\delta = \left(\left(\frac{R_{sample}}{R_{standard}} \right) - 1 \right) * 1000$$

where δ, reported in per mil notation (‰), represents the ratio of heavy to light isotopes within a sample (R_{sample}) relative to the ratio in an international standard ($R_{standard}$). All stable isotope analyses were performed at the Analytical Chemistry Laboratory at the Odum School of Ecology of the University of Georgia.

Statistical analyses

All analyses were run in R, version 3.5.3. Fire ant abundance (i.e. number of individuals) and ant species richness in pitfall traps were summed per plot and compared using generalised linear mixed-effects models with a Poisson or negative binomial distribution (with 'bobyqa' optimiser) for overdispersed data across four treatments ('Control', 'Dist', 'Amdro', 'Both') within each of the three sampling periods (May 2011, August 2011, August 2012). Fire ant abundance on tuna baits (or foraging activity) was similarly pooled per plot and compared across the four treatments within each of the three sampling periods, but models also included time since baits were deployed (10, 25, 40, 55 and 70 min) plus the interaction between treatment and time. Models included block as a random factor using the 'lme4' package (Bates *et al.*, 2015) and were thus defined as either [Fire ant pitfall abundance or species richness ~ Treatment + (1|Block)] or [(Fire ant bait abundance ~ Treatment * Time + (1|Block)]. Post-hoc contrasts between treatments were performed using the 'emmeans' package (Lenth, 2020). Ant community composition was compared using non-parametric multivariate analyses of variance (PERMANOVA) with 1000 permutations to test if ant communities changed across treatments within a sample period using the 'vegan' package (Oksanen *et al.*, 2019). PERMANOVA tests the null hypotheses of no difference among groups by using random permutations of the data with a pseudo *F*-statistic. For all comparisons, the Bray-Curtis index of dissimilarity was used. Significant relationships were visualised using non-metric multidimensional scaling (NMDS) ordinations. Pairwise treatment differences were further examined using PERMANOVA and significant results were compared using SIMPER to disentangle which species were contributing the most to the overall Bray–Curtis dissimilarity (Clarke, 1993). MANOVA was used to test for isotopic differences in fire ants across the four treatments. Standard ellipse area corrected for small sample size (SEA_c) was then calculated for fire ants within each treatment to determine the extent of diet breadth in $\delta^{13}C–\delta^{15}N$ bi-plot space in the 'SIBER' package (Jackson *et al.*, 2011).

Overlap of isotope values of co-occurring species and fire ants were visually inspected because of low sample sizes.

Results

Fire ant abundance in pitfall traps and on baits

Over 2 years, we collected 6385 fire ants in pitfall traps and observed 152 231 fire ants on baits across all 36 plots. In pre-treatment controls, pitfall traps captured 51.6 (±10.6 SE) fire ants per plot; capture rates were not different among plots (Fig. 1a; $\chi^2 = 4.23$, df = 3, $P = 0.238$). An average of 434.8 (±32.7 SE) fire ants per plot accumulated on baits after 70 min during the pre-treatment survey with a significant time (Fig. 1b; $\chi^2 = 67.05$, df = 4, $P < 0.001$), but not a significant treatment ($\chi^2 = 2.84$, df = 3, $P = 0.417$) or treatment by time interaction ($\chi^2 = 3.20$, df = 12, $P = 0.994$). Three months post-treatment, fire ant abundance in pitfall traps remained similar across plots (Fig. 1c; $\chi^2 = 5.15$, df = 3, $P = 0.161$), but their abundance on baits decreased 64.3% on 'Amdro' and 56.0% on 'Both' plots, compared to 'Control' plots (Fig. 1d; $\chi^2 = 66.59$, df = 3, $P < 0.001$). The interaction between treatment and time remained not significant ($\chi^2 = 5.67$, df = 12, $P = 0.932$). After 15 months, fire ant abundance in pitfall traps decreased 32.9% on 'Amdro' plots and 51.1% on 'Both' plots when compared to 'Control' plots (Fig. 1e; $\chi^2 = 17.27$, df = 3, $P < 0.001$). Similarly, fire ant abundance on baits during the last sampling event remained low in 'Amdro' and 'Both' plots (Fig. 1f) with significant treatment ($\chi^2 = 373.01$, df = 3, $P < 0.001$) and time effects ($\chi^2 = 55.54$, df = 4, $P < 0.001$), but not a significant interaction ($\chi^2 = 7.47$, df = 12, $P = 0.825$).

