



Trophic interactions among dead-wood-dependent forest arthropods in the southern Appalachian Mountains, USA

R.C. Garrick^{a,*}, D.K. Reppel^a, J.T. Morgan^a, S. Burgess^a, C. Hyseni^a, R.J. Worthington^a, M.D. Ulyshen^b

^a Department of Biology, University of Mississippi, Oxford, MS 38677, USA

^b USDA Forest Service, Southern Research Station, Athens, GA 30602, USA

ARTICLE INFO

Article history:

Received 8 June 2018

Received in revised form 16 November 2018

Accepted 11 December 2018

Keywords:

Appalachian Mountains

Dead wood

Food web

Mitochondrial DNA

Molecular taxonomic assignment

Saproxylic

Species interaction

Temperate forest

ABSTRACT

Food webs based on dead organic matter have received relatively little research attention. Here we focus dead-wood-dependent (saproxylic) arthropod communities—an overlooked component of forest biodiversity that contributes to decomposition of fallen trees and nutrient cycling. First, we summarized information on factors that impact saproxylic arthropod biodiversity via a descriptive mini-review of the literature, given that the structure of food webs should be contingent upon local community composition, species richness, and/or species abundances within and among neighboring rotting logs. Next, we coupled intensive fieldwork with molecular approaches to taxonomic identification of saproxylic arthropods sampled from rotting logs in the southern Appalachian Mountains, and synthesized information on their feeding ecology, in order to infer trophic interactions. Our mini-review highlighted major influences of local-scale (site-specific) factors affecting biodiversity, and by extension, food web structure; a pronounced publication bias toward saproxylic beetles from evergreen forests in Europe was also evident. Our empirical data on community composition of rotting logs at intermediate to late stages of decay revealed a complex food web structure. This was comprised of internal and external primary nutrient sources (dead wood within logs vs. nearby living trees and fallen leaves), a diverse suite of primary consumers (wood-feeding detritivores, leaf litter-feeding detritivores, as well as herbivores and fungivores), several secondary consumer functional groups (omnivorous scavengers and ectoparasites or parasitoids), and top-level carnivorous predators that were mostly made up of spiders, opiliones, and centipedes. We close by discussing persistent challenges and limitations, and suggest future research directions.

© 2019 Elsevier Inc. All rights reserved.

1. Introduction

Compared to green food webs, brown food webs (i.e., those based on dead rather than living autotrophs) remain understudied, despite the critical role the associated organisms play in nutrient cycling and related processes. Organisms associated with dead wood are of particular interest given their diversity and vulnerability to forest management decisions. Between one fifth and one third of all forest invertebrate species are saproxylic, meaning they depend directly or indirectly on dying or dead wood (Speight, 1989). Declines of saproxylic insect diversity constitute one of the biggest threats to biodiversity in many intensively managed landscapes, raising concerns about how basic forest functions may change as a result. For example, saproxylic insects are known to play important roles in decomposition and controlling forest pest populations (Ulyshen and Šobotník, 2018) and these services may be affected by losses of species. Dobson et al. (2006) suggested that ecosystem degradation results in a shortening of food chain length and that services

provided by upper trophic levels will likely be lost before those provided by species lower in the food chain. Consistent with this, saproxylic parasitoids are believed to be more sensitive to disturbances than their hosts (Hilszczański, 2018), and recent work indicates this may also be true for predatory saproxylic beetles (Ranlund and Victorsson, 2018). These findings suggest that predation may be one of the first services provided by saproxylic communities to be affected by habitat degradation, thus highlighting the importance of understanding trophic interactions among saproxylic invertebrates.

Although many studies have investigated the biodiversity of invertebrates in dead wood, few have explored the entire community of interacting taxa. Ironically, some of the earliest studies of saproxylic insect communities provide some of the most complete descriptions of insect communities occurring in dead wood (Blackman and Stage, 1924; Savely, 1939; Howden and Vogt, 1951; also see Harmon et al., 1986). By contrast, more recent inventories (Grove and Forster, 2011)—and especially experimental studies—have targeted particular subsets of this fauna with most focusing on beetle assemblages associated with recently dead wood. More efforts to collect baseline information about the composition of invertebrate communities in dead wood, especially

* Corresponding author.

E-mail address: rgarrick@olemiss.edu (R.C. Garrick).

in wood at advanced stages of decomposition (Ferro, 2018), are needed before it will be possible to characterize the trophic relationships necessary to understand the dynamics of these communities.

Despite various functional group classification systems (Bouget et al., 2005; Stokland, 2012) and growing interest in insect-insect (Brin and Bouget, 2018) and insect-fungal (Birkemoe et al., 2018) interactions, detailed descriptions of saproxylic food webs based on complete lists of coexisting species are rare. One of the first attempts at this was made by Savely (1939) who prepared a diagram showing the "food relations" among insect species observed in dead pine and oak logs in the southeastern United States. Similarly, Howden and Vogt (1951) created generalized food webs based on invertebrates collected from standing dead pine trees. Such studies continue to be hindered by the taxonomic impediment as well as uncertainties about the diets of most species. Once these limitations are overcome, there is considerable scope for making inferences about trophic interactions based on co-occurrence data and information on feeding ecology. Molecular approaches offer a solution to both challenges, first by providing a way to quickly and accurately identify specimens sampled at any life stage, including the separation of cryptic species (Garrick and Bouget, 2018), and second by providing insights into the trophic position of species using stable isotope analysis. Here we focus on the first of these two steps, applied to arthropods collected from rotting logs at intermediate to late stages of decay in the southern Appalachian Mountains. This temperate forest region is a well-known biodiversity hotspot for amphibians (Rissler and Smith, 2010), and its topographic complexity may promote high local endemism in low-mobility invertebrates (Garrick, 2011; Garrick et al., 2017). Indeed, the southern Appalachians are expected to harbor high levels of as-yet unknown arthropod biodiversity (Carlton and Bayless, 2007), and the region's saproxylic fauna are largely unstudied (but see Ferro et al., 2012a, 2012b).

Saproxylic invertebrates usually have very poor dispersal abilities due to winglessness and/or rapid desiccation as a consequence of adaptations to life in a rotting log (e.g., Vargo, 2003; Jackson et al., 2009; Garrick et al., 2012; Tini et al., 2018). Accordingly, the structure of food webs can be expected to be contingent upon local community composition, species richness, and/or species abundances within and among neighboring rotting logs. In this paper, our first goal was to summarize information on factors that impact saproxylic arthropod biodiversity at local and regional spatial scales. Within this context, our second goal was to present new data on the composition of this community in a temperate montane forest and to synthesize available information on feeding ecology of species that were identified in order to infer trophic interactions among them.

2. Materials and methods

2.1. Descriptive mini-review of the literature

2.1.1. Key word searches and synthesis of findings

To summarize basic information on factors that affect saproxylic community composition, species richness, and/or species abundances—and thus impact trophic interactions—literature searches were conducted using Scopus (www.elsevier.com, accessed 6th November 2018). A preliminary assessment of several combinations of search terms appearing in the title, abstract and/or keywords of published articles and those in press indicated that the majority of potentially relevant papers could be most efficiently captured using each of the following two search strings and associated Boolean operators: 1) *sapro** AND *insect** AND *interaction**, and 2) *sapro** AND (*insect** OR *arthropod**) AND *communit** AND *trophic** (where the asterisk is a wildcard character). From the resulting hits, exemplar papers reporting on different influential factors were identified and summarized. Although coarse, outcomes provide a general overview of how stochastic and deterministic processes may affect trophic interactions in dead wood microhabitats.

