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Biological Invasions

ISSN 1387-3547

Biol Invasions

DOI 10.1007/s10530-018-1737-9



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Red imported fire ants (*Solenopsis invicta*) and seasonality influence community refuge use

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Received: 25 August 2017 / Accepted: 16 April 2018

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Abstract Refuges are fundamental to animal ecology as refuge availability affects many levels of biological organization—from the behavior and physiology of individuals to the interspecific dynamics of a community. Although frequently studied in the context of predator–prey interactions, refuges may also mediate interspecific competition between native and invasive taxa given the role of refuges as a valuable resource. Because interspecific interactions (e.g., competition and predation) can be modulated by temporal and biotic (e.g., trophic level) factors, we used a manipulative approach to investigate community-wide refuge-use patterns in the context of two important ecological factors: invasive species and seasonality. We surveyed refuge (artificial cover object) use of ants and vertebrates in a forest community for 2 years, and we systematically suppressed an established invasive species (red imported

fire ant, *Solenopsis invicta*) to examine its impact on community refuge use. Native *Camponotus* ants appeared to co-exist and share refuges with *S. invicta*, but we found evidence for a negative effect of *S. invicta* on vertebrate refuge use that was also influenced by season. Vertebrates were more abundant under refuges undergoing suppression of *S. invicta*, and they were less abundant under refuges during the fall (the season characterized by the highest occupancy of refuges by *S. invicta*). Thus, researchers must continue to examine the entire community and to incorporate the effects of season when assessing the impact of invasive species (e.g., at our site, a survey conducted only in the summer or only on native ants would have indicated a negligible effect of *S. invicta* on community refuge use).

Keywords *Camponotus* · Coastal plain · Cover object · Invasive · Reptile

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10530-018-1737-9>) contains supplementary material, which is available to authorized users.

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Introduction

Refuges play an important role in animal ecology as the availability of refuges influences several biological levels of organization (Berryman and Hawkins 2006). Animals utilize refuges or shelter structures to improve predator avoidance (e.g., Dill and Fraser 1997; Cowlshaw 1997), thermoregulation (Roper et al. 2001; Goldsbrough et al. 2004; Stahlschmidt

et al. 2012), and energy balance (e.g., ambush hunting: Bevelander et al. 2006), and refuges can be important in mediating competition (Turchin and Kareiva 1989; reviewed in Wieters et al. 2009). In addition, artificial refuge structures can be used to monitor biodiversity and abundance related to conservation efforts (Fitch 1992; Wilson et al. 2007; Arida and Bull 2008; Grillet et al. 2010). Thus, as the number of natural refuge opportunities fluctuates due to various factors (e.g., human alteration of habitats), surveys of artificial refuges use can inform a range of biological dynamics—from the behavior of individuals to the assemblages of communities across a landscape.

Invasive species comprise one factor that will continue to have disruptive effects on organisms (e.g., animal behavior), species, and ecosystems (Kenis et al. 2009; Strayer 2010; Vila et al. 2011; reviewed in Mack et al. 2000; Thawley and Langkilde 2017). The impact of an established invasive species on animals and their refuge use patterns may exhibit at least two types of variation. First, ecological effects may vary throughout a year given animals' season-specific requirements for food (reviewed in Cook et al. 2011), thermoregulation (Diaz et al. 2006; Mortensen et al. 2007), and space (Madsen and Shine 1996; Chaverri et al. 2007; Bauder et al. 2016). As such, the relative frequency of interactions with a given invasive species may vary with season. Second, the relative impact of invasive species may vary among members of the native community. For example, invasive species typically have relatively larger (more negative) effects on species to which they are more similar in terms of phylogeny and/or functional role in the ecosystem (Vila et al. 2011). Therefore, seasonality and a range of trophic levels must be considered when determining the ongoing ecological impact of an invasive species on animal refuge-use behavior and biodiversity, in general.

We used a manipulative approach to assess the impact of red imported fire ants (*Solenopsis invicta*) on the seasonal refuge use of a forest animal community (as determined by the abundance, biodiversity, and community assemblages of animals using artificial refuges). *Solenopsis invicta* is a widespread invasive species that can now be found in 13 U.S. states and seven other countries, and it can influence vertebrate behavior, physiology, and mortality (reviewed in Allen et al. 2004; Graham et al. 2017). Additionally, *S. invicta* can alter arthropod communities via

