UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

THE WORLD BELOW: HOW RESOURCES AND HABITAT INFLUENCE INSECT

COMMUNITIES ACROSS SPACE AND THROUGH TIME

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

Degree of

DOCTOR OF PHILOSOPHY

By

KARL A. ROEDER
Norman, Oklahoma
2019

THE WORLD BELOW: HOW RESOURCES AND HABITAT INFLUENCE INSECT

COMMUNITIES ACROSS SPACE AND THROUGH TIME

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF BIOLOGY

BY

Dr. Michael E. Kaspari, Chair

Dr. Jeffrey F. Kelly

Dr. Michael A. Patten

Dr. Eli S. Bridge

Dr. Kirsten M. de Beurs

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

vi

LIST OF TABLES

LIST OF FIGURES

Chapter 4

Abstract

Insects are one of the most abundant, diverse, and ecologically important clades of organisms. They represent up to 5-10 million species, accounting for approximately 46% of the animal biomass on Earth. Yet, despite the importance of insects for terrestrial ecosystems—as consumers, detritivores, herbivores, and predators—little information is known about the natural history of most species and questions remain on how abiotic conditions and biotic interactions between such species structure ecological communities. In this dissertation, I answer four questions using a variety of techniques on ways in which organismal and ecosystem traits influence the spatio-temporal dynamics of insect communities across a variety of scales.

In chapter one, I used elemental and isotopic methods to explore how the invasive red imported fire ant, *Solenopsis invicta*, achieves its status as a trophic generalist. Across 31 *S. invicta* nests, I found that colonies ranged from 1°-consumer to 2°-predator, a range equivalent to whole ant communities in agroecosystems, grasslands, remnant woodlands, and tropical rainforests. I found that a colony's trophic rank was stable throughout multiple sampling events during the year, and that colonies eating at higher trophic levels consumed more C_3-based resources. While colony size was not a good predictor of trophic position, individual worker $\delta^{15}N$ increased with mass. Combined, I show how intraspecific trait variation contributes to the trophic breadth of *S. invicta* and posit additional hypotheses that may further explain why a colony's trophic signature varied across space but remained stable over time.

In chapter two, I focused on how one abiotic factor, temperature, regulated the activity of *S. invicta* and consequently its impact on an urban ant assemblage. I did this by quantifying *S. invicta*'s ability to outcompete native species for resources across ca. 75% of the hours in a summer day, primarily during the hours that were below its thermal maximum of 49°C.

Exclosure experiments revealed that *Dorymyrmex flavus*, a native ant species with similar body size and thermal tolerance to *S. invicta*, was competitively suppressed by the invasive, but likely persisted in this habitat due to dietary differences. While thermal and dietary traits were useful in predicting how species coexist in this invaded assemblage, one key to *S. invicta*'s success is likely its ability to forage in all but six hours of a summer's day.

In chapter three, I resurveyed 33 ant communities, quantifying 20-year differences in the incidence of 28 genera, toward testing the prediction that genera with higher average thermal limits tended to increase at the expense of those with lower limits. In doing so, I found that average temperatures had increased ~1°C during these 20 years and that about 2/3 of the ant genera had increased in more than half of their communities. Consistent with prediction, we found a positive decelerating relationship between critical thermal maxima (CT_{max}: the high temperatures at which ants lose muscle control) and the proportion of sites in which a genus had increased. Critical thermal minima (CT_{min}: the low temperatures at which ants first become inactive), by contrast, was not a useful predictor of change. Thermal functional traits like CT_{max} have thus proved useful in predicting the supposed "winners and losers" of climate change in this dominant and diverse insect taxon.

In chapter four, I move away from ants and instead search for rules by which organismal traits dictate the abundance and distribution of three common litter invertebrates across six North American forests. I used a novel image analysis method to quantify the abundance and body size distributions of mites, springtails, and spiders in 21-m^2 plots per forest. I test three hypotheses finding little support for any relationship between body size or litter depth and abundance, but documenting that abundance regularly constrained species richness ca. 82% of the time, often mediating any effect that body size and litter depth may have had on species richness. My results

suggest that body size and habitat space in brown food webs are rarely good predictors of

abundance, but that diversity is generally well predicted by abundance.

CHAPTER ONE

FROM CRYPTIC HERBIVORE TO PREDATOR: STABLE ISOTOPES REVEAL CONSISTENT VARIABILITY IN TROPHIC LEVELS IN AN ANT POPULATION

Karl A. Roeder[1] and Michael Kaspari[1,2]

[1] Department of Biology, Graduate Program in Ecology and Evolutionary Biology, University of Oklahoma, Norman, OK, 73019, USA

[2] Smithsonian Tropical Research Institute, Balboa, Republic of Panama

Abstract

Populations may collectively exhibit a broad diet because individuals have large diet breadths and/or because subpopulations of specialists co-occur. In social insect populations, the diet of the genetic individual—the colony—may similarly arise because workers are diet generalists or castes of specialists. We used elemental and isotopic methods to explore how the invasive red imported fire ant, *Solenopsis invicta*, achieves its status as a trophic generalist. In one 0.5-ha old field, 31 *S. invicta* colonies ranged from 1°-consumer to 2°-predator (δ^{15}N's 0.35‰ - 7.38‰), a range comparable to that shown in sampled ant communities. Moreover, a colony's trophic rank was stable despite δ^{15}N's fluctuating 2.98‰ over the year. Colonies that fed at higher trophic levels were not larger, but consumed more C_3-based resources. Individual worker mass, however, did increase with δ^{15}N ($r^2 = 0.29$, $p < 0.001$). The 9-fold variation in worker mass within a colony generated trophic variance ca. 15% of the population of colonies. Combined, we show how intraspecific trait variation contributes to the trophic breadth of *S. invicta*, and suggest mechanisms that further explain how their trophic signature varies across space, but remains stable over time.

Keywords: fire ant, invasive species, niche breadth, trait, trophic ecology

Introduction

A key question in ecology remains the origin and maintenance of trait diversity among species, populations, and individuals (Nylin and Gotthard 1998, Bolnick et al. 2011). Increasingly, individual variation is quantified and found to be a significant component of niche variation (Bolnick et al. 2003, Sih et al. 2004, Roeder and Behmer 2014). In behavioral studies, for example, trait diversity can lead to a variety of behavioral types, personalities, or even

2

syndromes (Sih et al. 2004, Pruitt and Ferrari 2011, Jandt et al. 2014). Likewise, individual level niche variation may provide a key mechanism promoting coexistence (Clark et al. 2007). These variable traits play important roles in deciding the outcome of intra- and interspecific interactions that shape population and community dynamics (McGill et al. 2006, Agrawal et al. 2007).

Diet breadth is one common trait used to distinguish between species, as dietary generalists and specialists are frequently found among related species (Lee et al. 2006, Smith et al. 2006). Furthermore, a population may collectively have a broad diet for two reasons: individuals have large diet breadths, or subpopulations vary widely in their diet preferences (Estes et al. 2003, Caut et al. 2008, Tinker et al. 2008). To measure this variance, trophic studies have adopted stable isotope techniques as one powerful tool to track resource assimilation and estimate a population's niche breadth (Bearhop et al. 2004, Newsome et al. 2007). A key challenge is quantifying this variation within populations (Fry 2006, Boecklen et al. 2011) and other traits underlying that variation.

Body size, an important functional trait (Kaspari and Weiser 1999, McGill et al. 2006), can covary with isotope signature within and across species (Kelly 2000). For example, insects that are more enriched in $\delta^{15}N$ and hence higher order consumers are frequently larger (Warren and Lawton 1987) with higher concentrations of nitrogen (Fagan et al. 2002). Under these assumptions, we propose the trophic-size hypothesis which posits that (1) as body size increases so does the requirement for more nitrogen per capita (e.g., to build more muscle mass) and (2) this acquisition of more nitrogen results in an enriched $\delta^{15}N$ signature and subsequent occupation of a higher trophic level.

A second trait of interest is sociality. Social taxa such as ants (Hymenoptera: Formicidae) consist of often-specialized individuals that collectively forage as a "superorganism" for a

variety of potential resources (Wheeler 1911, Hölldobler and Wilson 2009). Moreover, larger colonies forage over larger and more resource diverse areas (Hölldobler and Wilson 1990, Tschinkel et al. 1995). Both worker and colony size thus have the potential to contribute to a social insect population's diet breadth and trophic position.

Polymorphic ants like the red imported fire ant (S. invicta, hereafter RIFA) are a model system to explore worker and colony-level contributions to population variability. RIFA range in dry mass from 0.13 mg to 2.21 mg, and belong to colonies comprised of 10 to over 250,000 workers (Tschinkel 2006). In addition, the monogyne form of RIFA (i.e. single queen) has discrete territorial boundaries, in which the foraging area is not only correlated with colony size but also rarely entered into by workers from other conspecific monogyne colonies (Tschinkel 2006). Here we test the trophic-size hypothesis by exploring the interactions of body mass, nitrogen content, and trophic level across workers within colonies and across colonies at different times of the year. Our ultimate aim was to quantify the trophic breadth of a population of this species across time and space. Our results suggest that whereas larger workers within colonies are enriched in δ^{15}N, colony-level trophic position is independent of colony mass but highly variable among individual colonies—a pattern that remains constant over an annual cycle.

Methods

Study site

All samples were collected in a 0.5-ha field at the University of Oklahoma Biological Station (Oklahoma, USA, 33.88° N, 96.80° W, 204 m elevation) in July and November of 2015 as well as March of 2016. Yearly air temperatures range from -16.1°C to 44.4°C with a mean annual rainfall of 1027.94 mm (Oklahoma Climatological Survey). This location, while

4

undergoing secondary succession after decades of row crop agriculture, is dominated by the

monogyne form of RIFA.

Using stable isotopes to quantify trophic variation in ants

   Carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) stable isotope analyses were used to estimate the

source of dietary carbon and relative trophic position. After collection in the field, each sample

was frozen at -20°C. The petiole, post-petiole, and gaster were removed as they can contain

residual food particles, potentially altering the isotopic signature (Tillberg et al. 2006).

Replicates consisted of five homogenized workers with the same head width, measured in mm,

which was necessary to attain the minimum weight required for analysis. For this reason,

reported mass values are the actual measurements divided by five. Samples were dried to

constant mass at 60°C for 48 hours and weighed in tin capsules to the nearest 0.001 mg on a

Cahn microbalance (Cahn Instruments, Cerritos, CA). 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 ($R_{standard}$). Trophic position, *TP*,

was determined as:

$$TP = \lambda + \frac{(\delta^{15}N_{ant} - \delta^{15}N_{base})}{\Delta N}$$

where λ was equal to the trophic level of the basal food source (e.g. autotroph = 1), $\delta^{15}N_{ant}$

values were directly measured, and $\delta^{15}N_{base}$ was calculated by averaging the $\delta^{15}N$ values from

vegetation in the 0.5-ha field. ΔN represents the standard enrichment per trophic level of 3.4‰

(Kelly 2000, Post 2002). All stable isotope analyses were performed at the University of

California Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer

interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

Between colony isotopic, trophic, and temporal variation

Thirty-one RIFA colonies were sampled to assess how carbon and nitrogen isotope

values were affected by the size of a colony (estimated from mound volume as per Tschinkel

(1993, 2006), Appendix S1). For each colony, two environmental variables that may affect a

colonies' isotopic signature were also measured: (1) average daily temperature and (2) mean

vegetation height (see Appendix S1 for supplemental methods). To evaluate how colony size

affected a colony's elemental and isotopic signature, worker δ-values and C:N ratios from three

size classes—small (0.7 mm head width), medium (1.0 mm head width), and large (1.3 mm head

width)—were averaged and compared to estimates of mound volume with environmental

variables added to ascertain if temperature and vegetation height had an impact. Diet breadth

(Bearhop et al. 2004) was then determined by plotting $\delta^{13}C$ and $\delta^{15}N$ values in isotopic space (δ-

space) and calculating the relative trophic position for each colony using the $\delta^{15}Nbase$ of the

food web. Of the 31 RIFA colonies that were originally sampled, 23 were active the following

November and March, and resampled to ascertain the amount of temporal isotopic and trophic

variation (see Appendix S1 for supplemental methods).

Within colony elemental and isotopic variation

To evaluate how body size affected an individual's elemental and isotopic signature, the scaling exponents (hereafter *b*) of log-log regressions were calculated between an individual's mesosoma dry mass (hereafter body dry mass) and the amount of body carbon or nitrogen. Deviations from isometry were determined by comparing 95% confidence intervals around the calculated slope to the predicted scaling exponent for isometric growth ($b = 1$). If a relationship was allometric, isotope values were regressed against body mass to see if enriched δ-values were associated with increasing body size. The ratio of the amount of body carbon to body nitrogen (hereafter C:N ratio) was also compared to body mass.

Statistical analysis

All analyses were run in R, version 3.2.2. Variables were checked for normality using the Shapiro-Wilks test and log transformed when non-normal. Regressions were tested for heteroscedasticity. Standardized major axis (SMA) regression was used to compare carbon and nitrogen dry mass to body dry mass while polynomial regressions were used to compare C:N ratios and $\delta^{15}N$ values to body dry mass. An information theoretic approach was used to rank all possible intercolonial regression models by Akaike's information criterion (AIC). ΔAIC values for each model were calculated from the difference of the AIC of *i*th model and the model with the lowest AIC value. Akaike weights (w_i) were then calculated and represent a weight of evidence that model *i* was the best fit. For multiple regression models, predictor variables were checked for multicollinearity using a variance inflated factor (VIF) cutoff $= 3$. Ordinary least squares (OLS) regression was used to compare δ-values across colonies. Spatial autocorrelation of $\delta^{13}C$, $\delta^{15}N$, and intercolonial predictor variables was examined using Moran's *I*. Finally,

repeated measures ANOVA was used to determine if $\delta^{13}C$, $\delta^{15}N$, and trophic position changed across time periods per colony.

Results

The 31 colonies in this 0.5-ha old field fed across three trophic levels and assimilated nutrients acquired from C_3 and C_4 plants. During July, colonies varied from 0.35‰ to 7.38‰ in $\delta^{15}N$, from -24.69‰ to -16.69‰ in $\delta^{13}C$, and from 3.95 to 4.38 in their C:N ratio. Moreover, trophic position decreased linearly as colonies incorporated resources that contained C_4 plants in their diet (Fig. 1). We tested a variety of correlated but not collinear predictor variables (VIF: Temperature = 1.54, Vegetation Height = 2.14, Mound Volume = 2.18; Appendix S2: Fig. S1) that might account for this variation in $\delta^{13}C$, $\delta^{15}N$ and C:N ratio. Mean vegetation height was the only predictor found in each model within two AIC of the top model for colony $\delta^{15}N$ and $\delta^{13}C$ (Appendix S2: Table S1).

Over three samples across the year, the isotopic signature of colonies varied by up to 2.98‰ in $\delta^{15}N$ (repeated measures ANOVA: $F_{2,44} = 18.70$, $P < 0.001$; Appendix S2: Fig. S2) and 4.58‰ for $\delta^{13}C$ (repeated measures ANOVA: $F_{2,44} = 9.41$, $P < 0.001$; Appendix S2: Fig. S3). And while colonies isotopically fluctuated, their relative trophic position did not (repeated measures ANOVA: $F_{2,44} = 1.16$, $P = 0.3216$; Fig. 2), primarily due to an enrichment in the $\delta^{15}N_{base}$ (July = -3.05‰, November = -1.88‰, March = -1.80‰). Within a colony, worker dry mass varied 9-fold (range = 0.09 mg to 0.85 mg) with larger workers being elementally enriched in nitrogen and more isotopically enriched in $\delta^{15}N$ (Appendix S2: Fig. S4).

Discussion

Here we confirm that a population of the red imported fire ant is, collectively, a trophic generalist. We show that the population achieves this by supporting a range of trophic specialists: colonies varied 3-fold in trophic level, a ranking that persisted in the summer, winter and spring of a seasonal cycle. The trophic-size hypothesis was not as useful at predicting between colony differences: predators in this population did not represent the upper end of a 100-fold gradient in colony size. It was, however, useful in accounting for trophic variation across the 9-fold variation in body mass within colonies; a colony's workers spanned, on average only 15% of the δ^{15}N found among colonies in this old field. Combined, we show an unprecedented, consistent variation in trophic level among colonies as a chief cause for a commonly observed trait of a widespread, invasive species—its generalized diet.

Mechanisms underlying colony-level differences in trophic biology

We originally posited that because larger colonies maintain larger territories, and thus have access to a greater diversity of resources, a colony's size may be a good predictor of its isotopic signature. Yet despite a 100-fold difference within our population, colony size was not a good predictor. Moreover, the environmental variables of average daily temperature and mean vegetation height accounted for only a small amount of the isotopic variation across colonies. We propose two alternative, but not mutually exclusive, hypotheses to account for this variation across the 31 sampled RIFA colonies in this 0.5ha old field.

The first states that colonies with low trophic positions feed on plant-derived honeydew. Many ants tend fluid feeding hemipterans (Tillberg et al. 2007, Wilder et al. 2011). Honeydew, the by-product of these hemipterans, is rich in carbohydrates and only slightly isotopically

enriched in $\delta^{15}N$ compared to the plant from which it was produced (Sagers and Goggin 2007).

For this reason, hemipteran-tending ants have a lower $\delta^{15}N$ value and relative trophic position as they are essentially herbivores (Davidson et al. 2003). We predict that colonies near the trophic level expected for a fluid feeding hemipteran ($TP = 2$) incorporate a large amount of honeydew in their diet.

The second states that colonial $\delta^{15}N$ tracks plant chemistry. Different types of vegetation vary in their elemental composition and nutritional value (Marschner 1995, Aerts and Chapin 2000). For example, forbs, which are mainly C_3 plants, have a higher nutritional value and lower C:N ratio than grasses, which are generally C_4 plants (Joern et al. 2012). As we observed a decreasing linear relationship between a plant's $\delta^{15}N$ and C:N ratio (Appendix S2: Fig. S5) perhaps differences in the elemental profiles of plants may account for a proportion of the variation in colonial $\delta^{15}N$ values, even though colony $\delta^{15}N$ values were not spatially autocorrelated (Appendix S2: Table S2). Furthermore, temporal shifts in the isotopic signature of baseline material may explain a paralleled increase in the $\delta^{15}N$ of ants resulting in a constant relative trophic position for colonies through time.

Within colony variation due to polymorphism

We have found only two studies (Smith et al. 2008, Smith and Suarez 2010), both using the Florida harvester ant, *Pogonomyrmex badius*, that tested how worker body size is related to its isotopic signature: both found larger workers had lower C:N ratios and were $\delta^{15}N$ enriched. In RIFA, worker size is determined during the 3rd larval instar where individuals that reach a critical size are physiologically reprogrammed to extend their development by two to four days (Tschinkel 2006). As only late instar larvae can digest solid food particles, this extended

development time allows for the assimilation of more nitrogen from solid dietary items such as arthropod tissue (Petralia and Vinson 1978). However, while worker polymorphism accounted for worker variance in trophic level, it was small relative to inter-colony variation.

Conclusions

Many studies have quantified the average isotope values of communities of ant species. Here we reveal that much of a community's variance may be harbored by a generalist species that is in fact a collection of trophic specialists. When we reviewed 35 studies that incorporated ants and stable isotopes, 74% focused on across species comparisons (details in Appendix S1 and Appendix S2: Table S3). Our results, a 7.03‰ difference in $\delta^{15}N$ across RIFA colonies, was broader than 58% of these studies that used more than one ant species (Appendix S2: Fig. S6) and was comparable to ranges of values for entire ant communities from agroecosystems (Ottonetti et al. 2008, Platner et al. 2012), grasslands (Ness et al. 2009, O'Grady et al. 2010), pastures and remnant woodlands (Gibb and Cunningham 2010), and tropical rainforests (Blüthgen et al. 2003, Kaspari et al. 2012). By quantifying within-population trait variation at multiple spatial and temporal scales we reveal a hidden biodiversity akin to that associated with cryptic species complexes (Smith et al. 2006). In addition, dietary specialization among conspecifics may allow for increased abundance due to decreased intraspecific competition for resources, as seen in sea otters (Estes et al. 2003). It is hard not to speculate that this flexibility in diet breadth may have aided RIFA in becoming one of the most widespread invasive species throughout the southeastern United States.

Acknowledgments

Literature Cited

Aerts, R., and F. S. Chapin. 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Advances in Ecological Research 30:1–67.

Agrawal, A. A., D. D. Ackerly, F. Adler, A. E. Arnold, C. Caceres, D. F. Doak, E. Post, P. J. Hudson, J. Maron, K. A. Mooney, M. Power, D. Schemske, J. Stachowicz, S. Strauss, M. G. Turner, and E. Warner. 2007. Filling key gaps in population and community ecology. Frontiers in Ecology and the Environment 5:145–152.

Bearhop, S., C. E. Adams, S. Waldron, R. A. Fuller, and H. Macleod. 2004. Determining trophic niche width: a novel approach using stable isotope analysis. Journal of Animal Ecology 73:1007–1012.

Blüthgen, N., G. Gebauer, and K. Fiedler. 2003. Disentangling a rainforest food web using stable isotopes: dietary diversity in a species-rich ant community. Oecologia, 137:426–435.

Boecklen, W. J., C. T. Yarnes, B. A. Cook, and A. C. James. 2011. On the use of stable isotopes in trophic ecology. Annual Review of Ecology, Evolution and Systematics 42:411–440.

Bolnick, D. I., R. Svanbäck, J. A. Fordyce, L. H. Yang, J. M. Davis, C. D. Hulsey, and M. L. Forister. 2003. The ecology of individuals: Incidence and implications of individual specialization. The American Naturalist 161:1-28.

Bolnick, D. I., P. Amarasekare, M. S. Araujo, R. Bürger, J. M. Levine, M. Novak, V. H. W. Rudolf, S. J. Schreiber, M. C. Urban, and D. A. Vasseur. 2011. Why intraspecific trait variation matters in community ecology. Trends in Ecology and Evolution 26:183-192.

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

Clark, J. S., M. Dietze, S. Charkraborty, P. K. Agarwal, I. Ibanez, S. LaDeau, and M. Wolosin. 2007. Resolving the biodiversity paradox. Ecology Letters 10:647–662.

Davidson, D. W., S. C. Cook, R. R. Snelling, and T. H. Chua. 2003. Explaining the abundance of ants in lowland tropical rainforest canopies. Science 300:969–972.

Estes, J. A., M. L. Riedman, M. M. Staedler, M. T. Tinker, and B. E. Lyon. 2003. Individual variation in prey selection by sea otters: patterns, causes, and implications. Journal of Animal Ecology 72:144-155.

Fagan, W. F., E. Siemann, C. Mitter, R. F. Denno, A. F. Huberty, H. A. Woods, and J. J. Elser. 2002. Nitrogen in insects: implications for trophic complexity and species diversification. The American Naturalist 160:784–802.

Fry, B. 2006. Stable isotope ecology. Springer-Verlag, New York, USA.

Gibb, H., and S. A. Cunningham. 2010. Habitat contrasts reveal a shift in the trophic position of ant assemblages. Journal of Animal Ecology 80:119–127.

Hölldobler, B., and E. O. Wilson. 1990. The ants. Springer-Verlag, Berlin, Germany.

Hölldobler, B., and E. O. Wilson. 2009. The superorganism: the beauty, elegance, and

      strangeness of insect societies. W.W. Norton and Company, New York, USA.

Jandt, J. M., S. Bengston, N. Pinter-Wollman, J. N. Pruitt, N. E. Raine, A. Dornhaus, and A. Sih.

      2014. Behavioral syndromes and social insects: personality at multiple levels. Biological

      Reviews 89:48–67.

Joern, A., T. Provin, and S. T. Behmer. 2012. Not just the usual suspects: insect herbivore

      populations and communities are associated with multiple plant nutrients. Ecology

      93:1002–1015.

Kaspari, M., and M. D. Weiser. 1999. The size-grain hypothesis and interspecific scaling in ants.

      Functional Ecology 13:530–538.

Kaspari, M., D. Donoso, J. A. Lucas, T. Zumbusch, and A. D. Kay. 2012. Using nutritional

      ecology to predict community structure: a field test in Neotropical ants. Ecosphere 3:93.

Kelly, J. F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian

      trophic ecology. Canadian Journal of Zoology 78:1–27.

Lee. K. P., S. T. Behmer, and S. J. Simpson. 2006. Nutrient regulation in relation to diet breadth:

      a comparison of *Heliothis* sister species and a hybrid. Journal of Experimental Biology

      209:2076–2084.

Marschner, H. 1995. Mineral nutrition of higher plants. Academic Press, San Diego, USA.

McGill, B. J., B. J. Enquist, E. Weiher, and M. Westoby. 2006. Rebuilding community ecology

      from functional traits. Trends in Ecology and Evolution 21:178–185.

Ness, J. H., W. F. Morris, and J. L. Bronstein. 2009. For ant-protected plants, the best defender is

      a hungry offense. Ecology 90:2823–2831.

Newsome, S. D., C. Martinez del Rio, S. Bearhop, and D. L. Phillips. 2007. A niche for isotopic ecology. Frontiers in Ecology and the Environment 5:429–436.

Nylin, S., and K. Gotthard. 1998. Plasticity in life-history traits. Annual review of Entomology 43:63-83.

O'Grady, A., O. Schmidt, and J. Breen. 2010. Trophic relationships of grassland ants based on stable isotopes. Pedobiologia 53:221–225.

Ottonetti, L., L. Tucci, G. Chelazzi, and G. Santini. 2008. Stable isotopes analysis to assess the trophic role of ants in a Mediterranean agroecosystem. Agricultural and Forest Entomology 10:29–36.

Petralia, R. S., and S. B. Vinson. 1978. Feeding in the larvae of the imported fire ant, *Solenopsis invicta*: behavior and morphological adaptations. Annals of the Entomological Society of America 71:643–648.

Platner, C., J. Pinol, D. Sanders, and X. Espadaler. 2012. Trophic diversity in a Mediterranean food web–Stable isotope analysis of an ant community of an organic citrus grove. Basic and Applied Ecology 13:587–596.

Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703–718.

Pruitt J. N., and M. C. O. Ferrari. 2011. Intraspecific trait variants determine the nature of interspecific interactions in a habitat-forming species. Ecology 92:1902–1908.

Roeder, K. A., and S. T. Behmer. 2014. Lifetime consequences of food protein-carbohydrate content for an insect herbivore. Functional Ecology 28:1135–1143.

Sagers, C. L., and F. L. Goggin. 2007. Isotopic enrichment in a phloem-feeding insect: influences of nutrient and water availability. Oecologia 151:464–472.

Sih, A., A. Bell, and J. C. Johnson. 2004. Behavioral syndromes: an ecological and evolutionary overview. Trends in Ecology and Evolution 19:372–378.

Smith, C. R., and A. V. Suarez. 2010. The trophic ecology of castes in harvester ant colonies. Functional Ecology 24:122–130.

Smith, C. R., K. E. Anderson, C. V. Tillberg, J. Gadau, and A. V. Suarez. 2008. Caste determination in a polymorphic social insect: Nutritional, social, and genetic factors. The American Naturalist 172:497–507.

Smith, M. A., N. E. Woodley, D. H. Janzen, W. Hallwachs, and P. D. N. Hebert. 2006. DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). Proceedings of the National Academy of Sciences of the United States of America 103:3657–3662.

Tillberg, C. V., D. P. McCarthy, A. G. Dolezal, and A. V. Suarez. 2006. Measuring the trophic ecology of ants using stable isotopes. Insectes Sociaux 53:65–69.

