UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

NUTRIENT AVAILABILITY AND THE STRUCTURE AND FUNCTION OF BROWN FOOD WEBS

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

Degree of

DOCTOR OF PHILOSOPHY

By

NATALIE ANNE CLAY
Norman, Oklahoma
2013

NUTRIENT AVAILABILITY AND THE STRUCTURE AND FUNCTION OF BROWN FOOD WEBS

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF BIOLOGY

BY

Dr. Michael Kaspari, Chair

Dr. May Yuan

Dr. Gary Wellborn

Dr. Edith Marsh-Matthews

Dr. Jeffrey Kelly

I dedicate this dissertation to my Grandparents: Joseph and Anne Taylor. Thank you for your endless support.

Acknowledgements

First and foremost, I would like to thank my advisor Mike Kaspari. His high expectations, the freedom he gave me, and his support, guidance, and generosity made this dissertation possible. His passion for science and discovery were truly inspiring. Second, I thank my collaborators, Steve Yanoviak, Adam Kay, Jane Lucas, David Donoso and Ricky Lehrter for their invaluable insights, support, and logistical help. Working in the field with them was always a pleasure. I give special thanks to my committee members, Jeff Kelly, Edie Marsh-Matthews, Gary Wellborn and May Yuan for valuable discussions and insights, for their generosity in time, and for their generosity in use of their resources that were critical during my dissertation. Sincere thanks to Nina Velasco and Taylor Zumbusch for help in the field and for being all around fantastic people; their assistance made those field seasons successful. Thank you to Deana Flatt, Kendra Tholt, Michael O'Brien, Dustin Rinehart, and Josh Burks for their hard work in the laboratory and enthusiasm about bugs. Thanks to Barbara McCray and everyone else at the Oklahoma State University Soil Analytical Laboratory and Tim Maddox at the Stable Isotope Laboratory of the University of Georgia Odum School of Ecology and Jeff Kelly and Rick Maynard at the University of Oklahoma Stable Isotope Facility. Thanks to Liz Cooley, Robbie Stinchcomb, Marie Brooks, Carol Baylor, Kaye Carter, George Davis and Cheryl Stellner for all the logistical support within the Biology Department and for being by far the best staff any department could have. Thanks to M. Kaspari for supporting me on RA for the majority of my PhD. Thanks to the University of Oklahoma: Department of Biology, College of Arts and Science, Graduate College, and Graduate Student Senate, as well as the National

Science Foundation and Graduate Assistance in Areas of National Need Fellowship for funding my dissertation research and its dissemination. Thank you to all my past and current AntLab-mates, Jon Shik, David Donoso, Jesse Czekanski-Moir, Jelena Bujan, Jackson Helmes, Brittany Benson, Jane Lucas, and Karl Roeder for great discussions, fun and support in the field and lab, collaborations, and late night ridiculousness after too many hours spent looking into a microscope. Special thanks to Stephanie Strickler, Diane Landoll, Jon Mui, Judith Barkstedt, Carla Atkinson, Jelena Bujan, Alex Barnard, and Emily Khazan for friendship and support throughout grad school. Profound thanks to Donald Shepard for being a wonderful friend, love, and inspiration, for laughing with me and giving me strength, and for talking science; your support kept everything sunny through both the grey skies and the blue skies. Lastly, I thank all my family and friends for their love and support before and during my PhD.

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Abstract

Understanding individual nutritional requirements can generate good predictions for how communities should be structured and how ecosystems function over gradients of nutrient availability. Aboveground consumers can shape belowground processes by serving as conduits for resources through excreta, and these nutrient pulses can profoundly impact ecosystem processes. Excreta like urine and feces is a frequently deposited source of nitrogen (N) and potassium (K), and sodium (Na) that can create enduring spatial heterogeneity in the forest floor by creating nutrient hotspots. Unlike N and K, Na is unique in that it is relatively unimportant for plants, which concentrate very little Na in their tissues, but critical for consumers, which concentrate Na well above plant levels. This results in the trophic bioaccumulation of Na. Thus, plant consumers are likely Na-limited but their predators, which consume salty prey, are likely not. Omnivores are a common trophic group, eating both plant and animal tissue, that have Na tissue concentrations intermediate between herbivores and predators; but what determines the ratio of plants:prey is not well understood. Sodium is generally abundant near oceans, but its deposition in oceanic aerosols sharply decreases to zero moving inland away from coastlines. From this framework, I made predictions about how brown (detrital) food webs should be structured and function across Na gradients from tropical to boreal forests. I added Na subsidies to a Na-poor tropical forest and used stable isotope analysis to quantify the trophic positions of brown food web invertebrates and found that microbi-detritivores rapidly and predictably recruited to short-term Na subsidies but predators did not. In coastal Na-rich Panama I found that nutrient-rich excreta in the form of ant refuse from *Azteca trigona*, a dominant

Neotropical canopy ant that feeds on honeydew and insects and rains refuse out of its hanging nests onto the leaf litter below, was a nutritional conduit between the canopy and forest floor. Refuse enhanced decomposition and supported larger brown food webs than leaf litter without refuse. I then used synthetic urine to separate the effects of Na from urine's other nutrients and contrasted their roles in promoting decomposition and microbi-detritivore recruitment in both a Na-poor inland Ecuadorian and a Na-rich coastal Panamanian tropical forest. I found that urine was an important source of Na in the Na-poor but not Na-rich tropical forest that attracted termites and increased wood decomposition. Lastly, I tested whether omnivores become more carnivorous in Na-poor forests across a gradient of Na availability in ten paired (inland and coastal) lowland forests from Georgia to Maine, USA and also used laboratory *Solenopsis invicta* colonies. Omnivorous ants were up to an entire trophic level higher in Na-poor forests compared to conspecifics in Na-rich paired forests. Sodium gradients best predicted the difference in trophic position between conspecifics, and Na-starved *S. invicta* colonies increased Na and prey-seeking behavior 6-fold above control colonies. My results suggest that gradients of nutrient availability such as Na strongly impact brown food web structure and function. Across these gradients foraging animals facilitate heterogeneity in these leaf litter communities by acting as nutritional conduits between aboveground and belowground systems, but the impacts of these nutrient subsidies on brown food web structure and function depend on nutrient limitations of individual organisms. Lastly, my results suggest that there is a geography of omnivory that can be partially understood as a response to gradients in Na.

Chapter 1: Short-term sodium inputs attract microbi-detritivores and their predators

(Formatted for *Soil Biology & Biochemistry*)

Abstract

Understanding individual nutritional requirements can generate good predictions for how communities should be structured over gradients of nutrient availability. Sodium (Na) bioaccumulates from plants to predators: it is relatively unimportant for plants, which concentrate very little Na in their tissues, but critical for consumers, which concentrate Na well above plant levels. Thus, plant consumers are likely Na-limited but their predators, which consume salty prey, are likely not. From this framework, we can make predictions about how an entire community should respond to Na subsidies in Na-poor environments. We tested these predictions in an interior Amazonian brown (detrital) food web, where we quantified the responses of microbi-detritivores and their predators to experimental pulses of Na by adding 250 ml 0.5% NaCl solution that mimicked patchy urine deposition or river water as controls to 55 paired 0.25 m^2 plots every other day and regularly harvested plots over a 19 day period. Consistent with the hypothesis of Na-limitation among plant consumers, the response (effect size) to Na addition by microbi-detritivores like termites was nearly double that of predators. Moreover, consistent with the bioaccumulation of Na, fewer predatory invertebrates increased on +NaCl plots, thus trophic position alone was a good predictor of response. Our results support the Na bioaccumulation hypothesis and suggest that patchy, short-term Na inputs (like urine) facilitate heterogeneity in these leaf litter communities. Further, this study demonstrates that combining principles from nutritional and community ecology can generate predictions about how communities should be structured over gradients of nutrient availability with good accuracy.

Introduction

Nutritional ecology combines principles of physiology and nutrient availability to predict how a given organism will respond to changes in essential nutrients (Aumann and Emlen, 1965; Raubenheimer et al., 2009). Low nutrient availability relative to the requirement of the organism forces organisms to spend more time acquiring these nutrients or suffer performance deficits (Arms et al., 1974; Sterner and Elser, 2002; Simpson et al., 2006; Geerling and Loewy, 2008). In this way, the stoichiometric imbalances between consumers and their food can affect the composition and ecological services provided by food webs (White, 1993; Wardle, 2002). Nutritional ecology often focuses on individuals, but a species rarely exists in solitude, and thus it is more realistic, although infrequent, to examine nutrient limitation at the community level of organization (Simpson et al., 2010). Identifying and quantifying the imbalances between consumers and their diet are necessary to understand how nutrient limitation of an individual ramifies to the community and ecosystem levels (e.g., Hawlena et al. 2012).

Within a community, herbivores and predators often face different nutritional challenges. This is evident even in the relative ability to acquire and maintain a balance of micronutrients; heterotrophs, more so than autotrophs, represent nutritionally balanced diets for their consumers (Galef, 1996). This pattern is particularly apparent for the micronutrient sodium (Na). Plants require and concentrate very little Na in general, but heterotrophs need to concentrate Na well above plant levels to maintain neural function, osmotic balance, reproduction and development (Ganguli et al., 1969; Bursey and Watson, 1983; Blumwald et al., 2000; Geerling and Loewy, 2008).

Schowalter and colleagues (1981) measured plant, herbivore, and predator Na concentrations at 0.02, 0.31, 0.66 % Na respectively, which demonstrates the large stoichometric disparity between plants and herbivores. Herbivores and detritivores, as plant consumers, are thus potentially the most vulnerable to Na-limitation. Predators, in contrast, likely do not suffer from Na-deficits as their diet (other heterotrophs) is already rich in Na (Schowalter et al., 1981; Simpson et al., 2006; Kaspari et al., 2008). In fact, predators are generally insensitive to Na in choice tests (Bradshaw et al. 1996), but herbivores can taste and respond to Na even at concentrations as low as 0.005 M (Bell and Kitchel, 1966)! Thus, Na bioaccumulates from plants to predators, but we know of no studies that compare Na-limitation among plant consumers and their predators in a given community.

In general, Na is abundantly available near coastlines due to its deposition in rainfall, but away from oceanic sources there is a Na shortfall (Stallard and Edmond, 1981; National Atmospheric Deposition Program, 2011; Kaspari et al., 2009). However, local historic, geological, biotic, or anthropogenic factors may create important sources of Na in otherwise Na-poor environments. These include salt or clay licks (Tracy and McNaughton, 1995; Dormaar and Walker, 1996; Powell et al., 2009), urine (McNaughton et al., 1997), and road salts (Canadian Environmental Protection Act, 2001; Kaspari et al., 2010). Larger animals such as sheep are able to travel to reliable salt licks (Poole and Bachmann, 2008), but smaller organisms like consumers of the brown food web (BFW) likely rely on patchy and ephemeral Na subsidies such as urine, feces and corpses. BFWs are donor-controlled and comprised of microbes, microbi-detritivores that consume plant detritus and associated microbes, and their predators that interact and ultimately decompose ca. 90% of the productivity (uneaten plant biomass) in green food webs (Fittkau and Klinge, 1973; Coleman et al., 1996; Wardle, 2002; Decaëns, 2010). Consequently, understanding Na-limitation and its effects on BFWs can facilitate our understanding of broad geographic community and decomposition patterns.

Here we evaluate the responses of an interior Amazonian BFW to experimental patches of NaCl that simulate ephemeral Na subsides. In Na-poor environments like interior Amazon rainforests (Stallard and Edmond, 1981; Kaspari et al., 2008; Dudley et al., 2012), microbi-detritivores, but not their predators, should seek Na (Geerling and Loewy, 2008). We test the prediction that microbi-detritivores will accumulate in response to NaCl, but predators, which should be less Na-limited (via the bioaccumulation of Na), should not. By comparing BFWs of paired Control and +NaCl plots over a period of 19 days, we tested for a difference in community composition between paired plots and we further tested whether trophic position (i.e., microbi-detritivore, 1° predator, 2° predator) via the bioaccumulation hypothesis was a good predictor of response. We document how short-term Na subsidies create predictable changes in community composition.

Materials and Methods

Study site—We ran experimental plots from December 18, 2008 to January 6, 2009 at the Amazon Conservatory of Tropical Studies (ACTS) field station 67 km NE of Iquitos, in Loreto Province, Peru (3.25°S, 72.91° W). ACTS is a lowland tropical rainforest that receives ca. 3000 mm average rainfall per year and is characterized by variable soils in terms of geologic origins and fluvial inputs, but mainly consist of clay oxisols and ultisols (Madigosky and Vatnick, 2000; Mäki et al., 2001).

Experimental design—We used a split-plot design (Lindquist, 1953) with Day sampled as the wholeplot factor, Blocks as the subplot factor, and within blocks were paired Treatment plots (Figure 1). We used 55 paired 0.25 m^2 (0.5 x 0.5 m) plots that consisted of a +NaCl and a Control plot separated by 3 m and Blocks were separated by ≥ 5 m. Control plots received 250 ml stream water (see Kaspari et al., 2009 for stream description and water chemistry) and +NaCl plots received 250 ml 0.5% NaCl solution by weight in stream water from the same source. Although we added sodium in the form of NaCl, previous experiments separating the two elements demonstrate that sodium, and not chloride, is driving consumer response and decomposition (see Kaspari et al., 2009). Plots were fertilized on day 0 and every other day until the termination of the experiment on Day 19.

We destructively harvested 5 random Blocks on Days 1, 2, 4, 7, 9, 14, 15, 16, 17, 18, and 19. This allowed us to examine both rapid effects that mimic a migrating mammal excretion event (1 day), and longer term subsidies that mimic latrines where mammals such as howler monkeys (as well as other primates) common throughout the Neotropics display excretion site-fidelity and deposit daily both urine and feces (Feely, 2005; Dos Santos Neves et al., 2010). Prior to each harvest, we measured litter depth 1 cm inside the four plot corners of both Treatment plots using a wire gently inserted into the litter down to mineral soil. Invertebrates were collected from each 0.25 m^2 Treatment plot by collecting all leaf litter down to the topsoil and sifting it through a 1 cm^2 metal mesh. We then discarded the coarse leaf litter and extracted invertebrates from the siftate (the fine leaf litter and inverts that fell through the 1 cm^2 mesh) over 24 hours using Winklers (Bestelmeyer et al., 2000).

Trophic position—We used stable isotope analysis to assign 32 focal invertebrate taxa to a trophic level (see Fig. 2 for taxa list). These focal invertebrates accounted for 93% of individuals collected and are frequent subjects in other BFW studies (e.g., Wardle, 2002; Coleman et al., 1996; Chen and Wise, 1999; Decaëns, 2010). Ants (Hymenoptera: Formicidae) accounted for 21% of the total arthropod abundance and twelve of the common ant genera were included among the 32 focal invertebrates (see Fig. 2 for focal ant genera). N isotope values are reported using delta notation ($\delta^{15}N$) where: $\delta = ((R_{\text{sample}}/R_{\text{standard}}) - 1) \times 1000$. R = ratio of heavy to light isotope ($^{15}N/^{14}N$ for nitrogen stable isotopes) of the sample and standard. Delta N values are expressed in ‰ (per mil notation). All invertebrates were preserved in 95% ethanol prior to stable isotope analysis; as such, we only used N stable isotope values that are unaffected by ethanol (Fábián, 1998). A homogenate of multiple individuals was used in each sample to best represent each invertebrate's respective trophic position because many of the invertebrates are represented at the Class, Order, or Family level. Samples were dried at 60°C and ca. 500 µg of each homogenate was encapsulated in tin capsules. We included plants (grass, shrubs, and tree leaves) haphazardly sampled from the experimental site to provide the basal $\delta^{15}N$ values within the system. Nitrogen stable isotopes were analyzed using a Costech Elemental Analyzer interfaced through a ConFlo III open split valve with a Thermo Finnigan Delta V isotope ratio mass spectrometer (Costech Analytical Technologies Inc., Valencia, CA, USA). $\delta^{15}N$ values and precision were determined using a laboratory standard referenced against the international standard for N of atmospheric nitrogen (international standards calibrated with USGS 40 and 41). Nitrogen stable isotope precision was ± 0.128‰ ($N = 8$).

Trophic positions were calculated as: Trophic Position $= \lambda + (\delta^{15}N_{\text{secondary consumer}} - \delta^{15}N_{\text{base}}) / \Delta N$. We used the average plant value (2.876 ± 1.875 ‰) as $\delta^{15}N_{\text{base}}$, λ was 1 (the trophic level of the organisms used to estimate the base, i.e., autotrophs), $\delta^{15}N_{\text{secondary consumer}}$ was directly measured, and we used the standard 3.4 ‰ enrichment per trophic level as our ΔN (Fry, 1988; Post, 2002). Organisms occupying Trophic Position 2 are hereafter referred to as microbi-detritivores, those in Trophic Position 3 as 1° predators and those in Trophic Position 4 as 2° predators.

Statistical Analysis

We collected invertebrates on +NaCl and Control plots to test two predictions: 1) NaCl plots attract more invertebrates and 2) this effect diminishes with higher trophic position. In BFWs, leaf litter functions both as consumer habitat and food (Wardle 2002). Litter depth is patchy and as with aquatic systems, deeper litter (i.e., a great volume of leaf litter) as habitat is positively correlated with invertebrate abundance and richness (Angermeier and Schlosser, 1989; Kaspari and Yanoviak, 2009; Shik and Kaspari, 2010; Ettema and Wardle, 2002). Average litter depth on plots varied from 0.28 to 13.37 cm, which is representative of the natural heterogeneity in tropical forest litter depth (Fauth et al., 1989; N.A. Clay, *unpublished data*). However, NaCl addition enhances decomposition and litter depth decreased on +NaCl plots (average litter depth of Control: 5.18 ± 3.15 vs. +NaCl: 4.18 ± 3.30 cm; Wilcoxon Signed Ranks Test, d.f. = 55, $p = 0.007$, see Kaspari et al., 2009 for further discussion). Consequently, litter depth on +NaCl plots accounted for 24.1% of the variation in invertebrate abundance

($\log_{10}$Abundance = 0.606*$\log_{10}$LitterDepth + 1.871, $R^2 = 0.241$, $p = 0.0001$), and 8.6% on Control plots ($\log_{10}$Abundance = 0.632*$\log_{10}$LitterDepth + 1.568, $R^2 = 0.086$, $p = 0.03$). To account for this variation, control for habitat size-abundance relationships, and reduce potential type II error from natural and non-random, treatment-derived variation in litter depth, we thus used abundance per habitat volume: the number of invertebrates m^{-3} (plot area x litter depth) as is common in aquatic research (Downing and Leibold, 2002; Bagge et al., 2004).

Testing differences in Community Structure—To determine if communities on +NaCl plots differed from Control plots we used Primer 6 (version 6.1.11) computer program (Primer-E Ltd., Plymouth, United Kingdom) with the PERMANOVA add-on package (Anderson et al., 2008). Permanova is a mulitvariate permutational analysis of variance designed for community data (Anderson, 2001). We square-root transformed the abundance data from the 32 invertebrate taxa prior to analysis to control for rare taxa and used Bray-Curtis dissimilarities as the distance measure. The between-blocks effects were Day (wholeplot: n=11) and Block (n=5) and the within-blocks effects were Treatment (subplot: n=2) and the Day x Treatment interaction (Kirk, 1982; Gotelli and Ellison 2004, Fig. 1). We used 9999 permutations to test the null hypotheses of 1) no difference in BFW communities among Days and Blocks with Day at the wholeplot level, 2) no difference in BFW communities between Treatments (pairs) at the subplot level, and 3) no interaction between Treatment and Day (Blocks w. Day) at the subplot level. A significant effect of Treatment was followed by *a posteriori* Wilcoxon Signed Ranks Tests on the 32 focal invertebrates to determine which invertebrates within communities responded to Treatment effects and calculate their effect sizes.

Testing for diminished responses among predators—We then tested whether trophic position was a good predictor of response with the prediction from the Na bioaccumulation hypothesis that microbi-detritivores but not predators should recruit to +NaCl addition. First, we determined whether microbi-detritivores had a stronger response to +NaCl addition than predators using a t-test to test the null hypothesis of no difference in effect size between microbi-detritivores and 1° predators. We only compared the microbi-detritivores and 1° predators because only 1 invertebrate taxon occupied the 2° predators position (2° predator Hemiptera: Heteroptera, Fig. 2). We calculated each invertebrate taxon's effect size (r) from Wilcoxon Signed Rank Tests: $r = Z/\sqrt{N}$, where N is the total number of the samples. Effect size (r) was normally distributed (Kolmogorov-Smirnov, d.f. = 31, $p = 0.641$) with equal variance (Levene's Test of Equality of Variance, $p = 0.105$). Second, we tested whether r was a good predictor of group membership and how well it correctly classified invertebrate taxa to their respective trophic position using logistic regression (SPSS v. 10.0). We used r of the 31 invertebrates in microbi-detritivore and 1° predator groups as our predictor variable and our binomial response was yes or no invertebrate is a microbi-detritivore.

Results

We collected 16,367 invertebrates from the 55 paired +NaCl and Control plots. +NaCl plots had more invertebrates (Wilcoxon Signed Ranks, d.f. = 55, $p < 0.0001$). Median total invertebrate densities were 91 (IQR = 54-182) m^{-2} on +NaCl plots versus 68 (IQR = 32-114) m^{-2} on Control plots. The 32 focal invertebrates spanned 3 Trophic Positions: 15 microbi-detritivores, 16 1° predators, and 1 2° predator (Fig. 2).

Effects of NaCl on BFW composition—The addition of NaCl (Treatment) changed community composition (Within-blocks, Treatment: $p = 0.0127$; Table 1). At the wholeplot level, the community composition also differed among sample days (Between-blocks, Day: $p = 0.0001$), but the effects of NaCl addition on community composition were consistent regardless of the sampling Day (Treatment x Day: $p = 0.5208$; Table 1). In other words, as is characteristic of a dynamic tropical forest, there was daily variation in community composition for both +NaCl and Control plots, but the magnitude of the Treatment effect between plots remained constant over time (i.e., no interaction).

Do invertebrates lower in trophic position respond more strongly to NaCl?—Microbi-detritivores had a stronger response to +NaCl than 1° predators ($t_{29} = 1.990$, $p = 0.056$). The mean effect sizes (*r*) for microbi-detritivores and 1° predators were 0.28 and 0.17 respectively (Fig. 2). Further, *r* was a good predictor of trophic position (logistic regression, d.f. =1, $\chi^2 = 4.105$, $p = 0.043$). The model correctly classified 64.5% of invertebrates to their respective trophic position, yielding a 1.19 fold increase in probability of being a microbi-detritivore for every 0.1 increase in *r*. These results are further supported by the Wilcoxon Signed Ranks Tests (Table 1). As predicted, nearly all invertebrates feeding low in the food web (Microbi-detritivores) increased on +NaCl plots ($p \leq 0.044$; Lepidoptera larvae marginally increased, $p = 0.079$, Fig. 2). In contrast, most 1° predators did not increase on NaCl addition plots. The exceptions to this pattern were predatory mites (Acari: Mesostigmata, Prostigmata, Trombidioidea), and the ant genera *Strumigenys*, *Hylomyrma*, *Pheidole*, and *Solenopsis* ($p \leq 0.033$, Fig. 2). The large responses of microbi-detritivores and the small responses of 1° predators support our predictions of how Na-limitation is structured within a food web: large bottom-up effects within the detrital community in response to direct consumer resource provisioning. Additionally, the one 2° predator, Heteroptera (Hemiptera), increased on +NaCl plots ($p = 0.021$, Fig. 2).