2.2. Trophic interactions reconstructed from empirical data on community composition

2.2.1. Field collections and specimen curation

From 17 May to 30 June 2016, saproxylic arthropods were sampled from upland hardwood or mixed forests in Bankhead National Forest, Alabama (16 rotting logs from 5 sites), and Great Smoky Mountains National Park, Tennessee and North Carolina (16 logs from 5 sites; Fig. 1; Appendix 1). Briefly, Bankhead National Forest was established in 1918, at which time it had a long history of fire suppression and had been heavily logged; however, the Sipse Wilderness within Bankhead still contained some old growth forest (USDA, 2003). Management practices focused on reclaiming abandoned farmland and previously clear-cut areas, and reintroduction of fire via controlled burning. By the 1960s, upland hardwood forests were being replaced with loblolly pine owing to their fast growth rate and commercial logging value. Today, approximately 51% of forested areas are comprised of southern pines, with the remaining 49% being hardwoods (USDA, 2003). In contrast, the Great Smoky Mountains National Park was established in 1926 (later also designated as a World Heritage Site in 1983), had experienced less extensive land clearing and logging activities than Bankhead, and as much as 30% of forests within the park are old growth (NPS, 2006). Management focuses on protecting biodiversity (>19,000 species documented to date, including 11,000 invertebrates) with ~89% of the park classified as wilderness, as well as enhancing ecotourism, recreation and educational experiences for the ~11 million visitors per year (NPS, 2006). Owing to the environmental heterogeneity associated with the ancient, rugged and steep terrain, the Great Smoky Mountains National Park comprises over 70 vegetation communities, including spruce-Fraser fir, beech, hardwood and mixed forests (NPS, 2006).

All sampled logs were hardwoods at an intermediate to late stage of decay with an intact outer shell and heartwood colonized by brown-rot fungi, in direct contact with soil, with little or no indications of having been recently burned. Macroscopic arthropods (i.e., those of sufficient size that genomic DNA could be readily extracted and amplified) at any life stage were exhaustively sampled from a 0.9 m long section of each rotting log (removed using a chainsaw then carefully dismantled in an enclosure), and preserved in 95% ethanol. In the lab, specimens were separated from woody debris, and sorted into morphotype-based groups. The sorting procedure was conducted without any cross-referencing of morphotypes sampled in different logs (i.e., each sampled rotting log was essentially an independent unit), such that there was a tendency to initially over-split taxa. Subsequently, one representative of each morphotype per log was selected for molecular analyses. Putative duplicate specimens were stored at -80°C .

2.2.2. DNA extraction and mitochondrial COI amplification

Genomic DNA was extracted using a DNeasy Blood and Tissue kit (Qiagen, Valencia, CA) from single legs to whole bodies, depending on specimen size. After aerating tissue samples to facilitate ethanol evaporation, extraction procedures followed the manufacturers' recommendations. Next, a portion of the mitochondrial DNA (mtDNA) cytochrome c oxidase subunit I (COI) gene was amplified via polymerase chain reaction (PCR) in 15 μL volumes, containing the following: 3.0 μL 5 \times PCR Buffer (Promega, Madison, WI), 1.2 μL MgCl_2 (25 mM, Promega), 2.4 μL dNTPs (1.25 mM, Promega), 0.75 μL Bovine Serum Albumin (10 mg/mL, New England BioLabs), 4.5 μL dH_2O , 0.75 μL of each primer (10 μM), 0.15 μL *Go-Taq* (5 U/ μL , Promega), and 1.5 μL of genomic DNA. Owing to the considerable taxonomic diversity of sampled saproxylic arthropods, several combinations of forward and reverse primer pairs were often trialled. Broadly useful primer pair combinations were LCO-1490 and HCO-2198 (Folmer et al., 1994), C1-J-1718 and C1-N-2329 (Simon et al., 1994), and LCO-1490 and C1-N-2329. PCR amplifications were performed using a Bio-Rad T100 Thermal Cycler with the following profile: 95 $^{\circ}\text{C}$ for 2 min (1 cycle), 95 $^{\circ}\text{C}$ for

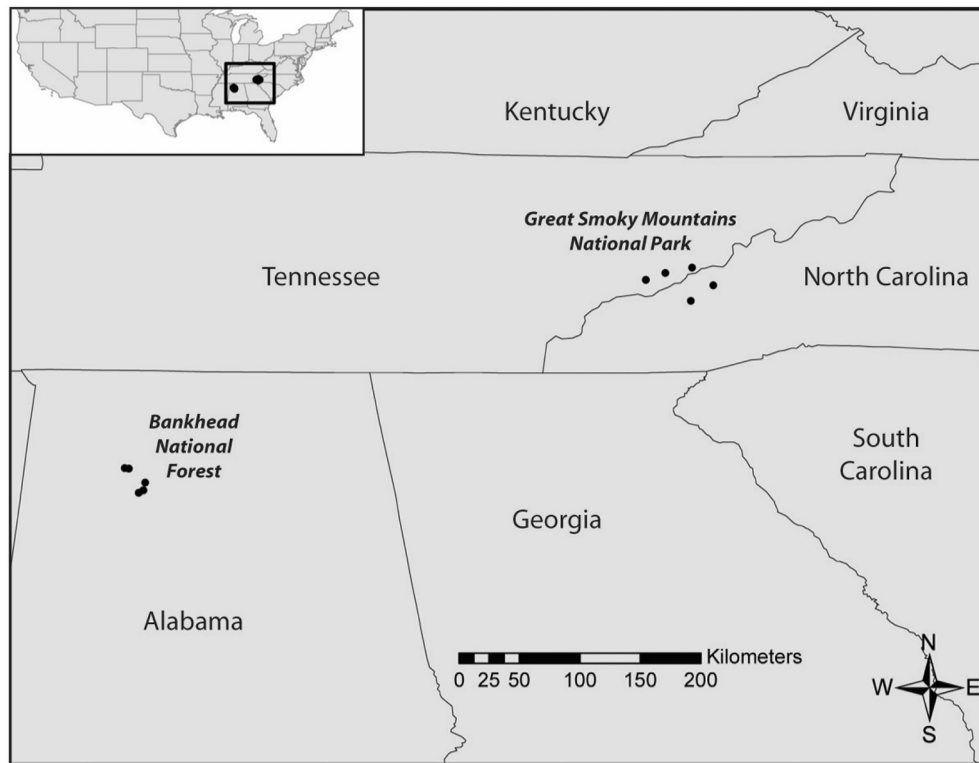


Fig. 1. Geographic distribution of rotting logs (16 logs across 5 sites in each of two forest regions; Bankhead National Forest and Great Smoky Mountains National Park) from which southern Appalachian saproxylic arthropods were sampled. Specimens were then sequenced for the purpose of molecular taxonomic identification, followed by reconstruction of trophic interactions. Inset: map of USA showing location of the study region.

30 s, 49 °C for 30 s, and 72 °C for 1 min (40 cycles), and final extension of 72 °C for 2 min (1 cycle). All PCRs were run with a negative control. Amplified products were viewed following electrophoresis on a 1.5% agarose gel, purified using ExoSAP-IT (Affymetrix, Santa Clara, CA), and sequenced on an Applied Biosystems 3730× Genetic Analyzer at Yale University's DNA Analysis Facility on Science Hill.