Tillberg, C. V, D. A. Holway, E. G. Lebrun, and A. V. Suarez. 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.

Tinker, M. T., G. Bentall, and J. A. Estes. 2008. Food limitation leads to behavioral diversification and dietary specialization in sea otters. Proceedings of the National Academy of Sciences of the United States of America 105:560–565.

Tschinkel, W. R. 1993. Sociometry and sociogenesis of colonies of the fire ant *Solenopsis invicta* during one annual cycle. Ecological Monographs 63:425–457.

Tschinkel, W. R. 2006. The fire ants. The Belknap Press of Harvard University, Massachusetts, USA.

Tschinkel, W. R., E. S. Adams, and T. Macom. 1995. Territory area and colony size in the fire ant *Solenopsis invicta*. Journal of Animal Ecology 64:473–480.

Warren, P. H. and J. H. Lawton. 1987. Invertebrate predator-prey body size relationships: an explanation for upper triangular food webs and patterns in food web structure? Oecologia 74:231–235.

Wheeler, W. M. 1911. The ant-colony as an organism. Journal of Morphology 22:307–325.

Wilder, S. M., D. A. Holway, A. V. Suarez, E. G. LeBrun, and M. D. Eubanks. 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.

Figure Legends

Figure 1. Inter- and intracolonial variation of RIFA colonies in δ-space. The large panel shows across colony variation with an r^2 and p-value corresponding to the linear relationship between colonial $\delta^{15}N$ and $\delta^{13}C$ (y = - 0.67x - 10.83). The inset bar plot represents the amount of variation across 90 polymorphic workers within a single colony, denoted by the red point, and scaled to the y-axis of the larger panel.

Figure 2. Temporal variation in the trophic position of RIFA colonies. The large panel shows changes in trophic position of each colony, connected by — lines, over three time periods. Small panels depict correlations of colonial trophic position between each combination of time periods with inset linear equations and r-values.

Figure 1.

$r^2 = 0.78$, $p < 0.001$
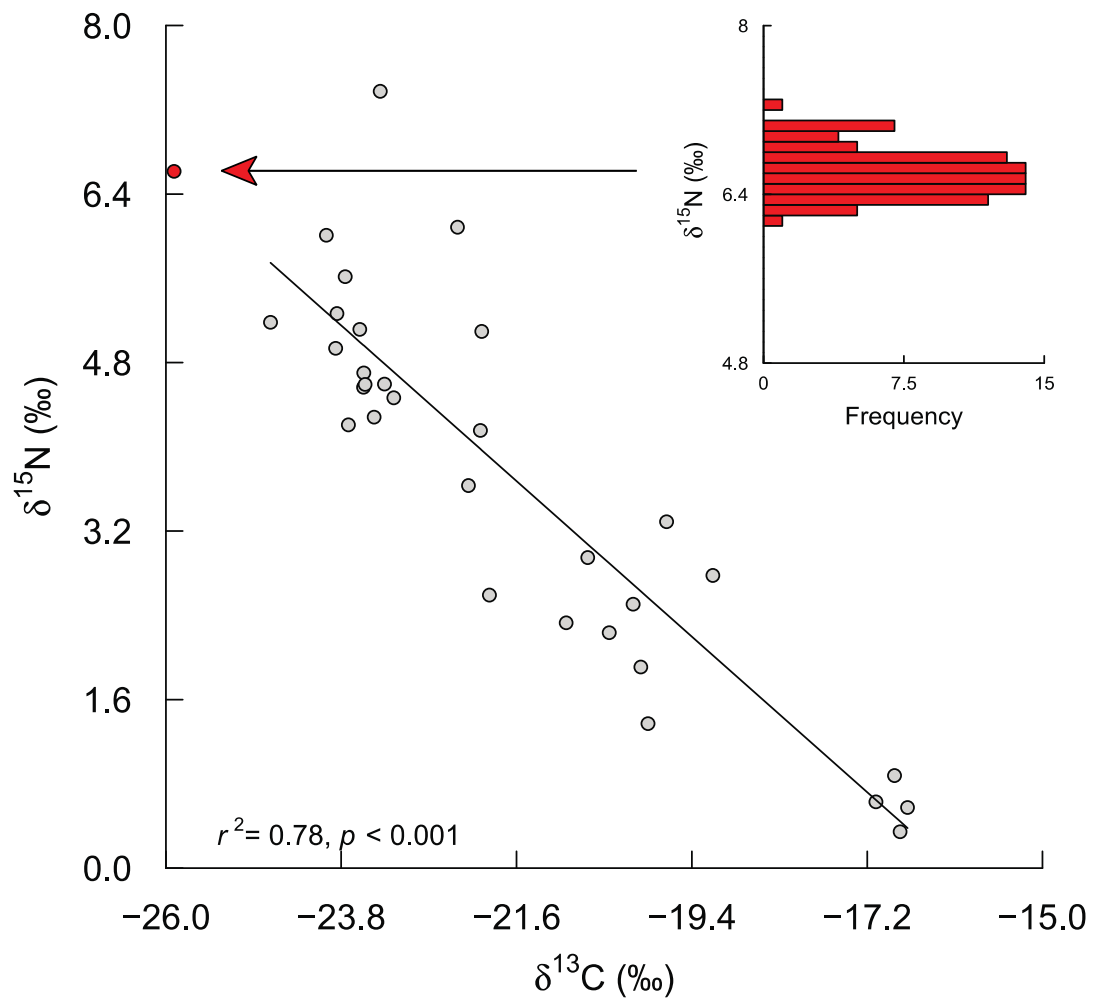
Figure 2.
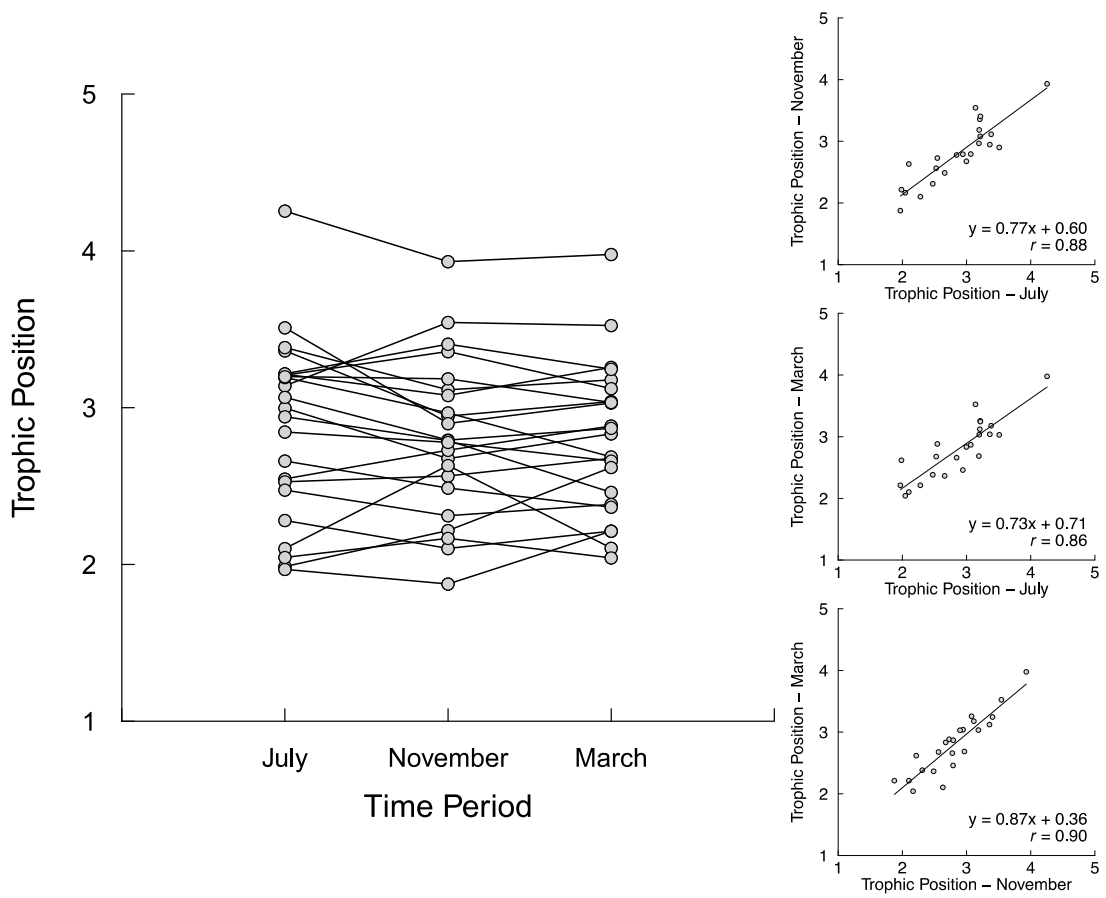
Chapter 1: Appendix S1. Supplemental Methods.

Mound volume calculation

RIFA mound volume, *V*, was estimated in liters using the equation for a prolate spheroid:

$$V = \left(\frac{4}{3} * \pi \right) * \left(\frac{a}{2} \right) * \left(\frac{b}{2} \right) * c$$

where *a*, *b*, and *c* represent the length, width, and height of the dimensional axes of the area

occupied by a colony's nest. This value is highly correlated with a colony's biomass, and can

accordingly be used as a proxy for colony size (Tschinkel 1993, Tschinkel 2006).

Measuring environmental variable

For each colony, two environmental variables were measured: (1) average daily

temperature and (2) mean vegetation height. Temperature, which has been shown to affect

isotopic fractionation (Barnes et al. 2007), was recorded at the top of every mound with HOBO

temperature data loggers (Onset Computer Corporation, Bourne, MA) for three consecutive days

during the sampling period. Vegetation height, which represents a plant's ability to compete for

light (Falster and Westoby 2003), was measured at the cardinal points on the edge of each

mound, averaged per colony, and reported in centimeters.

Measuring and calculating the base of the food web

$\delta^{15}N_{base}$ was determined by collecting plant species across five equidistant points along a

North-South transect through the center of the field. This was done for each time period to

calculate the relative trophic position for each colony.

Temporal Sampling

Of the 31 RIFA colonies that were originally sampled, 23 were active the following November and March. From these colonies, ants that had a head width size of 0.7 mm were analyzed for $\delta^{13}C$ and $\delta^{15}N$. These collection events were spaced four months apart to represent a length of time that is longer than the average life span for RIFA workers of this body size (Tschinkel 2006).

Literature review of ant stable isotope studies

We conducted a literature review using the key words "ant" and "stable isotope". From this we found 35 studies that focused on either a community or population. Here we define a community as any study that included more than one ant species. The majority of these studies (26 out of 35) focused on interspecific instead of intraspecific isotopic differences (Appendix S2: Table S3). To compare the ranges of reported $\delta^{15}N$ values from these studies to the range of $\delta^{15}N$ we measured in our RIFA population, we extracted data from the text or used ImageJ (https://imagej.nih.gov/ij/) to estimate values. For some studies, multiple sites were used and as such we used the highest reported range for our comparison. When only mean and SD or SE were provided, we estimated the range of $\delta^{15}N$ for ants using 2 standard deviations. We then plotted the ranges of $\delta^{15}N$ from community studies as a histogram (Appendix S2: Fig. S6) and compared this to the amount of variation we observed within a colony and across colonies of our population of RIFA.

Literature Cited

Barnes, C., C. J. Sweeting, S. Jennings, J. T. Barry, and N. V. C. Polunin. 2007. Effect of temperature and ration size on carbon and nitrogen stable isotope trophic fractionation. Functional Ecology 21:356–362.

Falster, D.S., and M. Westoby. 2003. Plant height and evolutionary games. Trends in Ecology & Evolution 18:337–343.

Tschinkel, W. R. 1993. Sociometry and sociogenesis of colonies of the fire ant *Solenopsis invicta* during one annual cycle. Ecological Monographs 63:425–457.

Tschinkel, W.R. 2006. The fire ants. The Belknap Press of Harvard University, Cambridge, Massachusetts, USA.

Chapter 1: Appendix S2. Supplemental Data.

Results of multiple regression models and spatial autocorrelation

When Akaike weights were summed by predictor across all models, mean vegetation height was also almost twice as high as the other predictor variables for colony $\delta^{13}C$ (Summed w_i: Vegetation = 0.713, Temperature = 0.364, Volume = 0.315) and $\delta^{15}N$ (Summed w_i: Vegetation = 0.764, Temperature = 0.386, Volume = 0.331). None of the predictors explained a significant proportion of colony C:N ratio. Spatially, colonial $\delta^{13}C$ but not $\delta^{15}N$ values were clustered (Table S2), due to the distinct isotopic signatures of the two plant types (C$_3$ plant: n = 13, $\bar{x}$ = -29.677, SE = 0.39; C$_4$ plant: n = 2, $\bar{x}$ = -12.331, SE = 0.02).

Within colony variation

Carbon and nitrogen scaled with worker mass but in different ways (Fig. S4a). The dry mass of carbon did not diverge from isometry (95% CI: b = 0.998 – 1.011). In contrast, the scaling of nitrogen to worker mass yielded an exponent >1 (95% CI b = 1.030 – 1.056). Larger workers of RIFA had lower C:N ratios, that is, were enriched in elemental nitrogen (Fig. S4b). $\delta^{15}N$ scaled as a positive accelerating relationship and larger workers were more isotopically enriched (Fig. S4c).

Table S1. AIC scores and weights of multiple linear regression models of average temperature (°C), mean vegetation height (cm), and mound volume (L) on $\delta^{15}N$ and $\delta^{13}C$ values of RIFA colonies.

	$\delta^{15}N$				$\delta^{13}C$		
Model	AIC	ΔAIC	w_i	Model	AIC	ΔAIC	w_i
Vegetation	122.73	0	0.37	Vegetation	141.72	0	0.36
Temperature + Vegetation	124.13	1.40	0.18	Temperature + Vegetation	143.41	1.69	0.15
Vegetation + Volume	124.70	1.97	0.14	Vegetation + Volume	143.66	1.94	0.14
Temperature	125.77	3.04	0.08	Temperature	144.25	2.53	0.10
Volume	126.00	3.27	0.07	Volume	144.98	3.26	0.07
Temperature + Vegetation + Volume	126.13	3.40	0.07	Null	145.01	3.29	0.07
Temperature + Volume	126.67	3.94	0.05	Temperature + Vegetation + Volume	145.23	3.51	0.06
Null	127.77	5.04	0.03	Temperature + Volume	145.85	4.13	0.05

Table S2. Similarity as a function of spatial distance between RIFA colonies for δ-values and environmental variables. Moran's *I* signifies either spatial clustering (positive) or over dispersion (negative). Bold * indicates a significant departure from random ($p < 0.05$).

Variable	Moran's *I*	SD	*p*-value
$\delta^{13}C$	**0.144**	**0.062**	**0.004***
$\delta^{15}N$	0.071	0.062	0.092
Average Temperature	0.067	0.062	0.107
Mean Vegetation Height	-0.011	0.061	0.714
Mound Volume	0.040	0.059	0.214

Table S3. Estimated $\delta^{15}N$ ranges from thirty-five published studies that measured ant communities or populations across regions and habitats. For some studies, multiple sites were used and as such we used the highest reported range for our comparison. Note: * indicates estimate from 2 standard deviations.

Citation	Region	Habitat	Number of Species	Estimated $\delta^{15}N$ Range
Davidson et al. 2003	Peru	Lowland tropical rainforest	112	9.50
Bihn et al. 2010	Brazil	Secondary tropical forest	99	8.86
Pfeiffer et al. 2013	Borneo	Primary lowland forest	93	10.15
Blüthgen, Gebauer and Fiedler 2003	Australia	Rainforest	50	7.10
Fiedler et al. 2007	Germany	Mixed across geography	43	10.57
Kaspari et al. 2012	Panama	Lowland tropical rainforest	40	5.20
Tillberg et al. 2007	Argentina	Riparian woodlands/pastures	36	12.08
Gibb and Cunningham 2010	Australia	Pasture and remnant woodland	26	9.90
Jacquemin et al. 2014	Ecuador	Evergreen premontane forest	20	10.16
Tillberg et al. 2006	Argentina	Riparian forest/wetlands	19	6.00
Platner et al. 2012	Spain	Citrus plantation	17	10.67
LeBrun et al. 2007	Argentina	Savanna, subtropical forest and wetlands	15	7.95
Clay et al. 2014	Peru	Lowland tropical rainforest	12	3.34
Ness et al. 2009	Arizona	Sonoran Desert and grassland	8	6.50
O'Grady et al. 2010	Ireland	Limestone grassland	8	2.59
Bennett and Hobson 2009	Saskatchewan	Boreal forest	7	6.00*
Penick et al. 2015	New York	City park	6	4.80

Ottonetti et al. 2008	Italy	Olive orchard	5	4.40
Sanders and Platner 2007	Germany	Dry grassland and meadow	5	2.30
Tillberg 2004	Costa Rica	Lowland tropical wet forest	4	4.42
Palmer 2003	Kenya	Wooded grassland	4	3.45
McNatty et al. 2009	Tokelau	Corral rubble island	3	4.61
Trimble and Sagers 2004	Costa Rica	Cercropia on roadside	2	7.96
Menke et al. 2010	California	chaparral and coastal sage	2	2.90
Vasconcelos and Davidson 2000	Brazil	Upland Amazonia rainforest	2	2.60
Schuch et al. 2008	Germany	Dry grassland	2	1.20
Mooney and Tillberg 2005	Colorado	Ponderosa pine forest	1	7.66*
Smith and Suarez 2010	Florida	Sparse longleaf pine	1	7.02*
Feldhaar et al. 2010	Lab	Lab	1	5.35
Sagers et al. 2000	Trinidad	Cercropia on roadside	1	5.30*
Resasco et al. 2012	South Carolina	Pine plantation/longleaf pine savanna	1	5.16
Smith et al. 2010	Florida	Sparse longleaf pine	1	4.47
McGlynn et al. 2015	Costa Rica	arboretum	1	2.40*
Tillberg and Breed 2004	Costa Rica	Lowland tropical rainforest	1	2.38
Lach et al. 2010	Mauritius	Coralline limestone islet	1	0.66*

Supplemental Figure Legends

Figure S1. Correlative relationships between environmental variables. Panel (a) shows the relationship between mound volume and mean vegetation height. Panel (b) shows the relationship between mound volume and average temperature. Panel (c) shows the relationship between mean vegetation height and average temperature. All panels contain inset r-values.

Figure S2. Temporal variation in $\delta^{15}N$ values of RIFA colonies. The large panel shows changes in $\delta^{15}N$ of each colony, connected by — lines, over three time periods. Small panels depict correlations of colonial $\delta^{15}N$ between each combination of time periods with inset linear equations and r-values.

Figure S3. Temporal variation in $\delta^{13}C$ values of RIFA colonies. The large panel shows changes in $\delta^{13}C$ of each colony, connected by — lines, over three time periods. Small panels depict correlations of colonial $\delta^{13}C$ between each combination of time periods with inset linear equations and r-values.

Figure S4. Intracolonial variation in the elemental composition and $\delta^{15}N$ of RIFA workers. Panel (a) shows the scaling relationship of body dry mass and the amount of carbon ($y = 1.01x - 0.31$) or nitrogen ($y = 1.04x - 0.90$) within an ant. Panel (b) shows the ratio of the amount of carbon to nitrogen regressed against body dry mass ($y = -1.48x^2 - 1.87x + 3.64$). Panel (c) shows the $\delta^{15}N$ values of ants regressed against body dry mass ($y = 0.92x^2 - 1.38x + 7.01$). All relationships were significant and contain inset r^2 and p-values.

Figure S5. Vegetation δ-values and their carbon to nitrogen ratios. Panel (a) shows the relationship between $\delta^{15}N$ and C:N ratio (y = - 0.20x + 2.35). Panel (b) shows the relationship between $\delta^{13}C$ and C:N ratio. Both panels contain inset r^2 and *p*-values.

Figure S6. Reported and estimated ranges of $\delta^{15}N$ from 26 ant community stable isotope studies. Dashed (---) lines represent either the intra- or intercolonial range of $\delta^{15}N$ that we observed in our population of RIFA.

