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Social buffering in a eusocial invertebrate: termite soldiers reduce the lethal impact of competitor cues on workers

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Abstract. While the impact of predator-induced stress on prey has received considerable attention, there has been far less research into the effect of competitors. Cues from aggressive competitors should be particularly likely to evoke behavioral and/or physiological responses, since they may be indicative of both direct (interference) and indirect (exploitative) threats. The danger posed by such competitors, and the "fear" they evoke, should be reduced at lower competitor densities and by the presence of individual conspecifics specialized for defense. We assessed how *Reticulitermes flavipes* termite workers and soldiers were affected by cues from conspecific nestmates, conspecific non-nestmates, and the heterospecific competitor *R. virginicus*. Competitor cues altered *flavipes* worker and soldier behavior, decreasing worker growth and increasing their mortality. The presence of *flavipes* soldiers largely ameliorated these negative impacts: adding even a single soldier (5% of *flavipes* individuals) decreased worker mortality by 50–80%. Although worker mortality increased with competitor density, increased soldier densities did not increase the benefit to workers. The small number of soldiers required to substantially alter cue-mediated interactions suggests that this caste, in addition to providing direct defense, also occupies a "keystone role" by providing homeostatic feedback to workers functioning in stressful environments.

Key words: eusociality; interference competition; nonlethal effects; risk cues; soldiers; stress.

Introduction

Organisms often react to the presence of predators, competitors, or other stressors with an array of behavioral and physiological changes that reduce the probability of being injured or killed. While adaptive in acutely risky situations, chronic activation of these responses can have a number of negative effects (Beckerman et al. 1997, McCauley et al. 2011). Behaviorally, chronic risk-induced reductions in foraging and other activities often decrease growth and fecundity (Creel et al. 2009, Adamo and Baker 2011). At the population level, the cumulative impact of such non-consumptive effects can equal or exceed that of direct predator-induced mortality (Preisser et al. 2005).

The impact of predator cues on prey suggests that some organisms may respond similarly to cues from dangerous heterospecific competitors. Interference competition, especially during territory defense, often results in intraguild killing (Dickman et al. 2014). When interspecific interactions have density-dependent outcomes, intraspecific aggregation can provide an numerical advantage against competitors in a manner similar to that found in predator-prey interactions (Jungwirth et al. 2015). Researchers have documented social buffering, the ability of nearby conspecifics to reduce the negative impact of stressors on individuals, in a wide range of vertebrate taxa (reviewed in Hennessy et al. 2009). Although this suggests that the ability to alter risk responses in response to conspecifics is advantageous, a similar response has not been documented in invertebrates.

Termites (Blattodea: Termitoidae) provide an ideal system for exploring cue-mediated impacts of heterospecific competitors, the factor(s) altering their magnitude of these impacts, and social buffering. These colonially living insects communicate via chemical and vibrational cues, and their almost exclusive reliance on cellulose for nutrition prevents them from using other termite species as a food source. The two dominant termite castes are workers, the primary foragers and nest caretakers who are often injured or killed during interspecific interactions (Shelton and Grace 1996), and soldiers, defensive specialists who provide little foraging benefit (Tian and Zhou 2014). Because a single location often contains multiple termite species that compete both directly and indirectly for the same habitats or food sources, foraging workers are chronically exposed to risk cues (Evans et al. 2009, Li et al. 2010). The continued presence of workers in such risky habitats led us to suspect that soldiers might play a "keystone role" (Modlmeier et al. 2014) by acting as social buffers whose presence reduces worker sensitivity and susceptibility to stressors.

We assessed how *Reticulitermes flavipes* termite workers were affected by cues from conspecific nestmate, conspecific non-nestmate, and heterospecific (*R. virginicus*) workers, and how the presence of an *R. flavipes*

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soldier altered the response of *R. flavipes* workers. We separated adjacent colonies using a semipermeable barrier that prevented physical contact but allowed cue transmission, allowing us to isolate the impact of cues on *R. flavipes* workers. We also explored how worker responses were affected by a conspecific soldier and by different densities of soldiers and competitors. In addition to their direct role in colony defense, we show that soldiers reduce the impact of competition stress on the relatively vulnerable worker caste.

Materials and Methods

Reticulitermes *colonies*

We used workers and soldiers from 11 field-collected *R. flavipes* colonies (A1–A5, A7–A8, R1–R4) in this study. Only a subset of three colonies was used in each experiment based on the availability of *R. flavipes* colonies in the field by the time the experiment was carried out. Workers from one field-collected *R. virginicus* colony (A9) were used as the heterospecific competitor while workers from two *R. flavipes* colonies (A6 and R5) were used as the conspecific non-nestmate competitor. The distribution of these congeneric species overlaps throughout North America, and each is agonistic towards the other (Polizzi and Forschler 1998). We collected "A"-prefix colonies from the University of Kentucky Arboretum (Lexington, KY), and "R"-prefix from Daniel Boone National Forest (Winchester, KY). We used *R. flavipes* colonies within one week of their collection to minimize the impact of isolation from their original colony; they were maintained in growth chambers (complete darkness at 27 ± 1°C, 80% ± 1% RH) and provisioned with pine wood mulch and fine pine wood logs. We identified termite species by a combination of soldier morphology and 16S mitochondrial ribosomal gene sequencing (Szalanski et al. 2003).