Discussion

Sodium has long been known to impact the behavior and performance of individual organisms (Arms et al., 1974; Belovsky and Jordan, 1981; Simpson et al., 2006; Kaspari et al., 2008), but its role in structuring communities had remained unexplored. Here we demonstrated that by using the principles of nutritional ecology at the individual level, we could predict with good accuracy how an entire community should respond to subsidies of a limiting nutrient. Because Na bioaccumulates from plants to predators, heterotrophs low in trophic position like microbi-detritivores rapidly recruited to pulses of NaCl that mimicked urine deposition in a Na-poor forest, whereas predators in general did not (Fig. 2). NaCl addition attracted > 33% more invertebrates than Control plots largely driven by microbi-detritivores, which had nearly double the average effect size of 1° predators (Fig. 2), and this pattern remained consistent over time regardless of daily variation in this dynamic tropical forest (Table 1). Further, Trophic Position alone was a good predictor of invertebrate response (effect size: *r*). These results document the strong effects of NaCl additions on a large fraction of the BFW and demonstrate that we can largely predict community composition changes in response to nutrient addition using principles of nutritional ecology pertaining to individual invertebrates.

Although from the Na bioaccumulation hypothesis, predators are not predicted to increase on +NaCl plots from Na-limitation, predictions from classic food web ecology provided an obvious mechanism for predator increase: increases in prey. Our experimental design and time scale were not adequate to test this directly, but four of the five predators that responded were ants, which forage widely and rapidly and strongly recruit to increased prey availability (Lach et al., 2010). Increased prey may also explain the increase in the only 2° predator, Heteroptera (Hemiptera, including Reduviids, Aradids, and Mirids, Fig. 2). Future studies should combine principles from nutritional and energy-based trophic ecology to fully understand the multiple mechanisms that shape community structure based on resource availability (Simpson et al., 2010).

In contrast to predators, microbi-detritivores strongly recruited to +NaCl plots (Fig. 2). Here we only examined invertebrate response and did not explore changes in the microbial community. Increased microbial necromass or biomass from Na application could be an indirect mechanism for increases in microbi-detritivores. However, microbi-detritivores responded from day 1 and remained consistent over time (Table 1). As such, sustained microbi-detritivore recruitment to microbial necromass seems unlikely as the necromass is finite. While increased microbial biomass may play a role in microbi-detritivore recruitment, numerous studies have used Na-soaked substances and demonstrated that Na itself is sufficient to attract herbivores (e.g., Arms et al., 1974; Barrows, 1974; Kaspari et al., 2008, 2010), and microbi-detritivores would still obtain Na by increasing consumption of salty organisms (e.g., Simpson et al., 2006).

Although microbi-detritivores increased nearly 1.5 fold on +NaCl over Controls plots, a handful of microbi-detritivores had similar abundances between treatments: Psocoptera, Blattaria, Diptera larvae, Staphylinidae (Coleoptera), Isopoda, and Pselaphidae (Coleoptera). Together, these invertebrates represented only 5% of the total microbi-detritivore abundance. A suite of mechanisms could potentially cause this pattern such as increased predation pressure, ontogenetic constraints, competitive exclusion, physiological Na adaptations, or a cryptic predatory behavior among others. However, without testing each specifically, it is impossible to discern the mechanism from our results. But, these exceptions provide opportunities to delve deeper into individual differences in Na physiology and species interactions to better understand when we should and should not predict Na-seeking behavior.

At the time scale of weeks, increases in invertebrates on +NaCl plots most likely arose from immigration rather than population increases. The rapid response of BFW invertebrates in this study suggests that they have evolved to quickly capitalize on ephemeral resources like NaCl pulses. Rapid recruitment of plant consumers to NaCl has also been documented in bees (Barrows, 1974), lepidoptera (Arms et al., 1974), and ants (Kaspari et al., 2008) among other invertebrates and vertebrates. Na is lost during any metabolic water loss process such as sweating or excretion and these processes increase with temperature (Peters, 1983). Thus, in Na-poor environments, particularly in the hot tropics, short-term Na subsidies are likely vital to consumers maintaining a Na-balance (Dudley et al., 2012). Further experiments investigating the role of urine deposition in structuring BFWs and their ecological services like decomposition over a gradient of Na availability will contribute to a multi-scale understanding of the patchiness of BFWs.

The increased microbi-detritivores like termites and Collembola on +NaCl plots likely drove the pattern of decreased litter depth with the addition of NaCl. Here we tested Na's effects on food web structure and invertebrate response and controlled for differences in habitat space between treatments, but Kaspari and colleagues (2009) demonstrated that the addition of Na increases both leaf litter and experimental filter paper decomposition and documented a similar response in termite recruitment to NaCl treated cellulose. The inverse relationship between habitat space and decomposition rates in BFWs—the "more food-less habitat effect" (Shik and Kaspari, 2010; Jacquemin et al., 2012)—suggests a dynamic mix of limiting factors for invertebrates as litter decomposes. Our results and those of Kaspari and colleagues (2009) demonstrate that even ephemeral patchy Na subsidies can have rapid and large effects on both BFW structure and function in Na-poor environments.

Our study is the first we know of to apply concepts of Na-limitation to entire food webs and it demonstrates how nutritional ecology can provide a predictive framework for community ecology. Over 80% of earth's terrestrial surface is potentially Na-poor as oceanic inputs (i.e., "salty rain") exponentially decrease with distance inland (Stallard and Edmond, 1981; National Atmospheric Deposition Program, 2011; Kaspari et al., 2009). Na should be included as one of the major nutritional resources foci in future research in community ecology, biogeography and classic trophic theory. Lastly, future research should include effects of both short- and long- term Na subsidies on food webs at both macro- and micro- spatial grains to fully understand how Na availability impacts individuals to ecosystem level processes.

Acknowledgements

We thank the Amazon Conservatory for Tropical Studies, Pam Bucur, and Steve Madigosky for facilitating fieldwork and the Peruvian Instituto Nacional de Recursos Naturales for permits. Special thanks to Kendra Tholt, Michael O'Brien, and Deana Flatt for assistance in the lab. Thanks to NAC's dissertation committee May Yuan, Gary Wellborn, Edie Marsh-Mathews, Jeff Kelly, as well as Don Shepard and Zoology Ecology and Evolutionary Biology Discussion Group for valuable discussions of this manuscript. Additionally, special thanks to Rick Maynard and the University of Oklahoma stable isotope facility, J. Kelly and E. Marsh-Matthews for the use of equipment. This project was funded by grants from the National Geographic Society and NSF DEB-0948762 to MEK, and funding from the Graduate Student Senate, University of Oklahoma.

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Table 1. Results of the split-plot permutational manova in Primer-E with PERMANOVA add-on based on Bray-Curtis dissimilarities for the abundances of 32 invertebrates within an Amazonian brown food web (see Fig. 1 for experimental design). Invertebrate abundance (number of individuals) was analyzed as per volume (m^{-3} = plot area x litter depth).

Source	d.f.	SS	MS	F	P	eta^2
Between-block effects (Wholeplot level)						
Day	10	23518	2351.8	3.590	0.0001	0.432
Blocks w. Day	4	4670.2	1167.2	1.782	0.0009	0.086
Residual	40	26203	655.08			
Within-block effects (Subplot level)						
Treatment	1	5619.1	5619.1	5.884	0.0127	0.034
Blocks	54	108780	2014.5	2.053	0.0001	0.650
Treatment x Blocks w. Day	4	3819.8	945.94	0.973	0.5208	0.023
Residual	50	49064	981.28			
Total	109	167290				

Figure Legends

Figure 1. Schematic diagram of the split-plot experimental design. The between-block effects at the wholeplot level are Day (n=11), which is the day when 5 random blocks were destructively sampled, and Blocks w. Day (n=5), which are the 5 random blocks sampled on each day. In a split-plot design, these effects are indistinguishable. The within-block effects at the subplot level are the paired Treatment plots (n=2), Blocks (n=55), which contain each paired Treatment plots, and the Treatment x Day (Blocks w. Day) interaction (see Table 1).

Figure 2. Trophic position (black circles) and effect size (r) based on Wilcoxon Signed Ranks Tests (grey diamonds) of the 32 invertebrates measured within the Amazonian brown food web. Vertical solid lines designate trophic position divides (autotrophs, microbi-detritivores, 1° predators, 2° predators, see Methods for how determined). Horizontal dashed lines are the mean r for each trophic position excluding the 2° predators that had only one invertebrate member. Stars in front of invertebrate labels represent significant ($p \leq 0.05$) increases in abundance on +NaCl plots determined from Wilcoxon Signed Ranks tests (Lepidoptera: $p = 0.079$). Homoptera represents: Hemiptera: Stenorrhyncha and Auchenorrhyncha.

Figure 1

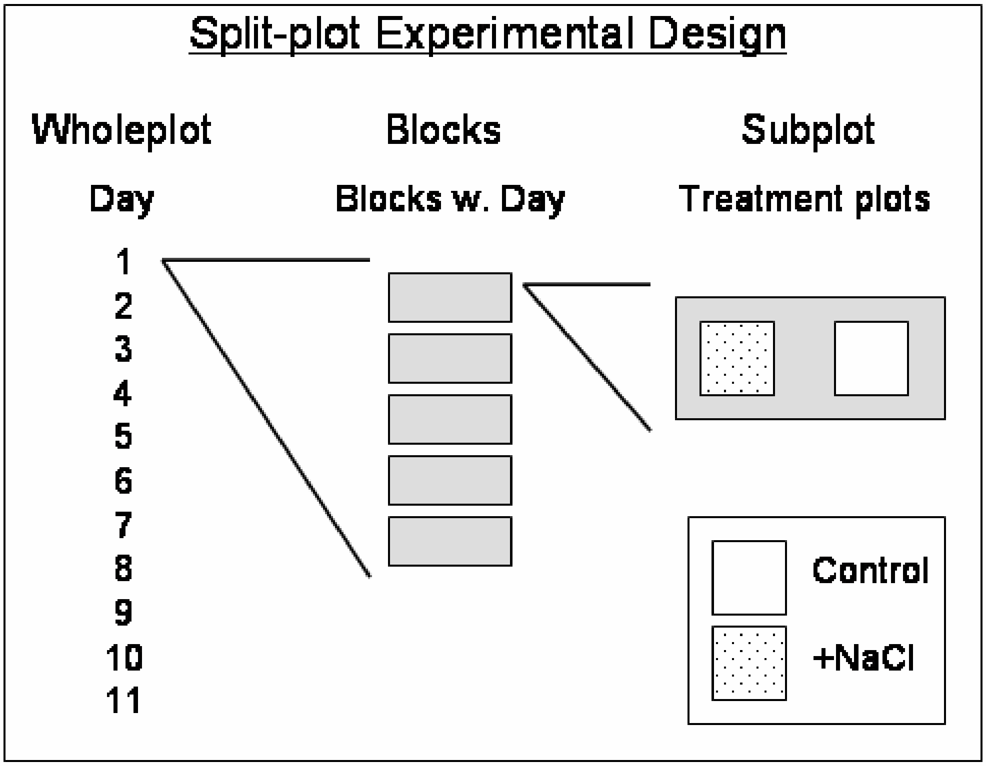
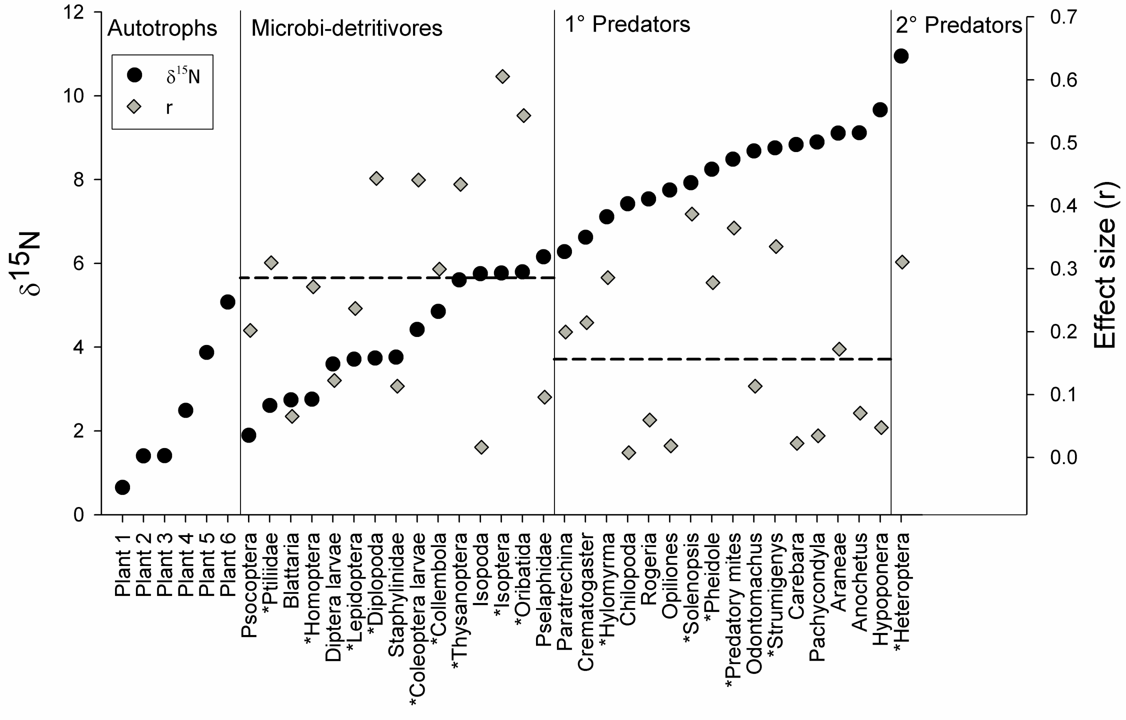

Split-plot Experimental Design
Wholeplot
Blocks
Subplot
Day
Blocks w. Day
Treatment plots
1
2
3
4
5
6
7
8
9
10
11
Control
+NaCl

Figure 2



Autotrophs
Microbi-detritivores
1° Predators
2° Predators
δ15N
r
δ15N
Effect size (r)
0
2
4
6
8
10
12
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Plant 1
Plant 2
Plant 3
Plant 4
Plant 5
Plant 6
Psocoptera
*Ptiliidae
Blattaria
*Homoptera
Diptera larvae
*Lepidoptera
*Diplopoda
Staphylinidae
*Coleoptera larvae
*Collembola
*Thysanoptera
Isopoda
*Isoptera
*Oribatida
Pselaphidae
Paratrechina
Crematogaster
*Hylomyrma
Chilopoda
Rogeria
Opiliones
*Solenopsis
*Pheidole
*Predatory mites
Odontomachus
*Strumigenys
Carebara
Pachycondyla
Araneae
Anochetus
Hypoponera
*Heteroptera

Chapter 2: Manna from heaven: Refuse from an arboreal ant links aboveground and belowground processes in a lowland tropical forest

(Formatted for *Ecosphere*)

Citation: Clay, N. A., J. Lucas, M. Kaspari, and A. D. Kay. 2013. Manna from heaven: Refuse from an arboreal ant links aboveground and belowground processes in a lowland tropical forest. Ecosphere 4(11):141. http://dx.doi.org/10.1890/ES13-00220.1.

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Abstract

Aboveground consumers can shape belowground processes by serving as conduits for resources. Social insects dominate in terms of biomass in tropical forests, but compared to studies on large mammals, or aggregate solitary insects, we know relatively little about the role of social insects as nutrient conduits particularly in complex environments like tropical forests. Social insects like ants in the tropical forest canopy can connect aboveground and belowground food webs by producing a nutrient stream (excreta) from large, long-lived and stationary nests. The excreta, in turn, would create enduring spatial heterogeneity in the forest floor. Here we evaluate this scenario in a lowland Neotropical forest using *Azteca trigona*, a dominant canopy ant that feeds on honeydew and insects and rains refuse out of its hanging nests onto the leaf litter below. We investigate decomposition rates and detrital communities associated with areas near nests versus 10 m away. Further, we directly test refuse's impact on decomposition and detrital communities in a common garden experiment. Relative to leaf litter, refuse is enriched 7-fold in P, 23-fold in K, and 3-fold in N, all elements shown to limit decomposition in this forest. Accordingly, both artificial substrates and natural leaf litter substrates decomposed over 1.5 and 1.2 fold faster respectively below *A. trigona* nests and areas under nests supported more invertebrate detritivores and predators compared to controls 10 m away. These decomposition results were replicated in a 6 wk common garden experiment, but the changes in detrital invertebrate composition were not. Canopy ants like *A. trigona* act as dependable nutritional conduits to patches of the forest floor, transferring significant quantities of aboveground

exudates and necromass. The general capacity for such social insect colonies to generate ecosystem heterogeneity remains an open question.

Introduction

Linkages between above- and below-ground processes have widespread impacts on terrestrial ecosystems (Bardgett et al. 1998, Scheu 2001, Van der Putten et al. 2001). Litterfall is one obvious biochemical flux that connects the canopy to the detrital decomposer microbes and invertebrates (Tian et al. 1992, Cadisch and Giller 1997). Additionally, consumer activity such as insect herbivory in forests can influence belowground processes via changes in vegetation structure and composition (Trumble et al. 1993), nutrient leaching rates (Tukey and Morgan 1963) and the quantity and quality of litterfall (Chapman et al. 2003, Classen et al. 2007, Kay et al. 2008).

Consumer byproducts such as excreta are another important linkage between above- and below-ground systems (McNaughton et al. 1997, Steinauer and Collins 2001, Mikola et al. 2009, Lovett and Ruesnik 1995, Reynolds and Hunter 2001, Kagata and Ohgushi 2012) and are a concentrated source of bioavailable nutrients (Ruess and McNaughton 1984, Sørensen et al. 2003). Herbivore byproducts (excreta and cadavers) fall in quantity to the forest floor (Schowalter 2000, Hunter 2001, Frost and Hunter 2004, 2007, 2008) and can accelerate decomposition (Wardle 2002, Frost and Hunter 2004), increase plant growth (Haines 1978, Feeley 2005) and create persistent landscape heterogeneity (Whitford 2000, Fox-Dobbs et al. 2010). However, the ecosystem consequences of insect excreta are often difficult to study experimentally because deposition is frequently diffuse and transitory. This is particularly true in complex environments like tropical forests where these organic inputs may exist as transfers between distant canopy and forest floor layers.

Ants and other social insects are model organisms for examining consumer-mediated above- and below-ground linkages because they regularly concentrate their excreta around large, stationary and long-lived colonies (Weiss 2006). However, surprisingly few studies have examined their ecosystem-level impacts (Wagner 1997, Whitford 2000, O'Dowd et al. 2003, Freyman et al. 2010, Wardle et al. 2011), despite their prominence as consumers in many systems including tropical forest canopies (Fittkau and Klinge 1973) where they dominate in terms of numbers and animal biomass (Tobin 1997, Dial et al. 2006). Ants have considerable promise as conduits between canopy and litter; they are voracious consumers that concentrate excreta, carcasses, and other 'trash' into refuse piles in or near their nests, which can create nutrient hot spots that increase nutrient mineralization and modify soil biota communities (Rojas 1989, Wagner 1997, Weiss 2006, Fox-Dobbs et al. 2010). Such central place foragers present an opportunity to directly measure connections between above- and below-ground systems even in complex environments like tropical forests where there is a notable gap in our knowledge (Rinker et al. 2001, but see Schowalter et al. 2011). This is particularly germane given much of the terrestrial primary productivity (Melillo et al. 1993) and insect biomass and diversity (Nadkarni 1994) exists in these forests.

In the Neotropics, the canopy ant *Azteca trigona* creates large (~0.5-4 m) hanging, conical-shaped nests (Wheeler 1986) that can persist upwards of 15 years (M. Kaspari, *personal observation*; Fig 1). The tapered bottom of the nest is open, regularly raining copious amounts of refuse that resembles used coffee grounds down to the forest floor. *Azteca trigona* feeds mainly on hemipteran honeydew and arboreal insects

(Wheeler 1986, Adams 1994, Davidson et al. 2004). *A. trigona* colonies are polydomous (having multiple nests per colony) and these nests are abundant; in Barro Colorado National Monument, Panama one of us recorded one *A. trigona* colony with 1-5 large nests every 40m^2 (Clay unpublished data). Given, their abundance and persistence, we hypothesized that *A. trigona* is an important nutrient conduit between the Neotropical canopy and forest floor that stimulates decomposition by enhancing the microbial and invertebrate breakdown of leaf litter.

Here we use multiple experiments to test the hypothesis that *A. trigona* act as nutritional conduits between the canopy and forest floor. Specifically, we test the predictions that 1) *A. trigona* refuse is nutritionally enriched relative to the litter on which it falls, 2) refuse addition stimulates decomposition relative to non-refuse addition plots, and 3) refuse addition further affects invertebrate abundance and community composition of the detrital food webs relative to non-refuse addition plots. Using *A. trigona* as a model species we were able to investigate the impact aboveground social insects can have on belowground systems in a complex tropical ecosystem.

Materials and Methods

We conducted fieldwork from mid-May to mid-July in 2009, 2010, and 2012 on Barro Colorado Island (BCI), Panama. BCI is managed by the Smithsonian Tropical Research Institute and is a seasonal lowland tropical rain forest receiving ca. 2600 mm rain annually, the majority of which falls in the rainy season from mid-April to mid-December (Wieder and Wright 1995).

Decomposition experiments

Artificial substrates—In 2010 we tested whether areas below *A. trigona* nests were associated with higher decomposition rates using standard artificial substrates (wood dowels and filter paper) to facilitate comparison to results from other studies. We located 10 nests and placed 3 decomposition bags at each of 3 locations: directly below the nests, 1 m from below the nest, and 10 m from the nests. For bags placed away from nests, we placed the 1st bag in a random direction, and then placed the second and third bags at the same distance from nests but at 120° and 240° degrees from the 1st bag. We constructed decomposition bags using solid polyester material on the bottom and 0.75 cm diameter polyester mesh on the top. This allowed macrofauna access and insured we fully collected all of the remaining substrates on collection days. In each bag we included one pre-weighed wood dowel (white birch: *Betula papyrifera*) and two pre-weighed folded pieces of 9 cm grade P8 filter paper (Fisher Scientific, www.fishersci.com). We collected one decomposition bag from each distance (0, 1 and 10m) every 2 weeks for 6 weeks; we failed to recover one bag from the 10m distance. After collection, we gently rinsed dirt and debris from substrates and dried them at 60° C for 48hrs (sufficient time for complete drying).

Natural substrates—In 2012, we used leaf litter from two temperate North American species not found on BCI: Post Oak (*Quercus stellata*) and Red Maple (*Acer rubrum*) and two resident BCI species: *Cecropia peltata* and *Astronium graveolens*. We collected recently fallen Post Oak litter from Oklahoma and Red Maple litter from Massachusetts in early fall 2012 and we collected *Cecropia* and *Astronium* litter from the forest floor in early June 2012. We used the temperate species because it allowed us to have large quantities of litterfall of consistent quality. We opportunistically used *Cecropia* and *Astronium* because they, unlike most BCI species, produced significant litterfall in early June (start of our field season). We located 15 nests and placed 8 decomposition bags directly under nests (0m) and 10m away from nests. Decomposition bags were made of 0.75 cm diameter polyester mesh that contained ca. 5 g of dried, sterilized, and pre-weighed leaf litter of a single species. At 3 and 6 weeks we collected 4 decomposition bags (1 of each species) from all 0 and 10 m distances. Litter was extracted, gently rinsed, dried at 60 °C until weight stabilized and reweighed. The use of all of these substrates (artificial sources, temperate litter, and native litter) allows us to assess the generality of *A. trigona* nest effects on decomposition.