Figure S1.
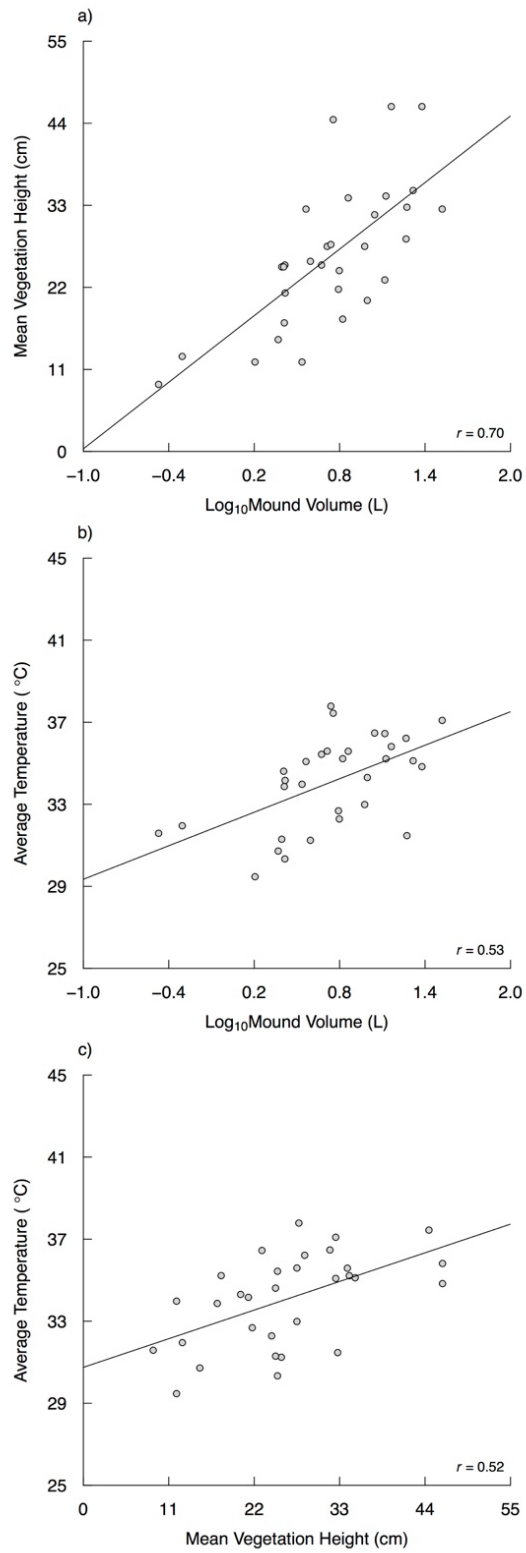
Figure S2.
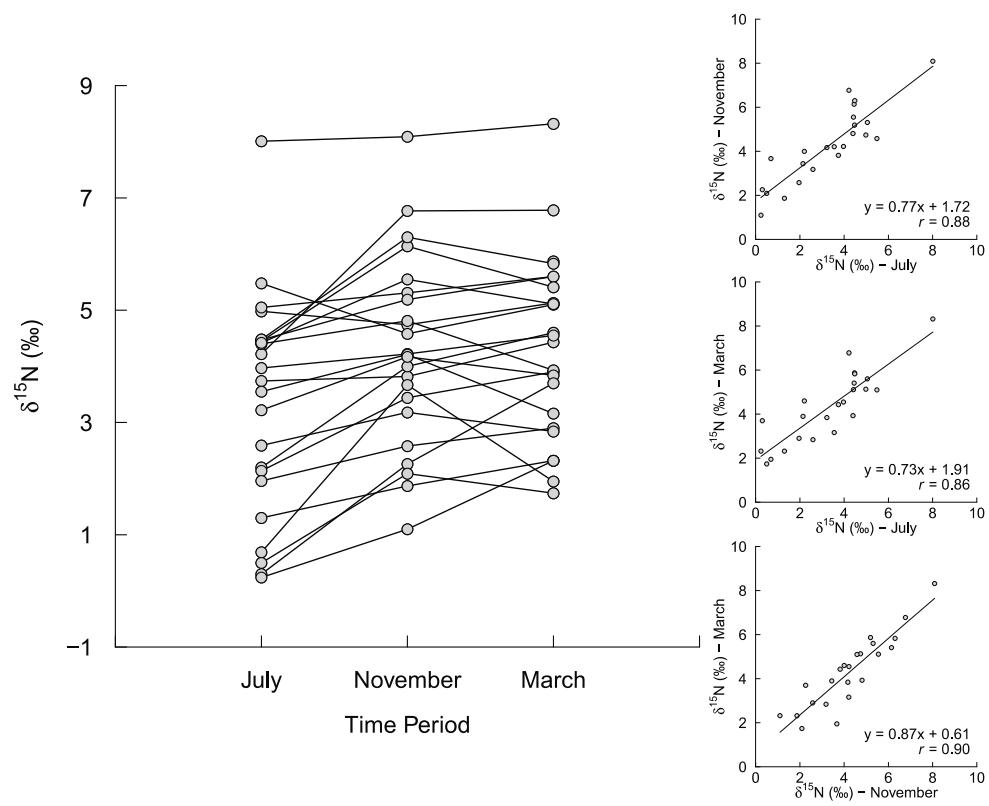
Figure S3.
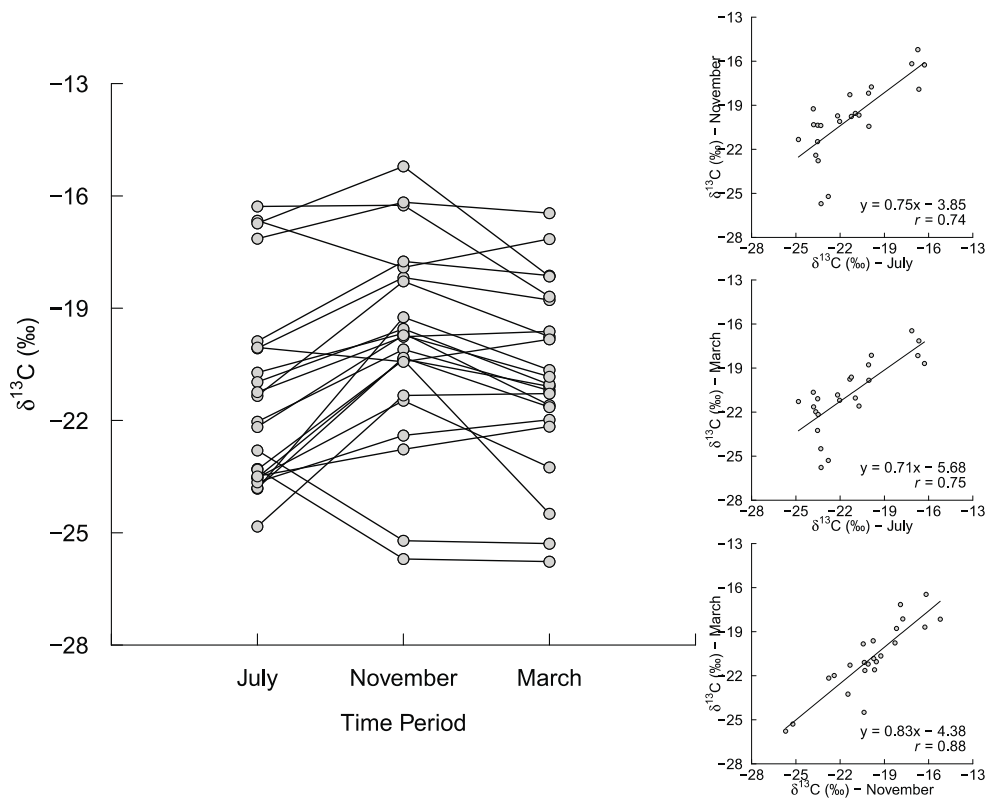
Figure S4.
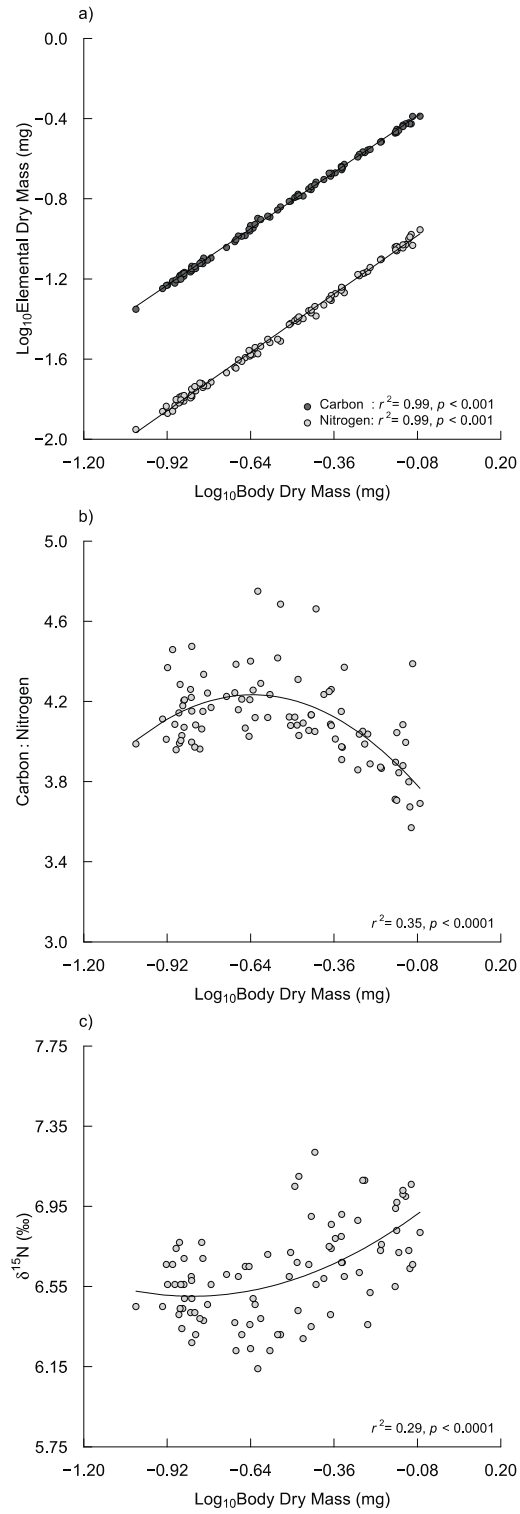
Figure S5.
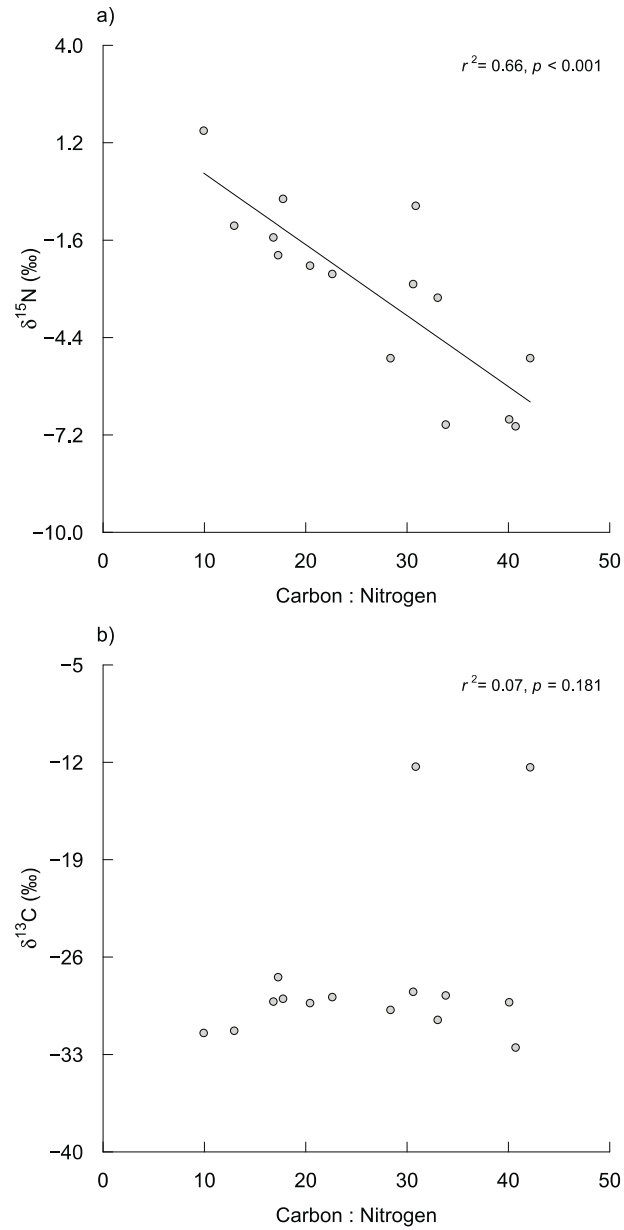
Figure S6.
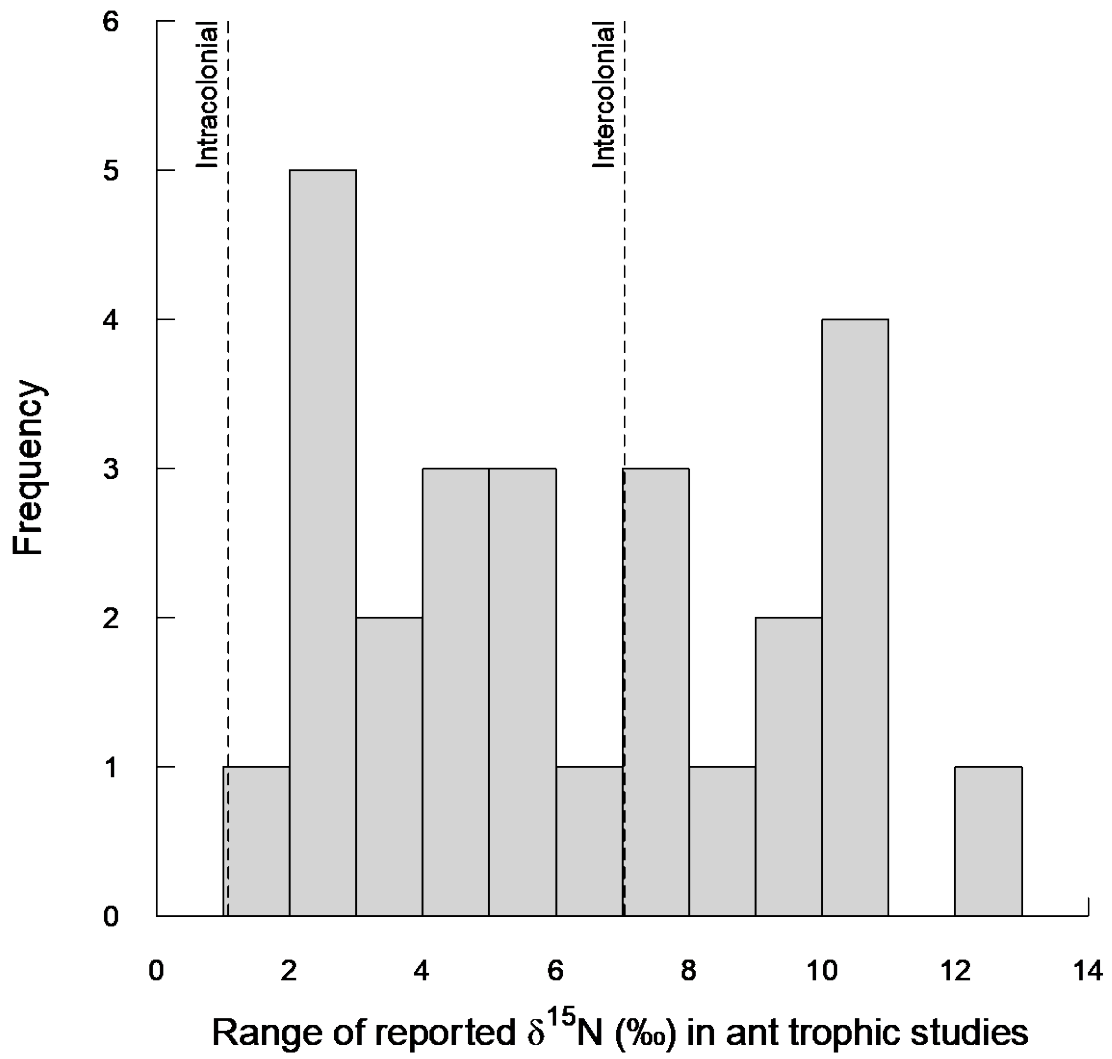
CHAPTER TWO

THE ROLE OF TEMPERATURE IN COMPETITION AND PERSISTENCE OF AN

INVADED ANT ASSEMBLAGE

Karl A. Roeder[1], Diane V. Roeder[2], and Michael Kaspari[1]

[1] Department of Biology, Graduate Program in Ecology and Evolutionary Biology, University of

Oklahoma, Norman, OK, 73019, USA

[2] Department of Agriculture, Biology and Health Sciences, Cameron University, 2800 W Gore

Blvd, Lawton, OK, 73505, USA

Abstract

1. To achieve numerical dominance, an ectotherm consumer requires a sizeable abiotic window in which it can forage. Here we explore how one abiotic factor, temperature, provides opportunity and regulates the impact of the invasive red imported fire ant, *Solenopsis invicta*, on an urban ant assemblage.

2. We first quantified *S. invicta*'s ability to outcompete native species by contrasting its foraging biomass to that of its potential competitors. In doing so, we found that *S. invicta* deployed more ant biomass at baits than the estimated whole colony biomass of three of the four co-occurring native species. It did so across ca. 75% of the hours in a summer day, those hours below its thermal maximum of 49°C. Higher thermal maxima allowed two native species to avoid encountering workers of *S. invicta*.

3. Exclosure experiments revealed that a third species, *Dorymyrmex flavus*, more similar in body size and thermal tolerance to *S. invicta*, was competitively suppressed by the invasive. Carbon and nitrogen stable isotope analyses suggest that *D. flavus*' persistence is likely due to dietary differences.

4. While thermal and dietary traits help predict how species coexist in this invaded assemblage, one key to *S. invicta*'s success is likely its ability to forage in all but six hours of a summer's day.

Key-words: fire ant, invasive species, stable isotopes, thermal tolerance, temperature, traits

Introduction

The composition and relative abundance of species in an assemblage arises in part from the match of their individual abiotic and biotic requirements to environmental availability—their

niche (Grinnell, 1917; Elton, 1927; Hutchinson, 1957; Chase & Leibold, 2003). In a warming world, temperature is one factor that regulates where species live and when they are active (Janzen, 1967; Huey & Stevenson, 1979; Adolph & Porter, 1993; Sanders et al., 2007). And while the abiotic environment may fundamentally limit where species can live, it does not alone necessarily determine if certain species live there as both intra- and interspecific interactions can be important factors in structuring communities (Paine, 1969; Tilman, 1977; Schoener, 1983; Parr, 2008; Diamond et al., 2016).

A potential invader whose niche space encompasses an ecosystem's abiotic center (the suite of common abiotic conditions for a given environment) can achieve numerical dominance and subsequently disrupt community structure through competitive displacement and behavioral dominance (Holway, 1999; Mooney & Cleland, 2001; Simberloff, 2013). However, opportunity for native species may occur on the abiotic periphery. For example, many dominant species are limited more so by abiotic conditions than interspecific interactions [e.g. the amount of soil moisture required by the Argentine ant, *Linepithima humile* (Holway et al., 2002)]. Thus, if invasive species are limited by extreme physical conditions, a diversity of traits and tolerances is one way that native species can specialize and promote coexistence. Here we explore how differences in functional traits like thermal tolerance and diet combine to predict biotic interactions in an invaded urban ant assemblage.

Ants are an ideal clade to explore how the abiotic environment and biotic interactions shape the niches of species within an assemblage. Ants are abundant and widespread, with over 200 introduced species throughout the world—five of which occupy spots on the IUCN's top 100 worst invasive species (Bertelsmeier et al., 2017). Ant species vary widely in diet (Blüthgen et al., 2003; King, 2016; Roeder & Kaspari, 2017), and species differences in thermal tolerance

in the lab (Diamond et al., 2012; Baudier et al., 2015; Kaspari et al., 2015; Bishop et al., 2017; Penick et al., 2017) frequently reflect overlap in ant activity in the field (Talbot, 1934; Kaspari, 1993; Cerdà et al., 1998; Bestelmeyer, 2000; Stuble et al., 2013). Yet we know of no studies that have looked at the combined effects of diet and thermal tolerance on the impact of an invasive ant.

The red imported fire ant, *Solenopsis invicta* (hereafter RIFA), is a devastating invasive that dominates disturbed landscapes across the southeastern United States (Porter & Savignano, 1990; Tschinkel, 2006; Resasco et al., 2014). We explore how RIFA impacts an urban ant assemblage by first quantifying its potential power to outcompete native species by contrasting its foraging biomass to that of its potential competitors. Next, we measure how both native and invasive ant species array their foraging activities across a 41°C range in temperature during a 24-hour summer cycle. We use two functional traits—thermal tolerance and body size—to predict the most likely competitor with RIFA. Finally, we use an experimental exclosure to test for competition among these two species and show evidence for dietary divergence using carbon and nitrogen stable isotope values.

Methods

Study site

All samples were collected at the University of Oklahoma Biological Station (hereafter UOBS; Oklahoma, USA, 33.88° N, 96.80° W, 204 m elevation) in July and August, a period of peak activity for RIFA in Oklahoma (Vogt et al., 2003). Yearly air temperatures range from -16.1°C to 44.4°C, with a mean annual rainfall of 1027.94 mm (Oklahoma Climatological Survey). Most of the area surrounding the UOBS has been modified into pastures, row crop

agriculture, or human residences with disturbed habitats being occupied by the monogyne form of RIFA.

Foraging biomass of fire ant colonies

We estimated the foraging biomass of RIFA colonies across a range of naturally occurring temperatures by baiting twenty nests at four different times of the day (06:00, 12:00, 18:00, and 24:00 Central Daylight Time). During each of these time periods and for each colony, we first placed a 3.55 ± 0.05 g piece of Bar-S® hotdog into a 50ml centrifuge bait tube. We then positioned four of these bait tubes at the cardinal points, 0.5m from the edge of each RIFA nest, retrieving one vial at 10, 20, 30, and 60 minutes after the start of each trial. Workers were dried to constant mass at 60°C for 48 hours and weighed to the nearest 0.01 mg using a R 200D electronic semi-microbalance (Sartorius Research, Gottingen, Germany). We report activity per colony as the total dry mass of collected individuals at 06:00, 12:00, 18:00, and 24:00. Surface temperature was recorded every minute, and then averaged per hour, at the top of each RIFA nest using HOBO temperature loggers for two days (Onset Computer Corporation, Massachusetts, USA).

Temperature as a structuring mechanism of ant assemblages

In late July/early August, we tested how temperature regulated activity patterns of ants during a daily cycle in two ways. First, we set out 100 1.5ml micro centrifuge vials (VWR International, Pennsylvania, USA) filled with cotton that was saturated with a 10% sucrose solution. Vials were evenly distributed along five 40m transects that were spaced 40m apart. This was done twenty-four times over a three-week period on the lawn of the UOBS. Each temporal

41

replicate represented a different hour of the day with sampling periods spaced at least twelve hours apart. We recorded activity by counting the number of vials that were occupied by ants after a one hour period. Individuals were then identified to species to quantify foraging activity, as a proportion of the total number of vials, for both invasive and native ants. Surface temperature was recorded every five minutes, and then averaged per hour, for each sampling period using a HOBO temperature logger for twenty days (Onset Computer Corporation, Massachusetts, USA).

Second, we located five colonies for all collected species to test if thermal tolerance was a mechanism behind the observed foraging patterns. For each colony, we performed thermal tolerance assays on five individual workers within two hours of collection to see when individuals lost muscle control and reached their critical thermal maximum, or CT_{max}. Assays were conducted by first placing an individual into a 1.5ml micro centrifuge tube that had been modified with modelling clay to remove a potential thermal refuge in the cap (as in Bujan et al., 2016). Vials were than randomly placed into a Thermal-Lok 2-position dry heat bath (USA Scientific, Florida, USA; advertised accuracy $\pm 0.2°C$) that was prewarmed to 36°C. Three additional vials per colony were kept at ambient temperature during thermal assays as a control, all of which survived. Every ten minutes, individuals were checked to see if they had reached their CT_{max} by rotating the tubes and looking for a righting response (as in Diamond et al., 2012). The dry bath temperature was then increased by 2°C and this process was repeated until all ants had reached their CT_{max}. For each run, the interior temperature of one unused vial was confirmed using a thermocouple attached to an Extech MN35 Digital Mini MultiMeter (Extech Instruments, Massachusettes, USA). Ants were then dried to constant mass at 60°C for 48 hours and weighed to the nearest 0.001 mg on a Cahn microbalance (Cahn Instruments, California, USA).

Competitive interactions between invasive and native ant species

The CT$_{max}$ of each individual was plotted against that ant's dry mass as species which overlap in trait space may be more likely to compete for resources. For the two species with the largest trait overlap, we setup 20 exclosure / 20 non-exclosure replicates (i.e. a total of 40 colonies for each species were used) on the lawn of the UOBS to test if the observed activity patterns were in part driven by competitive interactions. For each exclosure, we modified a Sterilite® plastic container (Height = 177.8 mm, Width = 361.95 mm, Depth = 444.5 mm) by removing the bottom, then positioning the container around an ant's nest. Four tent stakes were placed near the outside corners of the container and then connected, diagonally, with bungie cords to apply downward pressure to keep the container flush with the ground. As an additional measure of control, a light coat of Johnson's® baby powder was applied at the bottom of the container to prevent exterior ants from entering. When the temperature stabilized at 25°C, a single 1.5ml micro centrifuge vial filled with cotton that was saturated with a 10% sucrose solution was placed 5 cm away from each nest entrance in a random direction. After one hour vials were collected and the species inside were recorded. We predict that if RIFA is competitively suppressing native species, it will occupy baits both near their own nests and near the nests of the co-occurring native species. However, by restricting RIFA foraging with exclosures, we posit that native ants will forage on the provided resource given the opportunity.

We used carbon (^{13}C/^{12}C) and nitrogen (^{15}N/^{14}N) stable isotope analyses for 10 colonies per species to determine if the competing species were consuming similar dietary resources. Individuals were collected directly from nests before any bait studies were performed, and then frozen at -20°C. The petiole, post-petiole, and gaster were removed to eliminate any residual

43

food particles that could alter the isotopic signature (Tillberg et al., 2006). Each replicate

consisted of five homogenized workers with the same head width to attain the minimum weight

required for analysis. As RIFA has a polymorphic worker caste, we used individuals that had 0.7

mm head widths to reduce isotopic variation from body size (Roeder & Kaspari, 2017). 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 ($R_{standard}$). Ants were dried to

constant mass at 60°C for 48 hours and weighed to the nearest 0.001 mg on a Cahn microbalance

(Cahn Instruments, California, USA). All stable isotope analyses were performed at the

University of California Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL

elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon

Ltd., Cheshire, UK).

Statistical Analysis.

All analyses were run in R, version 3.3.0. A GLMM with colony identity as a random

factor was used to compare differences in the biomass of ants foraging across different time

periods. Beta regression was used to compare ant activity, as a continuous proportion, to surface

temperature. A GLMM with colony identity as a random factor was also used to compare CT_{max}

and body size values, with Tukey post-hoc comparisons used to test for differences among

species. Standard ellipse areas corrected for small sample size (SEA$_c$) were calculated to determine if species overlapped in trait space in the R package "SIBER" (Jackson et al., 2011). GLMs with binomial distributions were used to compare occupation (i.e. presence/absence) of baits near colonies with and without exclosures. MANOVA was used to calculate isotopic differences between the two competing species, and SEA$_c$s were calculated to determine the extent of overlap in δ^{13}C–δ^{15}N bi-plot space.

Results

Foraging biomass of fire ant colonies

Temperature at different times of day regulated the foraging biomass of RIFA (GLMM: $F = 40.77$, d.f. $= 3$, $P < 0.001$; Fig. 1) with random effects suggesting individual colonies vary up to 160.1 mg ($= 1$SD) higher or lower than the group average around 68% of the time. At the coolest temperatures—24.10°C (06:00) and 27.37°C (24:00)—colonies on average allocated 391.84 and 385.74 mg of biomass, respectively, towards baits. This investment level increased by ca. 32% (511.92 mg) at 18:00 when temperatures increased to 44.05°C. At 12:00, however, high surface temperatures (48.43°C) were associated with a significantly lower foraging biomass per colony ($\bar{x} = 20.52$ mg).

Temperature as a structuring mechanism of ant assemblages

Over a normal summer day at the UOBS, surface temperature ranged from 20.2°C to 61.0°C (Fig. 2a). Total ant activity declined with increasing temperature (beta regression: estimate $= -0.117$, pseudo $r^2 = 0.77$, $P < 0.001$), but this result was driven by a reduction in the foraging activity of the invasive and abundant RIFA (beta regression: estimate $= -0.158$, pseudo

$r^2 = 0.84$, $P < 0.001$; Fig. 2b). In contrast, activity of native ant species increased with temperature (beta regression: estimate = 0.085, pseudo $r^2 = 0.74$, $P < 0.001$; Fig. 2b) due to the presence of three thermophilic species: *Dorymyrmex flavus*, *Forelius pruinosus* and *Monomorium minimum*. The lone outlier, *Nylanderia terricola*, was collected in <1% of vials, generally at night or early morning and never at temperatures above 30°C.

Ants in this assemblage differed in thermal tolerance and body size

Of the five ant species found on the lawn of the UOBS, most had different CT_{max} values (GLMM: $F = 146.24$, d.f. = 4, $P < 0.001$; Fig. 2a) and three of the four native species had higher CT_{max} values than RIFA (Table 1). Only two species, *F. pruinosus* and *M. minimum*, had similar thermal tolerance values (Table 1). These ants also differed by up to an order of magnitude in body size from the low 0.047 ± 0.002 mg of *M. minimum* to 0.237 ± 0.020 mg of RIFA (GLMM: $F = 64.70$, d.f. = 4, $P < 0.001$; Table 1).

Competitive interactions between invasive and native ant species

When the CT_{max} of each individual was plotted against that ant's dry mass, only 10.6% of all ellipse area overlapped in trait space (Fig. S1). This overlap occurred between two species, RIFA and *D. flavus*. We, therefore, examined these two species for competitive interactions by testing bait occupation near nests, with and without exclosures. Exclosures did not reduce RIFA foraging near their own nests (GLM: $\chi^2 = 0.37$, df = 1, $P = 0.545$; Fig. 3a), and RIFA occupied both a similar number of baits and the majority of baits near their nests (95%) and near *D. flavus* nests (80%) when exclosures were not present (GLM: $\chi^2 = 2.18$, df = 1, $P = 0.139$). When *D. flavus* nests were enclosed, their occupation rate of baits increased from 20% to 85% (GLM: $\chi^2 =$

18.43, df = 1, $P < 0.001$; Fig. 3a), and the number of baits occupied by RIFA decreased from 80% to 5% (GLM: $\chi^2 = 26.59$, df = 1, $P < 0.001$).

Stable isotope values revealed dietary differences between RIFA and *D. flavus* (MANOVA: Pillai's Trace = 0.392, $F_{2,17} = 5.48$, $P = 0.015$; Fig. 3b), with only 5.93% standard ellipse overlap despite both species occupying a similar amount of total area in $\delta^{13}C$–$\delta^{15}N$ bi-plot space (SEA$_c$: RIFA = 3.99‰^{2}; *D. flavus* = 3.42‰^{2}; Fig. 3b). For carbon, both species spanned the continuum of C_3 to C_4 plants ($\delta^{13}C$ $_{RIFA}$ = -26.2‰ to -18.0‰; $\delta^{13}C$ $_{D. flavus}$ = -22.4‰ to -15.8‰) and did not differ (ANOVA: $F_{1,18} = 4.32$, $P = 0.052$), although $\delta^{15}N$ values between the species did vary (ANOVA: $F_{1,18} = 11.57$, $P = 0.003$) and ranged from 7.93‰ to 9.91‰ in RIFA and 9.28‰ to 10.94‰ in *D. flavus*.

Discussion

Here we show how the abiotic environment and biotic interactions regulate coexistence of species in an invaded urban ant assemblage. Traits such as thermal tolerance and body size combine to shape a species' niche which in turn affect activity patterns of invasive and native ants over a wide range of conditions. RIFA used its immense workforce to competitively suppress the native species, *D. flavus*, whose thermal tolerance and body size were most similar on baits. However, stable isotope analysis suggests that *D. flavus*' persistence is likely due to dietary differences. Combined, we suggest that using multiple components of species' niches— including morphology, physiology, behavior, and nutrition—will provide a more complete picture of how native and invasive species are able to coexist in similar environments.

Temperature as a structuring mechanism

Throughout large portions of the southern United States, the five species we observed at the UOBS commonly occur and overlap in their distribution (Guénard et al., 2017). Most of these species excel in disturbed landscapes and are opportunistic, perhaps, allowing them to monopolize an increasingly common habitat—a lawn. Urban areas continue to expand, and in doing so create heat islands which present novel thermal environments to both native and invasive species (Oke, 1982; Angilletta Jr. et al., 2007). As urban temperatures may reach levels higher than the thermal limits of species, this turnover in habitat may result in a lower diversity of taxa (McKinney, 2006). For example, a summer daily cycle on the lawn of the UOBS occurs over a 41°C range —a range that is likely problematic for some tiny ground dwelling ectotherms, and opportunistic for others. Yet in a forest patch a mere 200 m to the west of the UOBS lawn, tall elms and oaks shade an environment that not only peaks at 11°C lower than the lawn but also harbors almost three times the number of ant species in a similar-sized area (Table S1).

Competition and coexistence

We originally predicted that species in this urban locale should have little overlap in traits like thermal tolerance to reduce biotic interactions during a daily cycle. Our data suggest that when compared to RIFA, this is the case for three of the four native ant species (Table 1). However, the one native species that had similar traits, *D. flavus*, was dominated at sucrose baits both near and far away from their nest. One way RIFA could accomplish this feat was their immense workforce, as colonies were able to mobilize an incredible amount of biomass; an amount that on average was larger than the estimated colony biomass for three of the four native species on the lawn (Table 1). Yet for RIFA this level of investment is miniscule, an amount equal to less than 1% of an average colony's mass and likely an underestimate of a colony's

foraging workforce. Despite these competitive outcomes, *D. flavus* persists in this environment, perhaps due to a third niche axis—nutrition.