Changes in ant community composition

We collected a total of 54 ant species from 18 genera over 2 years in pitfall traps. Plots contained an average of 10.4 (±0.7 SE) species and richness was not different across treatments at the start of the experiment (Fig. 2a; $\chi^2 = 0.58$, df = 3, $P = 0.901$), nor was richness different after 3 months (Fig. 2b; $\chi^2 = 4.02$, df = 3, $P = 0.259$). When compared to 'Control' plots, richness declined on 'Amdro' and 'Both', but not 'Dist', treatments after 15-months (Fig. 2c; $\chi^2 = 16.27$, df = 3, $P < 0.001$). Specifically, 'Amdro' treatments contained 4.4 fewer species and 'Both' treatments had 4.7 fewer species.

Ant community composition was not different among plots at the start of the experiment (pseudo $F = 0.69$, df = 3, $P = 0.847$), nor 3 months after application of treatments (pseudo $F = 1.44$, df = 3, $P = 0.114$). However, community composition was different after 15 months (Fig. 3; pseudo $F = 1.93$, df = 3, $P = 0.011$), primarily due to separate sets of species occurring in 'Control' compared to 'Amdro' (pseudo $F = 3.11$, df = 1, $P = 0.006$) and 'Both' treatments (pseudo $F = 3.31$, df = 1, $P = 0.006$). All other pairwise comparisons were not significant ($P > 0.05$). SIMPER results suggest six ('Control' vs 'Amdro') and seven ('Control' vs 'Both') species combined to contribute at least 70% to the overall community dissimilarity between treatments (Table 1). *Brachymyrmex patagonicus*
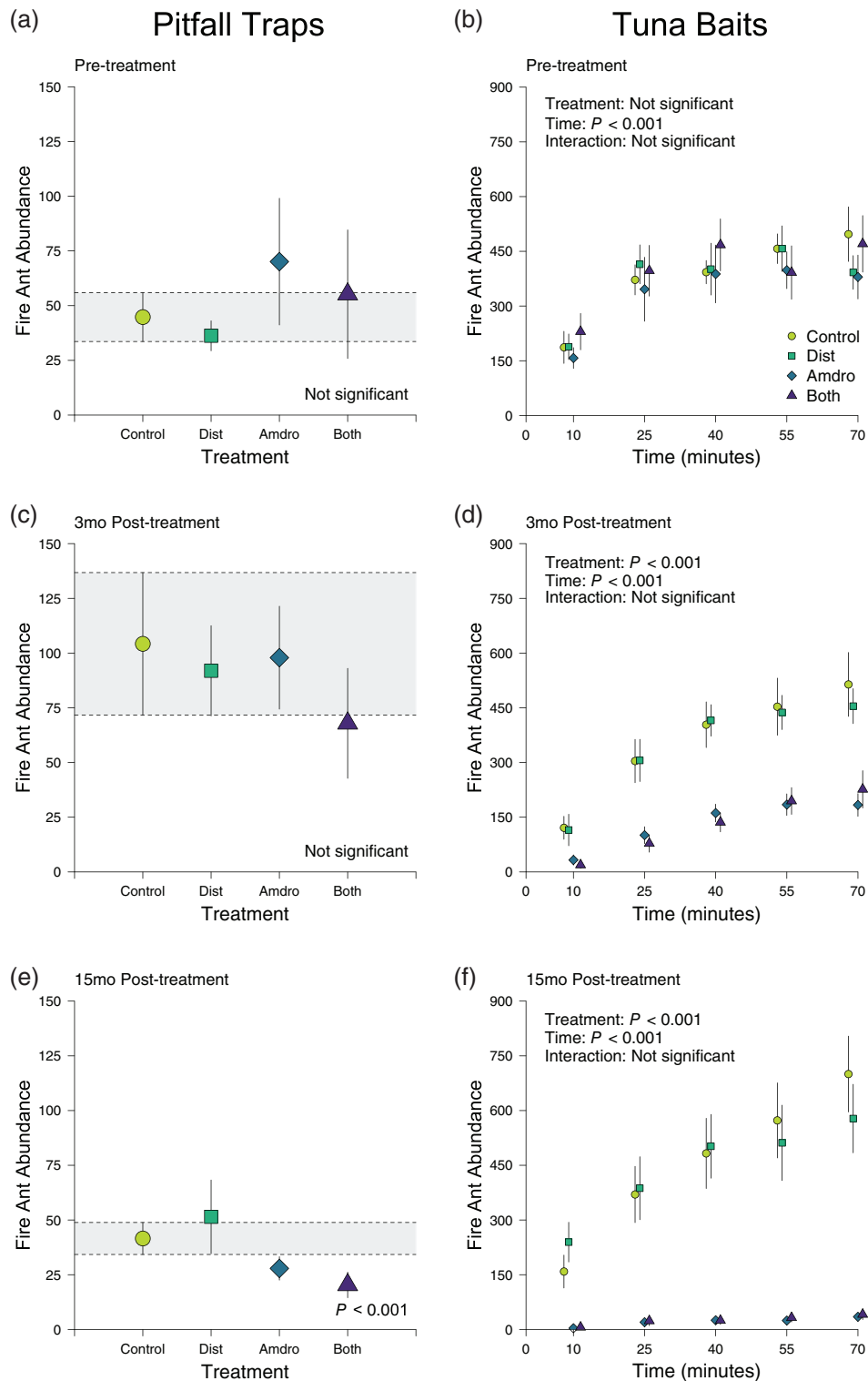
Fig. 1. Average abundance (±SE) per plot of red imported fire ants, *Solenopsis invicta*, in pitfall traps and on tuna baits across treatments and time. Panel (a) and (b) were before treatments were applied in May 2011, panels (c) and (d) were from August 2011, and panels (e) and (f) were from August 2012. Horizontal dashed lines that border grey polygons in panels (a), (c) and (e) represent the range of SE of control plots.
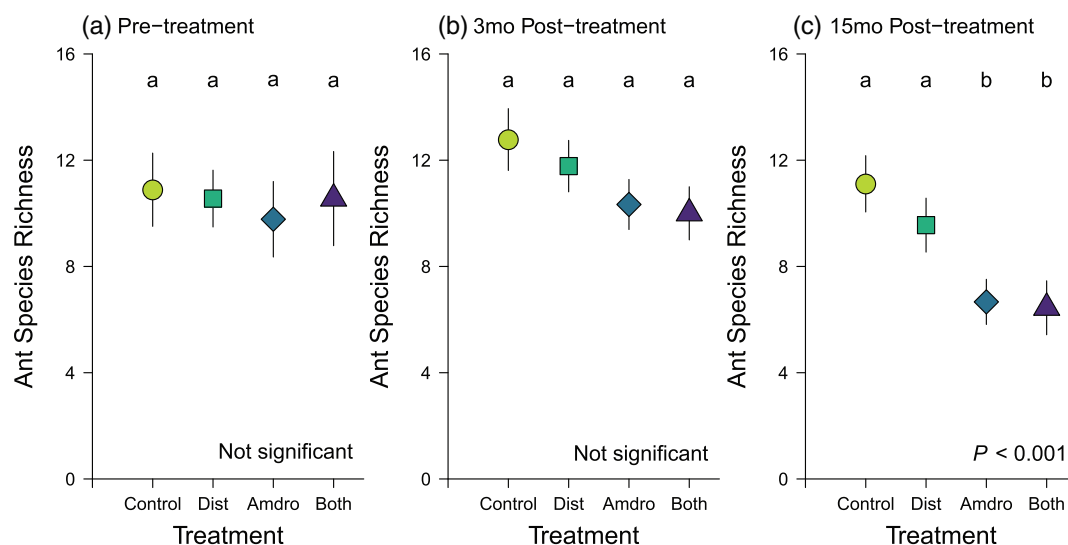
Fig. 2. Average species richness of ants (±SE) per plot across treatments and time. Panel (a) was before treatments were applied in May 2011, panel (b) was from August 2011, and panel (c) was from August 2012. Different letters indicate significant differences between treatments ($P < 0.05$).
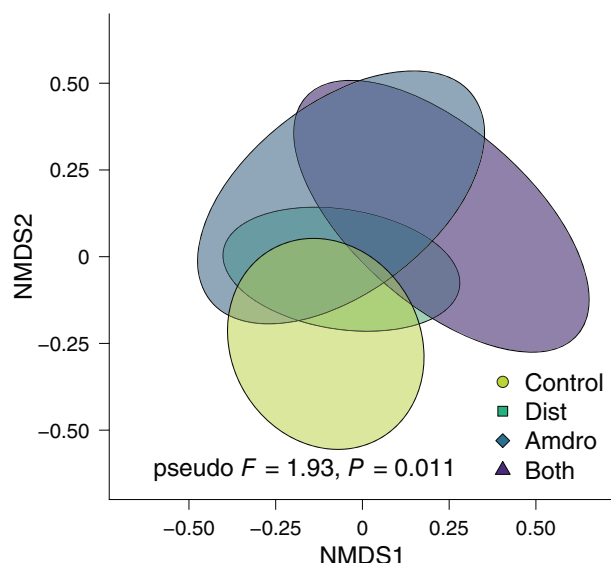