2.2.3. Sequence editing and molecular taxonomic assignment

DNA sequence chromatograms were edited and aligned using MEGA v6 (Tamura et al., 2013). Recovery of true mtDNA (cf. nuclear-mitochondrial pseudogenes) was assessed by translating DNA to amino acids and checking for open reading frames. In order to tentatively assign genus- or species-level identifications to sequenced specimens, nucleotide Basic Local Alignment Search Tool (Altschul et al., 1990) searches were conducted using tools available via the National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov) using default settings. Close matches to between the query (i.e., unknown saproxylic arthropod sequence) and subject (i.e., named database accession) were first rank-ordered based on percent similarity, and then by percent query coverage, in order to determine the best match. Following Telfer et al. (2015), we used thresholds of $\geq 98\%$ similarity for tentative species-level assignment, and $\geq 95\%$ similarity for genus-level assignment. For the purpose of inferring food web structure based on sampled taxa, family-level identifications were considered too coarse.

2.2.4. Feeding ecology data and food web construction

Following molecular identifications, information on feeding ecology was obtained for each taxon from a combination of peer-reviewed journal articles and books, unpublished PhD dissertations, or on-line resources curated by professional entomologists and qualified citizen scientists. For example, classification of major functional groups represented by each of the sampled beetle taxa was largely based on Evans (2014), whereas classification of ant and centipede species was based on AntWiki (www.antwiki.org) or AntWeb (www.antweb.org) and

ChiloBase (www.chilobase.biologia.unipd.it). The BugGuide searchable database hosted by Iowa State University (www.bugguide.net) was a useful source of feeding ecology data for other taxonomic groups. Where possible, we also checked for concordance with information other on-line sources (see Appendix 2). Collectively, these data were used to reconstruct likely trophic interactions among major functional groups of saproxylic arthropods from the southern Appalachian Mountains.

3. Results and discussion

3.1. Descriptive mini-review of the literature

A total of 30 and 136 papers matched our title, abstract and/or keyword search criteria using each of the two search strings and operators described above (see Materials and methods); 27 of these papers were relevant to the goals of this study. As a group, these studies indicated a strong taxonomic and geographic bias, in which saproxylic beetles from evergreen forests in Europe have attracted the most research attention (Table 1). Nonetheless, they are still informative about the kinds of factors that influence saproxylic arthropod community composition, species richness, and/or species abundances, and by extension, how the structure of saproxylic food webs may vary in space and over time. Briefly, across broad spatial scales, factors such as forest structural complexity, openness, connectivity and coarse woody debris (CWD) abundance are likely to have impacts on the number and types of trophic interactions that can occur within dead wood habitats. In forests that are harvested, management practices (e.g., timber extraction procedures, and logging cycles) may also influence saproxylic arthropod community composition and/or species abundances, presumably owing to changes in the spatial and temporal continuity of dead wood. Most notably, however, our mini-review highlighted the major influence that local-scale site-specific factors can have on food web structure within rotting log microhabitats (Table 1). These include abiotic factors

Table 1
Summary of exemplar studies that report on factors affecting saproxylic arthropod community composition, species richness, and/or species abundances (note: coarse woody debris is abbreviated as CWD).

Factor affecting saproxylic arthropods	Forest type and study region	Focal taxonomic groups	Major findings	References
Forest structure and/or CWD abundance	Cool temperate forests (beech, fir, spruce) in Germany	Coleoptera	CWD abundance is a more important determinant of beetle species richness than habitat patch size or isolation.	Seibold et al. (2017)
	Mediterranean montane forests (beech, conifers) in Italy	Coleoptera	Beetle assemblages are influenced by abundance, diversity and decay stage of CWD, forest structural complexity, and microhabitat occurrence.	Parisi et al. (2016)
	Mediterranean forests (oak, ash) in Spain	Coleoptera	Forest structural complexity positively influences saproxylic diversity and interaction complexity.	Quinto et al. (2015)
Management history	Cool temperate forests (beech, oak, pine, fir, spruce) in France	Coleoptera	Forest stand openness and CWD availability influence saproxylic beetle species richness and composition.	Bouget et al. (2014)
	Cool temperate boreal forests (aspen, balsam fir, poplar, white/black spruce, jack pine) in Canada	Not applicable (all saproxylic spp.)	Logging history does not influence arthropod abundance, but most saproxylic insect families are more abundant in late-decay wood.	Dennis et al. (2018)
	Cool temperate/boreal forests (oak) in Sweden	Coleoptera	Species richness and abundance were higher at sites with short histories of low-intensity logging (cf. 500 yr high intensity history)	Pilskog et al. (2018)
	Mediterranean Maulino (beech) forest in Chile	Coleoptera	Native forest supports greater species abundance and richness of all trophic guilds, compared to clear cuts replanted with exotic <i>Pine</i> and <i>Eucalyptus</i> .	Fierro et al. (2017)
	Cool temperate boreal forests (white spruce) in Canada	Coleoptera	Large patches of living trees retained in harvested areas support beetle assemblages similar to intact forest, smaller patches suffer from edge effects.	Lee et al. (2015)
	Cool temperate/boreal forests (spruce) in Sweden	Coleoptera	Compared to old growth or mature managed stands, clear cuts have distinct assemblages with lower beetle species richness but higher abundances.	Johansson et al. (2007)
	Cool temperate/boreal forests (oak) in Sweden	Coleoptera	Experimental placement of wood mold boxes at varied distances from hollow veteran trees showed that small-scale isolation led to reductions in biodiversity.	Mestre et al. (2018)
Local-scale factors	Mediterranean Maulino (beech) forest in Chile	Coleoptera	Across protected and unprotected native forests, dead wood volume and mean tree diameter strongly affected species abundances and distributions.	García-López et al. (2016)
	Temperate forests (oak, hickory) in the USA	Not applicable (all saproxylic spp.)	Downslope redistribution of dead wood creates topographic zones of high connectivity, which may be local hotspots of diversity.	Oberle et al. (2016)
	Cool temperate forests (beech) in Europe	Coleoptera	The relationship between dead wood amount and species richness is impacted by temperature.	Müller et al. (2015)
	Cool temperate forests (pine, oak, beech, fir) in France	Coleoptera	Mid-canopy vs. forest floor dead wood harbor beetle communities that differ in species richness and/or abundance of some trophic guilds among forest types.	Bouget et al. (2011)
	Cool temperate/boreal forests (spruce) in Sweden	Coleoptera	Experimentally burnt or shaded logs generally have lower beetle abundances or species richness after 1–2 years of field exposure.	Johansson et al. (2007)
	Cool temperate forests (oak, beech) in Belgium	Coleoptera and Diptera	Longhorn beetles favored stands dominated by mature oak with many snags, but hoverflies preferred open stands with herbs.	Fayt et al. (2006)
	Cool temperate/boreal forests (beech, spruce) in Switzerland	Coleoptera and Diptera	Over small spatial scales (150 m), high connectivity of dead wood was associated with higher species richness	Schiegg (2000)
	Not applicable (all temperate areas)	Not applicable (all saproxylic invertebrates)	Over time, there is a successional change from host-plant-specific to decay-stage-specific CWD species communities.	Harmon et al. (1986)
Priority effects	Cool temperate forests (oak, pine, spruce, hornbeam) in Poland	Lepidoptera	Saproxylic moth abundance/diversity is dependent on wood-decaying fungi species, number of fruiting bodies, and indirectly, CWD.	Jaworski et al. (2016)
	Cool temperate forests (beech) in England	Coleoptera	Fungal volatile organic compounds are sufficiently potent for detection by beetles. Eleven beetle species exhibit fungal preference.	Leather et al. (2014)
	Cool temperate/boreal forests (pine) in Spain	Coleoptera	Bark beetle (<i>Ips sexdentatus</i>) aggregation pheromones attract assemblage of beetles, including predators and competitors.	Etxebeste et al. (2013)
	Cool temperate/boreal forests (spruce) in Sweden	Coleoptera	Colonization of logs by certain early-succession beetle species, mediated by wood-decaying fungi, either facilitates or inhibits subsequent occurrence (10 yr later) of a rare beetle.	Weslien et al. (2011)
	Cool temperate/boreal old growth forests (spruce) in Sweden	Coleoptera	Odors emitted by some wood-rotting fungi attract dominant early-succession bark and rove beetle species.	Johansson et al. (2006)
Ecosystem engineers	Cool temperate forests (mixed) in Canada	Coleoptera	Activities of beavers concentrate dead wood, leading to higher activity and reproductive output of longhorn and bark beetles.	Mourant et al. (2018)
	Mediterranean forest (oak) in Spain	Coleoptera and Diptera	The feeding activities of Cetoniidae beetle larvae modify dead wood substrates, enhancing Syrphidae fly growth and fitness.	Sánchez-Galván et al. (2014, 2018)
	Cool temperate forests (oak) and pastures in Germany	Coleoptera	Activities of longhorn beetle larvae may lead to higher species richness (including red-listed taxa) in beetle communities.	Buse et al. (2008)
	Cool temperate Bavarian forests (spruce, beech, fir) in Germany	Coleoptera	Outbreaks of the spruce bark beetle create gaps in forests that lead to increased beetle species abundance and diversity.	Müller et al. (2008)