A nutritional way forward with niches

In homogeneous environments like lawns, nutrients may be controlled by dominant species or individuals. It has been hypothesized that carbohydrate resources in particular are important for fueling activity and producing workers, especially for the large colonies that invasive ant species typically maintain (Lach, 2005; Tilberg et al., 2007; Wilder at al., 2011; Wills et al., 2015). Moreover, carbohydrate-rich honeydew from fluid feeding hemipteran is a prime resource over which ant colonies often compete (Blüthgen et al., 2004; Wilder at al., 2013) and is only slightly enriched in $\delta^{15}N$ compared to the plant from which it was produced (Sagers & Goggin, 2007). When dominant ants monopolize such resources, as we hypothesize colonies of RIFA do at our study site, they isotopically appear as cryptic herbivores (Davidson et al., 2003; Roeder & Kaspari, 2017). In contrast, less dominant ants like *D. flavus* are often competitively displaced from these carbohydrate resources (Wilder et al., 2013).

Despite displacement, energy can be obtained by catabolizing protein or consuming the lipids of insect prey (Slansky & Rodriguez, 1987). Thus, increased consumption of nitrogen-rich arthropods, either through predation or scavenging, may lead to enriched $\delta^{15}N$ values—a result we observed in the *D. flavus* at the UOBS. Dietary divergence, revealed through natural variation in stable isotope values, may not only reveal the outcome of past competitive interactions between species but may also be important for understanding how species that are similar morphologically or physiologically are able to coexist.

Conclusions

Numerous studies have quantified (1) how the abiotic environment affects ant species (Porter & Tschinkel 1987; Kaspari, 1993; Cerdà et al., 1998; Bestelmeyer, 2000; Holway et al., 2002; Vogt et al., 2003; Lessard et al., 2009; Wittman et al., 2010; Stuble et al., 2013), or (2) the biotic interactions between invasive and native ant species (Porter & Savignano, 1990; Human & Gordon 1996; Suarez et al., 1998; Holway, 1999; Sanders et al., 2003; LeBrun et al., 2012; Resasco et al., 2014). Our work builds on this body of knowledge by focusing on trait based niche differences towards a better understanding of how species coexist in a shared habitat. Given that urbanization and habitat modification continue to create environments on which invasive species excel (Shochat et al., 2010), our findings suggest future outcomes for areas currently under development. Furthermore, if nutrition is the missing link for trait-based studies, then simply quantifying differences is not enough. Instead, focus should be placed on the biological mechanisms underlying those nutritional sources that are facilitating the persistence of invasive species like RIFA in novel environments.

Literature Cited

Adolph, S. C. & Porter, W. P. (1993) Temperature, activity, and lizard life histories. *The American Naturalist*, **142**, 273–295.

Angilletta Jr., M. J., Wilson, R. S., Niehaus, A. C., Sears, M. W., Navas, C. A. & Ribeiro, P. L. (2007) Urban physiology: city ants possess high heat tolerance. *PLoS One*, **2**, e258.

AntWeb. Available from http://www.antweb.org. Accessed 8 May 2017.

Baudier, K. M., Mudd, A. E., Erickson, S. C. & O'Donnell, S. (2015) Microhabitat and body size effects on heat tolerance: implications for responses to climate change (army ants: Formicidae, Ecitoninae). *Journal of Animal Ecology*, **84**, 1322–1330.

Bertelsmeier, C., Ollier, S., Liebhold, A. & Keller, L. (2017) Recent human history governs global ant invasion dynamics. *Nature Ecology & Evolution*, **1**, 0184.

Bestelmeyer, B. T. (2000) The trade-off between thermal tolerance and behavioural dominance in a subtropical South American ant community. *Journal of Animal Ecology*, **69**, 998–1009.

Bishop, T. R., Robertson, M. P., Rensburg, B. J. & Parr, C. L. (2016) Coping with the cold: minimum temperatures and thermal tolerances dominate the ecology of mountain ants. *Ecological Entomology*, **42**, 105–114.

Blüthgen, N., Gebauer, G. & Fiedler, K. (2003) Disentangling a rainforest food web using stable isotopes: dietary diversity in a species-rich ant community. *Oecologia*, **137**, 426–435.

Blüthgen, N., Stork, N. E. & Fiedler, K. (2004) Bottom-up control and co-occurrence in complex communities: honeydew and nectar determine a rainforest ant mosaic. *Oikos*, **106**, 344–358.

Bujan, J., Yanoviak, S. P. & Kaspari, M. (2016) Desiccation resistance in tropical insects: causes and mechanisms underlying variability in a Panama ant community. *Ecology and Evolution*, **6**, 6282–6291.

Cerdá, X., Retana, J. & Cros, S. (1998) Critical thermal limits in Mediterranean ant species: trade-off between mortality risk and foraging performance. *Functional Ecology*, **12**, 45–55.

Chase, J. M. & Leibold, M. A. (2003) *Ecological niches: linking classical and contemporary approaches.* University of Chicago Press, Chicago, USA.

Davidson, D. W., Cook, S. C., Snelling, R. R. & Chua, T. H. (2003) Explaining the abundance of ants in lowland tropical rainforest canopies. *Science*, **300**, 969–972.

Diamond, S. E., Sorger, D. M., Hulcr, J., Pelini, S. L., Del Toro, I., Hirsch, C., Oberg, E. & Dunn, R. R. (2012) Who likes it hot? A global analysis of the climatic, ecological, and evolutionary determinants of warming tolerance in ants. *Global Change Biology*, **18**, 448–456.

Diamond, S. E., Nichols, L. M., Pelini, S. L., Penick, C. A., Barber, G. W., Cahan, S. H., Dunn, R. R., Ellison, A. M., Sanders, N. J. & Gotelli, N. J. (2016) Climatic warming destabilizes forest ant communities. *Science Advances*, **2**, e1600842.

Elton, C. (1927) *Animal Ecology*. Sidgwick & Jackson, London, UK.

Grinnell, J. (1917) The niche-relationships of the California Thrasher. *Auk*, **34**, 427–433.

Guénard, B., Weiser, M. D., Gómez, K., Narula, N. & Economo, E. P. (2017) The Global Ant Biodiversity Informatics (GABI) database: synthesizing data on the geographic distribution of ant species (Hymenoptera: Formicidae). *Myrmecological News*, **24**, 83–89.

Holway, D. A. (1999) Competitive mechanisms underlying the displacement of native ants by the invasive Argentine ant. *Ecology*, **80**, 238–251.

Holway, D. A., Suarez, A. V. & Case, T. J. (2002) Role of abiotic factors governing susceptibility to invasion: a test with Argentine ants. *Ecology*, **83**, 1610–1619.

Huey, R. B. & Stevenson, R. D. (1979) Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. *American Zoologist*, **19**, 357–366.

Human, K. G. & Gordon, D. M. (1996) Exploitation and interference competition between the invasive Argentine ant, *Linepithema humile*, and native ant species. *Oecolgia*, **105**, 405–412.

Hutchinson, G. E. (1957) Concluding remarks. *Cold Spring Harbor Symposium on Quantitative Biology*, **22**, 415–427.

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.

Janzen, D. H. (1967) Why mountain passes are higher in the tropics. *The American Naturalist*, **101**, 233–249.

Kaspari, M. (1993) Body size and microclimate use in Neotropical granivorous ants. *Oecologia*, **96**, 500–507.

Kaspari, M. & Valone, T. J. (2002) On ectotherm abundance in a seasonal environment—studies of a desert ant assemblage. *Ecology*, **83**, 2991–2996.

Kaspari, M., Clay, N. A., Lucas, J., Yanoviak, S. P. & Kay, A. (2015) Thermal adaptation generates a diversity of thermal limits in a rainforest ant community. *Global Change Biology*, **21**, 1092–1102.

King, J. R. (2016) Where do eusocial insects fit into soil food webs? *Soil Biology and Biochemistry*, **102**, 55–62.

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

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.

Lessard, J. P., Dunn, R. R. & Sanders, N.J. (2009) Temperature-mediated coexistence in temperate forest ant communities. *Insectes Sociaux*, **56**, 149–156.

McKinney, M. L. (2006) Urbanization as a major cause of biotic homogenization. *Biological Conservation*, **127**, 247–260.

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.

Oke, T. R. (1982) The energetic basis of the urban heat island. *Quarterly Journal of the Royal Meteorological Society*, **108**, 1–24.

Paine, R. T. (1969) A note on trophic complexity and community stability. *The American Naturalist*, **103**, 91–93.

Parr, C. L. (2008) Dominant ants can control assemblage species richness in a South African savanna. *Journal of Animal Ecology*, **77**, 1191–1198.

Penick, C. A., Diamond, S. E., Sanders, N. J. & Dunn, R. R. (2016) Beyond thermal limits: Comprehensive metrics of performance identify key axes of thermal adaptation in ants. *Functional Ecology*, **31**, 1091–1100.

Porter, S. D. & Tschinkel, W. R. (1987) Foraging in *Solenopsis invicta* (Hymenoptera: Formicidae): effects of weather and season. *Environmental Entomology*, **16**, 802-808.

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., Haddad, N. M., Orrock, J. L., Shoemaker, D., Brudvig, L. A., Damschen, E. I., Tweksbury, J. J. & Levey, D. J. (2014) Landscape corridors can increase invasion by an exotic species and reduce diversity of native species. *Ecology*, **95**, 2033–2039.

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.

Sagers, C. L. & Goggin, F. L. (2007) Isotopic enrichment in a phloem-feeding insect: influences of nutrient and water availability. *Oecologia*, **151**, 464–472.

Sanders, N. J., Gotelli, N. J., Heller, N. E. & Gordon, D. M. (2003) Community disassembly by an invasive species. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 2474–2477.

Sanders, N. J., Lessard, J. P., Fitzpatrick, M. C. & Dunn, R. R. (2007) Temperature, but not productivity or geometry, predicts elevational gradients in ants across spatial grains. *Global Ecology and Biogeography*, **16**, 640–649.

Schoener, T. W. (1983) Field experiments on interspecific competition. *The American Naturalist*, **122**, 240–285.

Shochat, E., Lerman, S. B., Anderies, J. M., Warren, P. S., Faeth, S. H. & Nilon, C. H. (2010) Invasion, competition, and biodiversity loss in urban ecosystems. *BioScience*, **60**, 199–208.

Simberloff, D. (2013) *Invasive species: what everyone needs to know*. Oxford University Press, New York, USA.

Slansky, F. & Rodriguez, J. G. (1987) *Nutritional ecology of insects, mites, spiders and related invertebrates*. Wiley, New York, USA.

Smith, M. R. (1965) *House-infesting ants of the eastern United States: their recognition, biology, and economic importance* (No. 1326). United States Department of Agriculture, Washington D.C., USA.

Stuble, K. L., Rodriguez-Cabal, M. A., McCormick, G. L., Jurić, I., Dunn, R. R. & Sanders, N. J. (2013) Tradeoffs, competition, and coexistence in eastern deciduous forest ant communities. *Oecologia*, **171**, 981–992.

Suarez, A. V., Bolger, D. T. & Case, T. J. (1998) Effects of fragmentation and invasion on native ant communities in coastal southern California. *Ecology*, **79**, 2041–2056.

Talbot, M. (1934) Distribution of ant species in the Chicago region with reference to ecological factors and physiological toleration. *Ecology*, **15**, 416–439.

Tillberg, C. V., McCarthy, D. P., Dolezal, A. G. & Suarez, A. V. (2006) Measuring the trophic ecology of ants using stable isotopes. *Insectes Sociaux*, **53**, 65–69.

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.

Tilman, D. (1977) Resource competition between plankton algae: an experimental and theoretical approach. *Ecology*, **58**, 338–348.

Tschinkel, W. R. (1988) Colony growth and the ontogeny of worker polymorphism in the fire ant, Solenopsis invicta. *Behavioral Ecology and Sociobiology*, **22**, 103–115.

Tscinkel, W. R. (2006) *The fire ants*. Harvard University Press, Cambridge, Massachusetts, USA.

Van Pelt Jr., A. F. (1958) The ecology of the ants of the Welaka reserve, Florida (Hymenoptera: Formicidae). Part II. Annotated list. *American Midland Naturalist*, **59**, 1–57.

Vogt, J. T., Smith, W. A., Grantham, R. A. & Wright, R. E. (2003) Effects of temperature and season on foraging activity of red imported fire ants (Hymenoptera: Formicidae) in Oklahoma. *Environmental Entomology*, **32**, 447–451.

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.

Wilder, S. M., Barnum, T. R., Holway, D. A., Suarez, A. V. & Eubanks, M. D. (2013) Introduced fire ants can exclude native ants from critical mutualist-provided resources. *Oecologia*, **172**, 197–205.

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.

Wittman, S. E., Sanders, N. J., Ellison, A. M., Jules, E. S., Ratchford, J. S. & Gotelli, N. J. (2010) Species interactions and thermal constraints on ant community structure. *Oikos*, **119**, 551–559.

Table 1. Traits of workers and colonies for the five ant species found on the lawn of the University of Oklahoma Biological Station. Worker mass and critical thermal maximum, CT_{max}, are reported as means $\pm$ SE with letters indicating significant differences between species from Tukey post-hoc tests ($P < 0.05$). Colony sizes are estimated from the listed reference. Biomass is calculated by multiplying the mean worker mass by the estimated number of workers per colony.

Species	CT_{max} (°C)	Worker mass (mg)	Estimated workers per colony	Estimated biomass per colony (mg)	Colony size reference
Solenopsis invicta	49.0 ± 0.4c	0.237 ± 0.020a	~220000	52140	Tschinkel, 1988
Forelius pruinosus	56.0 ± 0.3a	0.099 ± 0.004b	~100000	9900	Kaspari & Valone, 2002
Monomorium minimum	55.1 ± 0.2a	0.047 ± 0.002c	~3000	141	Van Pelt Jr., 1958
Dorymyrmex flavus	51.5 ± 0.5b	0.216 ± 0.012a	~1000	216	Kaspari & Valone, 2002
Nylanderia terricola	46.7 ± 0.2d	0.078 ± 0.004bc	~300	23	Smith, 1965

Figure Legends

Figure 1. Temporal differences in mean foraging biomass (± SE) of 20 red imported fire ant colonies (*Solenopsis invicta*; RIFA) across four different times of day on the University of Oklahoma Biological Station lawn.

Figure 2. Temperature, thermal tolerance and activity of ant species during a daily cycle on the University of Oklahoma Biological Station lawn. Panel (a) represents the average surface temperature and 95% confidence interval (black line with grey polygon, respectively) recorded from 20 days in July and August. Horizontal lines represent the critical thermal maximum for each species collected at the location. Panel (b) shows the proportion of 10% sucrose baits occupied by either invasive red imported fire ants (*Solenopsis invicta*; RIFA) or native ants across time. Native species are represented by dark grey (—) lines, while invasive RIFA are represented by light grey (---) lines. Ant images were taken by April Noble and Jen Fogarty from www.AntWeb.org.

Figure 3. Competitive interactions and dietary differences between invasive red imported fire ants (*Solenopsis invicta*; RIFA; light grey) and the native *Dorymyrmex flavus* (dark grey). Panel (a) shows the number of 10% sucrose baits, out of 20, occupied by either species (above the midline = RIFA, below = *D. flavus*) near nests with or without exclosures. Panel (b) shows the mean ± SE and spread of nitrogen and carbon stable isotope values from 10 colonies of each species. Convex hulls connect the outermost points and are denoted by --- lines, while maximum likelihood standard ellipses are denoted by — lines and contain approximately 40% of the points.

Figure 1.

Figure 2.

a)

b)

Invasive RIFA

Native

Forelius pruinosus, 56.0 ºC

Monomorium minimum, 55.1 ºC

Dorymyrmex flavus, 51.5 ºC

Solenopsis invicta, 49.0 ºC

Nylanderia terricola, 46.7 ºC
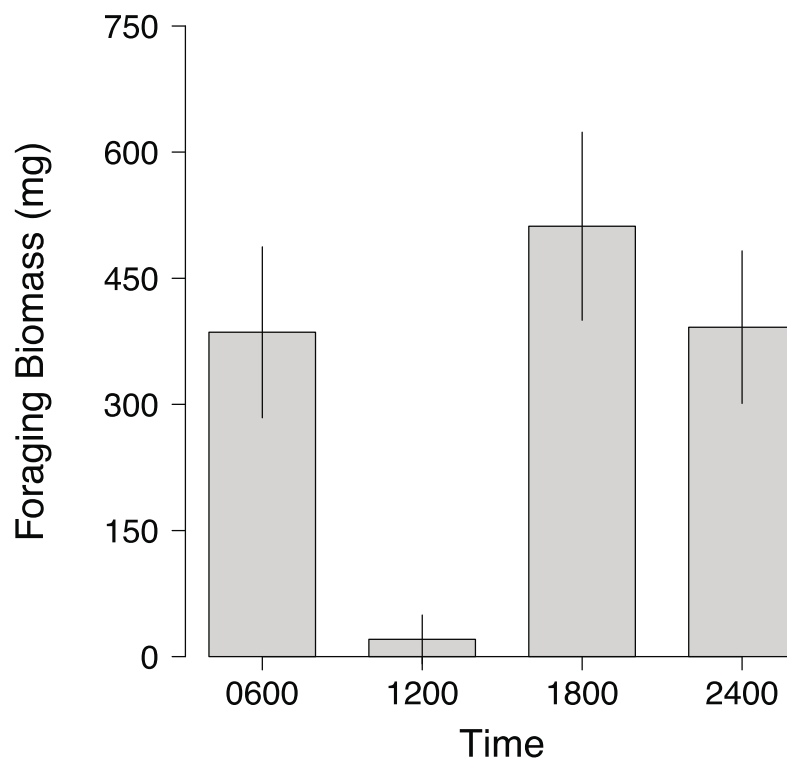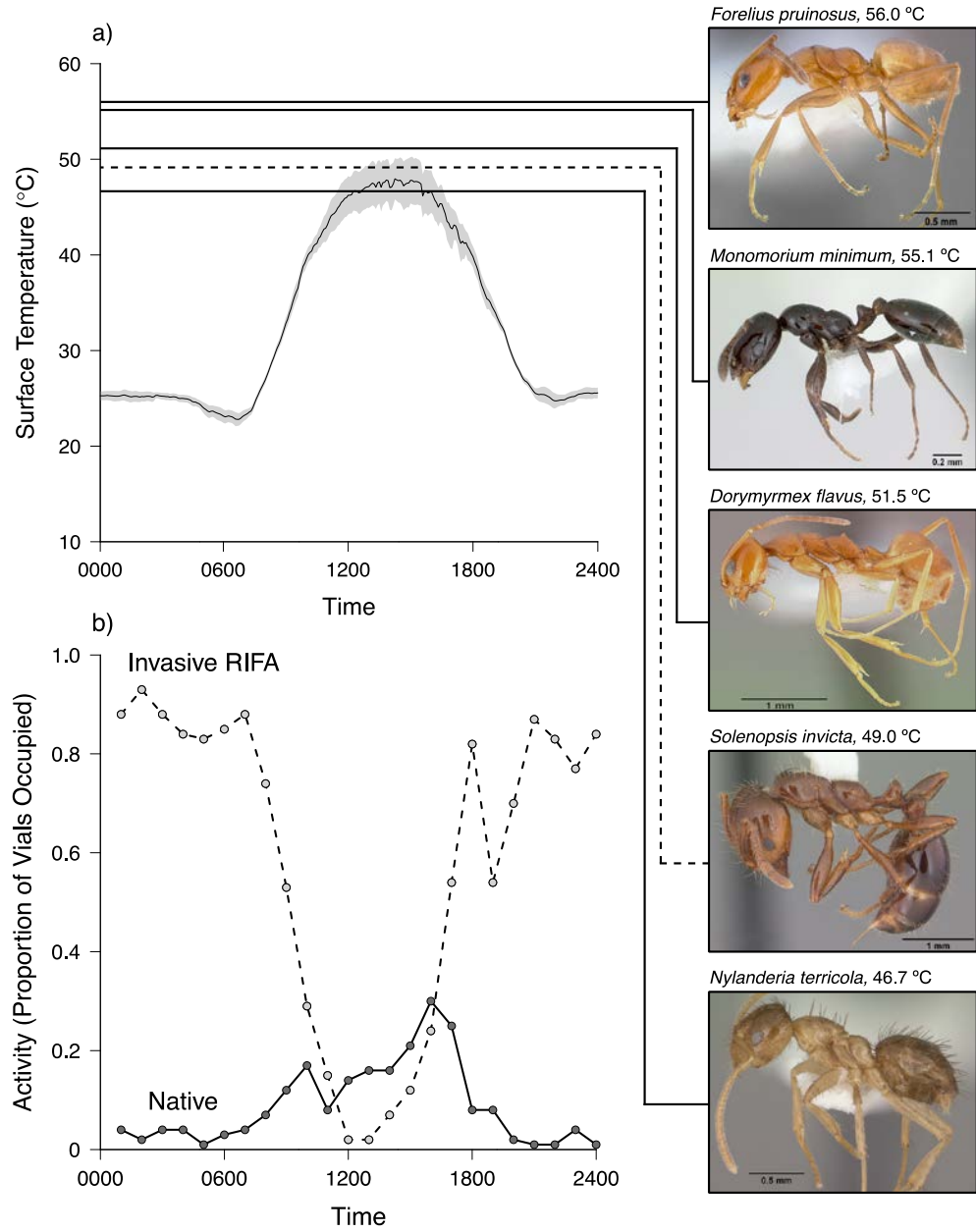
Figure 3.
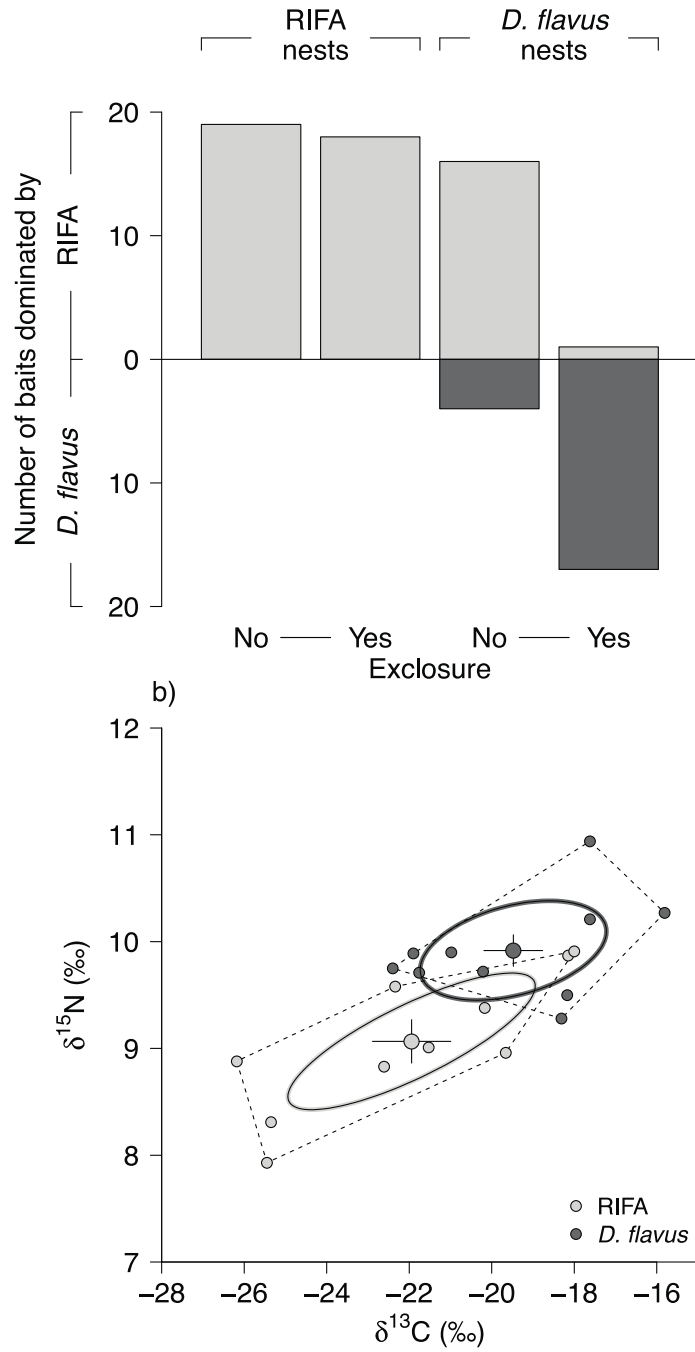
Chapter 2: Appendix S1. Supplemental Data.

Table S1. List of ant species from a 30x30m plot in lawn and forest habitat of the University of Oklahoma Biological Station. Individuals were collected off cricket baits (n=16) spaced 10m apart, and by hand collecting in each habitat for 90 minutes. An (x) indicates presence and a (—) indicates absence.

Taxon	Lawn	Forest
DOLICHODERINAE		
Dorymyrmex flavus McCook, 1879	x	—
Forelius pruinosus (Roger, 1863)	x	—
FORMICINAE		
Camponotus americanus Mayr, 1862	—	x
Camponotus pennsylvanicus (De Geer, 1773)	—	x
Nylanderia terricola (Buckley, 1866)	x	x
MYRMECINAE		
Aphaenogaster texana Wheeler, 1915	—	x
Crematogaster ashmeadi Mayr, 1886	—	x
Crematogaster laeviuscula Mayr, 1870	—	x
Crematogaster lineolata (Say, 1836)	—	x
Monomorium minimum (Buckley, 1867)	x	x
Myrmecina americana Emery, 1895	—	x
Pheidole dentata Mayr, 1886	—	x
Pheidole metallescens Emery, 1895	—	x
Pheidole pelor Wilson, 2003	—	x
Solenopsis invicta Buren, 1972	x	—
Solenopsis molesta (Say, 1836)	—	x
PONERINAE		
Hypoponera opacior (Forel, 1893)	—	x
SPECIES RICHNESS	5	14

Supplemental Figure Legends

Figure S1. Critical thermal maximum and body size (i.e. dry mass) differences across the five ant species on the lawn of the University of Oklahoma Biological Station. For each species, convex hulls connect the outermost points and are denoted by --- lines, while maximum

Figure S1.
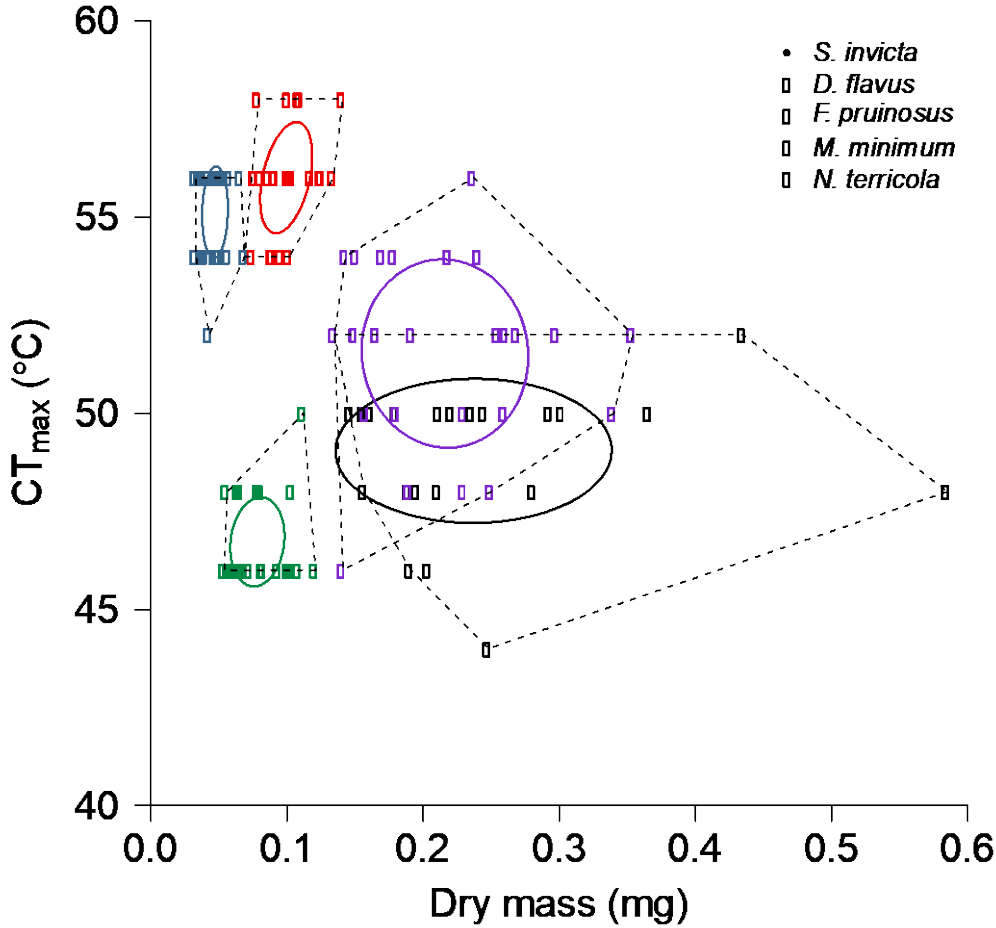
CHAPTER THREE

Formatted for publication in the *Journal of Animal Ecology*

THERMAL TRAITS PREDICT THE WINNERS AND LOSERS OF CLIMATE CHANGE: AN

EXAMPLE FROM NORTH AMERICAN ANT COMMUNITIES

Karl A. Roeder[1], Jelena Bujan[2], Kirsten de Beurs[3], Michael D. Weiser[1], and Michael Kaspari[1]

[1] Department of Biology, Geographical Ecology Group, University of Oklahoma, Norman, OK,

73019, USA

[2] Department of Biology, University of Louisville, Louisville, KY, 40292

[3]Department of Geography and Sustainability, University of Oklahoma, Norman OK, 73019

Abstract

1. Across the globe, temperatures are predicted to increase with consequences for many taxonomic groups. Arthropods, which are mostly ectotherms, are particularly at risk as temperature imposes physiological constraints on growth, survival and reproduction. Given that arthropods may be disproportionately affected in a warmer climate—the question becomes which taxa are vulnerable and can we predict the supposed winners and losers of climate change?

