
From field to fat

Integrating approaches to unveil use of trophic
resources by tropical and temperate ant species
(Hymenoptera: Formicidae)

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“It's a dangerous business, Frodo, going out your door.
You step onto the road, and if you don't keep your feet,
there's no knowing where you might be swept off to.”

- Samwise Gamgee



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1. Summary

The use of food resources is one of the most important aspects of ecosystem functioning. Trophic relationships determine fluxes of matter and energy, shape interactions between organisms and ultimately direct the evolution of the species themselves. Competition is a fundamental biotic interaction, and niche partitioning constitutes an important mechanism to allow species coexistence. However, many other factors influence community structuring, and may change or supplant the outcomes of competition. Ants are one of the most abundant, widespread and ecologically relevant terrestrial organisms. On the ground of tropical forests, dozens of species may coexist, which raises the question: how similar are they? Behavioral and environmental mechanisms of coexistence have been proposed for ants, but the use of resources itself is surprisingly understudied, and the trophic niches of most species is unknown. In this thesis, I used three complementary methods, representing a gradient of source-specificity/time-representativity, to describe patterns of resource use in a tropical and a temperate ant community. In the first study, I reviewed the available literature on resource use for the identified tropical species and compared it to field data obtained with baiting. Previous information was scant or inexistent for most species. Ants broadly used most resources available, but with quantitative differences between species. *Wasmannia auropunctata* has the most specialized niche, using only feces, a new behavior for the species. In the second study, my coauthors and I performed a laboratory experiment to describe fatty acid assimilation in ants. Two temperate ant species (*Formica fusca* and *Myrmica rubra*) displayed similar patterns and dynamics in composition, although amounts were influenced by their reproductive status. The main fatty acids (C16:0, C18:0 and C18:1n9) were extensively synthesized from sugars, but we observed some diet-specific ones that might work as biomarkers (C18:2n6, C18:3n3, C18:3n6). The experiment fulfilled a basic knowledge gap and set the ground for application of fatty acid analysis in an ecological context. In the third study, we put together field observations, fatty acid and stable isotope analyses to describe overall patterns of resource use and species' niches in both communities. We observed a consistent picture of high, and quantitatively equivalent, generalism in both communities, regardless of species richness. Temperate species presented fatty acid patterns distinct from tropical ones, which may be related to environmental factors. Similarities in bait attendance, fatty acid compositions and isotope

signatures were all correlated in Brazil, thus all methods corresponded in their characterization of species' niches to some extent, and were robust enough to detect differences even in a highly generalized community. Method complementarity was particularly important to understand the behavior of the most specialized species. In Germany, no correlations were observed, likely due to the small number of species available. Fatty acid analysis emerges as a powerful tool and may be applied to answer many questions related to resource use in ants, but use of fatty acids as biomarkers seems to be limited. In general, the results of this thesis agreed with the recent view that specialization does not increase with species richness towards the tropics. Several coexistence mechanisms may act to structure ant communities, with trophic niche partitioning playing a relatively small role in the ones we studied. No mechanism appears to be universal and community structure may be better understood on a case-by-case basis, at local scale.

2. Zusammenfassung

Die Nutzung von Nahrungsressourcen ist einer der wichtigsten Aspekte der Funktionalität von Ökosystemen. Trophische Beziehungen bestimmen Materie- und Energieflüsse, prägen Interaktionen zwischen Organismen und lenken letztlich die Evolution der Arten. Die Konkurrenz ist eine fundamentale biotische Interaktion und die Nischenaufteilung ist ein wichtiger Mechanismus der die Koexistenz von Arten ermöglicht. Allerdings beeinflussen viele andere Faktoren die Strukturierung von Biozönosen und können die Auswirkungen von Konkurrenz verändern oder sogar verdrängen. Ameisen gehören zu den häufigsten, am weitesten verbreiteten und ökologisch relevantesten Landorganismen. Auf dem Boden tropischer Wälder koexistieren viele Ameisenarten, was die Frage aufwirft: Wie ähnlich sind sie untereinander? Als Erklärung für die Koexistenz dieser Arten wurden Verhaltens- und Umweltmechanismen herangezogen, aber die Ressourcennutzung ist bis heute überraschend wenig erforscht, und die trophischen Einnischung der meisten Arten sind nicht bekannt. In meiner Dissertation verwendete ich drei komplementäre Methoden, entlang eines Gradienten von Quellenspezifität/Zeit-Repräsentativität, um die Muster der Ressourcennutzung in tropischer und temperater Ameisengemeinschaften zu beschreiben. In der ersten Studie analysierte ich die vorhandene Literatur zur Ressourcennutzung für die vorgefundenen tropischen Arten und verglich diese mit über Köderfallen erhobenen Felddaten. Die Literaturrecherche offenbarte einen spärlichen Kenntnisstand in Bezug auf die einzelnen Arten. Die Ameisenarten nutzten überwiegend den Großteil aller verfügbaren Ressourcen, jedoch mit quantitativen Unterschieden zwischen den Arten. *Wasmannia auropunctata* benutzte nur Faeces, was bisher gänzlich unbekannt war, und es besetzt die spezialisierteste Nische. In der zweiten Studie führten meine Koautoren und ich ein Laborexperiment durch, um die Fettsäureassimilation bei Ameisen aufzuklären. Zwei temperate Ameisenarten (*Formica fusca* und *Myrmica rubra*) zeigten ähnliche Muster und Dynamiken bei der Zusammensetzung ihrer Fettsäuren, obwohl die absoluten Mengen von dem jeweiligen reproduktiven Status beeinflusst wurden. Die Hauptfettsäuren (C16:0, C18:0 und C18:1n9) wurden überwiegend aus Zuckern synthetisiert, aber wir identifizierten einige diätspezifische Fettsäuren, die als Biomarker fungieren könnten (C18:2n6, C18:3n3, C18:3n6). Das Experiment füllte eine entscheidende Wissenslücke und lieferte die Grundlage für die Anwendung der Fettsäureanalyse in einem ökologischen

Kontext. In der dritten Studie führten wir eine stabile Isotopenanalyse durch und brachten die Ergebnisse mit unseren Feldbeobachtungen und Fettsäureanalysen in Zusammenhang, um die Ressourcennutzung und die Einnischung der Arten in beiden Gemeinschaften aufzuklären. In beiden Gemeinschaften zeigte sich unabhängig von der Artenzahl ein hoher und quantitativ äquivalenter Grad an Generalismus. Temperate Arten zeigten Fettsäuremuster, die sich von denen der tropischen unterscheiden, was eine Konsequenz der divergierenden Umweltfaktoren sein könnte. Die Ähnlichkeiten in Bezug auf Ködernutzung, Fettsäurezusammensetzung und Isotopensignatur waren bei der tropischen Gemeinschaft miteinander korreliert, so dass bei allen Methoden die Charakterisierung der jeweiligen Artnische übereinstimmte. Die Methoden waren auch robust genug um Unterschiede selbst in einer hoch generalisierten Gemeinschaft zu erkennen. Methodenkomplementarität war besonders wichtig, um das Verhalten der spezialisiertesten Arten zu verstehen. Bei der temperaten Gemeinschaft wurden jedoch keine derartigen Korrelationen festgestellt, was wahrscheinlich in der kleineren Anzahl an Arten begründet lag. Die Fettsäureanalyse stellt somit ein adäquates Werkzeug dar, um viele Fragen in Zusammenhang mit der Ressourcennutzung bei Ameisen aufzuklären. Die Verwendung von Fettsäuren als Biomarker hat allerdings Grenzen. Insgesamt stimmen die Ergebnisse dieser Arbeit mit der neueren Ansicht überein, dass der Grad der Spezialisierung in Biozöosen eben nicht mit einer höheren Artenvielfalt, wie in den Tropen, zunimmt. Verschiedene Koexistenzmechanismen können Ameisengemeinschaften strukturieren, wobei die trophische Nischenpartitionierung in den untersuchten Gemeinschaften nur eine untergeordnete Rolle spielt. Kein Mechanismus scheint universell zu sein, und die Struktur einer Gemeinschaft kann nur auf lokaler Ebene und einzelfallbezogen verstanden werden.

3. Introduction

What do ants eat? This is a simple, and even a bit silly, question. Most people will have a ready answer to it, such as “the sugar in my kitchen, for sure” or “the plants in my yard, of course”. Or one may look to the trails running all over their walls and cry out “these little pests are everywhere, eating everything!”. They are not wrong: small tramp ants flourish in urban habitats, while New World leaf-cutter ants have eaten more than one rose garden or agricultural plot.

However, simple questions often have complicated answers. There are thousands of ant species described, and many still undescribed. These species occupy almost all terrestrial ecosystems, and their numeric abundance and biomass rivals all other animal groups (together with another people’s friends, termites). Thus, the answer to “what does this ant eat in my house?” might be easy. “What does this ant eat in nature?” gets a bit more complicated. And “what are the trophic niches of ant species in this community?”, a truly challenge.

However, before actually delving into that, I must answer a much harder question: “why did you spend years of your life studying what ants eat?” ...

3.1. Getting the big picture: use of trophic resources and ecosystem functioning

The use and partitioning of food resources is a central aspect of community functioning. Trophic interactions govern the flux of matter and energy in ecosystems, through complex food webs with several pathways leading from producers to consumers and detritivores (Polis and Strong 1996). Other fundamental interactions, such as competition and mutualism, direct result of resource use, or highly influenced by it (Schoener 1974).

Niche partitioning is considered one of the most important mechanisms allowing species coexistence, and may ultimately link to evolutionary processes of adaptation and character displacement (Schluter 2000, Reitz and Trumble 2002). The logic behind niche partitioning is summarized in the so-called “principle of competitive exclusion”: two species with the same niche and distinct competitive abilities cannot coexist, because,

given enough time, competitive pressure would lead to the exclusion of the weaker competitor (Hardin 1960). Thus, species must adapt their niches to reduce competition, or be locally extinct. Interspecific competition has long been a hot topic among ecologists (Connell 1983). The *existence* of competition is a given for any natural scientist that observed an agonistic interaction between two animals, a tree struggling to develop under the other's shade, or a cat stealing food from the dog's bowl. The *relevance* of competition to structure communities and guide biological evolution, however, is often debated. In a vacuum, makes sense to expect competitive exclusion when species use the same resources, but one does it more efficiently. In reality, several mechanisms act together to structure communities, and in certain cases competition may be relegated to a secondary role (Cerdá et al. 2013).

The complex and context-dependent nature of biological communities results in few robust generalizations and poor predictive power at this ecological level (Lawton 1999). Far from being a "weakness", this only means that community ecology works better at local scales, where patterns and processes are more easily explained and the role of individual units (e.g. species) can be better understood. Such "case studies" are useful by themselves, and ultimately contribute to the progress of scientific knowledge in general (Simberloff 2004).

One earlier generalization related to competition and resource use was proposed by MacArthur (1972). He suggested that more stable environmental conditions in the tropics diminish constraints for evolutive change, and allow more specialized species to arise there, compared to temperate ecosystems. Specialization eases competitive pressure and, as consequence, more species can coexist. This would lead to a positive relationship between species richness and specialization towards the tropics. However, this idea was put in question by recent studies (Vázquez and Stevens 2004, Schleuning et al. 2012, Morris et al. 2014, Frank et al. 2018). Specialization may be similar, or even increase in temperate regions. Again, this relationship might be context-dependent and vary according to the study system. Tropical vs. temperate levels of specialization in resource use is an ongoing debate that would benefit from more diverse comparisons.

3.2. *Getting the focus: trophic biology of ants*

Ants (Hymenoptera: Formicidae) are among the most abundant groups of invertebrates in terrestrial ecosystems (Kaspari 2000). Currently there are 13452 species and 1909 subspecies described (Bolton 2018). Although this number pales in comparison to some other insect families, their eusocial organization leads to a huge number of individuals and large contribution to ecosystem biomass. They also present a comparatively high number of different behaviors, and, in most terrestrial ecosystems, can be found in all strata (underground, inside leaf-litter, ground's surface¹, understory, canopy). They attain their peak on tropical forests, where dozens of species may coexist at the same spot. Litter samples of 1 m² routinely yield 20-30 or more species (Ryder Wilkie et al. 2010, Silva and Brandão 2010). This represents thousands of individuals sharing the same space, which raises the question: how different are these species?

Hölldobler and Wilson (1990) famously stated that “competition is the hallmark of ant ecology”. The combination of high local species richness with high biomass is expected to translate into strong competition, and to evolutive pressure for more diversified and/or specialized niches. Some behavioral mechanisms of coexistence have been proposed, and their generality confirmed or debunked (Fellers 1987, Andersen 2000, Parr and Gibb 2012). Other factors have been shown to disrupt competitive relationships in specific contexts, such as different tolerances to temperature or preferential action of parasitoids on dominant species (Cerdá et al. 1997, Feener et al. 2008).

Trophic niche partitioning is less explored, and the use of food resources itself is surprisingly understudied. In general terms, most ants are regarded as omnivorous, feeding on a combination of living prey, dead arthropods, seeds and plant exudates (Kaspari 2000, Blüthgen and Feldhaar 2010, Lanan 2014). Several specialized behaviors are known, such as the famous leaf-cutter ants, mushroom harvesters, specialized granivory, and specialist predation (Davidson 1977, Baroni Urbani and De Andrade 1997, Brandão et al. 2012, Beeren et al. 2014). Even though these specialists are highly interesting from a scientific viewpoint, the truth is that the trophic niches of most species remain poorly

¹ Epigeic (or “ground-dwelling”) ants are the ones that forage primarily on the ground's surface. Many epigeic species also forage on vegetation or inside the leaf-litter. However, they are distinct from “true” leaf-litter ants that forage almost exclusively inside this stratum, which demands particular adaptations (Kaspari and Weiser 1999, Silva and Brandão 2010).

know. This is particularly true for the dozens of species of the “boring generalist” genera such as *Camponotus*, *Pheidole* and *Solenopsis*, which make up the largest share of tropical communities (Kaspari 2000, and Chapter 5 in this thesis). More is known for temperate species, but not always. Lanan (2014) stressed that even for the abundant, widespread and thoroughly studied *Lasius niger* (Linnaeus 1758), there is relatively little information reported in the literature about its behavior and natural history in the wild.

Part of the issue is the widespread use of morphospecies in myrmecology. Morphospecies (or "parataxonomic units" - Krell 2004) are informal groups separated by external morphology and identified only to a higher taxonomy category, such as *Pheidole* sp.1 or Coleoptera sp.367. They are less used in temperate communities, because taxonomy of these ants is more established (e.g. Seifert 2007). On the other hand, the taxonomy of many of the most important tropical genera is far from being solved, even in light of valuable recent efforts (Wilson 2003, Wild 2007, Bolton and Fisher 2011, LaPolla et al. 2011). Community lists with high proportion of morphospecies still are an everyday reality for tropical myrmecologists. When produced with care and a certain level of taxonomic expertise, morphospecies lists might be useful to study general patterns (Oliver and Beattie 1996, but see Krell 2004 for a critical view). However, this also leads to a significant waste of information, since the data on a given morphospecies is only applicable to a particular study. It cannot be compared, compiled or used as reference for future works. Currently, morphospecies are a necessary evil, but this can be alleviated by allocating effort to the identification step, and/or establishing partnerships with taxonomists.

Another reason for this knowledge gap is the simple lack of direct observation. Many species present cryptic behaviors, particularly the ones that live above surface or on tree canopies. Even for the more conspicuous epigeic species, most biological information comes from collection data that can be gathered in a typical biodiversity assessment, e.g. geographical records, nesting sites and colony structure (e.g. AntWeb 2018). Aspects that demand specific study designs, such as use of trophic resources, are less known. All things considered, use of trophic resource remains an open topic in ant ecology.

Are ants a good “model” to study competition and resource use in terrestrial arthropods? Not likely. Ants are a minute part of the ca. 1 million described insect species

(Stork 2018). Although some authors speculate that Hymenoptera might be the richest insect order, the far majority of these species are solitary parasitic wasps (Forbes et al. 2018). True social insects are few and far between. Ants as a group have traits that make them different from many other animals, regarding resource use:

(1) Colonies are sessile: ant colonies are limited to the resources available around the nest. Foraging distances might extend to a few hundred meters in ants with large colonies and medium/large body size (Carroll and Janzen 1973, Bowers and Porter 1981). Most species, however, have smaller ranges, so much that distances of 5-10 m are considered a good compromise between sample independence and field effort in biodiversity inventories (Fisher 1999, Agosti and Alonso 2000). Colonies are not completely static, though: they can change nest location under disturbance or unfavorable conditions, and residence times are highly variable (Hölldobler and Wilson 1990). Most of these changes are within a couple of meters (Smallwood 1982), but this may be enough to change the available resources for small ants. Army ants of the subfamily Dorylinae, which have no fixed nest and migrate for long distances while foraging, are another clear exception. However, more often than not, nests are fixed, and local resources have stronger influence on ants than on free-living organisms.

(2) Workers have a diet more limited than larvae: as all holometabolous insects, ants acquire most of their structural biomass during the larval stages (Blüthgen and Feldhaar 2010). The only part that changes size in adults is the gaster²: in short term, it greatly expands when workers ingest high amount of liquid food; in medium term, storage or use of lipid reserves in the fat body may change their body weight. Hymenopterans have a digestive tract adapted to liquid feeding: crop and midgut are linked by the proventriculus, which work as a pump, and limit the passage of solid particles with larger size (Eisner 1957). In ants, the crop acquired a role of storage organ, and the proventriculus also acts as a dam, holding the stored food, which otherwise would proceed into the midgut and be digested. In the subfamilies Formicinae and Dolichoderinae, the proventriculus acquired such a specialized structure for holding large

² Ant body parts use a different nomenclature due to the fact that, during embryonic development, the first abdominal segment is fused to the thoracic ones. Thus, the visual/functional equivalents of thorax and abdomen are called mesosoma (or alitrunk) and gaster, respectively. The second abdominal segment (or the second and third in some subfamilies) is modified to become an independent segment linking those two body parts, the petiole (or petiole and post-petiole).

amounts of liquids that the passage of any solid particle seems to be impossible (Eisner and Happ 1962). In any case, adult workers have limited assimilation of solid foods. Larvae, on the other hand, do not have these morphological limitations and possess more diversified enzymes. For these reasons, they are sometimes called the “digestive caste” of the colony (Hölldobler and Wilson 1990, Erthal et al. 2007).

(3) Ants share food: larvae and workers presumably have different diets. However, food can be shared through trophallaxis (regurgitation of stored food) between workers and larvae. Trophallaxis is more widespread in phylogenetically derived subfamilies such as Formicinae and Myrmicinae, but species of primitive subfamilies present other mechanisms of food sharing (Hölldobler and Wilson 1990). Thus, the gathered nutrients can be shared among individuals of diverse castes and ages within the colony (Howard and Tschinkel 1981).

(4) Ants have different foraging modes: eusociality does not mean that ants work together all the time. Some species only forage solitarily, while others present different levels of recruitment to food sources (Lanan 2014). Recruitment may be as simple as one individual recruiting a single nest mate (tandem recruitment), to the establishment of foraging trails with huge worker mobilization (mass recruitment). Two methodological caveats arise from this. First, number of individuals in a pitfall trap or bait is a poor predictor of species abundance or preference for a resource. Individual-based data can be useful for ecosystem analyses (e.g. removal rates of a given resource), but, for species analyses, frequency of occurrence on samples is used as a proxy for true abundance/preference (Gotelli et al. 2011). Second, the functional role of a species cannot be inferred from worker size, as sometimes it is made for other groups (Scheffer et al. 2018). A colony of small, mass foraging Myrmicinae can retrieve larger items than it would be expected for their size, and may consume resources in a higher rate than a colony of large, solitary foraging Ponerinae.

All things considered, extrapolation of biological patterns from ants to other organisms, or vice-versa, should be done very carefully. The same is valid for methods, which should be adapted to these particularities. Being a “bad model” means that we should focus our research effort elsewhere? Not at all. Simple personal interest for a biological group is a good incentive to perform basic science, although it might raise a few eyebrows in funding agencies. From a more objective point of view, the importance of ants

in terrestrial ecosystems is reason enough for their study (Folgarait 1998). Few other animal groups combine such cosmopolitan distribution, with high abundance of biomass, presence in all strata and many different functions performed. Understanding ants is understanding a huge, albeit peculiar, part of any terrestrial ecosystem.

3.3: Getting the answers: one method to rule them all?

To understand a single species' niche is far from a trivial task. The multidimensional nature of such niches makes it challenging to measure all relevant variables and, after getting odd results, researchers may always ask themselves what factor they missed, or whether their judgment of "relevance" was correct. Each available method provides certain kinds of reliable data and deals with a subset of factors, while being less suitable in other contexts. Researchers also are subject to pragmatic trade-offs: they have to choose whether they want broader geographical coverage, or a more time-representative dataset, or to get a larger portion of the variation in the study object (e.g. more species, strata or vegetation types in a sampling site), and so on.

A logical consequence of these considerations is that, for every research question, there is a more suitable method to be chosen (Birkhofer et al. 2017). This approach fits well works with one or few defined hypotheses to be tested. On the other hand, another conclusion is that methods may be combined to complement each other's strengths and weaknesses (Bestelmeyer et al. 2000). This approach is particularly useful in descriptive studies, when the purpose is to get an in-depth picture of the study system (and they still allow hypothesis testing, but usually of a more restricted nature).

In this thesis, I adopted the second approach: three methods with different properties were applied to assess resource use in ants. These methods, described in detail in the respective chapters, were: baiting (Chapters 5 & 7), fatty acid analysis (Chapters 6 & 7) and stable isotope analysis (Chapter 7). They represent a gradient of source-specificity/time-representativity trade-offs. Baiting (as field observations in general) brings direct information on resources used, but only as a temporal "snapshot": unless repeated over and over again, there is no guarantee that today's observations represent what ants ate yesterday, or will eat tomorrow. On the other hand, the isotopic composition

of a holometabolous insect is a long-term representation of its diet, but brings little information on actual sources or behaviors. Fatty acids are intermediate: the composition of stored fat is relatively stable, and is influenced both by amounts and sources of food. While the two first techniques have a long story in ant research, fatty acid analysis still is incipient for terrestrial organisms. It was never applied to ants in an ecological context, and little previous information on lipid metabolism existed for them.

3.4: Getting to work: resource use in tropical and temperate ants

In this thesis, my coauthors and I took a descriptive approach to finally answer what ants eat. We focused on describing local patterns on a tropical and a temperate community, which strongly differed in species richness and composition. We applied a field protocol adapted from previous works, and complemented it with stable isotope and fatty acid analyses. Also, I dedicated special effort to get the best taxonomic resolution possible, and to see how our results contribute to understand the role of individual species. In Chapter 5, I performed a literature review on trophic niche of the identified tropical species and compared this previous knowledge (or lack of it) with the observed field data. In Chapter 6, we experimentally assessed dynamics and patterns of fatty acid assimilation in ants, to fill a basic knowledge gap and set the ground to apply fatty acid analysis to this group. Finally, in Chapter 7, we put together all data to get a source-specific and time-representative view on resource use, and to describe and compare both communities.

4. Study sites

(for maps of the sites, see Annex I)

4.1. Brazil

Desterro Conservation Unit (UCAD) is located in Florianópolis, southern Brazil (27°31'38" S, 48°30'15" W, altitude ca. 250 m). Regional climate is humid subtropical (Cfa – Köppen). Average annual temperature and precipitation is 20.5 °C and 140 mm per month (EPAGRI/CIRAM).

Vegetation consists of Atlantic forest *sensu stricto* (= ombrophilous dense forest), a stripe of dense forests that runs along eastern Brazilian coast (Oliveira-Filho and Fontes 2000). The Atlantic Forest is considered the fourth “hottest” global hotspot of biodiversity (Myers et al. 2000). However, due to its coastal distribution, it has one of the longest and most intensive history of antropogenic disturbance among Brazilian ecosystems. An estimated 12-16% of the original vegetation remains, often in a very fragmented state. (Soares-Filho et al. 2014). Parts of UCAD were selectively logged or converted into pastures in the past. The current forest has 50-60 years of relatively undisturbed regeneration (Ladwig 1998).

The site is located in the subtropics, and near the southern boundary of the ombrophilous dense forest distribution. However, the high rainfall rate along the coast allows high productivity, and provides a tropical aspect for the Atlantic forest even at higher latitudes (ant species richness, for instance, increases towards the south – Silva and Brandão 2014). Additionally, most species included in this work are widespread across the Neotropics (Janicki et al. 2016).

Four plots of 4 x 4 sample points were set up across a hillside. Sample points were separated by 10 m. Plots were separated by 30-100 m and covered relatively homogeneous vegetation, although they were chosen to represent some variation in canopy heights and understory structure. Sampling was performed during the day and the night, as a way of increasing sample intensity and collect species with preference for a given period (although few species had any preference – see Chapter 5).

4.2 Germany

The Prinzenberg is a hill in Eberstadt-Darmstadt, southeastern Germany (49°50'14"N, 08°40'01"E, altitude ca. 250 m). Regional climate is temperate (Cfb – Köppen). Average annual temperature and precipitation is 10.1 °C and 64 mm per month (Deutsche Wetterdienst).

The site is part of the Naturpark Bergstrasse, which covers a large area between the rivers Main, Neckar and Rhine (www.geo-naturpark.net). It is a public park used for a wide range of activities related to recreation, local economy and biodiversity protection. The vegetation is variable, including patches of mixed forests, beech forests, meadows, pastures and orchards.

In Germany, lower ant species richness and abundance were expected, thus the sampling was directed to assess the highest number possible. No distinct nocturnal community was expected, thus the sampling was performed only during the day, but with an increased effort of 80 sample points. Three plots were established to cover different vegetations: (1) a patch of mixed forest dominated by oaks; (2) a patch of nearly-monotypic beech forest; (3) an open meadow/apple orchard.

My exploratory samples revealed ca. 20 ant species across the site, but only seven provided enough records to be included in this work (Chapter 7). These seven included *Formica fusca* and *Myrmica rubra*, thus we chose them for the fatty acid experiment (Chapter 6), although in this case colonies were obtained from a specialized store (Antstore, www.antstore.net).

5. Natural history of ants: what we (do not) know about trophic and temporal niches of Neotropical species

Félix Baumgarten Rosumek. 2017. Published in *Sociobiology* 64(3): 244-255 (full text in Annex II).

Synopsis: Our understanding of the natural history of Neotropical ants is limited, due to lack of descriptive efforts and widespread use of morphospecies in literature. Use of trophic resources and period of activity are two central niche aspects little explored for most species. This work aimed to broadly review the literature and provide empirical field data on these aspects for 23 species. The fieldwork was carried out in the Atlantic forest of southern Brazil. Trophic and temporal niches were assessed with pitfall traps and seven kinds of bait representing natural resources. Crushed insects were the preferred resource, whereas bird feces and living prey were less exploited. Most species broadly used the resources, but pronounced quantitative differences were found. *Odontomachus chelifer* (Latreille, 1802) and *Pachycondyla striata* Smith, 1858 were relatively well studied and field data matched previous accounts. They were the only species that consistently used large prey, and avoided oligosaccharides. *Wasmannia auropunctata* (Roger, 1863) differed remarkably from previous studies, using feces as its sole trophic resource. The six Pheidole species had no previous records and presented quantitative differences in resource use. Most species had no strong preference for period of activity. *Camponotus zenon* Forel, 1912 was nocturnal and *Crematogaster nigropilosa* Mayr, 1870, *Linepithema iniquum* (Mayr, 1870) and *Linepithema pulex* Wild, 2007 were diurnal. Complementary methods, context-dependence and descriptive studies have a central role in the understanding of ant natural history. Community assessments can contribute significantly to this knowledge if researchers also pay attention to the individual species involved.

6. Patterns and dynamics of neutral lipid fatty acids in ants – implications for ecological studies

Félix Baumgarten Rosumek, Adrian Brückner, Nico Blüthgen, Florian Menzel and Michael Heethoff. 2017. Published in *Frontiers in Zoology* 14:36 (full text in Annex III).

Synopsis: Trophic interactions are often difficult to observe directly. Several indirect techniques, such as fatty acid analysis, were developed to assess these interactions. Fatty acid profiles may indicate dietary differences, while individual fatty acids can be used as biomarkers. Ants are among the most important terrestrial animal groups, but little is known about their lipid metabolism, and no study so far used fatty acids to study their trophic ecology. We set up a feeding experiment with high- and low-fat food to elucidate patterns and dynamics of neutral lipid fatty acids (NLFAs) assimilation in ants. We asked whether dietary fatty acids are assimilated through direct trophic transfer, how diet influences NLFA total amounts and patterns over time, and whether these assimilation processes are similar across species and life stages. Ants fed with high-fat food quickly accumulated specific dietary fatty acids (C18:2n6, C18:3n3 and C18:3n6), compared to ants fed with low-fat food. Dietary fat content did not affect total body fat of workers or amounts of fatty acids extensively biosynthesized by animals (C16:0, C18:0, C18:1n9). Larval development had a strong effect on the composition and amounts of C16:0, C18:0 and C18:1n9. NLFA compositions reflected dietary differences, which became more pronounced over time. Assimilation of specific dietary NLFAs was similar regardless of species or life stage, but these factors affected dynamics of other NLFAs, composition and total fat. We showed that ants accumulated certain dietary fatty acids via direct trophic transfer. Fat content of the diet had no effect on lipids stored by ants, which were able to synthesize high amounts of NLFAs from a sugar-based diet. Nevertheless, dietary NLFAs had a strong effect on metabolic dynamics and profiles. Fatty acids are a useful tool to study trophic biology of ants, and could be applied in an ecological context, although factors that affect NLFA patterns should be taken into account. Further studies should address which NLFAs can be used as biomarkers in natural ant communities, and how factors other than diet affect fatty acid dynamics and composition of species with distinct life histories.

7. Unveiling community patterns and trophic niches of tropical and temperate ants using an integrative framework of field data, stable isotopes and fatty acids

Félix Baumgarten Rosumek, Nico Blüthgen, Adrian Brückner, Florian Menzel, Gerhard Gebauer and Michael Heethoff. 2017. Published in *PeerJ* 6:e5467 (full text in Annex IV).

Synopsis: The use and partitioning of trophic resources is a central aspect of community function. On the ground of tropical forests, dozens of ant species may be found together and ecological mechanisms should act to allow such coexistence. One hypothesis states that niche specialization is higher in the tropics, compared to temperate regions. However, trophic niches of most species are virtually unknown. Several techniques might be combined to study trophic niche, such as field observations, fatty acid analysis and stable isotope analysis. In this work, we combine these three techniques to unveil partitioning of trophic resources in a tropical and a temperate community. We describe patterns of resource use, compare them between communities, and test correlation and complementarity of methods to unveil both community patterns and species' niches. Resource use was assessed with seven kinds of bait representing natural resources available to ants. Neutral lipid fatty acid (NLFA) profiles, and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope signatures of the species were also obtained. Community patterns and comparisons were analyzed with clustering, correlations, multivariate analyses and interaction networks. Resource use structure was similar in both communities. Niche breadths (H') and network metrics (Q and H_2') indicated similar levels of generalization between communities. Stable isotope signatures and NLFA profiles also indicated high generalization, although the latter differed between communities, with temperate species having higher amounts of fat and proportions of C18:1n9. Bait use and NLFA profile similarities were correlated, as well as species' specialization indices (d') for the two methods. Similarities in $\delta^{15}\text{N}$ and bait use, and in $\delta^{13}\text{C}$ and NLFA profiles, were also correlated. Our results agree with the recent view that specialization levels do not change with latitude or species richness. Partition of trophic resources alone does not explain species coexistence in these communities, and might act together with behavioral and environmental mechanisms. Temperate species presented NLFA patterns distinct from tropical ones, which may be related to environmental factors. All methods corresponded in their characterization of species' niches to some extent, and were robust enough to detect differences even in highly

generalized communities. However, their combination provided a more comprehensive picture of resource use. Method complementarity was particularly important to understand individual niches of the most specialized species, such as *Wasmannia auropunctata* and *Lasius fuliginosus*. Fatty acid analysis was applied here for the first time in ant ecology, and proved to be a valuable tool due to its combination of specificity and temporal representativeness. We propose that a framework combining field observations with chemical analysis is valuable to understand resource use in ant communities.

8. Conclusions

In this work, I and my coauthors described resource use in two ant communities. I started from the basics, observing what they do in the field and assessing what was known about them (Chapter 5). Then we investigated lipid metabolism of ants, to allow a proper application of fatty acid analysis to this group (Chapter 6). Finally, we put together data from the three methods applied and described resource use patterns of both communities, compared them, and discussed in detail individual niches of some species (Chapter 7).

“Yes, yes... But what do ants eat, after all?”

Well, the overall picture we obtained is not much different from the answer of one of our friends at the beginning: ants eat everything. By this, I mean that one of our main results is the high level of generalism and strong overlap in resource use, observed in both communities. This study has a limited geographic scope, and we cannot straightforwardly extrapolate our results to other communities. However, the observed patterns of resource use, network metrics, niche breaths and stable isotopes signatures were not only qualitatively, but quantitatively very similar between these two communities, which strongly differed in their species richness, taxonomic composition and habitat structure. This is a hint that similar patterns can occur elsewhere.

It should be no coincidence that five of the six³ largest ant genera are predominantly generalists (*Camponotus*, *Pheidole*, *Polyrhachis*, *Tetramorium* and *Crematogaster*). Generalists are less vulnerable to scarcity or fluctuations in particular resources, and can more easily establish colonies in different contexts. For a species to become a specialist, it should have faced strong competitive pressure in the past, and adapted to avoid competition with superior generalists or be extinct. Hence, specialization *per se* is not an advantageous trait, but simply consequence of “the ghost of lousiness past”. Naturally, we still observed several specialist species in the tropical community, but their overall abundance paled in comparison to the core of generalist species. The same is true for the temperate community: *Lasius fuliginosus* is the most specialized species there, but only a single colony was found in our study grids.

³ The exception is *Strumigenys*, the third largest ant genus, with many species considered specialist predators of collembolans or other arthropods. However, direct observation of these minute leaf-litter ants in the field is barely possible, and few species were studied in laboratory.

Another finding that might be general is the differences in fatty acids between temperate and tropical ants. The amounts and compositions we observed in temperate field samples (Chapter 7) were similar to the ones we observed in colonies of two species of the laboratory study (Chapter 6), which were not from of the same location and lived under completely different conditions. The generality of such patterns might be easily tested with more sampling, as well as the mechanisms we proposed for the strong differences from tropical species.

The temperature-unsaturation hypothesis is particularly intriguing. There is no physiological “need” for the observed levels of C18:1n9 during summer. However, do ants need to change something, in the first place? Neutral fatty acids are stored in droplets inside cells of the fat body (Roma et al. 2010). They share the same properties of membrane phospholipids regarding fluidity and temperature effects (Jagdale and Gordon 1997). If such droplets are too solid, enzyme access and mobilization may be hindered. Contrary to membrane lipids, would an “excessively fluid” fatty body be a problem? If there is also no real need to increase saturation during summer, temperate ants might keep high levels of C18:1n9 all the time, and avoid the energetic cost of re-synthesize them every year.

8.1. What does allow coexistence between ant species?

High overlap does not mean that all species have the same niches and preferences, as showed in Chapter 5. Some preferences may still play a role in the coexistence of similar species. It means, however, that each resource is used by many species, so there is strong competition for them. Hence, trophic niche partitioning does not seem to be one of the main mechanisms of coexistence between epigeic ants. What might be, then?

Behavioral coexistence is relatively well-studied in ants. Andersen (1995, 1997) applied a behavioral hierarchy to Australian ants, and later compared it to North American communities. “Dominant” ants mass-recruit to food sources and aggressively defend their territory, excluding weaker colonies of dominant competitors. However, “subordinate” ants, which forage individually and/or move fast enough to dodge the dominants, are able to access the resources simultaneously, or before it get completely occupied by them. Savolainen and Vepsäläinen (1988) described an analogous hierarchy

for a boreal community. Something similar happens in “ant mosaics”, which were proposed for arboreal ants: dominant ants would exclude each other, creating patches where just one species is found, while the subordinate species may still coexist with them (Blüthgen and Stork 2007).

One of the most “popular” mechanisms of coexistence proposed in the last decades was the “discovery-dominance tradeoff” (Fellers 1987). Sharing similarities with the previous ones, this hypothesis states that dominant species are slower to find and recruit to food sources, which gives time to faster species to explore the resource before complete domination. The hypothesis has drawn much attention since its proposal (Davidson 1998, Adler et al. 2007), but apparently the trade-off is not predominant among communities, and domination and discovery abilities are often correlated (Parr and Gibb 2012).

In some cases, it was shown that an external factor disrupts competitive hierarchies, reducing the competitive pressure caused by dominant species. Lower tolerance of dominant species to higher temperature allows coexistence of subordinate, but more tolerant, species in Mediterranean communities (Cerdá et al. 1997). Preference of phorid parasitoids by dominant ants, which are attacked during foraging and recruitment to the resource, was also suggested as lowering competition in some contexts (Feener et al. 2008).

Finally, dispersion and recruitment effects still are little explored for ants. In this sense, ants share relevant traits with plants, more than with free-living animals. Colonies are sessile and restricted to the resources around the nests. However, they have winged reproductives and their dispersion is less constrained. Mature colonies have a strong local impact, and may easily displace or starve to death younger colonies (Adams 2016). Dispersion allows species to find free spots in the habitat and, given enough time to grow, an established colony may persist and avoid the establishment of theoretical stronger competitors, thus allowing long-term coexistence of all these species in the community. Stochastic dispersion combined with equivalent competitive abilities is at the core of “neutral” theories of community organization (Rosindell et al. 2011). These models were successful in describing patterns in some communities, but many other studies failed to support them (Scheffer et al. 2018). So far, they have not been empirically tested in ants. Studying dispersion and recruitment dynamics of ants brings its own methodological

challenges, though: established colonies are relatively long-lived, and newly founded nests are difficult to track.

In the end, there are so many mechanisms showed to enhance ant coexistence in diverse spatial and temporal scales that is hard to generalize them (Andersen 2008). This does not constitute a problem *per se*. Community ecology is, at its core, a science of case studies (Simberloff 2004). The background of studies in community organization allows a researcher to explore several hypotheses to explain how and why a particular community is organized. Trophic niche partitioning plays a relatively small role in organization of the two communities in this thesis. New studies may test other hypothesis and, ultimately, integrate results to test the relative weights of each mechanism on these specific communities.

8.2. Resource use and conservation of ecosystems

And for what would we bother with all of this? I took a “basic science” approach in this thesis, studying trophic niche of ants for the simple sake of knowing how a few elements of the universe work. However, due to the central importance of resource use for all aspects of ecosystem functioning, the findings have direct implications to conservation and management. High overlap in resource use (and its independence from species richness) shows that many species contributes for each processes (predation, scavenging, nectar use, etc.) related to flow of matter and energy. From a utilitarian viewpoint, what matter are not the species or individuals themselves, but the maintenance of the ecosystem processes and the services it provides⁴ (Rosumek and Martins 2010). Given the similarities among the abundant generalists, the loss of some species would not affect these processes. The rare species, although often having specialized niches, have a relatively small contribution to this.

A case might be made for *Pachycondyla striata* and *Odontomachus chelifer* in Brazil: these were the only two species which consistently hunted large prey. Their loss could open a functional gap in the system. *Wasmannia auropunctata* has a unique niche, but,

⁴ “Services for whom?” The ones asking themselves why conserve and manage to begin with, naturally (in this case, humans).

although frequently recorded, they seem to have very small colonies in that community⁵. Thirty-one other species used feces, accounting for 91% of the records, so *Wa. auropunctata* is not a key species in this process.

In Germany, *Lasius fuliginosus* has a specialized niche and strong local impact, although nests are scattered around the community. Resource use was very similar among other species, but *Lasius niger* had a notable presence: it recruited an average of 60 individuals per bait (and sometimes many hundreds), two times more than the second strongest recruiter, *Lasius platythorax*. I did not analyze recruitment rates in this work, and comparisons cannot be made so straightforwardly, but it is a crucial aspect to analyze resource use from the ecosystem perspective.

All these species are quite widespread, abundant and not particularly vulnerable to extinction. Exactly because of this, they are fundamental to the conservation of ecosystem processes, and might represent a robust buffer against species loss. Logically, one of the main problems of discussing species redundancy is the restricted scope in time, space and number of functions assessed in ecological studies, including ours (Rosenfeld 2002). Other factors should be taken into account before taking managing actions to ignore or enhance the survival of any particular species.

8.3. Next steps: resource use and ant morphology

After assessing ant's actions in the field and their body chemistry, our next step will be to look at their morphology. The morphology of an organism generally is linked to its ecology (Hespenheide 1973). For ants, the mouthparts understandably are the structures most directly related to resource use (Paul 2001). However, they must also serve to a number of activities, such as carrying the brood and digging nest galleries. Mandibles and their associated muscles must combine speed, strength and precision for both demanding and delicate tasks. Other morphological traits influence resource use as well: the neck joint supports all the load of a food item carried by ant ants often smaller than it (Nguyen et al. 2014); the previously cited proventriculus determines if an ant can digest solid food or not

⁵ Few individuals were collected in the baits (mean of 1.9, with maximum of 7), and intensive search during the second round of fieldwork failed to reveal any colony.

(Eisner 1957); and basic traits such as size and body shape determine what an individual can do.

Due to the hindrances of obtaining accurate field measurements for natural communities, morphology sometimes is advocated as a proxy for understanding ecological phenomena (Ricklefs and Travis 1980). This approach has been applied in studies of functional ecology of ants (Kaspari and Weiser 1999, Weiser and Kaspari 2006, Bihn et al. 2010, Silva and Brandão 2010, 2014). Usually the functionality of morphological traits in these studies is inferred, rather than measured. Although convenient and informative, *a priori* grouping based on inferences can lead to artificial assemblages (Blaum et al. 2011). Only recently quantitative correlations between ant morphology and niche traits started to be explored (Gibb and Parr 2013, Gibb et al. 2015).

If there is a strong link between morphology and resource use, some traits may then serve as "morphological markers", which directly reflect a function, but are easier to measure than the function itself (Violle et al. 2007). However, such relationships may be not so strong, particularly in ants, where eusociality also affects the types and sizes of food items gathered (Traniello 1989). Morphology may influence, but not predict, functionality.

Regardless of the use of inference or empirical data, almost all works linking morphology to function in ants use linear measures, obtained with a stereomicroscope. Such measures are rooted in taxonomical studies, with the purpose of having equivalent measures to compare and classify species. An important example is the mesosoma length, or "Weber's length" (Weber 1938), used as a proxy for total size of ants (Weiser and Kaspari 2006, Silva and Brandão 2010, 2014, Gibb and Parr 2013, Gibb et al. 2015). Mesosoma length is easier to measure than total length, and the mesosoma size is relatively stable, while the gaster may be expanded in specimens conserved in ethanol and/or will full crops. However, although easily comparable and convenient for taxonomic purposes, it is seldom tested whether it accurately represents body length or volume. In a later work with a single species, Weber (1946) stated that there was a 0.9 correlation between mesosoma length and total body length, but chose to use the latter. Kaspari and Weiser (1999) proposed different measurements as best predictors of body mass for each subfamily, but did not test mesosoma length. For functional morphology,

measurements should be comparable, but it is also important to know how much they reflect actual body size, volume or shape.