Common garden—We conducted a common garden experiment in June 2010 to determine if *A. trigona* refuse addition affects decomposition. We demarcated 45 0.5 x 0.5 m (0.25 m^2) plots on the forest floor and assigned plots to one of three treatments: refuse addition, soil addition (a control for the addition of habitat space via particulate matter), or control plots that were unmanipulated. We selected 15 nests not used in the observational study and measured nest length (distance from the nest's point of attachment to the tree to the bottom of the nest) and height above surface (distance from the ground to the bottom of the nest) using a clinometer. We collected refuse from nests by placing 0.7 m (0.39 m^2) diameter plastic buckets propped off the ground on 1.5 m PVC poles directly below nests. Bucket bottoms had two 2 cm diameter holes so rain did not gather in buckets and bucket tops were covered in removable fine mesh nylon that collected the falling refuse (Fig. 1). Every 3 days, we removed refuse from the cloth and measured the wet refuse volume for each nest using a graduated cylinder. The 15 refuse addition plots received equal volumetric proportions of refuse pooled from all 15 nests every 3 days. Similarly, 15 soil addition plots received soil (top soil was collected >100 m away from experimental plots) of equal volume as the refuse deposited on refuse addition plots. Prior to treatment, we placed 3 decomposition bags (as described above) on each of the 45 plots (Refuse, Soil and Control). Every 2 weeks for 6 weeks we collected 1 decomposition bag from each plot and cleaned, dried and reweighed wood and filter paper as described above in *Decomposition experiments: Artificial substrates*.

Chemical Analysis—In 2010, we collected refuse using buckets (as described above) from 3 nests not used in the observational or experimental studies herein. Refuse was analyzed for lignin content, the metals Ca, K, Mg, Na, Fe, Zn, Cu, Mn, Ni, and macronutrients S, C (total C), N (total N) and P. The Soil Analytical Laboratory at Oklahoma State University (Stillwater, Oklahoma, USA) conducted analyses using a combination Lachat QuickChem 8000 flow injection autoanalyzer (Lachat, Loveland, Colorado, USA), Spectro CirOs ICP [Inductively Coupled Plasma] spectrometer (Spectro, Kleve, Germany), and an Ankom fiber analyzer (Ankom, Macedon, New York, USA). Litterfall is a common conduit of nutrients between the canopy and forest floor. Here we compared the decomposition of substrates in leaf litter vs. refuse. Thus, we further compared the chemistry of leaf litter (n=25) and refuse to determine how refuse- and plant-mediated nutrient deposition differed. Leaf litter was gathered from the forest floor in locations throughout the island and analyzed in 2004 as part of a previous study that examined the relationship between litter depth and phosphorous (see Kaspari and Yanoviak 2008 for leaf litter collection and chemistry analysis methods).

Detrital invertebrate communities

Natural communities—In July 2009 we tested whether *A. trigona* refuse piles were associated with detrital invertebrate communities distinct from those in leaf litter 10 m away from nests. We collected the detrital communities from 0.25 m^2 (0.5 x 0.5 m) areas directly below 20 nests and from 0.25 m^2 areas 10 m from nests in a random direction; these nests were different from those used in the decomposition study. We collected all leaf litter on plots down to the topsoil into a large sifter with a 1 cm^2 metal mesh. After sifting, we discarded the coarse leaf litter and kept the fine 'siftate' containing the invertebrates below the metal mesh (Bestelmeyer et al. 2000). We placed a random 400 ml subset of the siftate into a Berlese funnel and after 24 hrs we collected the invertebrates that had fallen into the cup with 95 % ethanol at the bottom of the funnel. Invertebrates were identified to at least Class and represented the 33 focal taxa that were most common in this study and others (Wardle 2002; Decaëns 2010). We deposited vouchers in the Museo de Invertebrados Fairchild, Panama and MK's collection at the University of Oklahoma.

Common garden communities—We tested whether refuse addition is associated with distinct invertebrate communities within this common garden experiment. After 6 weeks, we harvested detrital invertebrate communities from the each plot (Refuse, Soil and Control plots) and extracted and identified invertebrates as described above in *Detrital invertebrate communities: Natural communities*.

Statistical analysis

Artificial and natural decomposition experiments—We tested for substrate decomposition differences among refuse piles, 1 m and 10 m (artificial substrates) or 0 and 10m (natural substrates) using Profile Analysis (SPSS v. 10.0, Chicago, IL). Profile Analysis tests three null hypotheses: 1) Slopes do not differ from 0 over time (i.e., flatness: a measure of within-group main effect), 2) No difference among groups over time (i.e., equal levels: a measure of between-group main effect), and 3) No difference among slopes over time (i.e., parallelism: a measure of within-group x between-group interaction). We used arcsine percent mass loss of leaf litter, filter paper or wood as the dependent variable sampled over time (within-group) and distance as the independent (between-group) variables. We used percent mass loss because numerous samples had 0 mass remaining after 6 weeks (our last collection time). In these cases we substituted all 100% mass loss values with 100 – 1/4n, where n was the sample size (Bartlett 1947, Sahu 2013). We report Wilk's lambda unless within-subject data did not meet assumptions of sphericity (Mauchly's Test of Sphericity: $p \leq 0.05$), in which case we report Greenhouse-Geisser test statistics. If any of these null hypotheses were rejected we performed Tukey HSD (between-group) or ANOVA (within-group) post hoc tests.

Common garden decomposition experiment—We quantified the effect of nest length and height above surface on the amount of refuse captured in buckets using linear regression. We then tested whether decomposition rates (arcsine percent mass loss of filter paper or wood) sampled over time differ among treatments (Refuse, Soil, or Control) in our common garden experiment using Profile Analysis. We preformed post hoc tests when any of the null hypotheses were rejected.

Chemical Analysis—We tested if refuse chemistry differed from leaf litter using GLM-MANOVA in SPSS (v. 10.0, Chicago IL). We used a posteriori ANOVAs to determine which chemistry parameters differed between refuse and leaf litter. We had insufficient samples to correct refuse for dry mass so we report results with caution. If refuse has higher nutrient content than leaf litter for a given element, these results are reliable because correcting for % dry matter increases the relative elemental quantity. Because leaf litter values are already corrected for dry mass, if we could correct refuse for dry mass this would result in an even greater difference between refuse and leaf litter. However, results that indicate no difference or a depletion in a given element should be interpreted with caution.

Natural detrital invertebrate communities—We tested whether invertebrate communities differed between locations (below nests vs. 10 m away) using Permanova in PC-ORD v. 6 (McCune and Mefford 2011). Permanova is a non-parametric multivariate analysis of variance that was designed for community analyses that violate the assumptions of parametric MANOVAs (Anderson 2001). Permanova tests the null hypothesis of no difference among groups (in this study: 0 vs. 10 m and Block: paired distances) using random permutations of the data. Permanova uses a Pseudo-F statistic and tests significance by permuting the data (for details see Anderson 2001). We used Bray-Curtis dissimilarities and 9999 permutations ($\alpha = 0.05$). We used a posteriori Wilcoxon tests to determine which taxa differed.

Common garden detrital invertebrate communities—We used Permanova to test for differences in invertebrate communities among Refuse, Soil, or Control plots as described above in *Statistical analysis: Natural detrital invertebrate communities*.

Results

We collected refuse from *A. trigona* nests that ranged from 0.7-3.1 m in length and 0.7-3.9 m in height above surface. Together, nest height above surface and length accounted for 64 % of the variation in refuse quantity collected below *A. trigona* nests. From a single nest, we captured on average 11.1 ± 9.03 ml (0.69 ± 0.39 g ml^{-1} wet wt) refuse day^{-1} $nest^{-1}$ (an underestimate given that our apparatus did not collect all refuse, particularly for nests higher in the canopy). The amount of refuse collected in buckets increased with nest length: a proxy for nest size ($p = 0.002$, Fig. 2a), and decreased with nest height above surface: a proxy for how diffusely the refuse fell ($p = 0.031$, Fig. 2b).

Decomposition experiments

Artificial and natural decomposition experiments—Decomposition rate generally increased with proximity to *A. trigona* nests for artificial substrates (Profile Analysis test of equal levels—main effect of distance: $p \leq 0.014$, Fig. 3) and natural substrates ($p \leq 0.058$) except for *Astronium graveolens* that had similar decomposition rates between distances and lost very little mass during the 6 week period ($p = 0.462$, Fig. 4, Table S1). After 6 weeks, mass loss averaged 1.5 fold higher below nests than 10 m away for artificial substrates (Tukey's HSD: $p < 0.05$) and 1.2, 1.3, and 1.4 for *A. rubrum*, *C. petlata*, and *Q. stellata* respectively. Substrates continued to lose mass over time (Profile Analysis test for flatness—Time: artificial substrates, *A. rubrum*, *C. petlata*, and *Q. stellata* $p \leq 0.001$; Table S1) and consistently for artificial and natural substrates (Figs. 3 & 4; Profile analysis test for parallelism—Time x Distance: $p \geq 0.074$; Table S1). Decomposition only increased under nests for *C. petlata* after 3 weeks ($p = 0.014$). These effects were evident for filter paper after 2 weeks, after 4 weeks for wood (Fig. 3), and after 6 weeks for *A. rubrum*, *C. petlata*, and *Q. stellata* (Fig. 4).

Common garden decomposition experiment—Over 6 weeks, *A. trigona* refuse added as a fertilizer in a common garden experiment, enhanced filter paper mass loss up to 1.3 fold more than soil addition or control plots (Profile analysis main effect of treatment: $p < 0.001$, Table S2; Fig. 5). The ability to detect this effect increased over time (Profile Analysis test for flatness: $p < 0.001$ and parallelism: $p = 0.050$, Table S2) and by 6 weeks, filter paper in refuse piles had virtually disappeared (Fig. 5a). Refuse addition marginally enhanced wood decomposition (Profile analysis test of equal levels: $p = 0.068$, Table S2). After 4 weeks, wood mass loss was marginally higher in refuse piles ($p = 0.049$) than controls but not soil plots, but treatment effects were smaller ($p > 0.05$) after 6 weeks (Profile Analysis test of flatness: $p < 0.001$ and parallelism: $p = 0.293$, Table S2, Fig. 5b).

Chemical Analysis—*A. trigona* refuse was enriched relative to leaf litter ($F_{12,15} = 96.579$, $p < 0.0001$) in a variety of elements shown in other tropical systems to limit decomposition (Table 1). Refuse was nearly 3-fold higher in total N, 6-fold in P, 13.5-fold in K, 9-fold in Na, and 2-fold in S. Refuse was depleted in a variety of nutrients relative to leaf litter: Fe by 41-fold, Ca by 2.5-fold, and Mn by 8-fold. There was no difference between *A. trigona* refuse and BCI leaf litter for Mg, Zn, Cu or Total C (Table 1).

Detrital invertebrate communities

Natural detrital invertebrate communities—Compared to 10m away, 14 of the 33 taxa quantified were more abundant in refuse piles and only Thysanoptera was less abundant (Fig. 6). Refuse communities averaged more arthropods (refuse: 736 ± 465, control: 322 ± 208; paired t-test $t_{18} = 3.67$, $p = 0.002$) and 2.4 fold more microbi-detritivores and predators compared to 10 m away (Permanova: $p = 0.0015$, Fig. 5, Table S3).

For example, the numerically dominant microbi-detritivores: Oribatids, Collembola and Diptera larvae, were respectively 2.1, 3.3, and 5.3 fold more abundant below nests compared to paired controls 10 m away (Fig. 6). Likewise, the less abundant Enchytraeids and Lumbricids (Annelida), Lepidoptera larvae and Nematoda also increased at least 3-fold under nests. Results of these latter taxa should be interpreted with caution as Berlese Funnel methods are not typically used to measure their abundance. Of the predators, Pseudoscorpions averaged 8.7 fold more abundant below *A. trigona* nests, and a variety of other predacious taxa were also more abundant, including the generalist ant predator *Wasmannia* (5-fold), Staphylinid beetles (4-fold), spiders (2.7 fold) and a 2.2 fold increase in predatory mites, which included Mesostigmata, Trombidioidea and Prostigmata (Fig. 6).

Common garden detrital invertebrate communities—The 6 weeks of fertilizing with *A. trigona* refuse did not alter invertebrate communities (Permanova, $p = 0.271$,

Table S4). Refuse addition plots trended toward higher arthropod abundance (refuse = 818 ± 642, soil = 477 ± 348, control = 422 ± 261; Permanova, $p = 0.266$), but with only a small effect size (partial eta^2 = 0.055).

Discussion

Here we demonstrate that social insects can generate long-term, concentrated nutrient conduits between the canopy and litter. We show that the Neotropical ant *A. trigona* produces a stream of C, N, P, K and other nutrients in the form of a rain of refuse that is at least 1.3 fold more concentrated in these decomposition-enhancing nutrients (Kaspari et al. 2008, Kaspari et al. 2009, Barantal et al. 2012) than leaf litterfall. Barro Colorado Island is 1,500 ha; given the density of *A. trigona* (3.7 ± 2.6 large nests 40m^{-2}, Clay unpublished data), this suggests that *A. trigona* deposit ca. 10.6 tons day^{-1} (12,488 liters day^{-1}) of refuse at this site. Litterfall on Gigante Pennisula, part of the Barro Colorado Island National Monument, averages 987.4 ± 123.9 g m^{-2} yr^{-1} (Sayer et al. 2012): ca. 40.6 tons day^{-1} on BCI. This suggests that this one species of ant deposits in refuse the equivalent of 25% of the total litterfall of a Neotropical forest! This nutrient stream is associated with a doubling of common microbi-detritivores like Oribatids and Collembolans and predators like Pseudoscorpions and a commensurate doubling of filter paper decomposition, a 1.5 fold increase in wood decomposition, and 1.2-1.4 fold increases in decomposition of three species of leaf litter compared to areas 10 m away. Further, marginally significant effects can extend up to 1 m from nests (Fig. 2) suggesting *A. trigona* produce 3m^2 patches of higher activity and abundance on the tropical forest floor. These results suggest that social aggregation in the canopy can generate heterogeneity in consumer productivity on the forest floor below.

The role of social insect nutrient translocation at ecosystem-level spatial scales has received surprisingly little attention (Wagner 1997, Whitford 2000, O'Dowd et al. 2003, Freyman et al. 2010, Wardle et al. 2011) despite other well documented excretion impacts on ecosystem function by consumers occurring in high densities (McNaughton et al. 1997, Beard et al. 2002, McIntyre et al. 2008, Atkinson et al. 2013, Feeley 2005). In kind and similar to insect frass additions in temperate forests (Lovett and Ruesnik 1995, Reynolds and Hunter 2001, Kagata and Ohgushi 2012), our common garden experiment confirmed the stimulatory effects of *A. trigona* refuse on artificial and natural substrate decomposition (Figs. 4 and 5). However, ours is one of a subset that follows the nutrient addition through to the decomposer communities themselves (but see Bardgett et al. 1998, Wardle 2002). Refuse piles that have likely persisted for multiple years supported distinct communities (Fig. 5). This was not the case in the experimental refuse addition plots after six weeks. A number of possibilities may account for our ability to replicate decomposition patterns but not invertebrate densities. First, six weeks may have been inadequate time to drive invertebrate population growth and recruitment. Second, it is highly plausible that refuse enhanced microbial activity or biomass (Allen and Schlesing 2004). Alternatively, increased invertebrate activity could have yielded the same result. More detailed information on refuse biochemistry and detrital community metabolism and composition is needed to identify the aspects of *A. trigona* refuse that impact nutrient cycling in this system.

Many aboveground insects produce excreta in pulses (seasonal or outbreaks: Lam and Dudgeon 1985, Lovett and Ruesnik 1995) and are not stationary, however, *A. trigona* nests have been known to persist 15 years or more, and as such, they generate long-standing productivity hot spots, landscape heterogeneity, and are dependable parts of the forest environment. Such dependability should have consequences for the surrounding plants. Accelerated decomposition frees more nutrients from standing crop dead organic matter that can stimulate plant growth and reproduction (Wardle 2002). The majority of *A. trigona* nests are attached near or on tree trunks, and the large quantities of refuse falling are concentrated at the base of host trees (N. A. Clay, *personal observation*). Given that *A. trigona* nests are stationary, long-lived, and nest refuse substantially enhances decomposition, host trees may benefit from their association with *A. trigona* through increased mineralization and nutrient availability. We predict that trees, especially the *A. trigona* nest host tree, will allocate roots to take advantage of these resources. Plants concentrate their nutrient uptake through fine roots and this response of tree-produced dense fine root mats occurs in howler monkey (*Alouatta*) excreta sites (Feeley 2005). Such nutrient exchange paired with *A. trigona*'s predatory behavior (Adams 1994) could lead to coevolution between *A. trigona* and host trees. However to our knowledge no studies have examined host tree fidelity in this species, but a closely related species *Azteca chartifex* shows preference for *Goupia glabra* (Goupiaceae) trees (Dejean et al. 2008). Further investigation into the relationship between *A. trionga* and host trees will likely reveal complex interactions.

The ecologies of the canopy and of the forest floor are often studied independently; research at the canopy-forest floor interface largely focuses on the roles of plants in litterfall. As important as litterfall chemistry is as a process linking above- and below-ground food webs (Wardle 2002), consumer excreta can also leave a substantial ecosystem footprint (Fonte and Schowalter 2005, Schowalter et al. 2011). The long-term impact of such subsidies from species like *A. trigona* are likely profound, especially when considered collectively with the diverse contributions of excreta from other canopy consumers.

Acknowledgements

We thank Taylor Zumbusch, David Donsoso, Matt Sweeney, Jon Shik, and Deana Flatt for assistance in the field and D. Flatt and Kendra Tholt for laboratory assistance. We also thank Oris Acevedo and Belkys Jiménez and STRI staff on BCI, Panama, ANAM for permits and OSU soil lab. ZEEB Discussion Group (OU) provided useful comments on earlier versions of the manuscript. Funding was provided by the Zoology Department (OU), the College of Arts and Sciences (OU), Graduate Student Senate (OU), and NSF DEB 0842038 (PI M. Kaspari, A. Kay).

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Table 1. Chemistry results from the Manova and univariate post hoc tests of refuse and leaf litter substrates from BCI, Panama (see Kaspari and Yanoviak 2008 for leaf litter collection and chemistry analysis methods). Parentheses next to substrates indicate sample size. Refuse values are uncorrected for % dry mass.

	Refuse (3)	Leaf litter (25)		
Chemical	Mean ± std	Mean ± std	F	P
P (%)	0.559 ± 0.081	0.091 ± 0.010	960.318	<0.0001
Ca (%)	0.840 ± 0.074	2.056 ± 0.536	14.930	0.001
K (%)	2.578 ± 0.630	0.127 ± 0.065	468.204	<0.0001
Mg (%)	0.305 ± 0.044	0.317 ± 0.126	0.026	0.872
Na (%)	0.074 ± 0.015	0.008 ± 0.002	513.693	<0.0001
S (%)	0.360 ± 0.052	0.188 ± 0.026	93.494	<0.0001
Fe (ppm)	772.10 ± 516.77	31547.36 ± 19190.31	8.230	0.008
Zn (ppm)	67.27 ± 17.27	77.33 ± 20.12	0.684	0.416
Cu (ppm)	46.57 ± 12.88	52.40 ± 17.63	0.304	0.586
Mn (ppm)	117.60 ± 41.64	957.22 ± 600.70	5.667	0.025
Total N (%)	5.032 ± 0.381	1.717 ± 0.233	481.422	<0.0001
Total C (%)	44.567 ± 1.850	39.258 ± 5.714	2.483	0.127

Figure Legends

Figure 1. Photo of *Azteca trigona* nest and the experimental bucket setup. Photo by Jane Lucas.

Figure 2. Amount of refuse collected in buckets propped on 1.5 m poles below 15 *Azteca trigona* nests. Refuse volume (ml) produced from each nest was measured every three days. Each point is the average of all 14 refuse measurements collected over 6 weeks for an individual nest related to that nest's length (a) and height above surface (b). Bars represent standard deviation.

Figure 3. Average percent mass loss of filter paper (a) and wood (b) directly below *A. trigona* nests (0m), 1m or 10m distance over time. Bars represent standard deviation and letters indicate significant differences among mass loss by distance within a sampling time.

Figure 4. Average percent mass loss of 2 BCI (a and b) and two temperate (c and d) species. * in c indicates p = 0.064. Bars are standard deviation and letters indicate significant differences among mass loss by distance within a sampling time.

Figure 5. Average percent mass loss of filter paper (a) and wood (b) in Refuse, Soil, and Control plots over time. Bars represent standard deviation and letters indicate significant differences among mass loss by treatment within a sampling time. *'s in A indicate p = 0.057.

Figure 6. Average abundance on a $\log_{10}$ scale of the 33 BFW taxa collected from $0.25m^2$ plots below nests in refuse or 10 m away. Taxa are divided into functional trophic groups. Bars represent positive standard deviation and *'s indicate significant difference in abundance (Wilcoxon, $\alpha = 0.05$) between refuse and 10m plots.