such as log-specific exposure to fire and shade, local temperatures, the vertical stratum in which dead wood microhabitats occur, and local topography of the forest floor. Furthermore, the decay stage of CWD is correlated with internal structural complexity, where late-stage logs may have well-developed gallery systems, and are generally more heterogeneous than early-stage logs. Also at the local scale, biotic factors such as priority effects (i.e., the lasting legacy of initial colonization events that are largely driven by chance) and the presence or absence of ecosystem engineers (i.e., species that significantly modify CWD) may be important determinants of saproxylic food web structure.

Kuuluvainen and Siitonen (2014) characterized the saproxylic food web in unmanaged Fennoscandian boreal forests as a complex adaptive system, and highlighted the importance of local-scale factors that generate and maintain a high diversity of species, and interactions among them. Those authors noted that multifaceted species interaction networks may be promoted by heterogeneous dead wood substrates within and among logs at a site, by the spatiotemporal patchiness of CWD occurrence, and by extension, colonization/succession dynamics. These findings also extend to South American forests; Fierro et al. (2017) reported on the importance of local factors such as substrate type, decay stage, and microclimate for saproxylic beetle diversity, abundance and trophic structure in Chile. Ultimately, each rotting log represents a discrete habitat patch that at any given time supports only a subset of saproxylic arthropods present in a forest. Additionally, this subset changes over time, as does the level of interaction network specialization (Wende et al., 2017). Based on these ideas, coupled with findings from the mini-review summarized above, we suggest that any characterization of a food web for this ecological community may need to be qualified by explicit reference to the decay stage of dead wood. Accordingly, inferences from empirical data presented in this paper specifically apply to intermediate to late stage logs in temperate forests.

3.2. Trophic interactions reconstructed from empirical data on community composition

Overall, we collected and sequenced saproxylic arthropods from 32 rotting logs across 10 sites in two forest regions in the southern Appalachian Mountains. On average each sampled log yielded ~10 different morphotype-based groups. Species-level molecular taxonomic assignments were achieved for 40 different taxa (14 beetles, 11 spiders, 7 ants, 2 termites, 2 harvestmen, and a cockroach, cricket, millipede and moth species; Appendix 2). An additional 8 taxa could be assigned to genus-level only (3 spiders, 2 centipedes, and a cockroach, scorpion and millipede). Of these 48 taxa, 25 of them were sampled from only one rotting log, thus precluding partitioning of the data set prior to downstream analyses. Basic information on feeding ecology was available for all 48 saproxylic arthropod taxa that were identified, and trophic interactions among major functional groups represented by them are depicted in Fig. 2. Because some of the sampled species are known to routinely forage outside of the rotting logs that they occupy, we explicitly represented these *ex situ* sources of nutrients.

3.2.1. Primary nutrient sources

The information on feeding ecology available for southern Appalachian saproxylic arthropods indicated that there are three primary sources of nutrient inputs into the food web: the intermediate- to late-stage rotting log itself, nearby dead and decomposing leaf litter, and living trees (i.e., sap, seeds with or without elaiosomes, and leaves). Wood is a notoriously recalcitrant and nutrient-poor substrate and this has given rise to a high degree of specialization and symbioses within saproxylic communities. The enzymes necessary to break down lignocellulose are mostly provided by fungi and bacteria, and cord-forming fungi are known to translocate large amounts of nutrients into decomposing logs from external sources (Filipiak, 2018). As a result, a large proportion of dead wood biomass consists of fungi and bacteria

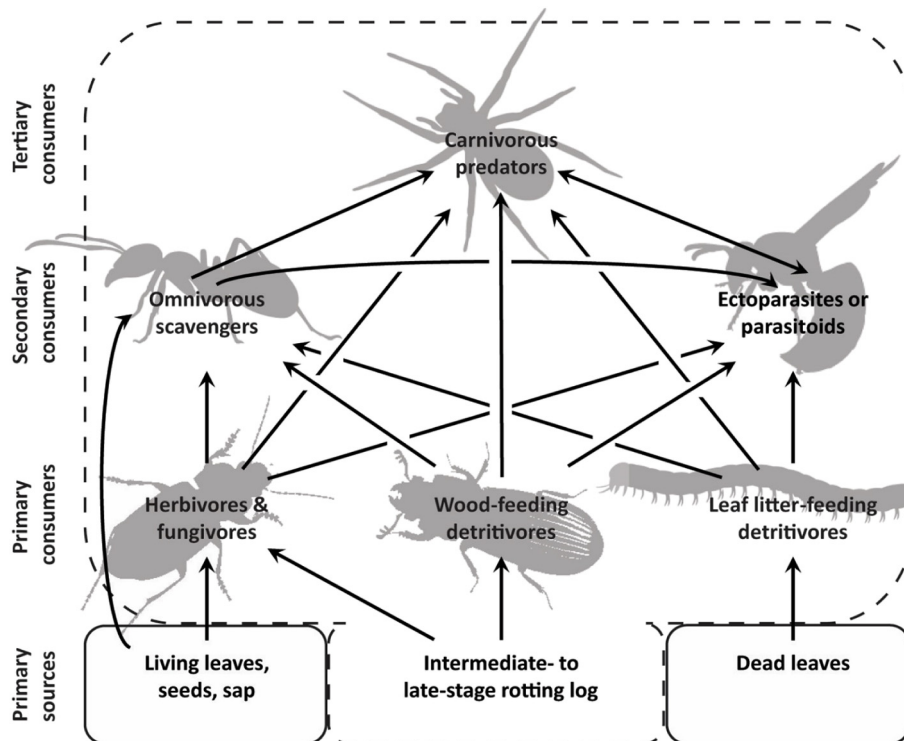


Fig. 2. Summary of trophic interactions among major functional groups of saproxylic arthropods based on feeding ecology information available for 48 taxa sampled from 32 rotting logs in the southern Appalachian Mountains, identified to genus- or species using molecular tools. Silhouettes of arthropods represent the most common taxonomic order associated with each functional group and trophic level (see Appendix 2 for a complete taxon list).

and these organisms form a much more important part of “wood-feeding” insect diets than previously recognized (Filipiak, 2018). This appears to be a general characteristic of brown food webs (Steffan et al., 2017) and, as discussed below (see Conclusions and future directions), this has important implications for the assignment of trophic positions to saproxylic insects.