As a part of this thesis, we acquired X-Ray microtomographies using synchrotron facilities at ANKA (University of Karlsruhe) and DESY (University of Hamburg). These tomographies allow construction of tridimensional models of external body surface and internal structures of organisms. We obtained them for most species collected, as well as other species from South America, Africa and Southeast Asia, to get a broad taxonomic and geographic representation of ant morphology. They will be used to: (i) establish the best linear predictors, if any, of tridimensional ant morphology; and (ii) relate morphological traits to our observed field and chemical data, focusing in mandibles, head muscles and other structures related to resource use.

8.4. Concluding remarks

The main contributions of this thesis were: the description of trophic niches of several, mostly unknown, tropical species (Chapter 5 and 7); the first description of fatty acid assimilation in ants (Chapter 6); the first application of fatty acid analysis in an ecological context for ants, and the finding that fatty acid compositions represent similarities in trophic niches (Chapter 7); and the observation of similar patterns of resource use and generalization in two very distinct ant communities (Chapter 7).

Local descriptive works are nowadays somewhat devalued in many scientific spheres. However, they constitute the building blocks from where hypotheses may be raised, and the first step to understand and organize the universe (Greene 2005). This is fully applicable here: the basic knowledge my coauthors and I gathered adds to our basic scientific knowledge, but also opens many new research avenues. From now on, we may ask more specific questions and tests biological hypothesis related to niche of some species (why is the little fire ant *so strange* in that community after all?), mechanisms behind NLFA compositions of temperate ants, and the generality (or not) of the observed patterns. Finally, we look forward to improve and expand the application of fatty acid analysis in ecology, which can be used to answer many questions not only for ants, but for terrestrial organisms in general.

9. References

- Adams, E. S. 2016. Territoriality in ants (Hymenoptera: Formicidae): a review. *Myrmecological News* 23:101–118.
- Adler, F. R., E. G. LeBrun, and D. H. Feener Jr. 2007. Maintaining diversity in an ant community: modeling, extending, and testing the dominance–discovery trade–off. *The American Naturalist* 169:323–333.
- Agosti, D., and L. E. Alonso. 2000. The ALL Protocol: a standard protocol for the collection of ground-dwelling ants. Pages 204–206 in D. Agosti, J. D. Majer, L. E. Alonso, and T. R. Schultz, editors. *Ants, Standard Methods for Measuring and Monitoring Biodiversity*. Smithsonian Institution Press, Washington, DC.
- Andersen, A. N. 1995. A Classification of Australian Ant Communities, Based on Functional Groups Which Parallel Plant Life-Forms in Relation to Stress and Disturbance. *Journal of Biogeography* 22:15–29.
- Andersen, A. N. 1997. Functional groups and patterns of organization in North American ant communities: a comparison with Australia. *Journal of Biogeography* 24:433–460.
- Andersen, A. N. 2000. A global ecology of rainforest ants: functional groups in relation to environmental stress and disturbance. Pages 25–34 in D. Agosti, J. D. Majer, L. E. Alonso, and T. R. Schultz, editors. *Ants: standard methods for measuring and monitoring biodiversity*. Smithsonian Institution Press, Washington, DC.
- Andersen, A. N. 2008. Not enough niches: non-equilibrium processes promoting species coexistence in diverse ant communities. *Austral Ecology* 33:211–220.
- AntWeb. 2018. *AntWeb*. <http://www.antweb.org/>.
- Baroni Urbani, C., and M. L. De Andrade. 1997. Pollen eating, storing, and spitting by ants. *Naturwissenschaften* 84:256–258.
- Beeren, C. V., M. M. Mair, and V. Witte. 2014. Discovery of a second mushroom harvesting ant (Hymenoptera: Formicidae) in Malayan tropical rainforests. *Myrmecological News* 20:37–42.
- Bestelmeyer, B. T., D. Agosti, L. E. Alonso, C. R. F. Brandão, W. L. Brown, J. H. C. Delabie, and R. Silvestre. 2000. Field techniques for the study of ground-dwelling ants. Pages 122–144 in D. Agosti, J. D. Majer, L. E. Alonso, and T. R. Schultz, editors. *Ants: standard methods for measuring and monitoring biodiversity*. Smithsonian Institution Press, Washington, DC.

-
- Bihn, J. H., G. Gebauer, and R. Brandl. 2010. Loss of functional diversity of ant assemblages in secondary tropical forests. *Ecology* 91:782–792.
- Birkhofer, K., H. Bylund, P. Dalin, O. Ferlian, V. Gagic, P. A. Hambäck, M. Klapwijk, L. Mestre, E. Roubinet, M. Schroeder, J. A. Stenberg, M. Porcel, C. Björkman, and M. Jonsson. 2017. Methods to identify the prey of invertebrate predators in terrestrial field studies. *Ecology and Evolution* 7:1942–1953.
- Blaum, N., E. Mosner, M. Schwager, and F. Jeltsch. 2011. How functional is functional? Ecological groupings in terrestrial animal ecology: towards an animal functional type approach. *Biodiversity and Conservation* 20:2333–2345.
- Blüthgen, N., and H. Feldhaar. 2010. Food and shelter: How resources influence ant ecology. Pages 115–136 in L. Lach, C. L. Parr, and K. L. Abbott, editors. *Ant Ecology*. Oxford University Press, Oxford.
- Blüthgen, N., and N. E. Stork. 2007. Ant mosaics in a tropical rainforest in Australia and elsewhere: a critical review. *Austral Ecology* 32:93–104.
- Bolton, B. 2018. *An online catalog of the ants of the world*. <http://antcat.org/>.
- Bolton, B., and B. L. Fisher. 2011. Taxonomy of Afrotropical and West Palaearctic ants of the ponerine genus *Hypoponera* Santschi (Hymenoptera: Formicidae). *Zootaxa* 2843:1–118.
- Bowers, M. A., and S. D. Porter. 1981. Effect of foraging distance on water content of substrates harvested by *Atta columbica* (Guerin). *Ecology* 62:273–275.
- Brandão, C. R. F., R. R. Silva, and J. H. C. Delabie. 2012. Neotropical ants (Hymenoptera) functional groups: Nutritional and applied implications. Pages 213–236 in A. R. Panizzi and J. R. P. Parra, editors. *Insect Bioecology and Nutrition for Integrated Pest Management*. CRC Press, Boca Raton.
- Carroll, C. R., and D. H. Janzen. 1973. Ecology of Foraging by Ants. *Annual Review of Ecology and Systematics* 4:231–257.
- Cerdá, X., X. Arnan, and J. Retana. 2013. Is competition a significant hallmark of ant (Hymenoptera: Formicidae) ecology? *Myrmecological News* 18:131–147.
- Cerdá, X., J. Retana, and S. Cros. 1997. Thermal disruption of transitive hierarchies in Mediterranean ant communities. *The Journal of Animal Ecology* 66:363.
- Connell, J. H. 1983. On the prevalence and relative importance of interspecific competition: evidence from field experiments. *The American Naturalist* 122:661–696.
-

-
- Davidson, D. W. 1977. Foraging ecology and community organization in desert seed-eating ants. *Ecology* 58:711-724.
- Davidson, D. W. 1998. Resource discovery versus resource domination in ants: a functional mechanism for breaking the trade-off. *Ecological Entomology* 23:484-490.
- Eisner, T. 1957. A comparative morphological study of the proventriculus of ants. *Bulletin of the Museum of Comparative Zoology at Harvard College* 116:439-490.
- Eisner, T., and G. M. Happ. 1962. The Infrabuccal pocket of a Formicine ant: a social filtration device. *Psyche* 69:107-116.
- Erthal, M., C. Peres Silva, and R. Ian Samuels. 2007. Digestive enzymes in larvae of the leaf cutting ant, *Acromyrmex subterraneus* (Hymenoptera: Formicidae: Attini). *Journal of Insect Physiology* 53:1101-1111.
- Feener, D. H., M. R. Orr, K. M. Wackford, J. M. Longo, W. W. Benson, and L. E. Gilbert. 2008. Geographic variation in resource dominance-discovery in Brazilian ant communities. *Ecology* 89:1824-1836.
- Fellers, J. H. 1987. Interference and exploitation in a guild of woodland ants. *Ecology* 68:1466-1478.
- Fisher, B. L. 1999. Improving inventory efficiency: a case study of leaf-litter ant diversity in Madagascar. *Ecological Applications* 9:714-731.
- Folgarait, P. 1998. Ant biodiversity and its relationship to ecosystem functioning: a review. *Biodiversity And Conservation* 7:1221-1244.
- Forbes, A. A., R. K. Bagley, M. A. Beer, A. C. Hippee, and H. A. Widmayer. 2018. Quantifying the unquantifiable: why Hymenoptera, not Coleoptera, is the most speciose animal order. *BMC Ecology* 18:21.
- Frank, K., F.-T. Krell, E. M. Slade, E. H. Raine, L. Y. Chiew, T. Schmitt, C. S. Vairappan, P. Walter, and N. Blüthgen. 2018. Global dung webs: high trophic generalism of dung beetles along the latitudinal diversity gradient. *Ecology Letters* 21:1229-1236.
- Gibb, H., and C. L. Parr. 2013. Does structural complexity determine the morphology of assemblages? An experimental test on three continents. *PLoS ONE* 8:e64005.
- Gibb, H., J. Stoklosa, D. I. Warton, A. M. Brown, N. R. Andrew, and S. A. Cunningham. 2015. Does morphology predict trophic position and habitat use of ant species and assemblages? *Oecologia* 177:519-531.
- Gotelli, N. J., A. M. Ellison, R. R. Dunn, and N. J. Sanders. 2011. Counting ants

-
- (Hymenoptera: Formicidae): biodiversity sampling and statistical analysis for myrmecologists. *Myrmecological News* 15:13–29.
- Greene, H. 2005. Organisms in nature as a central focus for biology. *Trends in Ecology & Evolution* 20:23–27.
- Hardin, G. 1960. The competitive exclusion principle. *Science* 131:1292–1297.
- Hespenheide, H. A. 1973. Ecological inferences from morphological data. *Annual Review of Ecology and Systematics* 4:213–229.
- Hölldobler, B., and E. O. Wilson. 1990. *The Ants*. 1st ed. Harvard University Press, Massachusetts.
- Howard, D., and W. Tschinkel. 1981. The flow of food in colonies of the fire ant, *Solenopsis invicta*: a multifactorial study. *Physiological Entomology* 6:297–306.
- Jagdale, G. B., and Gordon, R. 1997. Effect of temperature on the composition of fatty acids in total lipids and phospholipids of entomopathogenic nematodes. *Journal of Thermal Biology* 22(4–5):245–251.
- Janicki, J., Narula, N., Ziegler, M., Guénard, B., and Economo, E. P. 2016. Visualizing and interacting with large-volume biodiversity data using client-server web-mapping applications: The design and implementation of antmaps.org. *Ecological Informatics* 32: 185–193.
- Kaspari, M. 2000. A primer on ant ecology. Pages 9–24 in D. Agosti, J. D. Majer, L. E. Alonso, and T. R. Schultz, editors. *Ants: standard methods for measuring and monitoring biodiversity*. Smithsonian Institution Press, Washington, DC.
- Kaspari, M., and M. D. Weiser. 1999. The size-grain hypothesis and interspecific scaling in ants. *Functional Ecology* 13:530–538.
- Krell, F.-T. 2004. Parataxonomy vs. taxonomy in biodiversity studies – pitfalls and applicability of ‘morphospecies’ sorting. *Biodiversity and Conservation* 13:795–812.
- Ladwig, N. I. 1998. *As unidades de conservação ambiental e o cadastro técnico multifinalitário - estudo de caso: UCAD/UFSC (Unidade de Conservação Ambiental Desterro)*. Master thesis: Universidade Federal de Santa Catarina.
- Lanan, M. 2014. Spatiotemporal resource distribution and foraging strategies of ants (Hymenoptera: Formicidae). *Myrmecological News* 20:53–70.
- LaPolla, J. S., P. G. Hawkes, and B. L. Fisher. 2011. Monograph of Nylanderia (Hymenoptera: Formicidae) of the world, part I: *Nylanderia* in the afrotropics. *Zootaxa*
-

3110:10–36.

- Lawton, J. H. 1999. Are there general laws in ecology? *Oikos* 84:177–192.
- MacArthur, R. H. 1972. *Geographical ecology: patterns in the distribution of species*. Princeton University Press, Princeton.
- Morris, R. J., S. Gripenberg, O. T. Lewis, and T. Roslin. 2014. Antagonistic interaction networks are structured independently of latitude and host guild. *Ecology Letters* 17:340–349.
- Myers, N., N. Myers, R. a Mittermeier, R. a Mittermeier, G. a B. Fonseca, G. a B. Fonseca, J. Kent, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403:853–858.
- Nguyen, V., B. Lilly, and C. Castro. 2014. The exoskeletal structure and tensile loading behavior of an ant neck joint. *Journal of Biomechanics* 47:497–504.
- Oliveira-Filho, A. T., and M. A. L. Fontes. 2000. Patterns of floristic differentiation among Atlantic Forests in southeastern Brazil and the influence of Climate. *Biotropica* 32:793–810.
- Oliver, I., and A. J. Beattie. 1996. Invertebrate morphospecies as surrogates for species: a case study. *Conservation Biology* 10:99–109.
- Parr, C. L., and H. Gibb. 2012. The discovery-dominance trade-off is the exception, rather than the rule. *Journal of Animal Ecology* 81:233–241.
- Paul, J. 2001. Mandible movements in ants. *Comparative Biochemistry and Physiology* 131:7–20.
- Polis, G. A., and D. R. Strong. 1996. Food web complexity and community dynamics. *The American Naturalist* 147:813–846.
- Reitz, S. R., and J. T. Trumble. 2002. Competitive displacement among insects and arachnids. *Annual Review of Entomology* 47:435–465.
- Ricklefs, R. E., and J. Travis. 1980. A morphological approach to the study of avian community organization. *The Auk* 97:321–338.
- Roma, G. C., Bueno, O. C., and Camargo-Mathias, M. I. 2010. Morpho-physiological analysis of the insect fat body: a review. *Micron* 41:395–401.
- Rosenfeld, J. S. 2002. Functional redundancy in ecology and conservation. *Oikos* 98:156–162.
- Rosindell, J., S. P. Hubbell, and R. S. Etienne. 2011. The unified neutral theory of

-
- biodiversity and biogeography at age ten. *Trends in Ecology & Evolution* 26:340–349.
- Rosumek, F. B., and R. P. Martins. 2010. Ecologia, filosofia e conservação. *Natureza & Conservação* 8:87–89.
- Ryder Wilkie, K. T., A. L. Mertl, and J. F. A. Traniello. 2010. Species diversity and distribution patterns of the ants of Amazonian Ecuador. *PLoS ONE* 5:e13146.
- Savolainen, R., and K. Vepsäläinen. 1988. A Competition hierarchy among boreal ants: impact on resource partitioning and community structure. *Oikos* 51:135–155.
- Scheffer, M., E. H. van Nes, and R. Vergnon. 2018. Toward a unifying theory of biodiversity. *Proceedings of the National Academy of Sciences* 115:639–641.
- Schleuning, M., J. Fründ, A. M. Klein, S. Abrahamczyk, R. Alarcón, M. Albrecht, G. K. S. Andersson, S. Bazarian, K. Böhning-Gaese, R. Bommarco, B. Dalsgaard, D. M. Dehling, A. Gotlieb, M. Hagen, T. Hickler, A. Holzschuh, C. N. Kaiser-Bunbury, H. Kreft, R. J. Morris, B. Sandel, W. J. Sutherland, J. C. Svenning, T. Tschardt, S. Watts, C. N. Weiner, M. Werner, N. M. Williams, C. Winqvist, C. F. Dormann, and N. Blüthgen. 2012. Specialization of mutualistic interaction networks decreases toward tropical latitudes. *Current Biology* 22:1925–1931.
- Schluter, D. 2000. Ecological character displacement in adaptive radiation. *The American Naturalist* 156:S4–S16.
- Schoener, T. W. 1974. Resource partitioning in ecological communities. *Science* 185:27–39.
- Seifert, B. 2007. *Die Ameisen Mittel- und Nordeuropas*. Lutra, Tauer.
- Silva, R. R., and C. R. F. Brandão. 2010. Morphological patterns and community organization in leaf-litter ant assemblages. *Ecological Monographs* 80:107–124.
- Silva, R. R., and C. R. F. Brandão. 2014. Ecosystem-wide morphological structure of leaf-litter ant communities along a tropical latitudinal gradient. *PLoS ONE* 9:e93049.
- Simberloff, D. 2004. Community ecology: is it time to move on? *The American Naturalist* 163:787–799.
- Smallwood, J. 1982. Nest relocations in ants. *Insectes Sociaux* 29:138–147.
- Soares-Filho, B., R. Rajão, M. Macedo, A. Carneiro, W. Costa, M. Coe, H. Rodrigues, and A. Alencar. 2014. Cracking Brazil's forest code. *Science* 344:363–364.
- Stork, N. E. 2018. How many species of insects and other terrestrial arthropods are there on Earth? *Annual Review of Entomology* 63:31–45.
- Traniello, J. F. A. 1989. Foraging strategies of ants. *Annual Review of Entomology* 34:191–210.
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-
- Vázquez, D. P., and R. D. Stevens. 2004. The latitudinal gradient in niche breadth: concepts and evidence. *The American Naturalist* 164:E1–E19.
- Violle, C., M. L. Navas, D. Vile, E. Kazakou, C. Fortunel, I. Hummel, and E. Garnier. 2007. Let the concept of trait be functional! *Oikos* 116:882–892.
- Weber, N. A. 1938. The biology of the fungus-growing ants. Part IV. Additional new forms. Part V. The Attini of Bolivia. *Revista de Entomologia* 9:154–206.
- Weber, N. A. 1946. Dimorphism in the African *Oecophylla* worker and an anomaly (Hym.: Formicidae). *Annals of the Entomological Society of America* 39:1–10.
- Weiser, M. D., and M. Kaspari. 2006. Ecological morphospace of New World ants. *Ecological Entomology* 31:131–142.
- Wild, A. L. 2007. *Taxonomic revision of the ant genus Linepithema (Hymenoptera:Formicidae)*. University of California Press, Berkeley.
- Wilson, E. O. 2003. *Pheidole in the New World: a dominant, hyperdiverse ant genus*. Harvard University Press, Cambridge, Mass.

10. Acknowledgements

Well, that was a wild ride...

This work went through so many turns, ups and downs that I have a lot of people to acknowledge for helping me to stay on the track. Even with some bumps and disappointments in the way, it was an awesome experience, full of scientific learning, and a good dive into German culture. When the very first day of work finished with a beer in the balcony to celebrate the arrival of warm, sunny skies after winter, I knew things were going to be good.

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Brazilian fieldwork; Cristina Sánchez with the feeding experiment; and Anna Ruppenthal with the fatty acid analysis of temperate ants.

In both countries I had help from grown-ups, too. Andrea Hilpert and Karin Römer were fundamental to deal with logistics and bureaucracy in Germany. I'm grateful that they had the patience, willingness and good mood to deal with somebody with the language skills of a true German (shepherd). Karla Scherer was supportive as always in Brazil, as well as Benedito Lopes, the former head of the Ant Lab in UFSC, which is a friend and professional partner since I tried to mount my first *Camponotus* (seriously, everybody wants to quit at the first mounting).

A proper taxonomy was one of my main concerns in this work, but I'm not a taxonomist (yet). Fortunately, I had great assistance from specialists to confirm or correct my attempts. In Brazil, Rodrigo Feitosa, Thiago Silva and Alexandre Ferreira shared the fun of reviewing *Solenopsis*, *Pheidole* and the other beautifully crappy Neotropical genera. Taxonomy of temperate species surprised me, both for being very well-resolved and very well-complicated to deal with. Karsten Mody gave me some good beginner tips ("is this a *Formica* or a *Lasius*?") and Francisco Hita-Garcia helped to review my IDs. Naturally, any misidentification is my own responsibility.

My learning was not limited to the thesis itself. I had the opportunity to take part in great courses and learn from the big sources what often is not written in the literature. Brian Fisher, Bill Ward, Flávia Esteves and all the instructors and students of the Ant Course 2016 in Mozambique contributed to make it one of the best professional and personal experiences of my life. I also had opportunity to learn more about the mysterious temperate species with David Nash, Christos Georgiades, Riitta Savolainen and the other participants of Euro Ants 2017 in Copenhagen.

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Enough science, let's go personal. One of the first lessons they say to foreigners is that "Freund" has a somewhat restrict use in German, compared to other languages. Well, Brazilians don't care too much about this, and I can say for sure that I made many friends during these years. Besides the ones already mentioned, I crossed with many nice people in our research group: Jule, Meli, Kevin, Florian, Karsten, Phil, Arturo, Alba, Andrew. A special thanks to Anne, for closing the office windows when it was too cold and withstanding the ventilator noise when it was too hot. Also the people from our little Brazilian invasion, particularly Fernanda and Inácio, who brought a bit of homely warmth to the North. I also had the luck to have great old friends already established in Germany, Juliana and Tatiane (and this journey would not have been the same without the muddy metal festivals). I had a great time sharing an apartment with Nana and Yuri. Finally, I shared good times with a lot more people, sometimes for a few months, sometimes for a single congress evening. This is the nature of our vagrant stay in another reality: people come and people go, we just have to enjoy the time together.

Who were always there, distantly close, were my beloved ones in Brazil. My long-time friends and, above all, my family. They were fundamental to celebrate together in the good times, and support me in the not-so-good ones. I am extremely happy that I was actually able to share a bit of this European experience with them. I came here for science, music and beer. I know that I did very well at least two out of three. I'm pretty sure I'm still not done with it, but now it's time to go back home.

11. Curriculum vitae

M.Sc. Félix Baumgarten Rosumek

Date of birth: 25th March 1984

Place of Birth: Blumenau, Brazil

Nationality: Brazilian

Education and professional experience

04/2015-10/2014: Ph.D. candidate at Technische Universität Darmstadt, Germany. Thesis: "From field to fat – Integrating approaches to unveil use of trophic resources by tropical and temperate ant species (Hymenoptera: Formicidae)". Supervisor: PD Dr. Michael Heethoff. With scholarship from Conselho Nacional de Pesquisa e Desenvolvimento, Brazil.

01/2011-present: handling and academic editor for the journal Biotemas. Subjects: ecology and myrmecology.

02/2010-present: permanent position as researcher biologist at Universidade Federal de Santa Catarina, Brazil.

09/2009-12/2009: assistant professor in the bachelor course of Agricultural Sciences at Universidade Federal de Santa Catarina, Brazil. Course: Agricultural Ecology.

07/2009-12/2009 and 07/2010-12/2010: assistant of distance learning in the licentiate course of Biological Sciences at Universidade Federal de Santa Catarina, Brazil. Course: Introduction to Ecology.

02/2009-03/2009: basic education teacher at Escola Básica Municipal Anísio Teixeira. Course: Sciences.

03/2007-02/2008: master in Ecology at Universidade Federal de Minas Gerais, Brazil. Thesis: "Conservation? Why? Discussing the arguments and goals of the environmentalist

ideology" [in Portuguese]. Supervisor: Prof. Dr. Rogério Parentoni Martins. With scholarship from Coordenação de Aperfeiçoamento do Pessoal do Ensino Superior, Brazil.

01/2003-02/2007: internship at Laboratório de Mamíferos Aquáticos, Universidade Federal de Santa Catarina. Subject: ecology of terrestrial vertebrates.

10/2003-03/2005: internship at Laboratório de Biologia de Formigas, Universidade Federal de Santa Catarina. Subject: taxonomy and biodiversity of Formicidae.

04/2002-02/2007: bachelor and licentiate in Biological Sciences at Universidade Federal de Santa Catarina, Brazil. Thesis: "Ecology of *Liolaemus occipitalis* (Squamata: Tropiduridae) in sand dunes of Joquina beach, Florianópolis, Brazil" [in Portuguese]. Supervisor: Dr. Maurício Eduardo Graipel. With scholarship from Programa de Educação Tutorial-Biologia.

Publications

(9) Rosumek, F. B., Blüthgen, N., Brückner, A., Menzel, F., Gebauer, G., and Heethoff, M. 2018. Unveiling community patterns and trophic niches of tropical and temperate ants using an integrative framework of field data, stable isotopes and fatty acids. *PeerJ* 6:e5467.

(8) Klunk, C. L., Giehl, E. L. H., Lopes, B. C., Marcineiro, F. R., and Rosumek, F. B. 2018. Simple does not mean poor: grasslands and forests harbor similar ant species richness and distinct composition in highlands of southern Brazil. *Biota Neotropica* 18(3):e20170507.

(7) Rosumek, F. B. 2017. Natural history of ants: what we (do not) know about trophic and temporal niches of Neotropical species. *Sociobiology*, 64(3): 244-255.

(6) Rosumek, F. B., Brückner, A., Blüthgen, N., Menzel, F., and Heethoff, M. 2017. Patterns and dynamics of neutral lipid fatty acids in ants - implications for ecological studies. *Frontiers in Zoology* 14:36.

(5) Ulysséa, M. A., Cereto, C. E., Rosumek, F. B., Silva, R. R., and Lopes, B. C. 2011. Updated list of ant species (Hymenoptera, Formicidae) recorded in Santa Catarina State,

southern Brazil, with a discussion of research advances and priorities. *Revista Brasileira de Entomologia* 55:603-611, 2011.

(4) Rosumek, F. B., and Martins, R. P. 2010. Ecology, philosophy and conservation. *Natureza & Conservação* 8:87-89 [in Portuguese].

(3) Rosumek, F. B., Silveira, F. A. O., Neves, F. S., Barbosa, N. P. U., Diniz, L., Oki, Y., Pezzini, F., Fernandes, G. W., Cornelissen, T. 2009. Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. *Oecologia* 160:537-549.

(2) Rosumek, F. B. 2008. Association of *Eremanthus erythropappus* (DC.) McLeish (Asteraceae) with ants and its relationship with ground-dwelling ants, in an altitude forest, central Minas Gerais state. *Lundiana* 9:41-47 [in Portuguese].

(1) Rosumek, F. B., Ulysséa, M. A., Lopes, B. C., Steiner, J., and Zillikens, A. 2008. Ground and bromeliad-nesting ants in an Atlantic Forest area, Santa Catarina Island, south Brazil: species inventory and new records. *Biotemas* 21: 81-89 [in Portuguese].

Additional contributions

2014: evaluation of ants for the official list of threatened species in Brazil. Organizer: Instituto Chico Mendes de Conservação da Biodiversidade.

2010: evaluation of ants for the official list of threatened species in Santa Catarina state, Brazil. Organizer: IGNIS and Fundação do Meio Ambiente.

2009: evaluation of ecological impacts of the building of SC-280 highway on reptiles and amphibians. Organizer: Prosul and Fundação do Meio Ambiente.

Participation in 38 scientific conferences, with 7 talks and 24 posters presented.

Peer-reviewing for the journals: *Biotemas*, *Oecologia Australis*, *Sociobiology*, *Revista Brasileira de Entomologia*, *European Journal of Entomology*, *Insectes Sociaux*, *Anais da Academia Brasileira de Ciências*, *Pedobiologia*, *Journal of Natural History*.

12. Ehrenwörtliche Erklärung

Ich erkläre hiermit ehrenwörtlich, dass ich die vorliegende Arbeit entsprechend den Regeln guter wissenschaftlicher Praxis selbstständig und ohne unzulässige Hilfe Dritter angefertigt habe.

Sämtliche aus fremden Quellen direkt oder indirekt übernommenen Gedanken sowie sämtliche von Anderen direkt oder indirekt übernommenen Daten, Techniken und Materialien sind als solche kenntlich gemacht. Die Arbeit wurde bisher bei keiner anderen Hochschule zu Prüfungszwecken eingereicht.

Darmstadt, den 23. August 2018

Félix Baumgarten Rosumek

Annex I

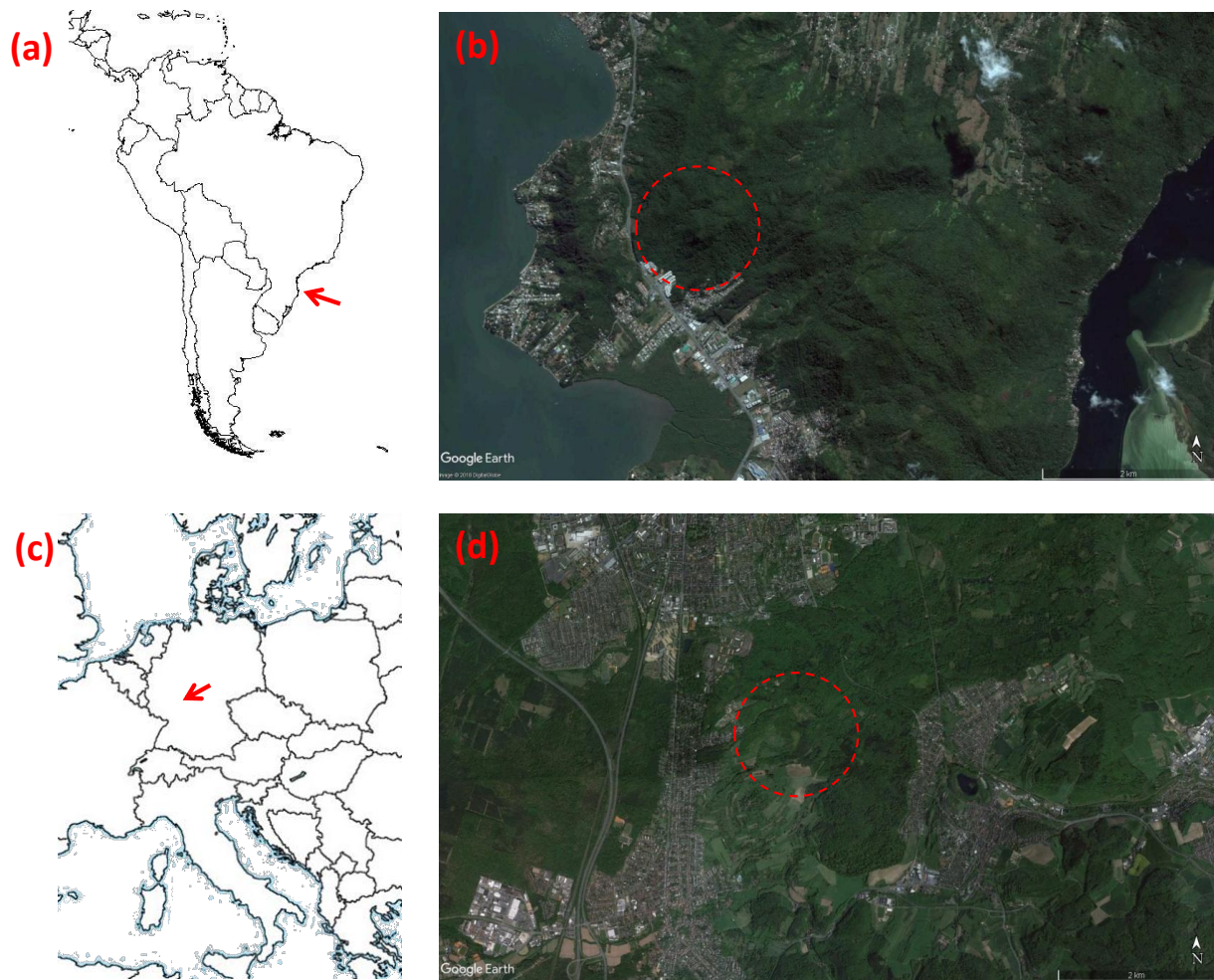


Figure 1: maps of the study sites. In (a) and (c) the setae indicate location of the sites in South America and Europe. In (b) and (d) the circles indicate the study sites within the local context in Brazil and Germany, respectively.

Annex II

Natural history of ants: what we (do not) know about trophic and temporal niches of Neotropical species

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RESEARCH ARTICLE - ANTS

Natural History of Ants: What We (do not) Know about Trophic and Temporal Niches of Neotropical Species

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Abstract

Our understanding of the natural history of Neotropical ants is limited, due to lack of descriptive efforts and widespread use of morphospecies in literature. Use of trophic resources and period of activity are two central niche aspects little explored for most species. This work aimed to broadly review the literature and provide empirical field data on these aspects for 23 species. The fieldwork was carried out in the Atlantic forest of southern Brazil. Trophic and temporal niches were assessed with pitfall traps and seven kinds of bait representing natural resources. Crushed insects were the preferred resource, whereas bird feces and living prey were less exploited. Most species broadly used the resources, but pronounced quantitative differences were found. *Odontomachus chelifer* (Latreille, 1802) and *Pachycondyla striata* Smith, 1858 were relatively well studied and field data matched previous accounts. They were the only species that consistently used large prey, and avoided oligosaccharides. *Wasmannia auropunctata* (Roger, 1863) differed remarkably from previous studies, using feces as its sole trophic resource. The six *Pheidole* species had no previous records and presented quantitative differences in resource use. Most species had no strong preference for period of activity. *Camponotus zenon* Forel, 1912 was nocturnal and *Crematogaster nigropilosa* Mayr, 1870, *Linepithema iniquum* (Mayr, 1870) and *Linepithema pulex* Wild, 2007 were diurnal. Complementary methods, context-dependence and descriptive studies have a central role in the understanding of ant natural history. Community assessments can contribute significantly to this knowledge if researchers also pay attention to the individual species involved.

Introduction

Ants (Hymenoptera: Formicidae) are among the most abundant groups of invertebrates in terrestrial ecosystems, presenting a wide variety of feeding habits, nesting sites, and interactions with organisms from all trophic levels (Kaspari, 2000). They are the subject of extensive and diversified research, in basic and applied science. Despite this, in all tropical regions, the biology of most species is virtually unknown, due to a combination of high richness, taxonomic

uncertainty, lack of descriptive studies and widespread use of morphospecies in literature (Krell, 2004; Greene, 2005).

Two fundamental aspects that remain elusive for many tropical species are trophic and temporal niche. Ants in general are regarded as omnivorous, feeding on a combination of live prey, dead animals, seeds and plant exudates, with some notorious specialized behaviors such as fungus cultivation and pollen consumption (Kaspari, 2000; Blüthgen & Feldhaar, 2010). At genus or species level, they are sometimes classified in broad groups or guilds like “generalist predators” or simply



“generalists” (Brandão et al., 2012). These generalizations have an important role to understand communities, but also leave out the remarkable variation among species in nature. This specific information can be assessed from indirect sources such as body ratios of stable isotopes (Blüthgen et al., 2003) and remains found in nests (Lattke, 1990), and direct observation such as interaction with plant resources (Byk & Del-Claro, 2010) and items carried to nests (Medeiros & Oliveira, 2009; Raimundo et al., 2009). Every approach has its focus and limitations, and adds a piece to the puzzle that is the niche of a species.

In turn, studies on temporal niche of ants are common in open areas and/or temperate habitats, where the fluctuations in abiotic factors could have a stronger effect on ant activity. This variation is often linked with temporal niche partitioning and coexistence of competing species (Lessard et al., 2009; Anjos et al., 2016). Less information is available for tropical forests (e.g. Medeiros & Oliveira, 2009; Raimundo et al., 2009; Feitosa et al., 2016). Inside a forest, less variation would be expected, because daily changes in temperature, humidity and wind are smaller. However, temporal niche could still be affected by the existing fluctuations or by competitive pressure.

In view of the importance of both use of trophic resources and period of activity, and considering the lack of information available for most Neotropical species, this work aims to quantify use of trophic resources and period of activity of ground-dwelling ants from a Neotropical forest. An extensive literature review was performed to assess how much is known about these individual species and compare results from the viewpoint of complementarity of methods and ecological context-dependence. These case studies highlight that descriptive studies are still fundamental for tropical faunas. In this sense, broader ecological assessments can give a significant contribution, if researchers also pay attention to the individual species involved.

Methods

Study area and sample design

Fieldwork was carried out in Desterro Conservation Unit, Florianópolis, south Brazil (27°31'38" S, 48°30'15" W, altitude ca. 250 m), between December 2015 and January 2016. Average annual temperature and precipitation is 20.5 °C and 140 mm per month (data from meteorological station of EPAGRI/CIRAM). Vegetation consists of secondary Atlantic forest *sensu stricto* (= ombrophilous dense forest) with at least 60 years of relatively undisturbed regeneration. This work was conducted in accordance with Brazilian laws, under authorization SISBIO number 51173-1.

The sampling was based on the recent design of Houadria et al. (2015) to assess community patterns on resource use and daily activity, but here its suitability to understand individual species is explored. Four plots with 4 x 4 sample points (16 per plot) were set up, with distances from

30 to 50 m between plots. Distance between sample points was 10 m. The baits were set up in transparent plastic boxes with diameter of 10 cm and ground-level slits that allowed the entrance of ants, and retrieved after 90 minutes. All individuals were collected from the baits in laboratory and stored in ethanol 70% for subsequent sorting.

Seven baits were offered as proxies for common resources available to ants (Table 1; see [Supplementary Material](#) for details on bait display and rationale for their choice). In each round, only one bait was offered per sample point, and bait types were mixed among points, with a similar number of points receiving each type (8-9 per round). Fourteen baiting rounds were performed, with only one period sampled each day, at daytime (around 13:00-15:30) or nighttime (around 21:00–23:30). In total, 896 baits were applied in the 64 sample points, with all seven baits being offered in each sample point two times (one at day and one at night). This design is suitable to assess multidimensional trophic niches, which are inferred from how often ants use each resource. Hence, “preferences” means simply relative high use of certain resources. Distance between each colony and the bait does not change from one resource to another, and the use of one resource does not affect the other. Thus, it differs from a typical cafeteria experiment, which is designed to assess preferences through choices among different resources offered at the same time (Krebs, 1999).

An independent community assessment was performed with three rounds of pitfall trapping, alternated with bait rounds. The plastic cups were 6 cm wide and contained propylene glycol 50% and a small amount of neutral detergent. Cups were buried previously and replaced after each round to avoid the digging-in effect. Pitfalls stayed opened for 10 hours during the day and 9 hours during the night (due to short summer nights), then an extra 3-hour nocturnal round was performed. One nocturnal and one diurnal pitfall round were performed in sequence, separated by intervals to avoid dusk and dawn times.

Specimen processing and identification

For each sample point, at least one individual per morphospecies was mounted. They were identified to genus level with Baccaro et al. (2015) and to species level with taxonomic revisions, and comparison to identified specimens in collections and Antweb images (AntWeb, 2016). The taxonomic sources used were: *Crematogaster* – Longino (2003); *Cyphomyrmex* – Kempf (1965) and Snealling and Longino (1992); *Gnamptogenys* – Lattke (1995); *Hylomyrma* – Kempf (1973); *Linepithema* – Wild (2007); *Odontomachus* and *Pachycondyla* – Fernández (2008); *Pheidole* – Wilson (2003); *Wasmannia* – Longino and Fernández (2007). *Camponotus* and *Strumigenys* were identified just by comparison with collections. The identifications were partially confirmed by taxonomists of the Laboratory of Ant Systematics and Biology, Federal University of Paraná, Brazil (see Acknowledgements). Vouchers were deposited at

the Laboratory of Ant Biology, Federal University of Santa Catarina, Brazil, and at the Ecological Networks research group, Technical University of Darmstadt, Germany.

Analysis

For analysis and literature review of trophic niche, all species with at least 10 bait records were included, and for daily activity all species with at least 6 records on baits and pitfalls. The systematic literature review included the following sources: search for species names in Google Scholar; original descriptions; taxonomic revisions and references therein; references found in AntWiki (AntWiki, 2016). In case of species subject to name changes, older versions were also considered. Taxonomic history and current nomenclature of species were checked with AntCat (Bolton, 2016). Distribution records were retrieved from AntMaps (Janicki et al., 2016). A representative, species-specific, literature on trophic and temporal niche for these species was gathered. Artificial breeding diets for laboratory colonies were not included, and use of generic baits (e.g. tuna, cookies) was considered just when relevant to discuss trophic niche.

Data is shown as proportions of records in each bait type/period relative to the total records for that species. For bait use, day and night records were not pooled. Records for the pitfall replicas of each period were pooled for every point. Differences were tested with two-tailed exact multinomial and binomial goodness-to-fit tests against a hypothesis of no preference, that is, equal proportions expected for each bait (1/7 or 0.14) or period (1/2 or 0.5). Tests were run in R 3.3.0 (R Core Team, 2016). Exact tests are the most appropriate for nominal variables with small sample sizes (McDonald, 2014), but are limited nonetheless. A non-significant result could mean either low sample size or very generalist diet/activity. Species with low number of records and non-significant results are discussed more briefly, since their results may not quantitatively represent their trophic niche.

Results

Seventy-six morphospecies were collected. It was possible to name 46 species, of which 23 had at least 6 records and 15 at least 10 (Table 2; see Supplementary Material for records of the remaining identified species).

A similar number of species was recorded in most bait types, but they differed greatly in the number of records and individuals attracted (Table 1). Crushed insects not only attracted ants more often, but also triggered larger recruitments. Seeds were extensively used by many species, but no specialized granivory was detected. Crickets attracted less species and were the resource less exploited overall. Feces and termites also presented a lower number of records and small recruitments compared to other resources.

Almost all common species (= frequent in pitfalls and/or in sample points) were well represented in baits (Table 2,

Supplementary Material). The only species conspicuously absent was *Pachycondyla harpax* (Fabricius, 1804) and, to a lesser extent, *Cyphomyrmex rimosus* (Spinola, 1851) and *Hylomyrma reitteri* (Mayr, 1887).

The use of trophic resources is discussed in the following sections (Fig 1). Literature review, results and discussion are presented for every individual species or genus. Period of activity is presented afterwards, for all species combined (Fig 2). General aspects are explored in a final Discussion section.

Gnamptogenys striatula Mayr, 1884 (Ectatomminae)

This species (or species complex – Arias, 2008; G. P. Camacho, UFPR, personal communication) is a rare example of a Neotropical ant extensively studied in the laboratory, covering many aspects of its biology (e.g. Giraud et al., 2000; Kaptein et al., 2005). However, the only information available about its trophic niche in the wild comes from Lattke (1990), who reports remnants of several insect orders inside nests, and posteriorly called it “a generalist epigeic forager of humid forests” (Lattke, 1995). A recent account recorded it rarely on experimental vertebrate carcasses left to rot in a forest, predating the larvae and pupae of necrophagous insects (Paula et al., 2016).

In accordance with this short background, the species was observed using termites frequently, but crushed insects, feces and sucrose were important as well (Fig 1). Hence, the species will scavenge and consume sugar when given the opportunity (but notice the lower use of melezitose, discussed in the next section). The relatively high use of feces, a less preferred resource overall, is a noteworthy feature that differentiates *G. striatula* from most other species of this study, particularly the two “generalist predators” discussed next.