Behavioral survey: R. flavipes *responses to conspecifics and heterospecifics*

We assessed whether the presence of soldiers altered the behavioral responses of *R. flavipes* workers to the non-lethal presence of conspecifics or a heterospecific competitor (*R. virginicus*). Prior to the survey, *R. flavipes* workers from the same colony were individually marked as follows. Workers were transferred into a 55-mm Petri dish containing a moist filter paper disk. As individual workers walked on the disk, the dorsal side of their head, thorax or abdomen was marked with two different colors of permanent marker. To reduce the potential for injury, each body part on a given individual was only marked once. Marked workers were transferred into another Petri dish; workers that sustained injury during marking were discarded.

Survey design.—We added color-coded *R. flavipes* workers to a 35-mm Petri dish ('test') placed at the center of a 55-mm Petri dish ('periphery'; Fig. 1). Before adding workers, we cut 16 evenly-spaced 1-mm slits into the wall of the 35-mm dish that transmitted chemical cues and allowed antennal contacts, but were too narrow for damaging/lethal interactions to occur. The survey began when we added *R. flavipes*, either 20 workers or 19 workers and one

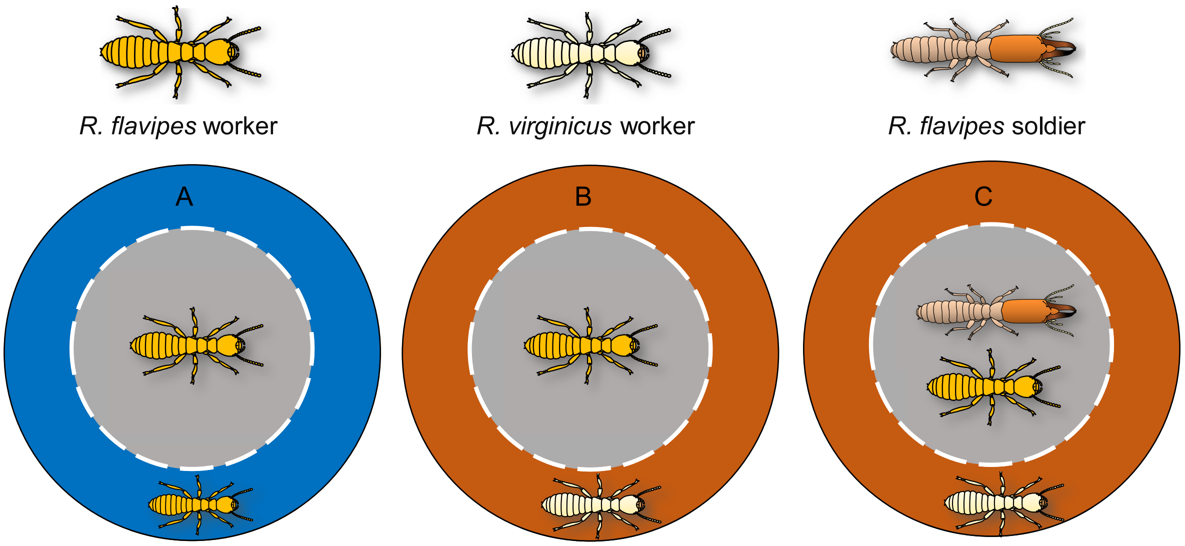

Fig. 1. Schematic drawing of the experimental setup. *Reticulitermes flavipes* workers (in yellow), with/without a soldier, were confined in the inner ring (in grey). Wall of the center dish was cut vertically to make 1 mm-wide silts. Competition risk was perceived by *R. flavipes* workers by antennation through the slits. In control (A), nestmate workers of *R. flavipes* were placed in the outer ring (areas in blue), while for treatments, *R. virginicus* workers or *R. flavipes* workers from other colonies (non-nestmates), the presumed competitors, were placed in the outer ring (areas in orange) without (B) or with (C) the soldier caste. [Colour figure can be viewed at wileyonlinelibrary.com]

R. flavipes soldier, to a test area provisioned with moistened paper disks for the termites to consume. After a 24-h acclimation period, we stocked the periphery with either 40 conspecific *R. flavipes* workers from the same colony or 40 heterospecific *R. virginicus* workers. This created four treatments: *R. flavipes* with conspecific cues without soldiers ("Conspecific") and with soldiers ("Conspecific+S"), with heterospecific cues without soldiers ("Heterospecific") and with soldiers ("Heterospecific+S"). The Conspecific and Conspecific+S treatments tested whether the workers were responding to heterospecific competitors or termite density per se, and whether the effect of soldier presence differed for of conspecific vs. heterospecific cues. We used *R. flavipes* colonies A1, A8, and R1, with one petri-dish replicate per colony for each of the four treatments, for a total of 12 replicates (three colonies × four treatments).