3.2.2. Primary consumers

Primary consumers can be classified into three main functional groups: 1) wood-feeding detritivores, 2) leaf litter-feeding detritivores, and 3) organisms feeding on material from living plants or fungi (i.e., herbivores and fungivores; Fig. 2). The southern Appalachian wood-feeding detritivores included termites (*Reticulitermes* spp.), stag beetles (*Ceruchus piceus*, *Lucanus elaphus*), long-horned beetles (*Graphisurus fasciatus*, *Orthosoma brunneum*), darkling beetles (*Hymenorus picipennis*), horned passalus beetles (*Odontotaenius disjunctus*), and an endemic cockroach species (*Cryptocercus punctulatus*). In our collections, there were few leaf litter-feeding detritivores (i.e., those that forage outside of the rotting log in which they reside). Most of these were millipedes (*Oxidus gracilis*, and *Cherokia* sp.), although there was also one cockroach taxon (*Parcoblatta* sp.) represented in this functional group. Although not evident from the sampling conducted in the present study, *Narceus americanus* is often found within rotting logs in the southern Appalachians (RC Garrick pers. obs.; Walker et al., 2009) and so the diversity of millipedes is likely to be higher than it appears here. Indeed, there is an extraordinary diversity of the millipede genus *Brachoria* in the region (Marek, 2010). Herbivores and fungivores form a functional group of primary consumers that directly feed on components of living plants (e.g., leaves, seeds or sap) outside of rotting logs, or components of living fungi inside or on the outer surfaces of rotting logs (e.g., mycelia, spores, fruiting bodies). Although few in number, species of herbivores were taxonomically diverse and included a moth species (*Baileya ophthalmica*) sampled in its pupal stage of development, and false long-horn beetle (*Cephaloon unglare*). Conversely, all fungus-feeders were beetles (*Mallodrya subaenea*, *Scaphisoma rubens*, and *Xylopinus saperdioides*), but from different families.

3.2.3. Secondary consumers

Functional groups that were collectively classified as secondary consumers include omnivorous scavengers, carnivorous predators, and ectoparasites or parasitoids (Fig. 2). Given that our sampling and mtDNA sequencing efforts focused only on macroscopic arthropods of sufficient size that genomic DNA could be readily extracted and amplified, none of the specimens that were collected fall in the latter group. However, we did observe mites on many specimens. Indeed, as many as 12 different species or mites occur on the horned passalus beetle (*O. disjunctus*), two of which are thought to be parasitic on the host, with the others being commensals that include predatory species (Pearse et al., 1936). The fly, *Zelia vertebrata*, is an arthropod parasitoid associated with *O. disjunctus* (Gray, 1946), as well as other wood-feeding detritivores that we collected, such as the giant stag beetle *L. elaphus* (Ulyshen et al., 2017). Other documented cases of insect parasitoids attacking some of the saproxylic arthropods that we sampled were encountered during examination of information on feeding ecology (e.g., the spider wasp, *Priocnemis minorata*, attacking the funnel weaving spider, *Wadotes hybridus*; Kurczewski et al., 2017). Thus, for completeness, ectoparasites or parasitoids were included. However more comprehensive inferences can be made regarding the species composition of omnivorous scavenger and carnivorous predator functional groups, given that numerous taxa within these categories were sampled in this study.

Omnivorous scavengers were most prominently represented by ants in the family Formicidae (4 genera) and the genus *Aphaenogaster* (4 species; Appendix 2). Other members of this functional group that were sampled from rotting logs in the southern Appalachians included two click beetle species (*Alaus oculatus* and *Ampedus mixtus*), a net-

winged beetle (*Dictyoptera aurora*), and a cricket (*Ceuthophilus gracilipes*). Interestingly, the latter species is also known to inhabit caves, which like rotting logs, are dark, humid, thermally buffered and temporally stable microhabitats (Cockley et al., 1977). The woodland ant *Aphaenogaster rudis* exemplifies the broad array of food web connections that omnivorous scavengers can have (Fig. 2). For example, this species (or members of this putative species complex; Umphrey, 1996) generally consumes small invertebrates, including the eastern subterranean termite *Reticulitermes flavipes* (Buczowski and Bennett, 2007). However, it also opportunistically feeds on fungal fruiting bodies (Epps and Penick, 2018), and eliasome-bearing seeds (as does *A. picea*; Clark and King, 2012). Given that foraging distances of *A. rudis* are typically short, with mean and maximum recorded distances from the nest of 57 cm and 140 cm respectively (Lubertazzi, 2012), workers generally operate within close proximity to the rotting log that they inhabit. Additional connections between saproxylic omnivorous scavengers and *ex situ* nutrient resources are mediated by foraging activities of black carpenter ant (*Camponotus pennsylvanicus*) workers, who tend aphids harvesting the phloem of living plants, and collect honeydew produced by them.

3.2.4. Tertiary consumers

At the highest trophic level, the carnivorous predator functional group was mostly comprised of arachnids. There was considerable taxonomic diversity among true spiders (8 families, and 12 genera/species; Appendix 2), but two representatives of harvestmen (*Leiobunum aldrichi*, and *Sabacon cavicolens*) were also present. Some of these predators are known to target specific prey, such as the jumping spider *Naphrys pulex*, which has specialized prey-catching techniques and strong feeding preference toward ants (Daiqin et al., 1996). However, the majority of predator species that we sampled from rotting logs in the southern Appalachian Mountains are thought to have broad diets. Indeed, arachnids prey on one another, including cannibalism (e.g., the fishing spider, *Dolomedes tenebrosus*; Schwartz et al., 2014). Interestingly, one of the spiders that we sampled, the hacklemesh weaver (*Callobius bennetti*), preys on spider wasps (i.e., a common parasitoid; Barlow, 2011). Other members of the carnivorous predator functional group that were present in our collections include a scorpion (*Vaejovis* sp.), two centipedes (*Hemiscolopendra* sp. and *Scolopocryptops* sp.), and a ground beetle (*Pterostichus tristis*). As with arachnids, centipedes exhibit predation upon one another, and some may also be cannibalistic (e.g., *Scolopocryptops* sp., Hickerson et al., 2005; Auerbach, 1951). Given the considerable abundance of top-level predators in the rotting logs that we sampled (i.e., they account 42% of all taxa identified to genus or species; Appendix 2), intra-guild predation and cannibalism may be particularly important components of saproxylic arthropod food webs. Very high abundance of top-level predators in rotting logs has also been reported for some temperate Southern Hemisphere forests (Barclay et al., 2000), suggesting that our findings may be typical.