Fig. 3. Non-metric multidimensional scaling (NMDS) ordination of ant communities across treatments in August of 2012 (i.e. 15 months post-treatment). Ellipses represent standard deviation of points per treatment.

and *Dorymyrmex bureni* were more abundant in 'Amdro' and 'Both' plots, while *Myrmecina americana*, *Pheidole crassicornis*, *Solenopsis invicta* and *Solenopsis molesta* were more abundant in 'Control' plots (Table 1).

Diet breadth of fire ants

Carbon and nitrogen stable isotope values revealed similarities in diet for fire ants between treatments after 15 months (Fig. 4;

Pillai's Trace = 0.094, $F = 0.53$, $P = 0.786$). There was substantial variation, however, in the overall amount of occupied $\delta^{13}C - \delta^{15}N$ bi-plot space with standard ellipse areas increasing from $0.84\%_o^2$ in 'Control' to $3.94\%_o^2$ in the 'Both' treatment (Fig. 4). As fire ant diet breadth increased, more co-occurring species were consequently encapsulated in the isotopic standard ellipses in which fire ants regularly consumed resources (Number of species within fire ant SEA_c: 'Control' = 2, 'Dist' = 7, 'Amdro' = 10, 'Both' = 12).

Discussion

Over 2 years, Amdro®-based treatments reduced fire ant abundance 42% in pitfall traps and 95% on baits, but native ant richness did not increase. Furthermore, the disturbance treatment alone had minimal effects on fire ants and co-occurring species. The recovery of some native species, however, through an increase in their abundance relative to control plots suggests strict 'driver' and 'passenger' models may not capture the full complexity of change that occurs in invaded ecosystems.

Fire ants as drivers, passengers, or both?

MacDougall and Turkington's 'driver' and 'passenger' models (2005) provide alternative hypotheses for understanding the ecological causes and consequences of establishment by invasive species. Under strict interpretation, our data might suggest that fire ants are 'passengers', not 'drivers', because when we reduced fire ant abundance, native species richness did not increase. Yet, there are several aspects of our study that affect interpretation and contrast with an experimental study in pine flatwoods of northern Florida that concluded fire ants were best described as 'passengers' (King & Tschinkel, 2008).

Table 1. Similarity percentage analysis of ant species contributing the most to overall differences between treatments after 15 months.

(a) 'Control' vs 'Amdro' treatment overall dissimilarity = 0.620

Species	Control abundance	Amdro abundance	Δ Abundance	Average contribution (%)	Cumulative contribution (%)
Solenopsis invicta	41.56	27.89	−	29.01	29.01
Pheidole crassicornis	10.89	0.33	−	11.45	40.46
Dorymyrmex bureni	3.78	12.11	+	11.34	51.80
Solenopsis molesta	10.33	2.22	−	10.65	62.45
Brachymyrmex patagonicus	3.33	4.11	+	5.73	68.18
Myrmecina americana	3.56	0.44	−	4.32	72.49

(b) 'Control' vs 'Both' treatment overall dissimilarity = 0.684

Species	Control abundance	Both abundance	Δ Abundance	Average contribution (%)	Cumulative contribution (%)
Solenopsis invicta	41.56	20.33	−	32.92	32.92
Pheidole crassicornis	10.89	0.33	−	11.15	44.07
Solenopsis molesta	10.33	2.56	−	10.57	54.64
Dorymyrmex bureni	3.78	4.44	+	5.89	60.53
Hypoponera opacior	0.89	3.89	+	4.38	64.91
Brachymyrmex patagonicus	3.33	3.44	+	4.37	69.28
Myrmecina americana	3.56	0.67	−	4.16	73.44

Panel (a) compares 'Control' to 'Amdro' while panel (b) compares 'Control' to 'Both' (see Materials and methods for treatment description). Abundance values are averages per plot from pitfall traps with Δ (delta) representing either an increase (+) or decrease (−) compared to 'Control'. Species are listed in descending order by their overall contribution to Bray−Curtis dissimilarity (contribution %).