competition or predation (Wickings and Ruberson 2011; reviewed in Wittman 2014), as well as indirectly influence several other interspecific interactions [e.g., parasitism (Castellanos et al. 2016), predation (Morrow et al. 2015), and mutualism (Wilder et al. 2013)]. Therefore, our first hypothesis was that *S. invicta* influences refuge use across several trophic levels and functional groups. From this hypothesis, we first predict that refuge use of native ants and vertebrates will be greater under refuges that are naturally unoccupied by *S. invicta*. We further predict that native ants and vertebrates will increase their use of refuges that have been treated to reduce *S. invicta* occupancy. Because patterns of foraging or abundance in *S. invicta* can vary with season (Vogt et al. 2003; Lu et al. 2012), our second hypothesis was that refuge use is influenced by season. From these hypotheses we predict *S. invicta* occupation and season will have additive effects on refuge use under artificial cover objects. Specifically, *S. invicta* occupancy of refuges will be particularly high during certain seasons, and these seasons will correspond to reduced refuge use by vertebrates and native ants.

Materials and methods

Study site

The sea island, Spring Island, is a private residential community in the coastal plain (Beaufort County, South Carolina, U.S.A.) that is characterized by a humid subtropical climate. Four surveying locations in a southern mixed deciduous-evergreen broadleaf forest were used in this study. These locations are managed via prescribed burning every 1–2 years, which is notable given *S. invicta* invasion is often associated with habitat disturbance (King and Tschinkel 2008). At each location, sheets of steel (approx. 1 m × 2 m) were used as artificial cover objects ($n = 108$ total) (sensu Joppa et al. 2009). Artificial cover objects can effectively estimate refuge use and biodiversity (e.g., Grant et al. 1992; Houze and Chandler 2002; Lettink and Patrick 2006; Costall and Death 2010; MacNeil and Williams 2014; Todd 2016) with relatively minimal disturbance to animals (e.g., other methods include trapping and/or handling). At least 1 year prior to the study, cover boards ($n = 20$ –42 at each location) were laid on the ground

at intervals approximately ≥ 5 m from one another within each location, and locations were separated from one another by 0.5–3 km. *Solenopsis invicta* has been present in Beaufort County since 1959–1964 (National Agriculture Pest Information System), uses artificial cover objects at our study site, and has mounds that are easy to observe (i.e., hundreds or thousands of individuals). In Beaufort County, *S. invicta* is the monogyne form (Kintz-Early et al. 2003), which is more common, territorial, and have lower mound densities than the polygyne form (Macom and Porter 1996).

Experimental design

For over a half-century, ecologists have been using manipulative approaches to examine the ecology of competitive release (e.g., Connell 1961; Jutila and Grace 2002; Bodey et al. 2009), and these experimental methods have also been used to examine the effects of invasive species on community dynamics beyond competition (e.g., reviewed in Wittman 2014; Castellanos et al. 2016). After an initial survey of the community, an invasive species can be systematically excluded or suppressed. To determine the invasive species' impact, the community can be re-surveyed, and the community's abundance or biodiversity can be compared from pre-suppression to post-suppression, as well as to a reference (control) community over the same time period. Despite its potential, this approach has not been implemented to assess the effects of invasive species on refuge-use patterns across a community over time.

Thus, approximately twice per month for 2 years (September 2014–August 2016), each cover board at our study was overturned to document the presence of vertebrate genera, as well as *S. invicta* mounds. At the last survey in August 2015 (i.e., after a 1 year baseline, control period), the hydramethylnon-containing bait Amdro[®] was administered to half of the cover boards across all four locations to selectively suppress *S. invicta*. Amdro[®] is not known to affect our focal native ant species (*Camponotus floridanus* and *C. pennsylvanicus*), but we sought to limit the spread of Amdro[®] to areas around or outside of cover boards by directly administering it under 'treated' cover boards, each of which was > 10 m from the nearest 'control' board. Amdro[®] was re-applied as needed throughout the year (e.g., if an *S. invicta* mound persisted or appeared

under a 'treated' board). From September 2015 through August 2016, both 'treated' and 'control' boards were surveyed for vertebrates, *S. invicta*, and the presence of nests of native ants (*C. floridanus* and *C. pennsylvanicus*)—that is, native ants were only surveyed in the second year.