Odontomachus chelifer (Latreille, 1802) and *Pachycondyla striata* Smith, 1858 (Ponerinae)

These two widespread species radically differ in morphology, but are similar in many aspects, therefore is appropriate to discuss both together. They are one of the most conspicuous elements of the southern Atlantic forest ground fauna, due to their abundance, solitary foraging mode and large size. Also, they are two of the most well-known species included in this study, and several account showed a multitude of functional roles and a broad trophic niche for them. Observation of nest entrances showed that 80-90% of the items carried by *Pa. striata* were arthropod parts, mostly termites and other ants, the remaining consisting of plant material (Giannotti & Machado, 1991; Medeiros & Oliveira, 2009). Through direct observation, Medeiros and Oliveira (2009) also showed that scavenging accounts for more than 80% of its foraging behavior. On the other hand, Fowler (1980) reported *O. chelifer* preferences for certain termite species in laboratory, and qualitatively stated that in the field prey

consisted almost entirely of termites. In the Atlantic forest, all items carried to nests were arthropods, mainly termites, but other animal groups accounted for 60% of them (Raimundo et al., 2009). Scavenging was also cited in this study, although not quantified. Both *O. chelififer* and *Pa. striata* were recorded on experimental carcasses predated by the larvae and pupae of necrophagous insects (Paula et al., 2016).

Other important items used by the two species are seeds with elaiosomes and other fallen diaspores rich in proteins and lipids, frequently collected from the ground in the Atlantic forest (Pizo & Oliveira, 2000; Passos & Oliveira, 2002, 2004). Field records on use of liquid sugars are scant and qualitative. *Odontomachus chelififer* was not observed using extra-floral nectaries (EFNs) by Raimundo et al. (2009), while there is one record for *Pa. striata* (in Cerrado, the Brazilian savannah – Byk & Del-Claro, 2010).

The results presented here are mostly consistent with this broader picture (Fig 1). Both species used more frequently dead insects, sucrose and large prey. In fact, they were the only two species consistently recorded on crickets. The low frequency in termite baits is unexpected and probably represents a methodological artifact. These large solitary foragers were observed quickly collecting termites (even glued ones) and leaving the baits in a few minutes, contrary to ants of smaller species that were recruited to them. In cricket baits, however, the two species spent more time, trying to carry out the tied cricket or dismembering it. Smaller ants frequently took advantage of this to grab the remains or lick spilled hemolymph. This largely contributed to the richness found in this bait (Table 1) and could happen in nature, whenever predators kill prey too large to carry them out at once.

Besides predation, scavenging was a common behavior, and *P. striata* in particular would prioritize it whenever possible, in accordance with what was observed by Medeiros and Oliveira (2009). This could be result of their morphology, because the triangular mandibles may be more suited to chop large carcasses than the trap-jaws of *O. chelififer*. In addition, it could be an effect of competition. The two species were never found at the same bait, and co-occurred in just 10 of the 62 points where they were recorded (Table 2). The two were previously reported to avoid each other, but, when

agonistic interactions occur, *Pa. striata* usually is the winner, and can steal the food or kill (and eat) *O. chelififer* (Medeiros & Oliveira, 2009; Raimundo et al., 2009). Thus, *Pa. striata* could displace *O. chelififer* and maintain control of a valuable resource such as dead arthropods through tandem recruitment (Medeiros & Oliveira, 2009; Silva-Melo & Giannotti, 2012), while cooperative foraging behavior was not observed in *O. chelififer* (Raimundo et al., 2009). Effectively, the average numbers of workers per bait was smaller for the latter species (*O. chelififer* = 1.4 ± 0.9 ; *Pa. striata* = 2.5 ± 2 ; Mann-Whitney, $z = -2.27$, $p = 0.02$).

Both species used sucrose frequently. They were never observed foraging on trees or low vegetation in this study, which fits previous accounts (Fowler, 1980; Medeiros & Oliveira 2009), so it is unlikely that they commonly use nectar as food source. This behavior also should limit honeydew use by them. Effectively, the difference between use of sucrose and melezitose is remarkable here. Ants differ in their sugar preferences/tolerances, and melezitose is highly attractive to some species, and less so for others (Völkl et al., 1999; Blüthgen & Fiedler, 2004). In some insects, weak or negative effect of melezitose on fitness was observed (Zoebelein, 1956; Chen & Fadamiro, 2006), and some evidence points out to reduced suitability of aphid oligosaccharides for predators (Wäckers, 2000). The low number of records for these ant species, even when melezitose was readily available, suggests a physiological constraint to the use of complex sugars. While some congeneric species are known to visit EFNs or tend hemipterans (e.g. *O. troglodytes* – Lachaud & Dejean, 1991), the main source of sugars for these ground foragers in the Atlantic forest is more likely to be fallen fruits rich on mono- and disaccharides.

Wasmannia auropunctata (Roger, 1863) (Myrmicinae)

This tiny species is native to the Neotropics, but infamous as an unpleasant guest worldwide. It is an exotic invader on many continents and islands, and also an indoor exotic species in colder places (Wetterer & Porter, 2003). A large body of knowledge describes how *W. auropunctata* dominate habitats and displace other ants, which often happens

Table 1 – Baits used to represent natural resources in this work, with total number of species (S), records (BA) and average number of individuals \pm S.D. (IN) recorded.

Bait	Resource represented	S	BA	IN
Living crickets	Larger and highly mobile prey	26	107	4 \pm 8
Living termites	Smaller and slower prey	31	203	4 \pm 15
Crushed insects	Dead arthropods	33	422	14 \pm 38
Chicken feces	Bird droppings	32	215	3 \pm 5
Seeds mixture	Seeds of diverse sizes and shapes, without elaiosomes	32	344	7 \pm 10
Melezitose	Oligosaccharides produced by sap-sucking insects	34	327	6 \pm 9
Sucrose	Dissaccharides present in extra-floral nectar and fleshy fruits	34	366	7 \pm 15

when the species is introduced or, within its native range, in crops and other open/disturbed areas. The species is portrayed feeding virtually on everything: scavenging; preying on small and large arthropods; collecting diversified plant parts; visiting extra-floral nectaries and tending honeydew-producing insects (Creighton, 1950; Kusnezov, 1952; Smith, 1954; Smith, 1965; Fabres & Brown, 1978; Clark et al., 1982; Deyrup et al., 2000; Wetterer & Porter, 2003; Longino & Fernández, 2007). Some of these authors suggest that honeydew is their main resource, such as Clark et al. (1982).

A comparatively small amount of information suggests that, inside forests within its native range, the species is not nearly as dominant (Majer & Delabie, 1999; Longino & Fernández, 2007). Very little is known about *W. auropunctata* habits in this context. Using generic baits, Orivel et al. (2009) showed a steep decline in bait use and nest density within a gradient from open areas to undisturbed forest. In Atlantic forest, ca. 1400 km north of the present study site, Santana et al. (2013) qualitatively showed it interacting with seven non-myrmecophorous diaspores on the ground.

In light of this literature record, it was really surprising to find the species to be a strict specialist in feces (Fig 1). In fact, it was the only species in this study that used a single resource. It was a comparatively frequent species (Table 2), but appeared

Table 2 – Species analyzed in this work. BA = total records in baits. PF = total records in pitfalls. PT = total records in sample points, considering both methods.

Species	BA	PF	PT
<i>Camponotus lespeii</i> Forel, 1886	9	1	4
<i>Camponotus zenon</i> Forel, 1912	14	0	10
<i>Crematogaster nigropilosa</i> Mayr, 1870	5	1	5
<i>Cyphomyrmex rimosus</i> (Spinola, 1851)	6	10	15
<i>Gnamptogenys striatula</i> Mayr, 1884	47	26	26
<i>Hylomyrma reitteri</i> (Mayr, 1887)	8	10	13
<i>Linepithema iniquum</i> (Mayr, 1870)	10	0	7
<i>Linepithema micans</i> (Forel, 1908)	16	1	6
<i>Linepithema pulex</i> Wild, 2007	14	1	5
<i>Odontomachus chelifer</i> (Latreille, 1802)	42	12	25
<i>Pachycondyla harpax</i> (Fabricius, 1804)	1	11	10
<i>Pachycondyla striata</i> Smith, 1858	88	58	47
<i>Pheidole angusta</i> Forel, 1908	6	1	4
<i>Pheidole aper</i> Forel, 1912	27	10	10
<i>Pheidole avia</i> Forel, 1908	9	2	5
<i>Pheidole lucretii</i> Santschi, 1923	50	10	13
<i>Pheidole nesiota</i> Wilson, 2003	89	14	19
<i>Pheidole risii</i> Forel, 1892	21	4	5
<i>Pheidole sarcina</i> Forel, 1912	51	13	12
<i>Pheidole sigillata</i> Wilson, 2003	91	25	35
<i>Strumigenys denticulata</i> Mayr, 1887	0	6	6
<i>Wasmannia auropunctata</i> (Roger, 1863)	19	0	16
<i>Wasmannia affinis</i> Santschi, 1929	20	3	6

always in low numbers and was not collected in pitfalls. This result differs from the widespread use of fleshy diaspores found in Santana et al. (2013), and also from the use of baits in Orivel et al. (2009). The latter authors suggested that abiotic factors play a role in the ecological shift of *W. auropunctata* from open to forest areas. A physiological constraint related to environmental conditions (e.g. temperature) could explain why the species has a limited role inside forests, and why, in a higher latitude, it shifts to a resource less preferred by other species. This intriguing behavior will be explored further and shows that there are open questions related to this important species, particularly outside the invasive context.

Wasmannia affinis Santschi, 1929 (Myrmicinae)

As a small genus (11 species) with one outstandingly famous representative, it is not unexpected to find very little information on the other *Wasmannia* (Longino & Fernández, 2007). That is the case for *W. affinis*, which has a geographic distribution apparently restricted to Atlantic forests of south and southwest Brazil. The single record about its feeding habits comes from Bieber et al. (2013), who reported it as the ant species most frequently interacting with fallen fruits of *Psychotria suterella* (Rubiaceae).

The results for this species were very distinct from *W. auropunctata* (Fig 1). *Wasmannia affinis* had a smaller incidence in the community, but used a broader range of resources. Feces were not particularly important, and having more records on termites, seeds and melezitose would make it unique among species of this work, although it was not possible to statistically confirm this pattern.

Pheidole (Myrmicinae)

With over a thousand species described (Bolton, 2016), *Pheidole* usually is the most rich, frequent and abundant genus on the ground of tropical and subtropical forests. This was also the case here, with 17 species, eight of them fitting previously described species. After the literature review, all the previous knowledge on these species can be summarized in Wilson's (2003) words: "Biology: unknown". Not surprising at all, taking into account its complicated taxonomic history that only recently began to be solved (Wilson, 2003; Longino 2009). However, identification is a time-consuming task, depends on the infrequently collected major workers, and it is still common to find new species, which could be the case for five morphospecies in the present work (A. C. Ferreira, UFPR, personal communication; see Supplementary Material).

Even if often labeled as "generalists", the little we know about *Pheidole* species shows a diversity of habits and functional roles (Wilson, 2003), which is expected for such a large genus. In accordance with this, differences were found among the six species with at least 10 records (Fig 1). *Pheidole lucretii* Santschi, 1923, *Pheidole nesiota* Wilson, 2003, and *Pheidole sigillata* (Wilson, 2003) had similar

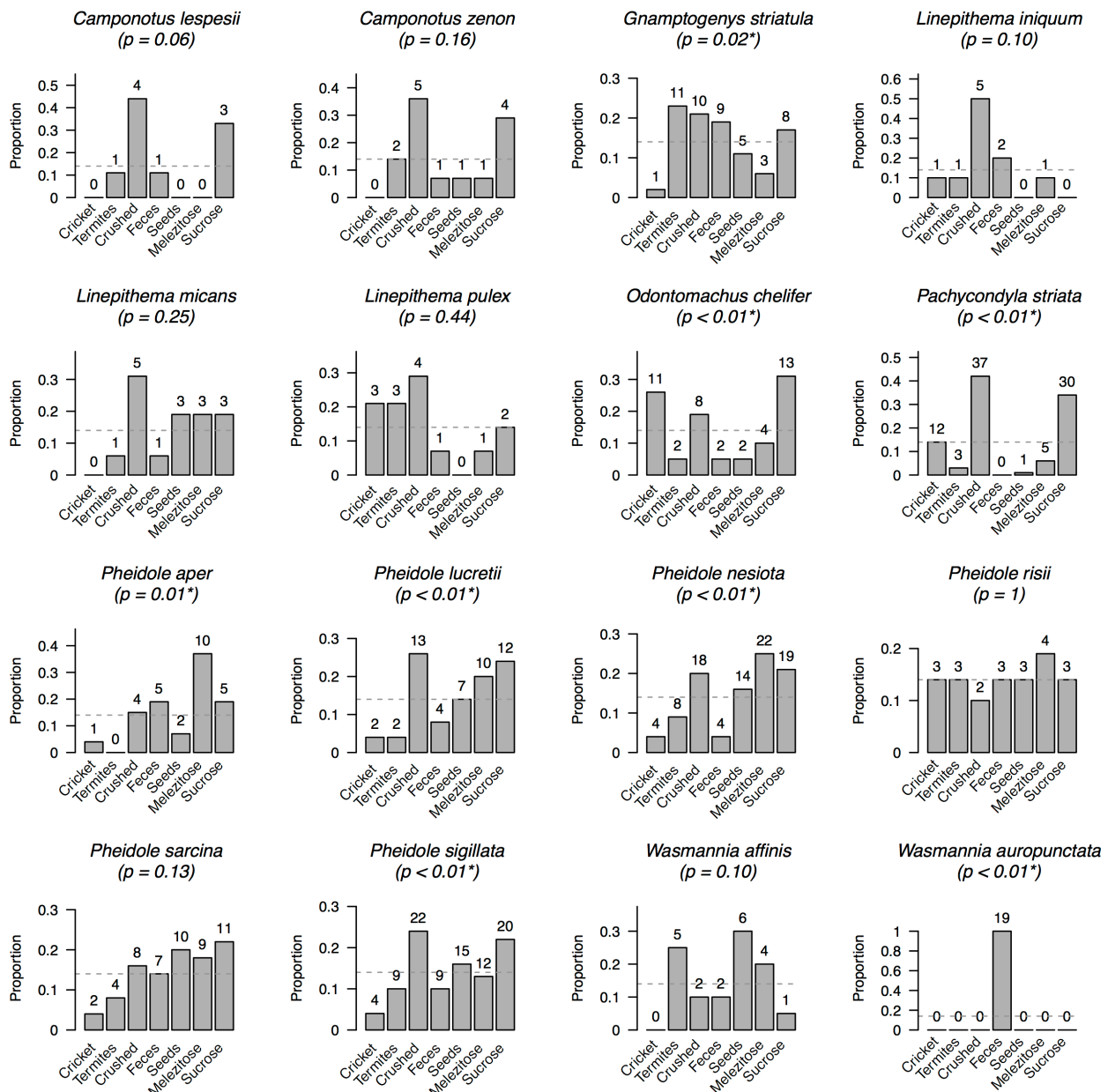


Fig 1 – Use of trophic resources by ant species in southern Brazil. Values above bars are numbers of records. The expected proportions in case of no preference for baits (= 0.14). Asterisks indicate statistical significant differences.

patterns and broadly used the most attractive resources. *Pheidole sarcina* Forel, 1912 included more seeds and feces than the others. *Pheidole aper* Forel, 1912 occupies a distinct niche, being the only species in this study that distinctively used more melezitose over other resources. Since honeydew is the only reliable source of this sugar in nature, interaction with sap-sucking insects should be important for this species. Finally, there is *Pheidole risii* Forel, 1892, the very definition of a generalist, which used all resources indiscriminately. The higher occurrence on living baits set it apart from its congeneric species.

Several mechanisms are proposed to explain the coexistence of dozens of ant species in a community, through a complex interplay of habitat structure, interspecific interactions and species traits (Cerdá et al., 2013). Behavioral

adaptations might be the main factor allowing coexistence among *Ph. lucretii*, *Ph. nesioti* and *Ph. sigillata*. But overall, the results also suggest that species-specific multidimensional trophic niches, presenting quantitative rather than qualitative differences, could play a role in coexistence, even among related species of the same “generalist” group.

Linepithema (Dolichoderinae)

The case of *Linepithema* is similar to *Wasmannia*. Besides the invasive and extensively studied *Linepithema humile* (Mayr, 1868), little is known about most species of the genus (Wild, 2007). *Linepithema micans* (Forel, 1908) is common in south Brazilian vineyards, strongly associated with sap-sucking insects (Morandi Filho et al., 2015). This

species certainly suffers from a misdiagnosed past, and Nondillo et al. (2013) suggest that many previous records of *L. humile* in infested vineyards should be *L. micans* instead. More is known about *Linepithema iniquum* (Mayr, 1870), mainly because it also appears as an exotic indoor species in North America and Europe. A few instances of honeydew and extra-floral nectaries use exist in the literature (Wheeler, 1929; Wild, 2007; Schmid et al., 2010) and Smith (1929) described it collecting arthropods, although without specifying if that meant scavenging or predation. Wild (2007) describes it as a primary arboreal ant, but clearly it also forages on the ground (Table 2). Finally, *Linepithema pulex* Wild, 2007 is one of the smallest and less-known representatives of the genus. It was recorded occasionally on experimental carcasses, predated the larvae and pupae of necrophagous insects (Paula et al., 2016).

None of these species showed statistically significant preferences, due to low number of records and use of several resources (Fig 1). *L. micans* and *L. pulex* seem to use resources more broadly than *L. iniquum*, which might descend to the ground mostly to scavenge animal resources. The small *L. pulex* may have stronger carnivorous tendencies and, in fact, twice they were able to recruit a few dozens of workers and predate crickets just by themselves, a remarkable feat considering its size.

Camponotus (Formicinae)

Camponotus is the only ant genus that currently rivals *Pheidole* in richness (Bolton, 2016), but still lacks comprehensive revisions at genus level. Accordingly, the biology of most tropical species remains unknown, such as the two recorded here. *Camponotus lespesii* Forel, 1886 is widespread in the Neotropics. Byk and Del-Claro (2010) recorded it qualitatively visiting extra-floral nectaries and Paula et al. (2016) observed it on experimental carcasses,

predating the larvae and pupae of necrophagous insects and feeding on the carcass itself. Conversely, *Camponotus zenon* Forel, 1912 has its distribution apparently restricted to southernmost Brazil and nothing is known about its biology.

The number of records was low for both species, precluding clear statistical results, even if their resource use was restricted (Fig 1; *Ca. lespesii* is included only 9 records due to its marginally significant result). The few records for both species were quite similar, both concentrated on crushed insects and sucrose.

Daily activity

In this work, most species have not displayed strong tendencies to be active at a particular time (Fig 2). In south Brazil, summer is both the warmest and wettest season, and any temporal preference that is linked to abiotic factors should be at its lowest. Still, some species showed preferences. Three species were exclusively, or almost exclusively, diurnal (*Crematogaster nigropilosa* Mayr, 1870, *L. iniquum* and *L. pulex*) and one nocturnal (*Ca. zenon*). *Gnamptogenys striatula* showed a moderate, statistically significant, preference for the night, and *Ph. nesioti* for the day. Not much previous information on daily activity for individual species was found. The ones with information available are discussed below.

A single account of “mainly diurnal activity” exists for *Ca. lespesii* (Byk & Del-Claro, 2010). In this work, performed in the Brazilian savannah, most *Camponotus* species were qualitatively classified as diurnals. This is rather distinct from the Atlantic forest, where *Ca. lespesii* had more records at night (and *Ca. zenon* displayed a truly nocturnal behavior). For *Cr. nigropilosa* in Costa Rica, Longino (2003) says “foragers may be found day or night”. However, in south Brazil the species seems to be a diurnal specialist. For *W. auropunctata*, the lack of preference was the same as previously observed in the invasive context (Clark et al., 1982).

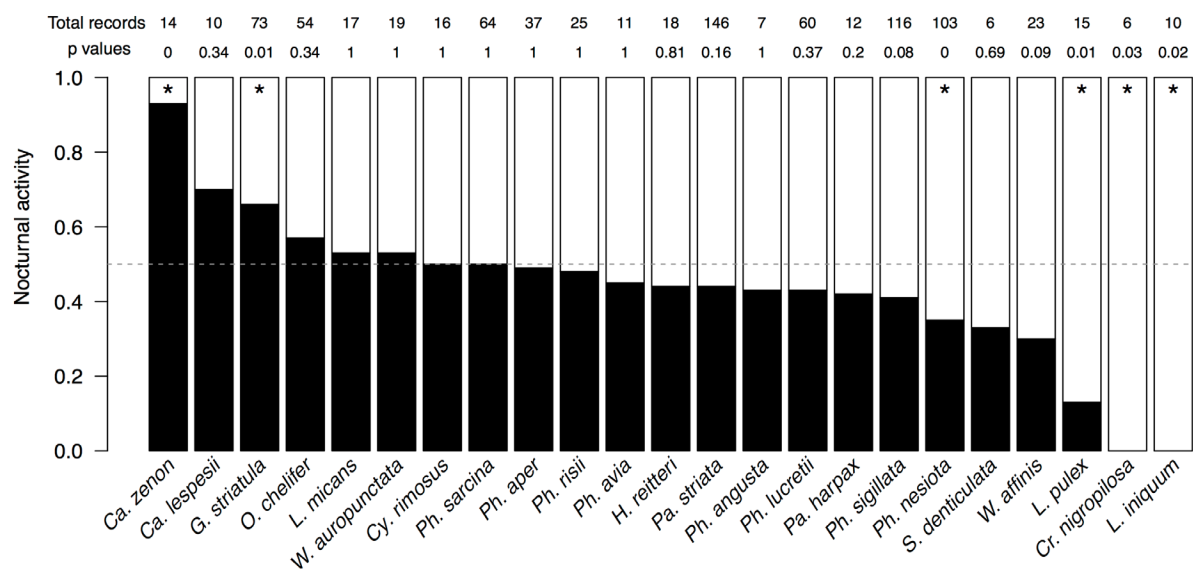


Fig 2 – Daily activity of ant species in southern Brazil. The dashed line shows expected proportions in case of no preference for period (= 0.5). Asterisks indicate statistical significant differences.

The studies in the Atlantic forest with *O. chelififer* and *Pa. striata* also assessed their period of activity (Medeiros & Oliveira, 2009; Raimundo et al., 2009). In these studies, *O. chelififer* showed a strong preference for nocturnal activity, and the inverse was found for *Pa. striata*. However, this clear pattern was not repeated in the present study. There was a slight inclination towards the same trends, but far lower than compared to the equivalent season in these studies. A response to variable weather conditions, community context or distinct behavioral adaptations to coexistence could generate such discrepancies. The interaction between these species inside and across communities is still an interesting and open topic for a detailed study.

Discussion

The life history of a species involves many aspects and is the result of a complex set of external variables and species traits. To fully understand one single history is not a trivial task. Methodology plays a key role on this, and results must be interpreted in the light of the advantages and caveats associated to every approach (Birkhofer et al., 2017). Since the bait method used in this study relies on proxies to broadly access resource use, the possibility of artificial, non-representative results must be considered. However, the results were consistent with previous accounts for two well-studied species (*O. chelififer* and *Pa. striata*), excluding the use of termites explained before. The unusual result for *W. auropunctata* is unlikely to be an artifact. The species proved to be relatively frequent in the community and, if it maintained its generalist habits, at least some records on other baits would have been expected. Also, this method does not evaluate extensively the natural variations for each resource, therefore is less suitable for detecting specialized behaviors, but focuses instead on the oft-neglected generalist species, which represent most of the community. The ants rarely recorded in baits were also uncommon in pitfalls, and many present known specialized behaviors or forage mainly on vegetation or inside leaf-litter (Table 2 and Supplementary Material). *Pachycondyla harpax* is the most notorious absence, and this could be due to a preference for specific termite species (García-Pérez et al., 1997), or also an artifact, as for the other two large Ponerinae. Finally, ants may be driven to more limited resources, instead of the ones they used more frequently. This may be particularly true for nitrogen-deprived arboreal ants (Kaspari & Yanoviak, 2001), and could be the reason behind the lack of melezitose use by *L. iniquum*. However, the two *Camponotus* species, which forage both on vegetation and ground, used sucrose and crushed insects similarly. Therefore, this deviation might be less relevant for ground-dwelling ants. In short, although with potential bias that must be considered, this bait method seems to appropriately assess the trophic niche of most species in the site and season in question, and the data it yields is useful to understand individual species.

Many methods to assess resource use cannot discriminate well between hunting and scavenging (e.g. nest excavation, observation of foragers on a nest entrance, barcoding of gut content, stable isotopes). On the other hand, this bait method assesses what species prefer to use, but not what they have available in the community. Carcasses are a rich and easy to gather resource, but in their absence ants have to fight their prey. Even taking this into account, and in light of the previous accounts (Medeiros & Oliveira, 2009; Raimundo et al., 2009), it is likely that resource use plays a role in the coexistence of the more scavenger *Pa. striata* and the more predator *O. chelififer*. This reduces niche overlap and may lead to distinct functional roles. On the other hand, species frequent on crushed insects, but not in living baits, probably are restricted to scavenging, independent of resource availability. That was the case for most *Linepithema*, *Pheidole* and *Camponotus*.

Sugar consumption is more frequently studied through observation of interactions with EFNs or hemipterans in plants, due to their role as main attractors in ant-plant interactions (Rosumek et al., 2009). Interactions of ground foragers with plant diaspores are assumed to be more associated with lipid-rich elaiosomes that mimic animal prey (Hughes et al., 1994; Giladi, 2006). However, the use of fallen fruits simply as sugar sources might be overlooked. This could partially explain the pronounced lack of use of carbohydrate resources by the “poneroid” clade observed by Lanan (2014). Few species in the present work were not attracted by sucrose, and some of them probably climb vegetation in search for EFNs (e.g. *Camponotus*). However, fallen fruits might be an occasional but disputed resource for species restricted to the ground. Preference for melezitose was less common, and it was conspicuously avoided by some species. Among physiological constraints that could reduce suitability of certain sugars to a species, particularly insect-synthesized oligosaccharides, are low gustatory perception, digestibility and nutritional value (Boevé & Wäckers, 2003). These constraints could be another source of niche partitioning among ant species.

Other fundamental aspect underlying the results of this work is the context-dependence of the patterns and processes studied in ecology, including the interaction between organisms and resources (Agrawal et al., 2007). The few well-studied species told different stories in a distinct context, like the trophic niche of *W. auropunctata* and daily activity of *Pa. striata* and *O. chelififer*. It is likely that the other, less-known species would exhibit such variation in different contexts, influenced by biotic interactions, abiotic factors and evolutionary history of the community.

Taking into account the lack of knowledge about most species, the complementary results given by different methodologies, and the variation under distinct contexts, it is clear that descriptive studies are still very much needed for tropical species, even if these studies are often relegated to second plane in modern science practice and funding (Greene,

2005). In this way, studies at community or larger scales could bring a considerable amount of information for individual species, above all when they are virtually unknown. This could be achieved first by spending time and effort on the taxonomic stage, and avoid use of morphospecies whenever possible. Second, researches should learn about their species to point out relevant findings, and prevent these to end up buried in a datasheet cell or the supplementary material. The large scale, pattern-driven enterprise is clearly important for the advancement of knowledge, but such basic aspects of natural sciences still are important. Claims for this “old-fashioned” natural history are not new (Jordan, 1916). A century later they remain valid, because we still have a lot to describe.

Supplementary Material

<http://periodicos.uefs.br/index.php/sociobiology/rt/suppFiles/1623/0>

<http://dx.doi.org/10.13102/sociobiology.v64i3.1623.s1740>

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References

- Agrawal, A.A., Ackerly, D.D., Adler, F., Arnold, A.E., Cáceres, C., Doak, D.F., Post, E., Hudson, P.J., Maron, J., Mooney, K.A. (2007). Filling key gaps in population and community ecology. *Frontiers in Ecology and the Environment*, 5: 145-152. doi: 10.1890/1540-9295(2007)5
- Anjos, D.V., Caserio, B., Rezende, F.T., Ribeiro, S.P., Del-Claro, K., Fagundes, R. (2016). Extrafloral-nectaries and interspecific aggressiveness regulate day/night turnover of ant species foraging for nectar on *Bionia coriacea*. *Austral Ecology*, 42: 317-328. doi: 10.1111/aec.12446.
- AntWeb. (2016). <http://www.antweb.org/>. (accessed date: 15 March, 2016)
- AntWiki. (2016). <http://www.antwiki.org/>. (accessed date: 10 November, 2016)
- Arias, T.M. (2008). Subfamilia Ectatomminae. In Jiménez, E., Fernández, F., Arias, T.M., Lozano-Zambrano, F.H. (Eds.), *Sistemática, biogeografía y conservación de las hormigas cazadoras de Colombia* (pp. 53-107). Bogotá: Instituto de Investigación de Recursos Biológicos Alexander von Humboldt.
- Baccaro, F.B., Feitosa, R.M., Fernández, F., Fernandes, I.O., Izzo, T.J., de Souza, J.L.P., Solar, R.R.C. (2015). *Guia para os gêneros de formigas do Brasil*. Manaus: Editora INPA. doi: 10.5281/zenodo.32912.
- Baker, H.G., Baker, I., Hodges, S.A. (1998). Sugar composition of nectars and fruits consumed by birds and bats in the Tropics and Subtropics. *Biotropica*, 30: 559-586. doi: 10.1111/j.1744-7429.1998.tb00097.x.
- Bieber, A.G.D., Silva, P.S.D., Oliveira, P.S. (2013). Attractiveness of fallen fleshy fruits to ants depends on previous handling by frugivores. *Écoscience*, 20: 85-89. doi: 10.2980/20-1-3573.
- Birkhofer, K., Bylund, H., Dalin, P., Ferlian, O., Gagic, V., Hambäck, P.A., Klapwijk, M., Mestre, I., Roubinet, E., Schroeder, M., Stenberg, J.A., Porcel, M., Björkman, C., Jonsson, M. (2017). Methods to identify the prey of invertebrate predators in terrestrial field studies. *Ecology and Evolution*, 7: 1942-53. doi: 10.1002/ece3.2791.
- Blüthgen, N., Feldhaar, H. (2010). Food and shelter: How resources influence ant ecology. In Lach, L., Parr, C.L., Abbott, K.L. (Eds.), *Ant Ecology* (pp. 115–136). Oxford: Oxford University Press.
- Blüthgen, N., Fiedler, K. (2004). Preferences for sugars and amino acids and their conditionality in a diverse nectar-feeding ant community. *Journal of Animal Ecology*, 73: 155-166. doi: 10.1111/j.1365-2656.2004.00789.x.
- Blüthgen, N., Gebauer, G., Fiedler, K. (2003). Disentangling a rainforest food web using stable isotopes: dietary diversity in a species-rich ant community. *Oecologia*, 137: 426-435. doi: 10.1007/s00442-003-1347-8.
- Boevé, J.L., Wäckers, F.L. (2003). Gustatory perception and metabolic utilization of sugars by *Myrmica rubra* ant workers. *Oecologia*, 136: 508-514. doi: 10.1007/s00442-003-1249-9.
- Bolton, B. (2016). An online catalog of the ants of the world. <http://antcat.org/>. (accessed date: 28 March 2017).
- Brandão, C.R.F., Silva, R.R., Delabie, J.H.C. (2012). Neotropical ants (Hymenoptera) functional groups: Nutritional and applied implications. In Panizzi, A.R., Parra, J.R.P. (Eds.), *Insect Bioecology and Nutrition for Integrated Pest Management* (pp. 213-236). Boca Raton: CRC Press.
- Byk, J., Del-Claro, K. (2010). Nectar- and pollen-gathering *Cephalotes* ants provide no protection against herbivory: a new manipulative experiment to test ant protective capabilities. *Acta Ethologica*, 13: 33-38. doi: 10.1007/s10211-010-0071-8.
- Cerdá, X., Arnan, X., Retana, J. (2013). Is competition a significant hallmark of ant (Hymenoptera: Formicidae) ecology? *Myrmecological News*, 18: 131-147.

- Chen, L., Fadamiro, H.Y. (2006). Comparing the effects of five naturally occurring monosaccharide and oligosaccharide sugars on longevity and carbohydrate nutrient levels of a parasitic phorid fly, *Pseudacteon tricuspis*. *Physiological Entomology*, 31: 46-56. doi: 10.1111/j.1365-3032.2005.00484.x.
- Clark, D.B., Guayasamin, C., Pazmino, O., Donoso, C., de Villacis, Y.P. (1982). The tramp ant *Wasmannia auropunctata*: Autecology and effects on ant diversity and distribution on Santa Cruz Island, Galapagos. *Biotropica*, 14: 196-207. doi: 10.2307/2388026.
- Creighton, W.S. (1950). The ants of North America. *Bulletin of the Museum of Comparative Zoology at Harvard College*, 104: 1-585.
- Deyrup, M., Davis, L., Cover, S. (2000). Exotic ants in Florida. *Transactions of the American Entomological Society*, 126: 293-326.
- Egerton, F.N. (2013). History of ecological sciences, part 47: Ernst Haeckel's ecology. *The Bulletin of the Ecological Society of America*, 94: 222-244. doi: 10.1890/0012-9623-94.3.222.
- Fabres, G., Brown, W.L. (1978). The recent introduction of the pest ant *Wasmannia auropunctata* into New Caledonia. *Australian Journal of Entomology*, 17: 139-142.
- Feitosa, R.M., Silva, R.R., Aguiar, A.P. (2016). Diurnal flight periodicity of a Neotropical ant assemblage (Hymenoptera, Formicidae) in the Atlantic Forest. *Revista Brasileira de Entomologia*, 60: 241-247. doi: 10.1016/j.rbe.2016.05.006.
- Fernández, F. (2008). Subfamilia Ponerinae s. str. In Jiménez, E., Fernández, F., Arias, T.M., Lozano-Zambrano, F.H. (Eds.), *Sistemática, biogeografía y conservación de las hormigas cazadoras de Colombia* (pp. 123–218). Bogotá: Instituto de Investigación de Recursos Biológicos Alexander von Humboldt.
- Fowler, H.G. (1980). Populations, prey capture and sharing, and foraging of the Paraguayan Ponerine *Odontomachus chelifer* Latreille. *Journal of Natural History*, 14: 79-84.
- García-Pérez, J., Blanco-Piñón, A., Mercado-Hernández, R., Badii, M. (1997). El comportamiento depredador de *Pachycondyla harpax* Fabr. sobre *Gnathamitermes tubiformans* Buckley en condiciones de cautiverio. *Southwestern Entomologist*, 22: 345-353.
- Giannotti, E., Machado, V.L.L. (1991). Notes on the foraging of two species of ponerine ants: food resources and daily activity (Hymenoptera, Formicidae). *Bioikos*, 5: 7–17.
- Giladi, I. (2006). Choosing benefits or partners: a review of the evidence for the evolution of myrmecochory. *Oikos*, 112: 481-492. doi: 10.1111/j.0030-1299.2006.14258.x.
- Giraud, T., Blatrix, R., Poteaux, C., Solognac, M., Jaisson, P. (2000). Population structure and mating biology of the polygynous ponerine ant *Gnamptogenys striatula* in Brazil. *Molecular Ecology*, 9: 1835-1841. doi: 10.1046/j.1365-294x.2000.01085.x.
- Greene, H. (2005). Organisms in nature as a central focus for biology. *Trends in Ecology and Evolution*, 20: 23-27. doi: 10.1016/j.tree.2004.11.005.
- Heath, R.J.; Rock, C.O. (2002). The Claisen condensation in biology. *Natural Product Reports*, 19: 581-596. doi: 10.1039/b110221b.
- Houadria, M., Salas-Lopez, A., Orivel, J., Blüthgen, N., Menzel, F. (2015). Dietary and temporal niche differentiation in tropical ants — Can they explain local ant coexistence? *Biotropica*, 47: 208-217. doi: 10.1111/btp.12184.
- Hughes, L., Westoby, M., Jurado, E. (1994). Convergence of elaiosomes and insect prey: evidence from ant foraging behaviour and fatty acid composition. *Functional Ecology*, 8: 358-365.
- Janicki, J., Narula, N., Ziegler, M., Guénard, B., Economo, E.P. (2016). Visualizing and interacting with large-volume biodiversity data using client-server web-mapping applications: The design and implementation of antmaps.org. *Ecological Informatics*, 32: 185-193. doi: 10.1016/j.ecoinf.2016.02.006.
- Jordan, D.S. (1916). Plea for old-fashioned natural history. *Bulletin of the Scripps Institution for Biological Research of the University of California*, 1: 3-6.
- Kaptein, N., Billen, J., Gobin, B. (2005). Larval begging for food enhances reproductive options in the ponerine ant *Gnamptogenys striatula*. *Animal Behaviour*, 69: 293-299. doi: 10.1016/j.anbehav.2004.04.012.
- Kaspari, M. (2000). A primer on ant ecology. In Agosti, D., Majer, J.D., Alonso, L.E., Schultz, T.R. (Eds.), *Ants: standard methods for measuring and monitoring biodiversity* (pp. 9-24). Washington: Smithsonian Institution Press.
- Kaspari, M., Yanoviak, S.P. (2001). Bait use in tropical litter and canopy ants – Evidence of differences in nutrient limitation. *Biotropica*, 33: 207-211.
- Kempf, W.W. (1965). A revision of the Neotropical fungus-growing ants of the genus *Cyphomyrmex* Mayr. Part II: Group of *rimosus* (Spinola) (Hym. Formicidae). *Studia Entomologica*, 8: 161-200.
- Kempf, W.W. (1973). A revision of the Neotropical myrmicine ant genus *Hylomyrma* Forel (Hymenoptera: Formicidae). *Studia Entomologica*, 16: 225-260.
- Krebs, C.J. (1999). *Ecological methodology*. 2nd ed. Menlo Park: Benjamin/Cummings, 620 p.
- Krell, F.T. (2004). Parataxonomy vs. taxonomy in biodiversity studies – pitfalls and applicability of “morphospecies” sorting. *Biodiversity and Conservation*, 13: 795-812. doi: 10.1023/B:BIOC.0000011727.53780.63.

- Kusnezov, N. (1952). El género *Wasmannia* en la Argentina (Hymenoptera, Formicidae). *Acta Zoologica Lilloana*, 10: 173-182.
- Lachaud, J.P., Dejean, A. (1991). Food sharing in *Odontomachus troglodytes* (Santschi): a behavioral intermediate stage in the evolution of social food exchange in ants. *Anales de Biología*, 17: 53-61.
- Lanan, M. (2014). Spatiotemporal resource distribution and foraging strategies of ants (Hymenoptera: Formicidae). *Myrmecological News*, 20: 53-70.
- Lattke, J.E. (1990). Revisión del género *Gnamptogenys* Roger en Venezuela (Hymenoptera: Formicidae). *Acta Terramaris*, 2: 1-47.
- Lattke, J.E. (1995). Revision of the ant genus *Gnamptogenys* in the New World (Hymenoptera: Formicidae). *Journal of Hymenoptera Research*, 4: 137-193.
- Lessard, J.P., Dunn, R.R., Sanders, N.J. (2009). Temperature-mediated coexistence in temperate forest ant communities. *Insectes Sociaux*, 56: 149–156. doi: 10.1007/s00040-009-0006-4.
- Longino, J.T. (2003). The *Crematogaster* (Hymenoptera, Formicidae, Myrmicinae) of Costa Rica. *Zootaxa*, 151: 1-150.
- Longino J.T. (2009). Additions to the taxonomy of New World *Pheidole* (Hymenoptera: Formicidae). *Zootaxa*, 2181: 1-90.
- Longino, J.T., Fernández, F. (2007). Taxonomic review of the genus *Wasmannia*. *Memoirs of the American Entomological Institute*, 80: 271–289.
- Majer, J.D., Delabie, J.H.C. (1999). Impact of tree isolation on arboreal and ground ant communities in cleared pasture in the Atlantic rain forest region of Bahia, Brazil. *Insectes Sociaux*, 46: 281-290. doi: 10.1007/s000400050147.
- McDonald, J.H. (2014). *Handbook of biological statistics*. 3rd ed. Baltimore: Sparky House Publishing, 299 p.
- Medeiros, F.N.S., Oliveira, P.S. (2009). Season-dependent foraging patterns case study of a Neotropical forest-dwelling ant (*Pachycondyla striata*; Ponerinae). In Jarau, S., Hrncir, M. (Eds.), *Food exploitation by social insects: ecological, behavioral, and theoretical approaches* (pp. 81-95). Boca Raton: Taylor & Francis Group.
- Morandi Filho, W.J., Pacheco-da-Silva, V.C., Willink, M.C.G., Prado, E., Botton, M. (2015). A survey of mealybugs infesting South-Brazilian wine vineyards. *Revista Brasileira de Entomologia*, 59: 251–254. doi: 10.1016/j.rbe.2015.05.002.
- Nondillo, A., Ferrari, L., Lerin, S., Bueno, O.C., Botton, M. (2014). Foraging activity and seasonal food preference of *Linepithema micans* (Hymenoptera: Formicidae), a species associated with the spread of *Eurhizococcus brasiliensis* (Hemiptera: Margarodidae). *Journal of Economic Entomology*, 107: 1385–1391. doi: 10.1603/EC13392.
- Nondillo, A., Sganzerla, V.M.A., Bueno, O.C., Botton, M. (2013). Interaction between *Linepithema micans* (Hymenoptera: Formicidae) and *Eurhizococcus brasiliensis* (Hemiptera: Margarodidae) in vineyards. *Environmental Entomology*, 42: 460-466. doi: 10.1603/EN13004.
- Oliveira, R.F., Silva, R.R., Souza-Campana, D.R., Nakano, M.A., Morini, M.S.C. (2015). Worker morphology of the ant *Gnamptogenys striatula* Mayr (Formicidae, Ectatomminae) in different landscapes from the Atlantic Forest domain. *Revista Brasileira de Entomologia*, 59: 21-27. doi: 10.1016/j.rbe.2015.02.002.
- Orivel, J., Grangier, J., Foucaud, J., Le Breton, J., Andrès, F.X., Jourdan, H., Delabie, J.H.C., Fournier, D., Cerdan, P., Facon, B. (2009). Ecologically heterogeneous populations of the invasive ant *Wasmannia auropunctata* within its native and introduced ranges. *Ecological Entomology*, 34: 504-512. doi: 10.1111/j.1365-2311.2009.01096.x.
- Passos, L., Oliveira, P.S. (2002). Ants affect the distribution and performance of seedlings of *Clusia criuva*, a primarily bird-dispersed rain forest tree. *Journal of Ecology*, 90: 517-528. doi: 10.1046/j.1365-2745.2002.00687.x.
- Passos, L., Oliveira, P.S. (2004). Interaction between ants and fruits of *Guapira opposita* (Nyctaginaceae) in a Brazilian sandy plain rainforest: ant effects on seeds and seedlings. *Oecologia*, 139: 376–382. doi: 10.1007/s00442-004-1531-5.
- Paula, M.C., Morishita, G.M., Cavarson, C.H., Gonçalves, C.R., Tavares, P.R.A., Mendonça, A., Suárez, Y.R., Antonioli-Junior, W.F. (2016). Action of ants on vertebrate carcasses and blow flies (Calliphoridae). *Journal of Medical Entomology*, 1: 1–9. doi: 119. 10.1093/jme/tjw119.
- Percival, M.S. (1961). Types of nectar in Angiosperms. *New Phytologist*, 60: 235–281.
- Pizo, M.A., Oliveira, P.S. (2000). The use of fruits and seeds by ants in the Atlantic Forest of Southeast Brazil. *Biotropica*, 32: 851-861. doi: 10.1111/j.1744-7429.2000.tb00623.x.
- R Core Team (2016). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. Retrieved from: <https://www.R-project.org/>
- Raimundo, R.L.G., Freitas, A.V.L., Oliveira, P.S. (2009). Seasonal patterns in activity rhythm and foraging ecology in the Neotropical forest-dwelling ant, *Odontomachus chelifer* (Formicidae: Ponerinae). *Annals of the Entomological Society of America*, 102: 1151–1157. doi: 10.1603/008.102.0625.
- Rosumek, F.B., Silveira, F.A.O., Neves, F. S., Barbosa, N.P.U., Diniz, L., Oki, Y., Pezzini, F., Fernandes, G.W., Cornelissen, T. (2009). Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. *Oecologia*, 160: 537-549. doi: 10.1007/s00442-009-1309-x.
- Santana, F.D., Cazetta, E., Delabie, J.H.C. (2013). Interactions

- between ants and non-myrmecochorous diaspores in a tropical wet forest in southern Bahia, Brazil. *Journal of Tropical Ecology*, 29: 71–80. doi: 10.1017/S0266467412000715.
- Schmid, S., Schmid, V.S., Kamke, R., Steiner, J., Zillikens, A. (2010). Association of three species of *Strymon* Hübner (Lycaenidae: Theclinae: Eumaeini) with bromeliads in southern Brazil. *Journal of Research on the Lepidoptera*, 42: 50–55.
- Smith, M.R. (1929). Two introduced ants not previously known to occur in the United States. *Journal of Economic Entomology*, 22: 241–243.
- Smith, M.R. (1954). Ants of the Bimini Island Group, Bahamas, British West Indies (Hymenoptera, Formicidae). *American Museum Novitates*, 1671: 1–16.
- Smith, M.R. (1965). House-infesting ants of the Eastern United States: Their recognition, biology and economic importance. Washington: United States Department of Agriculture, 105 p.
- Snealling, R.R., Longino, J.T. (1992). Revisionary notes on the Fungus-growing Ants of the genus *Cyphomyrmex*, *rimosus* group (Hymenoptera: Formicidae: Attini). In Quintero, D., Aiello, A. (Eds.), *Insects of Panama and Mesoamerica: selected studies* (pp. 479–494). Oxford: Oxford University Press.
- Völkl, W., Woodring, J., Fischer, M., Lorenz, M.W., Hoffmann, K.H. (1999). Ant-aphid mutualisms: the impact of honeydew production and honeydew sugar composition on ant preferences. *Oecologia*, 118: 483–491. doi: 10.1007/s004420050751.
- Wäckers, F.L. (2000). Do oligosaccharides reduce the suitability of honeydew for predators and parasitoids? A further facet to the function of insect-synthesized honeydew sugars. *Oikos*, 90: 197–201. doi: 10.1034/j.1600-0706.2000.900124.x.
- Wetterer, J.K., Porter, S.D. (2003). The little fire ant, *Wasmannia auropunctata*: distribution, impact, and control. *Sociobiology*, 42: 1–42.
- Wheeler, W. M. (1929). Two Neotropical ants established in the United States. *Psyche*, 36: 89–90.
- Wild, A.L. (2007). Taxonomic revision of the ant genus *Linepithema* (Hymenoptera: Formicidae). Berkeley: University of California Press, 151 p.
- Wilson, E.O. (2003). *Pheidole* in the New World: a dominant, hyperdiverse ant genus. Cambridge, Mass: Harvard University Press, 794 p.
- Zoebelein, G. (1956). Der Honigtau als Nahrung der Insekten: Teil I. *Zeitschrift für Angewandte Entomologie*, 38: 369–416.