After adding termites into the periphery area, we covered and sealed each 55-mm petri dish to decrease dehydration risk. We then used a Canon VIXIA HF G20 video camera to record the behavior of all *R. flavipes* workers and the soldier in each dish over the next 24 h. All three dishes were held under laboratory conditions (25 ± 1°C, 70% ± 1% RH) and illuminated by a ceiling-mounted fluorescent lamp. While we would have preferred to record termite behavior in total darkness, external lighting was necessary for our video-recording equipment. At the end of the 24-h sampling period, we analyzed the recorded footage using Observer (Noldus, Wageningen, The Netherlands), a behavior analysis program. At the beginning of the survey and every four hours thereafter (i.e., 0, 4, 8, 12, 16, 20, and 24 h), we analyzed a 3-min section of video for the time spent on behaviors by each marked worker and the soldier.

We recorded the following behaviors for each worker and for the soldier as per Korb and Schmidinger (2004): locomotion, resting, feeding, grooming (both itself and another individual), and vibration (rapid back-and-forward bodily movement). We also observed other behaviors (e.g., trophallaxis, defecation, and moving nestmate corpses) that were too infrequent to analyze.

Although we marked 20 termite workers in each petri dish prior to the start of observations, the markings on many workers were partially or totally rubbed off by the end. Because we only analyzed data from workers whose behavior could be tracked throughout the 24-h period, we observed a mean of 8.25 (range: 6–11) workers per replicate. We averaged worker data to calculate the per-replicate frequency of each of the six behavioral categories (summing to 100%). Replicates in the "soldier" treatments used data from the single soldier per replicate as the measurement of soldier behavior.

Experiment I: Short-term impact of soldiers on worker survival in response to cues from heterospecific competitors

Over a 2-d period, we assessed whether the presence of soldiers affected the survival of *R. flavipes* workers exposed to cues produced by two different *R. virginicus* worker densities. As in the behavioral survey, the test area contained either 20 *R. flavipes* workers ('Heterospecific'), or 19 workers and one nest-mate soldier ('Heterospecific+S'); none of the workers were color-coded. In both this experiment and experiment III, the first part of the treatment name, i.e., 'Heterospecific', denotes the type of termite cue that *R. flavipes* workers experienced; the second part of the treatment name, i.e., '+S', indicates the presence of an *R. flavipes* soldier with the workers. Immediately after placing *R. flavipes* in the test area, we placed either 20 (1:1 ratio) or 40 (2:1 ratio) *R. virginicus* workers in the periphery area. We provided termites in both the test and periphery areas with a moistened paper disk for food. The petri dishes were kept in an incubator (27 ± 1°C, 80% ± 1% RH) in complete darkness for two days, then removed and surviving workers counted. We used *R. flavipes* colonies A2, A4, and A5 in order to assess the potential for colony-level differences in termite responses. There were five replicates per colony for each of the four treatments (1:1 Heterospecific, 1:1 Heterospecific+S, 2:1 Heterospecific, 2:1 Heterospecific+S), for a total of 60 replicates (three colonies × four treatments × five replicates).

Experiment II: Short-term impact of soldiers on worker survival in response to cues from nestmate conspecifics and non-nestmate conspecifics

Over a 2-d period, we assessed whether the presence of soldiers affected the survival of *R. flavipes* workers exposed to cues produced by *R. flavipes* nestmates or *R. flavipes* non-nestmates. It was identical in design to experiment I except for the following differences. Immediately after placing *R. flavipes* workers (and, in the appropriate treatments, a single soldier) in the test area, we placed either 20 *R. flavipes* nestmate workers or 20 *R. flavipes* non-nestmate workers in the periphery area. This generated four treatments: nestmates (N), nestmates plus soldier (N+S), non-nestmates (NN), and non-nestmates plus soldier (NN+S). We used *R. flavipes* colonies R3, R4, and A6; workers from colony A7 were used as non-nestmate conspecifics for colonies R3 and R4, and workers from colony R5 were used as non-nestmate conspecifics for colony A6. There were three replicates per colony for each of the four treatments, for a total of 36 replicates (three colonies × four treatments × three replicates).