3.2.5. Challenges and limitations

While the empirical data generated in this study contributes to filling long-standing knowledge gaps, several caveats warrant consideration. First, our sampling focused only on macroscopic arthropods that were of sufficient size that genomic DNA could be readily extracted and amplified. As such, some log-dwelling taxa such as mites and springtails were overlooked. Not only may these organisms be quite abundant and thus constitute an important component of the saproxylic food web, but some of them may exemplify otherwise unrepresented functional groups (e.g., slime-mold-grazers; Garrick et al., 2004, 2007, 2008, 2012). Second, taxonomic identifications based on mitochondrial COI sequence similarity thresholds (Telfer et al., 2015) are not without error. Such criteria may fail when taxon sampling in the reference nucleotide database is sparse, or when dealing with recently diverged species (Cognato, 2006; Hickerson et al., 2006). Accordingly, not all of the formally named species that we sampled were ultimately included in the final dataset that underpinned

inferences about food web structure—this shortcoming underscores the indispensability of morphotaxonomic expertise. Indeed, the extent to which public sequence databases are populated with mitochondrial COI barcodes from voucher specimens that have been identified by experts is major determinant of assignment success (Hebert and Gregory, 2005). Furthermore, the existence of morphologically cryptic species presents an additional challenge, as cases of divergent lineages nested within one named species will undermine sequence-based identifications (see Garrick et al., 2018 for an example of where this may apply to a centipede taxon sampled in the present study). Finally, it can be difficult to unambiguously classify each identified taxon to a single functional group given that feeding ecology can vary across different life stages. For example, whereas larval giant stag beetles (*L. elaphus*) typically feed on dead wood within rotting logs on the forest floor, adults feed on sap from living trees high above the ground (Ulyshen et al., 2017). For this species, the extended larval development time (1–2 years) relative to the short-lived adult stage (1–3 months) provided a basis for making a classification, but this is nonetheless a simplification that can be expected to impact inferences about food web structure.

4. Conclusions and future directions

It is becoming increasingly apparent that the traditional method for assigning trophic levels to insect communities, based on interactions among the constituent species, can yield misleading or incomplete information about trophic positions. This is especially true in brown food webs where microbial biomass can form a more important part of the diet of consumers than the dead plant material itself. Steffan et al. (2017), for example, used stable isotopes to show that detritivorous microbes elevate the trophic position of detrital com-

plexes over time, with similar trophic inflation observed in metazoan consumers. This is likely to have major consequences for the trophic positions of saproxylic insects depending on the stage of wood decomposition and how important microbial biomass is to their diets. For example, the consumption of microbes may constitute predation while the consumption of dead wood colonized by microbes may be best described as omnivory or intra-guild predation (Digel et al., 2014). We therefore encourage future investigations into the trophic relationships among saproxylic insects to incorporate the molecular methods described here to facilitate identification, followed by stable isotope analyses (Hyodo, 2015) to ascertain trophic positions.

Conflict of interest

There are no conflicts of interest between the authors, the institution and fund donors.

Acknowledgements

This work was supported by grants from the Bay and Paul Foundations, Conservation and Research Foundation, Eppley Foundation for Research, National Geographic Society, and the Washington Biologists' Field Club. Sequencing was performed by staff at Yale University's DNA Analysis Facility on Science Hill. We thank Isis Da Costa Arantes, Chase Bailey, John Banusiewicz, Renan Bosque, Tyler Breech, Trey Dickenson III, Marcella Santos and Rebecca Symula for assistance with some lab and/or fieldwork. Scientific collecting permits were provided by USDA Forest Service, and the National Park Service (GRSM-2015-SCI-2242). We also thank three anonymous reviewers for comments that improved the manuscript.

Appendix A

Appendix 1

Region and site information, and GPS coordinates associated with rotting logs from which southern Appalachian saproxylic arthropods were sampled (abbreviations are: National Forest, NF; Great Smoky Mountains National Park, GSM NP).

Region	Site	Log ID	Latitude	Longitude	Elevation (m)	Date sampled
Bankhead NF	Houston Recreation Area	C01	34.12192	−87.29025	198	17-May-16
		C02	34.12176	−87.29032	202	17-May-16
		C11	34.12136	−87.29005	192	18-May-16
		C12	34.12120	−87.29002	208	18-May-16
	Little Natural Bridge Picnic Area	C03	34.17797	−87.27700	226	29-Jun-16
		C06	34.17792	−87.27605	220	30-Jun-16
	Sipsey River Picnic Area	C07	34.28280	−87.39816	219	30-May-16
		C10	34.28235	−87.39905	228	30-May-16
	Corinth Recreation Area	C13	34.10336	−87.32465	190	17-May-16
		C14	34.10343	−87.32438	186	16-May-16
		C15	34.10230	−87.32407	181	17-May-16
		C16	34.10151	−87.32454	182	17-May-16
	Sipsey Wilderness	C57	34.28418	−87.42854	275	30-Jun-16
		C58	34.28415	−87.42840	269	30-Jun-16
		C59	34.28410	−87.42831	279	30-Jun-16
		C60	34.28423	−87.42839	276	30-Jun-16
GSM NP	Big Witch Gap Overlook	C25	35.52532	−83.22337	1272	28-May-16
		C26	35.52518	−83.22374	1285	28-May-16
		C27	35.52557	−83.22472	1283	27-May-16
		C28	35.52561	−83.22472	1286	27-May-16
	Chataloochee	C29	35.64004	−83.05638	1109	27-May-16
		C31	35.63996	−83.05661	1109	27-May-16
		C37	35.77150	−83.21331	591	25-May-16
	Gunters Cemetery	C38	35.77148	−83.21333	591	25-May-16
		C39	35.77123	−83.21352	592	26-May-16
		C40	35.77142	−83.21355	582	26-May-16
	Greenbrier	C41	35.73247	−83.41083	441	25-May-16
		C42	35.73240	−83.41074	452	26-May-16
Little River	C44	35.73249	−83.41127	466	25-May-16	
	C45	35.67947	−83.55922	504	28-May-16	
	C47	35.67926	−83.55930	510	28-May-16	
	C48	35.67957	−83.55884	519	29-May-16	

Appendix 2

Molecular taxonomic identifications of saproxylic arthropods sampled from 32 rotting logs across two forest regions in the southern Appalachian Mountains. Sources of information on the feeding ecology of each taxon are abbreviated as follows:

AWe, AntWeb (www.antweb.org); AWi, AntWiki (www.antwiki.org); BG, BugGuide (www.bugguide.net); CA, Conference Abstract (Hladilek & Wise, ESA 2008, Milwaukee WI); CB, ChiloBase (www.chilobase.biologia.unipd.it); Diss, PhD dissertation; DL, Discover Life (www.discoverlife.org); EB, Encyclopedia Britannica (www.britannica.com); EOL, Encyclopedia of Life (www.eol.org); II, Insect Identification (www.insectidentification.org); JA, Journal Article; NW, Northern Woodlands (www.northernwoodlands.org); SOA, School of Ants (www.schoolofants.org); and SS, Science Source (www.sciencesource.com). For journal articles, books and PhD dissertation, the number in parentheses corresponds with the numbered reference list below.