SUPPLEMENTARY MATERIAL

Natural History of Ants: What We (do not) Know about Trophic and Temporal Niches of Neotropical Species*

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I. Bait composition and display

II. List of species with few records

III. References

I. Bait composition and display

(1) Living crickets: one individual of *Achaeta domestica* (Linnaeus, 1758) with 1-2 cm body size was tied between the first and second pair of legs with a 4 cm thread glued to the box's bottom. The cricket could not escape, but was still able to jump and move inside the trap.

Represents larger, mobile prey with harder integument.

(2) Living termites: 15 individuals were left free on a piece of mound. Previous testing showed that termites do not leave the mound piece after 90 minutes. Another 10 individuals were glued to a small wood stick, to increase the time spent by ants on the bait. Individuals from the subfamily Nasutitermitinae were used, which have specialized workers with chemical defenses that can affect ants (Prestwich, 1984), and both specialized and regular workers were placed in the trap. Thus, it represents prey smaller, slower and softer, but not defenseless.

(3) Crushed insects (2-3 g): crickets and mealworms (*Tenebrio molitor* Linnaeus, 1758).
Represents dead arthropods.

(4) Chicken feces (2-3 g): from organic breeding. Represents nitrogen-rich bird feces, which could be directly used by ants, or contain animal and vegetal remains collected by them (Leal & Oliveira, 1998; Jaffe et al., 2001; Sainz-Borgo, 2015).

(5) Seeds (2-3 g): a mix of soy, millet, sunflower, canary, barley, linseed, grass seeds and ground corn, representing different sizes and shapes of seeds. No seeds with elaiosomes were used, because these mimic animal prey and attract predacious species, not only granivorous ones (Hughes et al., 1994; Giladi, 2006).

(6) Sucrose solution (2-3 ml): at 20% concentration. Sucrose and its components, fructose and glucose, are the main nutritional sugars in plant exudates and fleshy fruits (Percival, 1961; Baker et al., 1998; Blüthgen et al., 2004).

(7) Melezitose solution (2-3 ml): at 20% concentration. Represents insect-synthesized oligosaccharides present on honeydew (Kiss, 1981; Wäckers, 2000). Although melezitose and other oligosaccharides occur naturally almost only on honeydew, some insects secrete honeydew with little modification, similar to plant exudates (Völkl et al., 1999; Blüthgen et al., 2004).

Therefore, preference for melezitose indicates use of honeydew, and avoidance suggests limited use, although not definitely excluding interaction with sap-sucking insects.

II. List of species with few records

The following table reports data on the identified species that had few records (less than 10 for resource use and/or 6 for daily activity) and were not discussed on the main text. The five undescribed *Pheidole* species are included, thus the data could be useful in case they are formally recognized and described. Although some of them are morphologically similar to described species, this does not necessarily mean that they share the same habits.

Table S1 – List of species with few records and not analyzed in the main text. Total records for day, night and sample points include records from pitfalls.

Species	Cricket	Feces	Melezitose	Seeds	Sucrose	Crushed insects	Termites	Day	Night	Points
<i>Acromyrmex aspersus</i> (Smith, 1858)	0	0	0	1	0	0	0	0	1	1
<i>Acromyrmex subterraneus</i> (Forel, 1893)	0	0	0	0	0	0	0	2	1	2
<i>Apterostigma acre</i> Lattke, 1997	0	0	0	1	0	0	0	1	2	2
<i>Crematogaster nigropilosa</i> Mayr, 1870	0	1	0	1	1	2	0	6	0	5
<i>Cyphomyrmex rimosus</i> (Spinola, 1851)	0	3	0	2	1	0	0	8	8	15
<i>Heteroponera dentinodis</i> (Mayr, 1887)	0	1	0	0	0	0	0	1	1	2
<i>Heteroponera dolo</i> (Roger, 1860)	0	0	0	0	0	0	0	1	0	1
<i>Hylomyrma reitteri</i> (Mayr, 1887)	1	0	1	1	2	3	0	10	8	13
<i>Linepithema leucomelas</i> (Emery, 1894)	0	0	0	0	0	0	0	1	1	2

<i>Neoponera crenata</i> (Roger, 1861)	0	0	0	0	0	0	0	1	0	1
<i>Neoponera villosa</i> (Fabricius, 1804)	0	0	0	0	0	0	0	0	1	1
<i>Nylanderia docilis</i> (Forel, 1908)	1	0	0	0	0	0	0	0	1	1
<i>Octostruma petiolata</i> (Mayr, 1887)	0	0	0	0	2	0	0	1	3	3
<i>Octostruma stenognatha</i> Brown & Kempf, 1960	0	0	0	0	0	0	0	0	1	1
<i>Odontomachus meinerti</i> Forel, 1905	0	0	0	0	0	0	1	2	0	2
<i>Oxyepoecus plaumanni</i> Kempf, 1974	0	0	0	0	0	0	0	0	1	1
<i>Pachycondyla harpax</i> (Fabricius, 1804)	0	0	0	0	1	0	0	7	5	10
<i>Pheidole schmalzi</i> Emery, 1894	0	0	0	0	0	0	0	1	0	1
<i>Pheidole aff. pubiventris</i>	3	10	17	18	21	19	13	68	53	21
<i>Pheidole complex bilimeki</i>	18	45	63	67	48	46	35	200	174	57
<i>Pheidole aff. subarmata</i>	4	15	11	11	16	16	5	37	69	23
<i>Pheidole aff. alpinensis</i>	1	0	1	1	1	1	0	5	2	2
<i>Pheidole</i> sp.	1	0	4	2	2	3	2	11	6	4
<i>Pseudomyrmex flavidulus</i> (Smith, 1858)	0	0	0	0	0	0	0	1	0	1
<i>Solenopsis subterranea</i> MacKay & Vinson, 1989	0	0	0	0	0	0	0	1	0	1
<i>Fulakora elongata</i> (Santschi, 1912)	0	0	0	0	0	0	1	1	0	1
<i>Strumigenys cosmostela</i> Kempf, 1975	0	0	0	0	0	0	0	1	0	1
<i>Strumigenys denticulata</i> Mayr, 1887	0	0	0	0	0	0	0	4	2	6
<i>Strumigenys elongata</i> Roger, 1863	0	0	0	0	0	0	0	0	1	1
<i>Strumigenys splendens</i> (Borgmeyer, 1954)	0	0	0	0	0	0	0	0	1	1
<i>Tapinoma atriceps</i> Emery, 1888	0	0	0	0	0	0	0	1	0	1
<i>Wasmannia lutzi</i> Forel, 1908	0	0	0	0	0	0	0	1	0	1

III. References

Blüthgen, N., Gottsberger, G., Fiedler, K. 2004. Sugar and amino acid composition of ant-attended nectar and honeydew sources from an Australian rainforest. *Austral Ecology*, 29: 418–429. doi: 10.1111/j.1442-9993.2004.01380.x.

Giladi, I. (2006). Choosing benefits or partners: a review of the evidence for the evolution of myrmecochory. *Oikos*. 112: 481–492. doi: 10.1111/j.0030-1299.2006.14258.x.

Hughes, L., Westoby, M., Jurado, E. (1994). Convergence of elaiosomes and insect prey: evidence from ant foraging behaviour and fatty acid composition. *Functional Ecology*, 8: 358–365.

Jaffe, K., Caetano, F.H., Sánchez, P., Hernández, J.V., Caraballo, L., Vitelli-Flores, J., Monsalve, W., Dorta, B., Lemoine, V.R. (2001). Sensitivity of ant (*Cephalotes*) colonies and individuals to antibiotics implies feeding symbiosis with gut microorganisms. *Canadian Journal of Zoology*, 79:1120–1124. doi: 10.1139/z01-079.

Kiss, A. (1981). Melezitose, aphids and ants. *Oikos*, 37: 382–382.

Leal, I.R., Oliveira, P.S. (1998). Interactions between fungus-growing ants (Attini), fruits and seeds in Cerrado vegetation in Southeast Brazil. *Biotropica*, 30: 170–178. doi: 10.1111/j.1744-7429.1998.tb00052.x.

Prestwich, G.D. (1984). Defense mechanisms of termites. *Annual Review of Entomology*, 29: 201–232.

Sainz-Borgo, C. (2015). Bird feces consumption by fire ant *Solenopsis geminata* (Hymenoptera: Formicidae). *Entomological News*, 124: 295–299. doi: 10.3157/021.124.0408.

Völkl, W., Woodring, J., Fischer, M., Lorenz, M.W., Hoffmann, K.H. (1999). Ant-aphid mutualisms: the impact of honeydew production and honeydew sugar composition on ant preferences. *Oecologia*, 118: 483–491. doi: 10.1007/s004420050751.

Wäckers, F.L. (2000). Do oligosaccharides reduce the suitability of honeydew for predators and parasitoids? A further facet to the function of insect-synthesized honeydew sugars. *Oikos*, 90: 197–201. doi: 10.1034/j.1600-0706.2000.900124.x.

Annex III

Patterns and dynamics of neutral lipid fatty acids in ants – implications for ecological studies

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Author contributions: MH provided the initial idea of the article. FBR, AB and MH designed the experiment¹. FBR and AB collected the data². AB performed data analyses. FBR drafted the manuscript. All authors contributed to the final manuscript. All authors read and approved the final manuscript.

¹FBR: choices of model species and resources provided;

²FBR: 80% of experimental data, with support of trainee students.

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RESEARCH

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Patterns and dynamics of neutral lipid fatty acids in ants – implications for ecological studies

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Abstract

Background: Trophic interactions are a fundamental aspect of ecosystem functioning, but often difficult to observe directly. Several indirect techniques, such as fatty acid analysis, were developed to assess these interactions. Fatty acid profiles may indicate dietary differences, while individual fatty acids can be used as biomarkers. Ants are among the most important terrestrial animal groups, but little is known about their lipid metabolism, and no study so far used fatty acids to study their trophic ecology. We set up a feeding experiment with high- and low-fat food to elucidate patterns and dynamics of neutral lipid fatty acids (NLFAs) assimilation in ants. We asked whether dietary fatty acids are assimilated through direct trophic transfer, how diet influences NLFA total amounts and patterns over time, and whether these assimilation processes are similar across species and life stages.

Results: Ants fed with high-fat food quickly accumulated specific dietary fatty acids (C18:2n6, C18:3n3 and C18:3n6), compared to ants fed with low-fat food. Dietary fat content did not affect total body fat of workers or amounts of fatty acids extensively biosynthesized by animals (C16:0, C18:0, C18:1n9). Larval development had a strong effect on the composition and amounts of C16:0, C18:0 and C18:1n9. NLFA compositions reflected dietary differences, which became more pronounced over time. Assimilation of specific dietary NLFAs was similar regardless of species or life stage, but these factors affected dynamics of other NLFAs, composition and total fat.

Conclusions: We showed that ants accumulated certain dietary fatty acids via direct trophic transfer. Fat content of the diet had no effect on lipids stored by ants, which were able to synthesize high amounts of NLFAs from a sugar-based diet. Nevertheless, dietary NLFAs had a strong effect on metabolic dynamics and profiles. Fatty acids are a useful tool to study trophic biology of ants, and could be applied in an ecological context, although factors that affect NLFA patterns should be taken into account. Further studies should address which NLFAs can be used as biomarkers in natural ant communities, and how factors other than diet affect fatty acid dynamics and composition of species with distinct life histories.

Keywords: Direct trophic transfer, Lipid metabolism, Dietary routing, Fatty acid biosynthesis, Trophic enrichment, Trophic ecology, Trophic markers, Formicidae, *Formica fusca*, *Myrmica rubra*

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Background

Trophic interactions play a central role in ecosystem processes, shaping complex food webs with multiple paths and levels [1]. The complexity of interactions within communities, however, makes it difficult to assess their nature and long-term outcome solely by field observations. Several complementary approaches were developed to address this issue, such as fatty acid analysis [2]. Fatty acids have been used to study trophic ecology of organisms in aquatic and terrestrial ecosystems [3, 4]. Variation in fatty acid profiles can answer basic questions about spatial and temporal variation in diets, as well as niche partitioning among species [3, 5, 6]. Also, fatty acids could be used as biomarkers, indicating qualitative and quantitative trophic relationships between organisms [7, 8]. Many recent studies using fatty acid analysis in terrestrial organisms focused on detritivores, such as Collembola and Nematoda [7, 9–14], which established the technique as a useful tool to analyze their feeding interactions in soil food webs [5, 15–17]. However, fatty acid patterns and dynamics depend on an organism's physiology and composition of its natural diet, which are variable among taxonomic groups. Therefore, basic information on lipid metabolism is needed before the application of fatty acid analyses to study trophic relations of a given animal group.

Ants (Hymenoptera: Formicidae) are among the most abundant groups of invertebrates in terrestrial ecosystems, with a wide variety of feeding habits, nesting sites, and interactions with organisms from all trophic levels [18]. Many ant species have a cryptic behavior, which is difficult to study directly (e.g., living underground, inside the leaf-litter or in tree canopies). Moreover, in diverse ecosystems, dozens of species can coexist simultaneously in a given stratum [19]. Thus, complementary techniques are needed to study their trophic ecology. Stable isotopes, for instance, have been extensively used to address many questions in ant ecology [20–22]. The application of DNA barcoding, another modern technique, is still incipient for ants [23–25]. Surprisingly, no study so far tested the applicability of fatty acids to understand trophic ecology of ants.

Ants in general are regarded as omnivorous, feeding on a combination of living prey, dead arthropods, seeds and plant exudates. Less common are specialized feeding habits such as fungus cultivation and predation exclusively upon certain arthropod groups, as well as use of unusual resources such as pollen, animal excrements or mushrooms [18, 26–29]. Fatty acids from the diet could be incorporated without modification (i.e. through direct trophic transfer), or actively modified in response to environmental factors and physiological needs [4, 30, 31]. Many ant species primarily feed on sugars usually obtained from floral and extra-floral nectar or honeydew

[32]. Like all higher organisms, they can synthesize a set of fatty acids from carbohydrates via a decarboxylative Claisen condensation [33]. Fatty acids are mainly stored as neutral lipid fatty acids (NLFAs), which mostly consist of triglycerides, the principal component of the insect fat body [30, 34]. The biosynthesis of saturated palmitic (C16:0) and stearic acids (C18:0) and monounsaturated oleic acid (C18:1n9) seems to be widespread among insects, and correspondingly these fatty acids are the most abundant in their bodies [30]. On the other hand, the ability to synthesize polyunsaturated fatty acids, such as linoleic acid (C18:2n6), is highly variable among species [35, 36]. However, the details of these physiological processes in ants are poorly understood, and there are no studies specifically addressing dynamics of dietary fatty acids assimilation in this important insect group. Knowing which fatty acids can be unambiguously related to food sources, and how well the overall fat composition of ants reflects their diet after any metabolic modification, are crucial steps to apply fatty acid analysis in an ecological context.

Considering the potential use of fatty acids to understand trophic relations, and the lack of information about lipid metabolism in ants, we aim to elucidate patterns and dynamics of neutral lipid fatty acids in ants. We provided ants with high- and low-fat food in a no-choice feeding experiment, and compared the fatty acid profiles of ant workers and larvae over a period of 8 weeks. We specifically ask: (1) whether NLFA amounts and compositions are affected by a high- and a low-fat diet; (2) whether dietary fatty acids are accumulated in the ants' body via direct trophic transfer; (3) how dietary fatty acids shape NLFA patterns over time; (4) whether these patterns and dynamics are the same in different species and life stages.

Methods

Studied species

The experiment was performed with colonies reared in the laboratory, during November and December 2016. We chose two species, common and widespread in the Northern hemisphere, which represent the largest Formicidae subfamilies: *Formica fusca* Linnaeus 1758 (Formicinae) and *Myrmica rubra* Linnaeus 1758 (Myrmicinae). Both have in nature a similar and generalized diet of living and dead arthropods, nectar and honeydew [37, 38], and can thus be reared in the laboratory with a single artificial diet. Six colonies of each species were purchased from Antstore (Berlin, Germany) where ants were fed on an unstandardized diet of honey and dead flies. All colonies had one queen and between 9 and 12 (*F. fusca*) and 15–20 (*M. rubra*) workers. Colonies of *M. rubra* were reproductive during the whole experiment, with lower numbers of eggs and larvae towards the end. For *F. fusca*, larvae were only

observed in two colonies in the last week of the experiment. Colonies were kept at a constant temperature of 25 °C and provided three times per week with water and food *ad libitum*.

Low- and high-fat treatments

Three colonies of each species received a low-fat treatment, whereas the remaining three received a high-fat treatment. As low-fat food we used a standardized recipe, suitable for breeding several ant species [39]. It contained 5 g agar, 1 g table salt (NaCl), 1 g vitamin-mineral mix powder (Altapharma, Burgwedel, Germany), 62 ml honey and 1 chicken egg homogenized in 500 ml hot water. The high-fat food followed the same recipe, with addition of 60 ml linseed oil (organic quality, Alnatura, Bickenbach, Germany). The mixture was stirred until it was cool and solid, to avoid separation of the aqueous and fatty phases. Both food mixtures were stored in a freezer at -20 °C until use, and food samples were taken for chemical analysis.

Experimental design

Before beginning the feeding experiment, we collected one worker per colony for fatty acid analysis (= week 0). Workers were chosen randomly from inside and outside the nest (a glass vial kept inside a plastic box). In addition, one larva of *M. rubra* was collected per colony. After starting to apply the treatments, we sampled one worker and one larva in the same way, every week for 8 weeks. Larva sample sizes were smaller from week 5 onwards, because some colonies were not reproductive anymore. In the last week, we also collected the queens for analysis (6 *F. fusca* and 5 *M. rubra*, since one queen died at the beginning of the experiment). All samples were immediately frozen at -20 °C until extraction.

Fatty acid analysis

Total lipids were extracted from the ants using 1 ml of a chloroform:methanol mixture, 2:1 (v/v) over a period of 24 h [40, 41]. Ants were directly refrozen after extraction and subsequently dried for 48 h at 50 °C and weighed with a microbalance (Mettler Toledo, XS3DU, Columbus, USA). The extracts were purified and separated according to the method described by Frostegård et al. [42]. SiOH-columns (Chromabond®) were washed and conditioned with 6 ml hexane. Subsequently, samples were applied on the column and elution of NLFAs (= mono-, di-, and triglycerides) was accomplished with 4 ml chloroform.

The chloroform fractions were evaporated to dryness under gentle nitrogen gas flow and residuals were redissolved in different concentrations of dichloromethane:methanol 2:1 (v/v) to adjust the samples to comparable concentration ranges: 1 ml for *F. fusca* queens and food

samples, 350 µl for workers of both species and *M. rubra* queens, and 50 µl for larvae. 50 µl aliquots (10 µl for high-fat food) were transferred to new glass vials with a conical inlet (150 µl) and 20 µl of internal standard (C19:0 in methanol; $\rho_i = 220$ ng/µl) were added. Samples were evaporated to dryness again, and finally derivatized to fatty acid methyl esters (FAMES) with 20 µl TMSH (trimethylsulfonium hydroxide; 0.25 M in MeOH from Fluka, Sigma-Aldrich, St. Louis, USA).

FAME samples of NLFAs were analyzed with a QP2010 Ultra GC/MS (Shimadzu, Duisburg, Germany). The gas chromatograph (GC) was equipped with a ZB-5MS fused silica capillary column (30 m × 0.25 mm ID, $df = 0.25$ µm) from Phenomenex (Aschaffenburg, Germany). Sample aliquots of 1 µl were injected by using an AOC-20i autosampler-system from Shimadzu into a PTV-split/splitless-injector (Optic 4, ATAS GL, Eindhoven, Netherlands), which operated in splitless-mode. Injection-temperature was programmed from initial 70 °C up to 300 °C and then an isothermal hold for 59 min, sampling-time was set to 3 min and hydrogen was used as carrier-gas with a constant flow rate of 1.3 ml/min. The temperature of the GC oven was raised from initial 60 °C for 1 min, to 150 °C with a heating-rate of 15 °C/min, to 260 °C with a heating-rate of 3 °C/min, to 320 °C with a heating-rate of 10 °C/min and then an isothermal hold at 320 °C for 10 min. Electron ionization mass spectra were recorded at 70 eV from m/z 40 to 650. The transfer line and ion source were kept at 250 °C.

Methyl esters of the NLFAs were identified by comparing gas chromatographic retention times and m/z fragmentation patterns with those of the Supelco® 37 Component FAME Mix standard and the Bacterial Acid Methyl Ester (BAME) Mix standards as commercially available fatty acids (all Sigma-Aldrich) and published literature data [31, 43, 44]. The identity of γ -linolenic acid was additionally confirmed by an iodine catalyzed dimethyl disulfide derivatization [45].

A technical problem during analysis resulted in the loss of a batch of samples. Therefore, we have no data of week 3 for *M. rubra* larvae, week 4 for *M. rubra* workers and week 5 for *F. fusca*.

Data analysis

In general we used two approaches to analyse our data: (1) linear mixed-effect models (LMM) to assess the trophic transfer of certain fatty acids; and (2) multivariate compositional data analysis to describe total NLFA patterns. Only fatty acids with >1% composition were included in our analyses. Queens were not statistically analyzed, since they were sampled just at the end of the experiment.

We used the absolute amount of NLFAs [μg] standardized by dry weight for ants or fresh weight for food [mg], thus reflecting the relative amounts of NLFAs in comparison to non-lipid components [$\mu\text{g}/\text{mg}$]. We additionally ran the analyses with absolute amounts and dry weight as a cofactor, and results were identical for workers, but different for larvae, due to their distinct dynamics (see S1 in Additional file 1, and results for larvae).

At first, we correlated the relative amounts of all NLFAs combined (= total NLFAs) with dry weights of larvae and workers of both species using Spearman's rank correlation. For adults, body weight reflects size polymorphism among workers. For larvae, body weight is a better indicator of larval development than the week of sampling, because queens lay eggs continuously during the reproductive time. Dry weights for workers did not differ between treatments and over time, while larval dry weight increased over time (see S2 in Additional file 1). Since time and size were correlated for larvae ($\rho_S = 0.63$, $p < 0.001$), we ran separated LMMs for each factor, with dry weight normalized by square-root transformation.

We statistically tested relative amounts of total NLFAs and of the three most abundant fatty acids (C16:0, C18:0, C18:1n9). We also tested a specific dietary NLFA (C18:2n6), which occurred in higher concentration in the high-fat diet, and was not conspicuously synthesized by the ants. We did not test the amounts of the other two specific dietary NLFAs (C18:3n3 and C18:3n6) and show their results only in plots, because both were always zero in the low-fat treatment and non-zero in the high-fat treatment. Remaining NLFAs that occurred only in very small amounts in ants and food and were not tested either.

Effects on relative amounts were tested with linear mixed-effect models (command `lme`) as implemented in the R package "nlme" [46] with feeding treatment and time as fixed factors and colony ID as random factor for each species separately. We checked for the normal distribution of the residuals and the homogeneity of variance prior to the analyses and transformed the data if necessary (see S3 in Additional file 1 for data transformation). We further investigated the total NLFA amount in *M. rubra* workers and larvae using a LMM with the same structure as before, but including life stage as a further fixed factor. The difference between workers and larvae was analyzed with a simultaneous test for general linear hypothesis using Tukey pairwise contrasts (package "multcomp"; [47]) of the previous LMM.

We furthermore analyzed whether the overall NLFA composition (i.e. percentages of all fatty acids) of *F. fusca*, *M. rubra* workers and *M. rubra* larvae changed in the different treatments over time. We tested Bray-Curtis similarities (BCS) based on compositional data using

permutational multivariate analysis of variance (PERMANOVA; [48]) for each species separately. Overall 10,000 permutations were performed with feeding treatment and time as fixed factors and colony ID as random factor. We checked the multivariate homogeneity of group dispersions before with a multivariate Levene's test (PERMDISP; all p values >0.1 ; [49]). These analyses were performed with PRIMER 7.0.12 [50].

Finally, NLFA compositional data were ordinated using principal component analyses (PCA) and according PCA biplots. We compared the differences of the overall NLFA composition in *F. fusca* and *M. rubra* who received the high-fat diet during the experimental time. We used the centered log-ratio transformation after replacing zero values to deal with the constant sum constraint of compositional data and make it suitable for PCA (R packages "zCompositions" and "compositions" [51, 52]). PCA biplots were constructed by plotting factor loadings of compounds that significantly contributed ($p < 0.01$) to the group separation onto the PCA scatter plots using the R package "vegan" [53]. For a detailed R script of this analysis, see [54]. LMMs and PCAs were performed with R version 3.3.1 [55].

Results

Fatty acid profiles of food and ants

The neutral lipid fatty acid (NLFA) profiles of ants and their food are summarized in Table 1 (for full dataset and value ranges, see Additional file 2). The high-fat food had about 40 times more total concentration of NLFAs than the low-fat food. The main component of the high-fat food was C18:3n3, but it also had notably higher amounts of C16:0, 18:0 and C18:2n6. Besides, it contained C:18:3n6, which was entirely absent from the low-fat food.

C16:0, C18:0 and C18:1n9 were the main fatty acids in ants (Table 1). C18:1n9 was the main component in all experimental workers and queens. On the other hand, larvae had comparatively high levels of C16:0 and C18:0. Ants from the high-fat treatment exhibited higher amounts of C18:2n6, and were the only ones with detectable levels of C18:3n3 and C18:3n6. Queens had less total NLFAs than workers. Samples were variable, thus the profiles in Table 1 do not exactly reflect temporal and treatment differences (particularly for the highly variable larvae); these effects are analyzed below.

Dynamics of total and individual NLFA amounts

For *F. fusca*, there was no difference between treatments in the total amount of NLFAs (Fig. 1a, Table 2). C16:0, C18:0, C18:1n9 and total NLFAs increased over time, but with no treatment effect (Fig. 1b-c, Table 2). On the other hand, we observed an increasingly higher amount of C18:2n6 in the high-fat treatment, while it remained

Table 1 – Fatty acid profiles of food and ants at the beginning and end of the experiment

NLFA	<i>Formica fusca</i>						<i>Myrmica rubra</i>						<i>Myrmica rubra</i> larvae				Food	
	Week 0		Week 8		Queens		Week 0		Week 8		Queens		Week 0		Week 8		+	-
	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-		
C12:0 lauric	0.1 (t)	0.1 (t)	0.1 (t)	0.1 (t)	0.1 (t)	0.1 (t)	0.6 (t)	0.4 (t)	0.2 (t)	0.3 (t)	0.1 (1)	0.2 (t)	3.9 (t)	2.4 (t)	0.4 (t)	1.0 (t)	t (t)	t (t)
C14:0 myristic	0.5 (t)	0.3 (t)	0.3 (t)	0.6 (t)	0.1 (t)	0.3 (t)	1.9 (1)	1.6 (t)	0.6 (1)	0.5 (1)	0.1 (1)	0.4 (1)	8.8 (1)	5.0 (1)	1.2 (1)	2.7 (1)	t (t)	t (t)
C16:0 palmitic	59.0 (25)	40.6 (25)	95.2 (12)	129.8 (18)	25.5 (11)	30.5 (18)	55.7 (18)	54.0 (18)	23.1 (32)	31.3 (30)	3.0 (26)	10.3 (17)	532.7 (54)	397.1 (59)	48.9 (33)	50.1 (25)	9.2 (8)	0.8 (32)
C16:1n9 palmitoleic	4.1 (1)	0.9 (t)	2.6 (t)	4.6 (1)	0.9 (t)	2.4 (1)	6.7 (2)	2.6 (1)	1.1 (1)	1.8 (1)	0.1 (1)	1.6 (3)	11.3 (1)	2.7 (t)	1.2 (1)	8.1 (4)	0.1 (t)	t (1)
C18:0 stearic	11.6 (10)	11.4 (12)	26.5 (3)	22.3 (3)	7.2 (3)	5.3 (3)	13.1 (5)	17.4 (6)	7.6 (15)	7.7 (13)	1.7 (15)	1.3 (3)	242.2 (25)	209.2 (31)	29.8 (20)	23.4 (12)	6.6 (6)	0.1 (4)
C18:1n9 oleic	284.7 (64)	224.6 (62)	523.7 (67)	570.3 (78)	151.4 (66)	135.4 (77)	273.5 (74)	251.7 (74)	80.0 (48)	129.2 (55)	4.4 (26)	46.1 (76)	202.6 (19)	51.5 (8)	39.4 (27)	112.2 (56)	1.8 (2)	1.5 (59)
C18:2n6 linoleic	0.4 (t)	0.4 (t)	4.2 (1)	0.2 (t)	3.0 (1)	0.5 (t)	1.3 (1)	1.6 (1)	2.4 (2)	0.3 (t)	1.1 (7)	0.4 (1)	3.2 (t)	2.1 (t)	3.4 (2)	1.3 (1)	5.3 (4)	0.1 (2)
C18:3n3 α-linolenic	0 (0)	0 (0)	138.8 (17)	0 (0)	30.8 (14)	0 (0)	0 (0)	0 (0)	3.9 (2)	0 (0)	3.1 (18)	0 (0)	0 (0)	0 (0)	20 (14)	0 (0)	80.9 (72)	t (1)
C18:3n6 γ-linolenic	0 (0)	0 (0)	22.5 (3)	0 (0)	9.1 (4)	0 (0)	0 (0)	0 (0)	1.3 (1)	0 (0)	0.9 (5)	0 (0)	0 (0)	0 (0)	1.7 (1)	0 (0)	8.9 (8)	0 (0)
C20:0 arachidic	0.1 (t)	0.1 (t)	0.1 (t)	0.1 (t)	0.1 (t)	0 (t)	0.2 (t)	0.2 (t)	0.1 (t)	0.1 (t)	0.1 (t)	0.1 (t)	0.7 (t)	0.6 (t)	0.2 (t)	0.1 (t)	t (t)	t (t)
Total	360.3	278.4	813.9	728.0	228.0	174.5	353.1	329.7	120.3	171.2	14.5	60.3	1005.5	670.6	146.3	198.9	112.8	2.6
Sample size	3	3	3	3	3	3	3	3	3	3	2	3	3	3	1	1	3	2

Average amounts are given in µg of NLFA/mg of dry weight (fresh weight for food). Values in brackets are average percentages of the total composition of NLFAs per sample. +: high-fat treatment; -: low-fat treatment; t: detected in trace amount (less than 0.1 µg/mg or 1% of composition)

small in the low-fat treatment (Fig. 1d, Table 2). Similarly, C18:3n3 and C18:3n6 increased remarkably in the high-fat treatment, but were never recorded in the low-fat treatment (Figs. 1e-f). *Formica fusca* presented considerable polymorphism (coefficient of variation [= CV] of dry weights = 41%), but there was no correlation between body size and total NLFA amount ($\rho_S = -0.06$, $p = 0.66$).

For *M. rubra*, the amounts of C18:2n6, C18:3n3 and C18:3n6 also increased in the high-fat treatment, and the last two NLFAs were completely absent in the low-fat treatment (Fig. 2d-f, Table 2). No time effect was observed for C18:2n6 in this species. There was no treatment effect in C16:0, C18:0, C18:1n9 and total NLFAs, but, opposite to *F. fusca*, we observed an overall decrease over time (Figs. 2a-b, Table 2). *Myrmica rubra* workers varied less in size (CV of dry weights = 17%) and, again, no correlation was found between body size and total NLFA amount ($\rho_S = 0.19$, $p = 0.19$).

Myrmica rubra larvae presented more complex dynamics, because they were influenced both by experimental time effect and their developmental stage. Nevertheless, since these variables were correlated, LMM results were similar, except for C18:1n9 (Table 2). The increasing trends for C18:2n6, C18:3n3 and C18:3n6 were the same

as in workers (Fig. 3d-f, Table 2). Total NLFAs also decreased with time (Fig. 3b, Table 2), but in a higher rate than in workers (Tukey pairwise contrasts, $z = 4.70$, $p < 0.001$, for full model see S4 in Additional file 1). Larvae from the high-fat treatment had more total NLFAs and C18:1n9 overall during the experiment (Figs. 3a-c, Table 2 [A]). However, as larvae increased in dry weight, C18:1n9 actually was higher in the low-fat treatment compared to the high-fat treatment (Table 2 [B]). There was a strong negative correlation between larval dry weight and relative NLFA amount (Fig. 4; Table 2 [B], $\rho_S = -0.72$, $p < 0.001$). The absolute amount of fat slightly increased with body size, but did not follow the growth in other body components, which resulted in lower concentration of NLFAs in larger and older larvae (Fig. 4). This decrease was mostly due to a decline on saturated fatty acids (C16:0 and C18:0, Table 2, see S5 in Additional file 1). Therefore, young larvae had relatively large fat storages and high ratios of saturated:unsaturated fatty acids, which both decreased during development.

Dynamics of overall fatty acid composition

The overall NLFA composition of the ants changed over time (Table 3). Treatment and time affected the composition of *F. fusca* and *M. rubra* larvae. For *M.*

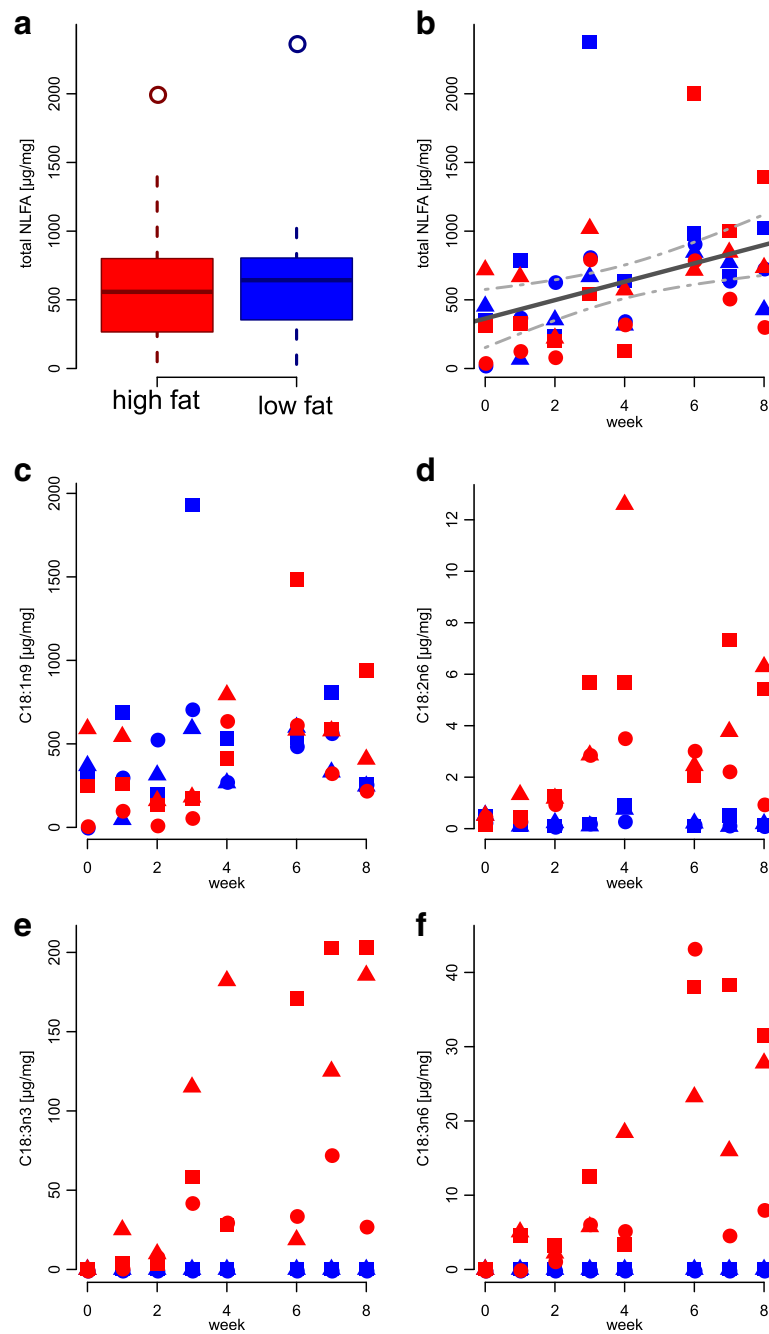


Fig. 1 Dynamics of NLFA total amount and individual fatty acids in *Formica fusca* workers. Symbols indicate distinct colonies. In (a) samples from all weeks and colonies are pooled, (b) total NLFA, (c) C18:1n9, (d) C18:2n6, (e) C18:3n3, (f) C18:3n6

rubra workers, no effect was found. However, this could be understood when the profile change of the high-fat colonies was analyzed with PCA (Fig. 5). For both species, we noticed a shift in composition over time, mainly driven by the dietary fatty acids. For *F. fusca*, C18:2n6, C18:3n3 and C18:3n6 altogether had a statistically significant effect on this shift. For *M. rubra*, only C18:3n3 (the main dietary fatty acid) had a

significant effect. The samples from week 8 were particularly odd, showing small proportions of C18:3n3 and C18:1n9 and relatively high proportions of C16:0 and C18:0. One individual from each treatment had unusually low amounts of total fat and oleic acid (below 20 µg/mg and 10% of composition, respectively; see Additional file 2), which added significant variation to the results. When week 8 was removed from

Table 2 Effects of time, treatment and larval dry weight on relative total amount [$\mu\text{g}/\text{mg}$] and individual amounts of fatty acids

	Total NLFAs			C16:0			C18:0			C18:1n9			C18:2n6						
	df	F	trend	p	df	F	trend	p	df	F	trend	p	df	F	trend	p			
<i>F. fusca</i> (n = 48)																			
Treatment	1	0.12		0.74	1	1.39		0.303	1	0.80		0.422	1	0.18		0.69			
Time	1	15.42	↑	< 0.001	1	22.30	↑	< 0.001	1	25.51	↑	< 0.001	1	10.52	↑	0.002	1	11.66	0.002
Treatment x Time	1	0.29		0.60	1	0.16		0.687	1	1.94		0.171	1	0.84		0.37	1	14.97	< 0.001
Residuals	44				44				44				44				44		↑ _{high} ↓ _{low}
<i>M. rubra</i> (n = 48)																			
Treatment	1	0.02		0.90	1	0.59		0.484	1	0.02		0.893	1	0.26		0.64	1	29.06	0.006
Time	1	6.34	↓	0.016	1	4.60	↓	0.038	1	9.85	↓	0.003	1	4.36	↓	0.043	1	2.98	0.09
Treatment x Time	1	0.07		0.79	1	0.23		0.634	1	0.01		0.956	1	0.02		0.89	1	2.12	0.15
Residuals	44				44				44				44				44		
<i>M. rubra</i> larvae [A] (n = 38)																			
Treatment	1	11.08	↑	0.029	1	1.36		0.308	1	3.20		0.148	1	9.93	↑	0.034	1	22.55	0.009
Time	1	12.27	↓	0.015	1	9.62	↓	< 0.001	1	37.08	↓	< 0.001	1	0.46		0.502	1	0.09	0.765
Treatment x Time	1	0.34		0.56	1	0.20		0.656	1	2.64		0.115	1	1.23		0.276	1	2.17	0.151
Residuals	33				33				33				33				33		
<i>M. rubra</i> larvae [B] (n = 38)																			
Treatment	1	25.78	↑	< 0.001	1	2.05		0.161	1	2.75		0.107	1	10.80		0.003	1	21.57	0.001
Dry weight	1	46.35	↓	< 0.001	1	53.59	↓	< 0.001	1	69.50	↓	< 0.001	1	0.15		0.700	1	0.62	0.434
Treatment x Dry weight	1	3.36		0.076	1	1.42		0.241	1	0.05		0.809	1	4.68	↑ _{low}	0.038	1	0.06	0.802
Residuals	33				33				33				33				33		↑ _{high} ↓ _{low}

Results of linear mixed-effect models. Trends indicate the direction of significant effects ($\alpha < 0.05$, in bold). For larvae, [A] = time as a factor, [B] = dry weight as a factor

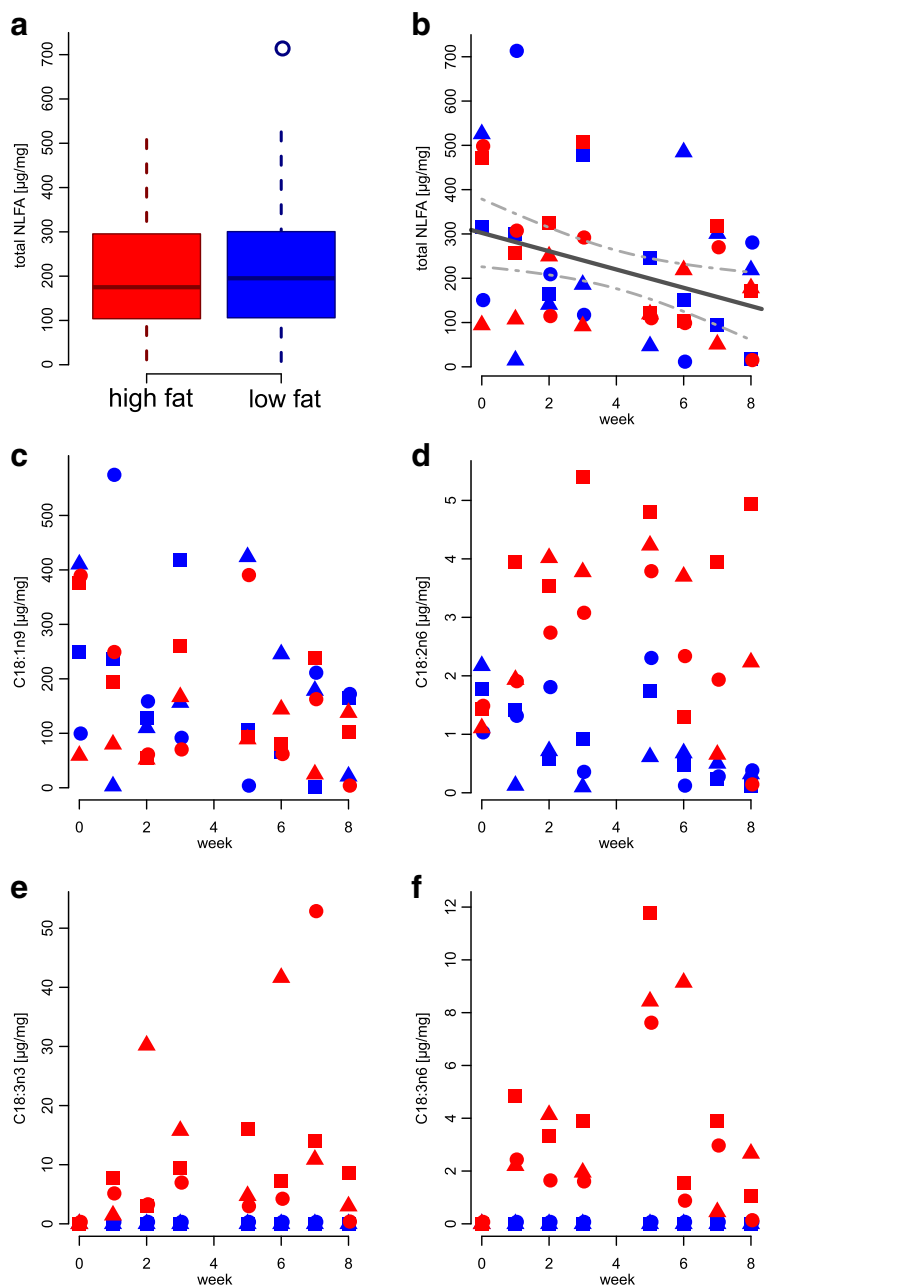


Fig. 2 Dynamics of NLFA total amount and individual fatty acids in *Myrmica rubra* workers. Symbols indicate distinct colonies. In (a) samples from all weeks and colonies are pooled, (b) total NLFA, (c) C18:1n9, (d) C18:2n6, (e) C18:3n3, (f) C18:3n6

the PERMANOVA, the treatment effect was noticeable (Table 3).