We assessed vertebrate refuge use by determining conventional metrics for assessing biodiversity, abundance, and community assemblages for the animals observed under cover boards. Richness (R, the number of taxa present), Shannon's Diversity (H), and Shannon's Evenness (E) for vertebrates were calculated during each season because R, H, and E are effective metrics for estimating biodiversity (Magurran 2004). To account for variation in surveying effort across seasons, the adjusted abundance of vertebrates (number of vertebrates observed/number of surveys) was determined for each cover board each season. To estimate the occupancy of ants, the proportion of observations of native ant nests and *S. invicta* mounds were determined for each cover board. For example, if *S. invicta* were observed occupying a given cover board three out of the six times it was surveyed during a given season, this cover board would be considered to have an *S. invicta* occupancy of 0.5 (i.e., occupied 50% of the times surveyed) during this season. Metrics of abundance, occupancy, and biodiversity at each cover board were compared across meteorological seasons [e.g., the total number of vertebrate genera observed under a given cover board during spring (March–May) 2015 vs. during summer (June–August) 2015]. To examine the potential role of temperature in refuge use, temperature data loggers (HOBO Pendant, Onset Computer Corp., Bourne, MA) recorded hourly temperature under each cover board in the second year of the study. Also in the second year, vertebrate communities occupying Amdro[®]-treated cover boards were compared to those occupying non-treated (control) cover boards using percent similarity indices (Czekanowski coefficients) at each location ($n = 4$) for every season.

Statistical analyses

Several linear mixed models were performed in SPSS (v.22 IBM Corp., Armonk, NY), data were log-transformed when necessary (i.e., generalized linear mixed models were used for the occupancy of native ants and the standard deviation in seasonal

temperature data), and two-tailed significance was determined at $\alpha = 0.05$. To better assess fixed effects, non-significant interactive effects were removed and models were re-run. To determine whether Amdro[®] treatment suppressed *S. invicta* occupancy and whether this effect was modulated by season, treatment (control; Amdro[®]), season, and treatment \times season were included as fixed effects on data from the 2nd year (i.e., the year in which Amdro[®] was administered). For this model and others, cover board identity nested within location was included as a random effect and post hoc pairwise comparisons among seasons were adjusted using sequential Bonferroni corrections (Holm 1979). We used another model to examine temporal effects on *S. invicta* occupancy because determining seasonal variation in *S. invicta* occupancy is important to understanding potential interactive or additive effects of *S. invicta* and season on refuge use by vertebrates and native ants (see below). This model was performed on data from control treatment cover boards (i.e., those not treated with Amdro[®]), and it included season, year (1: September 2014–August 2015; 2: September 2015–August 2016), and season \times year as fixed effects. Because Amdro[®] was only administered the 2nd year, five models were used to determine the effects of season, Amdro[®] treatment, and their interaction in the 2nd year (i.e., one model each for the R, H, E, and adjusted abundance of vertebrates and the occupancy by native ants). For these models, the following were included as fixed effects: treatment, season, and treatment \times season.

We used two statistical approaches to determine whether *S. invicta* occupancy was associated with reduced occupancy by vertebrates and native ants during the entire study. First, five models more were used on the seasonal data to determine the effects of time and Amdro[®] treatment across both years (i.e., one model each for the R, H, E, and adjusted abundance of vertebrates and the occupancy by native ants). For these models, the following were included as fixed effects: proportion of observations of *S. invicta* occupancy (covariate), year, season, and *S. invicta* occupancy \times season. The model for native ants only included data from the 2nd year as native ants were not surveyed the 1st year. Second, two generalized models were performed on the data for the cover board sampling points ($n = 4492$) rather than summarized data for each season (as described above). A

generalized binary logistic mixed model was used on the occupancy of native ants (presence vs. absence), and a Poisson model was used on vertebrate abundance. For these two models, *S. invicta* occupancy (presence vs. absence), season, and the interaction between *S. invicta* occupancy and season were included as fixed effects. Cover board identity was included as a random effect for both models, and year was included as a random effect for the vertebrate abundance model as native ants were only surveyed in the 2nd year.

A linear mixed model was performed on the mean and standard deviation in hourly temperature data at each cover board over each season for the second year. Treatment (to validate that Amdro[®]-treated cover boards exhibited similar thermal microclimates as control cover boards) and season were included as fixed effects. To examine whether treatment effects on vertebrate community assemblages at each location changed with season, a model was also performed on percent similarity indices (Czekanowski coefficients). Season was included as a fixed effect, and location was included as a random effect.