Experiment III: Long-term impact of soldiers on worker feeding, growth, and survival

Over a 15-d period, we assessed whether soldiers (either one or two individuals) altered the feeding rate, growth rate, and survival of *R. flavipes* workers exposed to *R. virginicus* cues. The test area contained either 20 *R. flavipes* workers ('Heterospecific'), 19 workers and one nest-mate soldier ('Heterospecific+S'), or 18 workers and two nest-mate soldiers (‘Heterospecific+2S’). Immediately after placing *R. flavipes* in the test area, we placed five *R. virginicus* workers in the periphery area. As in experiments I and II, we added another treatment in which five *R. flavipes* nestmate workers (“Conspecific” treatment) were placed in the periphery, and 20 *R. flavipes* workers were placed in the center. No soldier was added in the conspecific treatment. Termites in both areas were provisioned with a moistened paper disk that was replaced every three days. All Petri dishes were kept in an incubator as per experiment I. The 15-d length was chosen to simulate chronic exposure to neighboring colonies (a situation that often occurs between these two species; Polizzi and Forschler 1998).

Experiment III was conducted using individuals from three *R. flavipes* colonies. We initially set up 10 replicates for each colony. During the course of the 15-d assay, however, *R. flavipes* workers in some of the replicates were infected with mites. We decided to remove the replicates infested with mites because infection could potentially alter termite behavior and compromise their fitness. As a result, for colony A8, there were five replicates per treatment for each of the four treatments for a total of 20 replicates. For colony R5, there were seven replicates per treatment (total = 28), and for colony R6, there were nine replicates per treatment (total = 36).

We recorded worker mortality and removed dead workers each day for 15 days. While dead *R. flavipes* workers were not replaced, we did replace dead *R. flavipes* soldiers and dead *R. virginicus* workers to maintain constant conditions. At the start of the experiment and every third day, surviving workers were removed from each replicate, counted, and weighed to determine average worker weight. Percentage change was determined by subtracting the initial weight from the current measurement, dividing by the initial weight, and multiplying by 100.

We provisioned *R. flavipes* workers with a paper disk that had been oven-dried at 100°C for 1 h and weighed before being moistened with 100 mL deionized water and placed in the test area. Every third day, we replaced the partially consumed old disk with a new disk. We brushed the old disk to remove extraneous material, then dried and weighed it; paper consumption was calculated using the initial and final disk dry weights. We calculated paper consumption rate (‘PCR’; mg paper/mg termite/day) for each three-day period as follows: ((paper consumed, mg)/(total worker weight, mg))/3 d.

Statistical analysis

We analyzed the combined dataset on worker behavior using principal component analysis, a standard approach (e.g., Sitvarin et al. 2016). We followed recommended guidelines and retained all components whose eigenvalues exceeded 1.0 (Abdi and Williams 2010). We used two-way ANOVA to test for the main effects of cue type (conspecific, heterospecific), soldier presence, and their interaction on each principal component; colony was also included as a blocking variable. We used a similar procedure to analyze the dataset on soldier behavior.

Because the data in experiment I was not normally distributed, we assessed the individual effects of soldier presence, *virginicus:flavipes* ratio, and their interaction on *R. flavipes* mortality by fitting a generalized linear mixed model with a quasi-binomial error distribution (‘logit’ link function) using the penalized quasi-likelihood (PQL) ‘glmmPQL’ function in the MASS package in R (R Development Core Team 2010). Colony was used as a random effect in the model and χ^2 and *P*-values were obtained by performing a Wald χ^2 test on the model using the “ANOVA” function in the “car” package. The same procedure was also employed for experiment II to test the individual and interactive effects of nestmate status and soldier presence on *R. flavipes* mortality; *R. flavipes* source colony was a random effect.

A linear mixed effects modeling approach was taken to analyze PCR, percentage of weight change, and percentage of mortality data from experiment III. Linear mixed effects models were constructed for each of these response variables and treatment (i.e., Conspecific, Heterospecific, Heterospecific + S, and Heterospecific + 2S) nested within colony as fixed effects and sampling day as a random effect using the ‘lmer’ function as part of the ‘lme4’ package in R (R Development Core Team 2010). Chi-square and *P*-values were obtained for response variables as described for experiments I and II via a Wald χ^2 test.