First, King and Tschinkel (2008) chose habitat in which fire ants did not regularly occur and were consequently added to experimental plots. Because fire ants favour disturbed areas and did not otherwise colonise pine flatwoods, they concluded that fire ants follow the 'passenger' model (i.e. disturbance being the primary agent of change). Our study site, in contrast, was invaded by fire ants decades ago and the remaining ant community has already been modified by this invasive ant's presence (Resasco *et al.*, 2014; Resasco & Fletcher Jr., 2021). The regional species pool may likewise be reduced, limiting dispersal and recolonisation by native species. Alternatively, even if the regional species pool wasn't reduced, it may take longer than the 2 years of our study to detect native species recolonisation.

Second, while King and Tschinkel added fire ants to plots in 2008, we removed them using strategically positioned Amdro® baits. Often it is quite difficult to remove a well-established invasive species and, similar to others (King & Tschinkel, 2006; King & Tschinkel, 2013), we could not completely eliminate fire ants from our plots despite deploying Amdro® for 2 years. Such experimental caveats have drawn criticism as fire ant removal is not complete and the impact of fire ants at low densities can therefore not be ruled out (Stuble *et al.*, 2013).

Third, we saw no change in co-occurring ant species richness between control and disturbance plots without Amdro®. While the 'passenger' model posits that communities are primarily structured by factors other than interactions with invasive species, it also holds that environmental changes such as disturbance or climate are less constraining to invasive species than to native species. We might then have predicted that fire ants would increase in abundance in 'Dist' plots as they have historically excelled in disturbed environments (King & Tschinkel, 2008; Stuble *et al.*, 2011; LeBrun *et al.*, 2012). We did not observe this result (Fig. 1), perhaps because fire ants may have already reached a saturation point in this landscape.

The 'interacting drivers' model bridges the 'driver' and 'passenger' models, predicting partial recovery of some co-occurring species following removal or reduction in areas with already established invasive species (Didham *et al.*, 2005). Indeed, we observed *B. patagonicus and D. bureni*, quick scavengers on invertebrates and honeydew (MacGown *et al.*, 2007; Deyrup, 2016), increase in abundance in 'Amdro' and 'Both' plots, while predatory or granivorous species like *M. americana*, *P. crassicornis* and *S. molesta* decreased (Wilson, 2003; Ohyama *et al.*, 2020). But why did species differ in their response? One possibility is that non-target effects of Amdro® reduced the abundance of co-occurring taxa (e.g. thief ants that regularly visit baits, as seen in Mokkarala, 2002).

While experimental manipulations like ours can improve mechanistic understanding of drivers of ecological change, they have limitations. In this case, fire ant removal treatments could confound and underestimate the effects of fire ants on co-occurring species with similar trophic niches. We posit this might explain why specialists like *Hypoponera opacior*, a predator of leaf litter invertebrates and a species rarely observed on baits, could increase in 'Both' relative to 'Control' treatments. Alternatively, as fire ant abundance decreased, average colony size likely decreased and reduced the carbohydrate requirements of a large and growing work force. Dietary shifts by dominant species can have trophic implications for co-occurring species (Caut *et al.*, 2008) and we discuss below a working hypothesis that involves changes in diet breadth.
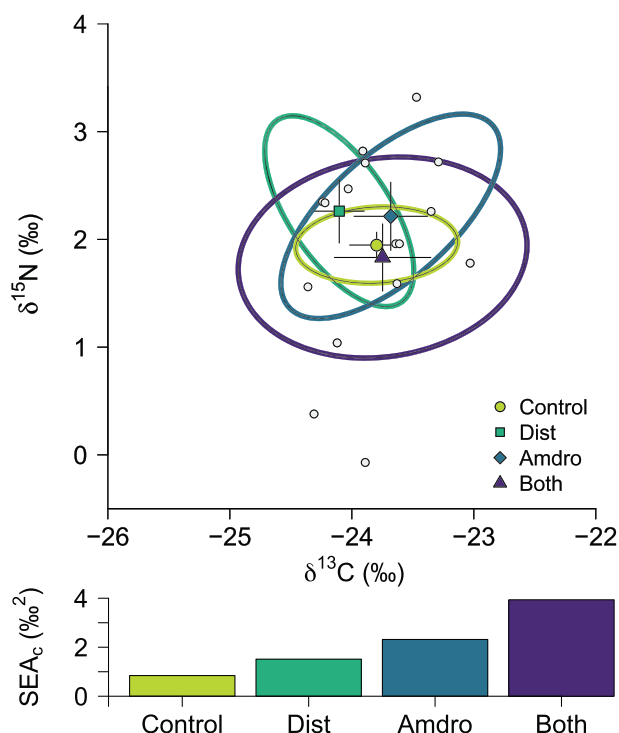
Fig. 4. Carbon and nitrogen stable isotope values of fire ants across treatments. In the top panel each treatment is represented by a mean ± SE value along with a maximum likelihood standard ellipse containing approximately 40% of the fire ant data. Grey points represent mean values for 16 native species that were collected in at least three plots (see Table S1 for species list). Fire ant diet breadth is represented by values in the lower panel as the amount of ellipse area (i.e. SEA_c) occupied in $\delta^{13}C-\delta^{15}N$ bi-plot space.