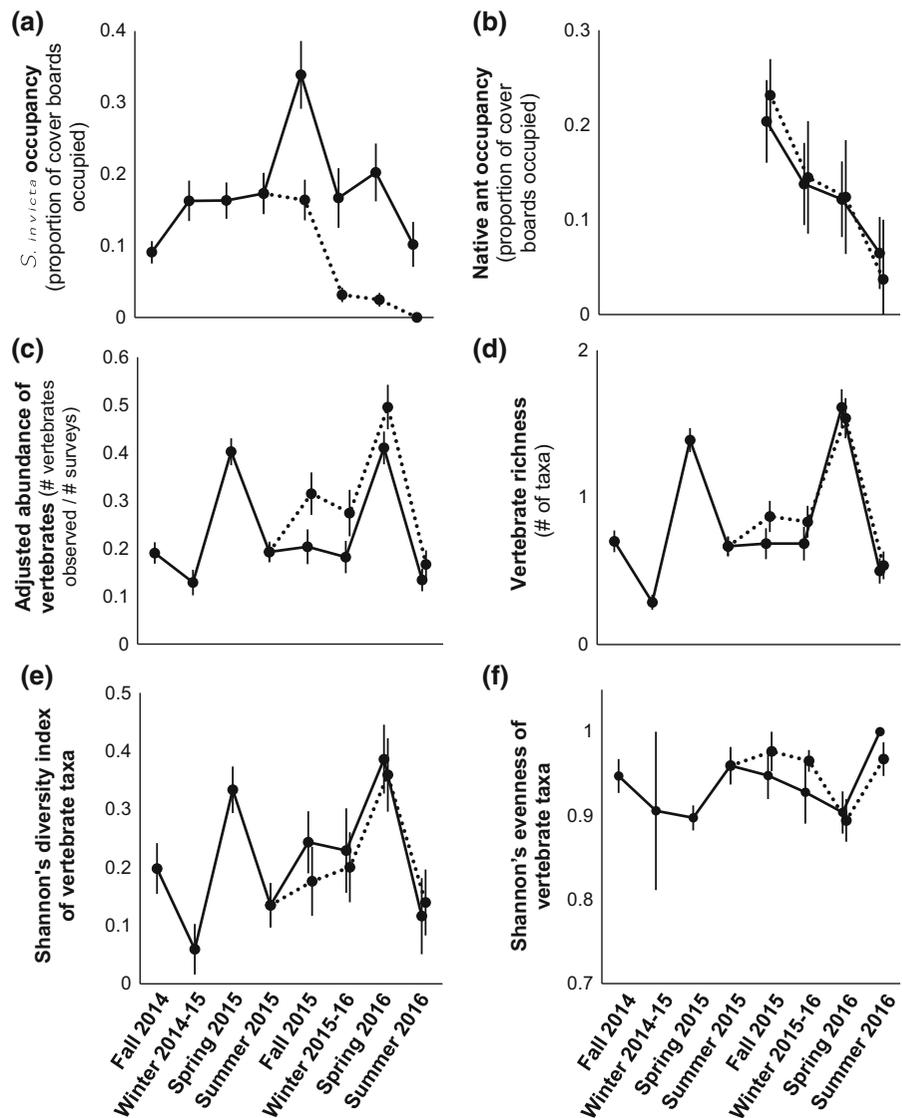
Results

During the 2nd year of the study, the occupancy of cover boards by *S. invicta* was influenced by treatment (Amdro[®] treatment reduced occupancy: $F_{1,106} = 19$, $P < 0.001$) and season (greatest occupancy during the fall, and spring occupancy was greater than the summer: $F_{3,318} = 31$, $P < 0.001$), but not by treatment \times season ($F_{3,318} = 1.3$, $P = 0.26$) (Fig. 1a). Across both years, *S. invicta* occupancy was not affected by year ($F_{1,318} = 2.1$, $P = 0.15$), but it was influenced by season (greater occupancy in fall relative to summer: $F_{3,749} = 3.6$, $P = 0.013$) and year \times season (fall-summer difference driven by effects in 2nd year: $F_{3,749} = 23$, $P < 0.001$) (Fig. 1a).

In both types models (i.e., either *S. invicta* occupancy or Amdro[®] treatment included as effect), the occupancy of cover boards by native (*Camponotus*) ants was only influenced by season (greater occupancy in fall relative to spring: $P < 0.007$; all other factors: $P > 0.07$) (Fig. 1b).