2. We resurveyed 33 ant communities, quantifying 20-year differences in the incidence of 28 genera, toward testing thermal performance theory's prediction that genera with higher average thermal limits will tend to accumulate at the expense of those with lower limits.

3. These North American ant communities were surveyed with 30 1-m^2 plots, and the incidence of each genus across the 30 plots was used to estimate changes in incidence between the original surveys from 1994-1997 and resurveys in 2016-2017. By this metric, about 2/3 of the genera had increased in more than half of their communities after 20 years. Average temperature had increased by 1°C in that time period (Range: -0.4°C to 2.5°C).

4. To test thermal performance theory, we used thermal dry baths to quantify critical thermal minima (CT_{min}: the low temperatures at which ants first become inactive) and maxima (CT_{max}: the high temperatures at which they lose muscle control) for common species at each site. Consistent with prediction, we found a positive decelerating relationship between CT_{max} and the proportion of sites in which a genus had increased.

CT_{min}, by contrast, was not a useful predictor of change. There was no evidence of phylogenetic signal in the genus means of these two variables.

5. Thermal functional traits like CT_{max} have thus proved useful in predicting patterns of long-term community change in a dominant, diverse insect taxon.

Keywords: ants, climate change, incidence, insects, temperature, thermal performance, thermal tolerance, traits

Introduction

As global temperatures increase, it is essential to build functional models that predict the responses of populations, communities, and ecosystems (Deutsch et al., 2008; Hellmann, Byers, Bierwagen, & Dukes, 2008; IPCC, 2014; Parmesan & Yohe, 2003; Walther et al., 2002). Ectotherms are often a focus of such efforts, given the clear link between temperature and the growth, survival and reproduction of organisms whose body temperatures track that of their environment (Calosi, Bilton, & Spicer, 2008; Chown & Nicolson, 2004; Colinet, Sinclair, Vernon, & Renault, 2015). Arthropods are (mostly) ectotherms that comprise ca. 46% of Earth's animal biomass (Bar-On, Philips, & Milo, 2018) and may represent up to 5-10 million of Earth's species (Ødegaard, 2000). Yet declines in arthropod abundance and species richness are already being documented with subsequent effects on ecosystem stability and the structure of food webs (Diamond et al., 2016; Eisenhauer, Bonn, & Guerra, 2019; Hallmann et al., 2017). In a Puerto Rican rainforest, for example, arthropod biomass has decreased four to eight times over the last 40 years resulting in a bottom-up trophic cascade (Lister & Garcia, 2018). The ability to predict

which arthropod taxa increase and which decrease—the "winners and losers"—is a crucial test for any theory predicting biotic responses to climate change.

Thermal performance theory (Angilletta, 2009; Kingsolver & Huey, 2008; Sinclair et al., 2016) posits unimodal, left skewed thermal performance curves: the fitness of an ectotherm should accelerate with temperature to a maximum before a quick descent ending in the ectotherm's death. Two of the variables from a thermal performance curve—the critical minimum temperature (CT_{min}) at which it loses mobility and the critical maximum temperature (CT_{max}) at which it loses muscle control—hold considerable promise for predicting the responses of taxa to warming since both vary geographically (Deutsch et al., 2008) and within ecological communities (Kaspari, Clay, Lucas, Yanoviak, & Kay, 2015). If, for example, populations that increase as environmental temperatures rise also have higher $CT_{max}s$—physiological thermal limits typically measured in the lab under controlled conditions—then these variables should have predictive value that may be scaled up to and across communities. Surprisingly, while we have long known that small temperature increases can increase generalist butterfly populations (Warren et al., 2001) and lead to asymmetric range expansions (Chen, Hill, Ohlemüller, Roy, & Thomas, 2011; Parmesan et al., 1999), we know of no data that bear on how the thermal limits of co-occurring populations in a warming environment predict which populations will increase and decrease.

The growing interest in thermal tolerance coincides with a growing number of studies reporting many fold changes in the biomass of different insect groups (Hallmann et al., 2017; Lister & Garcia, 2018; Sánchez-Bayo & Wyckhuys, 2019). Quantifying the thermal limits of a variety of widely distributed arthropod taxa should aid investigators in parsing the effects of warming temperatures from other potential causes including habitat destruction, pesticides, and

co-extinctions of mutualists (Eisenhauer et al., 2019; Cardinale, Gonzalez, Allington, & Loreau, 2018; Leather, 2018).

Ants (Hymenoptera: Formicidae) are a common, ecologically important group (Del Toro, Ribbons, & Pelini, 2012; Griffiths et al., 2018; Hölldobler & Wilson, 1990) with peak thermal maxima higher than many other model ectotherm taxa like flies and lizards (Diamond & Chick, 2018; Huey et al., 2009; Kellermann et al., 2012). Here we present two sets of data—an initial survey in the mid 1990s and a resurvey approximately 20 years later using the same methods—from 33 North American ant communities. Our goal was to quantify changes in local incidence of 28 common genera. At each site we measured CT_{min} and CT_{max} of common species. We combine these two datasets to test the prediction that a 20-year warming trend has favored genera with higher thermal maxima and minima, as predicted by thermal performance theory.

Methods

Study sites and their temperature

In 2016-2017, ants were resampled from April-September across North America at 33 locations previously sampled in 1994-1997 (Fig. 1; Kaspari, Alonso, & O'Donnell, 2000). These sites spanned 15.7° in latitude and 51.6° in longitude from warm southwestern deserts in southern California to cool northeastern deciduous forests in Massachusetts. Many of these locations occur at Long Term Ecological Research (LTER) stations, National Ecological Observatory Network (NEON) field sites, or within protected areas such as National or State parks and forests (see Fig. 1, Table S1).

Monthly temperature data from 1993-2017 was extracted from the Parameter-elevation Regressions on Independent Slopes Model (PRISM) datasets at Oregon State University. These

values were derived at a 4 km spatial resolution from the grid cell overlapping each of the 33 sampling locations. For each site, we calculated the average mean annual temperature (hereafter MAT) and the change in temperature between the two surveys' mean annual temperatures measured from June to May of the preceding year (hereafter ΔTemp). We then created average MAT and ΔTemp values for each genus from the sites where that genus occurred.

Sampling ants at each site

At each location, we used identical sampling methods from the original survey by setting out 30 1-m^2 plots, 10 m apart in a 330 m transect. Within each plot, ants were collected by hand while sifting through litter, debris, and vegetation. Each plot was then baited for 30 minutes with Keebler® Sandies® Pecan Shortbread cookies, a commonly used ant bait, and all ant strays were collected. Specimens were identified to genus using taxonomic keys (e.g. Fisher & Cover, 2007) and then compared to a reference collection maintained at the University of Oklahoma by the senior author from the original sampling event.

We quantified the change in incidence for each genus that occurred at 3 or more sample sites. We excluded genera that only occurred at one or two sites as these taxa were rare and usually only collected in the 2016-2017 resurvey (e.g. *Neivamyrmex* army ants occurring in one plot total). For both the original and resurvey, we counted for each genus the number of plots (out of 30) at a site where it was recorded. Our measure of change in incidence was the proportion of sites (0.0 to 1.0) where occurrences had increased in 2016-2017 compared to 1994-1997 for each genus.

Thermal tolerance and phylogenetic signal

71

We measured the critical thermal minima (CT_{min}) and maxima (CT_{max}) of ants using a ramping protocol commonly used to measure thermal tolerance (Bishop, Robertson, Van Rensburg, & Parr, 2017; Kaspari et al., 2015). For most of the common species at each location we collected at least ten live workers and placed these individuals into 1.5 ml microcentrifuge tubes that had been modified with modelling clay to remove a thermal refuge in the cap (as in Roeder, Roeder, & Kaspari, 2018a). For CT_{max}, we placed five vials with ants into a Thermal-Lok 2-position dry heat bath (USA Scientific, Orlando, FL, U.S.A.; advertised accuracy $\pm 0.2°C$) prewarmed to 36°C. Every 10 minutes, ants were checked to see if they had reached their critical thermal limit by rotating the vials and looking for a righting response. The temperature was then increased 2°C and the process was repeated until all ants had lost muscle control. Similarly, for CT_{min}, we placed five ants into an EchoTherm^{TM} IC20 chilling/heating dry bath (Torrey Pines Scientific, Carlsbad, CA, U.S.A.; advertised accuracy $\pm 1°C$) precooled to 20°C and followed the methods above except we decreased the temperature 2°C every 10 minutes. We averaged species values within a genus to create genus level CT_{max} and CT_{min} values.

We tested for phylogenetic signal in ant thermal traits by calculating Pagel's λ using an ant phylogeny (Moreau & Bell, 2013) pruned to include the genera for which we measured thermal tolerance. We added one genus that was not present in the original tree, *Ponera*, as a sister group to *Hypoponera* and ran separate analyses for CT_{max} and CT_{min} as we did not measure CT_{min} for the genus *Stigmatomma*. Pagel's λ considers how traits that are distributed on a phylogeny compare to those expected from Brownian motion by transforming the original tree using the parameter λ, which ranges from zero to one (Pagel, 1999). As λ approaches one, traits show greater phylogenetic signal.

Statistical analyses

All analyses were run in R, version 3.5.1. We used an information theoretic approach to rank multiple generalized linear regressions (GLM) with binomial error distributions that compared thermal traits (CT_{min} and CT_{max}) and environmental variables (MAT and ΔTemp) to the proportion of sites where incidence had increased between the two sampling periods. We tested CT_{max} and CT_{min} separately as we had additional data for the CT_{max} of the genus *Stigmatomma*.

Regression models were ranked by Akaike's information criterion (AIC) and ΔAIC values for each model were calculated from the difference of the AIC of *i*th model and the model with the lowest AIC value. Akaike weights (w_i) were then calculated and represent a weight of evidence that model *i* was the best fit (Burnham & Anderson, 2002). For multiple regression models, predictor variables were checked for multicollinearity using a variance inflation factor (VIF) cutoff = 3 in the "car" package. We also calculated Pearson correlation coefficients between (1) CT_{max} and CT_{min} to check for a tradeoff in thermal traits and (2) a genus' ΔTemp and MAT to examine if genera occurring in warm locales were experiencing greater changes in temperature. Cragg and Uhler pseudo r^2 values were determined using the "pscl" package. Pagel's λ was calculated to check thermal traits for a phylogenetic signal in the "ape" and "phylosignal" packages. Finally, regressions were visually inspected for non-linear relationships (i.e. quadratic and cubic relationships) and compared to their linear counterparts using AIC.

Results

Mean annual temperatures of our study sites ranged 24.2°C, from a cold Alpine tundra in Colorado (MAT = -1.0°C) to a warm desert in southern California (MAT = 23.2°C). Most sites

increased by an average of 1.0°C (± 0.1 SE) between the two sampling events [Range: -0.4°C in a New Mexico high desert to +2.5°C in a Missouri mixed hardwood forest (Fig. 1)]. Across these 33 sites and 990 1-m^2 plots, we collected 28 ant genera at three or more locations. Of these genera, about 64% had higher incidence in 2016-2017 than in the original sample 20 years prior.

Ant incidence increased with CT$_{max}$

CT$_{max}$s varied 14.4°C from a low of 38.8°C in *Brachymyrmex* to a high of 53.2°C in *Forelius.* CT$_{min}$s ranged less, from 0.2°C in the *Prenolepis* to 11.2°C in *Brachymyrmex.* A genus' CT$_{max}$ and CT$_{min}$ were not correlated (Pearson correlation: t = -0.90, *r* = -0.18, *P* = 0.375). We also found no phylogenetic signal that would account for the variation in CT$_{max}$ (λ = 0.38, *P* = 0.653) or CT$_{min}$ (λ < 0.01, *P* = 1) across these common genera.

While the ΔTemp experienced was correlated with MAT (Pearson correlation: t = -2.69, *r* = -0.47, *P* = 0.012), variance inflation factors suggest little collinearity between CT$_{max}$ (VIF = 1.4), MAT (VIF = 1.6) and ΔTemp (VIF = 2.1). We tested these correlated, but not colinear predictors, and found that as a genus' CT$_{max}$ value increased so did the proportion of communities in which its incidence—the number of plots occupied—likewise increased 20 years hence (Fig. 2a; Table 1). Moreover, CT$_{max}$ was the only parameter in each model within two AIC of the top model, accounting for 17.2% of the variation in the proportion of sites where genera increased. In other words, genera with high CT$_{max}$ values tended to occupy more plots at more sites after 20 years.

As the relationship with CT$_{max}$ appeared non-linear, we further tested and found that a quadratic and cubic fit were better than the linear model, accounting for an additional 12 to 15% of the variation for increasing incidence (Table 2). In the quadratic model, which had the lowest

AIC value, incidence peaked and then slightly decreased for thermally tolerant ant genera with CT_{max} values above the average of 46.1°C (Fig. 2a). In contrast, a genus' CT_{min} was unrelated to its 20-year change in incidence (Table 1) with CT_{min} values clustering around the genera mean of 5.7°C (Fig. 2b).

Discussion

Thermal performance curves are bracketed by two functional traits—CT_{min} and CT_{max}—of great potential utility for predicting changes in an ectotherm's performance, and hence its abundance, in a warming world. Here we realize that utility—predicting genera that are increasing and decreasing in incidence—by combining a new regional dataset on 20-year changes with a dataset on the thermal tolerances of common ant genera. To our knowledge, this is the first study to successfully combine long term community level changes and thermal traits to predict the winners and losers of global warming in a common and ecologically important arthropod group.

Genera with high CT_{max} are more likely to increase

A key focus of research in global change biology is the identification of functional traits—aspects of the phenotype linked to fitness (Kraft & Ackerly, 2010; McGill, Enquist, Weiher, & Westoby, 2006; Soudzilovskaia et al., 2013; Wong, Guénard, & Lewis, 2018)—that predict population responses to warming. We used a functional trait approach to dissect how diversity within the ants—for example CT_{max} varying 14.4°C across 28 common genera—informs how their community occurrence has shifted after 20 years. Our results reveal that CT_{max} is necessary, but not sufficient to predict winners and losers. An inspection of Figure 2a reveals

that genera that are increasing (i.e. occupying more plots in their respective communities) occur

across almost the entire range of CT_{max}; those that are decreasing are heavily biased toward low

thermal tolerance (i.e. CT_{max} <46°C). Some of these genera (e.g. *Brachymyrmex* and

Stigmatomma) declined 80% from the mid 1990s samples and were found in forests with

relatively high degrees of warming (+1.6°C).

The resulting nonlinearity suggests a few working hypotheses. The first suggests the

reasonable notion that more than one trait determines trends in an ant genus' incidence. In this

case, some aspect of natural history allows low CT_{max} genera like *Myrmecina*, *Ponera*, and

Strumigenys to increase while co-occurring *Brachymyrmex* and *Stenamma* decrease. At the same

time, some of the most thermophilic genera like *Monomorium* and *Forelius* ($CT_{max} = 53$°C) that

are active at the hottest parts of the day have incidence levels that are relatively stable compared

to large colony dominants like *Crematogaster*, *Formica*, and *Solenopsis* ($CT_{max} = 46$ to 51°C).

Here we suggest that more modest increases in temperature, especially in the already hottest

desert ecosystems, may provide no advantage to the thermophiles while increasing the

competitive ability of the dominant genera that forage in these communities for most hours of the

day, including night when warming is most prevalent (Alward, Detling, & Milchunas, 1999;

Cerdá, Retana, & Cros, 1998; Roeder et al., 2018a). Both of these hypotheses are likely informed

by tradeoffs in thermal and other functional traits (Bujan, Yanoviak, & Kaspari, 2016; Penick,

Diamond, Sanders, & Dunn, 2017).

Resistance to climate change in ecological communities

The relationship between thermal tolerance and abiotic conditions has been mixed for

arthropods: meta-analyses have generally shown a weak to nonexistent relationship between a

population's CT_{max} and the mean annual temperature of the habitat or geographic range (Addo-Bediako, Chown, & Gaston, 2000; Hoffmann, Chown, & Clusella-Trullas, 2013; Sunday, Dates, & Dulvy, 2011). Instead, upper thermal limits are often more phylogenetically constrained by evolutionary history, a result that has been documented for ants (Diamond et al., 2012) but one we did not observe in our analyses. We posit the lack of signal is partially due to reduced sample size—28 genera compared to the 156 species in Diamond et al. (2012)—which parallels a similar result observed in communities of montane ants in southern Africa (Bishop et al. 2017). But why do analyses of population data fail to find CT_{max}s tracking environmental temperature?

One possible answer is the diversity of CT_{max}s in any given ecological community potentially promotes the coexistence of some suite of species. For example, a moderately diverse grassland community in Oklahoma may support a low CT_{max} *Prenolepis* "winter ant", the nocturnal and crepuscular low CT_{max} *Lasius*, two dominant *Crematogaster* and *Solenopsis* species that maintain large colonies and forage most hours of the day, and the thermophile specialists like *Forelius* and *Monomorium* that emerge for a few superheated hours daily (Prather, Roeder, Sanders, & Kaspari, 2018; Roeder et al., 2018a). As a result, the mean annual temperature underestimates the available thermal niche space in many temperate North America locations. However, certain tropical species like army ants (Baudier, Mudd, Erickson, & O'Donnell, 2015; Baudier, D'Amelio, Malhortra, O'Connor, & O'Donnell, 2018) may be less resistant to moderate increases in temperature, if the more uniform temperatures in the tropics promote thermal specialization. If this scenario is true, we should expect community level comparisons of thermal tolerance across tropical sites to show better matches between environmental temperature and CT_{max}. This, indeed, seems to be the case in a Panama rainforest,

where the CT_{max}s of ants from the tropical canopy are consistently higher than those from the cooler shaded understory 30 m below (Kaspari et al., 2015).

Second, the functional traits CT_{max} and CT_{min} are uncorrelated in our dataset ($P = 0.375$) suggesting they are not constrained by a tradeoff, which allows for a diversity of thermal ranges (i.e. $CT_{range} = CT_{max} - CT_{min}$) to occur. Among the ants we studied, there are at least three reasons to evolve a broad thermal range. The first is to be an opportunistic generalist: *Tapinoma* is a cosmopolitan genus (Menke et al., 2010) which we collected from Massachusetts to New Mexico and maintains a thermal range from 3.1°C to 50.0°C; it also increased at 73% of our study sites. The second is to occupy a thermally variable nest site: the largely arboreal genus *Crematogaster* lives in twigs and branches that supercool in winter and superheat in summer (Bujan et al., 2016; Kaspari et al., 2015); its thermal range averaged 4.4°C to 51.2°C and its incidence increased at 58% of sites. Finally, genera common at high elevations also experience the greatest range of temperature: *Leptothorax*, which we primarily collected along elevational gradients in Colorado and Oregon, had a thermal range from 3.8°C to 51.4°C and increased at 67% of its sites. These three genera—*Tapinoma*, *Crematogaster*, *Leptothorax*—had among the lowest CT_{min}s but all increased in the 2016-2017 resurvey, likely in part due to their high CT_{max}s.

In contrast to CT_{max}, CT_{min} is unpredictive of genus-level change for North American ants. This is not for a lack of existing variation in CT_{min} which is similar (11.0°C) to CT_{max} (14.4°C). Yet this result is somewhat surprising as environmental temperatures can be important correlates of lower thermal tolerances in ants, at least across elevation and geography (Bishop et al., 2017; Diamond & Chick, 2018; Warren & Chick, 2013). Perhaps one reason for this discrepancy is the varying cost of a mismatch between the environmental temperature and either of the two thermal limits. Given the non-linear, accelerating nature of a thermal performance

curve, small increases in temperature near the peak of the curve have a stronger effect on performance and as a consequence, small discrepancies between the temperature at peak performance and CT_{max} potentially have a greater effect on an organism's performance—the rate it traverses the curve—than small changes in CT_{min}.

Caveats and next steps

The temporal dynamics of ecological communities arise from multiple factors acting at varying time scales (Christensen, Harris, & Ernest, 2018; Dornelas et al., 2014; Fukami & Nakajima, 2011; Roeder, Roeder, & Kaspari, 2018b; Schröder, Persson, & De Roos, 2005). We focused on large chunks of undisturbed habitat (reducing the potential effects of fragmentation) where relatively long-lived species frequently nest underground (and thus have a thermal refuge) while often collectively acting as dietary generalists (avoiding the complexities of ontogenetic niche shifts). The ants, by many lines of reasoning, are thermophilic ectotherms that should benefit from moderate warming.

Furthermore, given the importance of long-term data in understanding biotic change in the Anthropocene (Cardinale et al., 2018; Eisenhauer et al., 2019; Hallmann et al., 2017; Lister & Garcia, 2018), our dataset has both strengths and weakness. Strengths include using standardized methods to quantify 20-year differences in incidence for all the common ant genera across 33 habitats ranging from hardwood forest to warm desert to alpine tundra, and testing hypotheses from important theory using a new dataset on thermal tolerance. A weakness is that we examine two snapshots separated by 20 years rather than a series of annual surveys. As a consequence, we cannot distinguish long-term changes from those that arise, and could be reversed, on an annual time scale. That said, these 33 resurveys required two years and over

three-person years to collect and analyze. Resurveys with short between sample intervals [e.g. monthly or yearly as performed in iconic studies of rodents (Ernest et al., 2016) and trees (Hubbell, Comita, Lao, & Condit, 2012)] while still costly in money and person hours would undoubtedly enhance our ability to quantify the pattern and cause of changes in ant communities in North America.

Acknowledgments

Literature Cited

Addo-Bediako, A., Chown, S. L., & Gaston, K. J. (2000). Thermal tolerance, climatic variability and latitude. Proceedings of the Royal Society of London B: Biological Sciences, 267, 739–745.

Alward, R. D., Detling, J. K., & Milchunas, D. G. (1999). Grassland vegetation changes and nocturnal global warming. Science, 283, 229–231.

Angilletta Jr., M. J. (2009). Thermal adaptation: a theoretical and empirical synthesis. New York, NY: Oxford University Press.

Bar-On, Y. M., Philips, R., & Milo, R. (2018). The biomass distribution on Earth. Proceedings of the National Academy of Sciences of the United States of America, 115, 6506–6511.

Baudier, K. M., Mudd, A. E., Erickson, S. C., & O'Donnell, S. (2015). Microhabitat and body size effects on heat tolerance: implications for responses to climate change (army ants: Formicidae, Ecitoninae). Journal of Animal Ecology, 84, 1322–1330.

Baudier, K. M., D'Amelio, C. L., Malhortra, R., O'Connor, M. P., & O'Donnell, S. (2018). Extreme insolation: climatic variation shapes the evolution of thermal tolerance at multiple scales. The American Naturalist, 192, 347–359.

Bishop, T. R., Robertson, M. P., Van Rensburg, B. J., & Parr, C. L. (2017). Coping with the cold: minimum temperatures and thermal tolerances dominate the ecology of mountain ants. Ecological Entomology, 42, 105–114.

Bujan, J., Yanoviak, S. P., & Kaspari, M. (2016). Desiccation resistance in tropical insects: causes and mechanisms underlying variability in a Panama ant community. Ecology and Evolution, 7, 6282–6291.

Burnham, K. P., & Anderson, D. R. (2002). Model selection and multimodel inference: a practical information-theoretic approach. New York, NY: Springer.

Calosi, P., Bilton, D. T., & Spicer, J. I. (2008). Thermal tolerance, acclimatory capacity and

    vulnerability to global climate change. Biology Letters, 4, 99–102.

Cardinale, B. J., Gonzalez, A., Allington, R. H., & Loreau, M. (2018). Is local biodiversity

    declining or not? A summary of the debate over analysis of species richness time trends.

    Biological Conservation, 219, 175–183.

Cerdá, X., Retana, J., & Cros, S. (1998). Critical thermal limits in Mediterranean ant species:

    trade-off between mortality risk and foraging performance. Functional Ecology, 12, 45–

    55.

Chen, I., Hill, J. K., Ohlemüller, R., Roy, D. B., & Thomas, C. D. (2011). Rapid range shifts of

    species associated with high levels of climate warming. Science, 333, 1024–1026.

Chown, S. L., & Nicolson, S. W. (2004). Insect physiological ecology: mechanisms and patterns.

    New York, NY: Oxford University Press.

Christensen, E. M., Harris, D. J., & Ernest, S. K. M. (2018). Long-term community change

    through multiple rapid transitions in a desert rodent community. Ecology, 99, 1523–1529.

Colinet, H., Sinclair, B. J., Vernon, P., & Renault, D. (2015). Insects in fluctuating thermal

    environments. Annual Review of Entomology, 60, 123–140.

Del Toro, I., Ribbons, R. R., & Pelini, S. L. (2012). The little things that run the world revisited:

    a review of ant-mediated ecosystem services and disservices (Hymenoptera: Formicidae).

    Myrmecological News, 17, 133–146.

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., &

    Martin, P. R. (2008). Impacts of climates warming on terrestrial ectotherms across

    latitude. Proceedings of the National Academy of Sciences of the United States of

    America, 105, 6668–6672.

Diamond, S. E., & Chick, L. D. (2018). The Janus of macrophysiology: stronger effects of evolutionary history, but weaker effects of climate on upper thermal limits are reversed for lower thermal limits in ants. Current Zoology, 64, 223–230.