Expanding diet breadth with disturbance

Invasive ant species often follow a pattern: they establish, expand and consume invertebrate prey as the invasion front moves into neighboring areas (Porter & Savignano, 1990; O'Dowd *et al.*, 2003; Tillberg *et al.*, 2007). After initial establishment, colonies continue to grow, requiring extensive resources to fuel worker activity (Lach, 2005; Wilder *et al.*, 2011; Wills *et al.*, 2015; Roeder *et al.*, 2020). Previous research using carbon and nitrogen stable isotopes have revealed a shift in trophic position during this transition from predator to omnivore to cryptic herbivore as ants increasingly rely on carbohydrates from extrafloral nectaries or tending aphids for sugar-rich honeydew (Tillberg *et al.*, 2007).

We discovered an intriguing and possibly novel pattern that suggests a reversal of this trophic shift may occur as fire ant abundance decreases. While the average fire ant diet was similar across treatments, there was substantial variation in the overall amount of occupied $\delta^{13}C-\delta^{15}N$ bi-plot space with decreases in fire ant abundance linked to increases in their diet breadth by up to $3.1‰^2$ (Fig. 4). This increase in diet breadth (i.e. larger ellipse area in an isotope bi-plot) suggests fire ants in Amdro® plots consumed a greater variety of prey items and likely competed for

resources with more co-occurring species. We speculate that as Amdro® reduced colony sizes, dietary specialisation started to occur at the colony level because smaller colonies would likely sample and isotopically reflect a reduced portion of the patchy landscape. Intraspecific dietary specialisation and its reflection in isotopic signatures have been documented in several species (Martínez del Rio *et al.*, 2009; Newsome *et al.*, 2009; Helms IV *et al.*, 2021) and fire ants have large diet flexibility at both small and large spatial scales (Wilder *et al.*, 2011; Resasco *et al.*, 2012; Roeder & Kaspari, 2017). Alternatively, the observed shift in fire ant diet breadth may suggest changes in the invertebrate food web beyond our simple observations on the abundance and species richness of ants.

Conclusions and historical perspective

Upland habitat at the Savannah River Site likely harbored a richer ant fauna, as we found only 62% of the species reported in old field or pine forest habitat in the 1970s (Van Pelt & Gentry, 1985). Taxa that span broad functional and phylogenetic groups (*Dolichoderus*, *Lasius*, *Ponera*, *Pseudomyrmex* and *Tapinoma*) had been present but were not observed. These genera may persist, though, in the surrounding landscape (Resasco *et al.*, 2014; Resasco & Fletcher Jr., 2021). There is some evidence that native communities may rebound if fire ant abundance declines over time (Morrison, 2002). The extent to which this has or will occur remains uncertain.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1: Carbon and nitrogen stable isotope values for co-occurring ant species.

References

Ascunce, M.S., Yang, C., Oakey, J., Calcaterra, L., Wu, W., Shih, C. *et al.* (2011) Global invasion history of the fire ant *Solenopsis invicta*. *Science*, **331**, 1066–1068.

Bates, D., Maechler, M., Bolker, B. & Walkers, S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, **67**, 1–48.

Bertelsmeier, C., Avril, A., Blight, O., Confais, A., Diez, L., Jourdan, H. *et al.* (2015) Different behavioral strategies among seven highly invasive ant species. *Biological Invasions*, **17**, 2491–2503.

Buhs, J.B. (2004) *The Fire Ant Wars: Nature, Science, and Public Policy in Twentieth-Century America*. University of Chicago Press, Chicago, Illinois.

Caut, S., Angulo, E. & Courchamp, F. (2008) Dietary shift of an invasive predator: rats, seabirds and sea turtles. *Journal of Applied Ecology*, **45**, 428–437.

Clarke, K.R. (1993) Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, **18**, 117–143.

Deyrup, M. (2016) *Ants of Florida: Identification and Natural History*. CRC Press, Boca Raton, Florida.