Figure 1

Figure 2

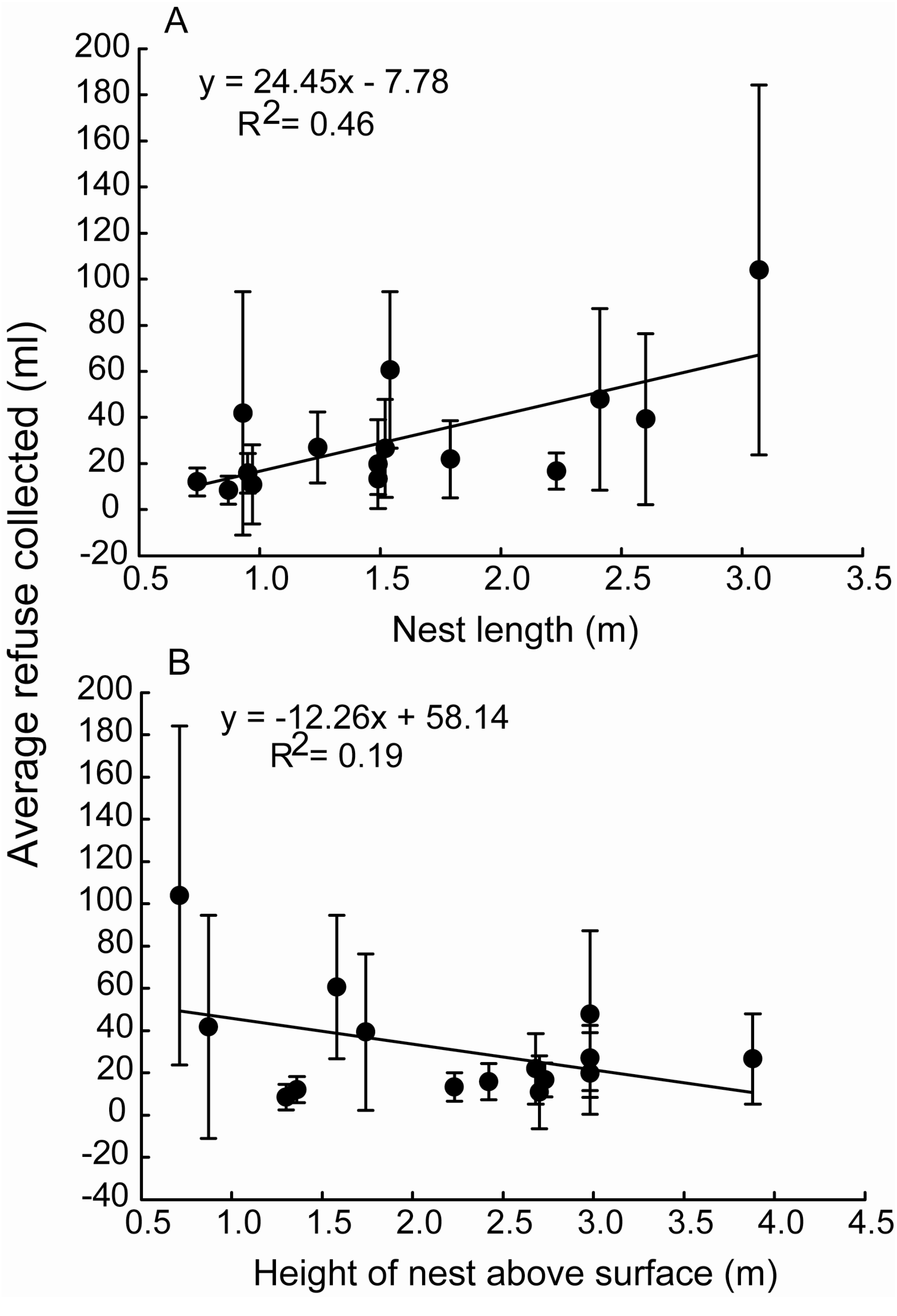

A
y = 24.45x - 7.78
R2= 0.46
200
180
160
140
120
100
80
60
40
20
0
-20
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Nest length (m)
Average refuse collected (ml)
B
y = -12.26x + 58.14
R2= 0.19
200
180
160
140
120
100
80
60
40
20
0
-20
-40
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
Height of nest above surface (m)

Figure 3

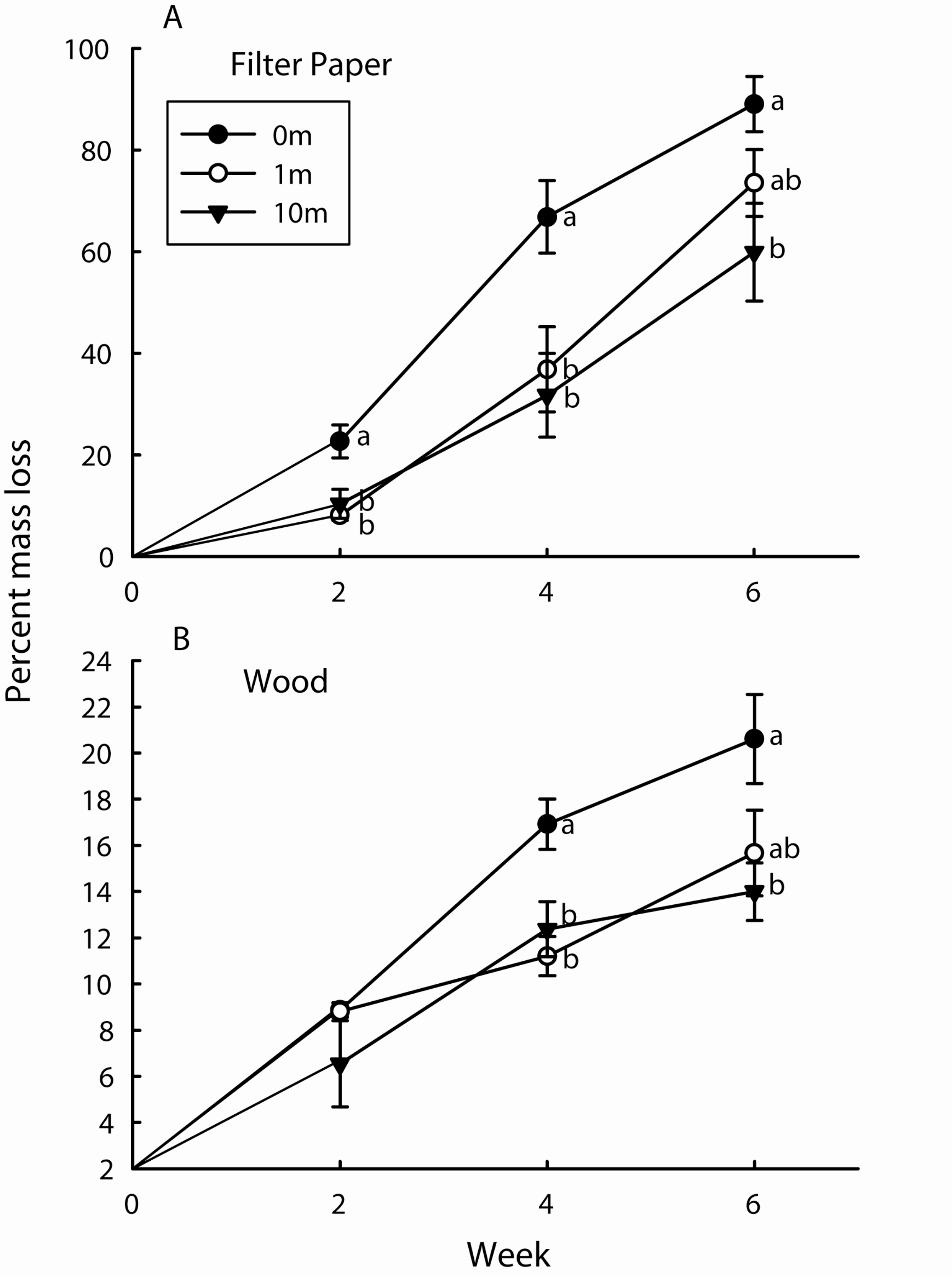

A
Filter Paper
0m
1m
10m
100
80
60
40
20
0
0
2
4
6
a
a
a
ab
b
b
b
b
b
B
Wood
24
22
20
18
16
14
12
10
8
6
4
2
0
2
4
6
a
a
ab
b
b
b
Percent mass loss
Week

Figure 4

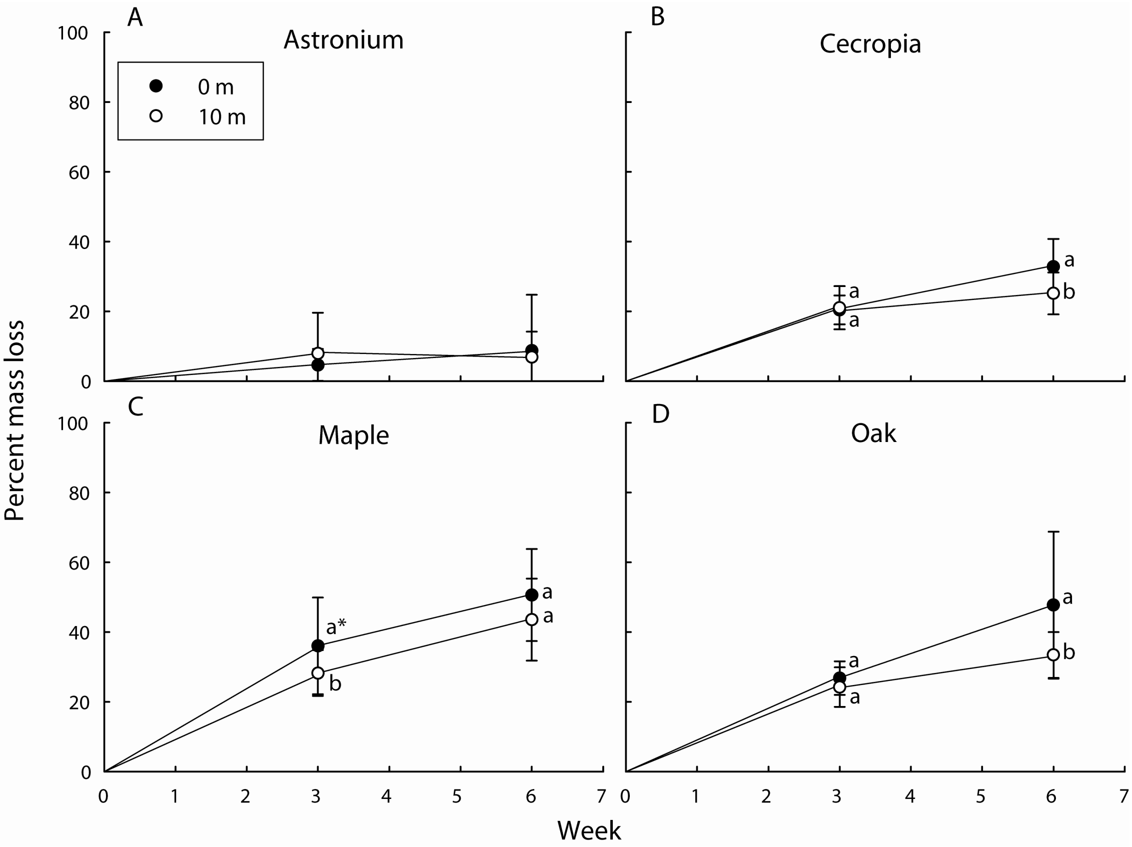

A
Astronium
B
Cecropia
C
Maple
D
Oak
● 0 m
○ 10 m
Percent mass loss
Week
0
20
40
60
80
100
0
1
2
3
4
5
6
7
a
b
a*

Figure 5

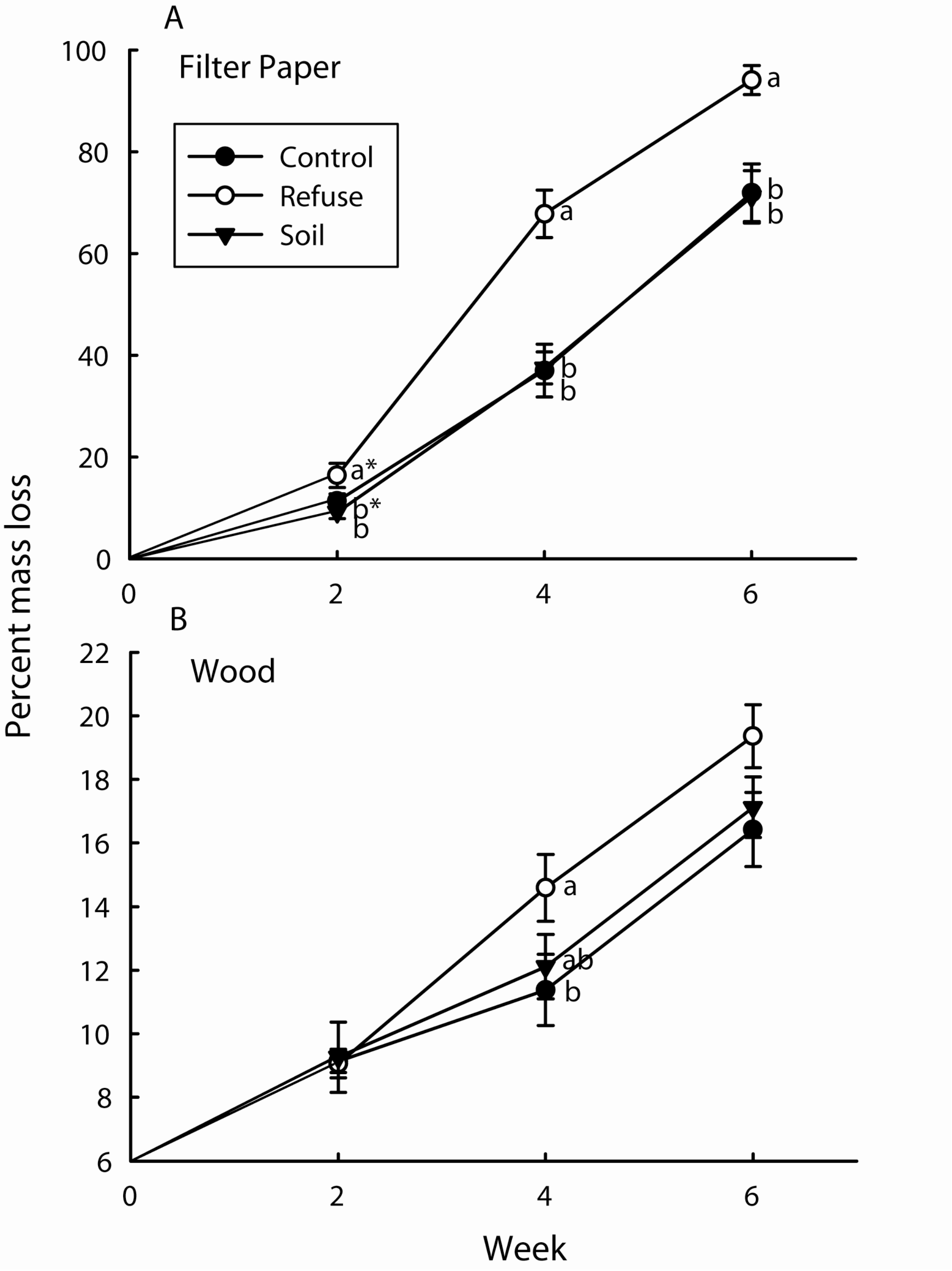
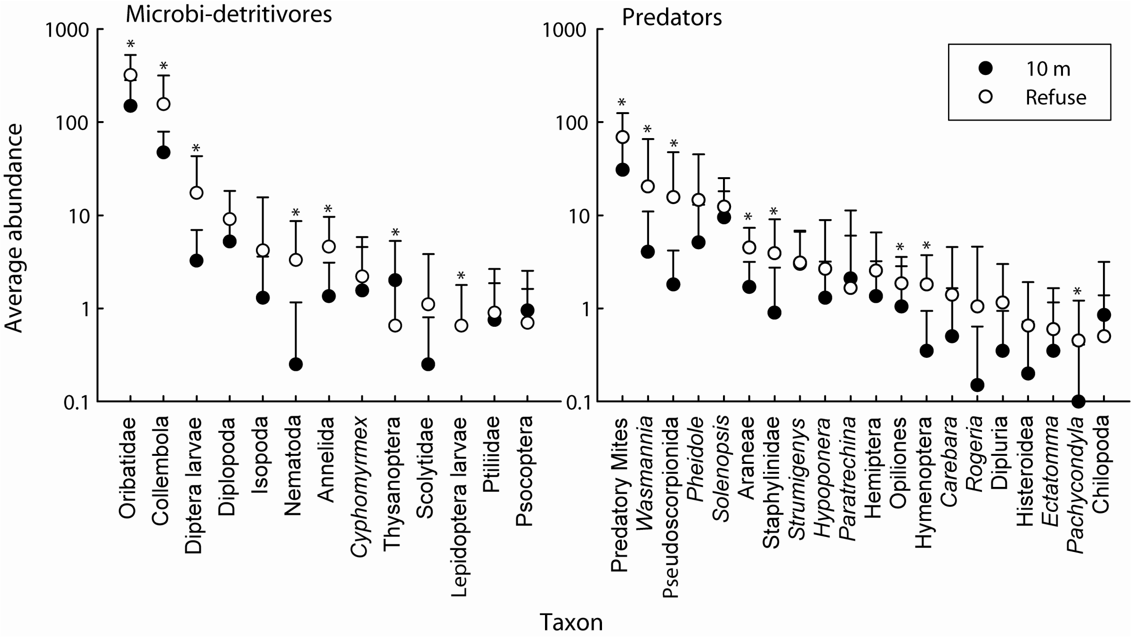

A
Filter Paper
100
80
60
40
20
0
Control
Refuse
Soil
a
a
a*
b
b
b
b
b*
b
0
2
4
6
B
Wood
22
20
18
16
14
12
10
8
6
a
ab
b
0
2
4
6
Percent mass loss
Week

Figure 6


Microbi-detritivores
Predators
Average abundance
1000
100
10
1
0.1
Taxon
Oribatidae
Collembola
Diptera larvae
Diplopoda
Isopoda
Nematoda
Annelida
Cyphomyrmex
Thysanoptera
Scolytidae
Lepidoptera larvae
Ptiliidae
Psocoptera
Predatory Mites
Wasmannia
Pseudoscorpionida
Pheidole
Solenopsis
Araneae
Staphylinidae
Strumigenys
Hypoponera
Paratrechina
Hemiptera
Opiliones
Hymenoptera
Carebara
Rogeria
Dipluria
Histeroidea
Ectatomma
Pachycondyla
Chilopoda
● 10 m
○ Refuse
*

Appendix

Supplementary Table 1. Results of the Profile Analysis testing *A. trigona* refuse effects associated with nests (Distance) on decomposition using both natural leaf litter (*Astronium graveolens*, *Cecropia peltata*, *Acer rubrum*, *Quercus stellata*), and artificial (filter paper and wood) substrates over Time. When assumptions of sphericity were violated we report Greenhouse-Geisser test statistics.

Source	*SS*	*df*	*MS*	*F*	*P*	Partial eta^2
Filter paper						
Between Subjects						
Distance	1.624	2	0.812	8.811	0.001	0.404
Error$_{BS}$	2.396	26	0.092			
Within Subjects						
Time	8.313	1.458	5.702	76.005	<0.001	0.745
Time x Distance	0.200	2.916	0.069	0.914	0.441	0.066
Error$_{WS}$	2.844	37.909	0.075			
Wood						
Between Subjects						
Distance	0.087	2	0.044	5.033	0.014	0.279
Error$_{BS}$	0.226	26	0.009			
Within Subjects						
Time	0.297	1.216	0.244	24.819	<0.001	0.488
Time x Distance	0.026	2.433	0.011	1.105	0.353	0.078

Error$_{WS}$	0.311	31.627	0.010			
Astronium						
Between Subjects						
Distance	0.030	1	0.030	0.556	0.462	0.019
Error$_{BS}$	1.502	28	0.054			
Within Subjects						
Time	0.001	1	0.001	0.017	0.898	0.001
Time x Distance	0.015	1	0.015	0.365	0.551	0.013
Error$_{WS}$	1.143	28	0.041			
Cecropia						
Between Subjects						
Distance	0.024	1	0.024	4.174	0.051	0.130
Error$_{BS}$	0.162	28	0.006			
Within Subjects						
Time	0.137	1	0.137	30.978	<0.001	0.525
Time x Distance	0.031	1	0.031	6.949	0.014	0.199
Error$_{WS}$	0.124	28	0.004			
Maple						
Between Subjects						
Distance	0.089	1	0.089	3.893	0.058	0.122
Error$_{BS}$	0.642	28	0.023			
Within Subjects						
Time	0.364	1	0.364	49.881	<0.001	0.640

Time x Distance	<0.001	1	<0.001	0.047	0.830	0.002
Error$_{WS}$	0.205	28	0.007			
Oak						
Between Subjects						
Distance	0.158	1	0.158	6.551	0.016	0.190
Error$_{BS}$	0.676	28	0.024			
Within Subjects						
Time	0.458	1	0.458	20.182	<0.001	0.419
Time x Distance	0.078	1	0.078	3.452	0.074	0.110
Error$_{WS}$	0.635	28	0.023			

Supplementary Table 2. Results of the Profile Analysis testing *A. trigona* refuse effects (Treatment: Refuse, Soil, or Control) on decomposition in the common garden experiment using artificial (filter paper and wood) substrates over Time. When assumptions of sphericity were violated we report Greenhouse-Geisser test statistics.

Source	*SS*	*df*	*MS*	*F*	*P*	Partial eta^2
Filter paper						
Between Subjects						
Treatment	1.877	2	0.938	18.297	<0.001	0.466
Error$_{BS}$	2.154	42	0.051			
Within Subjects						
Time	14.620	1.618	9.034	220.961	<0.001	0.840
Time x Treatment	0.355	3.237	0.110	2.679	0.050	0.113
Error$_{WS}$	2.779	67.970	0.041			
Wood						
Between Subjects						
Treatment	0.028	2	0.014	2.860	0.068	0.120
Error$_{BS}$	0.202	42	0.005			
Within Subjects						
Time	0.352	2	0.176	68.716	<0.001	0.621
Time x Treatment	0.013	4	0.003	1.259	0.293	0.057
Error$_{WS}$	0.215	84	0.003			

Supplementary Table 3. PERMANOVA results of BFW observational study comparing communities below nests (Refuse) and 10 m away (Control) (Nest effect: n = 20 each) using Bray-Curtis dissimilarities as the distance measure and 9999 permutations (α =0.05).

Source	d.f.	SS	MS	F	P
Nest effect	1	0.821	0.821	6.486	0.002
Block	19	2.274	0.120	0.946	0.589
Residual	19	2.404	0.127		
Total	39	5.499			

Supplementary Table 4. PERMANOVA results of BFW experimental study comparing communities in Refuse, Soil and Control plots (n= 15 each) using Bray-Curtis dissimilarities as the distance measure and 9999 permutations (α =0.05).

Source	d.f.	SS	MS	F	P
Treatment	2	3623.33	1811.67	1.211	0.271
Residual	42	62850.16	1496.43		
Total	44	66473.49			

Chapter 3: Urine as an important source of sodium increases decomposition in an inland but not coastal tropical forest

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Abstract

Nutrient pulses can profoundly impact ecosystem processes and urine is a frequently deposited source of nitrogen and potassium, and sodium (Na). Sodium is unimportant to plants, but its addition can increase decomposition and change invertebrate community structure in Na-poor tropical forests. Here we used synthetic urine to separate the effects of Na from urine's other nutrients and contrasted their roles in promoting decomposition and detritivore recruitment like termites in both a Na-poor inland Ecuadorian and a Na-rich coastal Panamanian tropical forest. Four treatments (250 ml of +Na, H_2O, Urine+Na, and Urine-Na) were applied every other day to 0.25 m^2 plots in a randomized block design. After 2 days and 2 weeks we sampled invertebrates and harvested litterbags containing cellulose and birch wood. Invertebrate communities did not vary among treatments at either site after only 2 days. But after 2 weeks, Ecuador wood, but not cellulose, decomposition was 2-fold higher on Urine+Na and +Na plots compared to H_2O and Urine-Na plots accompanied by > 20-fold increases in termite abundance on these plots. Panama, in contrast, showed no effect of Na on either cellulose or wood decomposition. In both forests, plots fertilized with urine (Urine+Na and Urine-Na) had nearly 2-fold decreases in detritivores after 2 weeks most likely due to a shock effect from ammonification. Moreover, the non-Na nutrients in urine did not enhance decomposition at this time scale. On control plots, Panama had higher decomposition rates for both cellulose and wood than Ecuador, but the addition of Na in Ecuador alleviated these differences. These results support the hypothesis that in Na-poor tropical forests, urine can enhanced wood decomposition and generate an important source of heterogeneity in the abundance and activity of brown food webs.