Class	Order	Family	Genus	Species	Authority	Functional group	Source(s) of information on feeding ecology	Life stage(s) when sampled		
Arachnida	Araneae	Agelenidae	<i>Wadotes</i>	<i>hybridus</i>	Emerton	Carnivorous predator	BG	Adult		
		Amaurobiidae	<i>Callobius</i>	<i>bennetti</i>	Blackwall	Carnivorous predator	NW, SS	Adult		
		Antrodiaetidae	<i>Antrodiaetus</i>	<i>unicolor</i>	Hentz	Carnivorous predator	JA (1), EOL	Adult		
		Clubionidae	<i>Elaver</i>	<i>excepta</i>	Koch	Carnivorous predator	CA	Adult		
		Cybaeidae	<i>Cybaeus</i>	<i>giganteus</i>	Banks	Carnivorous predator	BG	Adult		
		Hahniidae	<i>Cicurina</i>	sp.	Menge	Carnivorous predator	BG	Adult		
		Lycosidae	<i>Piratula</i>	<i>minuta</i>	Emerton	Carnivorous predator	BG	Adult		
			<i>Pirata</i>	<i>praedo</i>	Kulczyński	Carnivorous predator	BG	Adult		
				<i>Schizocosa</i>	<i>stridulans</i>	Stratton Chamberlin & Ivie	Carnivorous predator	JA (2)	Adult	
		Phrurolithidae	<i>Phrurotimpus</i>	sp.		Carnivorous predator	BG	Adult		
		Pisauridae	<i>Dolomedes</i>	<i>tenebrosus</i>	Hentz	Carnivorous predator	JA (3–4)	Adult		
		Salticidae	<i>Naphrys</i>	<i>pulex</i>	Hentz	Carnivorous predator	JA (5)	Adult		
			<i>Neon</i>	<i>reticulatus</i>	Blackwall	Carnivorous predator	BG	Adult		
			<i>Pelegrina</i>	sp.	Franganillo	Carnivorous predator	BG	Adult		
			Opiliones	Sabaconidae	<i>Sabacon</i>	<i>cavicolens</i>	Packard	Carnivorous predator	JA (6)	Adult
				Sclerosomatidae	<i>Leiobunum</i>	<i>aldrichi</i>	Weed	Carnivorous predator	JA (6–7), Book (8), BG	Adult
			Scorpiones	Vaejovidae	<i>Vaejovis</i>	sp.	Koch	Carnivorous predator	DL, II	Adult
		Chilopoda	Scolopendromorpha	Scolopendridae	<i>Hemiscolopendra</i>	sp.	Kraepelin	Carnivorous predator	CB, BG	Adult
				Scolopocryptopidae	<i>Scolopocryptops</i>	sp.	Newport	Carnivorous predator	CB, BG	Adult
	Diplopoda	Polydesmida	Paradoxosomatidae	<i>Oxidus</i>	<i>gracilis</i>	Koch	Leaf litter-feeding detritivore	EOL	Adult	
Xystodesmidae			<i>Cherokia</i>	sp.	Chamberlin	Leaf litter-feeding detritivore	BG	Adult		
Insecta	Blattodea	Cryptoceridae	<i>Cryptocercus</i>	<i>punctulatus</i>	Scudder	Wood-feeding detritivore	Book (9)	Adult		
		Ectobiidae	<i>Parcoblatta</i>	sp.	Hebard	Leaf litter-feeding detritivore	JA (10), BG	Adult		
		Rhinotermitidae	<i>Reticulitermes</i>	<i>flavipes</i>	Kollar	Wood-feeding detritivore	JA (11), BG	Adult (worker)		
	<i>Reticulitermes</i>		<i>virginicus</i>	Banks	Wood-feeding detritivore	JA (11), BG	Adult (worker)			
	Coleoptera	Carabidae	<i>Pterostichus</i>	<i>tristis</i>	Dejean	Carnivorous predator	Book (12)	Adult		
		Cerambycidae	<i>Orthosoma</i>	<i>brunneum</i>	Forster	Wood-feeding detritivore	JA (13), Book (12)	Juvenile & adult		
			<i>Graphisurus</i>	<i>fasciatus</i>	De Geer	Wood-feeding detritivore	Book (12)	Adult		
		Elateridae	<i>Alaus</i>	<i>oculatus</i>	Linnaeus	Omnivorous scavenger	Book (12), BG	Adult		
			<i>Ampedus</i>	<i>mixtus</i>	Herbst	Omnivorous scavenger	Book (12)	Juvenile		
		Lucanidae	<i>Ceruchus</i>	<i>piceus</i>	Weber	Wood-feeding detritivore	Book (12)	Juvenile & adult		
			<i>Lucanus</i>	<i>elaphus</i>	Fabricius	Wood-feeding detritivore	JA (14), Book (12), BG	Adult		
	Lycidae	<i>Dictyopectera</i>	<i>aurora</i>	Herbst	Omnivorous scavenger	Book (12), EB	Juvenile			
Passalidae	<i>Odontotaenius</i>	<i>disjunctus</i>	Illiger	Wood-feeding detritivore	Book (12), BG	Adult				

Appendix 2 (continued)

Class	Order	Family	Genus	Species	Authority	Functional group	Source(s) of information on feeding ecology	Life stage(s) when sampled
		Staphylinidae	<i>Scaphisoma</i>	<i>rubens</i>	Casey	Fungivore	Book (12)	Adult
		Stenotrachelidae	<i>Cephaloon</i>	<i>ungulare</i>	LeConte	Herbivore	Book (12)	Juvenile
		Synchroidea	<i>Mallogrya</i>	<i>subaenea</i>	Horn	unclassified detritivore	Book (12)	Adult
		Tenebrionidae	<i>Hymenorus</i>	<i>picipennis</i>	Casey	detritivore	JA (15), Book (12)	Juvenile & adult
			<i>Xylopinus</i>	<i>saperdioides</i>	Oliver	Fungivore	Book (12)	Juvenile & adult
	Hymenoptera	Formicidae	<i>Aphaenogaster</i>	<i>carolinensis</i>	Wheeler	Omnivorous scavenger	Diss (16), AWe, AWi	Adult
			<i>Aphaenogaster</i>	<i>fulva</i>	Roger	Omnivorous scavenger	Diss (16), AWe, AWi	Juvenile & adult
			<i>Aphaenogaster</i>	<i>picea</i>	Wheeler	Omnivorous scavenger	JA (17), Diss (16), AWe, AWi,	Adult
			<i>Aphaenogaster</i>	<i>rudis</i>	Enzmann	Omnivorous scavenger	JA (18–19), Diss (16), AWe, AWi,	Adult
			<i>Camponotus</i>	<i>pennsylvanicus</i>	De Geer	Omnivorous scavenger	Diss (20), AWe, AWi	Egg & adult
			<i>Lasius</i>	<i>alienus</i>	Förster	Omnivorous scavenger	AWe, AWi, SOA	Adult
			<i>Nylanderia</i>	<i>faisonensis</i>	Forel	Omnivorous scavenger	JA (21), AWe, AWi	Adult
	Lepidoptera	Nolidae	<i>Baileya</i>	<i>ophthalmica</i>	Guenée	Herbivore	BG	Juvenile
	Orthoptera	Rhaphidophoridae	<i>Ceuthophilus</i>	<i>gracilipes</i>	Haldeman	Omnivorous scavenger	BG	Adult