Diamond, S., E., Sorger, D. M., Hulcr, J., Pelini, S. L., Del Toro, I., Hirsch, C., … Dunn, R. R. (2012). Who likes it hot? A global analysis of the climatic, ecological, and evolutionary determinants of warming tolerance in ants. Global Change Biology, 18, 448–456.

Diamond, S. E., Nichols, L. M., Pelini, S. L., Penick, C. A., Barber, G. W., Cahan, S. H., … Gotelli, N. J. (2016). Climatic warming destabilizes forest ant communities. Science Advances, 2, e1600842.

Dornelas, M., Gotelli, N. J., McGill, B., Shimadzu, H., Moyes, F., Sievers, C., & Magurran, A. E. (2014). Assemblage time series reveal biodiversity change but not systematic loss. Science, 344, 296–299.

Eisenhauer, N., Bonn, A., & Guerra, C. A. (2019). Recognizing the quiet extinction of invertebrates. Nature Communications, 10, 50.

Ernest, S. K. M., Yenni, G. M., Allington, G., Christensen, E. M., Geluso, K., Goheen, J. R., … Valone, T. J. (2016). Long-term monitoring and experimental manipulation of a Chihuahuan desert ecosystem near Portal, Arizona (1977-2013). Ecology, 97, 1082.

Fisher, B. L., & Cover, S. P. (2007). Ants of North America: a guide to the genera. Berkeley, CA: University of California Press.

Fukami, T., & Najajima, M. (2011). Community assembly: alternative stable states or alternative transient states? Ecology Letters, 14, 973–984.

Griffiths, H. M., Ashton, L. A., Walker, A. E., Hasan, F., Evans, T. A., Eggleton, P., & Parr, C.
L. (2018). Ants are the major agents of resource removal from tropical rainforests.
Journal of Animal Ecology, 87, 293–300.

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., … de Kroon, H.
(2017). More than 75 percent decline over 27 years in total flying insect biomass in
protected areas. PLoS One, 12, e0185809.

Hellmann, J. J., Byers, J. E., Bierwagen, B. G., & Dukes, J. S. (2008). Five potential
consequences of climate change for invasive species. Conservation Biology, 22, 534–
543.

Hoffmann, A. A., Chown, S. L., & Clusella-Trullas, S. (2013). Upper thermal limits in terrestrial
ectotherms: how constrained are they? Functional Ecology, 27, 934–949.

Hölldobler, B., & Wilson, E. O. (1990). The ants. Cambridge, MA: Harvard University Press.

Hubbell, S., Comita, L., Lao, S., & Condit, R. (2014). Barro Colorado fifty hectare plot census of
canopy density, 1983-2012. http://dx.doi.org/10.5479/data.bci20140711.

Huey, R. B., Deutsch, C. A., Tewksbury, J. J., Vitt, L. J., Hertz, P. E., Pérez, H. J. A., & Garland,
T. (2009). Why tropical forest lizards are vulnerable to climate warming. Proceedings of
the Royal Society of London B: Biological Sciences, 276, 1939–1948.

IPCC. (2014). Climate change 2014: Synthesis report. Contribution of working groups I, II, III to
the fifth assessment report of the intergovernmental panel on climate change. Geneva,
Switzerland: IPCC.

Kaspari, M., Alonso, L., & O'Donnell, S. (2000). Three energy variables predict ant abundance
at a geographical scale. Proceedings of the Royal Society of London B: Biological
Sciences, 267, 485–489.

Kaspari, M., Clay, N. A., Lucas, J., Yanoviak, S. P., & Kay, A. (2015). Thermal adaptation
generates a diversity of thermal limits in a rainforest ant community. Global Change
Biology, 21, 1092–1102.

Kellermann, V., Overgaard, J., Hoffmann, A. A., Flojgaard, C., Svenning, J., & Loeschcke, V.
(2012). Upper thermal limits of Drosophila are linked to species distributions and
strongly constrained phylogenetically. Proceedings of the National Academy of Sciences
of the United States of America, 109, 16228–16233.

Kingsolver, J. G., & Huey, R. B. (2008). Size, temperature, and fitness: three rules. Evolutionary
Ecology Research, 10, 251–268.

Kraft, N. J. B., & Ackerly, D. D. (2010). Functional trait and phylogenetic tests of community
assembly across spatial scales in an Amazonian forest. Ecological Monographs, 80, 401–
422.

Leather, S. R. (2018). "Ecological Armageddon" - more evidence for the drastic decline in insect
numbers. Annals of Applied Biology, 172, 1–3.

Lister, B. C., & Garcia, A. (2018). Climate-driven declines in arthropod abundance restructure a
rainforest food web. Proceedings of the National Academy of Sciences of the United
States of America, 115, E10397–E10406.

McGill, B. J., Enquist, B. J., Weiher, E., & Westoby, M. (2006). Rebuilding community ecology
from functional traits. Trends in Ecology & Evolution, 21, 178–185.

Menke, S. B., Booth, W., Dunn, R. R., Schal, C., Vargo, E. L., & Silverman, J. (2010). Is it easy
to be urban? Convergent success in urban habitats among lineages of a widespread native
ant. PLoS ONE, 5, e9194.

Moreau, C. S., & Bell, C. D. (2013). Testing the museum versus cradle tropical biological

    diversity hypothesis: phylogeny, diversification, and ancestral biogeographic range

    evolution of the ants. Evolution, 67, 2240–2257.

Ødegaard, F. (2000). How many species of arthropods? Erwin's estimate revised. Biological

    Journal of the Linnean Society, 71, 583–597.

Pagel, M. (1999). Inferring the historical patterns of biological evolution. Nature, 401, 877–884.

Parmesan, C., & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts

    across natural systems. Nature, 421, 37–42.

Parmesan, C., Ryrholm, N., Stefanescu, C., Hill, J. K., Thomas, C. D., Descimon, H., … Warren,

    M. (1999). Poleward shifts in geographical ranges of butterfly species associated with

    regional warming. Nature, 399, 579–583.

Penick, C. A., Diamond, S. E., Sanders, N. J., & Dunn, R. R. (2017). Beyond thermal limits:

    comprehensive metrics of performance identify key axes of thermal adaptation in ants.

    Functional Ecology, 31, 1091–1100.

Prather, R. M., Roeder, K. A., Sanders, N. J., & Kaspari, M. (2018). Using metabolic and

    thermal ecology to predict temperature dependent ecosystem activity: a test with prairie

    ants. Ecology, 99, 2113–2121.

Roeder, K. A., Roeder, D. V., & Kaspari, M. (2018a). The role of temperature in competition

    and persistence of an invaded ant assemblage. Ecological Entomology, 43, 774–781.

Roeder, K. A., Roeder, D. V., & Kaspari, M. (2018b). Disturbance mediates homogenization of

    above and belowground invertebrate communities. Environmental Entomology, 47, 545–

    550.

Sanchez-Bayo, F., & Wyckhuys, K. A. G. (2019). Worldwide decline of the entomofauna: a

    review of its drivers. Biological Conservation, 232, 8–27.

Schröder, A., Persson, L., & De Roos, A. M. (2005). Direct experimental evidence for alternative

    stable states: a review. Oikos, 110, 3–19.

Sinclair, B. J., Marshall, K. E., Sewell, M. A., Levesque, D. L., Willett, C. S., Slotsbo, S., …

    Huey, R. B. (2016). Can we predict ectotherm responses to climate change using thermal

    performance curves and body temperatures? Ecology Letters, 19, 1372–1385.

Soudzilovskaia, N. A., Elumeeva, T. G., Onipchenko, V. G., Shidakov, I. I., Salpagarova, F. S.,

    Khubiev, A. B., … Cornelissen, H. C. (2013). Functional traits predict relationship

    between plant abundance dynamic and long-term climate warming. Proceedings of the

    National Academy of Sciences of the United States of America, 110, 18180–18184.

Sunday, J. M., Bates, A. E., & Dulvy, N. K. (2011). Global analysis of thermal tolerance and

    latitude in ectotherms. Proceedings of the Royal Society of London B: Biological

    Sciences, 278, 1823–1830.

Walther, G., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J. C., … Bairlein, F.

    (2002). Ecological responses to recent climate change. Nature, 416, 389–395.

Warren, M. S., Hill, J. K., Thomas, J. A., Asher, J., Fox, R., Huntley, B., … Thomas, C. D.

    (2001). Rapid responses of British butterflies to opposing forces of climate and habitat

    change. Nature, 414, 65–69.

Warren, R. J., & Chick, L. (2013). Upward ant distribution shift corresponds with minimum, not

    maximum, temperature tolerance. Global Change Biology, 19, 2082–2088.

Wong, M. K. L., Guénard, B., & Lewis, O. T. (2019). Trait-based ecology of terrestrial

    arthropods. Biological Reviews, in press.

Table 1. AIC scores and weights of multiple linear regression models testing how thermal traits (CT_{max} or CT_{min}), mean annual temperature (MAT), and the change in temperature between the two surveys (ΔTemp) affected the proportion of sites where genera have increased in incidence. Models are ordered by the lowest ΔAIC. **Bolded *** indicates a significant coefficient at $P < 0.05$.

Model	Intercept	Trait	MAT	ΔTemp	AIC	ΔAIC	w_i	pseudo r^2
Critical Thermal maxima								
CT_{max}	**-2.67***	**0.06***	—	—	105.87	0.00	0.35	0.17
CT_{max} + MAT	-2.45	**0.07***	-0.05	—	106.72	0.85	0.23	0.21
CT_{max} + ΔTemp	-2.76	0.07	—	0.03	107.87	2.00	0.13	0.17
CT_{max} + MAT + ΔTemp	-1.42	0.06	-0.07	-0.32	108.31	2.44	0.10	0.22
Null	**0.33***	—	—	—	109.05	3.18	0.07	—
ΔTemp	**0.70***	—	—	-0.40	109.73	3.86	0.05	0.05
MAT + ΔTemp	**2.00***	—	-0.08	-0.77	109.82	3.95	0.05	0.11
MAT	0.55	—	-0.02	—	110.89	5.02	0.03	0.01
Critical Thermal minima								
Null	**0.36***	—	—	—	104.15	0.00	0.31	—
ΔTemp	0.65	—	—	-0.32	105.29	1.14	0.18	0.03
MAT + ΔTemp	1.75	—	-0.06	-0.64	105.99	1.84	0.12	0.08
MAT	0.53	—	-0.01	—	106.05	1.90	0.12	< 0.01
CT_{min}	0.38	< -0.01	—	—	106.15	2.00	0.11	< 0.01
CT_{min} + ΔTemp	0.74	-0.01	—	-0.34	107.24	3.09	0.07	0.03
CT_{min} + MAT + ΔTemp	1.75	0.02	-0.07	-0.66	107.92	3.77	0.05	0.08
CT_{min} + MAT	0.52	0.01	-0.02	—	108.05	3.90	0.04	< 0.01

Table 2. Linear, quadratic, and cubic regression models testing relationships between ant genera critical thermal maxima (CT_{max}) and the proportion of sites where genera increased in incidence. Models are ordered by the lowest ΔAIC. **Bolded *** indicates a significant coefficient at $P < 0.05$.

Model	Intercept	x	x^2	x^3	AIC	ΔAIC	w_i	pseudo r^2
Quadratic	**-33.76***	**1.41***	**-0.01***	—	103.52	0.00	0.51	0.29
Cubic	163.37	-11.55	0.27	< -0.01	104.52	1.00	0.31	0.32
Linear	**-2.67***	**0.06***	—	—	105.87	2.35	0.16	0.17
Null	**0.33***	—	—	—	109.05	5.53	0.03	—

Figure Legends

Figure 1. Geographic distribution of 33 sampled ant communities that have increased in temperature by an average of 1°C after 20 years. The color of each point is scaled along a gradient from light blue, indicating a slight decrease in temperature, to bright red, indicating an increase in temperature.

Figure 2. Relationship between ant thermal traits and the proportion of sites where genera increased in incidence. Each point represents an ant genus. Panel (a) depicts CT_{max} and panel (b) depicts CT_{min} (model coefficients can be found in Table 1). The color of each point is scaled from light blue to bright red indicating decreasing to increasing average temperature change, respectively, at locations where that genus was found (see Fig. 1 for more details). Vertical dashed lines represent average CT_{max} or CT_{min} across all genera. Points above the horizontal dashed lines represent genera where incidence has increased at more than 50% of sites.
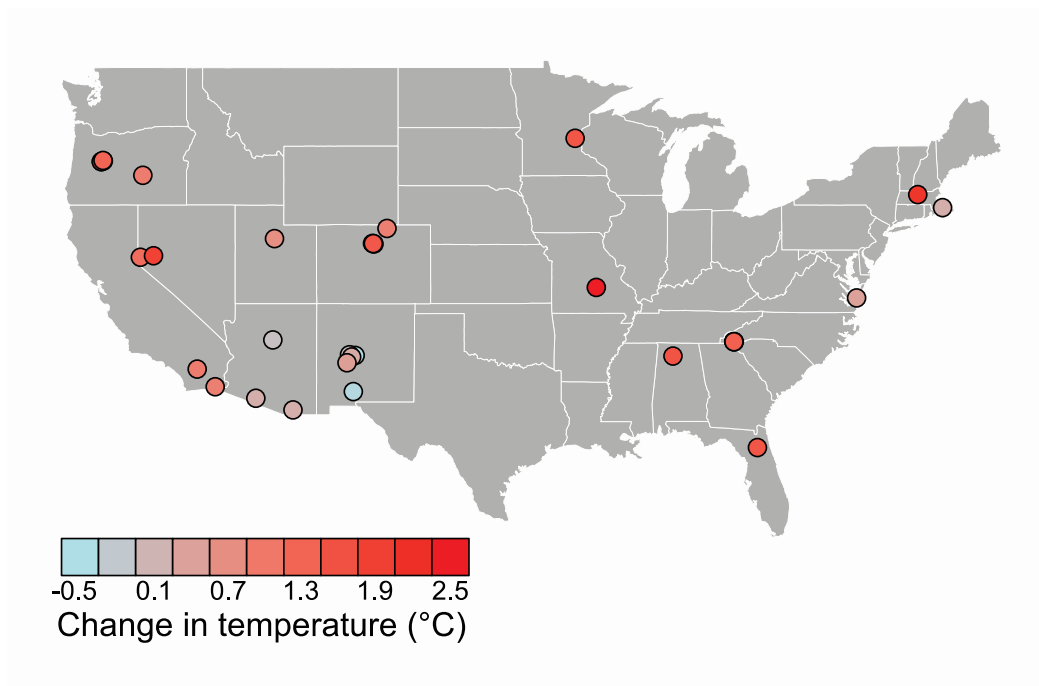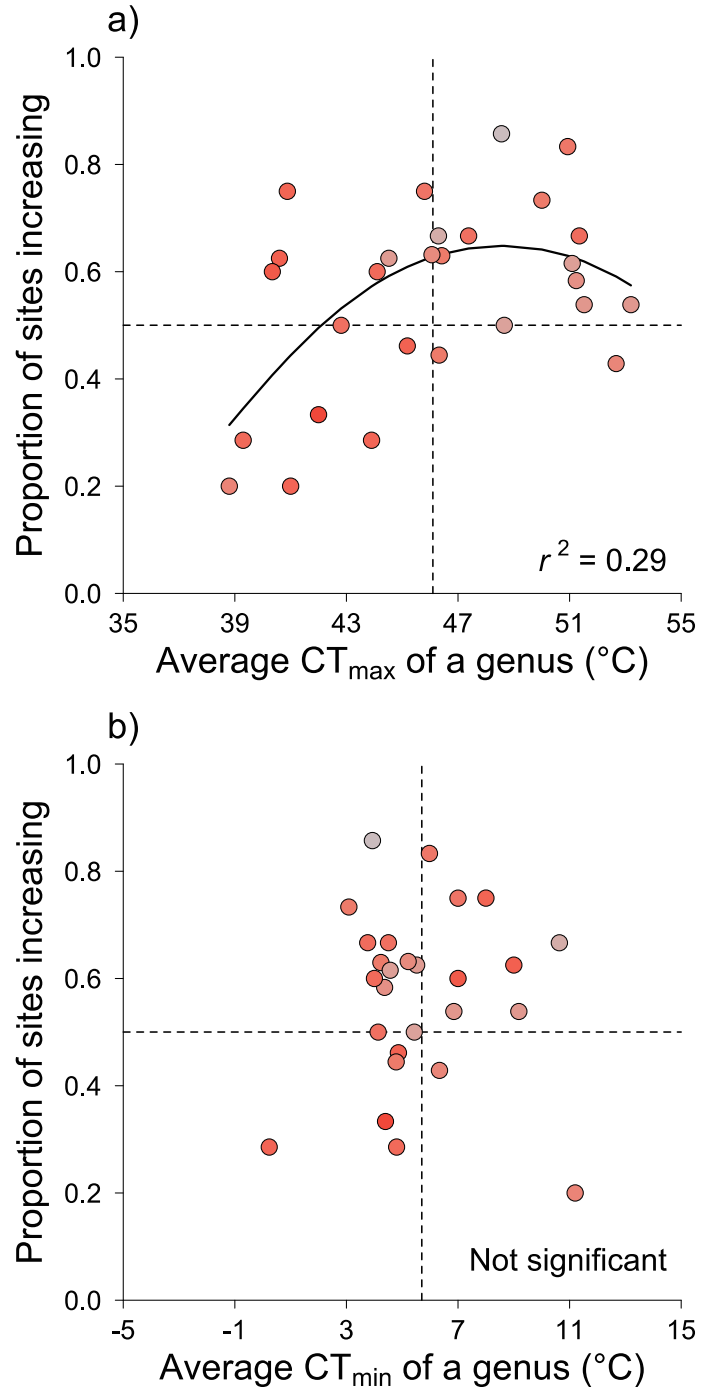
Figure 1.

Figure 2.

Chapter 3: Appendix S1. Supplemental Data.

Table S1. Locality and sampling date for 33 ant communities across North America.

Site	Latitude	Longitude	First Survey	Resurvey
Appleton-Whittell Research Ranch	31.593	-110.497	1996	2017
Bankhead National Forest	34.333	-87.187	1995	2017
Boyd Deep Canyon Desert Research Center	33.669	-116.371	1994	2017
Cedar Creek Ecosystem Science Reserve LTER	45.399	-93.195	1995	2017
Central Plains Experimental Range	40.822	-104.730	1994	2016
Coconino National Forest	35.153	-111.737	1996	2016
Coweeta LTER - High Transect	35.067	-83.462	1997	2016
Coweeta LTER - Low Transect	35.060	-83.429	1995	2016
Coweeta LTER - Mid Transect	35.060	-83.449	1997	2016
East Mesa Geothermal Area	32.770	-115.267	1994	2017
Fallon	39.433	-119.055	1994	2016
Galena Creek Regional Park	39.356	-119.857	1995	2016
H. J. Andrews LTER - Frissell Ridge	44.250	-122.127	1996	2016
H. J. Andrews LTER - Low Transect	44.213	-122.254	1995	2016
H. J. Andrews LTER - Vanilla Leaf Meadow	44.273	-122.149	1996	2016
H. J. Andrews LTER - Watershed 8	44.213	-122.255	1996	2016
Harvard Forest LTER	42.538	-72.177	1996	2017
Jornada Basin LTER	32.523	-106.797	1995	2016
Langmuir Laboratory for Atmospheric Research	33.991	-107.183	1997	2016
Mark Twain National Forest	37.819	-91.894	1996	2017
Myles Standish State Forest	41.874	-70.650	1995	2017
Niwot Ridge LTER - Alpine	40.059	-105.617	1996	2016
Niwot Ridge LTER - Pine	40.033	-105.530	1996	2016
Niwot Ridge LTER - Spruce	40.046	-105.572	1996	2016
Northern Great Basin Experimental Range	43.518	-119.710	1996	2016
Ordway-Swisher Biological Station	29.678	-82.014	1996	2017

Organ Pipe Cactus National Monument	32.183	-112.773	1996	2017
Sevilleta LTER - Deep Wells	34.366	-106.685	1997	2016
Sevilleta LTER - Red Tank	34.398	-107.037	1997	2016
Sevilleta LTER - Rio Salado	34.287	-106.904	1997	2016
Sevilleta LTER - Station	34.356	-106.885	1996	2016
Uinta National Forest	40.302	-111.628	1996	2016
Virginia Coast Reserve LTER	37.289	-75.929	1995	2017

CHAPTER FOUR

Formatted for publication in *Oikos*

PATCHINESS IN LITTER MITES, SPRINGTAILS, AND SPIDERS: TESTING THE ROLE

OF BODY SIZE AND LITTER DEPTH ACROSS SIX FORESTS IN NORTH AMERICA

Karl A. Roeder[1], Michael D. Weiser[1], and Michael Kaspari[1]

[1] Department of Biology, Geographical Ecology Group, University of Oklahoma, Norman, OK,

73019, USA

Abstract

Ecologists search for rules by which organismal traits dictate the abundance and distribution of species across environmental gradients. Here we search for rules that apply across three common litter invertebrates in six North American forests from Panama to Oregon. We use image analysis to quantify the abundance and body size distributions of mites, springtails, and spiders in 21-m^2 plots per forest. We contrast three hypotheses: two of which focus on trait-abundance relationships and a third linking abundance to species richness. Despite four orders of magnitude variation in body size, the predicted negative relationship between mean body size and abundance per m^2 occurred in only ca. 24% of cases—never for large bodied taxa like spiders. We likewise found only ca. 18% of tests supported our prediction that increasing litter depth allows for high abundance; 2/3 of which occurred at a single deciduous forest in Massachusetts. In contrast, invertebrate abundance constrained species richness ca. 82% of the time, often mediating any effect that body size and litter depth may have had on species richness. Our results suggest that size and habitat space in brown food webs are rarely good predictors of patchiness in abundance, but that patchiness in diversity is generally well predicted by abundance.

Keywords: abundance; body size; diversity; litter depth; richness; LTER

Introduction

The abundance and richness of species varies across ecological space and through evolutionary time. Understanding why and how these two components of diversity change has long been a core objective for ecologists (MacArthur 1965, Whittaker 1972, Huston 1979,

Ricklefs and Schluter 1993, Rosenzweig 1995). Numerous hypotheses about spatial and temporal drivers of diversity have been proposed throughout the years (Pianka 1966, May 1975, Tilman 1987, Cardinale et al. 2004).Yet in some systems like brown food webs [i.e. detritus, decomposers, and their consumers (Kaspari and Yanoviak 2009)], where an assortment of heterogeneous nutrients and structure support one of the most diverse biotas on earth (Swift et al. 1979; Wardle 2002), questions remain about how so many species can coexist without biotic mechanisms reducing diversity (Anderson 1975). Here we focus our efforts on two potential drivers of diversity in brown food webs—body size and litter depth—and test hypotheses on how these drivers shape patterns of abundance and richness in litter taxa across six forests in North America.

How body size and litter depth constrain abundance

The range of body sizes among animals spans more than twenty-one orders of magnitude (Smith and Lyons 2013). From the tiny crustacean, *Stygotantulus stocki* (<0.1 mm), to the colossal squid, *Mesonychoteuthis hamiltoni* (>12 m), invertebrates occupy a large portion of this size range and can increase throughout their ontogeny by as much as 43,000% (Klok and Chown 1999). It should not be surprising, thus, that size plays an integral role in determining an organism's temporal and spatial scale of operation (Peters 1983, Calder 1984, Schmidt-Nielsen 1984). Size, however, also underlies trade-offs as the demand for resources and habitat increases with body mass (Brown et al. 1995; Rosenzweig 1995). Indeed, body size has been linked to numerous traits and aspects of an organism's life history and ecology such as abundance (White et al. 2007), fecundity (Honek 1993), foraging (Mittelbach 1981), metabolism (Brown et al. 2004), range size (Gaston and Blackburn 1996), and trophic position (Roeder and Kaspari 2017).

A large effort has focused specifically on disentangling size-abundance relationships as they represent a link between an individual's traits and the structure of the community within which that individual resides (Blackburn et al. 1993; Russo et al. 2003; Ernest 2005; White et al. 2007). We propose two hypotheses below that focus on ways in which either body size or litter depth regulate the abundance of litter invertebrates.

The **body size-abundance hypothesis** posits that the number of individuals of a certain taxon is constrained by the average body size of that taxon. Within a given area there often resides a finite amount of resources which limits growth (Tilman 1982, Chase and Leibold 2003; Mittelbach 2012). When organisms are small, these resources can be divided among a large number of individuals. Conversely, when organisms grow and monopolize larger amounts of the resource pool, habitats consequently support fewer individuals. While this relationship may not be linear as metabolism, or the biological processing of energy, scales as ¾ powers of body mass (Peters 1993, Brown et al. 2004), we nonetheless predict that body size, indirectly through resource use, regulates abundance.

The **litter depth-abundance hypothesis** posits that a taxon's abundance is constrained by the size of the habitat in which it lives. Leaf litter is a complex substrate that, up to this point, has rarely been quantitatively indexed according to its habitat variety. For soil organisms, leaf litter qualifies as a resource because it contributes to the growth rate of the consumer population and is consumed, however, it is also the habitat because its complex three-dimensional structuring offers a number of potential niche axes to partition (Hansen and Coleman 1988, Wardle 2002, Wagner et al. 2003). Moreover, leaf litter can vary 16-fold in depth at the 1-m^2 level across forest stands in Panama and Peru creating a variety of habitats on which invertebrates can specialize (Kaspari and Yanoviak 2008). If resource availability is limited by

the size of the habitat (i.e. litter depth) and more resources can support more individuals, we predict that larger habitats should support more individuals.

Abundance as a constraint on species richness: the more-individuals hypothesis

Species richness represents another important property of communities that changes from place to place across the globe (Brown 1995; Rosenzweig 1995; Kaspari et al. 2000). In speciose locales, much debate has arisen on the abiotic and biotic constraints that determine the number of species (Hutchinson 1961; Anderson 1975; Wardle 2002; Lessard et al. 2016). One such constraint is the amount of available energy within a system: **the more-individuals hypothesis** or **MIH** (Wright 1983; Currie 1991; Srivastava and Lawton 1998; Kaspari et al. 2000; Hurlbert and Jetz 2010; Storch et al. 2018). The MIH posits that energy, often reported as net primary productivity or NPP, limits the number of individuals that an ecosystem can support. As populations require a certain quantity of individuals to remain viable (MacArthur and Wilson 1967), the number of species is consequently limited by abundance. Abundance can therefore constrain another property of taxocenes: their species richness.

While tests of the MIH are often performed across geographically defined gradients of productivity, here we test if abundance-richness relationships exist at a smaller spatial grain that is more appropriately scaled to our taxa of interest. Leaf litter systems are notoriously patchy (Yanoviak and Kaspari 2000; Wardle 2002; Richardson et al. 2005) and there has been little systematic study of the role of body size and litter depth in generating this community level patchiness. Moreover, litter communities are composed of a diversity of orders (Hattenschwiler et al. 2005; Kaspari and Yanoviak 2009; Clay et al. 2014). Using six surveys from alpine coniferous forests to tropical rainforests, we test the generality of the MIH alone and in

conjunction with our two abundance hypotheses to quantify the direct and indirect effects of body size and litter depth on the abundance and richness of leaf litter invertebrates.