Introduction

Foraging animals influence the quantity and quality of available resources for the microbes, detritivores, and their predators in belowground systems, and can thereby impact decomposition and nutrient mineralization (Fittkau and Klinge, 1973; Wardle 2002; Bardgett and Wardle 2003). Herbivores, for example, can affect both the quantity and quality of litterfall inputs to detrital systems (Persson et al. 2000) and can alter resources across the landscape through vegetation trampling (Persson et al. 2000), soil turnover (Eldridge and James 2009), and damming (Wright et al. 2002). Likewise, predators impact the behavior and densities of herbivores that can ultimately alter decomposition processes (Fortin et al. 2005; Hawlena et al. 2012). But animals also provide some of the most important nutrient inputs to belowground systems via defecation, excretion, and corpses, which supply a direct and often concentrated source of bioavailable nutrients (Steinauer and Collins 1995; Drake et al. 2005; Weiss 2006). The deposition of these organic nutrient sources may alleviate nutrient limitation in otherwise nutrient-poor environments (e.g., McNaughton et al. 1977a).

Urine, in particular, is plentiful in nitrogen (N), potassium (K), sodium (Na), and to a lesser extent micronutrients like calcium (Ca) and magnesium (Mg; Steinauer and Collins 1995). Its deposition rate can be roughly quantified via metabolic scaling. As urine deposition rates (ml 24 hr^{-1}) scale to Mass (M) as $\text{aM}^{0.75}$ (Peters 1983; Brown et al. 2004) and animal density (N km^{-2}) scales as = $\text{bM}^{-0.75}$, a corollary of the Energy Equivalence Rule (Damuth 1981, 2007; Nee et al. 1991) predicts mammal urine deposition as a product of species richness and the two normalization constants (Habeck and Meehan 2008): $a = 60.85$ (Edwards 1975), and $b = 4.06$ (Ebenman et al. 1995).

Thus, a lowland Ecuadorian forest with 199 non-volant, non-domesticated, terrestrial mammals (InfoNatura 2007) should receive ca. 49 L km^{-2} day^{-1}. These nutrients can enhance decomposition (Kaspari et al. 2008a; Kaspari et al. 2009; Waring 2012) and are rare in many environments (White 1993; Wright et al. 2011; Kaspari et al. 2008b). Thus, urine is likely an important stimulus for detrital food webs and their function.

In particular, urine is a rich source of Na and likely creates Na hotspots in Na-poor environments away from oceanic Na sources (Stallard and Edmond 1981; National Atmospheric Deposition Program 2008). Inland tropical forests are ideal candidates for Na limitation because they are far from oceanic inputs, have high degrees of leaching, and high temperatures increase metabolic Na loss (Peters 1983; Kaspari et al. 2009; Dudley et al. 2012). Plants require and concentrate very little Na in their tissues, but plant consumers concentrate Na 10-100 fold above plants for neural function, osmotic balance, reproduction and development (Ganguli et al. 1969; Bursey and Watson 1983; Blumwald et al. 2000; Geerling and Loewy 2008). This stoichiometric disparity between plants and plant consumers drives detritivores, as plant consumers, to seek Na (Schulkin 1991; Geerling and Loewy 2008; Dudley et al. 2012). Previous research demonstrated rapid community and decomposition responses to Na subsidies in inland forests that mimicked patchy urine deposition (Clay et al. *in review*, Kaspari et al. 2009); this suggests that these communities have likely evolved to capitalize on urine as a Na subsidy.

Most research has not separated urine into its nutritional components despite urine being a rich source of N, K, Na, Ca, and Mg, all of which can enhance decomposition processes (Steinauer and Collins 1995, 2001; Shand et al. 2000; Kaspari et al. 2008a; Kaspari et al. 2009; Waring 2012). Further, these nutrients may have additive or synergetic effects on belowground systems when one or more of these nutrients are limiting. Subsequently, the effects of urine deposition on decomposition processes may differ across sites depending on site-specific nutrient availability. For instance, in Na-poor inland tropical forests, urine deposition may enhance decomposition rates significantly more than in Na-rich coastal forests and these effects may be non-additive. Experimental factorial designs that use synthetic urine can discern the relative importance of these nutrients in driving decomposition processes by creating treatments that isolate and exclude specific nutrients.

We investigated the role of urine as a source of nutrients in two tropical forests using synthetic urine to separate the effects of Na from the other nutrients in urine. We tested the hypothesis that urine is an important source of Na for detrital communities and their function in inland but not coastal tropical forests with the predictions that 1) because inland detritivores are likely Na-limited (Clay et al. *in review*), Na addition should increase detritivore abundance in inland but not coastal forests, 2) Na addition should increase decomposition in inland but not coastal forests (e.g., Kaspari et al. 2009), and 3) because urine is also a source of many other bioavailable and often limiting nutrients, the addition of synthetic urine containing Na and all other nutrients may have additive or synergetic effects on detritivore abundance and decomposition. In both an inland and coastal tropical forest, we document the role of urine as a Na source for communities after 1 application simulating a migratory mammal and after 2 weeks simulating mammals with excretion site fidelity and the effects of the other nutrients in urine on detrital communities and their function. Further, we discuss how animal behavioral ecology through urine deposition can impact landscape heterogeneity and ecosystem function.

Materials and Methods

Fieldwork was conducted from June 4 to June 24 in Barro Colorado Island (BCI), Panama (2009) and Yasuni National Park (YNP), Ecuador (2010), both lowland tropical rainforests. We *a priori* chose BCI and YNP as likely Na-rich and Na-poor forests based on distance from an ocean (National Atmospheric Deposition Program 2008, Kaspari et al. 2008b, Kaspari et al. 2009). BCI is a seasonal tropical forest located between two oceans (9°09' N, °79'51 W) receiving ca. 2600 mm rain annually, the majority of which falls in the rainy season from mid-April to mid-December (Wieder and Wright 1995). The soils have been described as Frijoles Clay with alfisols and oxisols (Windsor et al. 1990). YNP is an aseasonal tropical rainforest located inland, west of the Andes (0°40' S, 76°24' W) that receives ca. 3000 mm rain annually. The soils are not well described, but have been characterized as typic hapludult and typic paleudults characterized by clayey, kaolinitic, udic, isohyperthermic, and rich in aluminium with affinities to inceptisols and oxisols in at least one site (Korning et al. 1994).

In each forest, we demarcated 25 blocks of four treatments plots: Na addition (+Na), urine with Na (Urine+Na), urine without Na (Urine-Na), and control (H_2O), which allowed us to separate effects of Na from those of the other nutrients in urine. Treatment plots within blocks were 0.25 m^2 (0.5 x 0.5 m) and separated by 3 m. Blocks were separated by $\geq$ 10 m. We made the synthetic urine the day of addition using the chemical formula from Steinauer and Collins (1995): 13.65 g/l Urea, 0.75 g/l $MgCl_2$-$6H_2O$, 0.73 g/l $MgSO_4$-$7H_2O$, 0.09 $CaCl_2$, 7.02 g/l KCl, and 6.83 g/l $KHCO_3$. In the Urine+Na treatment we increased the Na concentration to 5g/l NaCl. This value mimics the upper range of predator urine Na content (Clarke and Berry 1992) and other Na fertilization research (Kaspari et al. 2008b, Kaspari et al. 2009, Clay et al. *in review*). Prior to fertilization, we placed a polyester mesh litterbag with 0.75 cm mesh containing pre-weighed and folded Whatman #1 9 cm circular filter paper (Whatman^{TM}) for cellulose and the wooden dowels were 11.4 x 0.95 x 0.32 cm (l x w x d) white birch (*Betula papyrifera*). We used standard substrates to minimize substrate-derived variation and increase comparability between sites and with other studies (Harmmon et al. 1999). Every other day we added 250 ml of each respective treatment solution to treatment plots within each block.

Invertebrate communities—We randomly and destructively harvested 5 blocks on days 2, and 15 blocks after 2 weeks (days 15, 16, and 17). We harvested the 2 week plots over 3 days due to limited Berlese funnels and hereafter refer to these 15 blocks as one group (2 weeks). We harvested after 2 days (1 fertilizer application) to examine rapid effects that simulate a migratory mammal with patchy and ephemeral urine deposition. Plots harvested after 2 weeks simulated mammals with excretion site fidelity like many primates (Feeley 2005). We harvested the detrital invertebrates on each plot by collecting leaf litter down to the top soil into a sifter with a 1 cm^2 metal mesh. We discarded the coarse leaf litter such as leaves and twigs above the metal mesh, and retained the ‘siftate’ that contained the invertebrate communities within the fine leaf litter material that passed through the 1 cm^2 mesh (Bestemeyer et al. 2000). We recorded the volume of the siftate and transferred a random 400 ml subset of each siftate to Berlese Funnels were invertebrates were extracted into 95% ethanol over 24 hours. We tested the null hypothesis of no difference in the community structure within blocks among treatments for 21 focal invertebrate taxa (supplementary material) on day 2 and after 2 weeks using a blocked Permanova (Anderson 2001) in PC-ORD v.6 (McCune and Mefford 1999) with 9999 permutations (see Anderson 2001 for a detailed description of Permanova). When we rejected the null hypothesis, we used a blocked indicator species analysis (PC-ORD) to determine which invertebrates differed among treatments.

Decomposition—We harvested litterbags from 20 blocks after 2 weeks. We included the additional 5 blocks to increase power in detecting treatment effects on decomposition. We did not examine decomposition after 2 days because this is insufficient time and sample size to detect effects. After harvesting litterbags, we gently rinsed substrates of debris, dried them at 60°C until weight stabilized, and reweighed them. We used percent mass loss as our dependent variable and we tested the null hypothesis of no difference in substrate percent mass loss (both cellulose and wood) among treatments within blocks after 2 weeks. We used a blocked Permanova in PC-ORD with 9999 random permutations. We followed significant differences in decomposition among treatments with *a posteriori* ANOVAs.

Results

Invertebrate communities—After 2 days invertebrate communities were similar among all treatments in Ecuador (Treatment: $p = 0.819$, Block: $p = 0.245$) and Panama

(Treatment: p = 0.069, Block: p = 0.0002; Table S1). After 2 weeks, the detrital communities differed among treatments (Ecuador, Treatment: p = 0.0001; Block: p = 0.0042; Panama, Treatment: p = 0.0309; Block: p = 0.0009; Table S2). In both forests, detrital invertebrate abundance was similar between Control and +Na plots, but decreased nearly 2-fold on plots that were fertilized with urine (Urine+Na and Urine-Na; Figs. 1 and 2). Seven taxa differed with treatment in Panama (Fig. 1), and in six in Ecuador (Fig. 2).

Termites in Ecuador were a conspicuous exception to the above pattern. Inland Ecuadorian termites only recruited to plots that were fertilized with Na (Urine+Na and +Na plots; Fig. 2). Conversely, termites did not differ among treatments in coastal Panama.

Decomposition—The Panama and Ecuador forests differed in their decomposition rates; specifically, median decomposition rates on control plots were 1.5- and 1.4 fold higher for cellulose and wood respectively in the coastal Panama forest compared to the inland Ecuador forest (Mann-Whitney Test, cellulose: N = 20, U = 110, p = 0.015; wood: N = 20, U = 20, p < 0.001; Fig. 3). Median cellulose mass loss after 2 weeks was 21.1 and 14.2 % for Panama and Ecuador respectively, and wood mass loss was 11.4 and 8.2 %. Adding Na alone eliminated the differences between the two forests (cellulose: N = 20, U = 168, p = 0.387; wood: N = 20, U = 188, p = 0.745; Fig. 3). This was not the case when Na was added with other nutrients, although differences were smaller (cellulose: U = 127, p = 0.048; wood: U = 116, p = 0.023).

Within Ecuador, wood decomposition was nearly 2-fold greater on Urine+Na and +Na plots than on Urine-Na or Control plots after 2 weeks (Treatment: p = 0.007, Block: p = 0.189; Table S3; Fig. 3). Contrary to our prediction, cellulose decomposition was consistent among treatments (Treatment: pseudo-F = 1.401, p = 0.221, Block: pseudo-F = 1.718, p = 0.027; Fig. 3). This was not the case in Panama where 2 weeks of fertilization had no effect on decomposition for either wood (Treatment: p = 0.115, Block: p = 0.356) or cellulose (Treatment: p = 0.268, Block: p = 0.214; Table S4; Fig. 3).

Discussion

Here we demonstrated that urine is an important source of Na for inland tropical detrital systems. Both BCI, Panama and YNP, Ecuador are species rich tropical forests (InfoNatura 2007), but BCI receives significant Na deposition from oceanic aerosols, and YNP does not (Stallard and Edmond 1981; National Atmospheric Deposition Program 2008; Kaspari et al. 2008b). Adding synthetic urine containing Na to the detrital system in Ecuador increased median wood decomposition nearly 2-fold above Control and Urine-Na plots (Fig. 3). These decomposition effects were similar to those of just Na addition (Fig. 3), suggesting that the Na in urine drove these results. Termites increased >20-fold on plots containing Na in Ecuador (+Na and Urine+Na; Fig. 3). No other invertebrates responded to the Na treatments (Fig. 2). In contrast, the addition of Na as NaCl or in urine had no effects on decomposition or detrital community structure in coastal Panama as predicted (Figs. 1 and 3). Contrary to our predictions, urine addition with or without Na decreased detrital invertebrate abundance 2-fold in both Ecuador and Panama despite the bioavailable N, K, and micronutrients in urine. Our results support the hypotheses that inland but not coastal tropical forests environments are Na-limited, and that urine is an important source of Na in Na-poor forests.

Plant consumers in inland tropical forests experience Na-shortfalls in at least three ways: 1) in their diet (Na-poor plants: Stamp and Harmon 1991), 2) in environmental availability (Na-poor tropical forests: Stallard and Edmond, 1981), and 3) through temperature enhanced metabolic Na loss (Peters 1983). This generally drives rapid Na-seeking behavior (Arms et al. 1974; Barrows 1974; Kaspari et al. 2008b). For example, Clay and colleagues (*in review*) found that detritivores strongly recruited to Na addition after just one day in an interior Peruvian forest near Iquitos. Accordingly, we expected to see similar responses from detritivores on +Na and Urine+Na treatments in inland Ecuador after 2 days, but communities were unaffected by treatment. Flooding regularly occurs at YNP and these frequently flooded soils have high cation concentrations including Na+ (Tuomisto et al. 2003), which may alleviate strong Na-limitation at YNP. A comparison of soil cations between YNP, Ecuador and the Iquitos-Pebas region, Peru clearly demonstrates higher concentrations of cations in soils at YNP than Iquitos-Pebas, Peru (Vormisto et al. 2004) and may explain why we did not see evidence of Na-limitation for the majority of detritivores in Ecuador.

Contrary to our predictions, urine addition did not stimulate decomposition in either forest after 2 weeks (Urine+Na and Urine-Na treatments: Fig. 3; Tables S3 and 4), and these plots had 2-fold decreases in detrital invertebrate abundance (Figs. 1 and 2; Table S2). This was likely a “shock effect” to ammonia production from urea hydrolysis (Marshall 1974). Initial reductions in soil fauna after urea application are commonly reported and followed by rapid recoveries as soon as four weeks (Marshall et al. 1974; Lohm et al. 1977). Natural latrines are typically nutrient rich sites where nutrient mineralization and decomposition occur rapidly and shock effects are likely insignificant because cell-free ureases can accumulate in areas of frequent urine deposition and accelerate mineralization (McNaughton et al. 1997b; Dos Santos Neves et al. 2010). It is likely that we would have seen increased invertebrate abundance and enhanced decomposition on urine-fertilized plots had we sampled after a longer period.

As predicted, decomposition increased with the addition of Na in inland Ecuador (+Na and Urine+Na treatments: Fig. 3; Table S3), and was accompanied by >20-fold increases in termites (Fig. 2). Termites in Ecuador recruited to Na despite potential ammonia toxicity on Urine+Na plots, whereas coastal Panamanian termites did not differ among treatments. Kaspari and colleagues (2009) also demonstrated strong recruitment of termites to Na addition in an inland tropical forest while investigating Na-limitation of decomposition and hypothesized termites drove the enhanced decomposition rates. The termites collected in this study were *Nasutitermes* and *Reticulitermes*, both of which are wood-feeding termites (Jones and Eggleton 2011), and wood within litterbags on Na-fertilized plots had conspicuous termite tunneling and was often covered in termites (N.A. Clay, *personal observation*). This suggests that our enhanced wood mass loss on plots fertilized with Na resulted from termite recruitment to and consumption of the salted wood (Figs. 2 and 3). Wood-feeding termites may have a higher Na-demand than other detritivores because lignin fermentation by their symbionts is a Na-intensive process (Yoshimura et al. 2002; Boiangiu et al. 2005). If wood-feeding termites are tracking Na sources and increasing wood consumption in the presence of Na, this could have important implications for carbon cycling and pest management across micro- and macro- geographies of Na availability.

When organisms are nutrient limited, they often suffer performance deficits (Arms et al., 1974; Sterner and Elser, 2002). Decomposition was 50 and 40% higher in coastal Panama than inland Ecuador for cellulose and wood respectively. However, the addition of Na (+Na treatment) alleviated these differences, but not for Urine+Na treatments—likely because of the ephemeral shock effect. Given sufficient time, it is likely that urine deposition would have the same or even enhanced effects (from the other nutrients) on decomposition in inland tropical forests as +Na treatments. These results support the hypothesis that Na shortages slow the degradation of cellulose and lignin and promote carbon storage, ultimately constraining the carbon cycle in inland tropical forests (Kaspari et al. 2009). Increased replications of this design in more paired forests and across longer timeframes will provide a better understanding of urine's role as a Na source for inland detrital food web structure and function.

Together, our results suggest that mammals act as nutrient conduits between above- and belowground processes through urine deposition and that urine is an important source of Na in inland tropical forests shaping forest floor heterogeneity and ecosystem function. Our results further supported the hypothesis that Na-limitation constrains the carbon cycle of inland tropical forests likely due in part to Na-limitation of wood-feeding termites. Thus, patchy and long-term urine deposition can increase detritivore abundance and activity creating hotspots of productivity on the tropical forest floor. Understanding mammal behavioral ecology can increase our understanding of how tropical forest ecosystems function and patterns of landscape heterogeneity.

Acknowledgements

We thank D. Flatt, K. Tholt, J. Burks and D. Rinehart for help in the laboratory. Special thanks to N. Velasco and T. Zumbusch, who were invaluable in the field. We also thank Oris Acevedo and Belkys Jiménez and STRI staff on BCI, Panama, and ANAM for permit SEX/AP-3-09. Miguel Rodriquez and Alvaro Barragán provided logistical help in Quito and Yasuni. We thank Ministerio de Ambiente del Ecuador for research permit 0020-2010-IC-FUN-DPO-MA. Funding was provided from the Loren G. Hill Zoology Excellence Fund, and NSF DEB-0948762 (PI M. Kaspari).

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Figure Legends

Figure 1. The results of the blocked indicator species analysis showing the abundances of the seven Panamanian invertebrate taxa that differed among treatments after 2 weeks.

Figure 2. The results of the blocked indicator species analysis showing the abundances of the six Ecuadorian invertebrate taxa that differed among treatments after 2 weeks.

Figure 3. Wood (lower panels) and cellulose (upper panels) decomposition measured as percent mass loss after 2 weeks of fertilization with each respective treatment in Ecuador (left panels) and Panama (right panels).

Figure 1

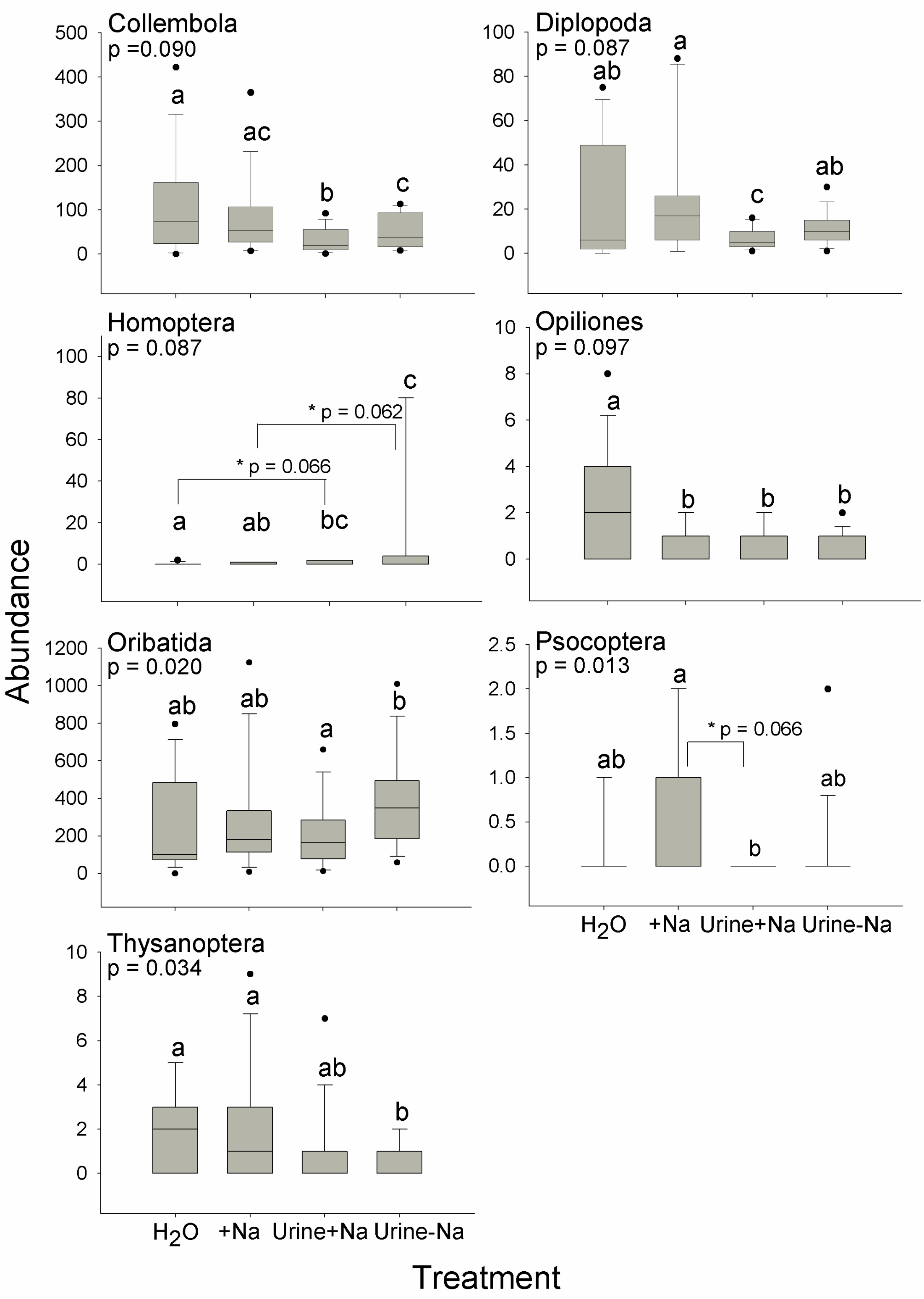

Collembola
p =0.090
500
400
300
200
100
0
a
ac
b
c
Diplopoda
p = 0.087
100
80
60
40
20
0
ab
a
c
ab
Homoptera
p = 0.087
100
80
60
40
20
0
* p = 0.062
* p = 0.066
a
ab
bc
c
Opiliones
p = 0.097
10
8
6
4
2
0
a
b
b
b
Oribatida
p = 0.020
1200
1000
800
600
400
200
0
ab
ab
a
b
Psocoptera
p = 0.013
2.5
2.0
1.5
1.0
0.5
0.0
ab
a
* p = 0.066
b
ab
Thysanoptera
p = 0.034
10
8
6
4
2
0
a
a
ab
b
Abundance
H2O
+Na
Urine+Na
Urine-Na
Treatment