Discussion

Fatty acid profiles of ants

Several factors influence the fatty acid composition of insects, such as flying activity, life stage, growth, reproductive status, environmental temperature, and diet [4, 30, 56]. Due to this complexity, Stanley-Samuelson et al. [30] argued against a “typical” insect profile, and

indeed a high variation is found among orders, families, and species [56, 57]. Just a few ant profiles are available in literature: *Myrmica incompleta* Provancher, 1881 (worker and pupae; [58]), *Lasius claviger* (Roger, 1862) (only pupae; [59]), *Myrmica rubra* (only the free fatty acid fraction from head extracts; [60, 61]) and *Polyrhachis dives* Smith, 1857 (sun-dried workers cultivated as food; [62]). These fatty acid profiles are not entirely comparable due to the multitude of goals and methods, but, together with our results, they indicate C18:1n9 as

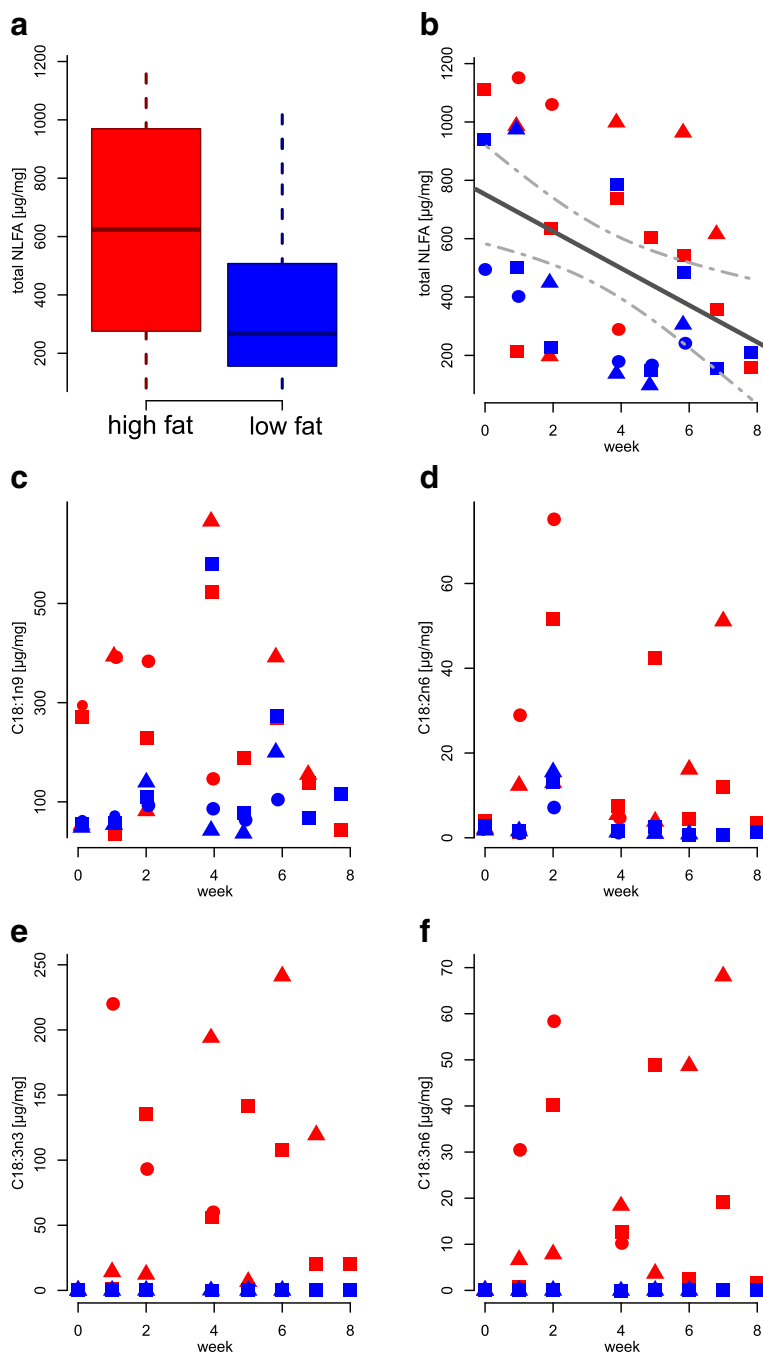


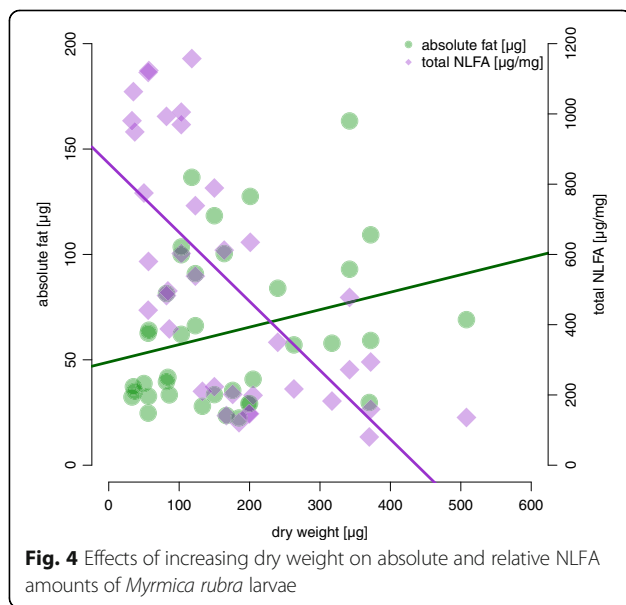
Fig. 3 Dynamics of NLFA total amount and individual fatty acids in *Myrmica rubra* larvae. Symbols indicate distinct colonies. In (a) samples from all weeks and colonies are pooled

the predominant NLFA in ant bodies, followed by C16:0 and C18:0. High levels of C18:1n9 are standard for Hymenoptera, but the abundance of other fatty acids varies within the order [56].

Dynamics of individual NLFAs and overall composition

Some fatty acids are extensively synthesized de novo by animals, while others are produced in small amounts,

or only by certain taxa [30, 36]. In our experiment, the food enrichment with linseed oil allowed us to observe the influence of diet on NLFAs found a priori in high, low and null amounts in ants' bodies. C18:3n3 and C18:3n6 were absent in week 0, and solely recorded in the high-fat treatment during the experiment. This suggests that ants are not able to synthesize them, or only in small doses which are directly incorporated in



the polar lipid fractions (i.e. phospholipids, glycolipids, free fatty acids). The amounts of C18:3n3 and C18:3n6 increased with the time ants fed on the diet, thus their concentration reflects how much/how often the ants consumed a resource. If these NLFAs are neither highly

Table 3 Effects of time and treatment on overall NLFAs composition

	df	pseudoF	p
<i>F. fusca</i>			
Treatment	1	8.87	< 0.001
Time	7	2.60	0.015
Treatment x time	7	0.91	0.543
Residuals	32		
<i>M. rubra</i> (week 8)			
Treatment	1	2.98	0.089
Time	7	1.44	0.197
Treatment x time	7	0.53	0.832
Residuals	32		
<i>M. rubra</i> (week 7)			
Treatment	1	4.70	0.025
Time	6	1.46	0.193
Treatment x time	6	0.87	0.871
Residuals	28		
<i>M. rubra</i> larvae			
Treatment	1	22.46	< 0.001
Time	7	12.45	< 0.001
Treatment x time	7	1.76	0.090
Residuals	23		

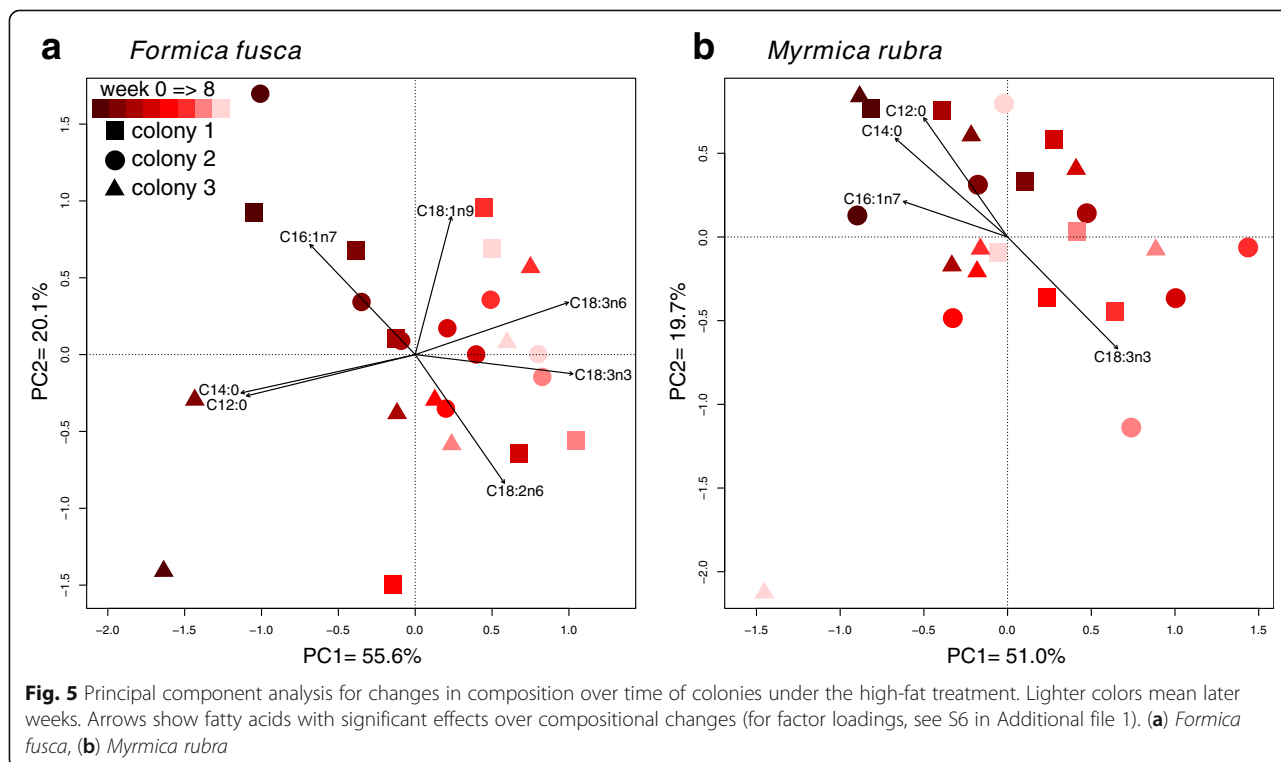
PERMANOVA results for overall composition (%) based on Bray-Curtis Similarities. Significant results ($p < 0.05$) are in bold

mobilized nor modified, they should mainly be stored in the fat body when acquired in considerable amounts from the diet, and thus detectable with neutral lipid fatty acid analysis.

C18:2n6 was found in smaller amounts in all samples of the low-fat treatment, but it is not clear whether this fatty acid was synthesized by ants de novo, was obtained from the small amounts in the food, or from the pre-experimental diet. About one third of reported insect species, from five different orders, are able to synthesis C18:2n6, but high interspecific variation was observed within orders [35, 36]. Regarding the Hymenoptera, C18:2n6 biosynthesis was not observed in the mason bee *Osmia lignaria* Say, 1837 (Megachilidae) [35], but it is known from the parasitoid *Nasonia vitripennis* (Walker, 1836) (Pteromalidae) [63]. Regardless of the actual ability of ants to synthesize C18:2n6, its amounts also increased with the diet and, in *F. fusca*, over time as well. In *M. rubra* and its larvae the time effect was not clear.

On the other hand, C16:0, C18:0 and C18:1n9 behaved similarly in both treatments. No treatment effect in C16:0 and C18:0 was noticed, even if they occurred in the high-fat food in levels higher than C18:3n6 and C18:2n6, respectively. Hence, it seems most likely that C16:0, C18:0 and C18:1n9 are synthesized de novo in large amounts from carbohydrates and constantly modified depending on physiological requirements. For example, the physiologically ideal fluidity of the fat body, which changes accordingly with environmental temperature, is achieved through a balanced ratio between saturated and unsaturated fatty acids [4]. Hence, the interplay between β -oxidation and Claisen condensation of these abundant NLFAs should be essential for this mechanism. The lack of a treatment effect on total NLFAs also suggests that, at least under *ad libitum* feeding conditions, ants have no significant energetic loss due to de novo fatty acid biosynthesis. Thus, ants with a sugar-based diet should not have a disadvantage compared to species that acquire most lipids from the diet. However, this may not be true under conditions with limited resources, and detectable differences in ratios could occur between ants that feed directly on lipids and ants that only synthesize them.

Our multivariate analyses showed that a shift in diet results in an equivalent shift in profile, and this difference was more pronounced when the ants fed longer on that resource (Table 3, Fig. 5). The main drivers of this compositional change were specific dietary NLFAs. Therefore, these profiles represent another way to assess dynamics of resource use or detect differences among species [3, 5, 6]. They could be particularly useful when the exact lipid composition of the food is not known, such as in samples collected from the field.



Factors affecting NLFA dynamics

Our results point out to several factors that affect lipid metabolism in ants, and could be important from biological and methodological points of view. First of all, one possible caveat of analytical methods that use ants' full body is that the undigested food stored in their crops could bias the results [20]. If this were the case in our experiment, we would expect higher total amount of lipids in ants of the high-fat treatment, and a conspicuous increase during the first week. Also, higher variance should occur in the high-fat treatment, due to the collection of workers with variable crop filling. However, (1) the amount of NLFAs did not differ between treatments, with the exception of larvae (which do not possess a crop; [64]); (2) we observed linear patterns for total fat and several NLFAs, consistent with lipid storage in the fat body; and (3) variances did not differ between treatments, in all cases (F test; *F. fusca* – $F = 1.01$, $p = 0.97$, *M. rubra* – $F = 1.58$, $p = 0.28$, larvae – $F = 1.67$, $p = 0.28$). Even if ants had undigested food in their crops, its contribution would have been relatively small. Thus, as far as the dietary component of interest does not occur in very high amounts in the food (e.g. ca. 10% NLFAs in our high-fat diet), full body extraction can be used to investigate the effect of diet in ants. In certain research contexts, however, it might be important to fully eliminate this factor, using a methodological alternative such as dissection of the fat body.

The reproductive status of the colonies influenced fatty acid dynamics. Feeding the brood can negatively affect the amount of fat stored by the workers, as observed in *Camponotus festinatus* (Buckley, 1866) [65], potentially explaining the decrease of NLFAs in *M. rubra*. On the contrary, *F. fusca* colonies were getting closer to reproduction mode during the experiment, and effectively we observed larvae in two colonies at the last week (this reproductive timing was also observed in non-experimental colonies kept in the same conditions). These colonies needed to accumulate reserves to fuel upcoming larval feeding and egg laying. Considering this, it is intriguing that queens of both species displayed a very low amount of fat at the end of the experiment.

We also observed an effect of development in compositions and dynamics of *M. rubra* larvae. The young larvae had large fat storages and amounts of saturated fatty acids. Earlier in their growth process, they quickly develop other tissues to build more complex organs [66], resulting in a proportionally smaller amount of NLFAs. The increase in C18:1n9 in the low-fat treatment with development may appear counterintuitive, but this was the only unsaturated NLFA ants were able to synthesize in large amounts. In turn, larvae from the high-fat treatment already received several polyunsaturated NLFAs from the diet. The shift to a more balanced composition between saturated and unsaturated NLFAs might enhance metabolic processes in a more complex body. In

contrat to workers, larvae seem to benefit from a high-fat diet from which they accumulate slightly more NLFAs. For *Solenopsis invicta* Buren, 1972 it has been demonstrated that sugars, lipids and proteins are differently allocated among worker subcastes, larvae and queens [67].

The distinct distribution of nutrients among individuals of a colony is not restricted to different life stages, but also among worker subcastes. Several studies observed higher fat storage in workers that stay inside the nest and take care of the brood (= nurses), and less in workers that spend more time in activities outside the nest (= foragers) [67, 68]. However, this pattern may not occur in a few species, and no difference was previously found in field samples of *F. fusca* [69]. In *M. rubra*, nurse and forager subcastes were identified in laboratory colonies smaller than ours, and their role was related to individual age and size [70]. Differences in worker size were unrelated to total amount of fat for both species in our data. Individual variation in fat storages could indicate behavioral subcastes, but it was the same in the reproductive *M. rubra* and the non-reproductive *F. fusca* (CV of NLFA total amounts for all samples = 72% in both species). Thus, we found no evidence for considerable differences in lipid storage across behavioral or morphological subcastes within these species, under our experimental conditions, although these effects may be minute in small colonies and need a specific setup to be detected.

Regardless of the variation across species and life stages in profiles and dynamics, the assimilation of specific dietary NLFAs (C18:2n6, C18:3n3 and C18:3n6) followed the same pattern. Thus, the physiological processes involved in NLFA metabolism should be conserved at least between the subfamilies Formicinae and Myrmicinae, which comprise about three quarters of all valid ant species [71]. It is likely that all ants behave similarly, but this needs to be tested with experiments using species with more diversified feeding behaviors and from more distant branches of the ant tree of life, such as the Ponerinae or Dorylinae [72].

Implications to the study of ant trophic ecology

In trophic ecology, fatty acids can basically be used in two ways: as overall profiles, whose variation indicates differences in diet; and as biomarkers, which indicate specific interactions between organisms [3, 4]. Our results suggest that both applications are suitable for ants. Profiles and individual NLFAs observed in ants changed in response to diet, and these shifts became more pronounced over time. Fatty acid analysis can provide a better resource resolution than stable isotopes, in a more quantitative way and representative timeframe

than barcoding of gut DNA [2]. However, these methods are complementary, rather than opposing, and could be coupled with field observations to provide a comprehensive perspective on ant trophic ecology.

The factors affecting NLFA amounts and composition that we observed should also be considered in an ecological context. A representative sample of castes and life stages is recommended if one is interested in detailed trophic ecology of a particular species. For a study at community level, profiles of forager workers sampled at a similar time may be enough to provide comparative information on resource partitioning, although distinct reproduction times could influence amounts and compositions.

In this study, we did not aim to survey prospective biomarkers for natural resources used by ants. However, the three specific dietary NLFAs (C18:2n6, C18:3n3 and C18:3n6) presented chemical properties of suitable biomarkers, as they were not produced by ants (or only in small amounts) and assimilated through direct trophic transfer, with little or no metabolic modification [4]. They can be found in natural diets of ants, such as in elaiosomes, seeds and other insects, in variable patterns that may allow detection of specific interactions [56, 73, 74]. Thus, they are good candidates for trophic markers. Since their assimilation was not affected by species identity, reproductive status or life stage, the biomarker approach seems to be quite promising for ants. Naturally, the actual relevance of these NLFAs would depend on context and occurrence within a community. On the other hand, since C16:0, C18:0 and C18:1n9 are synthesized from carbohydrates in large amounts, and highly modified to attend physiological needs, it would be difficult to relate their amounts to a particular resource or feeding behavior. Further research can provide more fatty acids useful as biomarkers, related to other resources used by ants, which would likely be distinct from the ones suggested for other groups (e.g. C18:1n9 as an indicator of herbivory in *Collembola* [5]).

Conclusions

We showed that ants accumulated fatty acids from their diet via direct trophic transfer, and that both, individual NLFAs and overall profiles reflect their diets. The fat content of the diet had no effect in lipids stored by ants, which shows that they are able to synthesize large amounts of NLFAs from sugars. Other factors such as reproductive status and life stage also affected total amounts and profiles of NLFAs. Specific dietary fatty acids were assimilated independent of species or life stage. Fatty acid analysis is a suitable technique to study feeding behavior of ants, and can become a valuable tool to study ant trophic ecology in the field. To this end, central points to be addressed by future research are which biomarkers are most informative of ant diets in natural communities, and

how factors other than diet affect fatty acid dynamics and composition of ant species with distinct life histories.

Additional files

Additional file 1: Supplementary information. (PDF 511 kb)

Additional file 2: Full dataset. (XLSX 42 kb)

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its Additional files.

Authors' contributions

MH provided the initial idea. FBR, AB and MH designed the experiment. FBR and AB collected the data. AB performed data analyses. All authors discussed the data. FBR drafted the manuscript. All authors contributed to the final manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

- Polis GA, Strong DR. Food web complexity and community dynamics. *Am Nat*. 1996;147:813–46.
- Birkhofer K, Bylund H, Dalin P, Ferlian O, Gagic V, Hambäck PA, et al. Methods to identify the prey of invertebrate predators in terrestrial field studies. *Ecol Evol*. 2017;7:1942–53.
- Budge SM, Iverson SJ, Koopman HN. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. *Mar Mammal Sci*. 2006;22:759–801.
- Ruess L, Chamberlain PM. The fat that matters: soil food web analysis using fatty acids and their carbon stable isotope signature. *Soil Biol Biochem*. 2010;42:1898–910.
- Ruess L, Schütz K, Haubert D, Häggblom MM, Kandeler E, Scheu S. Application of lipid analysis to understand trophic interactions in soil. *Ecology*. 2005;86:2075–82.
- Ferlian O, Scheu S, Pollierer MM. Trophic interactions in centipedes (Chilopoda, Myriapoda) as indicated by fatty acid patterns: variations with life stage, forest age and season. *Soil Biol Biochem*. 2012;52:33–42.
- Chamberlain PM, Bull ID, Black HJ, Ineson P, Evershed RP. Collembolan trophic preferences determined using fatty acid distributions and compound-specific stable carbon isotope values. *Soil Biol Biochem*. 2006;38:1275–81.
- Pollierer MM, Scheu S, Haubert D. Taking it to the next level: Trophic transfer of marker fatty acids from basal resource to predators. *Soil Biol Biochem*. 2010;42:919–25.
- Ruess L, Häggblom MM, Garcia Zapata EJ, Dighton J. Fatty acids of fungi and nematodes – possible biomarkers in the soil food chain? *Soil Biol Biochem*. 2002;34:745–56.
- Chamberlain PM, Bull ID, Black HJ, Ineson P, Evershed RP. Fatty acid composition and change in Collembola fed differing diets: identification of trophic biomarkers. *Soil Biol Biochem*. 2005;37:1608–24.
- Haubert D, Häggblom MM, Scheu S, Ruess L. Effects of fungal food quality and starvation on the fatty acid composition of *Protaphorura fimata* (Collembola). *Comp Biochem Physiol B Biochem Mol Biol*. 2004;138:41–52.
- Haubert D, Langel R, Scheu S, Ruess L. Effects of food quality, starvation and life stage on stable isotope fractionation in Collembola. *Pedobiologia*. 2005;49:229–37.
- Haubert D, Häggblom MM, Scheu S, Ruess L. Effects of temperature and life stage on the fatty acid composition of Collembola. *Eur J Soil Biol*. 2008;44:213–9.
- Haubert D, Pollierer MM, Scheu S. Fatty acid patterns as biomarker for trophic interactions: changes after dietary switch and starvation. *Soil Biol Biochem*. 2011;43:490–4.
- Ruess L, Schütz K, Migge-Kleian S, Häggblom MM, Kandeler E, Scheu S. Lipid composition of Collembola and their food resources in deciduous forest stands – implications for feeding strategies. *Soil Biol Biochem*. 2007;39:1990–2000.
- Haubert D, Birkhofer K, Fließbach A, Gehre M, Scheu S, Ruess L. Trophic structure and major trophic links in conventional versus organic farming systems as indicated by carbon stable isotope ratios of fatty acids. *Oikos*. 2009;118:1579–89.
- Ngosong C, Raupp J, Scheu S, Ruess L. Low importance for a fungal based food web in arable soils under mineral and organic fertilization indicated by Collembola grazers. *Soil Biol Biochem*. 2009;41:2308–17.
- Kaspari M. A primer on ant ecology. In: Agosti D, Majer JD, Alonso LE, Schultz TR, editors. *Ants: standard methods for measuring and monitoring biodiversity*. Washington, DC: Smithsonian Institution Press; 2000. p. 9–24.
- Agosti D, Majer J, Alonso L, Schultz T, editors. *Sampling ground-dwelling ants: case studies from the worlds' rain forests*. Perth: Curtin University of Technology; 2000.
- Blüthgen N, Gebauer G, Fiedler K. Disentangling a rainforest food web using stable isotopes: dietary diversity in a species-rich ant community. *Oecologia*. 2003;137:426–35.
- Davidson DW, Cook SC, Snelling RR, Chua TH. Explaining the abundance of ants in lowland tropical rainforest canopies. *Science*. 2003;300:969–72.
- Feldhaar H, Gebauer G, Blüthgen N. Stable isotopes: past and future in exposing secrets of ant nutrition (Hymenoptera: Formicidae). *Myrmecol News*. 2010;13:3–13.
- Fournier V, Hagler J, Daane K, de León J, Groves R. Identifying the predator complex of *Homalodisca vitripennis* (Hemiptera: Cicadellidae): a comparative study of the efficacy of an ELISA and PCR gut content assay. *Oecologia*. 2008;157:629–40.
- Muilenburg VL, Goggin FL, Hebert SL, Jia L, Stephen FM. Ant predation on red oak borer confirmed by field observation and molecular gut-content analysis. *Agric For Entomol*. 2008;10:205–13.
- Penn HJ, Chapman EG, Harwood JD. Overcoming PCR inhibition during DNA-based gut content analysis of ants. *Environ Entomol*. 2016;45:1255–61.
- Blüthgen N, Feldhaar H. Food and shelter: how resources influence ant ecology. In: Lach L, Parr CL, Abbott KL, editors. *Ant Ecology*. Oxford: Oxford University Press; 2010. p. 115–36.
- Brandão CRF, Silva RR, Delabie JHC. Neotropical ants (Hymenoptera) functional groups: nutritional and applied implications. In: Panizzi AR, Parra JRP, editors. *Insect bioecology and nutrition for integrated Pest management*. Boca Raton: CRC Press; 2012. p. 213–36.

28. von Beeren C, Mair MM, Witte V. Discovery of a second mushroom harvesting ant (hymenoptera: Formicidae) in Malayan tropical rainforests. *Myrmecol News*. 2014;20:37–42.
29. Sainz-Borgo C. Bird feces consumption by fire ant *Solenopsis geminata* (Hymenoptera: Formicidae). *Entomol News*. 2015;124:295–9.
30. Stanley-Samuelson DW, Jurenka RA, Cripps C, Blomquist GJ, de Renobales M. Fatty acids in insects: composition, metabolism, and biological significance. *Arch Insect Biochem Physiol*. 1988;9:1–33.
31. Brandstetter B, Ruther J. An insect with a delta-12 desaturase, the jewel wasp *Nasonia vitripennis*, benefits from nutritional supply with linoleic acid. *Sci Nat*. 2016;103
32. Davidson DW, Cook SC, Snelling RR. Liquid-feeding performances of ants (Formicidae): ecological and evolutionary implications. *Oecologia*. 2004;139:255–66.
33. Heath RJ, Rock CO. The Claisen condensation in biology. *Nat Prod Rep*. 2002;19:581–96.
34. Fast PG. Insect lipids: a review. *Mem Entomol Soc Can*. 1964;37:1–50.
35. Cripps C, Blomquist GJ, de Renobales M. De novo biosynthesis of linoleic acid in insects. *Biochim Biophys Acta BBA-Lipids Lipid Metab*. 1986;876:572–80.
36. Renobales M, Cripps C, Stanley-Samuelson DW, Jurenka RA, Blomquist GJ. Biosynthesis of linoleic acid in insects. *Trends Biochem Sci*. 1987;12:364–6.
37. Collingwood CA. The Formicidae (Hymenoptera) of Fennoscandia and Denmark. *Fauna Entomol Scand*. 1979;8:1–174.
38. Seifert B. Die Ameisen Mittel- und Nordeuropas. *Lutra: Tauer*; 2007.
39. Bhatkar A, Whitcomb WH. Artificial diet for rearing various species of ants. *Fla Entomol*. 1970;53:229–32.
40. Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem*. 1957;226:497–509.
41. Gómez-Brandón M, Lores M, Domínguez J. A new combination of extraction and derivatization methods that reduces the complexity and preparation time in determining phospholipid fatty acids in solid environmental samples. *Bioresour Technol*. 2010;101:1348–54.
42. Frostegård Å, Tunlid A, Bååth E. Microbial biomass measured as total lipid phosphate in soils of different organic content. *J Microbiol Methods*. 1991;14:151–63.
43. Zelles L. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biol Fertil Soils*. 1999;29:111–29.
44. Stein SE. Mass Spectra by NIST Mass Spec Data Center. In: Lindstrom PJ, Mallard WG, editors. *NIST Chemistry WebBook*. National Institute of Standards and Technology. 2015. <http://webbook.nist.gov>. Accessed 10 Oct 2016.
45. Dunkelblum E, Tan SH, Silk PJ. Double-bond location in monounsaturated fatty acids by dimethyl disulfide derivatization and mass spectrometry: application to analysis of fatty acids in pheromone glands of four Lepidoptera. *J Chem Ecol*. 1985;11:265–77.
46. Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. nlme: Linear and nonlinear mixed effects models. 2016. <http://CRAN.R-project.org/package=nlme>. Accessed 15 Dec 2016.
47. Hothorn T, Bretz F, Westfall P. Simultaneous inference in general parametric models. *Biom J*. 2008;50:346–63.
48. Anderson MJ. A new method for non-parametric multivariate analysis of variance. *Austral Ecol*. 2001;26:32–46.
49. Anderson MJ. Distance-based tests for homogeneity of multivariate dispersions. *Biometrics*. 2006;62:245–53.
50. Clarke KR, Gorley RN. *PRIMER v7: User Manual/Tutorial*. Plymouth: PRIMER-E; 2015.
51. Palarea-Albaladejo J, Martín-Fernández JA. zCompositions — R package for multivariate imputation of left-censored data under a compositional approach. *Chemom Intell Lab Syst*. 2015;143:85–96.
52. van den Boogaart KG, Tolosana R, Bren M. compositions: Compositional data analysis. 2014. <https://CRAN.R-project.org/package=compositions>. Accessed 15 Dec 2016.
53. Oksanen F, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, et al. vegan: Community ecology package. 2016. <https://CRAN.R-project.org/package=vegan>. Accessed 15 Dec 2016.
54. Brückner A, Heethoff M. A chemo-ecologists' practical guide to compositional data analysis. *Chemoecology*. 2017;27:33–46.
55. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing. 2016. <https://www.R-project.org/>. Accessed 15 Dec 2016.
56. Thompson SN. A review and comparative characterization of the fatty acid compositions of seven insect orders. *Comp Biochem Physiol Part B Comp Biochem*. 1973;45:467–82.
57. Hanson BJ, Cummins KW, Cargill AS, Lowry RR. Lipid content, fatty acid composition, and the effect of diet on fats of aquatic insects. *Comp. Biochem. Physiol. Part B Comp. Biochem*. 1985;80:257–76.
58. Stanley-Samuelson DW, Howard RW, Akre RD. Nutritional interactions revealed by tissue fatty acid profiles of an obligate myrmecophilous predator, *Microdon albicomatus*, and its prey, *Myrmica incompleta*, (Diptera: Syrphidae) (Hymenoptera: Formicidae). *Ann Entomol Soc Am*. 1990;83:1108–15.
59. Barlow JS. Fatty acids in some insect and spider fats. *Can J Biochem*. 1964;42:1365–74.
60. Brian MV, Blum MS. The influence of *Myrmica* queen head extracts on larval growth. *J Insect Physiol*. 1969;15:2213–23.
61. Brian MV. Caste control through worker attack in the ant *Myrmica*. *Insect Soc*. 1973;20:87–102.
62. Bhulaidok S, Sihamala O, Shen L, Li D. Nutritional and fatty acid profiles of sun-dried edible black ants (*Polyrhachis vicina* Roger). *Maejo Int J Sci Technol*. 2010;4:101–12.
63. Blaul B, Steinbauer R, Merkl P, Merkl R, Tschochner H, Ruther J. Oleic acid is a precursor of linoleic acid and the male sex pheromone in *Nasonia vitripennis*. *Insect Biochem Mol Biol*. 2014;51:33–40.
64. Wheeler WM. *Ants - their structure, development and behavior*. New York: Columbia University Press; 1910.
65. Rosell RC, Wheeler DE. Storage function and ultrastructure of the adult fat body in workers of the ant *Camponotus festinatus* (Buckley) (Hymenoptera : Formicidae). *Int J Insect Morphol Embryol*. 1995;24:413–26.
66. Wang YJ, Happ GM. Larval development during the nomadic phase of a nearctic army ant, *Neivamyrmex nigrescens* (Cresson) (Hymenoptera: Formicidae). *Int J Insect Morphol Embryol*. 1974;3:73–86.
67. Sorensen AA, Busch TM, Vinson SB. Control of food influx by temporal subcastes in the fire ant, *Solenopsis invicta*. *Behav Ecol Sociobiol*. 1985;17:191–8.
68. Tschinkel WR. Sociometry and sociogenesis of colonies of the harvester ant, *Pogonomyrmex badius*: worker characteristics in relation to colony size and season. *Insect Soc*. 1998;45:385–410.
69. Silberman RE, Gordon D, Ingram KK. Nutrient stores predict task behaviors in diverse ant species. *Insect Soc*. 2016;63:299–307.
70. Brian MV. Brood-rearing behaviour in small cultures of the ant *Myrmica rubra* L. *Anim Behav*. 1974;22:879–89.
71. Bolton B, Bolton A. An online catalog of the ants of the world. 2016. <http://antcat.org/>. Accessed 27 Feb 2017.
72. Kück P, Hita Garcia F, Misof B, Meusemann K. Improved phylogenetic analyses corroborate a plausible position of *Martialis heureka* in the ant tree of life. *Gilbert MTP, editor. PLoS ONE*. 2011;6:e21031.
73. Hughes L, Westoby MT, Jurado E. Convergence of elaiosomes and insect prey: evidence from ant foraging behaviour and fatty acid composition. *Funct Ecol*. 1994;8:358–65.
74. Reifenrath K, Becker C, Poethke HJ. Diaspore trait preferences of dispersing ants. *J Chem Ecol*. 2012;38:1093–104.

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Patterns and dynamics of fatty acids as trophic markers in Formicidae – implications to study ant ecology

Supplementary information

S1 – Linear mixed-effect models with absolute amounts of NLFAs

S2 – Dry weight of ants during the experiment

S3 – Data transformations for linear mixed-effect models

S4 – Linear mixed model with *M. rubra* workers and larvae

S5 – Effect of larval growth in saturated NLFAs

S6 – Factor loadings for Principal Components Analyses

S1 – Linear mixed-effect models with absolute amounts of NLFAs

The absolute amounts of NLFAs increased with dry weight for all ants (Table 1). For workers, significant results and trend directions were the same for LMMs with absolute amounts and dry weight as a cofactor, and with relative amounts of NLFAs (main text). For larvae, again LMMs with time and dry weight yielded similar results, with the exception of absolute total NLFAs and C18:2n6, which did not change with time, but increased with dry weight. As explained in the main text, this increase was small compared to growth of other body components, thus the relative total amount decreased. Since time and dry weight were correlated, but each factor influenced individual NLFAs in a different manner, some other relationships changed. The models with relative amounts in the main text are better to understand larvae dynamics overall, because they account for both factors in the same analysis (experimental time as a factor and variables standardized by dry weight).

Table 1 – Effects of dry weight, time and treatment on absolute total amount [μg] and individual amounts of fatty acids.

Total NLFAs				C16:0				C18:0				C18:1n9				C18:2n6					
	df	F	trend	p	df	F	trend	p	df	F	trend	p	df	F	trend	p	df	F	trend	p	
<i>F. fusca</i> (n=48)																					
Dry weight	1	7.91	↑	0.007	1	11.07	↑	0.002	1	13.64	↑	< 0.001	1	12.74	↑	< 0.001	1	3.27		0.077	
Treatment	1	0.03		0.855	1	1.95		0.170	1	1.50		0.226	1	1.00		0.322	1	77.99		< 0.001	
Time	1	5.71	↑	0.021	1	15.30	↑	< 0.001	1	16.33	↑	< 0.001	1	7.15	↑	0.010	1	12.66		0.001	
Treatment x Time	1	0.52		0.472	1	0.20		0.650	1	0.61		0.438	1	0.01		0.929	1	15.81	↑ _{high} ↓ _{low}	< 0.001	
Residuals	43				43				43				43				43				
<i>M. rubra</i> (n=48)																					
Dry weight	1	9.19	↑	0.004	1	5.46	↑	0.024	1	0.01		0.921	1	10.08	↑	0.003	1	0.08		0.768	
Treatment	1	0.02		0.870	1	0.98		0.327	1	0.02		0.875	1	0.18		0.671	1	42.94	↑	< 0.001	
Time	1	5.27	↓	0.027	1	5.26	↓	0.027	1	18.28	↓	< 0.001	1	5.38	↓	0.020	1	2.27		0.143	
Treatment x Time	1	0.00		0.984	1	0.14		0.708	1	0.71		0.401	1	0.00		0.988	1	0.51		0.482	
Residuals	43				43				43				43				43				
<i>M. rubra</i> larvae [A] (n=38)																					
Treatment	1	7.10	↑	0.011	1	0.33		0.567	1	3.83		0.059	1	4.37	↑	0.044	1	12.85	↑	0.001	
Time	1	1.42		0.241	1	1.88		0.179	1	17.13	↓	< 0.001	1	12.50	↑	0.001	1	1.19		0.282	
Treatment x Time	1	0.48		0.494	1	1.06		0.309	1	0.19		0.661	1	3.90		0.057	1	0.93		0.341	
Residuals	33				33				33				33				33				
<i>M. rubra</i> larvae [B] (n=38)																					
Treatment	1	8.22	↑	0.007	1	0.31		0.577	1	2.91		0.098	1	5.27	↑	0.028	1	14.09	↑	< 0.001	
Dry weight	1	6.48	↑	0.016	1	0.03		0.874	1	4.84	↓	0.035	1	23.72	↑	< 0.001	1	4.24	↑	0.048	
Treatment x Dry weight	1	0.51		0.482	1	1.27		0.269	1	0.15		0.701	1	3.05		0.090	1	1.38		0.248	
Residuals	33				33				33				33				33				

Results of linear mixed-effect models. Trend indicates the direction of significant effects ($p < 0.05$, in bold). For larvae, [A] = time as a factor, [B] = dry weight as a factor.

S2 – Dry weight of ants during the experiment

Overall, dry weight of ants did not differ between treatments (Table 2). It did not change also for workers, which do not grow in dimensions after emerging from pupae (although their total weight may change by accumulating or spending fat reserves). Larvae in general grew over time during the experiment (Fig. 1).