Over the course of the study, there were 1337 observations of vertebrate individuals. These individuals spanned 20 genera and every class of terrestrial

Fig. 1 Effects of *Solenopsis invicta* suppression treatment [control (no suppression): solid lines; Amdro[®] (suppression): dotted lines] and time on metrics of refuge (cover board) use: **a** occupancy of *S. invicta*, **b** occupancy of native ants, **c** adjusted abundance of vertebrates, **d** vertebrate richness, **e** Shannon's diversity index of vertebrates, and **f** Shannon's evenness of vertebrates. Values are displayed as mean \pm s.e.m. for 108 cover boards. No cover boards were treated with Amdro[®] (i.e., they were control boards) through the first year of the study, and half of the cover boards were then treated with Amdro[®] in the second year of the study



vertebrates. In sum, the animals we observed comprised ≥ 3 trophic levels (e.g., *Camponotus* ants are consumed by *Scincella lateralis* and *Plestiodon* lizards, which are consumed by *Coluber constrictor*: A. Mills unpublished) (Fig. 2). Taxa were identified to the species level with the exception of two genera that were difficult to further classify without disturbance (i.e., handling): *Plestiodon* (either *P. fasciatus*, *P. laticeps*, or *P. inexpectatus*) and *Hyla* (either *H. cinerea* or *H. squirella*). The vast majority (nearly 90%) of observed vertebrates were squamate reptiles (Fig. 2).

During the 2nd year of the study, the adjusted abundance of vertebrates under cover boards was influenced by treatment (Amdro[®] treatment increased abundance: $F_{1,106} = 6.8$, $P = 0.010$) and season (greatest abundance during the spring, and fall abundance was greater than the summer: $F_{3,318} = 31$, $P < 0.001$), but not by treatment \times season ($F_{3,318} = 0.44$, $P = 0.73$) (Fig. 1c).

Across both years, adjusted abundance was affected by the proportion of observations of *S. invicta* mounds (abundance negatively covaried with *S. invicta* occupancy: $F_{1,509} = 15$, $P < 0.001$), year (greater abundance in 2nd year: $F_{1,753} = 6.6$, $P = 0.010$), and

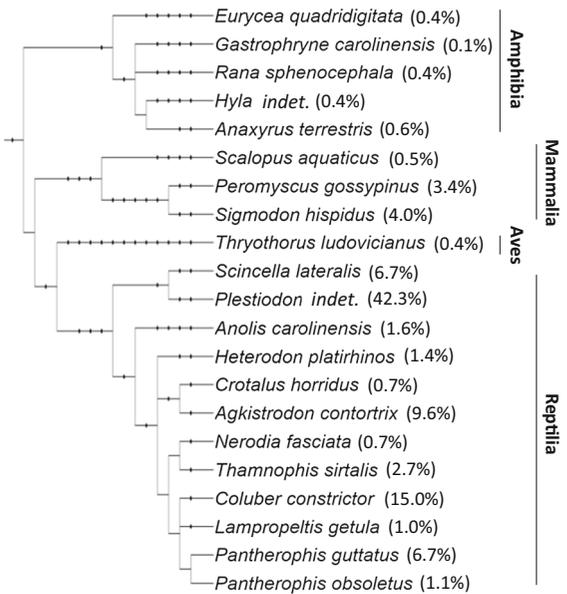


Fig. 2 Phylogenetic tree of the vertebrate community using refuges (cover boards) at our study site on Spring Island, SC, U.S.A. over the 2-year study. The percent of total observations of individual vertebrates are included next to each taxon. The tree was generated using a web-based software. Interactive Tree of Life, version 3: Letunic and Bork (2016)

season (greatest abundance in spring: $F_{3,764} = 41$, $P < 0.001$), but not by season \times *S. invicta* occupancy ($F_{3,808} = 1.9$, $P = 0.12$) (Fig. 1c). During the 2nd year of the study, the richness of vertebrates under cover boards was only influenced by season (greatest abundance during the spring: $F_{3,318} = 40$, $P < 0.001$; all other factors: $P > 0.41$) (Fig. 1d). Across both years, vertebrate richness was affected by the proportion of observations of *S. invicta* mounds (richness negatively covaried with *S. invicta* occupancy: $F_{1,544} = 10$, $P = 0.0014$), year (greater richness in 2nd year: $F_{1,753} = 8.6$, $P = 0.034$), and season (spring was richest, and fall was richer than winter), but not by season \times *S. invicta* occupancy ($F_{3,803} = 1.2$, $P = 0.32$) (Fig. 1d). In both types of models (i.e., either *S. invicta* occupancy or Amdro[®] treatment included as effect), the Shannon's diversity index and evenness of vertebrates under cover boards was influenced only by season (greatest diversity during the spring: $P < 0.001$; evenness lower in spring relative to fall and summer: $P = 0.008$; all other factors: $P > 0.20$) (Fig. 1e, f).