RESULTS

Termite cues altered worker and soldier behavior

Workers behaved very differently in the presence of conspecifics versus heterospecific cues, and in the presence or absence of a conspecific soldier (Fig. 2, left panel). The first principal component explained 43% of the variation in worker behavior, and reflected differences in worker resting, walking, and vibration (Appendix S1: Table S1). There was a main effect of both cue type ($F_{1,6}$ = 49.6, $P < 0.001$) and soldier presence ($F_{1,6}$ = 6.5, $P = 0.043$). Cues from *R. virginicus* workers increased the amount of time *R. flavipes* workers spent moving and vibrating and decreased the time they spent resting, while the presence of a *R. flavipes* soldier had the opposite effect (Fig. 2, left panel); the interaction, however, was not significant ($P = 0.5$). The second (21%) and third (20%) principal components reflected differences in feeding/walking/other and grooming/vibration/other behaviors, respectively (Appendix S1: Table S1), but neither component was affected by cue type or soldier presence (all $P > 0.2$). Colony identity affected the first principal component ($F_{2,6}$ = 8.4, $P = 0.018$), but not the second or third (both $P > 0.5$).

Although *R. flavipes* soldiers behaved differently than workers, they had similarly strong responses to

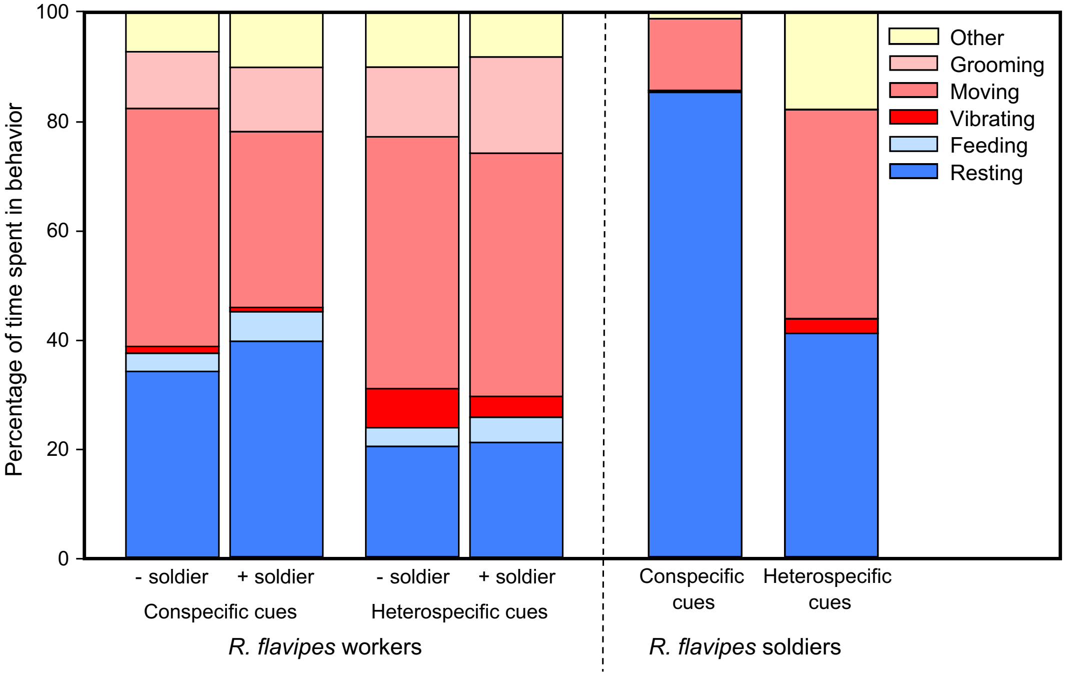

FIG. 2. Behavioral responses of *R. flavipes* workers and soldiers to conspecific or heterospecific cues. Left panel: behavioral responses of *R. flavipes* workers to cues from conspecific workers or heterospecific *R. virginicus* workers in the absence or presence of a single *R. flavipes* nest-mate soldier. Right panel: behavioral responses of *R. flavipes* soldiers to cues from conspecific nest-mate workers or heterospecific *R. virginicus* workers. Red bars: energetically costly activities (grooming, moving, and vibrating); blue bars: energetically beneficial activities (resting and feeding). [Colour figure can be viewed at wileyonlinelibrary.com]

heterospecific cues (Fig. 2, right panel). The first principal component captured 79% of the variation in soldier behavior, and reflected the fact that heterospecific cues decreased soldier resting and increased walking and vibration ($F_{1,2} = 25.6$, $P = 0.037$). Colony identity did not affect this response ($P = 0.56$).

Soldiers decreased the impact of heterospecific competitor cues on worker mortality

The mortality rate of *R. flavipes* workers increased as a function of *R. virginicus* density (Fig. 3A; 1:1 ratio = 10.1 ± 3.48 [SE]; 2:1 ratio = 21.9 ± 3.56; $\chi^2 = 6.96$, df = 1, $P = 0.008$). The presence of a single *R. flavipes* soldier reduced the negative impact of *R. virginicus*, decreasing worker mortality in both density treatments by >80% ($\chi^2 = 19.45$, df = 1, $P < 0.001$). The two-way interaction was not significant ($P > 0.05$).