Methods

Study sites

Samples were collected from six forests in North America: Andrews LTER, Oregon (AND); Barro Colorado Island, Panama (BCI); Coweeta LTER, North Carolina (CWT); Harvard Forest LTER, Massachusetts (HFR); Luquillo LTER, Puerto Rico (LUQ); and Niwot Ridge LTER, Colorado (NWT). These sites vary in type from tropical rainforest (BCI, LUQ) to temperate deciduous (CWT, HFR) or coniferous forests (AND, NWT) across approximately 35° of latitude (Fig. 1). At each site, 21 1-m^2 plots were arrayed in a cross design with plots placed 1, 10, 50, 100, and 200 m at each cardinal direction from a central plot (see Zhou et al. 2016 for more details).

Invertebrate sampling

Invertebrates were collected from each plot by sifting litter and approximately 0.5 cm of mineral soil through 1 cm mesh screens. Invertebrates were then extracted from the siftate over 48 hours in Tulgren funnels with 25 W bulbs (Weiser et al. 2018). Individuals were sorted taxonomically, counted, and assigned to morphospecies—which often represents a reliable estimate of species richness for invertebrate community analyses (Oliver and Beattie 1996; Roeder et al. 2018). Mites (Acari), springtails (Collembola), and spiders (Arachnida) were the most abundant invertebrate groups collected within most plots and we focus on differences amongst these three groups for our analyses.

Body size and litter depth measurements

For each plot, all individuals from the three focal groups listed above were photographed within a 10 mm x 10 mm box set at 10x magnification using a Leica EC3 digital camera with FireCam software version 3.4.1 (Leica Microsystems Inc., Wetzlar, Germany) mounted on a Leica S8 APO stereo microscope. After photos were cropped and resized using Adobe Photoshop CS3 version 10.0.1 (Adobe Systems Inc., San Jose, CA, USA), the length and width of each individual, representing the major and minor axes respectively, were measured in mm using ImageJ (U.S. National Institutes of Health, Bethesda, MD, USA). As the body shape of most invertebrates roughly resembles an ellipse which may be a better correlate of size (i.e. mass), we also calculated ellipse area using the equation:

$$\text{Ellipse Area} = \pi * \left(\frac{\text{Length}}{2}\right) * \left(\frac{\text{Width}}{2}\right)$$

To validate our methods, digital measurements from a subset of mites (N = 84) were compared to those from an ocular micrometer and found to be similar (Fig. S1; $r^2 = 0.9961$, $P < 0.0001$). We then dried those 84 mites to constant mass at 60°C for 48 h and weighed them to the nearest 0.001 mg on a Cahn microbalance (Cahn Instruments, Cerritos, California, USA). Of the three body size measurements, ellipse area was the best correlate to mass (Fig. S2; $r^2 = 0.88$, $P < 0.0001$) and subsequently used in all hypothesis tests. Litter depth, our measure of habit size for invertebrates, was measured 1 cm from the four corners of each plot and averaged. We exclude two plots from our analysis, one at AND due to litter depth not being recorded and the second at

NWT due to the absence of any individual from our selected taxa. This changes the total number of plots used to 124.

Statistics for hypothesis testing

Analyses were run in R, version 3.5.1. All variables were $\log_{10}$ transformed to meet the assumptions of normality. Predictor variables in multiple regression models were checked for multicollinearity using a variance inflation factor (VIF) cutoff = 3 (Zuur et al. 2010) in the "car" package. We focus on patterns of abundance and richness within sites, replicating analyses for each location (N = 6) and taxonomic group (N = 3) to determine the generality of our results.

Abundance—To test the body size-abundance and litter depth-abundance hypotheses, we used ordinary least squares (OLS) regression in an information theoretic approach to rank all possible regression models by Akaike's information criterion corrected for small sample size (AIC_c). ΔAIC_c values for each model were calculated from the difference of the AIC_c of ith model and the model with the lowest AIC_c value. Akaike weights (w_i) were then calculated and represent a weight of evidence that model i was the best fit (Burnham and Anderson 2002). AIC_c and Akaike weights (w_i) were calculated in the "MuMIn" and "qpcR" packages. We test the prediction that a smaller mean body size results in more individuals being supported within a given area and that deeper litter (i.e. more habitat) resulted in more individuals being supported. While we propose hypothetical reasons for why both body size and litter depth could individually affect abundance, we also test for interactions between these two variables as both may be working together to drive patterns of abundance.

Richness—To test the more-individuals hypothesis, we used OLS regressions to compare abundance to species richness across plots within sites. We further tested causal pathways to

determine whether abundance acts as a mediator between body size/litter depth and richness, or if body size/litter depth by itself was a better predictor of richness. We did this by fitting a structural equation model (SEM) to estimate the direct and indirect effects on richness in the "lavaan" package using only the previously determined size-abundance regression models within 2 ΔAIC_c of the top model and not within 2 ΔAIC_c of any null model. We predict that if we find strong support for the MIH (i.e. abundance is correlated with richness), that there will be a significant indirect effect in our mediation models such that body size or litter depth drive patterns of abundance which consequently drive patterns of richness.

Results

Across six forests in North America, we collected 46,898 mites, springtails, and spiders that varied in size across four orders of magnitude. These focal taxa were patchily distributed at the 1-m^2 scale, yet much of that patchiness could be found in any given forest. Tropical rainforests and temperate deciduous forests supported a larger variety of body sizes [Coefficient of Variation (hereafter CV): BCI = 5.09; CWT = 3.68; HFR = 3.36; LUQ = 3.18] than temperate coniferous forests (CV: AND = 1.14; NWT = 0.79). However, this was often dependent on the taxonomic group. Litter depth varied less within sites (CV: AND = 0.31, BCI = 0.51; CWT = 0.38; HFR = 0.54; LUQ = 0.37; NWT = 0.44) but a 3.3-fold difference in mean values was still observed across sites as deep litter banks were maintained at locations like AND (Mean = 11.9 cm) and CWT (Mean = 12.2 cm) compared to HFR (Mean = 3.7 cm) or LUQ (Mean = 5.1 cm).

Testing body size and litter depth-abundance hypotheses

The predicted negative relationship between mean body size and abundance per m^2 was observed in 3 of 6 the sites for mites, 2 of 6 the sites for springtails, and in none of the sites for spiders (Table 1). Similarly, the predicted decrease in abundance as litter depth declined was found in 2 of 6 sites for mites, and in 1 site each for collembola and spiders (Table 1). Although we occasionally found competing models that contained the combination of body size and litter depth as the top model or within <2 AIC$_c$ of the top model, there was no evidence of interaction between these two drivers (i.e. interaction models were always >2 AIC$_c$ of the top model).

Testing the more-individuals hypothesis

We found stronger support for the more-individuals hypothesis, as it was significant in 14 of the 17 (82%) taxa by site combinations we tested (Fig. 2). For mites, richness m^{-2} increased with abundance m^{-2} at 4 of the 6 sites (AND, BCI, CWT, NWT; Fig. 2a) with abundance accounting for 50 to 73% of the variation in species richness (P's < 0.001; Fig. 2a). Similarly, at every location besides NWT (P = 0.08), 26 to 83% of the species richness of springtails was accounted for by abundance (P's < 0.5; Fig. 2b). All abundance-richness relationships for spiders were significant (P's < 0.5, r^2's = 0.23-0.73) despite the fact that spiders occurred in fewer plots on average (Fig. 2c).

Disentangling body size and litter depth-abundance-richness relationships

We used the seven significant, single parameter models from our body size and litter depth hypothesis tests (Table 1) to build SEMs. Our goal was to determine if abundance acts as a mediator between size and richness, or if body size/litter depth by itself was a better predictor of

species richness. We discuss our four body size SEMs first and then the remaining three litter depth SEMs.

The direct effect of body size on richness was mediated by abundance in two of four SEMs: AND mites (Fig. 3) and BCI mites (Fig. S3a). In other words, these sites supported more individuals when those individuals were small, and in turn, more abundant sites had higher species richness. The direct effect of body size, however, was not fully mediated in HFR springtails (standardized path coefficient = 0.246, P = 0.022; Fig. S4a), suggesting that abundance alone does not account for all of the variation in species richness as this location. For LUQ springtails, we found inconsistent mediation as the direct (standardized path coefficient = 0.153) and indirect effects (standardized path coefficient = -0.521) contained opposing signs resulting in a non-significant overall model (net effect = -0.369, P = 0.343; Fig. 4c).

Abundance mediated the direct effect of litter depth on richness in two of three SEMs: CWT mites (Fig. S3b) and HFR springtails (Fig. S4b). Thus, at these two locations, deeper litter supported more individuals, which in turn supported more species. The one SEM where this was not the case was HFR spiders (Fig. S5), where the indirect effect only approached significance (standardized path coefficient = 0.416, P = 0.065) because of a low plot sample size (N = 14) and small path coefficients between litter depth and abundance (0.818), and between abundance and richness (0.508).

Discussion

Our body size and litter depth-abundance hypotheses assume that resources in the litter (e.g., food, predator free space) are important constraints to the fitness of taxa. Yet despite 4 orders of magnitude variation in body size and a 3.3 fold variation in litter volume across a range

of forest types, we generally found weak and inconsistent support for either of these models (~21%), especially in the largest sized taxa—spiders. In contrast, abundance itself regularly constrains species richness (~82% of all models) and often mediates any effect that body size and litter depth may have had on species richness. Combined, it suggests the 292-fold variation in abundance m^{-2} is a useful shortcut to estimating a brown food web's local richness. We discuss our results in terms of their generality towards ecological theory and why site- or taxa-level differences may result in such variable outcomes.

Why body size did not work but why it did best for mites

Evidence accumulates for patterns between body size and abundance (Damuth 1987) that are less clear at smaller spatial grains of observation (White et al. 2007). For example, despite large scale patterns existing between body size and population density (Cyr et al. 1997), body size was a poor predictor of abundance at the species level in assemblages of birds, insects, and aquatic invertebrates (Blackburn et al. 1993). In congruence, our data suggest that when a taxon was independently analyzed within a site (i.e. only mites, springtails, or spiders), body size alone was a weak predictor of abundance and consequently species richness (Table 1; Figs. 3, S3a, S4a, S4c). Community level patterns may therefore require a more complete "assemblage analysis" to sort out the mechanisms that govern how multiple taxa are organized (Gaston et al. 2008; Chown and Gaston 2010). We nevertheless examined the taxa-specific responses and discuss two reasons why they may occur.

First, a diverse biota of decomposers and their consumers exist in the brown food web (Hättenschwiler et al. 2005; Lavelle et al. 2006; Bardgett and van der Putten 2014; Griffiths et al. 2018). However, when species are lumped into broad taxonomic groups, such groups can contain

106

a functionally diverse subset of the overall food web. As different size-abundance relationships may occur within and across trophic levels (Kerr and Dickie 2001; White et al. 2007), perhaps this is one reason why we observed greater support for body size-abundance relationships in functionally diverse groups like mites—which contain detritivores, omnivores, and predators (Maraun and Scheu 2000)—compared to functionally restricted groups like spiders.

Second, body size-abundance relationships may be density dependent. Mites and springtails comprise up to 95% of the total number of microarthropods in many ecosystems (Seastedt 1984; Bardgett and Cook 1998). Spiders, in comparison, make up only a fraction of the abundance of these groups and are often more patchily distributed (Uetz 1975). We observed such discrepancies in abundance at sites like Harvard Forest where we recorded 84-fold less spiders (218) than mites (18348). If size-abundance relationships only become apparent when a certain number of individuals are present, then there may have not been enough power to test our hypotheses in the less abundant taxa like spiders.

Why litter depth did not work in general

We originally hypothesized greater amounts of litter would allow for more individuals to occur, in turn regulating the number of species as diversity is often correlated with ecosystem size (Post et al. 2000; Fukami 2004; Kaspari and Yanoviak 2009). Yet we found little support for a relationship between litter depth and abundance. Of the three significant models (Table 1), two occurred at HFR which had the least amount of litter (Mean = 3.7 cm). Thus, if litter depth is limiting abundance at HFR, but not elsewhere, then perhaps a threshold exists at which litter depth no longer restricts the number of individuals for particular taxa. Alternatively, as the litter at HFR had the highest coefficient of variation across plots (CV = 0.54), habitat heterogeneity

within a site could create patchy environments where taxa can increase when rare (Hansen and Coleman 1998; Rainey and Travisano 1998; Wardle 2002). For example, springtail and spider assemblages exhibit vertical stratification in leaf litter systems when microhabitat differences occur, increasing overall diversity through niche partitioning (Uetz 1975; Faber and Joose 1993; Wagner et al. 2003).

Why the more-individuals hypothesis worked best

Species richness was constrained by abundance at all sites, and for most taxonomic groups (Fig. 2). We posit one reason that such strong patterns occur is due to the intrinsic nature of how species richness scales with abundance—there cannot be more species than individuals in a given unit of area. Yet the abundance of mites, despite being 9.6 and 63.8-fold greater than springtails and spiders, surprisingly failed to predict richness at two locations: HFR and LUQ. Both sites maintained on average lower amounts of litter (HFR = 3.7 cm; LUQ = 5.1 cm), but plots within these sites that had deeper litter did not necessarily support a greater number of individuals. And while litter depth was a poor predictor of abundance, and consequently species richness, across all measured taxa, we acknowledge a simple observation: not all leaf litter is the same. Leaf litter can vary in elemental composition, moisture, and decomposition rate (Aerts 1997; Kaspari et al. 2008; Gora et al. 2018)—all of which may directly or indirectly affect the abundance and richness of invertebrates.

Conclusions

Animals and plants, along with their fundamental properties, change in abundance as you move from place to place (MacArthur 1972; Brown 1995; Swenson et al. 2012; Holt et al. 2018).

Often such changes have been measured along environmental gradients like moisture or temperature (Whittaker 1960; Kaspari et al. 2000; Mittelbach et al. 2001; Sanders et al. 2007). In doing so, macroecology looks for overarching principles that explain how sampled communities, on average, should appear and function across large spatial and temporal scales (Brown and Maurer 1989; Shade et al. 2018). Yet, the communities which make up these geographic patterns may operate differently from each other, resulting in a lack of clear signal when scaled down to within a single location or across a subset of sites.

We, thus, propose two working hypotheses that may account for the observed patchiness in litter invertebrate abundance. First, mites, springtails, and spiders make up a portion of leaf litter communities that are fluid in time. Our tacit assumption is that abundance m^{-2} is fixed, yet this is rarely the care as predacious spiders can be highly mobile (Clarke and Grant 1968) and disturbance by abiotic [e.g. rainfall (Frith and Frith 1990)] and biotic factors [e.g. army ant raids (Kaspari et al. 2011)] can drastically change invertebrate densities. Abundance may therefore be governed by a variety of factors working in concert that cannot be captured in a single snapshot. Second, while we did not focus on temperature variation within sites, as differences were minimal across plots, we acknowledge that mean annual temperature ranged from 2.5 to 25.7°C, a 10.3-fold difference, across our six sites (macroeco.lternet.edu). We posit that cold locales may have additional constraints on size or abundance that limited the utility of our hypotheses. In rodents, for example, the distribution of body sizes was recently found to be more similar across species at sites with lower minimum temperatures (Read et al. 2018). Thus, body size and litter depth may fail to predict abundance at locations that are abiotically challenging like NWT in Colorado, which had the coldest mean temperature and low but similar coefficients of variation in size for mites (CV = 0.76) and springtails (CV = 0.78).

Disentangling all of these site and taxa specific differences has led some to consider community ecology a "mess" (Lawton 1999) or incapable of containing general rules due to their complex nature (Simberloff 2004). Yet, we disagree with the notion that community ecology is incapable of containing general rules as clear abundance-richness relationships were observed within each site and for most taxonomic groups, often completely mediating the direct effect of body size or litter depth.

Acknowledgements

Literature Cited

Aerts, R. 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. – Oikos 79: 439–449.

Anderson, J. M. 1975. The enigma of soil animal species diversity. – In: Vaněk, J. (ed.), Progress in Soil Zoology. Springer, pp. 51-58.

Bardgett, R. D. and Cook, R. 1998. Functional aspects of soil animal diversity in agricultural grasslands. – Applied Soil Ecology. 10: 263–276.

Bardgett, R. D. and van der Putten, W. H. 2014. Belowground biodiversity andecosystem functioning. – Nature 515: 505–511.

Blackburn, T. M. et al. 1993. The relationship between abundance and body size in natural animal assemblages. – Journal of Animal Ecology 62: 519–528.

Brown, J. H. 1995. Macroecology. – The Univ. of Chicago Press.

Brown, J. H. and Maurer, B. A. 1989. Macroecology: the division of food and space among species on continents. – Science 243: 1145–1150.

Brown, J. H. et al. 1995. Spatial variation in abundance. – Ecology 76: 2028–2043.

Brown, J. H. et al. 2004. Toward a metabolic theory of ecology. – Ecology 85: 1771–1789.

Burnham, K. P. and Anderson, D. R. 2003. Model selection and multimodel inference: A practical information-theoretic approach. – Springer.

Calder, A. A. 1984. Size, function, and life history. – Harvard University Press.

Cardinale, B. J. 2004. Effects of species diversity on the primary productivity of ecosystems: extending our spatial and temporal scales of inference. – Oikos 104: 437–450.

Chase, J. M. and Leibold, M. A. 2003. Ecological niches: linking classical and contemporary approaches. – University of Chicago Press.

Chown, S. L. and Gaston, K. J. 2010. Body size variation in insects: a macroecological perspective. – Biological Reviews 85: 139–169.

Clarke, R. D. and Grant, P. R. 1968. An experimental study of the role of spiders as predators in a forest litter community. Part 1. – Ecology 49: 1152–1154.

Clay, N. A. et al. 2014. Short-term sodium inputs attract microbe-detritivores and their predators. – Soil Biology and Biochemistry 75: 248–253.

Currie, D. 1991. Energy and large-scale patterns of animal- and plant-species richness. – The

    American Naturalist 137: 27–49.

Cyr, H. et al. 1997. Population density and community size structure: comparison of aquatic and

    terrestrial systems. – Oikos 80: 139–149.

Damuth, J. 1987. Interspecific allometry of population-density in mammals and other animals –

    the independence of body-mass and population energy-use. – Biol. J. Linn. Soc. 31: 193–

    246.

Ernest, S. K. M. 2005. Body size, energy use, and community structure of small mammals. –

    Ecology 86: 1407–1413.

Faber, J. H. and Joose, E. N. G. 1993. Vertical distribution of Collembola in a *Pinus nigra*

    organic soil. – Pedobiologia 37: 336–350.

Frith, D. and Frith, C. 1990. Seasonality of litter invertebrate populations in an Australian upland

    tropical rain forest. – Biotropica 22: 181–190.

Fukami, T. 2004. Assembly history interacts with ecosystem size to influence species diversity. –

    Ecology 85: 3234–3242.

Gaston, K. J. and Blackburn, T. M. 1996. Range size-body size relationships: evidence of scale

    dependence. – Oikos 75: 479–485.

Gaston, K. J. et al. 2008. Ecogeographic rules: elements of a synthesis. – Journal of

    Biogeography 35: 483–500.

Gora, E. M. et al. 2018. Decomposition of coarse woody debris in a long-term litter manipulation

    experiment: a focus on nutrient availability. – Functional Ecology 33: 1128–1138.

Griffiths, H. M. et al. 2018. Ants are the major agents of resource removal from tropical

    rainforests. – Journal of Animal Ecology 87: 292–300.

Hansen, R. A. and Coleman, D. C. 1998. Litter complexity and composition are determinants of the diversity and composition of oribatid mites (Acari:Oribatida) in litterbags. – Applied Soil Ecology 9: 17–23.

Hättenschwiler, S. et al. 2005. Biodiversity and litter decomposition in terrestrial ecosystem. – Annual review of Ecology, Evolution, and Systematics. 36: 191–218.

Holt, B. G. et al. 2018. Environmental variation is a major predictor of global trait turnover in mammals. – Journal of Biogeography. 45: 225–237.

Honek, A. 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. – Oikos 66: 483–492.

Hurlbert, A. H. and Jetz, W. 2010. More than "more individuals": the nonequivalence of area and energy in the scaling of species richness. – The American Naturalist. 176: E50–E65.

Huston, M. 1979. A general hypothesis of species diversity. – The American Naturalist 113: 81–101.

Hutchinson, G. E. 1961. The paradox of the plankton. – The American Naturalist 95: 137–145.

Kaspari, M. and Yanoviak, S. P. 2008. Biogeography of litter depth in tropical forests: evaluating the phosphorus growth rate hypothesis. – Functional Ecology 22: 919–923.

Kaspari, M. and Yanoviak, S. P. 2009. Biogeochemistry and the structure of tropical brown food webs. – Ecology 90: 3342–3351.

Kaspari, M. et al. 2000. Three energy variables predict ant abundance at a geographical scale. – Proceedings of the Royal Society B. 267: 485–489.

Kaspari, M. et al. 2008. Multiple nutrients limit litterfall and decomposition in a tropical forest. – Ecology Letters 11: 35–43.

Kaspari, M. et al. 2011. Predation and patchiness in the tropical litter: do swarm-raiding army ants skim the cream or drain the bottle? – Journal of Animal Ecology 80: 818–823.

Kerr, S. R. and Dickie, L. M. 2001. Biomass Spectrum. – Columbia University Press.

Klok, C. J. and Chown, S. L. 1999. Assessing the benefits of aggregation: thermal biology and water relations of anomalous Emperor Moth caterpillars. – Functional Ecology 13: 417–427.

Lavelle, P. et al. 2006. Soil invertebrates and ecosystem services. – European journal of soil biology. 42: S3–S15.

Lawton, J. H. 1999. Are there general laws in ecology? – Oikos. 84: 177–192.

Lessard, J. P. et al. 2016. Process-based species pools reveal the hidden signature of biotic interactions amid the influence of temperature filtering. – The American Naturalist. 187: 75–88.

MacArthur, R. H. 1965. Population ecology of some warblers of northeastern coniferous forests. – Ecology 39: 599–619.

MacArthur, R. H. 1965. Patterns of species diversity. – Biological reviews. 40: 510–533.

MacArthur, R . H. 1972. Geographical ecology: patterns in the distribution of species. – Princeton Univ. Press.

MacArthur, R . H. and Wilson, E. O. 1967. The theory of island biogeography. – Princeton Univ. Press.

Maraun, M. and Scheu. S. 2000. The structure of oribatid mite communities (Acari, Oribatida): patterns, mechanisms and implications for future research. – Ecography 23: 374–382.

May, R. M. 1975. Patterns of species abundance and diversity. – In: Cody, M. L. and Diamond, J (eds.), Ecology and evolution of communities. Belknap Press of Harvard University, pp. 81-120.

Mittelbach, G. G. 1981. Foraging efficiency and body size: a study of optimal diet and habitat use by bluegills. – Ecology 62: 1370–1386.

Mittelbach, G. G. 1981. Community Ecology. – Oxford Univ. Press.

Mittelbach, G. G. et al. 2001. What is the observed relationship between species richness and productivity? – Ecology 82: 2381–2396.

Oliver, I. and Beattie, A. J. 1996. Invertebrate morphospecies as surrogates for species: a case study. – Conserv. Biol. 10: 99–109.

Peters, R. H. 1983. The ecological implications of body size. – Cambridge Univ. Press.

Pianka, E. R. 1966. Latitudinal gradients in species diversity: A review of concepts. – The American Naturalist 100: 33–46.

Post, D. M. et al. 2000. Ecosystem size determines food-chain length in lakes. – Nature 405: 1047–1049.

Rainey, P. B. and Travisano, M. 1998. Adaptive radiation in a heterogeneous environment. – Nature 394: 69–72.

Read, Q. D. et al. 2018. Among-species overlap in rodent body size distributions predicts species richness along a temperature gradient. – Ecography 41: 1718–1727.

Richardson, B. A. et al. 2018. Separating the effects of forest type and elevation on the diversity of litter invertebrate communities in a humid tropical forest in Puerto Rico. – Journal of Animal Ecology 74: 926–936.

Ricklefs, R. E. and Schluter, D. 1993. Species diversity: regional and historical influences. –
Univ. of Chicago Press.

Roeder, K. A. and 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. et al. 2018. Disturbance mediates homogenization of above and belowground
invertebrate communities. – Environmental Entomology 47: 545–550.

Rosenzweig, M. L. 1995. Species diversity in space and time. – Cambridge University Press.

Russo, S. E. et al. 2003. Size-abundance relationships in an Amazonian bird community:
implications for the energetic equivalence rule. – The American Naturalist 161: 267–283.

Sanders et al. 2007. Temperature, but not productivity or geometry, predicts elevational diversity
gradients in ants across spatial grains. – Global Ecology and Biogeography. 16: 640–649.

Schmidt-Nielsen, K. 1984. Scaling: why is body size so important. – Cambridge University
Press.

Seastedt, T. R. 1984. The role of microarthropods in decomposition and mineralization
processes. – Annual review of entomology. 29: 25–46.

Shade et al. 2018. Macroecology to unite all life, large and small. – Trends in Ecology and
Evolution. 33: 731–744.

Simberloff, D. 2004. Community Ecology: Is it time to move on? – The American Naturalist.
163: 787–799.

Smith, F. A. and Lyons, S. K. 2013. Animal Body Size: Linking pattern and process across
space, time, and taxonomic group. – The University Press of Chicago.

Srivastava, D. S. and Lawton, J. 1998. Why more productive sites have more species: an

    experimental test of theory using tree-hole communities. – The American Naturalist. 152:

    510–529.

Storch, D. et al. 2018. The more-individuals hypothesis revisited: the role of community

    abundance in species richness regulation and the productivity-diversity relationship. –

    Ecology Letters 21: 920–937.

Swenson, N. G. et al. 2011. The biogeography and filtering of woody plant functional diversity

    in North and South America. – Global Ecology and Biogeography 21: 798–808.

Swift, M. J. et al. 1979. Decomposition in terrestrial ecosystems. – University of California

    Press.

Tilman, D. 1982. Resource competition and community structure. – Princeton Univ. Press.

Tilman, D. 1987. Secondary succession and the pattern of plant dominance along experimental

    nitrogen gradients. – Ecological Monographs 57: 189–214.

Uetz, G. W. 1975. Temporal and spatial variation in species diversity of wandering spiders

    (Araneae) in deciduous forest litter. – Environmental Entomology 4: 719–724.

Wagner, J. D. 2003. Spatial stratification in litter depth by forest-floor spiders. – Journal of

    Arachnology 31: 28–39.

Wardle, D. A. 2002. Communities and ecosystems: linking the aboveground and belowground

    components. – Princeton University Press.

Weiser, M. D. et al. 2018. Toward a theory for diversity gradients: the abundance-adaptation

    hypothesis. – Ecography 41: 255–264.

White, E. P. 2007. Relationships between body size and abundance in ecology. – Trends in

    Ecology and Evolution 22: 323–330.

Whittaker, R. H. 1960. Vegetation of the Siskiyou mountains, Oregon and California. –
Ecological Monographs. 30: 279–338.

Whittaker, R. H. 1972. Evolution and measurement of species diversity. – Taxon. 21: 213–251.

Wright, D. 1983. Species-energy theory: an extension of species-area theory. – Oikos. 41: 496–
506.

Yanoviak, S. P. and Kaspari, M. 2000. Community structure and the habitat template: ants in the
tropical forest canopy and litter. – Oikos. 89: 259–266.

Zhou, J. et al. 2016. Temperature mediates continental-scale diversity of microbes in forest soils.
– Nature Communications 7: 12083.

Zuur, A. F. et al. 2010. A protocol for data exploration to avoid common statistical problems. –
Methods in Ecology and Evolution 1: 3–14.

Table 1. AIC_c scores and weights of top multiple linear regression models used to test relationships between body size (BS) or litter depth (LD) and abundance. Null models are reported when they were the top model or within 2 ΔAIC_c of the top model. No spiders were collected at NWT.