Didham, R.K., Tylianakis, J.M., Hutchinson, M.A., Ewers, R.M. & Gemmell, N.J. (2005) Are invasive species the drivers of ecological change? *Trends in Ecology & Evolution*, **20**, 470–474.

Elton, C.S. (1958) *The Ecology of Invasions by Animals and Plants*. Methuen & Co., London, U.K.

Greenslade, P.J.M. (1973) Sampling ants with pitfall traps: digging-in effects. *Insectes Sociaux*, **20**, 343–353.

Gurevitch, J. & Padilla, D.K. (2004) Are invasive species a major cause of extinctions? *Trends in Ecology & Evolution*, **19**, 470–474.

Helms, J.A. IV, Roeder, K.A., Ijelu, S.E., Ratcliff, I. & Haddad, N.M. (2021) Bioenergy landscapes drive trophic shifts in generalist ants. *Journal of Animal Ecology*, **90**, 738–750.

Jackson, A.L., Inger, R., Parnell, A.C. & Bearhop, S. (2011) Comparing isotopic niche widths among and within communities: SIBER–stable isotope Bayesian ellipses in R. *Journal of Animal Ecology*, **80**, 595–602.

King, J.R. & Tschinkel, W.R. (2006) Experimental evidence that the introduced fire ant, *Solenopsis invicta*, does not competitively suppress co-occurring ants in a disturbed habitat. *Journal of Animal Ecology*, **75**, 1370–1378.

King, J.R. & Tschinkel, W.R. (2008) Experimental evidence that human impacts drive fire ant invasions and ecological change. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 20339–20343.

King, J.R. & Tschinkel, W.R. (2013) Experimental evidence for weak effects of fire ants in a naturally invaded pine-savanna ecosystem in North Florida. *Ecological Entomology*, **38**, 68–75.

Lach, L. (2005) Interference and exploitation competition of three nectar-thieving invasive ant species. *Insectes Sociaux*, **52**, 257–262.

LeBrun, E.G., Tillberg, C.V., Suarez, A.V., Folgarait, P.J., Smith, C.R. & Holway, D.A. (2007) An experimental study of competition between fire ants and argentine ants in their native range. *Ecology*, **88**, 63–75.

LeBrun, E.G., Plowes, R.M. & Gilbert, L.E. (2012) Imported fire ants near the edge of their range: disturbance and moisture determine prevalence and impact of an invasive social insect. *Journal of Animal Ecology*, **81**, 884–895.

Lenth, R. (2020) *emmeans: Estimated margina means, aka least-square means. R package version 1.4.8.*

Lockwood, J.L., Hoopes, M.F. & Marchetti, M.P. (2007) *Invasion Ecology*. John Wiley & Sons, West Sussex, U.K.

MacDougall, A.S. & Turkington, R. (2005) Are invasive species the drivers or passengers of change in degraded ecosystems? *Ecology*, **86**, 42–55.

MacGown, J.A. (2003) *Ants (Formicidae) of the southeastern United States*. Mississippi Entomological Museum, Mississippi State University. Accessed 2011–2012.

MacGown, J.A., Hill, J.V.G. & Deyrup, M.A. (2007) *Brachymyrmex patagonicus* (hymenoptera: Formicidae), an emerging pest species in the southeastern United States. *Florida Entomologist*, **90**, 457–464.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M. & Bazzaz, F.A. (2000) Biotic invasions: causes, epidemimology, global consequences, and control. *Ecological Applications*, **10**, 689–710.

Martínez del Rio, C., Sabat, P., Anderson-Sprecher, R. & Gonzales, S.P. (2009) Dietary and isotopic specialization: the isotopic niche of three Cinclodes ovenbirds. *Oecologia*, **161**, 149–159.

Mokkarala, P. (2002) *Effects of red imported fire ant baits on some non-target ants*. MS Thesis. Texas A&M University.

Mooney, H.A. & Cleland, E.E. (2001) The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 5446–5451.

Morrison, L.W. (2002) Long-term impacts of an arthropod-community invasion by the imported fire ant, *Solenopsis invicta*. *Ecology*, **83**, 2337–2345.

Newsome, S.D., Tinker, M.T., Monson, D.H., Oftedal, O.T., Ralls, K., Staedler, M.M. *et al.* (2009) Using stable isotopes to investigate individual diet specialization in California sea otters (*Enhydra lutris nereis*). *Ecology*, **90**, 961–974.