When examining data for the cover board sampling points rather than summarized data for each season,

results were similar to the seasonal results regarding the effects of *S. invicta* occupancy and season on refuge use (see above). The occupancy of cover boards by native ants was influenced only by season (Wald $\chi^2 = 11$, $df = 3$, $P = 0.012$). The abundance of vertebrates under cover boards was influenced by *S. invicta* occupancy (Wald $\chi^2 = 17$, $df = 1$, $P < 0.001$) and season (Wald $\chi^2 = 64$, $df = 3$, $P < 0.001$).

The mean temperature under cover boards was influenced by season ($F_{3,248} = 4039$, $P < 0.001$), but not by treatment ($F_{1,100} = 2.5$, $P = 0.12$) or a season \times treatment interaction ($F_{3,248} = 0.82$, $P = 0.48$). Although cover boards in the spring and fall had similar mean temperatures to one another, all other season-season combinations were significantly different from one another (Fig. 3a). Likewise, the standard deviation in temperature under cover boards was influenced by season ($F_{3,248} = 33$, $P < 0.001$), but not by treatment ($F_{1,100} = 1.7$, $P = 0.19$) or a

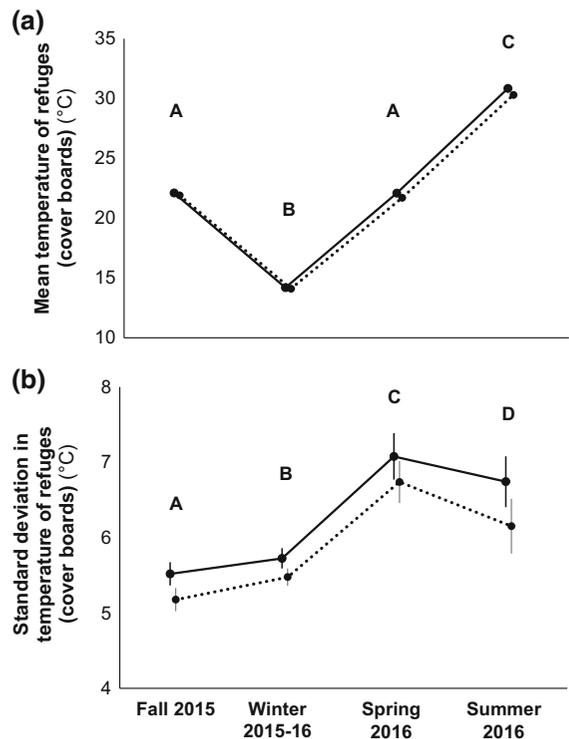


Fig. 3 Effects of *Solenopsis invicta* suppression treatment [control (no suppression): solid lines; Amdro[®] (suppression): dotted lines] and season on **a** mean and **b** standard deviation in temperature for each season in the second year of the study. Values are displayed as mean \pm s.e.m. for 108 cover boards, and letters denote significant differences in pair-wise comparisons of season (no effect of treatment)

season \times treatment interaction ($F_{3,248} = 0.40$, $P = 0.75$). The standard deviation in temperature was different in each season in the second year of the study (Fig. 3b). Season influenced the percent similarity of vertebrate communities at each location ($F_{3,9} = 9.5$, $P = 0.004$)—that is, differences in community assemblages due to Amdro[®] treatment varied across seasons in the second year of the study. Specifically, communities were much more similar to one another during the spring and summer relative to the fall and winter (Fig. 4).

Discussion

We used observational and manipulative approaches to examine the effects of an invasive species on the temporal patterns of refuge use by an animal community. Amdro[®] treatment (*S. invicta* suppression) or the occupancy of *S. invicta* influenced refuge use by vertebrates, but not by native ants (Fig. 1). Further, *S. invicta* occupancy of cover boards negatively covaried with vertebrate abundance and richness, but not with native ant occupancy. Thus, we have partial support for our first hypothesis (*S. invicta* influences refuge use across several trophic levels and functional groups). Our results in vertebrates also support our second hypothesis (refuge use is influenced by season)

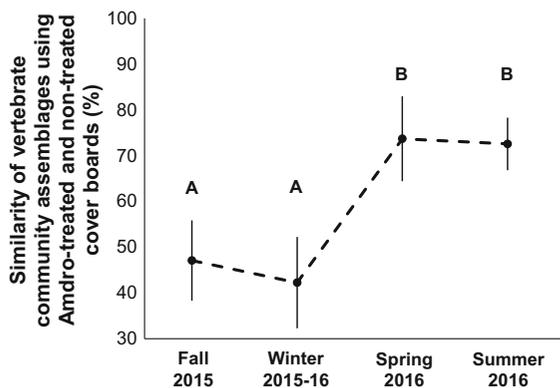


Fig. 4 Effects of season on the percent similarity (sensu Czekanowski coefficient) of vertebrate communities occupying Amdro[®]-treated (i.e., *Solenopsis invicta* suppression treatment) cover boards to those occupying non-treated (control) cover boards in the second year of the study. Values are displayed as mean \pm s.e.m. for four cover board arrays in different locations, and letters denote significant differences in pair-wise comparisons

because vertebrate abundance, and the richness, diversity, evenness, and similarity of vertebrate communities were affected by season (Figs. 1, 4). In sum, we demonstrate taxon- and season-dependent effects of a widespread invasive species on the refuge use patterns of an animal community.