Table 2 – Effects of treatment and time on dry weight of ants during the experiment time

	dry weight [mg]			
	df	F	trend	p
<i>F. fusca</i> (n=48)				
Treatment	1	1.08		0.31
Time	1	0.26		0.61
Treatment x time	1	0.22		0.64
Residuals	44			
<i>M. rubra</i> (n=48)				
Treatment	1	0.01		0.97
Time	1	1.30		0.26
Treatment x time	1	3.90		0.055
Residuals	44			
<i>M. rubra</i> larvae (n=38)				
Treatment	1	1.77		0.25
Time	1	19.30	↑	< 0.001
Treatment x time	1	2.90		0.099
Residuals	33			

Results of linear mixed-effect models. Trends indicate the direction of significant effects ($p < 0.05$, in bold).

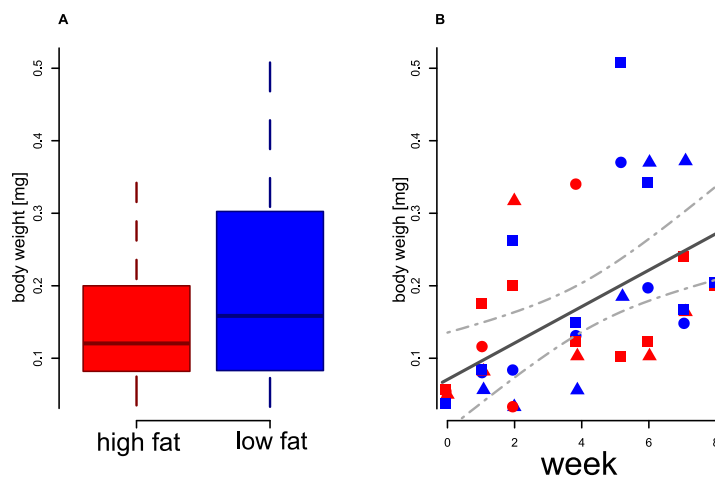


Fig. 1 – Increase in dry weight of *Myrmica rubra* larvae during the experiment.

S3 – Data transformations for linear mixed-effect models

Table 3 – Data transformations for linear mixed-effect models. For larvae, dry weight was also normalized by a square-root transformation (workers' weight had normal distributions and was not transformed).

	Total NLFAs	C16:0	C18:0	C18:1n9	C18:2n6
<i>F. fusca</i>	sqrt	log	-	log	log
<i>M. rubra</i>	sqrt	log	sqrt	sqrt	sqrt
<i>M. rubra</i> larvae	sqrt	sqrt	sqrt	log	log

sqrt = square-root transformation; log = logarithmic transformation; - = no transformation

S4 – Linear mixed model with *M. rubra* workers and larvae

	df	F	p
Treatment	1	2.30	0.148
Time	1	21.87	< 0.001
Life stage	1	22.14	< 0.001
Residuals	83		

S5 – Effect of larval growth in saturated NLFAs

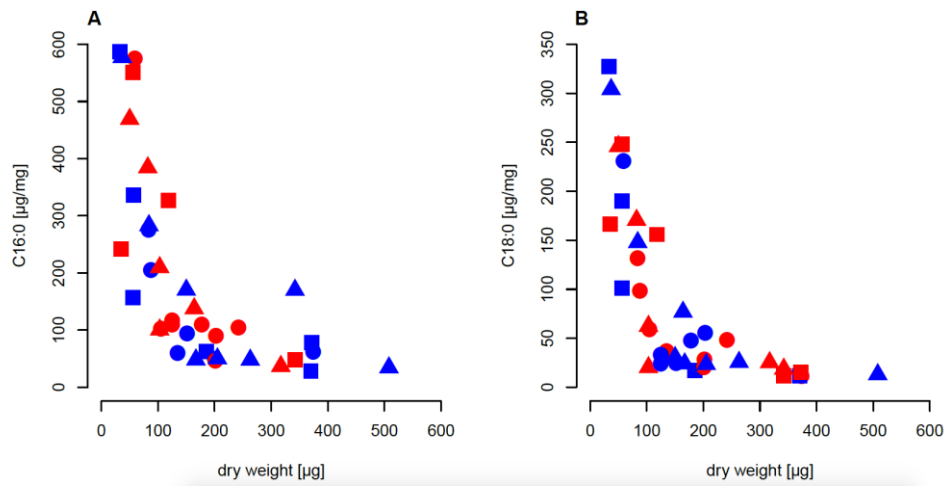


Fig. 2 – Decrease of relative amounts of saturated NLFAs with increasing body size in *M. rubra* larvae. Red = high-fat treatment. Blue = low-fat treatment.

S6 – Factor loadings for Principal Components Analyses

Formica fusca – main text, Fig. 5A

	PC1	PC2
C12:0	-1.1145	-0.2729
C14:0	-0.9414	-0.2072
C16:0	-0.1976	0.2512
C18:0	-0.2350	-0.1158
C20:0	-0.1617	-0.6004
C16:1n9	-0.7114	0.7445
C18:1n9	0.1906	0.7266
C18:2n6	0.5230	-0.7533
C18:3n3	1.4822	-0.1699
C18:3n6	1.1656	0.3972

Myrmica rubra – main text, Fig. 5B

	PC1	PC2
C12:0	-0.5150	-0.0592
C14:0	-0.6791	-0.0155
C16:0	-0.3305	-0.0970
C18:0	-0.5435	-0.3657
C20:0	-0.0761	-0.6645
C16:1n9	-0.8402	0.4124
C18:1n9	0.4310	1.0472
C18:2n6	0.4074	-0.0984
C18:3n3	1.6567	-0.2649
C18:3n6	0.4893	0.1057

Annex IV

Unveiling community patterns and trophic niches of tropical and temperate ants using an integrative framework of field data, stable isotopes and fatty acids

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Author contributions: FBR provided the initial idea of the article. FBR, NB, AB, FM and MH designed the experiments¹. FBR and GG collected the data². FBR and AB performed data analysis³. FBR drafted the manuscript. All authors contributed to the final manuscript. All authors read and approved the final manuscript.

¹FBR: choices of study sites and sample sizes; choice and design of resources offered (with FM).

²FBR: all fieldwork, sample sorting and fatty acid analysis, with support of trainee students.

³FBR: all taxonomic identifications and statistical analysis.

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Unveiling community patterns and trophic niches of tropical and temperate ants using an integrative framework of field data, stable isotopes and fatty acids

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ABSTRACT

Background: The use and partitioning of trophic resources is a central aspect of community function. On the ground of tropical forests, dozens of ant species may be found together and ecological mechanisms should act to allow such coexistence. One hypothesis states that niche specialization is higher in the tropics, compared to temperate regions. However, trophic niches of most species are virtually unknown. Several techniques might be combined to study trophic niche, such as field observations, fatty acid analysis (FAA) and stable isotope analysis (SIA). In this work, we combine these three techniques to unveil partitioning of trophic resources in a tropical and a temperate community. We describe patterns of resource use, compare them between communities, and test correlation and complementarity of methods to unveil both community patterns and species' niches.

Methods: Resource use was assessed with seven kinds of bait representing natural resources available to ants. Neutral lipid fatty acid (NLFA) profiles, and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope signatures of the species were also obtained. Community patterns and comparisons were analyzed with clustering, correlations, multivariate analyses and interaction networks.

Results: Resource use structure was similar in both communities. Niche breadths (H') and network metrics (Q and H_2') indicated similar levels of generalization between communities. A few species presented more specialized niches, such as *Wasmannia auropunctata* and *Lasius fuliginosus*. Stable isotope signatures and NLFA profiles also indicated high generalization, although the latter differed between communities, with temperate species having higher amounts of fat and proportions of C18:1n9. Bait use and NLFA profile similarities were correlated, as well as species' specialization indices (d') for the two methods. Similarities in $\delta^{15}\text{N}$ and bait use, and in $\delta^{13}\text{C}$ and NLFA profiles, were also correlated.

Discussion: Our results agree with the recent view that specialization levels do not change with latitude or species richness. Partition of trophic resources alone does not explain species coexistence in these communities, and might act together with

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page 25

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behavioral and environmental mechanisms. Temperate species presented NLFA patterns distinct from tropical ones, which may be related to environmental factors. All methods corresponded in their characterization of species' niches to some extent, and were robust enough to detect differences even in highly generalized communities. However, their combination provides a more comprehensive picture of resource use, and it is particularly important to understand individual niches of species. FAA was applied here for the first time in ant ecology, and proved to be a valuable tool due to its combination of specificity and temporal representativeness. We propose that a framework combining field observations with chemical analysis is valuable to understand resource use in ant communities.

Subjects Biodiversity, Ecology, Entomology

Keywords Formicidae, Trophic niche, Baits, Fatty acids, Stable isotopes, Atlantic forest, Temperate forest, Trophic ecology, Methodology, Food resources

INTRODUCTION

The use and partitioning of trophic resources is a central aspect of community functioning. Trophic interactions govern the flux of matter and energy in food webs, and lead to other fundamental interactions such as competition and mutualism (*Polis & Strong, 1996; Reitz & Trumble, 2002*). Trophic niche partitioning is one of the most important mechanisms allowing species coexistence, and may ultimately link to evolutionary processes of adaptation and character displacement (*Schluter, 2000*).

Ants (Hymenoptera: Formicidae) are among the most abundant groups of invertebrates in terrestrial ecosystems, presenting a wide range of feeding habits, nesting sites and interactions with organisms from all trophic levels. In general they are regarded as omnivorous, feeding on a combination of living prey, dead arthropods, seeds and plant exudates (*Blüthgen & Feldhaar, 2010; Lanan, 2014*). On the ground of tropical forests, dozens of species may coexist at the same spot, which raises the question: how ecologically different are these species? Although the role of interspecific competition in ant communities has recently been hotly debated (*Cerdá, Arnan & Retana, 2013*), the combination of high species richness with high biomass may lead to evolutionary pressure for more diversified niches. *MacArthur (1972)* suggested that specialization increases in tropical communities and, as a result, more species can coexist. However, this idea was put in question by recent studies (*Schleuning et al., 2012; Morris et al., 2014; Frank et al., 2018*). For ants, behavioral and environmental mechanisms of coexistence have been proposed (*Cerdá, Retana & Cros, 1997; Andersen, 2000; Parr & Gibb, 2012*). The use of food resources itself is surprisingly understudied, and trophic niches of most species remain poorly known. This is particularly evident in rich tropical communities (*Rosumek, 2017*), but also true for some temperate species (*Lanan, 2014*).

Field observations are the most straightforward way of gathering information, but there are trade-offs between the number of species studied (e.g., single species natural history vs. community patterns; *Medeiros & Oliveira, 2009; Houadria et al., 2015*),

the number of resources assessed (e.g., protein/sugar comparisons vs. all resources collected by workers; *Kaspari & Yanoviak, 2001; Lopes, 2007*) and the sampling intensity (e.g., seasonal studies vs. temporal “snapshots”; *Albrecht & Gotelli, 2001; Rosumek, 2017*). Moreover, many species present cryptic habits, and the sheer complexity of interactions makes the assessment of trophic niches a laborious task. Baiting is a method widely used in ant ecology to assess communities and infer resource use (*Bestelmeyer et al., 2000*), but it is affected by the aforementioned drawbacks.

Several techniques have been applied in ecology to deal with these issues, among them stable isotope analysis (SIA) and fatty acid analysis (FAA). Indirect techniques could be faster and reduce fieldwork effort, but also rely on several assumptions to interpret their results. Since every method has its assets and caveats, the choice depends on the nature of the questions being asked (*Birkhofer et al., 2017*). However, this also works the other way around: complementary methods can be combined to provide a detailed and integrative perspective on the community being studied.

Stable isotopes have been widely applied to address several questions in ant biology (*Feldhaar, Gebauer & Blüthgen, 2010*). Most commonly used are the relative abundance of heavy nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) (*Hyodo, 2015*). $\delta^{15}\text{N}$ increases predictably when one organism consumes another, thus indicating whether species are at the top or bottom of the food web (*Heethoff & Scheu, 2016*). $\delta^{13}\text{C}$ could be used to distinguish between main carbon sources at the bottom of the food web because C_3 , C_4 and CAM plants have different signatures (*O’Leary, 1988; Gannes, Del Rio & Koch, 1998*). SIA provides time-representative clues about trophic position, but limited information on specific food sources or feeding behaviors. For instance, if two species feed exclusively on primary consumers, they will have similar $\delta^{15}\text{N}$, regardless of what prey items they actually consume, or whether the food is obtained through predation or scavenging. As such, stable isotope signatures are not suitable to calculate niche breadth or overlap, or to be analyzed as species-resources interaction networks.

Fatty acids obtained from the diet are mainly stored as neutral lipid fatty acids (NLFAs) in the fat body of insects. Some fatty acids can be synthesized de novo by organisms, from carbohydrates or other fatty acids. Synthesis of C16:0, C18:0 and C18:1n9 (palmitic, stearic and oleic acids) is widespread, and they are the most abundant NLFAs in insects (*Stanley-Samuelson et al., 1988*; but see *Thompson, 1973*). Ability to synthesize other NLFAs is highly variable among taxonomic groups, such as C18:2n6 (linoleic acid; *Malcicka, Visser & Ellers, 2018*). When fatty acids are reliably assigned to specific food sources, they may act as biomarkers (*Ruess & Chamberlain, 2010*). Even when such biomarkers are not identified, all fatty acids assimilated without modification (i.e., through direct trophic transfer) influence the composition of the fat reserves, including the relative amounts of de novo-synthesized NLFAs. Hence, the stored fat preserves information on ingested carbon sources, and NLFA profiles can be compared to infer niche differences (*Budge, Iverson & Koopman, 2006*). However, the application of FAA in field studies of terrestrial organisms still is limited. Most studies focused on soil detritivores, such as collembolans and nematodes (*Ruess et al., 2007; Haubert et al., 2009; Ngosong et al., 2009*). So far, FAA was not used to study trophic ecology of ants.

In this work, we combine field observations with SIA and FAA to unveil the use and partitioning of trophic resources in a tropical and a temperate epigeic ant community. Our main goal is to describe patterns for each community and test differences between communities and methods. Specifically, we aim to: (1) assess use of multiple resources, stable isotope signatures and NLFA profiles of the most abundant species in both communities; (2) compare patterns between communities using descriptive and statistical approaches; (3) test whether different methods provide convergent or complementary information on patterns of resource use.

MATERIALS AND METHODS

Baiting

Fieldwork in Brazil was carried out in Florianópolis (Desterro Conservation Unit, 27°31'38"S, 48°30'15"W, altitude ca. 250 m), in December 2015 and January 2016, under sampling permit SISBIO 51173-1 (ICMBio), and export permits 15BR019038/DF and 17BR025207/DF (IBAMA). The vegetation consists of a secondary Atlantic forest with at least 60 years of regeneration. High rainfall rate along the coast results in high productivity, ant species richness and a tropical aspect for the Atlantic forest even at higher latitudes such as in our work (*Silva & Brandão, 2014*). In Germany, it was carried out in Darmstadt (Prinzenberg, 49°50'14"N, 08°40'01"E, altitude ca. 250 m), in July 2015 (no permits needed there). The vegetation consists of patches of mixed forest, beech forest and orchards, which were all covered by the sample grids.

Sampling design followed similar protocols in both sites ([Table 1](#)). Seven bait types were offered as proxies for resources that are widely used by ants in general (*Kaspari, 2000*; *Blüthgen & Feldhaar, 2010*; *Lanan, 2014*; for a full description of baits, see [Supplemental Document S1](#)). Sample points were distributed in grids and separated by 10 m. In each sampling session, only a single bait was offered per point, and bait types were randomized among points. Baits were set up in transparent plastic boxes and retrieved after 90 min. This procedure was repeated in different days until all bait types had been offered at each point (twice in Brazil). The design was based on *Houadria et al. (2015)* and evaluates use of multiple resources, differing from a typical cafeteria experiment, which is designed to assess preferred resources (*Krebs, 1999*).

Pitfall sampling

We performed a concomitant pitfall assessment to verify whether bait records represented well the epigeic community ([Table 1](#)). One vial per sample point was previously buried to avoid the digging-in effect (*Greenblade, 1973*), and replaced after collection for the next round. Pitfall and bait sampling were not performed simultaneously at the same point. Vials were buried at ground level, had six cm diameter and 150 ml volume, and contained 40 ml propylene glycol 50%.

Fatty acid analysis

In Brazil, samples were obtained from baits and complemented by colony sampling in November 2017. We only used ants from melezitose, sucrose and seed baits, to avoid

Table 1 Details of the sampling design applied in this study.

	Brazil	Germany
Sampling effort		
Sampling points	64	80
Period of the day	Day and night	Day
Baits	64 per resource per period (= 896 baits)	80 per resource (= 560 baits)
Pitfall sampling	Three 10-h rounds per period (= 60 h)	Three 12-h rounds (= 36 h)
Resource represented		
Larger, faster and harder prey		Living crickets (<i>Achaeta domesticus</i> Linnaeus, 1758)
Smaller, slower and softer prey	Living termites (Nasutitermitinae)	Living maggots (<i>Lucilia sericata</i> Meigen, 1826)
Dead arthropods	Crushed crickets and maggots/mealworms (<i>Tenebrio molitor</i> Linnaeus, 1758)	
Bird droppings	Chicken feces from organic breeding	
Seeds	Seed mixture of diverse sizes and shapes, without elaiosomes	Seeds of <i>Chelidonium majus</i> (L.), with elaiosomes
Oligosaccharides in honeydew	Melezitose 20%	
Disaccharides in nectar and fruits	Sucrose 20%	

interference of bait lipids. In Germany, they were obtained by colony sampling between July and August 2017. Samples were frozen at -18°C directly from the field. Total lipids were extracted from the ants whole body using one ml chloroform:methanol solution, 2:1 (v/v). The solution was applied to SiOH-columns and the neutral parcel (= mono-, di- and triglycerides) eluted with four ml chloroform. Samples were analyzed with gas chromatography–mass spectrometry, following the same procedures described in [Rosumek et al. \(2017\)](#). NLFA amounts were obtained comparing their proportions to an internal standard (C19:0 in methanol; $\rho_i = 220 \text{ ng}/\mu\text{l}$). Ants were subsequently dried to obtain their lean dry weight (= without lipids).

Stable isotope analysis

Ants collected in baits and conserved in ethanol 70% were used to analyze $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. C and N isotope abundances were measured in a dual element analysis mode with an elemental analyzer coupled to a continuous flow isotope ratio mass spectrometer as described in [Bidartondo et al. \(2004\)](#). Relative abundances were calculated following the equation: $\delta_x = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1,000$ [‰], where R denotes the ratio between heavy and light isotopes of samples and international standards (N_2 in the air and CO_2 in PeeDee belemnite). Gasters were removed prior to analysis to avoid interference of gut content ([Blüthgen, Gebauer & Fiedler, 2003](#)).

Taxonomic identification

Ants were identified with taxonomic revisions, and comparison to identified specimens in collections and AntWeb images ([AntWeb, 2016](#)). Updated names were checked with

Antcat (Bolton, 2018). Identifications were partially confirmed by taxonomists (see Acknowledgements).

In Brazil, ants were identified to genus level with Baccaro *et al.* (2015) and to species level with: *Acanthognathus*—Galvis & Fernández (2009); *Acromyrmex*—Gonçalves (1961); *Cephalotes*—De Andrade & Baroni Urbani (1999); *Crematogaster*—Longino (2003); *Cyphomyrmex*—Kempf (1965) and Snealling & Longino (1992); *Gnamptogenys*—Lattke (1995); *Hylomyrma*—Kempf (1973); *Linepithema*—Wild (2007); *Octostruma*—Longino (2013); *Odontomachus* and *Pachycondyla*—Fernández (2008); *Pheidole*—Wilson (2003); *Wasmannia*—Longino & Fernández (2007). *Camponotus* and *Strumigenys* were identified solely by comparison with collections.

In Germany, ants were identified to genus and species with Seifert (2007), Seifert & Schultz (2009) and Radchenko & Elmes (2010).

All material is stored in the collections of the Ecological Networks research group, Technische Universität Darmstadt, Darmstadt, Germany and Department of Ecology and Zoology, Federal University of Santa Catarina, Florianópolis, Brazil.

Data analysis

As a first step, we compared species' incidences in baits and pitfalls (i.e., number of sampling points where it was recorded with each method). We assumed incidence in pitfalls to represent abundance in the community, and qualitatively compared it to incidence in baits to check whether common species were underrepresented in baits. To account for different efficiencies between methods, expected incidences were indicated by a line of slope $m = I_{\text{baits}}/I_{\text{pitfalls}}$, where I is the sum of all incidences for each method (Houadria *et al.*, 2015).

Number of species, replicates and individuals per sample differed between methods, based on sample availability and ant size. In Brazil, we analyzed 24 species for baits, 41 for FAA and 31 for SIA. Method comparisons were performed only with 22 species considered in all three datasets. In Germany, seven species were analyzed with all methods. For a full list of recorded species and respective labels used in plots, see Table S1.

Unless noted otherwise, similarity matrices were based on unweighted Bray–Curtis dissimilarities, and Mantel tests and correlations used Spearman's coefficient (ρ). Analyses were run in R 3.4.3 (R Core Team, 2017) and PAST 3.14 (Hammer, Harper & Ryan, 2001).

For all bait analyses, we used proportion of occurrence on each bait type, relative to total records for each species. Only species with at least 10 records from five or more sample points were considered. In Brazil, day and night records were considered as independent to calculate proportions. For FAA, we calculated proportions of each NLFA relative to total composition, and used average proportions for each species. All NLFAs with average proportion >0.01% were considered. For SIA, we also used species' averages and analyzed $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ separately, using Euclidean distances to build similarity matrices. A special case was *Lasius fuliginosus* in Germany, which was represented by a single colony that foraged over a large area. Bait records from different sample points were considered independent, and chemical results represent the average of different samples from that colony.

To analyze resource use, we used clustering and network analysis. UPGMA clustering was used for species, to show functional groups based on similar use of resources, and for baits, to show the structure of resource use in each community. Statistical significance of clusters was tested with SIMPROF (Clarke, Somerfield & Gorley, 2008) using the package “clustsig” (Whitaker & Christman, 2015). A Mantel test was used to compare similarity matrices of Brazil and Germany.

For network analysis, we used quantitative modularity (Q) (Dormann & Strauss, 2014) and specialization indices for species/resources (d') and whole networks (H'_2) (Blüthgen, Menzel & Blüthgen, 2006), using the package “bipartite” (Dormann, Freund & Gruber, 2017). Modularity shows how compartmentalized is a community, that is, if there are groups of species that strongly interact with groups of resources. In turn, d' indicates whether individual species are specialized in certain resources, or resources that are used by a specialized group of species. H'_2 is an extension of d' and shows how specialized the network is overall. $H'_2 = 0$ would mean that all species used resources in the same proportions, and $H'_2 = 1$ that each species has its exclusive pattern of resource use.

Specialization indices were also used to analyze species \times fatty acids contingency tables. In this case, they indicate how exclusively NLFAs are distributed across species (Brückner & Heathoff, 2017). $H'_2 = 0$ would mean that all compounds occur in the same proportion in all species, and $H'_2 = 1$ that each species has its exclusive compounds. Correspondingly, relatively high d' represents NLFAs that occur more exclusively in certain species, or species with more exclusive proportions of certain NLFAs. Low d' means a compound that is widespread among species, or species with similarly generalized profiles. Additionally, we tested whether the two communities differed in their overall NLFA composition with PERMANOVA, using site as a fixed factor (Anderson, 2001). Homogeneity of multivariate dispersion was tested a priori with PERMDISP (Anderson & Walsh, 2013). To detect which NLFAs contributed to differences, we used SIMPER (Clarke, 1993). These tests were performed using package “vegan” (Oksanen et al., 2017).

To test whether niche breadths and NLFA profile diversity were different between communities at species level, we calculated Shannon diversity indices for each ant species as $H' = -\sum p_i \ln p_i$, where p_i is the proportion of each resource i used by the species, or NLFA found in its profile, and compared them with Mann–Whitney tests.

To test whether particular NLFAs were related to use of certain resources, we performed principal component analyses (PCA) using baits \times species contingency tables, replacing zeros by small values (0.000001) and using centered log-ratio transformation to deal with the constant-sum constraint (Brückner & Heathoff, 2017). PC axes were correlated with NLFAs using function “envfit” from package “vegan.” We also compared proportions, amounts (in $\mu\text{g}/\text{mg}$; the amount of fat divided by lean dry weight) and unsaturation indices (UI; the sum of percentages of each unsaturated NLFA multiplied by its number of double bounds) between Brazil and Germany with Mann–Whitney tests. We did this for total fat and the three most abundant NLFAs (C16:0, C18:0 and C18:1n9). To test whether there was a direct relationship between total fat amount and C18:1n9, or total amount and UI, we correlated values for all individual samples of each community (166 in Brazil, 32 in Germany).

Finally, to test whether the results yielded by the three methods were correlated, we performed Mantel tests between similarity matrices of species for each method. We also correlated species' d' values for baits and NLFAs, to test whether specialization levels were related.

RESULTS

Use of resources

Most common species were recorded in baits in proportions similar to the expected, given their frequency in the community (Fig. S1 and Table S1). A few species were underrepresented in baits (e.g., *Pachycondyla harpax*, *Myrmica scabrinodis*, *Stenammina debile*), but, in general, species with few bait records were also rare in pitfalls. Thus, we consider that a representative part of the epigeic communities was properly sampled. Despite strong variation in total number of records, the number of species recorded in each bait was similar, with exception of large prey (Table 2).

Similarities in resource use were correlated between Brazil and Germany (Fig. 1, Mantel test, $\rho = 0.63$, $p = 0.03$). In both communities, large prey was set apart from the other resources, being used less frequently and by fewer species. Seeds and melezitose changed positions between communities. In Germany, all ants used both sugars indiscriminately, while in Brazil several species used more sucrose (e.g., *Camponotus zenon*, *Gnamptogenys striatula*, *Pachycondyla striata*, *Odontomachus chelifer*, *Solenopsis* sp.6) and others used more melezitose (e.g., *Pheidole aper*, *Solenopsis* sp.8, *Wasmannia affinis*) (Table 2). Both modularity ($Q_{BR} = 0.16$, $Q_{GE} = 0.14$) and network specialization ($H'_{2BR} = 0.13$, $H'_{2GE} = 0.12$) were relatively low and similar between sites. Species used resources in different ways and a few were more specialized (see below), but there were no clear links between particular resources and species or groups of species.

In Brazil, *W. auropunctata* occupied a highly specialized niche, using only feces baits, which lead to the highest d' values for any species and resource. *Linepithema iniquum* also showed a relatively higher specialization level due to its preference for dead arthropods and low use of sugars. *P. striata* and *O. chelifer* used more large prey, dead arthropods and sucrose. *C. zenon* grouped with them based on use of dead arthropods and sucrose, but avoided large prey. *P. aper* was the only species to have melezitose as its preferred resource. Other species showed higher redundancy and clustered together, including all *Solenopsis* and most *Pheidole* (Fig. 1; Table 2).

In Germany, only *L. fuliginosus* showed a relatively high specialization level and clustered separately, due to its almost exclusive use of animal resources (living prey and dead arthropods). Other species showed low specialization and dissimilarity (Fig. 1; Table 2).

Niche breadths were similar between communities (Mann–Whitney, $p = 0.44$). Average Shannon index was 1.6 ± 0.4 SD in Brazil and 1.7 ± 0.1 SD in Germany (Table 2).

Fatty acids

Temperate species contained much higher amounts of total fat than tropical ones (Fig. 2). Fatty acid compositions changed between communities (PERMANOVA, $r^2 = 0.35$,

Table 2 Resource use of ant species in Brazil and Germany. Values for the seven baits are given in % of the total records for each species. Only species with at least 10 records from five sample points are listed.

Species	Large prey	Small prey	Dead arthropods	Feces	Seeds	Melezitose	Sucrose	d'	Shannon index	Records
Brazil										
<i>Camponotus zenon</i>	–	14	36	7	7	7	29	0.06	1.6	14
<i>Gnamptogenys striatula</i>	2	23	21	19	11	6	17	0.04	1.8	47
<i>Linepithema iniquum</i>	10	10	50	20	–	10	–	0.16	1.4	10
<i>Linepithema micans</i>	–	6	31	6	19	19	19	0.03	1.7	16
<i>Nylanderia</i> sp.1	7	10	25	6	9	23	21	0.03	1.8	267
<i>Odontomachus chelifer</i>	26	5	19	5	5	10	31	0.12	1.7	42
<i>Pachycondyla striata</i>	14	3	42	–	1	6	34	0.16	1.3	88
<i>Pheidole aper</i>	4	–	15	19	7	37	19	0.08	1.6	27
<i>Pheidole lucretii</i>	4	4	26	8	14	20	24	0.02	1.8	50
<i>Pheidole nesiota</i>	4	9	20	4	16	25	21	0.02	1.8	89
<i>Pheidole sarcina</i>	4	8	16	14	20	18	22	0.01	1.9	51
<i>Pheidole sigillata</i>	4	10	24	10	16	13	22	0.00	1.8	91
<i>Pheidole</i> sp.1	3	13	19	10	18	17	21	0.01	1.9	101
<i>Pheidole</i> sp.2	6	11	14	14	21	20	15	0.01	1.9	322
<i>Pheidole</i> sp.4	5	6	21	19	14	14	21	0.01	1.9	78
<i>Pheidole</i> sp.7	6	6	6	6	41	18	18	0.08	1.6	17
<i>Solenopsis</i> sp.1	4	14	18	13	26	12	13	0.01	1.8	141
<i>Solenopsis</i> sp.2	2	14	24	7	28	13	13	0.03	1.8	180
<i>Solenopsis</i> sp.3*	–	4	16	8	32	20	20	0.05	1.6	25
<i>Solenopsis</i> sp.4	1	5	21	10	31	14	18	0.03	1.7	96
<i>Solenopsis</i> sp.6	2	10	29	7	17	10	26	0.02	1.7	42
<i>Solenopsis</i> sp.8	7	11	29	7	25	14	7	0.03	1.8	28
<i>Wasmannia affinis</i>	–	25	10	10	30	20	5	0.09	1.6	20
<i>Wasmannia auropunctata</i> *	–	–	–	100	–	–	–	0.62	0	19
d'	0.17	0.09	0.09	0.24	0.14	0.07	0.09	$H'_2 = 0.13$		
Total richness [†]	26	31	33	32	32	34	34			
Total records [†]	107	203	422	215	344	327	366			
Germany										
<i>Formica fusca</i>	4	5	21	2	12	26	30	0.06	1.6	57
<i>Lasius fuliginosus</i>	20	27	33	13	–	–	7	0.31	1.5	15
<i>Lasius niger</i>	7	14	19	11	9	19	20	0.01	1.9	118
<i>Lasius platythorax</i>	–	–	17	17	4	30	30	0.11	1.5	23
<i>Myrmica rubra</i>	3	13	15	13	3	25	30	0.03	1.7	40
<i>Myrmica ruginodis</i>	–	10	27	14	6	18	24	0.03	1.7	49
<i>Temnothorax nylanderii</i>	–	4	21	14	18	21	22	0.06	1.7	165
d'	0.29	0.14	0.02	0.05	0.13	0.11	0.05	$H'_2 = 0.12$		
Total richness [†]	4	8	8	8	7	9	11			
Total records [†]	14	42	99	56	54	102	116			

Notes:

* Species not considered in comparisons between methods.

† Including species with less than 10 records.

–, Species not recorded in this bait.

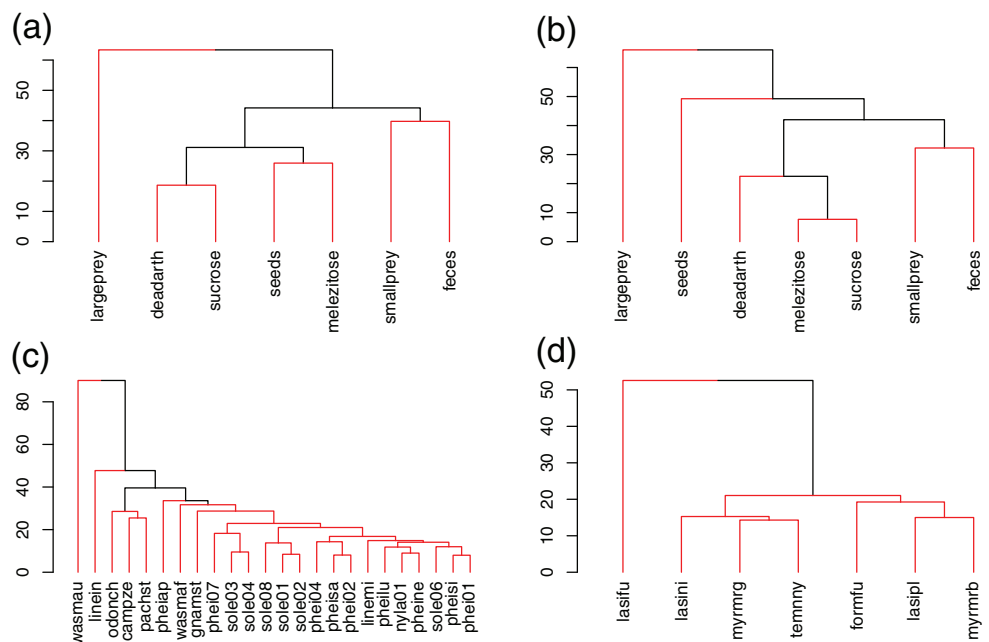


Figure 1 UPGMA clustering of resources and species in Brazil (A, C) and Germany (B, D), based on Bray–Curtis dissimilarities. Red lines link elements from the same statistically significant cluster (SIMPROF, $p < 0.05$). [Full-size !\[\]\(5fd6ef84f97f42d7f8b34275f1b65312_img.jpg\) DOI: 10.7717/peerj.5467/fig-1](https://doi.org/10.7717/peerj.5467/fig-1)

$p < 0.01$). Multivariate dispersion was heterogeneous, being higher in Brazil than in Germany (PERMDISP, $F = 11.32$, $p < 0.01$). This does not change the previous result because, in this case, PERMANOVA becomes overly conservative (Anderson & Walsh, 2013).

The main reason for this difference was the predominant role of C18:1n9 in temperate species (SIMPER, dissimilarity contribution = 47%, $p < 0.01$, Figs. 2 and 3; Table 3). In Brazil, composition was more balanced, which led to higher proportions of C18:0 (contribution = 21%, $p < 0.01$), although amounts were similar. C16:0 was proportionally the most abundant NLFA in Brazil and the difference from Germany was marginally significant (contribution = 20%, $p = 0.06$), although amounts again were higher in temperate species. A few other NLFAs had statistically significant, but very small contributions to the difference (Table S2).

Fatty acid compositions were generalized overall, but more homogeneous in Germany because of the predominance of C18:1n9 ($H'_{2BR} = 0.09$, $H'_{2GE} = 0.03$). Accordingly, NLFA profile diversity was higher in tropical species (average Shannon index = 1.5 ± 0.2 SD) than temperate ones (0.9 ± 0.2 SD) (Mann–Whitney, $p < 0.01$) (Fig. 3; Table 3).

In samples from Germany, there was no correlation between total amount of fat and percentage of C18:1n9 ($\rho = 0.19$, $p = 0.30$) or unsaturation index ($\rho = 0.24$, $p = 0.89$). In samples from Brazil, there was weak negative correlation between total fat and both C18:1n9 ($\rho = -0.16$, $p = 0.04$) and unsaturation index ($\rho = -0.22$, $p > 0.01$).

In Brazil, several fatty acids were related to resource use (Fig. 4; see Table S3 for PCA eigenvalues and full Envfit results). Species with higher C18:1n9 also used more dead

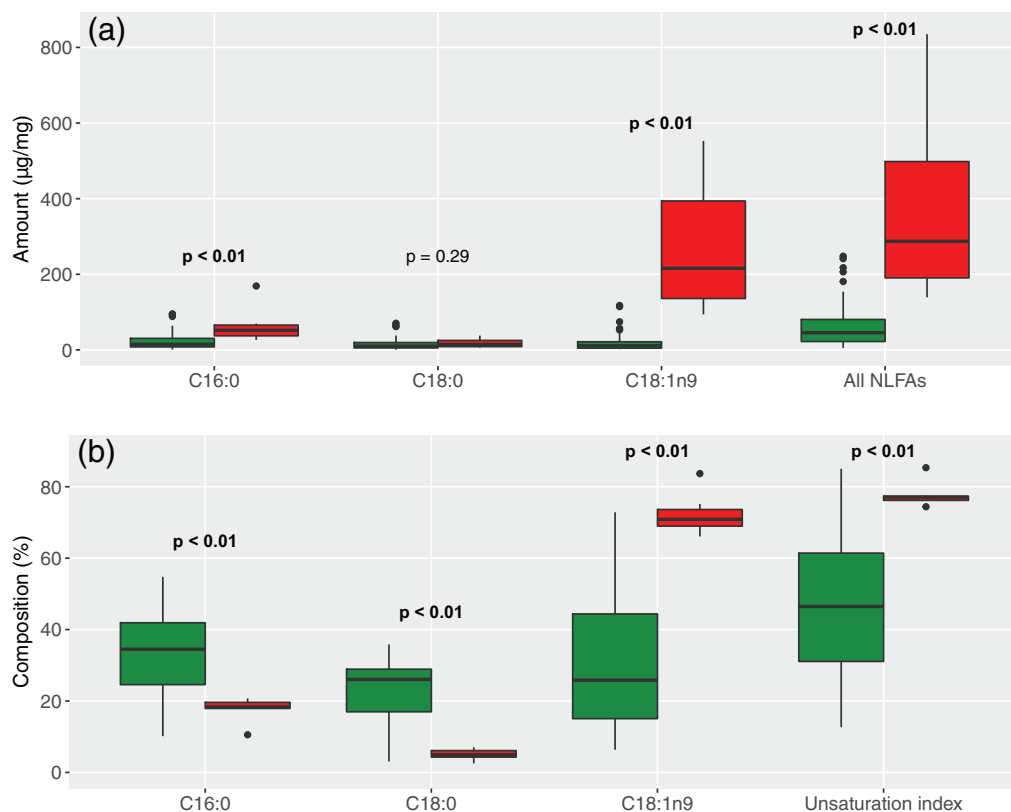


Figure 2 Comparison of the three most abundant NLFAs, total amounts and unsaturation indices between tropical and temperate species. (a) Amounts; (b) percentages. Green = tropical species; red = temperate species. Significant differences are in bold (Mann-Whitney test).

Full-size [DOI: 10.7717/peerj.5467/fig-2](https://doi.org/10.7717/peerj.5467/fig-2)

arthropods (Envfit, r^2 of the NLFA with PC axes = 0.32, $p = 0.02$), while C18:2unk1 (an unidentified NLFA) was related to use of sucrose and large prey ($r^2 = 0.31$, $p = 0.03$). C14:0 (myristic acid) tended to be higher in species that used more seeds, feces and small prey ($r^2 = 0.39$, $p = 0.01$). Notice that the first two Principal Components explained only 60% of the variance and linear regressions were not strong. In Germany, most variation was along the sugar-protein axis. C18:0 and C17:0 (margaric acid) were strongly correlated with PC axes ($r^2 = 0.84$, $p = 0.05$ and $r^2 = 0.78$, $p = 0.04$, respectively). Both were higher in species that used more sugars, and C17:0 also was related to use of feces, although its relative abundance was very low in all species (<0.5%, Table 3).

Stable isotopes

In Brazil, *W. auropunctata* presented distinctive signatures for both isotopes. It was the species with highest $\delta^{15}\text{N}$, while most species ranged from 5.8 to 8.2, and six showed conspicuously lower signatures. Besides *W. auropunctata*, $\delta^{13}\text{C}$ varied less, ranging from -24.1 to -27 (Fig. 5; Table 4).

In Germany, $\delta^{15}\text{N}$ was lower overall, ranging from 3.6 (*Lasius niger*) to -1.1 (*Lasius fuliginosus*). Species varied little in $\delta^{13}\text{C}$ (from -25.4 to -26.3), with values within the range of Brazilian species (Fig. 5; Table 4).

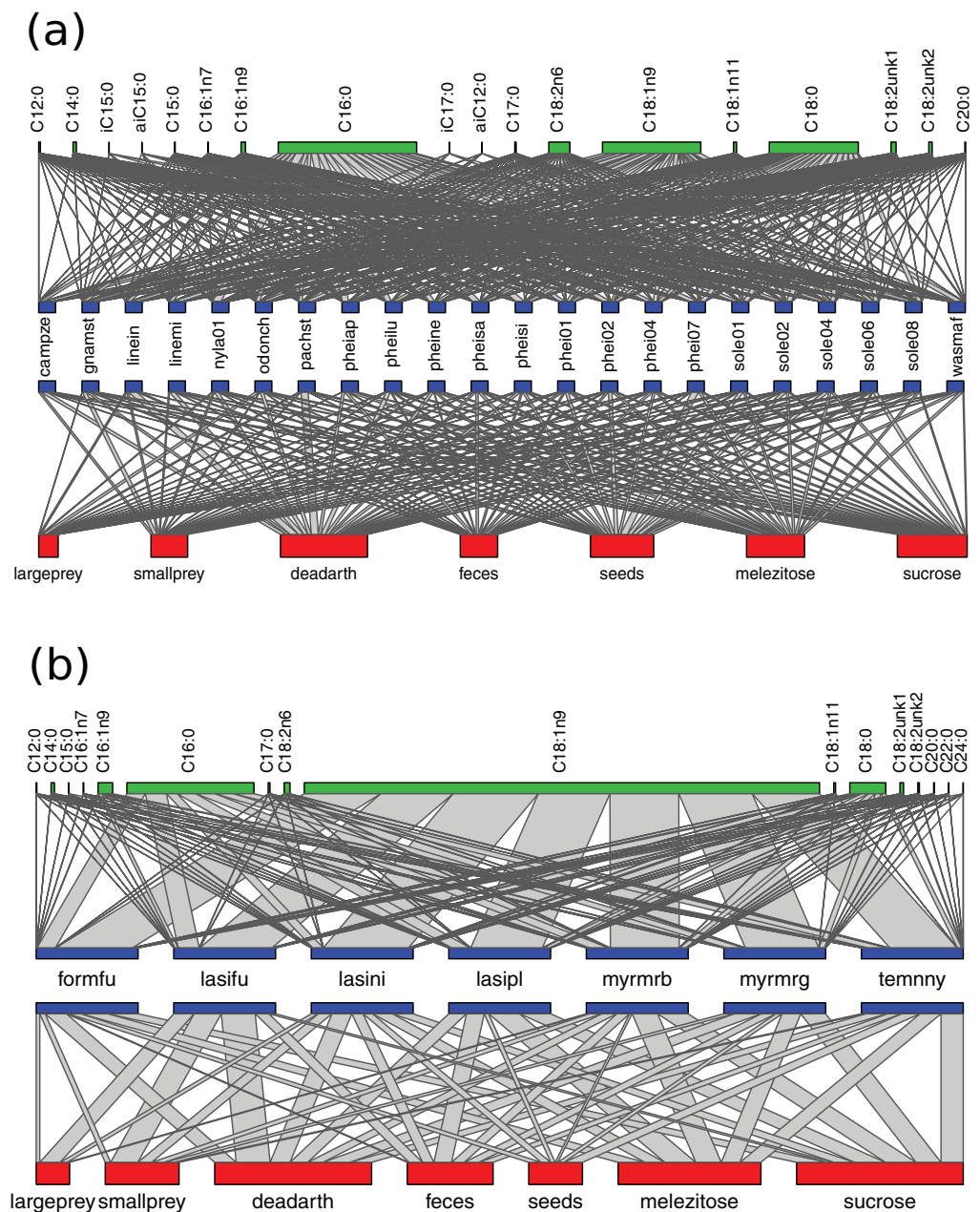


Figure 3 Networks of resource use and fatty acid composition in Brazil (A) and Germany (B). Species labels are standardized to represent 100% of resource use/NLFA composition. The width of connecting lines represents proportion of bait use/NLFA abundance for each species. Bait/NLFA labels show the sum of proportions for the whole community. Only species analyzed with all three methods are included.