Figure 2

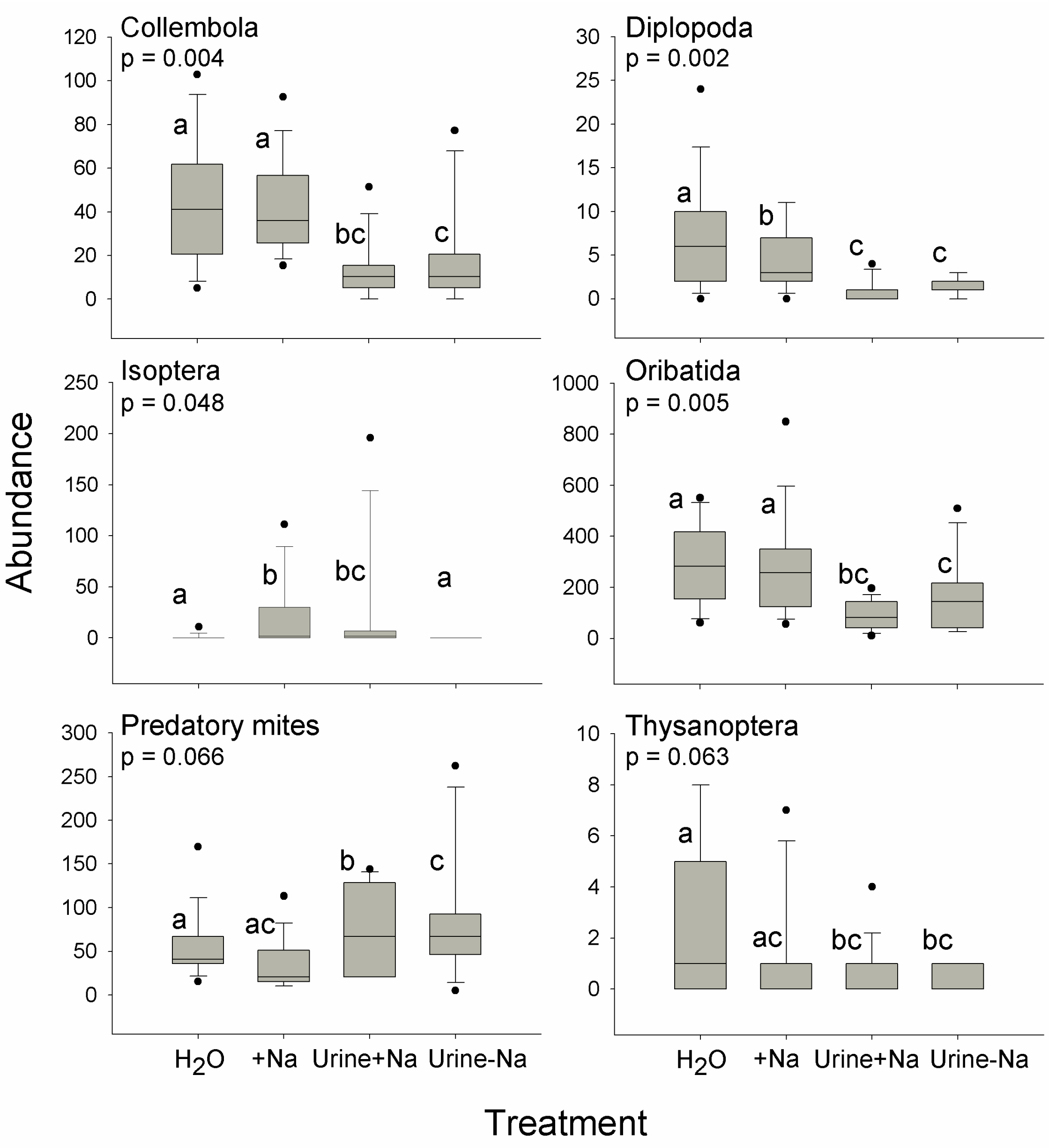
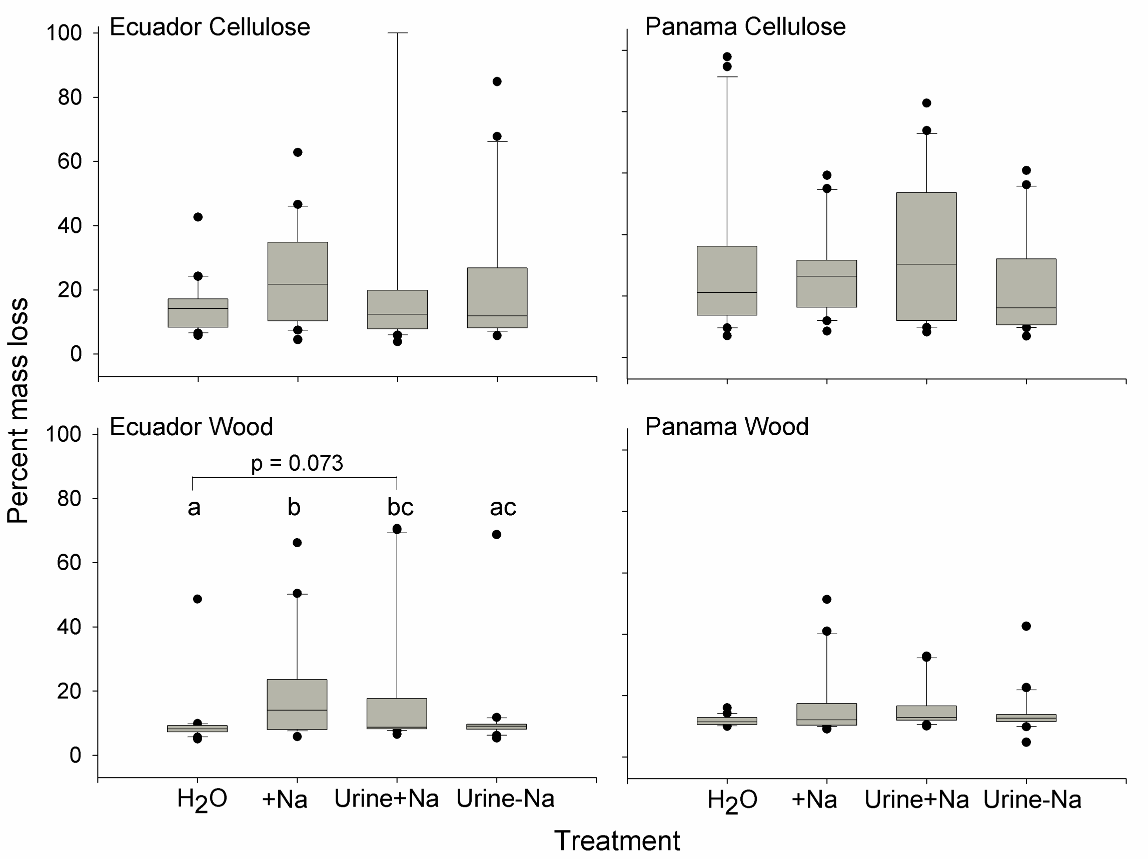

Collembola
p = 0.004
Diplopoda
p = 0.002
Isoptera
p = 0.048
Oribatida
p = 0.005
Predatory mites
p = 0.066
Thysanoptera
p = 0.063
a
a
bc
c
b
bc
ac
Abundance
Treatment
H_2O
+Na
Urine+Na
Urine-Na
0
20
40
60
80
100
120
5
10
15
20
25
30
50
150
200
250
300
400
600
800
1000
2
4
6
8

Figure 3


Ecuador Cellulose
Panama Cellulose
Ecuador Wood
Panama Wood
Percent mass loss
0
20
40
60
80
100
p = 0.073
a
b
bc
ac
H_2O
+Na
Urine+Na
Urine-Na
Treatment

Appendix

The 21 invertebrate taxa analyzed from detrital communities at each forest were: ants (Hymenoptera: Formicidae), spiders (Araneae), cockroaches (Blattaria), centipedes (Chilopoda), beetle larvae (Coleoptera larvae), beetles (Coleoptera), springtails (Collembola), fly larvae (Diptera larvae), millipedes (Diplopoda), flies (Diptera), pill bugs (Isopoda) caterpillars (Lepidoptera larvae), harvestmen (Opiliones), pseudoscorpions (Pseudoscorpionida), book lice (Psocoptera), termites (isopteran), thrips (Thysanoptera), predatory mites (Acari: Mesostigmata, Prostigmata, Trombidioidea), oribatids (Oribatida), true bugs (Hemiptera:Heteroptera), and homopterans (Hemiptera: Stenorrhyncha and Auchenorrhyncha).

Supplementary Table 1. Results of the Permanovas testing for differences in community structure among treatments after 2 days in Ecuador and Panama.

Source	d.f.	SS	MS	Pseudo-F	P
Ecuador					
Block	4	0.675	0.169	1.258	0.245
Treatment	3	0.237	0.079	0.588	0.819
Residual	12	1.609	0.134		
Total	19	2.520			
Panama					
Block	4	1.355	0.339	7.585	0.0002
Treatment	3	0.226	0.075	1.686	0.069
Residual	12	0.536	0.045		
Total	19	2.117			

Supplementary Table 2. Results of the Permanovas testing for differences in community structure among treatments after 2 weeks of fertilization in inland Ecuador and coastal Panama.

Source	d.f.	SS	MS	Pseudo-F	P
Ecuador					
Block	14	2.183	0.156	1.802	0.004
Treatment	3	1.374	0.458	5.296	0.0001
Residual	42	3.633	0.087		
Total	59	7.190			
Panama					
Block	14	3.106	0.222	2.036	0.0009
Treatment	3	0.644	0.215	1.971	0.031
Residual	42	4.577	0.109		
Total	59	8.327			

Supplementary Table 3. Results of the Permanovas testing for differences in percent mass loss of filter paper (cellulose) and white birch sticks (wood) among treatments after 2 weeks of fertilization in inland Ecuador.

Source	d.f.	SS	MS	Pseudo-F	P
Cellulose					
Block	19	2.637	0.139	1.718	0.027
Treatment	3	0.339	0.113	1.401	0.221
Residual	57	4.604	0.081		
Total	79	7.580			
Wood					
Block	19	1.526	0.080	1.290	0.189
Treatment	3	0.733	0.244	3.923	0.007
Residual	57	3.550	0.062		
Total	79	5.808			

Supplementary Table 4. Results of the Permanovas testing for differences in percent mass loss of filter paper (cellulose) and white birch sticks (wood) among treatments after 2 weeks of fertilization in coastal Panama.

Source	d.f.	SS	MS	Pseudo-F	P
Cellulose					
Block	19	1.895	0.100	1.263	0.214
Treatment	3	0.309	0.103	1.303	0.268
Residual	57	4.500	0.080		
Total	79	6.703			
Wood					
Block	19	0.577	0.030	1.090	0.356
Treatment	3	0.157	0.052	1.882	0.115
Residual	57	1.587	0.028		
Total	79	2.320			

Chapter 4: Toward a biogeography of omnivory: Omnivores increase carnivory when sodium is limiting

(Formatted for *Global Ecology and Biogeography*)

Abstract

Omnivores are a common trophic group, eating both plant and animal tissue, but what determines the ratio of plants:prey is not well understood. Toward understanding the geography of omnivory, we collected ants at Na, sugar, and H_2O baits in ten paired (inland and coastal) lowland forests from Georgia to Maine, USA. We tested three complementary hypotheses that predict abiotic drivers of plants:prey omnivores consume. The Sodium Limitation Hypothesis posits that omnivores consume proportionally more prey in sodium-poor environments because Na bioaccumulates from plants to predators, and thus, heterotrophs are rich Na-sources. The Nitrogen Limitation Hypothesis uses the same logic to predict lower plants:prey in N-poor environments. The Energy Limitation Hypothesis posits that high NPP environments support more organisms and trophic positions, and thus increase access to more prey. We used N stable isotope analysis to test each hypothesis by comparing $\delta^{15}N$ of 20 conspecifics of omnivorous ants shared between paired forests. Differences in $\delta^{15}N$ between conspecifics in paired forests ranged from 0.02‰ to 4.14‰ with a mean of 1.29‰. Sodium gradients accounted for 15% of this variation. We found no support for the Energy Limitation Hypothesis, but NPP, opposite to the predictions of the Energy Limitation Hypothesis, increased explanatory power 11% using stepwise multiple regression. We found no support for the Nitrogen Limitation Hypothesis. We then tested if Na-hunger and predation could be induced in a laboratory experiment. We fed *Solenopsis invicta* laboratory colonies Low, High, or Control Na diets to experimentally test the Sodium Limitation Hypothesis. *Solenopsis invicta* colonies fed Low-Na diets discovered crickets 6-times faster than Control colonies and sugar discovery (ant

activity) was consistent among diet treatments. Our results suggest that there is a geography of omnivory that can be partially understood as a response to gradients in Na and NPP.

Introduction

Omnivory, once thought to be rare, is now recognized as common in most ecosystems (McCann and Hastings 1997). Omnivory, here defined as consuming both plant and animal material, can increase food chain length (Sprules and Bowerman 1988), food web resilience and resistance (Fagan 1997), omnivores can serve as biological controls (González-Fernández et al. 2009), and their activity can link food webs and landscapes (Vanni et al. 2005). While the ecological consequences of omnivory have long been studied, we still know relatively little about how and why it is distributed in time and space.

The relative proportion of plant:prey consumed (prey consumption is defined here as any animal material consumed by scavenging, carnivory, or predation) by an omnivore is plastic (Landry 1981). A variety of abiotic and biotic factors can increase prey consumption such as temporal changes like those associated with reproductive seasons (Dam et al. 1994), ontogenetic changes from increased size or life stage (Polis et al. 1989), limiting nutrients (Denno and Fagan 2003), plant defenses (Agrawal et al. 1999), and competition (Brabrand 1985). Despite these many examples of omnivore diet plasticity, there is a distinct lack of a unifying theory or data for the biogeography of omnivory and the mechanisms controlling plant:prey consumption over the range of a given omnivore.

Toward a biogeography of omnivory, we investigate the explanatory power of three hypotheses that posit abiotic drivers of the relative proportion of plants:prey eaten by omnivores across the landscape:

The Sodium Limitation Hypothesis: Sodium is relatively unimportant for plants but critical for consumers. Plants concentrate very little Na in their tissues, but heterotrophs concentrate Na up to 100-fold more than plants in order to maintain neural function, osmoregulation, reproduction and development (Schulkin 1991, Geerling and Loewy 2008). The stoichiometric disparity between plants and herbivores results in a variety of adaptations to acquire Na such as puddling (Arms et al. 1974), visiting mineral licks (Dudley et al. 2012), and consuming low energy but salty plants (Botkin et al. 1973). Omnivores achieve Na tissue concentrations that are intermediate between herbivores and predators (Schowalter et al. 1981); this trophic bioaccumulation of Na renders heterotrophs as abundant sources of Na (Schowalter et al. 1981, Kaspari et al. 2008). The Sodium Limitation Hypothesis predicts that omnivores increase prey consumption when Na is scarce (e.g., Simpson et al. 2006).

The Nitrogen Limitation Hypothesis: Nitrogen is essential to both plants and animals, but is less abundant in plant tissue: the C:N ratio of plants is ca. 38 compared to 6 in herbivores and 4 in predators (Denno and Fagan 2003; Matsumura et al. 2004). This trophic bioaccumulation of N suggests N-limitation is most likely in plants, then herbivores, then predators (White 1993). Omnivores have N tissue concentrations that are intermediate between herbivores and predators (Matsumura et al. 2004). The Nitrogen Limitation hypothesis predicts that omnivores increase prey consumption in N-poor environments.

The Energy Limitation Hypothesis: Increases in the available energy of ecosystems (Net Primary Productivity: gC m^{-2} y^{-1}) are often accompanied by increases in species richness, the number of trophic levels, and food chain length (Jeanne 1979; McNaughton et al. 1989; Oksanen 1990; Kaspari et al. 2000; Kaspari 2001). The opportunity of omnivory (to feed on both plant and heterotrophs) thus increases with NPP. The Energy Limitation Hypothesis predicts that increases in NPP—and thus carbohydrates (CHOs), should provide the energy to support increased prey consumption by omnivores.

Ants are a model system for examining the geography of omnivory: they are ubiquitous, ecologically important, and although they occupy a diversity of trophic positions, are mainly omnivores (Hölldobler and Wilson 1990). Ants are easily manipulated in laboratory studies, and consistently implicated as indicators of Na limitation, N limitation, and energy limitation (Kaspari et al. 2000; Davidson 2005; Kaspari et al. 2008). Further, the trophic behavior of ant populations vary across communities (Davidson et al. 2003; Tillberg et al. 2007; Gibb and Cunningham 2011).

Here we test three hypotheses toward discovering the underlying mechanisms driving the geography of omnivory. We use both a field study that examines patterns of omnivory in 20 forests over 12° latitude and an experimental laboratory study that examines the mechanism driving increased prey consumption by omnivores from the hypothesis most supported in the field study.

Materials and Methods

Field component:

From May 21 to July 22, 2012 we sampled ants at 10 paired lowland forests from Georgia to Maine (Fig. 1). Coastal forests were 10-50 km from the coast and inland forests were on the same latitude but 200-400km from its paired coastal site (see Table S1 for forests and coordinates). Restricting locations to the east coast maximized the likelihood of collecting conspecifics, and limited other potentially confounding abiotic variables such as historic factors. The sampling sites represent a broad geographic range (ca. 12° latitude) that span multiple abiotic gradients such as mean annual temperature, but the paired design allowed us to separate latitudinally correlated effects from differences in Na, NH_4^+ and NPP between paired sites.

At each site we laid 12 100 m transects of 100 2.0 ml microcentrifuge vials between 10:00 and 17:00 h. Each vial was half filled with cotton and on the same day as deployment they were soaked in solution until the cotton was saturated, the excess solution poured out, vial snapped shut, and the outside rinsed. Solutions consisted of: NaCl at 0.1, 0.5, 1.0 % wt/vol, sugar at 1.0, 5.0, and 10.0% wt/vol, and H_2O as controls. Sugar usage is an indication of ant activity, while NaCl usage indicated the extent of Na-limitation. Each transect consisted of 15 vials of each concentration of NaCl and Sugar and 10 vials of H_2O. Upon deployment, one random vial was placed every meter with the cap open. After one hour, vials and ants were collected by snapping shut the vial with ants inside. This standardized ant collection while sampling a broad spectrum of ants in a short time. We identified ants to species and stored them in 35% by weight NaCl solution on ice.

For statistical analysis of bait usage we used 'hits' (number of vials that had at least 1 ant in it) rather than abundance (number of ants in a given vial) as the dependent variable, because strength of ant recruitment to resources is species-specific and the huge size variation among ants impacts the number of ants that can fit in a vial (e.g., *Camponotus* vs. *Monomorium*). We also tested for differences in ant species composition between paired sites to account for any systematic differences in species based on geographic distance from the coast. We used a blocked Permanova (Anderson 2001) in PC-ORD (McCune and Medford 1999) and 9999 permutations to test the null hypothesis of no difference between paired coastal and inland forests with Bray-Curtis dissimilarities as the distance measure.

From each site we collected leaf litter down to the top soil from four 0.25 m^2 plots and extracted arthropods from the litter using Winklers (Bestemeyer et al. 2000). The arthropods were kept in NaCl solution on ice, and leaf litter was dried in the field and stored with drierite. We chemically analyzed leaf litter samples for Na^+, NH_4^+, and NO_3^- and additionally measured Total N, Total C, ADF, ADL, P, Ca, K, Mg, S, Fe, Zn, Cu, Mn, to make test for additional nutritional differences between paired forests. The Soil Analytical Laboratory at Oklahoma State University (Stillwater, Oklahoma, USA) conducted analyses using a combination Lachat QuickChem 8000 flow injection autoanalyzer (Lachat, Loveland, Colorado, USA), Spectro CirOs ICP [Inductively Coupled Plasma] spectrometer (Spectro, Kleve, Germany), and an Ankom fiber analyzer (Ankom, Macedon, New York, USA). We tested the null hypothesis of no difference in chemistry between paired forests using a blocked permanova and 9999 permutations of the data.

We used stable isotope analysis to quantify trophic position of conspecific ants. N isotope values are reported using delta notation ($\delta^{15}N$) where $\delta = ((R_{\text{sample}}/R_{\text{standard}}) - 1) \times 1000$; R = ratio of heavy to light isotope ($^{15}N/^{14}N$ for nitrogen stable isotopes) of the sample and standard. Delta N values are expressed in ‰ (per mil notation). Nitrogen stable isotopes were analyzed using a Finnigan Delta Plus mass spectrometer (Thermo-Finnigan, Bremen, Germany) in the Stable Isotope Laboratory of the University of Georgia Odum School of Ecology. $\delta^{15}N$ values and precision were determined using a bovine protein (peptone) laboratory standard referenced against the international standard of atmospheric nitrogen. Nitrogen stable isotope precision was ± 0.08‰ (N = 8). We used average $\delta^{15}N$ values from each forest's leaf litter as a baseline for isotope values for forests (Ponsard and Arditi 2000; Nakagawa et al. 2007). We used average $\delta^{15}N$ for ant species in each forest so a given species is only represented once per forest.

Testing the Sodium Limitation Hypothesis: The terrestrial availability of Na is broadly determined through oceanic aerosol deposition (i.e., salty rain), which results in little or no oceanic Na input in land-locked environments (Botkin et al. 1973; National Atmospheric Deposition Program 2011a; Fig. 1). However, this pattern is not without exceptions, so we used %Na^+ in leaf litter samples to determine Na availability. We used Wilcoxon Signed Ranks Test to test the assumption that increased ant use of Na baits is a result of Na-limitation and not increased activity (determined by sugar use between paired forests). If percent ant use of Na is greater in the Na-poor paired forests, but sugar use does not vary between pairs, then we can reject the null hypothesis of no difference in Na-limitation between paired forests.

If Na-limitation drives increased carnivory, then the difference (inland-coastal) in the average δ^{15}N values ($\Delta\delta^{15}$N) between conspecific ants in paired coastal and inland forests should be related to the difference (coastal-inland) in Na availability (ΔNa) between paired forests (Fig. 2). $\Delta\delta^{15}$N is a measure of trophic shift or change in the relative consumption of plants:prey between conspecifics. We used linear regression to test the null hypothesis of no difference in conspecific δ^{15}N values ($\Delta\delta^{15}$N) between paired forests with different Na availability (ΔNa). If as the ΔNa increases (regardless of which paired forest was saltier), $\Delta\delta^{15}$N of conspecific ants increases resulting in the Na-poor forest having enriched ants with higher δ^{15}N values relative to the conspecifics in the Na-rich pair, then this supports Na-limitation driving increased carnivory by omnivores (Fig. 2).

Testing the Nitrogen Limitation Hypothesis: NH_4^+ and NO_3^- deposition is largely determined by rain patterns around industrial and agricultural areas and NH_4^+ and NO_3^- are both biologically available sources of N (Paerl et al. 2001; National Atmospheric Deposition Program 2011b; Fig. 1). We used NH_4^+ and NO_3^- (ppm) in leaf litter samples to determine NH_4^+ and NO_3^- availability. We first tested whether NH_4^+, NO_3^-, and Na^+ availability were correlated using partial correlation analysis. NH_4^+ and NO_3^- were uncorrelated with Na^+ ($p = 0.120$ and 0.090 respectively), but correlated with each other (Pearson Correlation = 0.865, $p \leq 0.001$). We therefore used only NH_4^+ as it had the highest explanatory power and least correlation with Na^+.