Taxon	Site	Model	Intercept	BS	LD	AIC_c	ΔAIC_c	w_i	r^2
Mites	AND	BS	1.16	-1.18	—	-6.90	0.00	0.79	0.47
	BCI	BS	0.27	-1.94	—	15.52	0.00	0.67	0.35
	BCI	BS + LD	0.06	-1.91	0.33	17.51	1.99	0.25	0.34
	CWT	BS + LD	-0.52	-1.81	1.16	22.90	0.00	0.42	0.29
	CWT	LD	1.05	—	1.19	23.26	0.36	0.35	0.20
	HFR	Null	2.90	—	—	1.95	0.00	0.33	—
	LUQ	Null	2.25	—	—	19.61	0.00	0.40	—
	NWT	Null	2.09	—	—	43.40	1.44	0.24	—
Springtails	AND	Null	1.09	—	—	35.01	0.00	0.31	—
	BCI	Null	1.15	—	—	22.39	0.00	0.64	—
	CWT	Null	1.64	—	—	16.40	0.20	0.38	—
	HFR	LD	0.53	—	1.64	39.36	0.00	0.30	0.25
	HFR	BS + LD	0.24	-0.82	1.12	39.48	0.12	0.28	0.32
	HFR	BS	0.54	-1.22	—	39.51	0.15	0.28	0.25
	LUQ	BS	-0.03	-1.36	—	21.86	0.00	0.57	0.25
	NWT	Null	1.74	—	—	24.02	0.00	0.70	—
Spiders	AND	Null	0.42	—	—	9.92	0.00	0.41	—
	BCI	Null	0.63	—	—	8.58	1.69	0.23	—
	CWT	Null	1.16	—	—	-3.72	0.00	0.61	—
	HFR	LD	0.57	—	0.82	-9.21	0.00	0.80	0.52
	LUQ	Null	0.60	—	—	16.83	0.00	0.43	—

Figure Legends

Figure 1. Sampling locations in North America covering tropical rainforests (BCI, LUQ), temperate deciduous forests (CWT, HFR), and temperate coniferous forests (AND, NWT).

Figure 2. Abundance-richness relationships testing the more-individuals hypothesis (MIH) across three taxa and six sites in North America. Panel (a) depicts mites, panel (b) depicts springtails, and panel (c) depicts spiders. Significant relationships are represented by solid (—) colored lines, while dashed (---) lines represent non-significant relationships.

Figure 3. Example of a structural equation model (SEM) showing the direct and indirect effects of body size on abundance and species richness in mites at H. J. Andrews (AND). Standardized path coefficients and *P* values are indicated next to arrows. Significant relationships are represented by solid arrows (—), while dashed arrows (---) represent non-significant relationships. Endogenous (dependent) variables have r^2 values listed below. In this example, there is a negative indirect effect of body size on species richness, mediated by abundance. Other SEMs for previously determined candidate models in Table 1 are located in Figs. S3-S5.
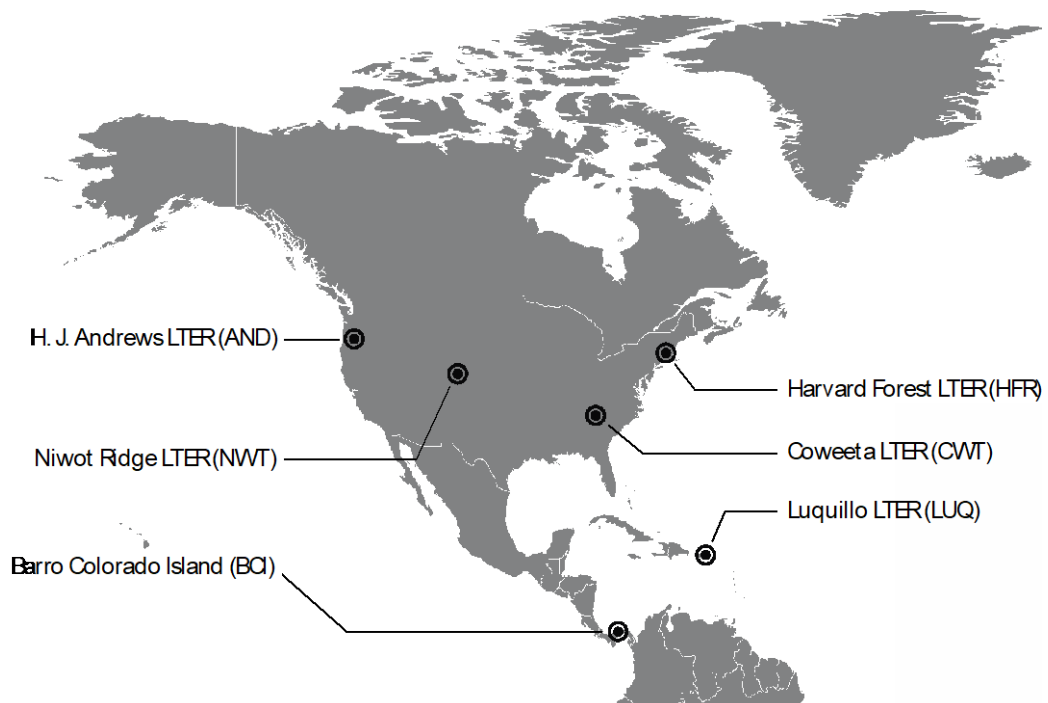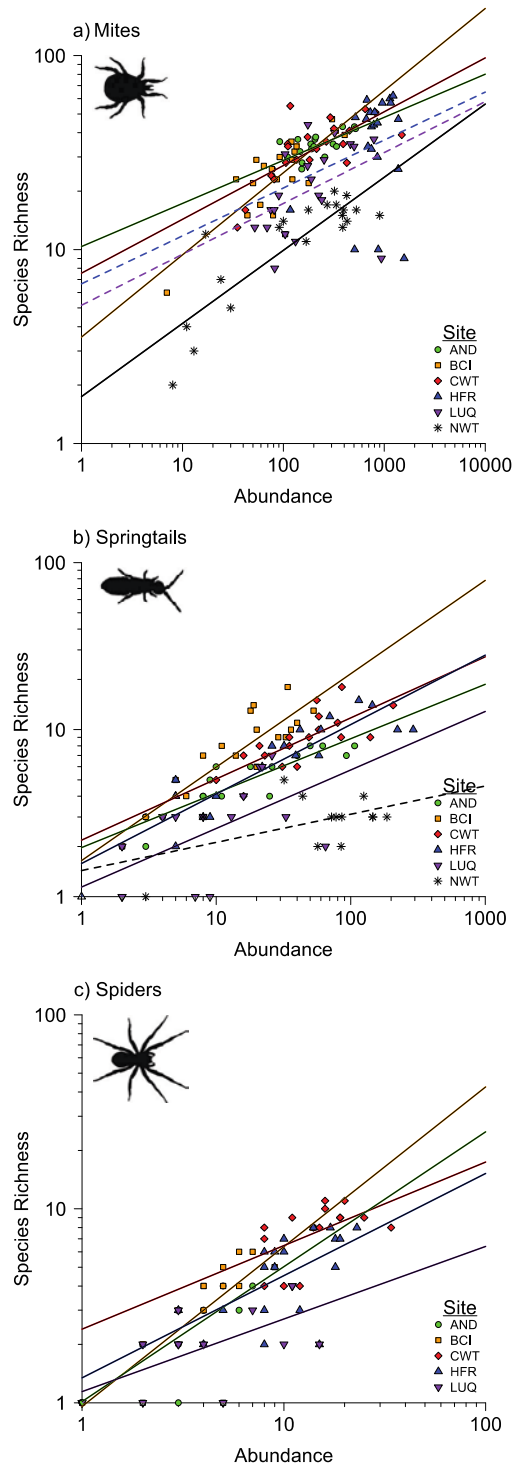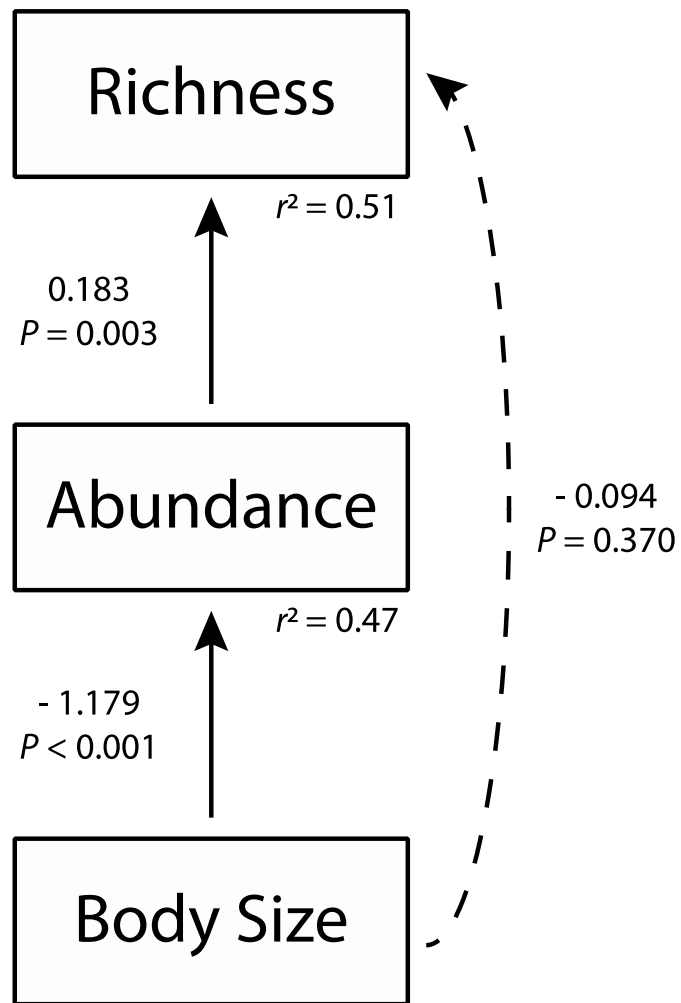
Figure 1.

Figure 2.

a) Mites

b) Springtails

c) Spiders

122

Figure 3.

Richness

$r^2 = 0.51$

0.183
$P = 0.003$

Abundance

$r^2 = 0.47$

- 0.094
$P = 0.370$

- 1.179
$P < 0.001$

Body Size

Indirect effect: - 0.216, $P = 0.014$

Net effect: - 0.310, $P = 0.001$

Chapter 4: Appendix S1. Supplemental Data.

Supplemental Figure Legends

Figure S1. Linear relationship between ImageJ and ocular micrometer measurements.

Figure S2. Linear relationship between mite ImageJ body size measurements ($\log_{10}$Area (mm^2)) and dry mass values ($\log_{10}$Dry Mass (mg)).

Figure S3. Structural equation models (SEMs) showing the direct and indirect effects of body size or litter depth on abundance and species richness in mites. Panel (a) depicts plots from BCI and panel (b) depicts plots from CWT. Standardized path coefficients and P values are indicated next to arrows. Significant relationships are represented by solid arrows (—), while dashed arrows (---) represent non-significant relationships. Endogenous (dependent) variables have r^2 values listed below.

Figure S4. Structural equation models (SEMs) showing the direct and indirect effects of body size or litter depth on abundance and species richness in springtails. Panels (a) and (b) depict plots from HFR and panel (c) depicts plots from LUQ. Standardized path coefficients and P values are indicated next to arrows. Significant relationships are represented by solid arrows (—), while dashed arrows (---) represent non-significant relationships. Endogenous (dependent) variables have r^2 values listed below.

Figure S5. Structural equation model (SEM) showing the direct and indirect effects of litter depth on abundance and species richness in spiders from Harvard Forest (HFR). Standardized

path coefficients and *P* values are indicated next to arrows. Significant relationships are represented by solid arrows (—), while dashed arrows (---) represent non-significant relationships. Endogenous (dependent) variables have r^2 values listed below.
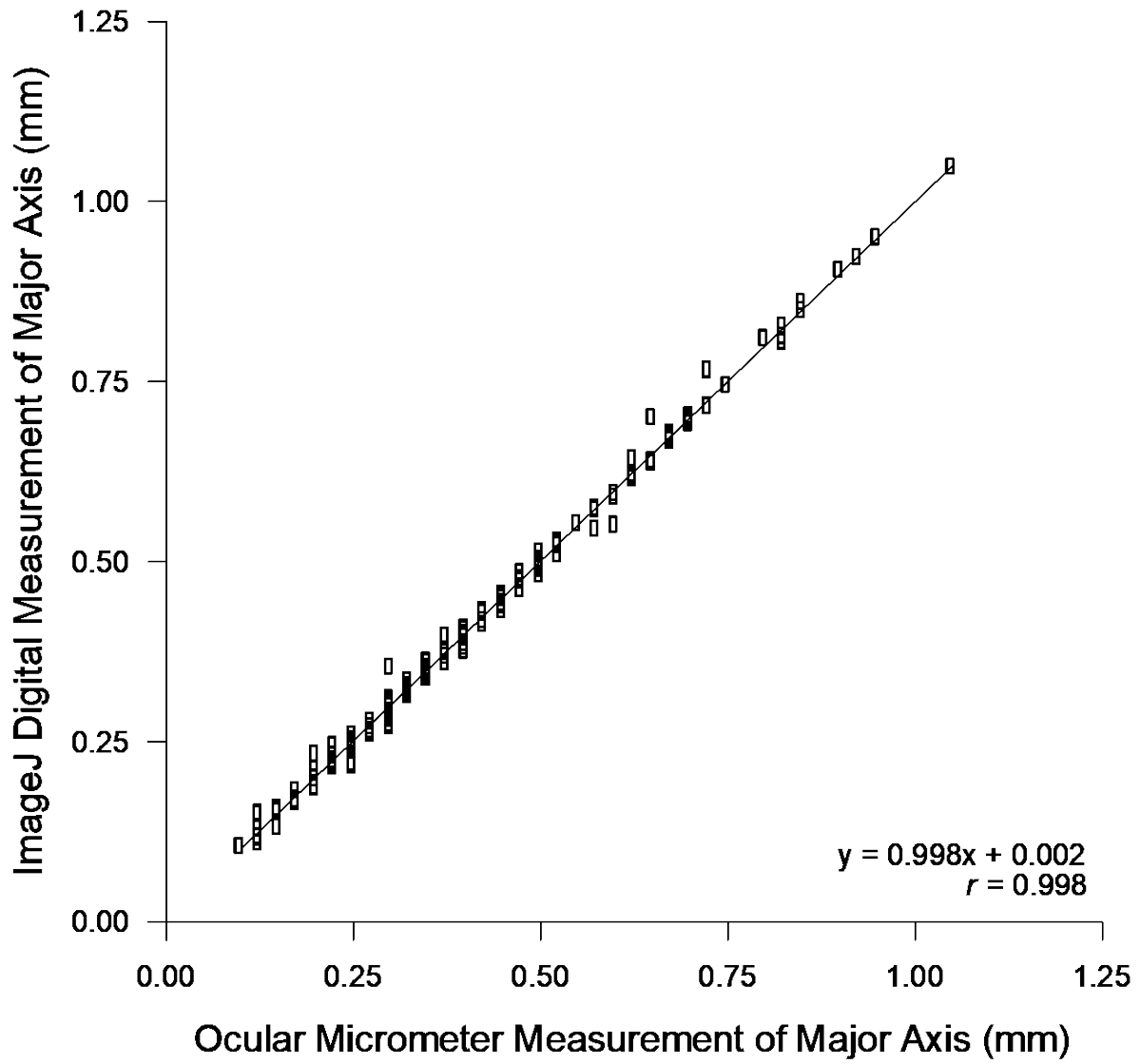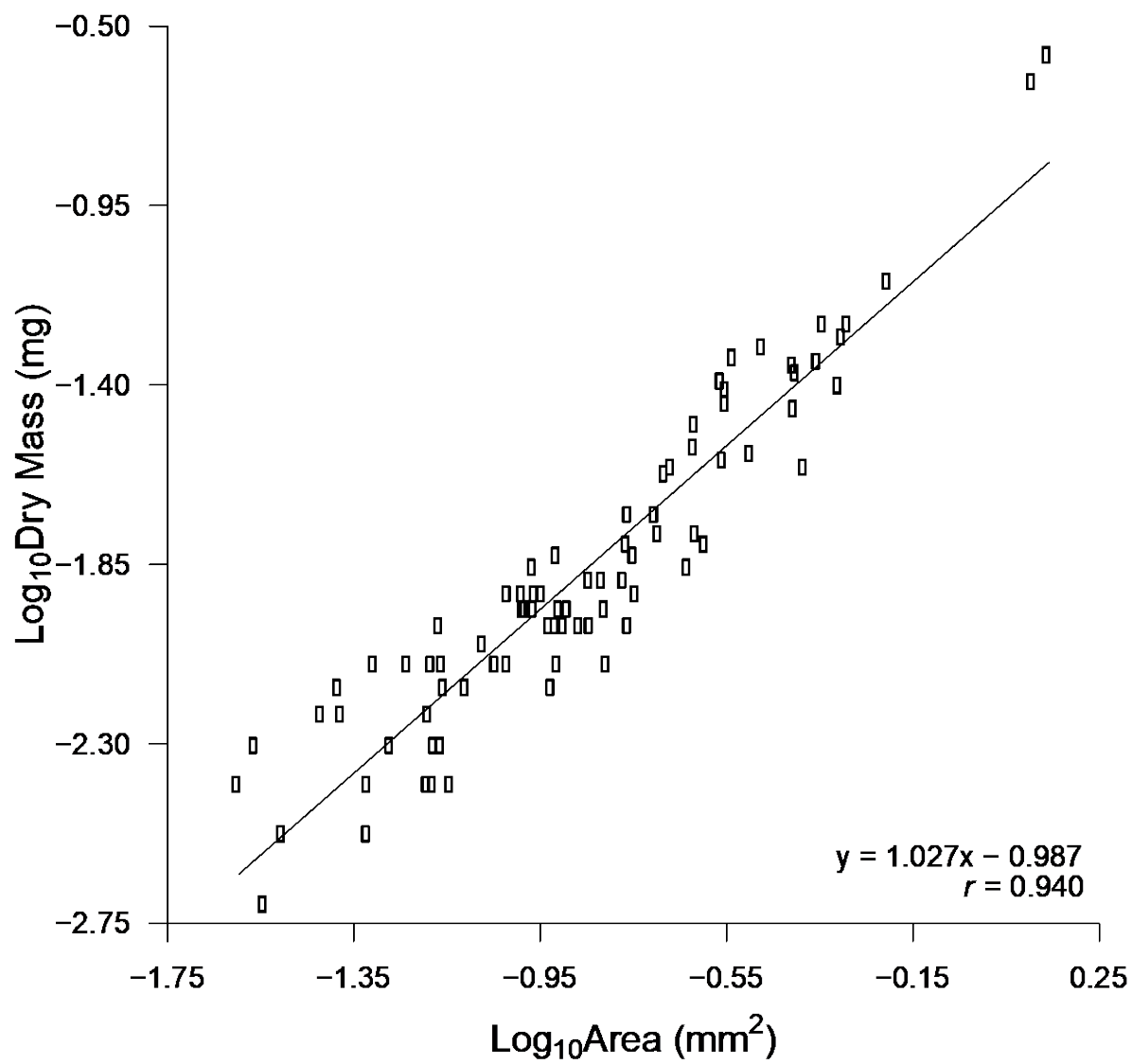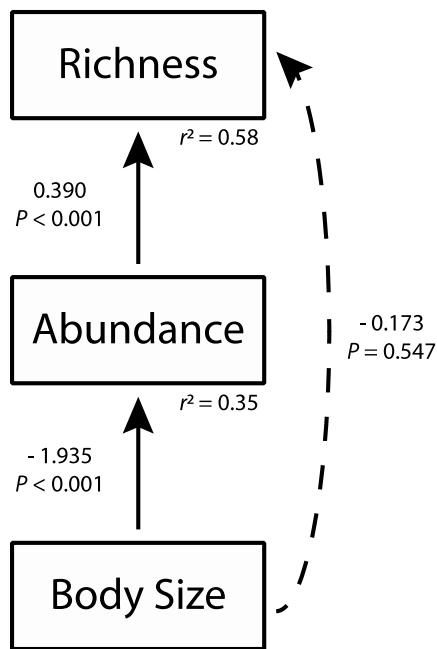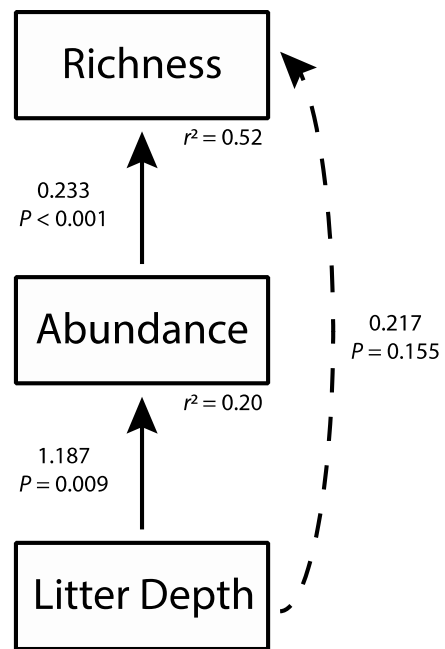
Figure S1.

$y = 0.998x + 0.002$
$r = 0.998$

Figure S2.

$y = 1.027x - 0.987$
$r = 0.940$

Figure S3.

a) Mites — BCI

Richness
$r^2 = 0.58$

0.390
$P < 0.001$

Abundance
$r^2 = 0.35$

- 1.935
$P < 0.001$

- 0.173
$P = 0.547$

Body Size

Indirect effect: - 0.755, $P = 0.006$
Net effect: - 0.928, $P = 0.003$

b) Mites — CWT

Richness
$r^2 = 0.52$

0.233
$P < 0.001$

Abundance
$r^2 = 0.20$

1.187
$P = 0.009$

0.217
$P = 0.155$

Litter Depth

Indirect effect: 0.277, $P = 0.034$
Net effect: 0.494, $P = 0.004$

128

Figure S4.

a) Springtails — HFR

Richness
$r^2 = 0.83$
0.474
$P < 0.001$
Abundance
$r^2 = 0.25$
- 1.223
$P = 0.004$
Body Size
0.246
$P = 0.022$

Indirect effect: - 0.580, $P = 0.006$
Net effect: - 0.334, $P = 0.136$

b) Springtails — HFR

Richness
$r^2 = 0.79$
0.404
$P < 0.001$
Abundance
$r^2 = 0.25$
1.638
$P = 0.004$
Litter Depth
0.063
$P = 0.693$

Indirect effect: 0.662, $P = 0.007$
Net effect: 0.726, $P = 0.007$

c) Springtails — LUQ

Richness
$r^2 = 0.21$
0.384
$P = 0.015$
Abundance
$r^2 = 0.25$
- 1.358
$P = 0.011$
Body Size
0.153
$P = 0.697$

Indirect effect: - 0.521, $P = 0.080$
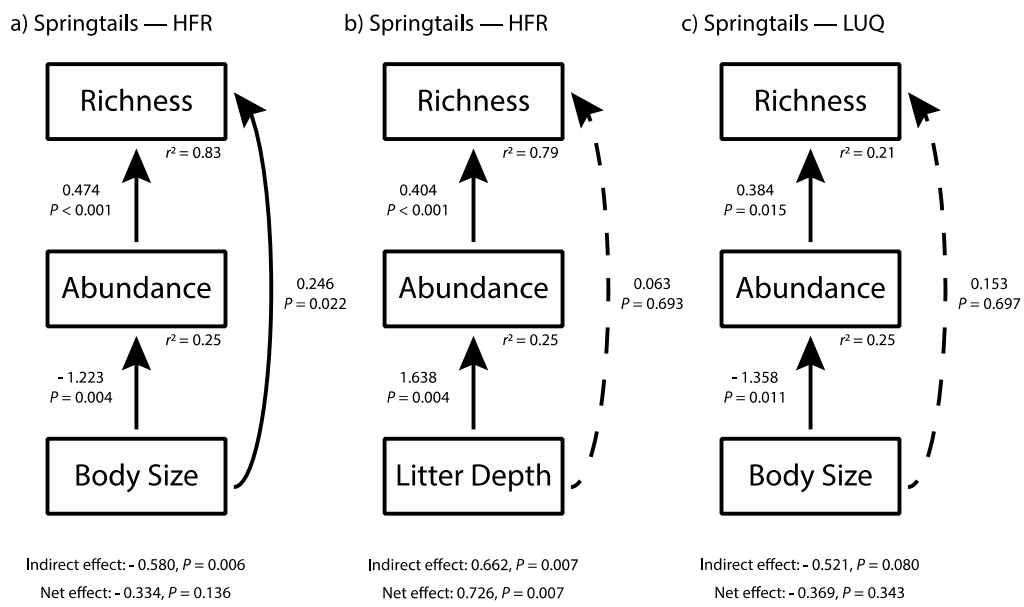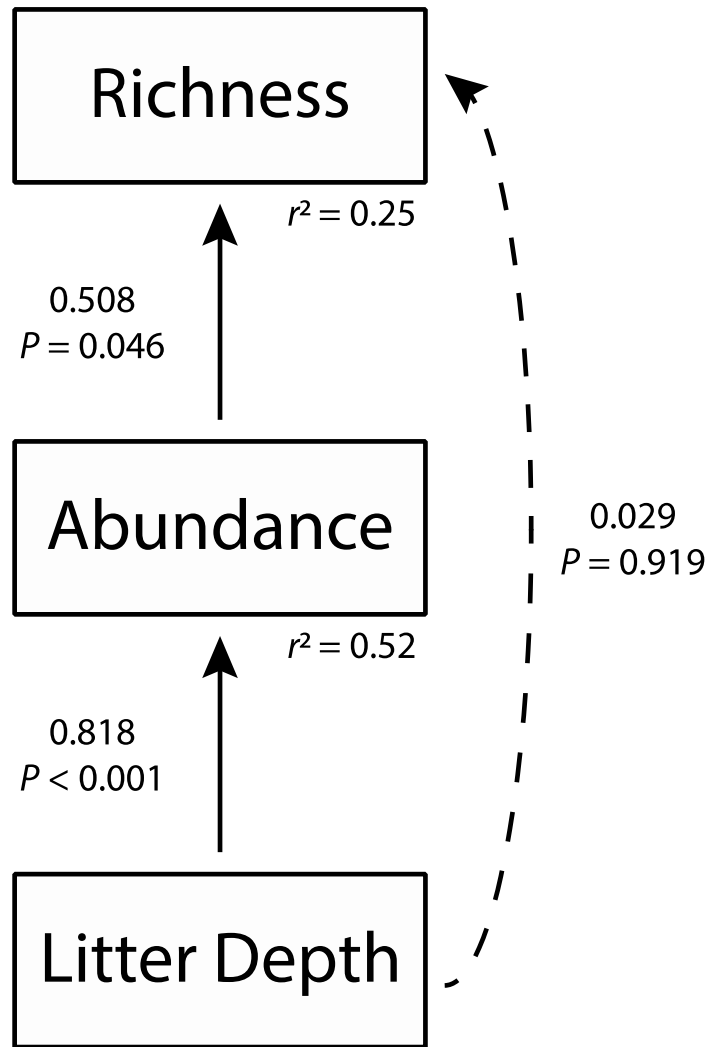Net effect: - 0.369, $P = 0.343$

129

Figure S5.

Indirect effect: 0.416, $P = 0.065$

Net effect: 0.445, $P = 0.033$