Full-size  DOI: [10.7717/peerj.5467/fig-3](https://doi.org/10.7717/peerj.5467/fig-3)

Isotope signatures were correlated for tropical species ($\rho = 0.43$, $p = 0.02$). For temperate species, the correlation lacked statistical significance ($\rho = -0.46$, $p = 0.3$), but their inclusion slightly strengthened the correlation for all species together ($\rho = 0.47$, $p < 0.01$).

Table 3 Fatty acid profiles of ant species in Brazil and Germany. Average individual NLFA abundances are given in % of the total composition.

Species	C12:0	C14:0	C15:0	alC15:0	iC15:0	C16:0	C16:1n7	C16:1n9	C17:0	alC17:0	iC17:0	C18:0	C18:1n9	C18:1n11	C18:2n6	C18:2nkl	C18:2nkk	C20:0	C22:0	C24:0	d'	Shannon	Amount (µg/mg)	UI	Samples
Brazil																									
<i>Acanthognathus brevicornis</i> *	0.3	1.0	0.4	-	0.3	28	0.3	0.3	0.5	0.04	0.1	24	24	2.0	11	3.6	2.8	0.8	-	-	0.01	1.8	60	61	2
<i>Acanthognathus ocellatus</i> *	0.4	0.9	0.3	0.01	0.04	38	0.1	0.4	0.4	-	-	30	21	1.1	5.4	1.4	1.0	0.5	-	-	0.01	1.5	65	38	2
<i>Acromyrmex aspersus</i> *	0.3	1.3	0.1	-	-	31	0.1	4.3	0.1	-	-	21	29	1.2	5.7	2.4	1.8	0.8	-	-	0.01	1.7	13	55	2
<i>Acromyrmex laticeps</i> *	0.2	0.2	0.1	-	-	10	-	0.2	0.5	-	-	16	37	1.1	2.3	6.0	5.1	0.5	-	-	0.09	1.7	8	106	1
<i>Acromyrmex lundii</i> *	0.2	0.4	0.0	-	-	13	-	0.4	0.2	-	-	21	44	1.8	11	4.2	3.7	0.4	-	-	0.04	1.6	6	84	1
<i>Acromyrmex subterraneus</i> *	0.1	0.2	0.1	0.01	0.03	10	0.2	0.5	0.4	0.03	0.01	18	44	1.2	17	4.0	3.6	0.5	-	-	0.06	1.6	30	95	2
<i>Azteca sp.2</i> *	0.5	0.3	0.0	-	-	13	0.1	0.4	0.1	-	-	10	73	0.8	1.0	0.7	0.5	0.2	-	-	0.10	1.0	62	78	3
<i>Camponotus lespeii</i> *	0.3	0.4	0.2	-	-	30	0.2	1.4	0.1	-	-	18	48	0.6	0.6	0.3	0.2	0.2	-	-	0.03	1.3	11	52	2
<i>Camponotus zenon</i>	0.6	1.0	0.2	-	-	28	0.2	2.8	0.3	-	-	15	50	0.8	0.8	0.2	0.1	0.1	-	-	0.03	1.3	5	56	2
<i>Cephalotes pallidicephalus</i> *	0.1	0.8	0.0	0.1	0.1	25	-	6.0	0.1	-	-	3	60	4.4	0.5	-	-	0.4	-	-	0.11	1.2	95	71	2
<i>Cephalotes pusillus</i> *	0.7	1.0	0.2	-	-	17	3.1	2.5	0.3	-	-	12	61	1.9	0.4	-	-	0.8	-	-	0.09	1.3	36	69	1
<i>Crematogaster nigropilosa</i> *	0.2	1.0	0.0	-	-	27	0.3	0.5	0.2	-	-	17	48	0.6	3.1	1.1	0.6	0.4	-	-	0.02	1.4	154	59	6
<i>Cyphomyrmex rimosus</i> *	0.5	1.6	0.2	-	-	38	0.1	2.7	0.3	0.04	-	34	15	1.0	3.7	1.0	0.8	0.5	-	-	0.02	1.5	86	30	4
<i>Gnamptogenys striatula</i>	0.2	1.0	0.3	0.01	0.3	27	0.5	1.1	0.6	0.1	0.2	18	39	2.4	6.0	1.9	1.4	0.7	-	-	0.01	1.7	56	61	6
<i>Hylomyrma reitteri</i> *	0.7	1.1	-	-	-	55	-	-	0.4	-	-	29	9	0.6	3.0	1.1	0.6	-	-	-	0.05	1.2	22	19	1
<i>Linepithema iniquum</i>	0.1	0.8	0.1	-	-	26	0.4	4.2	0.2	-	-	15	47	0.9	2.6	1.1	0.7	0.4	-	-	0.02	1.5	248	62	3
<i>Linepithema micans</i>	0.4	0.7	0.1	-	-	24	0.1	0.2	0.2	-	-	16	56	0.5	1.4	0.3	0.2	0.2	-	-	0.04	1.2	94	61	4
<i>Nyländeria sp.1</i>	0.4	0.7	0.2	0.01	-	49	0.1	2.1	0.2	-	-	28	15	0.7	3.1	0.4	0.3	0.1	-	-	0.03	1.3	31	25	11
<i>Octostruma petiolata</i> *	0.5	1.4	0.3	0.1	0.2	42	0.3	1.4	0.8	0.1	0.1	36	12	1.2	2.0	0.6	0.4	0.5	-	-	0.03	1.4	85	21	4
<i>Odontomachus chelifer</i>	0.2	0.7	0.2	0.1	0.1	23	0.3	1.9	0.6	0.1	0.1	16	38	1.7	9.4	4.2	2.5	0.8	-	-	0.02	1.8	17	74	10
<i>Pachycondyla harpax</i> *	0.3	0.5	0.1	0.1	0.1	19	0.1	0.3	0.6	-	-	22	40	0.8	9.0	3.1	2.9	0.3	-	-	0.02	1.6	12	72	1
<i>Pachycondyla striata</i>	0.1	0.5	0.1	0.02	0.1	19	0.1	1.2	0.4	0.03	0.1	13	46	1.1	13	3.7	2.0	0.4	-	-	0.04	1.6	47	85	16
<i>Pheidole aper</i>	0.6	0.8	0.1	-	-	38	0.1	0.3	0.3	-	-	25	23	0.3	9.1	1.5	1.3	-	-	-	0.01	1.5	27	47	9
<i>Pheidole lucretii</i>	0.3	0.7	0.2	-	-	34	0.1	0.4	0.4	-	-	21	32	1.2	5.7	1.9	1.5	0.2	-	-	<0.01	1.5	59	52	12
<i>Pheidole nesioti</i>	0.4	0.7	0.1	-	-	35	0.1	0.3	0.3	-	-	27	25	0.4	6.4	2.1	1.6	0.1	-	-	<0.01	1.5	58	46	6
<i>Pheidole risii</i> *	0.6	0.7	0.2	-	-	41	0.1	0.3	0.3	-	-	30	19	0.5	5.1	1.0	0.8	0.1	-	-	0.01	1.4	38	34	1
<i>Pheidole sarcina</i>	0.5	0.8	0.1	-	-	47	0.1	0.2	0.3	-	-	32	13	0.5	4.1	0.8	0.6	0.2	-	-	0.03	1.3	20	24	1
<i>Pheidole sigillata</i>	0.7	1.1	0.2	-	-	53	0.1	0.3	0.7	-	-	34	6	0.9	1.7	0.5	0.3	0.1	-	-	0.06	1.2	46	13	1
<i>Pheidole sp.1</i>	0.5	0.6	0.1	-	-	37	0.1	0.3	0.5	-	-	28	23	0.5	6.3	1.8	1.1	0.1	-	-	<0.01	1.5	18	42	1

(Continued)

Table 3 (continued).

Species	C12:0	C14:0	C15:0	alC15:0	iC15:0	C16:0	C16:1n7	C16:1n9	C17:0	alC17:0	iC17:0	C18:0	C18:1n9	C18:1n11	C18:2n6	C18:2unk1	C18:2unk2	C20:0	C22:0	C24:0	d'	Shannon	Amount ($\mu\text{g}/\text{mg}$)	UI	Samples
<i>Pheidole</i> sp.2	0.5	0.9	0.2	-	-	47	0.1	0.3	0.3	-	-	28	14	0.6	6.5	0.9	0.6	0.1	-	-	0.02	1.4	18	31	4
<i>Pheidole</i> sp.4	0.7	0.8	0.3	<0.01	0.01	40	0.1	0.9	0.2	-	-	26	23	0.8	3.8	2.0	0.5	0.1	-	-	<0.01	1.5	24	38	7
<i>Pheidole</i> sp.5*	1.9	1.0	-	-	-	27	-	-	1.0	-	-	26	30	1.6	6.6	3.2	2.3	-	-	-	0.01	1.7	34	55	2
<i>Pheidole</i> sp.6*	0.4	0.7	0.1	-	-	34	-	0.3	0.3	-	-	28	26	0.5	7.4	1.5	0.8	0.05	-	-	<0.01	1.5	43	46	4
<i>Pheidole</i> sp.7	0.4	0.8	0.1	-	-	52	0.1	0.1	0.2	-	-	34	7	0.4	4.1	0.8	0.3	0.1	-	-	0.06	1.2	181	18	4
<i>Solenopsis</i> sp.1	0.6	1.8	0.3	0.03	-	44	0.1	0.4	0.6	-	-	32	11	0.7	7.7	0.4	0.3	0.2	-	-	0.03	1.4	217	29	5
<i>Solenopsis</i> sp.2	0.7	1.6	0.4	0.03	-	43	0.04	0.4	0.5	-	-	31	11	0.6	8.6	0.8	0.7	0.2	-	-	0.03	1.5	206	33	5
<i>Solenopsis</i> sp.4	0.7	0.6	0.1	<0.01	0.05	45	0.1	0.6	0.2	0.01	0.03	26	18	0.5	6.7	0.9	0.7	0.1	-	-	0.01	1.4	79	35	4
<i>Solenopsis</i> sp.6	0.4	0.6	0.2	-	-	36	0.1	0.5	0.3	-	-	27	27	0.4	4.6	1.2	0.9	0.3	-	-	<0.01	1.5	41	42	3
<i>Solenopsis</i> sp.8	0.5	1.0	0.1	-	-	53	-	0.3	0.4	-	-	27	11	0.3	5.3	1.1	0.7	0.1	-	-	0.04	1.3	75	26	1
<i>Strumigeris denticulata*</i>	0.5	1.5	0.3	-	-	38	-	0.2	0.6	-	-	32	20	1.2	3.0	1.3	0.9	1.0	-	-	0.01	1.5	81	31	5
<i>Wasmannia affinis</i>	0.2	1.6	0.1	<0.01	0.02	21	0.2	7.6	0.2	0.01	0.01	7	47	2.2	7.9	2.0	1.5	0.4	-	-	0.06	1.6	241	80	5
d'	<0.01	<0.01	<0.01	<0.01	<0.01	0.07	<0.01	0.15	<0.01	<0.01	<0.01	0.05	0.13	0.04	0.09	0.07	0.08	<0.01	-	-	$H'_2 = 0.03$				
Germany																									
<i>Formica fusca</i>	0.03	0.1	0.02	-	-	18	0.1	0.5	0.1	-	-	4.9	75	0.01	0.4	0.2	0.1	0.1	0.1	0.01	<0.01	0.8	287	77	5
<i>Lasius fuliginosus</i>	0.1	0.4	0.05	-	-	21	0.3	3.4	0.03	-	-	2.5	71	0.1	0.6	0.7	0.1	0.02	-	-	<0.01	0.9	230	77	2
<i>Lasius niger</i>	0.05	0.1	0.01	-	-	11	0.04	1.1	0.04	-	-	4.0	84	0.3	0.1	0.03	0.02	0.02	0.01	-	0.02	0.6	660	85	5
<i>Lasius platythorax</i>	0.1	0.2	0.03	-	-	18	0.1	2.1	0.4	-	-	7.0	70	0.6	0.3	0.2	0.1	0.1	0.03	0.02	<0.01	1.0	336	74	5
<i>Myrmica rubra</i>	0.2	0.6	-	-	-	19	0.1	1.8	0.2	-	-	6.6	68	0.2	1.8	1.0	0.6	0.2	0.1	0.03	<0.01	1.1	139	76	5
<i>Myrmica ruginodis</i>	0.03	0.4	-	-	-	18	-	1.6	0.5	-	-	5.6	72	0.2	0.8	0.5	0.3	0.2	0.1	0.01	<0.01	0.9	151	77	5
<i>Tennothorax nylanderi</i>	0.2	1.5	<0.01	-	-	20	0.1	3.8	0.4	-	-	4.5	66	0.2	1.7	0.9	0.3	0.1	0.03	0.01	<0.01	1.1	835	76	5
d'	<0.01	<0.01	<0.01	-	-	0.01	<0.01	0.04	<0.01	-	-	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	$H'_2 = 0.03$				

Notes:

* Species not considered in comparisons between methods.

-, NLFA not detected in this species; UI, unsaturation index.

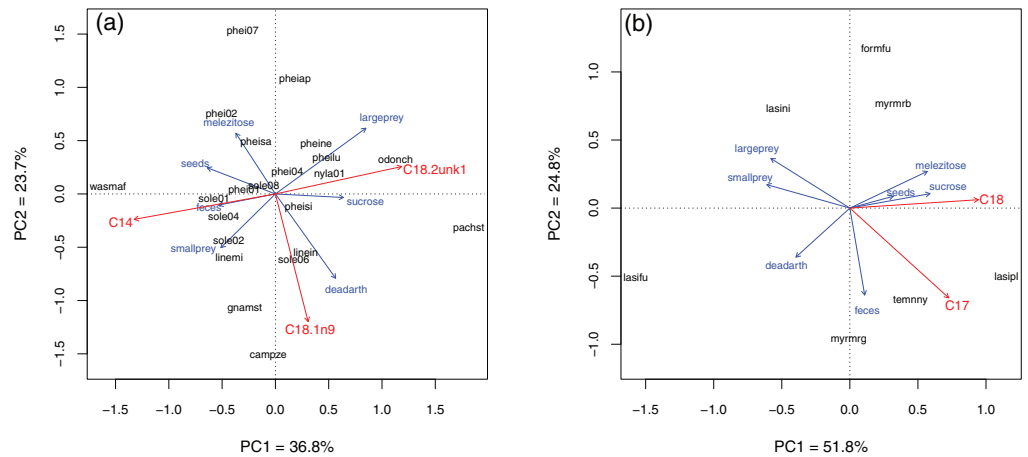


Figure 4 Principal component analysis of resource use in Brazil (A) and Germany (B). Blue setae = bait direction vectors. Red setae = NLFAs correlated to the two main PC axes (Envfit; only statistically significant relationships are plotted). Setae sizes indicate relative strength of the relationships, but are scaled independently for each plot. Only species analyzed with all three methods are included.

Full-size [DOI: 10.7717/peerj.5467/fig-4](https://doi.org/10.7717/peerj.5467/fig-4)

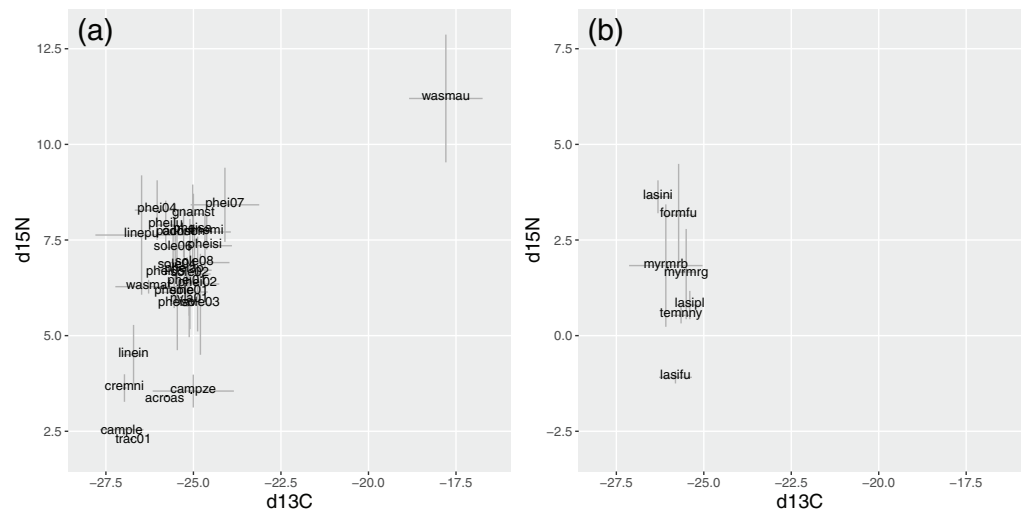


Figure 5 Stable isotope signatures of species in Brazil (A) and Germany (B). Gray bars display standard deviations for species averages. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ are displayed in ‰, following the equation described in the methods.

Full-size [DOI: 10.7717/peerj.5467/fig-5](https://doi.org/10.7717/peerj.5467/fig-5)

Dataset comparisons

In Brazil, similarities in bait use and NLFA profiles were correlated (Table 5). While $\delta^{15}\text{N}$ similarities were correlated with similarities in bait use, but not with NLFAs, the opposite was found for $\delta^{13}\text{C}$. That is, similar use of resources among species was reflected in similar body fat composition, and both were related to their long-term trophic position, albeit in different ways. In Germany, no such correlations were found between datasets (although it was marginally significant for NLFAs and $\delta^{15}\text{N}$).

Table 4 Stable isotope signatures of ant species in Brazil and Germany. Average $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ are given in ‰, following the equation described in the methods.

Species	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	Samples
Brazil			
<i>Acromyrmex aspersus</i> *	3.42	-25.76	1
<i>Camponotus lespesii</i> *	2.44	-26.99	3
<i>Camponotus zenon</i>	3.50	-25.06	4
<i>Crematogaster nigropilosa</i> *	3.63	-26.97	2
<i>Gnamptogenys striatula</i>	8.18	-25.00	5
<i>Linepithema iniquum</i>	4.50	-26.71	5
<i>Linepithema micans</i>	7.71	-24.62	4
<i>Linepithema pulex</i> *	7.63	-26.48	4
<i>Nylanderia</i> sp.1	5.94	-25.12	5
<i>Odontomachus chelifer</i>	7.69	-25.28	5
<i>Pachycondyla striata</i>	7.69	-25.52	5
<i>Pheidole aper</i>	6.71	-25.26	5
<i>Pheidole avia</i> *	5.84	-25.46	3
<i>Pheidole lucretii</i>	7.89	-25.79	3
<i>Pheidole nesiota</i>	6.13	-25.55	5
<i>Pheidole sarcina</i>	7.77	-25.02	4
<i>Pheidole sigillata</i>	7.35	-24.67	5
<i>Pheidole</i> sp.1	6.40	-25.18	5
<i>Pheidole</i> sp.2	6.35	-24.88	5
<i>Pheidole</i> sp.4	8.23	-25.98	5
<i>Pheidole</i> sp.5*	6.63	-25.79	1
<i>Pheidole</i> sp.7	8.42	-24.10	5
<i>Solenopsis</i> sp.1	6.14	-25.12	5
<i>Solenopsis</i> sp.2	6.61	-25.10	5
<i>Solenopsis</i> sp.3*	5.82	-24.80	3
<i>Solenopsis</i> sp.4	6.81	-25.47	5
<i>Solenopsis</i> sp.6	7.30	-25.58	5
<i>Solenopsis</i> sp.8	6.91	-24.97	3
<i>Trachymyrmex</i> sp.1*	2.29	-26.77	1
<i>Wasmannia affinis</i>	6.28	-26.28	4
<i>Wasmannia auropunctata</i> *	11.20	-17.79	4
Germany			
<i>Formica fusca</i>	3.15	-25.72	5
<i>Lasius fuliginosus</i>	-1.09	-25.81	5
<i>Lasius niger</i>	3.63	-26.31	5
<i>Lasius platythorax</i>	0.80	-25.40	5
<i>Myrmica rubra</i>	1.78	-26.03	5
<i>Myrmica ruginodis</i>	1.66	-25.56	5
<i>Temnothorax nylanderii</i>	0.53	-25.65	5

Notes:

* Species not considered in comparisons between methods.

Table 5 Correlations between methods in Brazil and Germany. Results are for Mantel tests using Spearman's rho, based on similarities matrices (Bray–Curtis for baits and NLFAs, Euclidean distances for isotopes). Asterisks indicate significant correlations.

Method	Baits		NLFAs	
	rho	<i>p</i>	rho	<i>p</i>
Brazil				
NLFAs	0.43	<0.01*		
$\delta^{13}\text{C}$	0.23	0.07	0.23	0.02*
$\delta^{15}\text{N}$	0.25	0.04*	0.14	0.08
Germany				
NLFAs	-0.23	0.77		
$\delta^{13}\text{C}$	-0.24	0.76	0.29	0.19
$\delta^{15}\text{N}$	0.37	0.12	0.46	0.06

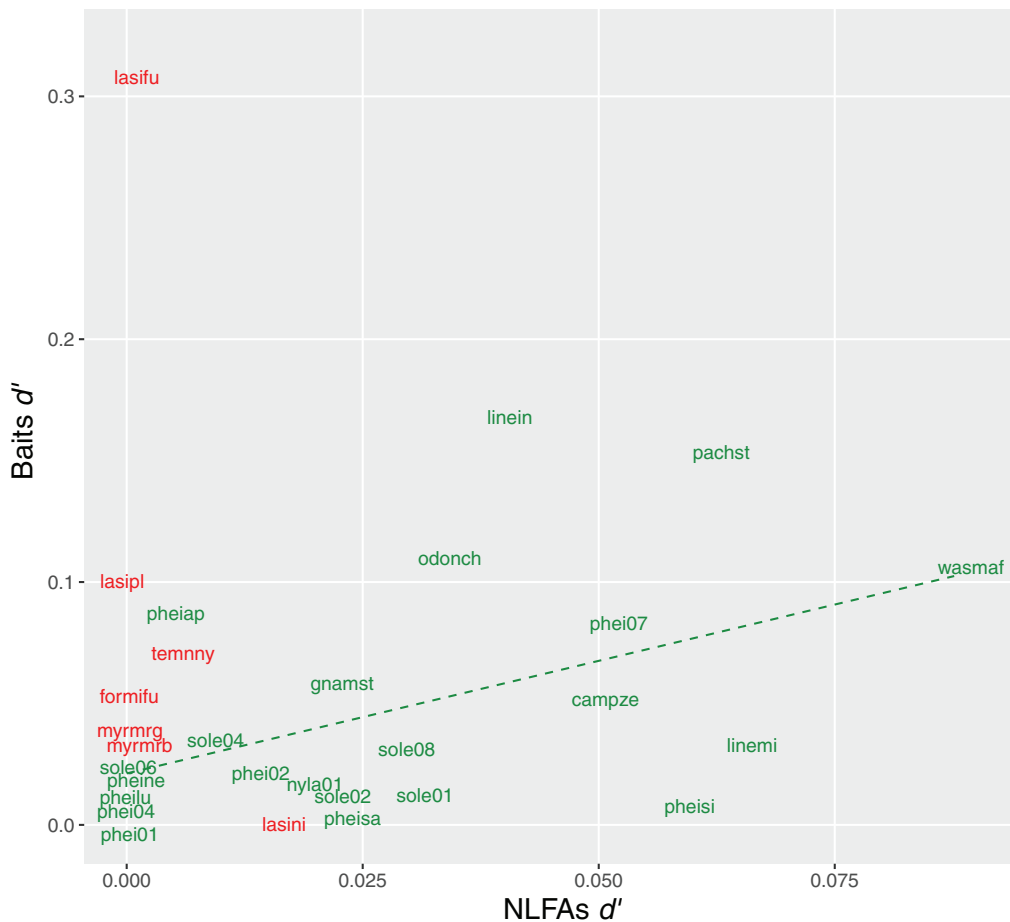


Figure 6 Relationship between specialization indices (d') for bait use and NLFAs. Green = tropical species, the dashed line indicates significant correlation; red = temperate species, no correlation observed. Only species analyzed with all three methods are included.

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Exclusiveness (d') of bait choices and NLFAs profiles were also correlated in Brazil ($\rho = 0.51$, $p = 0.02$), suggesting that specialization in resources was reflected in more specific compositions of fatty acids. No correlation was observed in Germany ($\rho = -0.07$, $p = 0.86$) (Fig. 6).

DISCUSSION

Our main findings in this work are: (1) patterns of resource use are similar in both communities, although the role of oligosaccharides is distinct; (2) both communities are similarly generalized in resource use, regardless of species richness; (3) temperate ants present higher amounts of fat and more homogeneous NLFA compositions; (4) composition and specialization in resource use and NLFAs are correlated, and are also related to species' trophic position; (5) some species show specialized behaviors that can be better understood by method complementarity.

The hypothesis proposed by *MacArthur (1972)* suggested that specialization is higher in tropical communities because the environmental stability allows species to adapt to more specialized niches without increasing extinction risk, thus allowing more species to coexist. However, this idea was put in question by recent studies, where the latitude-richness-specialization link was not confirmed, or an inverse trend was found (*Schleuning et al., 2012; Morris et al., 2014; Frank et al., 2018*). Our work is not an explicit test of this hypothesis, but several results agree with the view that specialization does not necessarily increase with higher richness toward the tropics: despite the different number of species, network metrics of resource use and niche breadths were similarly generalized in both communities; fatty acid compositions were also highly generalized, although in this case in different level, possibly due to other factors (see discussion on fatty acids below); cluster analysis of resource use showed similar patterns between communities and both species clusters and stable isotopes indicated strong overlap inside each community.

The bait protocol we applied is efficient to assess niches of generalists, and specialized species were seldom recorded. Nevertheless, these generalists represent the majority of the communities (as highlighted by our pitfall data), and one might expect more diversified niches to allow coexistence, but that was not the case. The differences we observed might still play a role in coexistence of some species, particularly when they share other traits, such as *O. chelifera* and *Pachycondyla striata*. Both are large, solitary foraging Ponerinae species, very common on the ground of the Atlantic forest, but *O. chelifera* is more predatory and *Pachycondyla striata* is more scavenging (*Rosumek, 2017*). Coexistence is result of a complex interplay of habitat structure, interspecific interactions and species traits and no single factor governs ant community organization (*Cerdá, Arnan & Retana, 2013*). Trophic niche alone does not explain coexistence of the common species in these two communities, but likely is one of the many factors structuring them.

Use of resources

Resource use in Brazil was discussed in detail in *Rosumek (2017)*, as well as the literature review on trophic niche of our identified tropical species. Large prey was the less used

resource, because size and mobility of the prey limits which species are able to overcome them. Small prey and feces were also relatively less used, the first because also is relatively challenging to acquire, and the second probably due to smaller nutritional value. On the other hand, the other resources are nutritive and relatively easy to gather, particularly dead arthropods, which was by far the most used resource. Considering the similarity in resource use patterns, most general remarks in that work apply to Germany as well. The two main differences we found are discussed below.

The role of insect-synthesized oligosaccharides seems to be distinct between temperate and tropical communities. In Brazil, the aversion to melezitose showed by some species could represent a physiological constraint, since tolerance to oligosaccharides differs among ant species (Rosumek, 2017). For ants without physiological constraints, melezitose use might be opportunistic and does not necessarily mean that they interact with sap-sucking insects. However, honeydew is the only reliable source of oligosaccharides in nature, so the few species that preferred this sugar may engage in such interactions (particularly *Pheidole aper*). In Germany, on the other hand, all species used both sugars similarly. The two *Myrmica*, *Lasius* and *Formica fusca* are known to interact with sap-sucking insects, and *Temnothorax nylander* uses honeydew opportunistically when droplets fall on the ground (Seifert, 2007).

Seeds were other resource used differently, but this probably is consequence of our methodological choice of seeds with elaiosomes in Germany. Elaiosomes are thought to mimic animal prey and attract predators and scavengers (Hughes, Westoby & Jurado, 1994), not only granivores. Effectively, elaiosomes of *Chelidonium majus* are attractive to a wide range of ants (Reifenrath, Becker & Poethke, 2012). However, seeds were more extensively used in Brazil. A higher diversity of shape and sizes of seeds was offered there, which allowed more ants to use them.

Fatty acids

Fatty acid compositions were generalized, but differed between communities. In Germany, C18:1n9 plays a prominent role, making up for more than 70% of the NLFAs stored by ants. The amounts of fat also differed remarkably: in average, temperate ants stored over five times more fat. Similarly high amounts of total fat and percentages of C18:1n9 were observed in laboratory colonies of *F. fusca* and *M. rubra* (Rosumek et al., 2017), which suggest that it might be a general trend for temperate species. In Brazil, NLFA abundance at community level was more balanced between C16:0, C18:0 and C18:1n9. Both amounts and proportions of C18:1n9 were variable among species.

Organisms can actively change their fatty acid composition in response to environmental factors and physiological needs (Stanley-Samuelson et al., 1988). Temperature and balance between saturated and unsaturated NLFAs are important, because the fat body should present a certain fluidity that allows enzymes to access stored nutrients (Ruess & Chamberlain, 2010). C18:1n9 seems to be the only unsaturated fatty acid that ants are able to synthesize by themselves in large amounts (Rosumek et al., 2017). However, there was no positive correlation between amount of fat and C18:1n9 or unsaturation index of samples, which would be expected if C18:1n9 synthesis was a direct

mechanism of individuals to balance saturation:unsaturation ratios (the weak negative correlation in Brazil also does not fit this hypothesis).

Therefore, we suggest that differences in C18:1n9 percentages and total amounts could be consequence of two distinct environmental factors. Under lower temperatures, higher proportion of unsaturated fatty acids is needed to maintain lipid fluidity (Jagdale & Gordon, 1997). Thus, temperate species might be adapted to synthesize and store more C18:1n9 to withstand the cold seasons. If this hypothesis were correct, the ants would maintain this high proportion throughout the year, since we collected in summer. In turn, high amount of fat could be a direct consequence of the marked seasonality in temperate regions. These species might be adapted to quickly acquire and accumulate energy reserves during the short warm season, while there is less pressure for this in regions where resources are available throughout the year.

We observed relationships between certain NLFAs and resources, although overall they were not strong and not necessarily result of direct trophic transfer. C18:1n9 was related to use of dead arthropods in Brazil. This NLFA is considered a “necromone,” a chemical clue for recognition of corpses by ants and other insects (Sun & Zhou, 2013), so it presumably increases in dead arthropods. However, only polyunsaturated fatty acids can be degraded to form C18:1n9 during decomposition, and C18:1n9 itself turns into C18:0 (Dent, Forbes & Stuart, 2004). Thus, for high C18:1n9 to be a direct result of scavenging, prey items should previously possess high levels of unsaturation. This might be an indirect effect as well: scavenger ants might be better at tracking and retrieving food items that are naturally rich in C18:1n9. No correlation was found in Germany, which could also be related to the special role of this NLFA in temperate species: its predominance due to environmental factors may override its dietary signal.

C18:2n6 occurs independently of diet only in very small amounts, and it is a potential biomarker (Rosumek et al., 2017). The differences we observed among species are direct result of diet. Its occurrence was more widespread in Brazil, but we observed no clear correlation with specific resources. C18:2n6 is found in elaiosomes, seeds and other arthropods in different amounts (Thompson, 1973; Hughes, Westoby & Jurado, 1994). Since it can come from different sources, C18:2n6 cannot be straightforwardly used as a biomarker for specific diets, but depends on a deeper analysis of the resources actually available in the habitat.

The biological significance of the correlations of NLFAs and resources in Germany is difficult to grasp. C18:0 does not appear to be preferably synthesized from carbohydrates, compared to C16:0 and C18:1n9 (Rosumek et al., 2017). Adding to the fact that such correlation was not found in Brazil, this might not represent a physiological link between sugar consumption and C18:0 synthesis. With low number of species in Germany, even strong correlations might be result of species-specific factors other than diet. The same might be said for C17:0, a fatty acid that occurs in very low amounts in several vegetable oils (Beare-Rogers, Dieffenbacher & Holm, 2001).

Interestingly, we did not observe any 18:3n3 or 18:3n6 (α - and γ -linolenic acids). Ants are not able to synthesize them, and they are assimilated through direct trophic

transfer (Rosumek *et al.*, 2017). In the studied communities, these fatty acids seem to be completely absent from food sources used by ants. This is an unexpected result, since their occurrence is well documented in elaiosomes and a wide range of insect groups that might serve as prey (Thompson, 1973; Hughes, Westoby & Jurado, 1994).

The use of fatty acids as biomarkers to track food sources is one of the greatest potentials of this method. However, it might be more suitable to detritivore systems, where the biomarkers are distinctive membrane phospholipids from microorganisms that decompose specific resources, and that end up stored in the fat reserves of the consumers (Ruess & Chamberlain, 2010). The NLFA profiles we observed are generalized, and the most relevant fatty acids could represent distinct sources and/or be synthesized *de novo* in large amounts. The biomarker approach might not be suitable at community level for ground ants, contrary to NLFA profiles (see Method Comparison below). However, it still might be useful to unveil species-specific interactions, or in contexts with less potential sources that can be better tracked (e.g., leaf-litter or subterranean species).

Stable isotopes

Trophic shift (i.e., the degree of change in isotopic ratios from one trophic level to another) varies among taxonomic groups and according to other physiological factors (McCutchan *et al.*, 2003). “Typical” values of ca. 3‰ for $\delta^{15}\text{N}$ and 1‰ for $\delta^{13}\text{C}$ were experimentally observed in one ant species (Feldhaar, Gebauer & Blüthgen, 2010). Establishing discrete trophic levels is unrealistic in most food webs, particularly for omnivores such as ants (Polis & Strong, 1996), but species within the range of one trophic shift are more likely to use resources in a similar way. $\delta^{15}\text{N}$ ranges of ca. 9‰ were observed for ant communities in other tropical forests, representing three trophic shifts (Davidson *et al.*, 2003; Bihn, Gebauer & Brandl, 2010). This is similar to our range of 8.9‰ but, discounting *W. auropunctata*, the remaining range of 6.1‰ is more similar to what was observed in an Australian forest (7.1‰; Blüthgen, Gebauer & Fiedler, 2003). In Germany, only *Lasius fuliginosus* presented a distinct signature. In both communities, most species fell within the range of one trophic shift.

$\delta^{13}\text{C}$ showed smaller, but meaningful, variations that were correlated to $\delta^{15}\text{N}$. $\delta^{13}\text{C}$ is less applied to infer trophic levels, as it is more sensitive to sample preservation method and diet composition (Tillberg *et al.*, 2006; Heethoff & Scheu, 2016). An average change of 0.61‰ was observed in samples stored in ethanol by Tillberg *et al.* (2006). However, we observed correlations (including with NLFAs—see below) despite this eventual change, and it would not affect the similarity among species and between communities. Primary consumers using distinct plant sources may present differences of up to 20‰, and this will influence the signature of secondary consumers (O’Leary, 1988; Gannes, Del Rio & Koch, 1998). However, in our case, only *W. auropunctata* presented such distinct value.

Again, both isotopes suggest that the core of these communities is composed by generalists that broadly use the same resources. Since we did not establish baselines, lower values in Germany do not necessarily mean lower trophic levels in this community. Isotope signatures for the same species are highly variable among sites in Europe

(Fiedler et al., 2007), and this variation can be the result of either different isotope baselines or actual changes in species' ecological roles.

Low $\delta^{15}\text{N}$ suggest that a species obtain most of their nitrogen from basal trophic levels, mainly plant sources (Blüthgen, Gebauer & Fiedler, 2003; Davidson et al., 2003). This fits the six species with lowest $\delta^{15}\text{N}$ in Brazil. Two were fungus-growing ants (*Acromyrmex aspersus*, *Trachymyrmex* sp.1), which use mostly plant material to grow its fungus. The others were species that forage frequently on vegetation, besides the ground (*Camponotus lespesii*, *Camponotus zenon*, *Crematogaster nigropilosa*, *Linepithema iniquum*). Arboreal species that heavily rely on nectar or honeydew usually present low $\delta^{15}\text{N}$, which may be the case for these species. *Linepithema* represents well this trend: the two mainly ground-nesting species, *Linepithema micans* and *Linepithema pulex*, presented higher signatures than the plant-nesting *Linepithema iniquum* (Wild, 2007).

Community patterns and method comparison

The correlations we observed between methods are interesting from both the methodological and the biological perspective. From a methodological viewpoint, for terrestrial animals, this is the first time an empirical relationship is shown between patterns of resource use and composition of stored fat in natural conditions, and that both relate to their long-term trophic position. Although differences between species were small, these relationships were robust enough to be detected by different methods. From a biological viewpoint, it highlights several physiological mechanisms involved in such relationships. We will discuss in the following some of these mechanisms, as well as caveats that are often cited for these methods. They probably still influence our results and correlations, but did not completely override the patterns.

A commonly cited caveat for using baits is that ants could be attracted to the most limited resources, instead of the ones they use more often. Evidence for this comes mainly from nitrogen-deprived arboreal ants (Kaspari & Yanoviak, 2001), and some cases are discussed below (see method complementarity). However, this effect might be less pronounced in epigeic species, and our results suggest that there is convergence between bait attendance, and medium- and long-term use of resources.

Diet may significantly change NLFA composition in a few weeks (Rosumek et al., 2017) and persist for a similar time (Haubert, Pollierer & Scheu, 2011). Therefore, the “snapshot” of resource use we observed with baits should represent at least the seasonal preferences of the species. A seasonal study on NLFA compositions can bring valuable information on resource use changes, or if they are stable throughout the year.

Adult ants are thought to feed mostly on liquid foods, due to the morphology of the proventriculus, which prevents solid particles to pass from the crop to the midgut (Eisner & Happ, 1962). Larvae are able to process solids and possess a more diversified suit of enzymes, and are sometimes called the “digestive caste” of the colony (Hölldobler & Wilson, 1990; Erthal, Peres Silva & Ian Samuels, 2007). Trophallaxis is an important mechanism of food sharing between workers and larvae. Our results suggest that the trophic signal of NLFAs is not lost in this processes, and that might be true even for solid

items such as arthropods or seeds. However, the similarities could be as well the result of direct digestion and assimilation of liquid sources (sugars, hemolymph).

We also found correlations with stable isotopes. They were weaker than between baits and NLFAs, and different for each isotope. For $\delta^{15}\text{N}$, it shows that patterns of resource use are more correlated with trophic level. Protein amino acids must be obtained from diet or synthesized from other nitrogenated compounds, so the signal relative to nitrogen sources should be more preserved. This also fits to the idea that $\delta^{15}\text{N}$ reflects larval diet, because it is in this stage that ants grow and build most of their biomass (Blüthgen & Feldhaar, 2010). On the other hand, it makes sense that the signal relative to carbon sources is related to NLFA composition. We should note that we removed the gaster of the ants used in SIA, so we observed only the signal of carbon incorporated in the other body parts. This is related to dietary carbon, but a stronger signal could be expected if the fat body is included.

The low source-specificity of stable isotope signatures might also lead to relatively weak correlations. *Pachycondyla striata* and *O. chelifer* had the same $\delta^{15}\text{N}$ despite their different preferences. Other species that appear to be mostly scavengers had similar or higher $\delta^{15}\text{N}$ than those two “predators,” such as *Linepithema micans*, *Pheidole sarcina*, *Pheidole lucretii* and *Pheidole* sp.4.

In Germany, no correlation was observed between methods. This is probably a consequence of the low number of species available in the community. The relationships found in Brazil might be valid for other communities, although ecological context and physiology might change their significance or strength.

Species niches and method complementarity

Niche differences were correlated at community level, but the use of different techniques allows better understanding of species’ niches. Method complementarity is particularly important if one is interested in the functional role of individual species, not only in overall patterns. Some cases are described below.

In Brazil, *W. auropunctata* was distinct from the remaining community, both in resource use and isotopic signature (unfortunately, no NLFA samples were obtained for this species). Strong preference for feces is a novel behavior for this species, known to invade and dominate disturbed habitats, but less dominant inside forests (Rosumek, 2017). Its isotopic signature confirms that they have a highly differentiated diet, and could be direct result of a feces-rich diet. In herbivorous mammals, feces are usually enriched in $\delta^{15}\text{N}$ relative to diet (Sponheimer et al., 2003; Hwang, Millar & Longstaffe, 2007). The proposed mechanism of ^{15}N enrichment along trophic levels states that this happens due to preferential excretion of ^{14}N , and it is assumed that most nitrogen is excreted in the urine, which is depleted in ^{15}N (Peterson & Fry, 1987; Gannes, Del Rio & Koch, 1998; but see Sponheimer et al., 2003). However, ^{15}N -enriched feces were also observed in uricotelic organisms, such as birds and locusts (Webb, Hedges & Simpson, 1998; Bird et al., 2008). Thus, high $\delta^{15}\text{N}$ is consistent with a diet based on ^{15}N -enriched feces from other consumers. The relationship with the $\delta^{13}\text{C}$ signature is less clear, but it also suggests high specialization.

This behavior might be a local adaptation, but also could indicate that *W. auropunctata* shifts to less disputed resources inside native forests (although the exact resources used may be context-dependent). In [Davidson \(2005\)](#), *Wasmannia* species (including *W. auropunctata*) presented relatively high $\delta^{15}\text{N}$ and were considered highly carnivorous. However, our result shows that high $\delta^{15}\text{N}$ should not be taken from granted to represent high-level consumers. It might be a solid generalization for communities, but other trophic pathways may lead to such signatures. Due to their lack of specificity, isotope signatures should be combined with field observations to provide reliable information at species level. As another example, the second highest $\delta^{15}\text{N}$ in our work was observed in *Pheidole* sp.7, a species that used mainly seeds and was seldom recorded in animal (or feces) baits.