O'Dowd, D.J., Green, P.T. & Lake, P.S. (2003) Invasional 'meltdown' on an oceanic Island. *Ecology Letters*, **6**, 812–817.

Ohyama, L., King, J.R. & Jenkins, D.G. (2020) Are tiny subterranean ants top predators affecting aboveground ant communities? *Ecology*, **101**, e03084.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R. B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E. & Wagner, H. (2019) Vegan: community ecology package. R package version 2.5–6.

Orians, G.H. (1986) Site characteristics favoring invasions. *Ecology of Biological Invasions of North America and Hawaii* (ed. by H. Mooney and J. Drake), pp. 133–148. Springer, New York, New York.

Parr, C.L., Andersen, A.N., Chastagnol, C. & Duffaud, C. (2007) Savanna fires increase rates and distances of seed dispersal by ants. *Oecologia*, **151**, 33–41.

Porter, S.D. & Savignano, D.A. (1990) Invasion of polygyne fire ants decimates native ants and disrupts arthropod community. *Ecology*, **71**, 2095–2106.

Resasco, J. & Fletcher, R.J. Jr. (2021) Accounting for connectivity alters the apparent roles of spatial and environmental processes on metacommunity assembly. *Landscape Ecology*. https://doi.org/10.1007/s10980-021-01203-z.

Resasco, J., Levey, D.J. & Damschen, E.I. (2012) Habitat corridors alter relative trophic position of fire ants. *Ecosphere*, **3**, 1–9.

Resasco, J., Pelini, S.L., Stuble, K.L., Sanders, N.J., Dunn, R.R., Diamond, S.E. *et al.* (2014) Using historical and experimental data to reveal warming effects on ant assemblages. *PLoS One*, **9**, e88029.

Roeder, K.A. & Kaspari, M. (2017) From cryptic herbivore to predator: stable isotopes reveal consistent variability in trophic levels in an ant population. *Ecology*, **98**, 297–303.

Roeder, K.A., Roeder, D.V. & Kaspari, M. (2018) The role of temperature in competition and persistence of an invaded ant assemblage. *Ecological Entomology*, **43**, 774–781.

Roeder, K.A., Prather, R.M., Paraskevopoulos, A.W. & Roeder, D.V. (2020) The economics of optimal foraging by the red imported fire ants. *Environmental Entomology*, **49**, 304–311.

Stuble, K.L., Kirkman, L.K., Carroll, C.R. & Sanders, N.J. (2011) Relative effects of disturbance on red imported fire ants and native ant species in a longleaf pine ecosystem. *Conservation Biology*, **25**, 618–622.

Stuble, K.L., Chick, L.D., Rodriguez-Cabal, M.A., Lessard, J.P. & Sanders, N.J. (2013) Fire ants are drives of biodiversity loss: a reply to King and Tschinkel (2013). *Ecological Entomology*, **38**, 540–542.

Tillberg, C.V., Holway, D.A., Lebrun, E.G. & Suarez, A.V. (2007) Trophic ecology of invasive Argentine ants in their native and introduced ranges. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 20856–20861.

Tschinkel, W.R. (2006) *The Fire Ants*. Harvard University Press, Cambridge, Massachusetts.

Turner, M.G. (2010) Disturbance and landscape dynamics in a changing world. *Ecology*, **91**, 2833–2849.

Van Pelt, A. & Gentry, J.B. (1985) *Ants (Hymenoptera: Formicidae) of the Savannah River Plant, South Carolina*. United States Department of Energy, Savannah River Site SRO-NERP-14: 1–56.

Vitousek, P.M., D'Antonio, C.M., Loope, L.L. & Westbrooks, R. (1996) Biological invasions as global environmental change. *American Scientist*, **84**, 468–478.

Wilder, S.M., Holway, D.A., Suarez, A.V., LeBrun, E.G. & Eubanks, M.D. (2011) Intercontinental differences in resource use reveal the importance of mutualisms in fire ant invasions. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 20639–20644.

Wills, B.D., Chong, C.D., Wilder, S.M., Eubanks, M.D., Holway, D.A. & Suarez, A.V. (2015) Effect of carbohydrate supplementation on investment into offspring number, size, and condition in a social insect. *PLoS One*, **10**, e0132440.

Wilson, E.O. (2003) *Pheidole in the New World: a Dominant, Hyperdiverse Ant Genus*. Harvard University Press, Cambridge, Massachusetts.

Wittman, S.E.W. (2014) Impacts of invasive ants on native ant communities (Hymenoptera: Formicidae). *Myrmecological News*, **19**, 111–123.