We used linear regression to test the null hypothesis of no difference in conspecific δ^{15}N values ($\Delta\delta^{15}$N: inland-coastal) between paired forests differing in NH_4^+ availability (ΔNH_4^+: coastal-inland). If as the ΔNH_4^+ increases (regardless of which paired forest was more N-rich), $\Delta\delta^{15}N$ increases, resulting in the N-poor forest having enriched ants with higher $\delta^{15}N$ values relative to their conspecifics in the N-rich pair, then this supports N-limitation driving increased carnivory by omnivores (Fig. 2).

Testing the Energy Limitation Hypothesis: If NPP (the availability of CHO) drives increased heterotroph consumption, then the difference in conspecific $\delta^{15}N$ values ($\Delta\delta^{15}N$: inland-coastal) should vary with ΔNPP (coastal-inland; Fig. 2). We used ArcGIS v. 10.2 to extract NPP values ($gCm^{-2}yr^{-1}$) for each forest site from the Moderate-Resolution Imaging Spectroradiometer (MODIS) MOD17 30-arcsec (1km spatial resolution) data for 2012.

We used linear regression to test the null hypothesis of no difference in conspecific $\Delta\delta^{15}N$ between paried forests differing in productivity (ΔNPP). If as the ΔNPP increases (regardless of which paired forest was more productive), $\Delta\delta^{15}N$ decreases, resulting in the more productive paired forest having enriched ants with higher $\delta^{15}N$ values relative to their conspecifics in the less productive paired forest, then this supports energy-limitation driving increased prey consumption by omnivores (Fig. 2).

Diet and $\delta^{15}N$ are plastic, but because we used multiple species to test each hypothesis and the amount of plasticity could be constrained by phylogeny we also used Bootstrap sampling with replacement on the linear regressions of all three hypotheses. We used 39 Bootstrap samples with 9999 replications, and 5% significance level (i.e., 95% confidence intervals). We tested the null hypothesis of $ß_1 = 0$, and we rejected the null if the 95% CI did not contain 0. We performed this analysis in R.2.15.2 (R

Development Core Team 2009) using Filiola's (2006) script (see supplementary material for script).

We further used stepwise multiple linear regression as a first step toward formulating a general model of abiotic controls of $\Delta\delta^{15}N$. Predictor variables were ΔNa, ΔNH_4^+, and ΔNPP (coastal-inland) and the dependent variable was $\Delta\delta^{15}N$ (inland-coastal).

Laboratory component:

If Na-limitation is driving increased carnivory, then the sodium-seeking behavior driven from Na shortfalls (Schulkin 1991; Geerling and Loewy 2008) should increase prey-seeking. We tested for plasticity and the extent to which Na-hunger can be manipulated using *Solenopsis invicta* (Red imported fire ant). *S. invicta* is sensitive to changes in diet and frequently displays diet shifts (Wilder and Eubanks 2010a; Wilder et al. 2011). We used 23 queen-right colonies collected from Norman, OK. We separated workers, queens, and brood from the soil using the water dripping method then reduced ants to 250 random workers and their queen and removed all brood. Colonies were kept in 31 x 21 x 6 cm plastic containers with talc powder around the rim to prevent ants from escaping. Containers were kept sealed with plastic lids that had two 9.5 x 1.3 cm rectangles cut out on either end for air circulation. These rectangular air slits were covered in fine 1 mm metal mesh to prevent ants from escaping. Each colonies was given a 50 ml plastic centrifuge tube containing 25 ml distilled H_2O and ca. 10 ml paper towel that absorbed the water and provided a continuously moist substrate for queens to colonize and for colonies to obtain water as needed. All queens colonized centrifuge tubes before the start of the experiment. Colonies were kept in 12:12 h light:dark schedule, and ants acclimated in the lab for at least 5 days fed a diet of tuna and honey with adlib water before the start of the experiment.

At the onset of the experiment, colonies were fed Low-Na diets (0.1%, n = 8), High-Na diets (0.9%, n = 8) and Control diets (0.4%, n = 7) based on Bhaktar and Whitcomb (1970) and Dussutour and Simpson (2008) artificial diets (see supplementary material for artificial diet methods and chemistry analysis: Table S2). We used goat milk and hemp protein to keep sodium concentrations low and we used fish oil as a source of lipids. Amino acids, carbohydrates, vitamins, minerals, and lipids amounts all approximated those in Dussutour and Simpson (2008; see supplementary material for detailed diet methods). We fed each colony an 8 cm^3 (2 x 2 x 2 cm) agar block of artificial diets in a 35 mm diameter dish. These dishes also contained 2 ml distilled water to keep food blocks moist and provide colonies with additional water. We replaced food blocks every other day.

After 5 weeks, we removed the food blocks and starved colonies for 5 days; colonies still had access to adlib water during this time. Colonies were offered a choice of cotton soaked in 5 % sugar solution or an immobilized cricket (PetSmart®). Sugar and Crickets were in separate 12.5 x 12.5 x 4.5 cm plastic containers and a Y-shaped bifurcating ramp connected the three containers (Colonies, Crickets, and Sugar). Ramps were made of poster paper and the central leg was 17 cm to the bifurcation with two 19 cm legs extending from the central leg at 60°. During trials each colony was placed in a white-walled arena where landmarks and other colonies were not visible. Position of the Cricket and Sugar were randomized and we measured Discovery Time for both Crickets and Sugar, which we defined as time after the start of trial when the first ant located and sampled Crickets and Sugar. After initial discovery, we measured Recruitment to Crickets and Sugar (the number of ants on either Resource) over a pseudo-log time scale starting at 5 minutes, then at 10, 20, 40, 60 and ending 80 minutes after initial discovery.

We analyzed colony Discovery Time to both Sugar and Crickets among diet treatments (Diet) using repeated-measures Anova (SPSS v. 10.0, Chicago, IL). To meet assumptions of equal variance and homogeneity we analyzed $\log_{10}(X+1)$ transformed Discovery Time. If the null hypothesis of no difference in time to discovery among diet treatments was rejected, we used one-way Anovas and Tukey HSD post hoc tests to determine which diet treatments differed in Discovery Time of Crickets and Sugar.

We similarly analyzed $\log_{10}(X+1)$ transformed recruitment to Crickets and Sugar (Resource) after initial discovery using repeated-measures Manova. We used mean Recruitment during eight 10 minute intervals (0-10, 11-20, 21-30….71-80 minutes) after initial discovery as our dependent variable. We tested the null hypotheses of no effect of time on recruitment (Time), no difference in recruitment between Crickets and Sugar (Resource), no difference in recruitment among Low-Na, High-Na and Control diets (Diet), and their interactions.

Results

The ten pairs of coastal and inland forests represented matched habitats for biogeochemistry, ant species composition, and activity. Leaf litter chemistry (Total N, Total C, ADF, ADL, P, Ca, K, Mg, Na, S, Fe, Zn, Cu, Mn, NH_4^+, NO_3^-) did not vary between paired coastal and inland forests (Permanova, Between pairs: $p = 0.190$, Block: $p = 0.309$; Table S4). Of the 18,198 ants collected in baited vials (72 species and 21 genera), species composition differed with latitude (Block: $p = 0.0006$; Table S3), but was consistent between paired forests (Permanova: Coastal vs. Inland: $p = 0.490$). Control (H_2O) bait usage was only 3% of the total vial usage (median = 4 hits per forest; IQR: 1 and 7) and was use was similar between paired sites (Wilcoxon Signed Ranks Test, W = 24, d.f. = 10, $p = 0.090$), as was use of sugar baits, which was a measure of activity (W = 41, d.f. = 10, $p = 0.169$). There were 39 instances of conspecifics (20 species from 10 genera) shared between paired inland and coastal forests (Fig. 3). Differences in $\delta^{15}N$ between conspecifics in paired forests ranged from 0.02 ‰ to 4.14 ‰ with a mean of 1.29 ‰.

Na concentrations varied from 0.006 to 0.1 % Na across the 20 sites; the median difference in Na between paired forests was 0.017 (IQR: 0.003 and 0.035). However, ants used Na baits 2-fold more overall in inland forests than in paired coastal forests (Wilcoxon Signed Ranks Test, d.f. = 10, W = 47.5 $p = 0.041$), with increased usage of 0.5 % Na in inland forests (W = 42, $p = 0.021$) but similar usage of 0.1 or 1.0 % Na baits between paired forests ($p \geq 0.113$). Na bait usage at all concentrations varied independently of leaf litter Na concentration (Pearson correlation, $p \geq 0.636$).

Consistent with the **Sodium Limitation Hypothesis** (Fig. 2), ants occupied higher trophic positions in the Na-poor forest compared to the Na-rich pair. Specifically, $\Delta\delta^{15}N$ increased with ΔNa, which accounted for 15 % of the variation in $\Delta\delta^{15}N$ (Linear Regression: $p = 0.015$; Fig. 3) and these results were supported by

Bootstrap analysis ($ß_1$ 95% CI: 1.2 and 42.8). At the extremes on the ΔNa axis (≥ 0.06 %), ants in the Na-poor forest were up to 4.1 ‰ enriched above conspecifics in the Na-rich paired forest (Fig. 3). One outlier, *Tapinoma sessile* (the odorous house ant), decreased in $\Delta\delta^{15}N$ by 4.1 ‰ in the Na-poor forest (Fig. 3), contrary to predictions. Without *T. sessile*, the Na gradient (ΔNa) accounted for 31 % of the variation in $\Delta\delta^{15}N$ ($\Delta\delta^{15}N = 27.9*\Delta Na + 0.007$, $R^2 = 0.311$, $p < 0.001$).

NH_4^+ concentrations varied from 74 to 372 ppm across the 20 sites with a median of 143.1 ppm (IQR: 107.1 and 203.7 ppm). The median difference in NH_4^+ between paired forests (ΔNH_4^+) was 77.0 ppm (IQR: 37.3 and 124.1 ppm). Contrary to the predictions of the **Nitrogen Limitation Hypothesis** (Fig. 2), there was no relationship between ΔNH_4^+ and $\Delta\delta^{15}N$ (Linear Regression, $p = 0.401$; Fig. 3). Bootstrap analysis supported these results ($ß_1$ 95% CI: -0.005 and 0.008).

Net Primary Productivity had a median value of 868 $gCm^{-2}yr^{-1}$ (IQR: 708 and 936 $gCm^{-2}yr^{-1}$) varying independently of distance from the ocean (Wilcoxon Signed Ranks Test, d.f.= 10, W = 41, $p = 0.169$). The median difference in NPP between paired forests (ΔNPP) was 71 $gCm^{-2}yr^{-1}$ (IQR: 22 and 214 $gCm^{-2}yr^{-1}$). Productivity had marginal predictive power for $\Delta\delta^{15}N$ (Linear regression $p = 0.081$; Fig. 3), but in the opposite direction of the predictions of the **Energy Limitation Hypothesis** (Fig. 2). Specifically, $\Delta\delta^{15}N$ increased with ΔNPP, meaning that conspecifics occupied a lower trophic position in the more productive forest. ΔNPP accounted for 8 % of the variation and at the extremes on the ΔNPP axis (-100 and 600), ants in the productive forest were up to 2.6 ‰ depleted below conspecifics in the relatively unproductive paired forest

(Fig. 3). Thus, we found marginal support for NPP (Bootstrap: $ß_1$ 95% CI: 7 x 10^{-6} and 0.004) in predicting $\Delta\delta^{15}N$, but not for the Energy Limitation Hypothesis.

We tested the combined predictive power of the three hypotheses using stepwise multiple regression (Table 1). ΔNa was uncorrelated with ΔNPP and ΔNH_4^+ ($p \geq 0.466$) but ΔNPP and ΔNH_4^+ were slightly correlated ($R^2 = -0.198$, $p = 0.012$). ΔNa best explained differences in $\Delta\delta^{15}N$ ($R = 0.385$) and variation explained by the model increased to 25.3% with the addition of ΔNPP. The model excluded ΔNH_4^+. We ran a second stepwise multiple regression with *Tapinoma sessile* excluded (Table 2). Again, ΔNa best explained differences in $\Delta\delta^{15}N$ ($R = 0.558$) and variation explained by the model increased to 40% with the addition of ΔNPP, but in this analysis, the most variation was explained (54%) when all three variables (including ΔNH_4^+) were included in the model.

Laboratory Experiment—For laboratory colonies of *S. invicta*, the Na content of the diet accounted for differences in prey discovery, but not subsequent recruitment to bait sources. *Solenopsis invicta* fed Low-Na diets discovered crickets 5.7-times faster than Controls (RM Manova, Diet: $p = 0.026$, Time: $p = 0.035$; Fig. 4; Table S5), but was similar to colonies fed High-Na diets ($p = 0.217$). Mean Cricket discovery time was 76 (± 77), 26 (± 26), and 14 (± 18) minutes for Control, High-Na, and Low-Na diets respectively. In contrast, Sugar discovery time averaged 15 (± 21) minutes and was consistent among diet treatments (Diet: $p = 0.615$). All colonies regardless of diet discovered Sugar at least 1.4-times faster than Crickets (Diet x Time: $p = 0.488$; Fig.4; Table S5).

After discovery, recruitment to Crickets and Sugar was consistent among diet treatments (Diet: $p = 0.686$) and over time (Time: $p = 0.279$; Diet x Time: $p = 0.430$; Table S6). Mean *S. invicta* recruitment to Crickets was ≥ 3.4-times stronger than to Sugar (Resource: $p < 0.001$) irrespective of diet (Diet x Resource: $p = 0.898$) or time (Time x Resource: $p = 0.383$; Figs. 4 and S1; Table S6).

Discussion

Omnivory is widespread, yet there is no general theory for predicting the proportion of plants:animals consumed in an omnivores diet across the landscape. Here we tested three potential hypotheses toward a predictive geography of omnivory: Sodium Limitation, Nitrogen Limitation, and Energy Limitation. We demonstrated that omnivorous ants (20 common species) from forests over a 12º span in latitude, were consistently higher in trophic position in relatively Na-poor forests than paired Na-rich forests. We further demonstrated in a laboratory experiment that Na-seeking behavior is inducible and that Na-starved ants seek prey and find it 6-times faster than ants given a sufficient Na supply (Fig. 4). In contrast to the Energy Limitation Hypothesis, the more productive forest in a pair supported omnivores that used proportionally more plant:animal tissue. Finally, we found no support for the Nitrogen Limitation Hypothesis as a driver of omnivory. In a stepwise multiple regression model, Na had the greatest predictive power (15%) and variation explained by the model increased to 25% when NPP was included. These data suggest that ants increase carnivory to maintain a Na-balance in relatively Na-poor environments.

Sodium regulating behavior is innate (Shulkin 1991; Geerling and Loewy 2008) and plant consumers and omnivores from termites to gorillas will travel long distances to sources (Tracy and McNaughton, 1995; Dormaar and Walker, 1996; Powell et al., 2009), consume urine (Weeks and Kirkpatick 1978; N.A. Clay *unpublished data*), shred logs (Rothman et al. 2006), and even resort to cannibalism (Simpson et al. 2006) to obtain this essential nutrient. By manipulating the diet of *S. invicta* colonies we demonstrated that Na-hunger can increase Na-seeking behavior 6-fold (Fig. 4; Table S5). Similar increases in Na-seeking behavior have been found in Na-starved laboratory rats (Zhang et al. 1984; Schulkin et al. 1985). The mechanism for higher discovery rates for Na in Na-starved ants is unclear as NaCl is not volatile. However, ants can detect prey through olfaction and can learn to associate odors with prey (Schatz and Hossaert-McKey 2010). Na-starved ants may have either been more sensitive to cricket odors or responded faster to cricket odors after initial detection. Further experimentation is needed determine the mechanism.

There were six species of ants of shared between the paired forests that showed the greatest disparity in Na content (ΔNa) and the ants in the Na-poor forests were an entire trophic level (3.4‰) above conspecifics in the Na-rich paired forest! Ants were trophically higher in the Na-poor forests even when inland forests were saltier than their coastal paired forest (Fig. 3). These ants could be increasing trophic position in at least one of two ways: through consumption of proportionally more prey or by feeding on prey that are high in trophic position (e.g., spiders). We have no data to distinguish between these possibilities.

Omnivory at the community level is a complex process, and Na gradients accounted for 15 % of the variation in $\Delta\delta^{15}N$; 31% if we excluded the single case of the species *T. sessile*. *Tapinoma sessile* is behaviorally subordinate (Fellers 1987); if the majority of ants in Na-poor forests are more carnivorous, *T. sessile* may be out-competed and may increasingly use sugar sources like hemipteran honeydew that are likely receiving less attention from the other ants. The most variation in $\Delta\delta^{15}N$ was located around 0 ΔNa (no difference between pairs; Fig. 3) indicating that when omnivores are not Na-limited, other factors become more important in determining the proportion of plants:prey consumed. These may include N- limitation, prey availability, resource quality and quantity, among a variety of other factors previously shown to influence prey consumption in omnivores (e.g., Brabrand 1985; Dam et al. 1994; Agrawal et al. 1999; Denno and Fagan 2003; Hin et al. 2011).

We found no support for the Energy Limitation hypothesis: ants in relatively productive forests were significantly lower in trophic position (not higher as predicted) than conspecifics in relatively unproductive forests. An alternative hypothesis is the Seasonality Hypothesis. The length of the growing season impacts variation in the availability of resources such as food as well as the ability to exploit essential resources (Boyce 1979); as the variation in temperature increases, the opportunity to acquire essential resources becomes limited to shorter time periods and the physiological need for higher quality and energy rich food sources increases (Vulla et al. 2009). The Seasonality Hypothesis predicts that omnivores increase prey consumption in more seasonal (variable) environments. Similarly, Vulla and colleagues (2009) found a positive correlation between increased carnivory (prey to plant consumption) and latitude in omnivorous mammals. They attributed this pattern to the shorter growing season in higher latitudes suggesting that omnivorous mammals likely increased their consumption of higher quality and energy-rich food to survive the winter and restore body condition after winter periods. Less productive environments may force omnivores to consume higher quality resources like protein (prey) to meet energy demands, thus environments that are Na-poor and relatively unproductive should have the most carnivorous omnivores. ΔNPP slightly increased the explanatory power of the multiple regression model when added to the Sodium Limitation Hypothesis (Table 1)

The Nitrogen Limitation Hypothesis has received the most attention in explaining the proportion of prey:plants consumed in an omnivores diet (White 1993, Fagan et al. 2002, Denno and Fagan 2003, Matsumura et al. 2004), but we found no support for N-limitation driving increased carnivory in omnivores. NH_4^+ is a biologically available source of N (Paerl et al. 2001), but examining N as a macromolecule in protein and amino acids may be a better predictor than N as an element (Wilder and Eubanks 2010b). As such the Nitrogen Limitation Hypothesis may be most applicable at the microscale, where local or temporal N availability (as amino acids and proteins) may cause omnivores to increase prey consumption.

These results provide a predictive framework toward understanding the geography of omnivory and the relative proportion of plants:prey omnivores consume across their range. The majority of research has been on invertebrate omnivores, but these principles should hold for vertebrates as well (e.g., Vulla et al. 2009). Omnivores in the most Na-poor forests increased their trophic position an entire trophic level above conspecifics in Na-rich paired forests; thus food chain length may also be higher in these forests. Increased carnivory could suppress prey density or activity and result in decreased herbivory (green food web) and detritivory (brown food web). This could result in a greener (e.g., HSS: Hairston et al. 1960) and browner (e.g., Wu et al. 2011) worlds in Na-poor environments.

Acknowledgements

We thank the many forests that gave us permits and permissions to collect. Special thanks to Edie Marsh-Matthews and Jack Longino for use of equipment, Jelena Bujan for help in the field, and Don Shepard and Ray Huey for valuable discussions. We also thank Tim Maddox at the Stable Isotope Laboratory of the University of Georgia Odum School of Ecology and Barbara McCray at Oklahoma State University Soil Analytical Lab. Funding was provided by an NSF DDIG DEB-1210336 (PI: M. Kaspari, Co-PI: N. Clay).

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Table 1. Correlation matrix and summary table of step-wise multiple correlation. The stepwise linear multiple regression excluded Variable 3 (ΔNH_4^+) from the final model.

Variable	1 $\Delta\delta^{15}N$	2 ΔNa	3 ΔNH_4^+	4 ΔNPP
1	1.000	0.385	0.138	0.283
2		1.000	0.120	-0.102
3			1.000	-0.395
4				1.000
Step number	Variable entered	R	R^2	Increase in R^2
1	2	0.385	0.148	0.148
2	4	0.503	0.253	0.105

Table 2. Correlation matrix and summary table of step-wise multiple regression with one outlier point *Tapinoma sessile* excluded from the model.

Variable	1 $\Delta\delta^{15}N$	2 ΔNa	3 ΔNH_4^+	4 ΔNPP
1	1.000	0.558	0.309	0.255
2		1.000	0.044	-0.377
3			1.000	-0.072
4				1.000

Step number	Variable entered	R	R^2	Increase in R^2
1	2	0.558	0.311	0.311
2	4	0.631	0.399	0.088
3	3	0.762	0.544	0.145

Figure Legends

Figure 1. Na^+ (A) and NH_4^+ (B) wet deposition (National Atmospheric Deposition Program 2011a and b) and Net Primary Productivity (C) for the USA and the ten paired forests sampled on the east coast (D).

Figure 2. Predictions of the Sodium Limitation, Nitrogen Limitation, and Energy Limitation Hypotheses with regards to $\Delta\delta^{15}N$ of conspecific ants between paired forests.

Figure 3. Results of the linear regression analyses testing the relationship between ΔNa, ΔNH_4^+, and ΔNPP and $\Delta\delta^{15}N$ of conspecific ants between paired forests. All x-axes are coastal-inland, and all y-axes are inland-coastal (see Fig. 2). Dashed lines are 95% confidence intervals and numbers in parentheses next to species names are the number paired forests that species was shared between.

Figure 4. Results of the Na dietary manipulation study on *Solenopsis invicta* with mean Discovery Time (minutes) and Recruitment (abundance) among colonies fed Low-Na, High-Na and Control diets. Bars represent positive standard deviation and letters differences in Cricket Discovery Time among diet treatments.

Figure 1.