Refuge use by vertebrates was negatively affected by *S. invicta* (reduced adjusted abundance: Fig. 1c; negative correlation between vertebrate richness and *S. invicta* occupancy). This result was not unexpected because a number of previous studies have indicated that *S. invicta* can increase mortality in all four of the classes of terrestrial vertebrates that we observed in our study (Fig. 2; reviewed in Allen et al. 2004). Many of the largest detrimental effects reported have been shown in animals in early life stages characterized by limited mobility (e.g., eggs or juveniles: Mueller et al. 1999; Allen et al. 2004; Thawley and Langkilde 2016; Darracq et al. 2017). Thus, fully mobile animals can mitigate the risks of *S. invicta* via behavioral adjustments, which have been demonstrated at the population-level (Langkilde 2009a; but see Langkilde 2009b). However, our results indicate vertebrates (most of which were small lizards: Fig. 2) may make decisions related to *S. invicta* at a relatively fine spatial scale in their natural environments—animals appeared to shift their preferences for refuges that were situated mere meters away from one another. Previous work has demonstrated that the hispid cotton rat (*Sigmodon hispidus*), which we found at our site (Fig. 2), also exhibits fine-scale plasticity in foraging behavior response to *S. invicta* (Darracq et al. 2016). Because refuges play a key role in a range of ecological processes (e.g., as a resource that mediates competition and predator–prey dynamics: reviewed in Berryman and Hawkins 2006), behavioral plasticity in refuge-use decisions may scale up and be an important component to population- or community-level resilience to this widespread invasive species.

Our results on the effects of *S. invicta* on native *Camponotus* ants failed to support our first hypothesis that *S. invicta* influences refuge use across several trophic levels and functional groups. Refuge use by this species of native ant was not affected by *S. invicta* or by an interaction between season and *S. invicta* (Fig. 1b). At the landscape level, *S. invicta* has been associated with population declines of a range of native ant species (Porter and Savignano 1990; Gotelli and Arnett 2000). Meanwhile, some ant species co-

exist with and/or aggressively compete with *S. invicta* (Rao and Vinson 2004; King and Tschinkel 2008; Warriner et al. 2008). Combined with our results with *Camponotus* and vertebrates, these results suggest invasive species may not always have greater impacts on species to which they are more closely related phylogenetically or trophically. Our results, in particular, indicate that some native ants at our study site do not exhibit behavioral avoidance (i.e., shifts in refuge use) with regard to *S. invicta*. This may be an adaptive strategy by *Camponotus* ants given *S. invicta* has been present at our study site in Beaufort County since 1959–1964 (National Agriculture Pest Information System). That said, we advocate for further research because we only surveyed for large, conspicuous native ants (*Camponotus*), and we do not know how the native ant community responded immediately following *S. invicta* invasion because the pre-invasion ant levels at our study site are unknown.

We detected significant effects of season on refuge use by both *S. invicta* and *Camponotus* in our study (Fig. 1a, b). Increased refuge use by *S. invicta* in the fall may simply reflect seasonal variation in this species' relative density. *Solenopsis invicta* mate when midday temperatures are 23–25 °C (April or May at our site), and colonies typically grow to several thousand individuals within 6 months of mating (Vinson and Sorenson 1986). Thus, we expect a large density of *S. invicta* at our study site during the fall, which would increase the conspicuousness of mounds and the likelihood of colonies choosing cover boards as refuges. Similarly, other populations of *Camponotus* exhibit bimodal oviposition (reviewed in Cannon and Fell 2002), and one bout of reproduction corresponds with the presence of brood (which we regularly observed under cover boards) in the fall. The choice by both ant taxa to increase occupancy of cover boards during the fall may have been due to the moderate thermal conditions of the cover boards in the fall (Fig. 3). Although the spring also exhibits moderate temperatures at our study site (Fig. 3), *Camponotus* may avoid using cover boards during this season as cover boards are heavily used by *Camponotus* predators (small lizards: *Plestiodon*, *Scincella*, and *Anolis*) in the spring. *Camponotus* may prefer to avoid predation risk in the spring at the expense of increased competition with *S. invicta* for refuge resources in the fall. Yet, both ant taxa may be under pressure to choose warmer (not moderate) temperatures—*S.*