Soldiers decreased the impact of conspecific non-nestmate cues on worker mortality

Non-nestmate *R. flavipes* workers increased worker mortality more than nestmate workers (Fig. 3B; $\chi^2 = 18.2$, df = 1, $P < 0.001$). There was less mortality in the presence of nestmate workers, regardless of soldier presence. In contrast, cues from non-nestmate workers increased mortality 25-fold relative to nestmate workers. Workers exposed to these non-nestmate cues benefitted greatly from the presence of a soldier; mortality rates were 75% lower in the soldier-present treatment than in the soldier-absent treatment ($\chi^2 = 8.28$, df = 1, $P < 0.004$; Fig. 3B). The two-way interaction was not significant ($P > 0.05$).

A single soldier buffered the chronic impact of competitor cues on workers

In the absence of soldiers, workers exposed to heterospecific cues consumed 32% less paper over the course of the experiment than did workers exposed to conspecific cues (0.075 vs. 0.111 mg/mg worker/day, respectively; Fig. 4A; $\chi^2 = 8.11$, df = 3, $P = 0.044$). While the presence of one *R. flavipes* soldier reduced the negative impact of *R. virginicus* workers, doubling the soldier percentage from ~5% (1/19) to ~11% (2/18) of total *R. flavipes* had no additional impact. Because the Heterospecific+S and Heterospecific+2S treatments had similar effects on all three measured variables, we hereafter focus on the Heterospecific+S treatment.

Despite different feeding rates, there were no treatment-level differences in mean worker weight (Fig. 4B; $\chi^2 = 1.35$, df = 3, $P = 0.718$). The absence of a statistically-significant difference is due to the fact that (A) The rapid death of smaller workers in the Heterospecific treatments left only the largest workers alive; and (B) when all of the workers in a replicate died, we excluded that replicate from our statistical analyses. The impact of including "dead" replicates is seen in Fig. 4B, where the

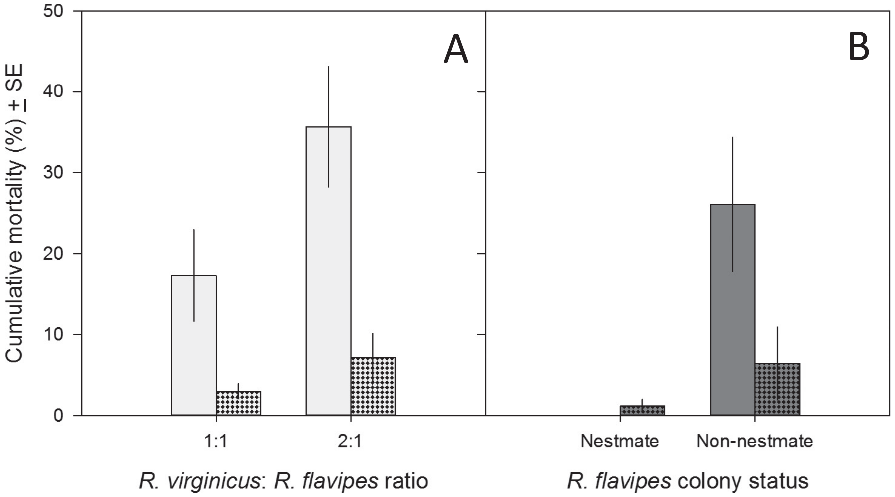

Fig. 3. Competitor-induced mortality in the absence and presence of a soldier. (A) Cumulative mortality of *R. flavipes* workers over 2 d when exposed to cues from *R. virginicus* workers in the absence and presence (checked bars) of a single *R. flavipes* nest-mate soldier. Left pair of bars: 1:1 *virginicus:flavipes* ratio; right pair of bars: 2:1 *virginicus:flavipes* ratio. (B) Cumulative mortality of *R. flavipes* workers over two days when exposed to cues from *R. flavipes* workers in the absence and presence (checked bars) of a single *R. flavipes* nest-mate soldier. Left pair of bars: nestmate *R. flavipes* workers; right pair of bars: non-nestmate *R. flavipes* workers. [Colour figure can be viewed at wileyonlinelibrary.com]

Heterospecific treatment diverged sharply from the two 'S' treatments on day 15. This divergence reflects the fact that in 7/21 replicates in the Heterospecific treatment had 100% *R. flavipes* mortality by day 15. In contrast, none of the 63 replicates in the other treatments had 100% *R. flavipes* mortality.

Worker mortality in the presence of conspecific cues was minimal: 6% over the 15-day experiment (Fig. 4C). While heterospecific cues from even a small number of *R. virginicus* workers (1:4 ratio of *virginicus* to *flavipes*) increased mortality tenfold in the absence of a soldier, the presence of a soldier reduced mortality from 65% (heterospecific cues without soldier) to 33% (heterospecific cues with soldier; $\chi^2 = 51.41$, df = 3, $P < 0.001$). There was no difference in mortality rates between the Heterospecific+S and Heterospecific+2S treatments.