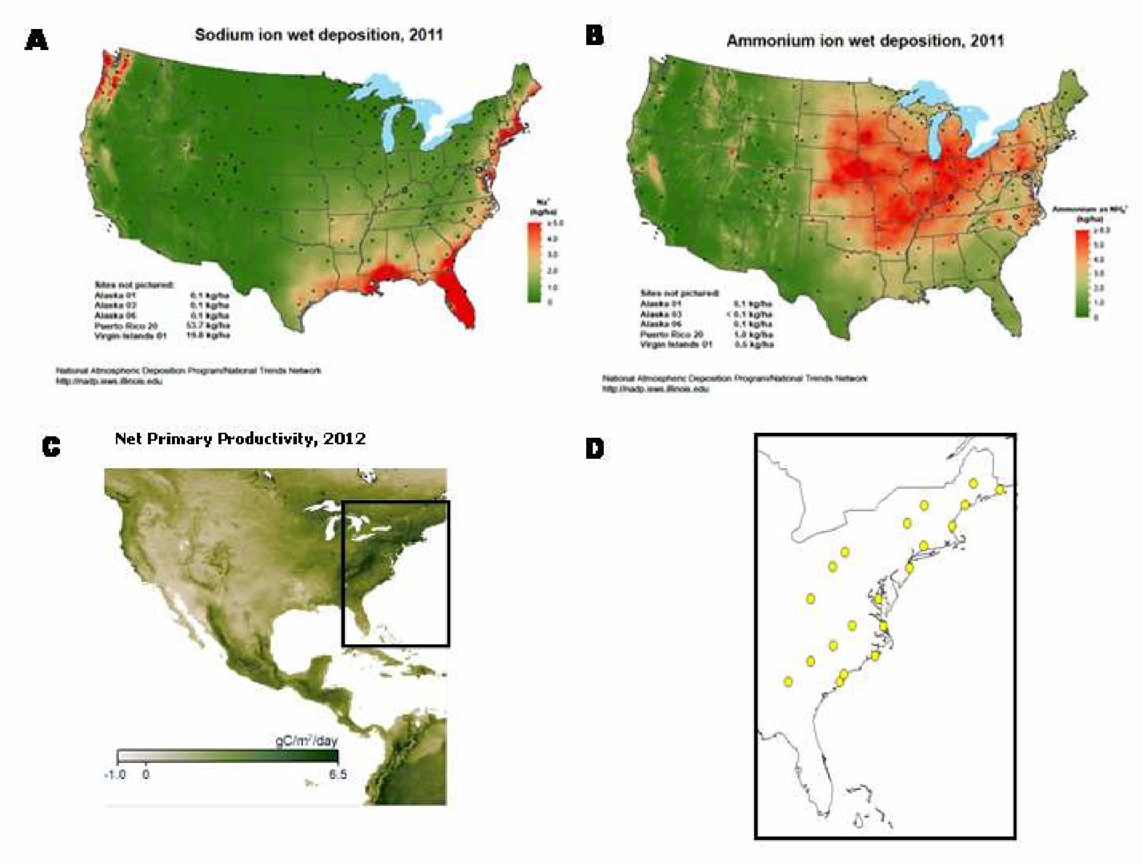

A
Sodium ion wet deposition, 2011
Na⁺ (kg/ha)
≥ 5.0
4.0
3.0
2.0
1.0
0
Sites not pictured:
Alaska 01 0.1 kg/ha
Alaska 03 0.1 kg/ha
Alaska 06 0.1 kg/ha
Puerto Rico 20 53.7 kg/ha
Virgin Islands 01 19.8 kg/ha
National Atmospheric Deposition Program/National Trends Network
http://nadp.isws.illinois.edu
B
Ammonium ion wet deposition, 2011
Ammonium as NH₄⁺ (kg/ha)
≥ 6.0
5.0
4.0
3.0
2.0
1.0
0
Sites not pictured:
Alaska 01 0.1 kg/ha
Alaska 03 < 0.1 kg/ha
Alaska 06 0.1 kg/ha
Puerto Rico 20 1.0 kg/ha
Virgin Islands 01 0.5 kg/ha
National Atmospheric Deposition Program/National Trends Network
http://nadp.isws.illinois.edu
C
Net Primary Productivity, 2012
gC/m²/day
-1.0
0
6.5
D

Figure 2.

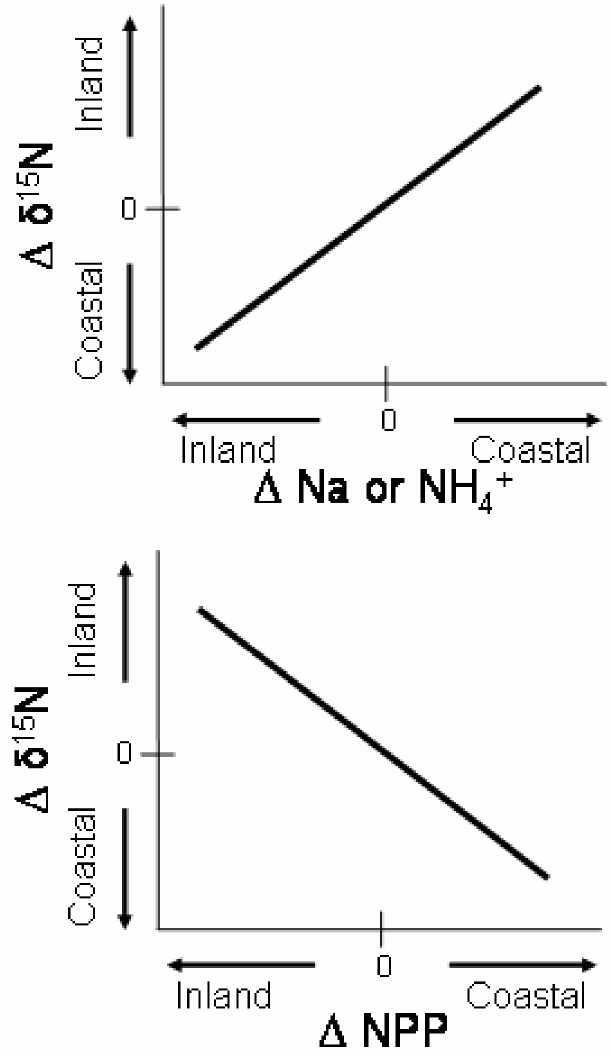

Δ δ15N
Inland
0
Coastal
Inland
0
Coastal
Δ Na or NH4+
Δ δ15N
Inland
0
Coastal
Inland
0
Coastal
Δ NPP

Figure 3.

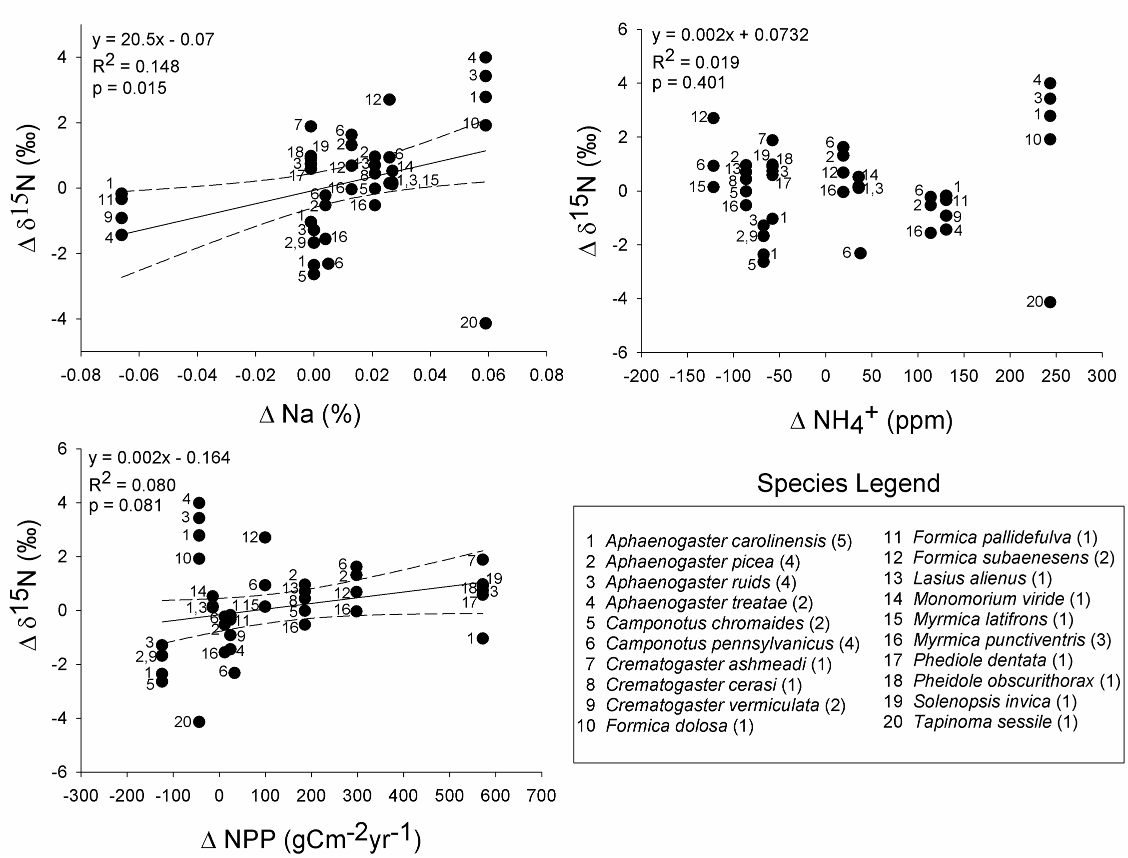
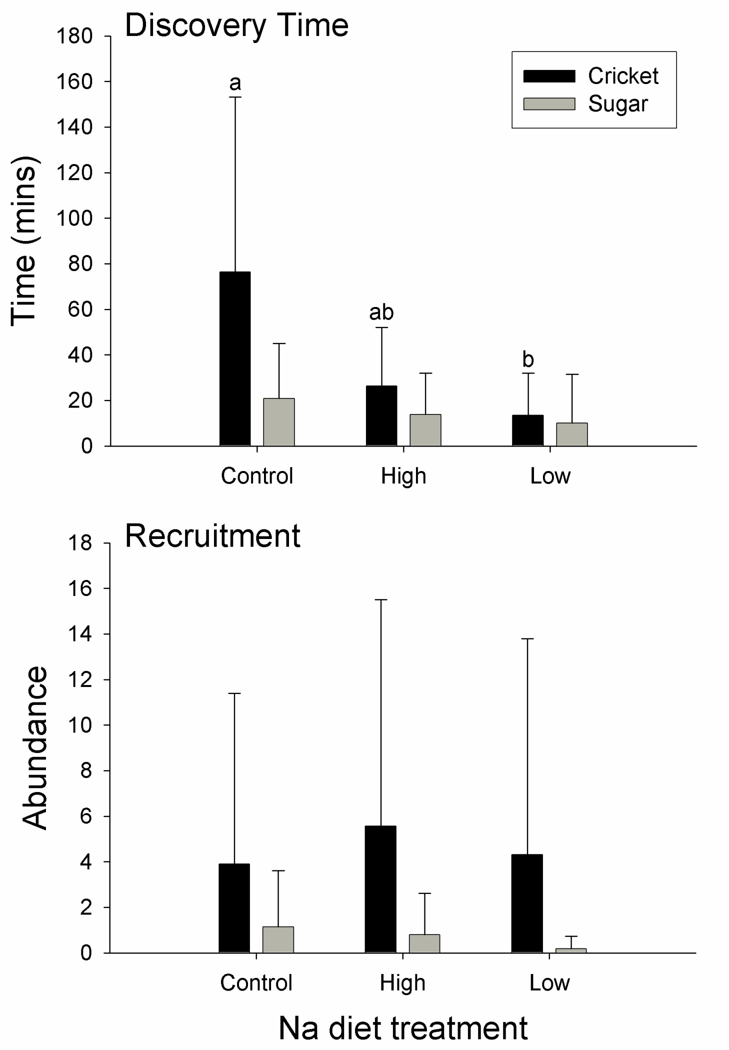

y = 20.5x - 0.07
R² = 0.148
p = 0.015
Δ δ15N (‰)
Δ Na (%)
-0.08 -0.06 -0.04 -0.02 0.00 0.02 0.04 0.06 0.08
y = 0.002x + 0.0732
R² = 0.019
p = 0.401
Δ δ15N (‰)
Δ NH4+ (ppm)
-200 -150 -100 -50 0 50 100 150 200 250 300
y = 0.002x - 0.164
R² = 0.080
p = 0.081
Δ δ15N (‰)
Δ NPP (gCm-2yr-1)
-300 -200 -100 0 100 200 300 400 500 600 700
Species Legend
1 Aphaenogaster carolinensis (5)
2 Aphaenogaster picea (4)
3 Aphaenogaster ruids (4)
4 Aphaenogaster treatae (2)
5 Camponotus chromaides (2)
6 Camponotus pennsylvanicus (4)
7 Crematogaster ashmeadi (1)
8 Crematogaster cerasi (1)
9 Crematogaster vermiculata (2)
10 Formica dolosa (1)
11 Formica pallidefulva (1)
12 Formica subaenesens (2)
13 Lasius alienus (1)
14 Monomorium viride (1)
15 Myrmica latifrons (1)
16 Myrmica punctiventris (3)
17 Phediole dentata (1)
18 Pheidole obscurithorax (1)
19 Solenopsis invica (1)
20 Tapinoma sessile (1)

Figure 4.


Discovery Time
Time (mins)
180
160
140
120
100
80
60
40
20
0
Cricket
Sugar
a
ab
b
Control
High
Low
Recruitment
Abundance
18
16
14
12
10
8
6
4
2
0
Control
High
Low
Na diet treatment

Appendix

Bootstrap linear regression in R

R code from Filiola (2006).

http://people.revoledu.com/kardi/tutorial/Bootstrap/Lyra/Bootstrap%20Simple%20Linear%20Regression.htm

```
simp.reg.boot<-function(x,y,b,k,alpha)

{

n<-length(x)

a1<-data.frame(x,y)

a<-data.matrix(a1)


inta<-n*sum(x*y)

intb<-sum(x)*sum(y)

intc<-n*sum(x^2)

intd<-(sum(x))^2

inte<-inta-intb

intf<-intc-intd

beta1<-inte/intf

ybar<-sum(y)/n

xbar<-sum(x)/n

beta0<-ybar-(beta1*xbar)
```

```
cat("Least Square Method","\n")

cat("beta zero =",beta0,"\n")

cat("beta one =",beta1,"\n")


yhat<-beta0+(beta1*x)

mse<-sum((y-yhat)^2)/(n-2)

varbeta1<-mse/sum((x-xbar)^2)

sebeta1<-sqrt(varbeta1)

sxx<-sum((x-xbar)^2)

varbeta0<-mse*(sum(x^2)/(n*sxx))

sebeta0<-sqrt(varbeta0)


cat("Classical Method","\n")

cat("The accuracy of beta zero","\n")

cat("bias of beta zero =",0,"\n")

cat("standard error of beta zero =",sebeta0,"\n")

cat(((1-alpha)*100),"% confidence interval for beta zero","\n")

lslobound0=beta0-(qt(1-(alpha/2),(n-2))*sebeta0)

lsupbound0=beta0+(qt(1-(alpha/2),(n-2))*sebeta0)


cat("lower bound =",lslobound0,"\n")

cat("upper bound =",lsupbound0,"\n")
```

```
cat("\n")


cat("The accuracy of beta one","\n")

cat("bias of beta one =",0,"\n")

cat("standard error of beta one =",sebeta1,"\n")

cat(((1-alpha)*100),"% confidence interval for beta one","\n")

lslobound1=beta1-(qt(1-(alpha/2),(n-2))*sebeta1)

lsupbound1=beta1+(qt(1-(alpha/2),(n-2))*sebeta1)


cat("lower bound =",lslobound1,"\n")

cat("upper bound =",lsupbound1,"\n")

cat("\n")



bootbeta0=NULL

bootbeta1=NULL

for(i in 1:b)

{

v<-sample(1:n,k,replace=TRUE)

indep<-a[v]

dep<-a[v,2]

int1<-k*sum(indep*dep)

int2<-sum(indep)*sum(dep)
```

```
int3<-k*sum(indep^2)

int4<-(sum(indep))^2

int5<-int1-int2

int6<-int3-int4

betaboot1<-int5/int6

depbar<-sum(dep)/k

indepbar<-sum(indep)/k

betaboot0<-depbar-(betaboot1*indepbar)

{

bootbeta0[i]=betaboot0

bootbeta1[i]=betaboot1

}

}


cat("\n")

cat("Bootstrap for Regression, Correlation Model","\n")


meanbetaboot0=mean(bootbeta0)

cat("Bootstrap's beta zero :",meanbetaboot0,"\n")

cat("The Bootstrap's accuracy measures of beta zero","\n")

biasbeta0=meanbetaboot0-beta0

cat("bootstrap's bias for beta zero=",biasbeta0,"\n")
```

```
varbetaboot0=var(bootbeta0)

sebetaboot0=sqrt(varbetaboot0)

cat("bootstrap's standard error for beta zero=",sebetaboot0,"\n")


cat(((1-alpha)*100),"% confidence interval for beta zero","\n")

lobound0=quantile(bootbeta0,(alpha/2))

upbound0=quantile(bootbeta0,(1-(alpha/2)))


cat("lower bound =",lobound0,"\n")

cat("upper bound =",upbound0,"\n")


cat("\n")


meanbetaboot1=mean(bootbeta1)

cat("Bootstrap's beta one :",meanbetaboot1,"\n")

cat("The Bootstrap's accuracy measures of beta one","\n")

biasbeta1=meanbetaboot1-beta1

cat("bootstrap's bias for beta one=",biasbeta1,"\n")


varbetaboot1=var(bootbeta1)

sebetaboot1=sqrt(varbetaboot1)

cat("bootstrap's standard error for beta one=",sebetaboot1,"\n")
```

```
cat(((1-alpha)*100),"% confidence interval for beta one","\n")
lobound1=quantile(bootbeta1,(alpha/2))
upbound1=quantile(bootbeta1,(1-(alpha/2)))

cat("lower bound =",lobound1,"\n")
cat("upper bound =",upbound1,"\n")

par(mfrow=c(1,2))
hist(bootbeta0)
hist(bootbeta1)
}
```

Diet

We used a diet based on Bhaktar and Whitcomb and Dussutour and Simpson 2008. To keep sodium levels low for Low-Na diets, we used Double Bonded Protein™ that was high-octane goat milk protein in dark chocolate flavor from Mt. Capra® and Living Harvest Tempt™ hemp protein. The amino acids and quantities approximated those in the artificial diet used by Dussutour and Simpson 2008. We also used Equate® Complete Multivitamin: A thru Z to provide the necessary vitamins and minerals and Now Foods® Lemon Flavored Omega-3 Fish Oil as a source of lipids and cholesterol. Lastly, we used honey to meet sugar requirements in a Sigma-Aldrich® Standard quality Agar.

We boiled 500 ml water and mixed in 10 g agar. During this time we blended 250 ml water with 52.5 g of the goat milk protein, 52.5 g hemp protein, 1 multivitamin, 170 g honey, 5 ml fish oil. We then mixed this blend into the agar solution. This solution was poured into three 15 x 2 cm (d x h) cylindrical dishes. These dishes contained either 0, 0.7, or 2.8 g NaCl for Low-Na, Control, and High-Na diets respectively and salt was mixed into solution. Dishes were covered and placed into the refrigerator to store.

Supplementary Table 1. Forest sites and coordinates where ants were sampled.

Forest	State	Coastal vs. Inland	Latitude	Longitude
Oconee National Forest	GA	Inland	33.20692	-83.40029
Francis Marion National Forest	SC	Coastal	33.21215	-79.47955
Sumter National Forest	SC	Inland	34.45005	-81.70036
Waccamaw National Wildlife Refuge	SC	Coastal	33.65934	-79.15001
Uwharrie National Forest	NC	Inland	35.41855	-79.98325
Croatan National Forest	NC	Coastal	34.77053	-76.75883
Occoneechee State Park	VA	Inland	36.63141	-78.52246
Northwest River Park	VA	Coastal	36.58231	-76.16412
Kanawha State Forest	WV	Inland	38.25214	-81.68057
Saint Mary's River State Park	MD	Coastal	38.24993	-76.53809
Mingo Creek County Park	PA	Inland	40.18825	-80.04912
Allaire State Park	NJ	Coastal	40.1427	-74.13933
Allegheny National Forest	PA	Inland	41.07037	-79.08192
Naugatuck State Forest	CT	Coastal	41.45955	-73.04893
Charleston State Forest	NY	Inland	42.82678	-74.32862
Willowdale State Park	MA	Coastal	42.67743	-70.89535
Green Mountain National Forest	VT	Inland	43.91605	-73.03013
Beatrice Baxter State Forest	ME	Coastal	43.96218	-69.8865

Peaks-Kenny State Park	ME	Inland	45.2627	-69.27937
Great Works Wildlife Management Area	ME	Coastal	44.88453	-67.27928

Supplementary Table 2. The nutritional content of the artificial diets (n = 9) used in *Solenopsis invicta* laboratory study. The Na content was 0.40 ± 0, 0.93 ± 0.06, and 0.13 ± 0.06 % for Control, High-Na and Low-Na diets (n = 3 ea.) respectively.

Source	Mean	Std
Ca (%)	0.778	0.083
Cu (ppm)	10.867	0.718
Fe (ppm)	175.911	13.952
K (%)	0.800	0.087
Mg (%)	0.256	0.053
Mn (ppm)	63.122	4.941
P (%)	0.822	0.083
Protein (%)	25.215	1.980
S (%)	0.344	0.053
Total C (%)	45.136	1.805
Total N (%)	4.034	0.317
Zn (ppm)	111.578	8.499

Supplementary Table 3. Permanova results testing for differences in ant species visiting baits between paired forests.

Source	d.f.	SS	MS	Pseudo-F	P
Block	9	4.213	0.468	2.328	0.0006
Between Pairs	1	0.196	0.196	0.973	0.490
Residual	9	1.809	0.201		
Total	19	6.218			

Supplementary Table 4. Permanova results testing for differences in leaf litter chemistry (Total N, Total C, ADF, ADL, P, Ca, K, Mg, Na, S, Fe, Zn, Cu, Mn, NH_4^+, NO_3^-) between paired forests.

Source	d.f.	SS	MS	Pseudo-F	P
Block	9	0.993	0.110	1.215	0.309
Between Pairs	1	0.171	0.171	1.882	0.190
Residual	9	0.818	0.091		
Total	19	1.982			

Supplementary Table 5. Results of the Repeated Measures Anova testing for differences in Discovery Time of Crickets and Sugar among diet treatments.

Source	*df*	*SS*	*MS*	*F*	*P*	Partial eta^2
Between Subjects						
Diet	2	4.806	2.403	4.428	0.026	0.307
Error$_{BS}$	20	10.854	0.543			
Within Subjects						
Time	1	2.222	2.222	5.092	0.035	0.203
Time x Diet	2	0.650	0.325	0.745	0.488	0.069
Error$_{WS}$	20	8.726	0.436			

Supplementary Table 6. Results of the Repeated Measures Manova testing for differences in Recruitment to Crickets and Sugar among diets and over time after initial discovery. The analysis did not meet the assumptions of sphericity so we report Greenhouse-Geisser statistics.

Source	*df*	*SS*	*MS*	*F*	*P*	Partial eta^2
Between Subjects						
Resource	1	5.097	5.097	15.333	<0.001	0.277
Diet	2	0.253	0.126	0.380	0.686	0.019
Resource x Diet	2	0.072	0.036	0.108	0.898	0.005
Error$_{BS}$	40	13.298	0.332			
Within Subjects						
Time	1.897	1.171	0.618	1.297	0.279	0.031
Time x Resource	1.897	0.868	0.457	0.961	0.383	0.023
Time x Diet	3.794	1.740	0.459	0.963	0.430	0.046
Time x Resource x Diet	3.794	2.331	0.614	1.290	0.282	0.061
Error$_{WS}$	75.878	36.138	0.476			

Supplementary Figure 1. Ant recruitment (mean abundance) with positive standard deviation bars over time (minutes) after initial discovery of both sugar and Crickets for each diet treatment. Times represent means over 10 minute intervals (e.g., 10 mins includes all recruitment over 0-10 minutes after initial discovery).