invicta colony growth is maximized at 25–32 °C (Porter and Tschinkel 1993), and other *Camponotus* nurse workers prefer 25–31 °C for their brood (Roces and Nunez 1995, 1996). Therefore, either biotic (life cycle) factors drove seasonal patterns of refuge use by ants at our site more than abiotic factors, or other, unmeasured abiotic factors played a key role in ants' refuge use (e.g., humidity: Stuble et al. 2009; Xu et al. 2009).

We found some evidence that the effects of *S. invicta* on vertebrate refuge use were influenced by season as the percent similarity of vertebrate communities between Amdro[®]-treated and non-treated (control) cover boards varied with season (Fig. 4). Specifically, vertebrate communities between these two types of cover objects was least similar during the fall and winter (i.e., *S. invicta* appeared to have the strongest effect on vertebrate refuge use during these seasons) (Fig. 4). Refuges can be an important resource for animals (Berryman and Hawkins 2006)—thus, refuges would be subject to the most competition or predation risk if or when they are limited in the environment. In support, our results indicate greater competition or predation risk (i.e., more negative effect of *S. invicta*) in the seasons in which either *S. invicta* or vertebrates exhibited high rates of refuge (cover board) use (i.e., fall and spring, respectively: Fig. 1). Conversely, interactions between *S. invicta* and vertebrates (competition or predation) under cover boards seemed to be lowest when *S. invicta* and vertebrate refuge use was lowest (i.e., summer: Fig. 1). Therefore, simple supply-and-demand dynamics may drive interspecific interactions under refuges between *S. invicta* and vertebrates.

However, similar to *S. invicta* (see above), the seasonality of vertebrate refuge (cover board) use may also be influenced by life-cycle patterns. Most of the vertebrate taxa we observed (squamate reptiles: Fig. 2) breed in the spring (Gibbon and Dorcas 2005; Gibbons et al. 2009) and, thus, are more active and conspicuous during this time. Thermoregulation during the breeding (reproductive) season is particularly important in reptiles (Shine 2012). Refuges (cover boards) may be more valuable to vertebrates during the spring because this is when the cover boards exhibited a large range of temperatures that could promote thermoregulation (Fig. 3). In support, community assemblages under Amdro[®]-treated (*S. invicta* suppressed) and control cover boards were most

similar in the spring (Fig. 4) meaning that (regardless of *S. invicta* presence) a particular community of vertebrates may have been highly motivated to occupy cover boards during this time.

Together, our manipulative study clarifies the temporal dynamics of community refuge-use patterns in the context of a prominent, established invasive species. Although we detected a strong effect of seasonality on community refuge use, we also demonstrate a range of effects on the community due to *S. invicta* (Figs. 1, 4). Our results suggest two responses to *S. invicta* that appear to be taxon-specific. First, native ants co-existed and shared refuges with *S. invicta* throughout the year (Fig. 1a, b). However, more thorough surveys of all ants at our site (rather than solely large, conspicuous *Camponotus* ants) and relevant control sites (i.e., those lacking *S. invicta*) are required to better understand the effects of *S. invicta* on native ants. Second, vertebrates in our study (albeit biased toward small lizards: Fig. 2) may exhibit temporal niche separation from *S. invicta* as they tended to largely use cover boards during a season in which *S. invicta* did not (Fig. 1). However, future work is required to determine whether this is an adaptive response to the establishment of *S. invicta*, or due to a combination of life-cycle patterns of observed vertebrates and the seasonality of microclimates (i.e., mean and variance in temperature) under cover objects. Related, further investigation is required to better understand the dynamics of the interspecific interactions related to *S. invicta* and refuge use (e.g., whether a native species competes with, preys upon, or is preyed upon by *S. invicta* is likely to influence its refuge-use response to *S. invicta* throughout a given year). In sum, our results highlight the importance of seasonality in understanding species' interactions with invasive taxa in relation to an important resource (i.e., refuge).

Acknowledgements We thank Spring Island Trust, Georgia Southern University, and University of the Pacific for funding. We also thank George Todd, Hayley Bryant, and Kristen Marshall Mattson for assistance in the field, and Jacob Stanley and Dan McNamee for assistance with data entry. Last, we appreciate insightful feedback on the manuscript by Neil Tsutsui and two anonymous reviewers.

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