Discussion

Cues from both heterospecific and non-nestmate conspecific competitors were rapidly lethal to *R. flavipes* termite workers, and their impact increased as a function of competitor density. Although other studies have documented lethal effects of chronic predator cue exposure in invertebrates (e.g., Schmitz et al. 1997, McCauley et al. 2011), we found that even 2 d of competitor cue exposure sharply increased worker mortality. Conspecific nestmate soldiers countered this effect, and substantially decreased worker mortality in both two-day experiments (Fig. 3A, B) and the 15-d experiment (Fig. 4). While worker mortality scaled with competitor density, the ameliorating impact of soldiers was unaffected by the worker: soldier ratio: a doubling of soldier densities had no impact (Fig. 4). Our results appear to provide the first evidence that social buffering, the ability of nearby conspecifics to reduce the negative impact of stressors on an individual (Hennessy et al. 2009), also occurs in invertebrates and appears to be associated with caste identity. Given the rarity of soldiers in *R. flavipes* colonies, they seem to play a "keystone role" (Modlmeier et al. 2014) via their amelioration of antagonistic cue effects on the numerically dominant worker caste.

The competitor-induced increase in *R. flavipes* workers' activity and vibratory behavior (Fig. 2, left panel) agrees with research finding that workers from four different *Reticulitermes* species vibrated/oscillated when exposed to threatening situations (Reinhard and Clément 2002), and with other work showing that termite soldiers decrease the magnitude of worker vibration/defensive responses (Roisin et al. 1990, Ishikawa and Miura 2012). The fact that *R. flavipes* mortality scaled with heterospecific density (Fig. 3A; also compare these mortality levels to the third-day numbers in Fig. 4C) showed that workers were responding to both the presence and magnitude of the threat (as per Van Buskirk et al. 2011). Although termite responses to heterospecific chemical, vibrational, and auditory cues have attracted considerable attention (reviewed in Costa-Leonardo and Haifig 2014), we are unaware of other work documenting that the cues themselves can prove lethal.

The most likely explanation for our results appears to be that the combined impact of increased energy expenditure (i.e., behavioral/physiological responses) and decreased energy intake (i.e., reduced feeding) lethally depleted worker resources. This interpretation is consistent with data from our behavioral survey, where workers exposed to conspecific nestmate cues spent approximately equal time in energetically-costly and