Another example where results seem to be contradictory is *L. fuliginosus*, which showed strong preference for animal baits, but low $\delta^{15}\text{N}$. In this case, the natural history of the species is well known, and it strongly interacts with aphids, particularly the giant oak aphid *Stomaphis quercus* ([Seifert, 2007](#)). This suggests that this aphid's honeydew is not enriched in ^{15}N and has a composition similar to the plants on which they feed. The honeydew supply should be abundant, since ants basically ignored sugar baits, but also relatively poor in nitrogen, which makes *L. fuliginosus* use animal sources whenever possible. A similar pattern may apply to *Linepithema iniquum* in Brazil, which also combined low $\delta^{15}\text{N}$ with preference for animal baits. This species is also known to use extra-floral nectaries and honeydew ([Rosumek, 2017](#)), but does not have such strong and specific interactions as its temperate counterpart.

CONCLUSIONS

In this work, we investigated two communities with three distinct methods, and provided information on community patterns of resource use and species' trophic niches. Our results agree with the view that ant communities are mostly composed by generalist species that share similar resources, and suggest that such patterns do not differ between tropical and temperate communities. Although high richness may lead to more specialists in the tropics, the generalist core of the community should be maintained by a combination of several factors.

Overall, we observed that the three methods corresponded in their characterization of the communities, but their combination provided a more comprehensive picture of resource use. However, the time and costs demanded should limit the broad application of this framework, and some techniques are more suitable to answer particular questions. We gave special focus on FAA in this work because it was the first time this method was applied to study ant ecology in the field. Considering that NLFA profiles provide a more time-representative snapshot than baits, and are more specific than stable isotopes, we suggest FAA as a powerful tool to study trophic niche relationships in species-rich ant communities. It allows the researcher to obtain quantitative data related to diet with relatively short fieldwork time, or from systems where direct observation is limited, and then use it to infer niche breadths, similarities and overlap. However, their use as biomarkers has yet to be developed, and seems to be limited for epigeic ant communities.

Combining NLFA compositions with field observations is strongly recommended if the researcher is interested in source-specificity. Finally, stable isotopes (particularly $\delta^{15}\text{N}$) might be added as a long-term representation of trophic position, which can corroborate or complement other results.

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Felix B. Rosumek conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Nico Blüthgen conceived and designed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
- Adrian Brückner conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, approved the final draft.

- Florian Menzel conceived and designed the experiments, authored or reviewed drafts of the paper, contributed reagents/materials/analysis tools, approved the final draft.
- Gerhard Gebauer performed the experiments, contributed reagents/materials/analysis tools, approved the final draft.
- Michael Heethoff conceived and designed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.

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Data Availability

The following information was supplied regarding data availability:

All raw data used for the analysis of this article are provided in [Tables 2–4](#), and [Table S1](#).

Supplemental Information

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REFERENCES

- Albrecht M, Gotelli NJ. 2001.** Spatial and temporal niche partitioning in grassland ants. *Oecologia* **126**(1):134–141 DOI [10.1007/s004420000494](https://doi.org/10.1007/s004420000494).
- Andersen AN. 2000.** A global ecology of rainforest ants: functional groups in relation to environmental stress and disturbance. In: Agosti D, Majer JD, Alonso LE, Schultz TR, eds. *Ants: Standard Methods for Measuring and Monitoring Biodiversity*. Washington, D.C.: Smithsonian Institution Press, 25–34.
- Anderson MJ. 2001.** A new method for non-parametric multivariate analysis of variance. *Austral Ecology* **26**(1):32–46 DOI [10.1111/j.1442-9993.2001.01070.pp.x](https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x).
- Anderson MJ, Walsh DCI. 2013.** PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: what null hypothesis are you testing? *Ecological Monographs* **83**(4):557–574 DOI [10.1890/12-2010.1](https://doi.org/10.1890/12-2010.1).
- AntWeb. 2016.** AntWeb. Available at <http://www.antweb.org/> (accessed 15 March 2016).
- Baccaro FB, Feitosa RM, Fernández F, Fernandes IO, Izzo TJ, de Souza JLP, Solar RRC. 2015.** *Guia para os gêneros de formigas do Brasil*. Manaus: Editora INPA.
- Beare-Rogers JL, Dieffenbacher A, Holm JV. 2001.** Lexicon of lipid nutrition (IUPAC Technical Report). *Pure and Applied Chemistry* **73**(4):685–744 DOI [10.1351/pac200173040685](https://doi.org/10.1351/pac200173040685).
- Bestelmeyer BT, Agosti D, Alonso LE, Brandão CRF, Brown WL, Delabie JHC, Silvestre R. 2000.** Field techniques for the study of ground-dwelling ants. In: Agosti D, Majer JD, Alonso LE, Schultz TR, eds. *Ants: Standard Methods for Measuring and Monitoring Biodiversity*. Washington, D.C.: Smithsonian Institution Press, 122–144.

- Bidartondo MI, Burghardt B, Gebauer G, Bruns TD, Read DJ. 2004.** Changing partners in the dark: isotopic and molecular evidence of ectomycorrhizal liaisons between forest orchids and trees. *Proceedings of the Royal Society B: Biological Sciences* **271(1550)**:1799–1806 DOI [10.1098/rspb.2004.2807](https://doi.org/10.1098/rspb.2004.2807).
- Bihn JH, Gebauer G, Brandl R. 2010.** Loss of functional diversity of ant assemblages in secondary tropical forests. *Ecology* **91(3)**:782–792 DOI [10.1890/08-1276.1](https://doi.org/10.1890/08-1276.1).
- Bird MI, Tait E, Wurster CM, Furness RW. 2008.** Stable carbon and nitrogen isotope analysis of avian uric acid. *Rapid Communications in Mass Spectrometry* **22(21)**:3393–3400 DOI [10.1002/rcm.3739](https://doi.org/10.1002/rcm.3739).
- Birkhofer K, Bylund H, Dalin P, Ferlian O, Gagic V, Hambäck PA, Klapwijk M, Mestre L, Roubinet E, Schroeder M, Stenberg JA, Porcel M, Björkman C, Jonsson M. 2017.** Methods to identify the prey of invertebrate predators in terrestrial field studies. *Ecology and Evolution* **7(6)**:1942–1953 DOI [10.1002/ece3.2791](https://doi.org/10.1002/ece3.2791).
- Blüthgen N, Feldhaar H. 2010.** Food and shelter: how resources influence ant ecology. In: Lach L, Parr CL, Abbott KL, eds. *Ant Ecology*. Oxford: Oxford University Press, 115–136.
- Blüthgen N, Gebauer G, Fiedler K. 2003.** Disentangling a rainforest food web using stable isotopes: dietary diversity in a species-rich ant community. *Oecologia* **137(3)**:426–435 DOI [10.1007/s00442-003-1347-8](https://doi.org/10.1007/s00442-003-1347-8).
- Blüthgen N, Menzel F, Blüthgen N. 2006.** Measuring specialization in species interaction networks. *BMC Ecology* **6**:9 DOI [10.1186/1472-6785-6-9](https://doi.org/10.1186/1472-6785-6-9).
- Bolton B. 2018.** An online catalog of the ants of the world. Available at <http://antcat.org/> (accessed 4 June 2018).
- Brückner A, Heethoff M. 2017.** A chemo-ecologists' practical guide to compositional data analysis. *Chemoecology* **27(1)**:33–46 DOI [10.1007/s00049-016-0227-8](https://doi.org/10.1007/s00049-016-0227-8).
- Budge SM, Iverson SJ, Koopman HN. 2006.** Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. *Marine Mammal Science* **22(4)**:759–801 DOI [10.1111/j.1748-7692.2006.00079.x](https://doi.org/10.1111/j.1748-7692.2006.00079.x).
- Cerdá X, Arnan X, Retana J. 2013.** Is competition a significant hallmark of ant (Hymenoptera: Formicidae) ecology? *Myrmecological News* **18**:131–147.
- Cerdá X, Retana J, Cros S. 1997.** Thermal disruption of transitive hierarchies in Mediterranean ant communities. *Journal of Animal Ecology* **66(3)**:363–374 DOI [10.2307/5982](https://doi.org/10.2307/5982).
- Clarke KR. 1993.** Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* **18(1)**:117–143 DOI [10.1111/j.1442-9993.1993.tb00438.x](https://doi.org/10.1111/j.1442-9993.1993.tb00438.x).
- Clarke KR, Somerfield PJ, Gorley RN. 2008.** Testing of null hypotheses in exploratory community analyses: similarity profiles and biota-environment linkage. *Journal of Experimental Marine Biology and Ecology* **366(1–2)**:56–69 DOI [10.1016/J.JEMBE.2008.07.009](https://doi.org/10.1016/J.JEMBE.2008.07.009).
- Davidson DW. 2005.** Ecological stoichiometry of ants in a New World rain forest. *Oecologia* **142(2)**:221–231 DOI [10.1007/s00442-004-1722-0](https://doi.org/10.1007/s00442-004-1722-0).
- Davidson DW, Cook SC, Snelling RR, Chua TH. 2003.** Explaining the abundance of ants in lowland tropical rainforest canopies. *Science* **300(5621)**:969–972 DOI [10.1126/science.1082074](https://doi.org/10.1126/science.1082074).
- De Andrade ML, Baroni Urbani C. 1999.** Diversity and adaptation in the ant genus *Cephalotes*, past and present. *Stuttgarter Beiträge zur Naturkunde. Serie B (Geologie und Paläontologie)* **271**:1–889.
- Dent BB, Forbes SL, Stuart BH. 2004.** Review of human decomposition processes in soil. *Environmental Geology* **45(4)**:576–585 DOI [10.1007/s00254-003-0913-z](https://doi.org/10.1007/s00254-003-0913-z).

- Dormann CF, Fruend J, Gruber B. 2017.** *bipartite: visualising bipartite networks and calculating some (ecological) indices*. Version 2.08. Available at <https://cran.r-project.org/package=bipartite>.
- Dormann CF, Strauss R. 2014.** A method for detecting modules in quantitative bipartite networks. *Methods in Ecology and Evolution* 5(1):90–98 DOI 10.1111/2041-210X.12139.
- Eisner T, Happ GM. 1962.** The Infrabuccal pocket of a Formicine ant: a social filtration device. *Psyche: A Journal of Entomology* 69(3):107–116 DOI 10.1155/1962/25068.
- Erthal M, Peres Silva C, Ian Samuels R. 2007.** Digestive enzymes in larvae of the leaf cutting ant, *Acromyrmex subterraneus* (Hymenoptera: Formicidae: Attini). *Journal of Insect Physiology* 53(11):1101–1111 DOI 10.1016/J.JINSPHYS.2007.06.014.
- Feldhaar H, Gebauer G, Blüthgen N. 2010.** Stable isotopes: past and future in exposing secrets of ant nutrition (Hymenoptera: Formicidae). *Myrmecological News* 13(3):13.
- Fernández F. 2008.** Subfamilia Ponerinae s. str. In: Jiménez E, Fernández F, Arias TM, Lozano-Zambrano FH, eds. *Sistemática, Biogeografía y Conservación de las Hormigas Cazadoras de Colombia*. Bogotá: Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, 123–218.
- Fiedler K, Kuhlmann F, Schlick-Steiner BC, Steiner FM, Gebauer G. 2007.** Stable N-isotope signatures of central European ants—assessing positions in a trophic gradient. *Insectes Sociaux* 54(4):393–402 DOI 10.1007/s00040-007-0959-0.
- Frank K, Krell F-T, Slade EM, Raine EH, Chiew LY, Schmitt T, Vairappan CS, Walter P, Blüthgen N. 2018.** Global dung webs: high trophic generalism of dung beetles along the latitudinal diversity gradient. *Ecology Letters* 21(8):1229–1236 DOI 10.1111/ele.13095.
- Galvis JP, Fernández F. 2009.** Ants of Colombia X. *Acanthognathus* with the description of a new species (Hymenoptera: Formicidae). *Revista Colombiana de Entomologia* 35:245–249.
- Gannes LZ, Del Rio CM, Koch P. 1998.** Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. *Comparative Biochemistry and Physiology* 119(3):725–737 DOI 10.1016/S1095-6433(98)01016-2.
- Gonçalves CR. 1961.** O gênero *Acromyrmex* no Brasil (Hym. Formicidae). *Studia Entomologica* 4:113–180.
- Greenslade PJM. 1973.** Sampling ants with pitfall traps: diggin-in effects. *Insectes Sociaux* 20(4):343–353 DOI 10.1007/BF02226087.
- Hammer Ø, Harper DAT, Ryan PD. 2001.** PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4:1–9.
- Haubert D, Birkhofer K, Fließbach A, Gehre M, Scheu S, Ruess L. 2009.** Trophic structure and major trophic links in conventional versus organic farming systems as indicated by carbon stable isotope ratios of fatty acids. *Oikos* 118(10):1579–1589 DOI 10.1111/j.1600-0706.2009.17587.x.
- Haubert D, Pollierer MM, Scheu S. 2011.** Fatty acid patterns as biomarker for trophic interactions: changes after dietary switch and starvation. *Soil Biology and Biochemistry* 43(3):490–494 DOI 10.1016/j.soilbio.2010.10.008.
- Heethoff M, Scheu S. 2016.** Reliability of isotopic fractionation ($\Delta^{15}\text{N}$, $\Delta^{13}\text{C}$) for the delimitation of trophic levels of oribatid mites: diet strongly affects $\Delta^{13}\text{C}$ but not $\Delta^{15}\text{N}$. *Soil Biology and Biochemistry* 101:124–129 DOI 10.1016/j.soilbio.2016.07.013.
- Hölldobler B, Wilson EO. 1990.** *The Ants*. Cambridge: Harvard University Press.

- Houadria M, Salas-Lopez A, Orivel J, Blüthgen N, Menzel F. 2015.** Dietary and temporal niche differentiation in tropical ants—can they explain local ant coexistence? *Biotropica* 47(2):208–217 DOI 10.1111/btp.12184.
- Hughes L, Westoby M, Jurado E. 1994.** Convergence of elaiosomes and insect prey: evidence from ant foraging behaviour and fatty acid composition. *Functional Ecology* 8(3):358–365 DOI 10.2307/2389829.
- Hwang YT, Millar JS, Longstaffe FJ. 2007.** Do $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of feces reflect the isotopic composition of diets in small mammals? *Canadian Journal of Zoology* 85(3):388–396 DOI 10.1139/Z07-019.
- Hyodo F. 2015.** Use of stable carbon and nitrogen isotopes in insect trophic ecology. *Entomological Science* 18(3):295–312 DOI 10.1111/ens.12128.
- Jagdale GB, Gordon R. 1997.** Effect of temperature on the composition of fatty acids in total lipids and phospholipids of entomopathogenic nematodes. *Journal of Thermal Biology* 22(4–5):245–251 DOI 10.1016/S0306-4565(97)00019-3.
- Kaspari M. 2000.** A primer on ant ecology. In: Agosti D, Majer JD, Alonso LE, Schultz TR, eds. *Ants: Standard Methods for Measuring and Monitoring Biodiversity*. Washington, D.C.: Smithsonian Institution Press, 9–24.
- Kaspari M, Yanoviak SP. 2001.** Bait use in tropical litter and canopy ants—evidence of differences in nutrient limitation. *Biotropica* 33(1):207–211 DOI 10.1646/0006-3606(2001)033[0207:BUITLA]2.0.CO;2.
- Kempf WW. 1965.** A revision of the neotropical fungus-growing ants of the genus *Cyphomyrmex* Mayr. Part II: Group of *rimosus* (Spinola) (Hym. Formicidae). *Studia Entomologica* 8:161–200.
- Kempf WW. 1973.** A revision of the neotropical myrmicine ant genus *Hylomyrma* Forel (Hymenoptera: Formicidae). *Studia Entomologica* 16:225–260.
- Krebs CJ. 1999.** *Ecological Methodology*. Menlo Park: Benjamin/Cummings.
- Lanan M. 2014.** Spatiotemporal resource distribution and foraging strategies of ants (Hymenoptera: Formicidae). *Myrmecological News* 20:53–70.
- Lattke JE. 1995.** Revision of the ant genus *Gnamptogenys* in the New World (Hymenoptera: Formicidae). *Journal of Hymenoptera Research* 4:137–193.
- Longino JT. 2003.** The *Crematogaster* (Hymenoptera, Formicidae, Myrmicinae) of Costa Rica. *Zootaxa* 151(1):1–150 DOI 10.11646/zootaxa.151.1.1.
- Longino JT. 2013.** A revision of the ant genus *Octostruma* Forel 1912 (Hymenoptera, Formicidae). *Zootaxa* 3699:1–61 DOI 10.11646/zootaxa.3699.1.1.
- Longino JT, Fernández F. 2007.** Taxonomic review of the genus *Wasmannia*. *Memoirs of the American Entomological Institute* 80:271–289.
- Lopes BC. 2007.** Ecologia do forrageio por *Cyphomyrmex morschii* Emery (Hymenoptera, Formicidae) em vegetação de restinga no Sul do Brasil. *Revista Brasileira de Zoologia* 24(1):52–56 DOI 10.1590/S0101-81752007000100006.
- MacArthur RH. 1972.** *Geographical Ecology: Patterns in the Distribution of Species*. Princeton: Princeton University Press.
- Malcicka M, Visser B, Ellers J. 2018.** An evolutionary perspective on linoleic acid synthesis in animals. *Evolutionary Biology* 45(1):15–26 DOI 10.1007/s11692-017-9436-5.
- McCutchan JH, Lewis WM, Kendall C, McGrath CC. 2003.** Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102(2):378–390 DOI 10.1034/j.1600-0706.2003.12098.x.

- Medeiros FNS, Oliveira PS. 2009. Season-dependent foraging patterns case study of a neotropical forest-dwelling ant (*Pachycondyla striata*; Ponerinae). In: Jarau S, Hrnir M, eds. *Food Exploitation by Social Insects: Ecological, Behavioral, and Theoretical Approaches*. Boca Raton: Taylor & Francis Group, 81–95.
- Morris RJ, Gripenberg S, Lewis OT, Roslin T. 2014. Antagonistic interaction networks are structured independently of latitude and host guild. *Ecology Letters* 17(3):340–349 DOI 10.1111/ele.12235.
- Ngosong C, Raupp J, Scheu S, Ruess L. 2009. Low importance for a fungal based food web in arable soils under mineral and organic fertilization indicated by *Collembola* grazers. *Soil Biology and Biochemistry* 41(11):2308–2317 DOI 10.1016/j.soilbio.2009.08.015.
- Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H. 2017. *vegan: community ecology package*. Version 2.5-2. Available at <https://cran.r-project.org/package=vegan>.
- O'Leary MH. 1988. Carbon isotopes in photosynthesis. *Bioscience* 38(5):328–336 DOI 10.2307/1310735.
- Parr CL, Gibb H. 2012. The discovery-dominance trade-off is the exception, rather than the rule. *Journal of Animal Ecology* 81(1):233–241 DOI 10.1111/j.1365-2656.2011.01899.x.
- Peterson BJ, Fry B. 1987. Stable isotopes in ecosystem studies. *Annual Reviews of Ecology and Systematics* 18:292–320 DOI 10.1146/annurev.es.18.110187.001453.
- Polis GA, Strong DR. 1996. Food web complexity and community dynamics. *American Naturalist* 147(5):813–846 DOI 10.1086/285880.
- R Core Team. 2017. *R: A Language and Environment for Statistical Computing*. Version 3.4.3. Vienna: the R Foundation for Statistical Computing. Available at <https://www.R-project.org>.
- Radchenko A, Elmes GW. 2010. *Myrmica Ants (Hymenoptera: Formicidae) of the Old World*. Warszawa: Natura optima dux Foundation.
- Reifenrath K, Becker C, Poethke HJ. 2012. Diaspore trait preferences of dispersing ants. *Journal of Chemical Ecology* 38(9):1093–1104 DOI 10.1007/s10886-012-0174-y.
- Reitz SR, Trumble JT. 2002. Competitive displacement among insects and arachnids. *Annual Review of Entomology* 47(1):435–465 DOI 10.1146/annurev.ento.47.091201.145227.
- Rosumek FB. 2017. Natural history of ants: what we (do not) know about trophic and temporal niches of neotropical species. *Sociobiology* 64(3):244–255 DOI 10.13102/sociobiology.v64i3.1623.
- Rosumek FB, Brückner A, Blüthgen N, Menzel F, Heethoff M. 2017. Patterns and dynamics of neutral lipid fatty acids in ants—implications for ecological studies. *Frontiers in Zoology* 14(1):36 DOI 10.1186/s12983-017-0221-1.
- Ruess L, Chamberlain PM. 2010. The fat that matters: soil food web analysis using fatty acids and their carbon stable isotope signature. *Soil Biology and Biochemistry* 42(11):1898–1910 DOI 10.1016/j.soilbio.2010.07.020.
- Ruess L, Schütz K, Migge-Kleian S, Häggblom MM, Kandeler E, Scheu S. 2007. Lipid composition of *Collembola* and their food resources in deciduous forest stands—implications for feeding strategies. *Soil Biology and Biochemistry* 39(8):1990–2000 DOI 10.1016/j.soilbio.2007.03.002.
- Schleuning M, Fründ J, Klein AM, Abrahamczyk S, Alarcón R, Albrecht M, Andersson GKS, Bazarian S, Böhning-Gaese K, Bommarco R, Dalsgaard B, Dehling DM, Gotlieb A, Hagen M, Hickler T, Holzschuh A, Kaiser-Bunbury CN, Kreft H, Morris RJ, Sandel B, Sutherland WJ, Svenning JC, Tscharrntke T, Watts S, Weiner CN, Werner M, Williams NM,

- Winqvist C, Dormann CF, Blüthgen N. 2012.** Specialization of mutualistic interaction networks decreases toward tropical latitudes. *Current Biology* **22(20)**:1925–1931 DOI [10.1016/j.cub.2012.08.015](https://doi.org/10.1016/j.cub.2012.08.015).
- Schluter D. 2000.** Ecological character displacement in adaptive radiation. *American Naturalist* **156(S4)**:S4–S16 DOI [10.1086/303412](https://doi.org/10.1086/303412).
- Seifert B. 2007.** *Die Ameisen Mittel-und Nordeuropas*. Tauer: Lutra.
- Seifert B, Schultz R. 2009.** A taxonomic revision of the *Formica rufibarbis* Fabricius, 1793 group (Hymenoptera: Formicidae). *Myrmecological News* **12**:255–272 DOI [10.1007/s12275-007-0115-6](https://doi.org/10.1007/s12275-007-0115-6).
- Silva RR, Brandão CRF. 2014.** Ecosystem-wide morphological structure of leaf-litter ant communities along a tropical latitudinal gradient. *PLOS ONE* **9(3)**:e93049 DOI [10.1371/journal.pone.0093049](https://doi.org/10.1371/journal.pone.0093049).
- Snealling RR, Longino JT. 1992.** Revisionary notes on the fungus-growing ants of the genus *Cyphomyrmex*, *rimosus* group (Hymenoptera: Formicidae: Attini). In: Quintero D, Aiello A, eds. *Insects of Panama and Mesoamerica: Selected Studies*. Oxford: Oxford University Press, 479–494.
- Sponheimer M, Robinson TF, Roeder BL, Passey BH, Ayliffe LK, Cerling TE, Dearing MD, Ehleringer JR. 2003.** An experimental study of nitrogen flux in llamas: is ^{14}N preferentially excreted? *Journal of Archaeological Science* **30(12)**:1649–1655 DOI [10.1016/S0305-4403\(03\)00066-9](https://doi.org/10.1016/S0305-4403(03)00066-9).
- Stanley-Samuelson DW, Jurenka RA, Cripps C, Blomquist GJ, de Renobales M. 1988.** Fatty acids in insects: composition, metabolism, and biological significance. *Archives of Insect Biochemistry and Physiology* **9(1)**:1–33 DOI [10.1002/arch.940090102](https://doi.org/10.1002/arch.940090102).
- Sun Q, Zhou X. 2013.** Corpse management in social insects. *International Journal of Biological Sciences* **9(3)**:313–321 DOI [10.7150/ijbs.5781](https://doi.org/10.7150/ijbs.5781).
- Thompson SN. 1973.** A review and comparative characterization of the fatty acid compositions of seven insect orders. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry* **45(2)**:467–482 DOI [10.1016/0305-0491\(73\)90078-3](https://doi.org/10.1016/0305-0491(73)90078-3).
- Tillberg CV, McCarthy DP, Dolezal AG, Suarez AV. 2006.** Measuring the trophic ecology of ants using stable isotopes. *Insectes Sociaux* **53(1)**:65–69 DOI [10.1007/s00040-005-0836-7](https://doi.org/10.1007/s00040-005-0836-7).
- Webb SC, Hedges RE, Simpson SJ. 1998.** Diet quality influences the d^{13}C and d^{15}N of locusts and their biochemical components. *Journal of Experimental Biology* **201**:2903–2911.
- Whitaker D, Christman M. 2015.** *clustsig: significant cluster analysis*. Version 1.1. Available at <https://CRAN.R-project.org/package=clustsig>.
- Wild AL. 2007.** *Taxonomic Revision of the Ant Genus Linepithema (Hymenoptera: Formicidae)*. Berkeley: University of California Press.
- Wilson EO. 2003.** *Pheidole in the New World: A Dominant, Hyperdiverse Ant Genus*. Cambridge: Harvard University Press.

Unveiling community patterns and trophic niches of tropical and temperate ants using an integrative framework of field data, stable isotopes and fatty acids

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Supplementary document S1: Bait composition and display (based on Houadria et al. 2015 and Rosumek 2017)

(1) Large prey: one individual of *Achaeta domesticus* (Linnaeus, 1758) with 1-2 cm body size, was tied between the first and second pair of legs to prevent it from escaping, but still be able to jump and move inside the trap. Represents larger, mobile prey with harder integument.

(2) Small prey: in Brazil, 15 termites were left free on a piece of mound, divided between workers and soldiers. Previous testing showed that termites do not leave the mound piece after 90 minutes. Another 10 individuals were glued to a small wood stick, to increase the time spent by ants on the bait. Individuals from the subfamily Nasutitermitinae were used, which have specialized workers with chemical defenses that can affect ants (Prestwich, 1984). In Germany, termites are rare, thus we used larvae of *Lucilia sericata* Meigen, 1826. Two maggots were pinned at the rear end, still able to move the body but not leave the bait. Previous testing showed

that they stayed alive for several hours under this condition. Both baits represent prey smaller, slower and softer, but not defenseless.

(3) Dead arthropods (2-3 g): crushed crickets and mealworms (*Tenebrio molitor* Linnaeus, 1758 – in Brazil) or maggots (*Lucilia sericata* Meigen, 1826 – in Germany).

(4) Bird feces (2-3 g): chicken feces from organic breeding. Represents nitrogen-rich bird feces, which could be directly collected by ants, or contain animal and vegetal remains used by them (Leal & Oliveira, 1998; Jaffe et al., 2001; Sainz-Borgo, 2015).

(5) Seeds (2-3 g): in Brazil, a mix of soy, millet, sunflower, canary, barley, linseed, grass seeds and ground corn, representing different sizes and shapes of seeds. We avoided seeds with elaiosomes because these mimic animal prey and attract predacious species, not only granivorous ones (Hughes et al., 1994; Giladi, 2006). However, in Germany, granivory is restricted to *Messor* ants, absent in our study site (Seifert, 2007). Thus, we used elaiosome seeds of *Chelidonium majus* (L.), known to be attractive to ants (Reifenrath, Becker & Poethke, 2012).

(6) Sucrose solution (2-3 ml): at 20% concentration. Sucrose and its components, fructose and glucose, are the main nutritional sugars in plant exudates and fleshy fruits (Percival, 1961; Baker et al., 1998; Blüthgen et al., 2004).

(7) Melezitose solution (2-3 ml): at 20% concentration. Represents insect-synthesized oligosaccharides present on honeydew (Kiss, 1981; Wäckers, 2000). Although melezitose and other oligosaccharides occur naturally almost only on honeydew, some insects secrete honeydew with little modification, similar to plant exudates (Völkl et al., 1999; Blüthgen et al., 2004).

Therefore, preference for melezitose indicates use of honeydew, and avoidance suggests limited use, although not definitive lack of interaction with sap-sucking insects.

References

Blüthgen N, Gottsberger, G, Fiedler K. 2004. Sugar and amino acid composition of ant-attended nectar and honeydew sources from an Australian rainforest. *Austral Ecology* 29:418–429 DOI 10.1111/j.1442-9993.2004.01380.x.

Giladi I. 2006. Choosing benefits or partners: a review of the evidence for the evolution of myrmecochory. *Oikos* 112:481–492 DOI 10.1111/j.0030-1299.2006.14258.x.

Houadria M, Salas-Lopez A, Orivel J, Blüthgen N, Menzel F. 2015. Dietary and temporal niche differentiation in tropical ants—can they explain local ant coexistence? *Biotropica* 47:208–217 DOI 10.1111/btp.12184.

Hughes L, Westoby M, Jurado E. 1994. Convergence of elaiosomes and insect prey: evidence from ant foraging behaviour and fatty acid composition. *Functional Ecology* 8:358–365.

Jaffe K, Caetano FH, Sánchez P, Hernández JV, Caraballo L, Vitelli-Flores J, Monsalve W, Dorta B, Lemoine VR. 2001. Sensitivity of ant (*Cephalotes*) colonies and individuals to antibiotics implies feeding symbiosis with gut microorganisms. *Canadian Journal of Zoology* 79:1120–1124 DOI 10.1139/z01-079.

Kiss A. 1981. Melezitose, aphids and ants. *Oikos* 37:382–382.

Leal IR, Oliveira PS. 1998. Interactions between fungus-growing ants (Attini), fruits and seeds in Cerrado vegetation in Southeast Brazil. *Biotropica* 30:170–178 DOI 10.1111/j.1744-7429.1998.tb00052.x.

Prestwich GD. 1984. Defense mechanisms of termites. *Annual Review of Entomology* 29:201–232.

Reifenrath K, Becker C, Poethke HJ. 2012. Diaspore trait preferences of dispersing ants. *Journal of Chemical Ecology* 38:1093–1104 DOI 10.1007/s10886-012-0174-y.

Rosumek FB. 2017. Natural history of ants: what we (do not) know about trophic and temporal niches of neotropical species. *Sociobiology* 66:244–255 DOI 10.13102/sociobiology.v64i3.1623.

Sainz-Borgo C. 2015. Bird feces consumption by fire ant *Solenopsis geminata* (Hymenoptera: Formicidae). *Entomological News* 124:295–299 DOI 10.3157/021.124.0408.

Seifert B. 2007. *Die Ameisen Mittel- und Nordeuropas*. Tauer: Lutra.

Völkl W, Woodring J, Fischer M, Lorenz MW, Hoffmann KH. 1999. Ant-aphid mutualisms: the impact of honeydew production and honeydew sugar composition on ant preferences. *Oecologia* 118:483–491 DOI 10.1007/s004420050751.

Wäckers FL. 2000. Do oligosaccharides reduce the suitability of honeydew for predators and parasitoids? A further facet to the function of insect-synthesized honeydew sugars. *Oikos* 90:197–201 DOI 10.1034/j.1600-0706.2000.900124.x.

Table S1: Incidence of species recorded in this work and labels used in plots.

Species	IB	IP	Label	Species	IB	IP	Labels
Brazil							
<i>Acanthognathus brevicornis</i> Smith, 1944	-	-	acanbr	<i>Pheidole schmalzi</i> Emery, 1894	-	1	pheisc
<i>Acanthognathus ocellatus</i> Mayr, 1887	-	-	acanoc	<i>Pheidole sigillata</i> Wilson, 2003	33	18	pheisi
<i>Acromyrmex aspersus</i> (Smith, 1858)	1	-	acroas	<i>Pheidole</i> sp.1	18	13	phei01
<i>Acromyrmex laticeps</i> (Emery, 1905)	-	-	acrola	<i>Pheidole</i> sp.2	57	38	phei02
<i>Acromyrmex lundii</i> (Guérin-Méneville, 1838)	-	-	acrolu	<i>Pheidole</i> sp.4	17	23	phei04
<i>Acromyrmex subterraneus</i> (Forel, 1893)	-	-	acrosu	<i>Pheidole</i> sp.5	1	2	phei05
<i>Apterostygma acre</i> Latteke 1997	1	-	apteac	<i>Pheidole</i> sp.6	4	2	phei06
<i>Azteca</i> sp.1	-	1	azte01	<i>Pheidole</i> sp.7	5	6	phei07
<i>Azteca</i> sp.2	-	-	azte02	<i>Pheidole</i> sp.8	-	1	phei08
<i>Brachymyrmex</i> sp.1	2	1	brac01	<i>Pheidole</i> sp.9	-	1	phei09
<i>Brachymyrmex</i> sp.2	-	1	brac02	<i>Pheidole</i> sp.10	1	-	phei10
<i>Camponotus lespepii</i> Forel, 1886	4	1	cample	<i>Pseudomyrmex flavidulus</i> (Smith, 1858)	-	1	pseufl
<i>Camponotus zenon</i> Forel, 1912	10	-	campze	<i>Solenopsis subterranea</i> MacKay & Vinson, 1989	-	1	solesu
<i>Cephalotes pallidicephalus</i> (Smith, 1876)	-	-	cephpa	<i>Solenopsis</i> sp.1	41	20	sole01
<i>Cephalotes pusillus</i> (Klug, 1824)	-	-	cephpu	<i>Solenopsis</i> sp.2	43	28	sole02
<i>Crematogaster nigropilosa</i> Mayr, 1870	4	1	cremni	<i>Solenopsis</i> sp.3	12	8	sole03
<i>Cyphomyrmex rimosus</i> (Spinola, 1851)	6	10	cyphri	<i>Solenopsis</i> sp.4	40	24	sole04
<i>Gnamptogenys striatula</i> Mayr, 1884	21	21	gnamst	<i>Solenopsis</i> sp.5	1	1	sole05
<i>Fulakora elongata</i> (Santschi, 1912)	1	-	fulael	<i>Solenopsis</i> sp.6	12	7	sole06
<i>Heteroponera dentinodis</i> (Mayr, 1887)	1	1	hetede	<i>Solenopsis</i> sp.7	-	1	sole07
<i>Heteroponera dolo</i> (Roger, 1860)	-	1	hetedo	<i>Solenopsis</i> sp.8	7	2	sole08
<i>Hypoconera</i> sp.1	-	2	hypo01	<i>Strumigenys cosmoptela</i> Kempf, 1975	-	1	struco
<i>Hypoconera</i> sp.2	-	1	hypo02	<i>Strumigenys denticulata</i> Mayr, 1887	-	6	strude
<i>Hypoconera</i> sp.3	-	1	hypo03	<i>Strumigenys elongata</i> Roger, 1863	-	1	struel
<i>Hypoconera</i> sp.4	1	-	hypo04	<i>Strumigenys splendens</i> (Borgmeyer, 1954)	-	1	strusp
<i>Hylomyrma reitteri</i> (Mayr, 1887)	7	9	hylore	<i>Tapinoma atriceps</i> Emery, 1888	-	1	tapiat
<i>Linepithema iniquum</i> (Mayr, 1870)	7	-	linein	<i>Trachymyrmex</i> sp.1	1	1	trac01
<i>Linepithema leucomelas</i> (Emery, 1894)	-	2	linele	<i>Wasmannia affinis</i> Santschi, 1929	6	2	wasmaf
<i>Linepithema micans</i> (Forel, 1908)	6	1	linemi	<i>Wasmannia auropunctata</i> (Roger, 1863)	16	-	wasmau
<i>Linepithema pulex</i> Wild, 2007	4	1	linepu	<i>Wasmannia lutzii</i> Forel, 1908	-	1	wasmlu
<i>Neoponera crenata</i> (Roger, 1861)	-	1	neopcr	Germany			
<i>Neoponera villosa</i> (Fabricius, 1804)	-	1	neopvi	<i>Formica cunicularia</i> (Latreille, 1798)	2	5	formcu
<i>Nylanderia docilis</i> (Forel, 1908)	1	-	nylado	<i>Formica fusca</i> Linnaeus, 1758	35	41	formfu
<i>Nylanderia</i> sp.1	54	23	nyla01	<i>Formica rufibarbis</i> Fabricius, 1793	-	1	formru
<i>Octostruma petiolata</i> (Mayr, 1887)	2	2	octope	<i>Lasius brunneus</i> (Latreille, 1798)	4	2	lasibr
<i>Octostruma stenognatha</i> Brown & Kempf, 1960	-	1	octost	<i>Lasius flavus</i> (Fabricius, 1782)	-	12	lasifl
<i>Octostruma</i> sp.1	1	1	octo01	<i>Lasius fuliginosus</i> (Latreille, 1798)	5	5	lasifu
<i>Odontomachus chelifer</i> (Latreille, 1802)	20	11	odonch	<i>Lasius niger</i> (Linnaeus, 1758)	25	26	lasini
<i>Odontomachus meinerti</i> Forel, 1905	1	1	odonme	<i>Lasius platythorax</i> Seifert, 1991	10	10	lasipl
<i>Oxyepoecus plaumanni</i> Kempf, 1974	-	1	oxyepl	<i>Myrmica lobicornis</i> Nylander, 1846	1	5	myrmlo
<i>Pachycondyla harpax</i> (Fabricius, 1804)	1	10	pachha	<i>Myrmica rubra</i> (Linnaeus, 1758)	15	22	myrmrb
<i>Pachycondyla striata</i> Smith, 1858	43	40	pachst	<i>Myrmica ruginodis</i> Nylander, 1846	25	16	myrmrg
<i>Pheidole angusta</i> Forel, 1908	3	1	pheian	<i>Myrmica rugulosa</i> Nylander, 1849	1	2	myrmrl
<i>Pheidole aper</i> Forel, 1912	9	7	pheiap	<i>Myrmica scabrinodis</i> Nylander, 1846	6	17	myrmrc
<i>Pheidole avia</i> Forel, 1908	5	2	pheiav	<i>Stenamma debile</i> (Foerster, 1850)	-	16	stende
<i>Pheidole lucretii</i> Santschi, 1923	13	8	pheilu	<i>Temnothorax affinis</i> (Mayr, 1855)	-	2	temnaf
<i>Pheidole nesioti</i> Wilson, 2003	19	11	pheine	<i>Temnothorax nylanderi</i> (Foerster, 1850)	53	52	temnny
<i>Pheidole risii</i> Forel, 1892	4	3	pheiri	<i>Tetramorium caespitum</i> (Linnaeus, 1758)	-	1	tetrca
<i>Pheidole sarcina</i> Forel, 1912	11	9	pheisa				

IB = incidence in baits (i.e. number of sample points where the species was recorded with this method); IP = incidence in pitfall traps; - = species was not recorded with this method (double "--" means that the species was only recorded by colony sampling).

Table S2: SIMPER analysis for ant fatty acids in Brazil and Germany.

NLFAs are ordered by decreasing contribution towards dissimilarity between sites (Bray-Curtis).

Asterisks indicate statistically significant differences.

NLFA	Average dissimilarity	SD	Average/SD ratio	Average % Brazil	Average % Germany	Cumulative contribution	P
C18:1n9	0.210	0.087	2.409	30.311	72.257	0.473	0.000*
C18:0	0.091	0.040	2.312	23.231	5.039	0.679	0.000*
C16:0	0.083	0.053	1.558	33.075	17.810	0.866	0.056
C18:2n6	0.026	0.022	1.185	5.919	0.808	0.924	0.222
C16:1n9	0.009	0.007	1.346	1.182	2.047	0.944	0.250
C18:2unk1	0.006	0.006	1.001	1.634	0.500	0.958	0.665
C18:2unk2	0.005	0.005	0.930	1.187	0.222	0.970	0.590
C18:1n11	0.004	0.004	1.060	1.032	0.245	0.979	0.211
C14:0	0.003	0.002	1.556	0.875	0.474	0.985	0.024
C12:0	0.002	0.001	1.229	0.451	0.087	0.990	0.154
C20:0	0.001	0.001	1.045	0.310	0.099	0.992	0.746
C17:0	0.001	0.001	1.316	0.373	0.256	0.995	0.325
C16:1n7	0.001	0.002	0.393	0.194	0.091	0.997	0.624
C15:0	0.001	0.000	1.454	0.158	0.016	0.999	0.040*
C22:0	0.000	0.000	1.384	0.000	0.039	0.999	0.000*
iC15:0	0.000	0.000	0.453	0.033	0.000	0.999	1.000
iC17:0	0.000	0.000	0.360	0.016	0.000	1.000	1.000
C24:0	0.000	0.000	1.182	0.000	0.011	1.000	0.000*
aiC17:0	0.000	0.000	0.430	0.010	0.000	1.000	1.000
aiC15:0	0.000	0.000	0.488	0.010	0.000	1.000	1.000

Table S3:**Principal component analysis of ant species × baits in Brazil and Germany.**

	PC1	PC2	PC3	PC4	PC5	PC6
Brazil						
Inertia/variance = 4.435						
Eigenvalue	1.61	1.05	0.85	0.46	0.29	0.17
% of variance explained	36.4	23.8	19.2	10.4	6.5	4
Germany						
Inertia/variance = 3.238						
Eigenvalue	1.676	0.804	0.519	0.151	0.084	0.004
% of variance explained	51.8	24.8	16	4.7	2.6	0.1

Regression values of NLFAs with the two first Principal Components in Brazil and Germany.

Asterisks indicate statistically significant differences.

NLFA	PC 1	PC 2	r ²	p	NLFA	PC 1	PC 2	r ²	p
Brazil					Germany				
C12:0	-0.96	0.29	0.11	0.33	C12:0	-0.73	-0.68	0.01	0.99
C14:0	-0.98	-0.18	0.39	0.01*	C14:0	0.35	-0.94	0.20	0.68
iC15:0	0.30	-0.96	0.12	0.29	C15:0	-0.94	-0.33	0.20	0.65
aiC15:0	0.99	-0.13	0.12	0.29	C16:1n7	-0.96	-0.27	0.52	0.18
C15:0	-0.07	-1.00	0.11	0.34	C16:1n9	-0.27	-0.96	0.49	0.28
C16:1n7	0.18	-0.98	0.19	0.15	C16:0	0.18	-0.98	0.18	0.74
C16:1n9	-0.73	-0.68	0.14	0.23	C17:0	0.74	-0.67	0.84	0.04*
C16:0	-0.41	0.91	0.26	0.06	C18:2n6	0.88	-0.48	0.08	0.79
iC17:0	0.45	-0.89	0.13	0.26	C18:1n9	-0.56	0.83	0.33	0.46
aiC17:0	0.90	-0.45	0.16	0.23	C18:1n11	0.89	-0.46	0.42	0.35
C17:0	0.79	-0.62	0.09	0.42	C18:0	1.00	0.07	0.78	0.05*
C18:2n6	0.68	0.73	0.13	0.29	C18:2unk1	-0.31	-0.95	0.08	0.84
C18:1n9	0.25	-0.97	0.32	0.02*	C18:2unk2	0.99	-0.15	0.08	0.85
C18:1n11	-0.15	-0.99	0.09	0.39	C20:0	0.99	-0.12	0.23	0.60
C18:0	-0.23	0.97	0.23	0.09	C22:0	0.91	0.42	0.33	0.43
C18:2unk1	0.98	0.21	0.31	0.03*	C24:0	1.00	0.04	0.60	0.19
C18:2unk2	0.97	0.25	0.22	0.09					
C20:0	0.58	-0.81	0.14	0.23					