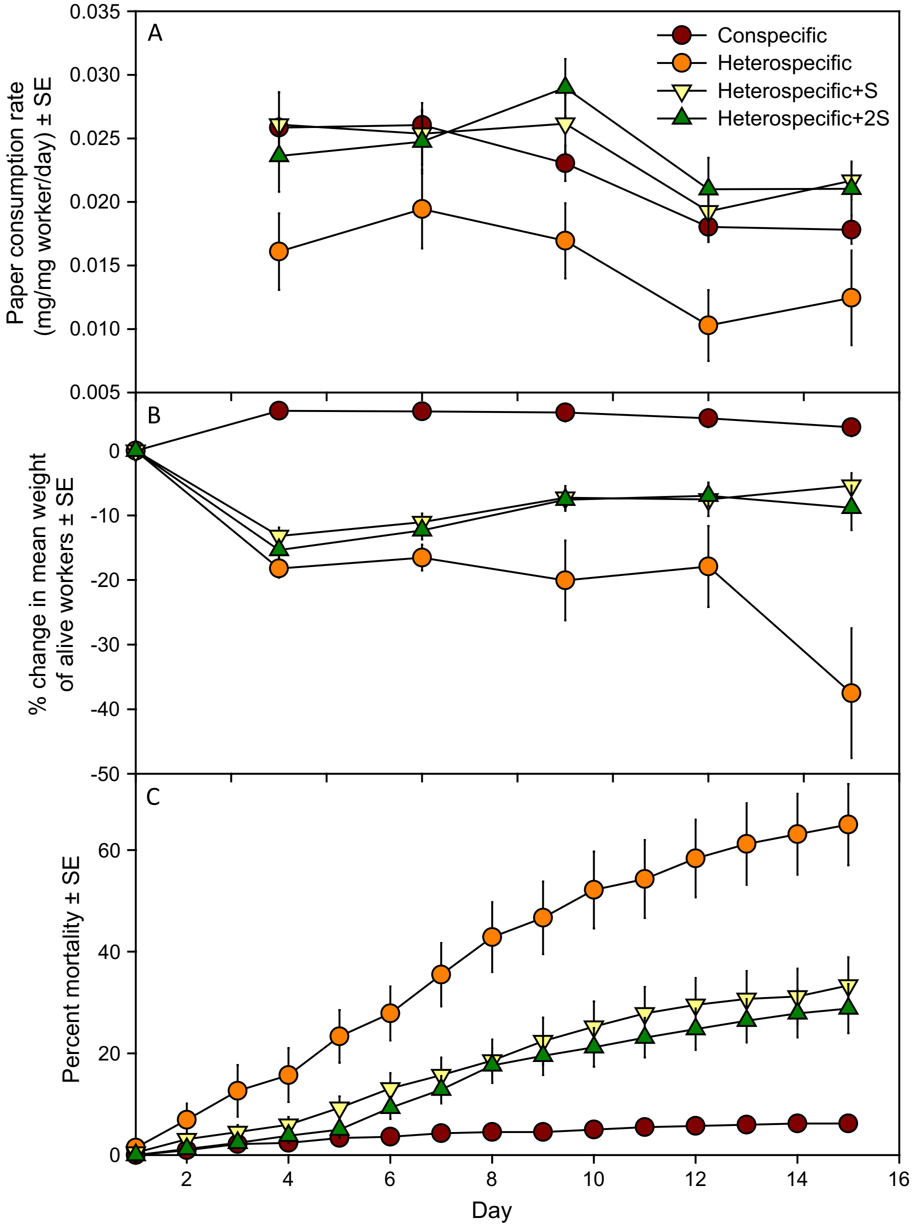

FIG. 4. Impacts of competitor cues on workers. Worker feeding rate (A), mean percent weight change of alive workers relative to day 0 (B), and percent mortality (C) over a 15-d experimental period in the presence of cues from conspecific workers (red circles), *R. virginicus* workers (orange circles), or *R. virginicus* workers and also one (yellow inverted triangles) or two (green triangles) *R. flavipes* nest-mate soldiers. The large drop in percent weight change on day 15 for the Heterospecific treatment is due to the fact that there was 100% mortality in 7/21 replicates; no other treatments had any replicates with 100% mortality. [Colour figure can be viewed at wileyonlinelibrary.com]

–beneficial activities (Fig. 2, left panel; red versus blue cross-sections). Competitor cues increased the ratio of costly to beneficial activities and decreased the fraction of time spent resting and feeding. Similar cessation of feeding has also been documented in the grasshopper *Melanoplus femurrubrum,* where exposure to spider cues increases starvation risk (Schmitz et al. 1997).

The ability of a single termite soldier to buffer the lethal effects of competitor cues suggests a previously unrecognized degree of complexity in caste relationships. While soldiers play a critical role in colony defense, they are only ~2% of the individuals in *R. flavipes* colonies and spend much of their time immobile (Howard and Haverty 1981, Reinhard and Clément 2002). We found that soldiers exposed to conspecific cues spent >80% of their time resting and were never observed grooming (Fig. 2, left panel). In contrast, the numerical dominance of workers makes this caste likely to first encounter threats;

R. flavipes workers are responsible for triggering soldier aggregation and defense (Hu et al. 2003). The importance of worker-derived cues is indicated by the fact that soldiers from several other *Reticulitermes* species respond more strongly to worker alarm cues than to the threat itself, to the point of ignoring the threat when workers are absent (Reinhard and Clément 2002).

While worker behaviors like rapid vibration may be required to quickly alert soldiers to a potential threat, these energetically costly actions should decrease once soldiers have responded. Worker alarm/defensive behavior in the termite *Nasutitermes princeps*, for instance, virtually stops once soldiers arrive at a threat (Roisin et al. 1990, also see Ishikawa and Miura 2012). If the cessation of alarm behavior depends on soldier presence rather than the concentration of their cues, then amelioration of worker responses should be relatively insensitive to soldier density. This hypothesis is consistent with the fact that doubling soldier densities had no additional impact on workers (Fig. 4). In the absence of soldiers, however, the energetic cost of continued alarm behavior may eventually prove lethal to the signaling workers.

In addition to demonstrating a strong impact of competitor cues, our work also offers insight into how caste identity might affect social buffering in eusocial invertebrates. While many solitary animals exhibit a negative correlation between morphological defense and the magnitude of their behavioral responses to risk, the presence of specialized castes may allow this tradeoff to occur at the colony level in eusocial species (Tian and Zhou 2014). While such specialization provides important benefits, a high degree of inter-caste coordination is necessary to ensure rapid and appropriate responses to biotic and abiotic stressors (Bignell et al. 2011). A cost of this interdependence is evident in high worker mortality rates when soldiers are absent, while its benefit (i.e., providing workers 'peace of mind') is found in the reduced impact of cues from competitors. Eusocial organisms span a wide range of taxa and include species that are highly-successful inhabitants of both natural and human-modified environments; it seems likely that social buffering plays a similar role in many of these systems.

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