References

- Hendrixson, B.E., Bond, J.E. (2005) Two sympatric species of *Antrrodiaetus* from southwestern North Carolina (Araneae, Mygalomorphae, Antrrodiaetidae). *Zootaxa*, 872, 1–19.
- Roualdes, E.A., Bonner, S.J., Whitney, T.D., Harwood, J.D. (2016) Formal modelling of predator preferences using molecular gut-content analysis. *Environmental and Ecological Statistics*, 23, 317–336.
- Nyffeler, M., Symondson, W.O.C. (2001) Spiders and harvestmen as gastropod predators. *Ecol. Entomol.*, 26, 617–628.
- Schwartz, S.K., Wagner Jr., W.E., Hebets, E.A. (2014) Obligate male death and sexual cannibalism in dark fishing spiders. *Animal Behaviour*, 93, 151–156.
- Li, D., Jackson, R.R., Cutler, B. (1996) Prey-capture techniques and prey preferences of *Habrocestum pulex*, an ant-eating jumping spider (Araneae, Salticidae) from North America. *Journal of Zoology*, 240, 551–562.
- Edgar, A.L. (1971) Studies on the Biology and Ecology of Michigan Phalangida (Opiliones). *Miscellaneous Publications Museum of Zoology, University of Michigan*, 144, 1–64.
- Shultz, J.W. (2000) Skeletomuscular anatomy of the harvestman *Leiobunum aldrichi* (Weed, 1893) (Arachnida: Opiliones: Palpatores) and its evolutionary significance. *Zoological Journal of the Linnean Society*, 128, 401–438.
- Pinto-da-Rocha, R., Machado, G., Giribet, G. (2007) Harvestmen: The Biology of Opiliones. Harvard University Press, USA.
- Bell, W.J., Roth, L.M., Nalepa, C.A. (2016) Cockroaches: Ecology, behavior, and natural history. The Johns Hopkins University Press, USA.
- Schal, C., Gautier, J.Y., Bell, W.J. (1984) Behavioural ecology of cockroaches. *Biological Reviews*, 59, 209–254.
- Vargo, E.L., Husseneder, C. (2009) Biology of subterranean termites: Insights from molecular studies of *Reticulitermes* and *Copotermes*. *Annual Review of Entomology*, 54, 79–403.
- Evans, A.V. (2014) Beetles of Eastern North America. Princeton University Press, New Jersey, USA.
- Jones, O.T., Coaker, T.H. (1978) A basis for host plant finding in phytophagous larvae. *Entomol. Exp. Appl.*, 24, 472–484.
- Ulyshen, M.D., Zachos, L.G., Stireman, J.O., III, Sheehan, T.N., Garrick, R.C. (2017) Insights into the ecology, genetics and distribution of *Lucanus elaphus* Fabricius (Coleoptera: Lucanidae), North America's giant stag beetle. *Insect Conservation and Diversity*, 10, 331–340.
- Majka, C.G., Bouchard, P., Bousquet, Y. (2008) Tenebrionidae (Coleoptera) of the Maritime Provinces of Canada. *Canadian Entomologist*, 140, 690–713.
- Carroll, J.F. (1975) Biology and ecology of ants in the genus *Aphaenogaster* in Florida. University of Florida, USA.
- Clark, R.E., King, J.R. (2012) The ant, *Aphaenogaster picea*, benefits from plant elaiosomes when insect prey is scarce. *Environmental Entomology*, 41, 1405–1408.
- Epps, M.J., Penick, C.A. (2018) Facultative mushroom feeding by common woodland ants (Formicidae, *Aphaenogaster* spp.). *Food Webs*, 14, 9–13.
- Lubertazzi, D. (2012) The biology and natural history of *Aphaenogaster rudis*. *Psyche*, 752815.
- Cannon, C.A. (1998) Nutritional ecology of the carpenter ant *Camponotus pennsylvanicus* (De Geer): Macronutrient preference and particle consumption. Virginia Tech, USA.
- Bewick, S., Stuble, K.L., Lessard, J.-P., Dunn, R.R., Adler, F.R., Sanders, N.J. (2014) Predicting future coexistence in a North American ant community. *Ecol. Evol.*, 4, 1804–1819.

References

- Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. *J. Mol. Biol.* 215, 403–410.
- Auerbach, S.I., 1951. The centipedes of the Chicago area with special reference to their ecology. *Ecol. Monogr.* 21, 97–124.
- Barclay, S., Ash, J.E., Rowell, D.M., 2000. Environmental factors influencing the presence and abundance of a log-dwelling invertebrate, *Euperipatoides rowelli* (Onychophora: Peripatopsidae). *J. Zool.* 250, 425–436.
- Barlow, V., 2011. Forest spiders. Northern Woodlands. 68, p. 33.
- Birkemoe, T., Jacobsen, R.M., Sverdrup-Thygeson, A., Biedermann, H.W., 2018. Insect-fungus interactions in dead wood systems. In: Ulyshen, M.D. (Ed.), *Saproxyllic Insects: Diversity, Ecology and Conservation*. Springer, pp. 377–427.
- Blackman, M.W., Stage, H.H., 1924. On the succession of insects living in the bark and wood of dying, dead and decaying hickory. Technical Publication No. 17. New York State College of Forestry, pp. 3–269.
- Bouget, C., Brustel, H., Nageleisen, L.M., 2005. Nomenclature of wood-inhabiting groups in forest entomology: synthesis and semantic adjustments. *C. R. Biol.* 328, 936–948.
- Bouget, C., Brin, A., Brustel, H., 2011. Exploring the “last biotic frontier”: are temperate forest canopies special for saproxyllic beetles? *For. Ecol. Manag.* 261, 211–220.
- Bouget, C., Larrieu, L., Brin, A., 2014. Key features for saproxyllic beetle diversity derived from rapid habitat assessment in temperate forests. *Ecol. Indic.* 36, 656–664.

- Brin, A., Bouget, C., 2018. Biotic interactions between saproxylic insect species. In: Ulyshen, M.D. (Ed.), *Saproxylic Insects: Diversity, Ecology and Conservation*. Springer, pp. 471–514.
- Buczowski, G., Bennett, G., 2007. Protein marking reveals predation on termites by the woodland ant, *Aphaenogaster rudis*. *Insect. Soc.* 54, 219–224.
- Buse, J., Ranius, T., Assmann, T., 2008. An endangered longhorn beetle associated with old oaks and its possible role as an ecosystem engineer. *Conserv. Biol.* 22, 329–337.
- Carlton, C., Bayless, V., 2007. Documenting beetle (Arthropoda: Insecta: Coleoptera) diversity in Great Smoky Mountains National Park: beyond the halfway point. *South-east. Nat.* 1, 183–192.
- Clark, R.E., King, J.R., 2012. The ant, *Aphaenogaster picea*, benefits from plant elaiosomes when insect prey is scarce. *Environ. Entomol.* 41, 1405–1408.
- Cockley, D.E., Gooch, J.L., Weston, D.P., 1977. Genic diversity in cave dwelling crickets (*Ceuthophilus gracilipes*). *Evolution* 31, 313–318.
- Cognato, A.I., 2006. Standard percent DNA sequence difference for insects does not predict species boundaries. *J. Econ. Entomol.* 99, 1037–1045.
- Daiqin, L.I., Jackson, R.R., Cutler, B., 1996. Prey-capture techniques and prey preferences of *Habrocestum pulex*, an ant-eating jumping spider (Araneae, Salticidae) from North America. *J. Zool.* 240, 551–562.
- Dennis, R.W.J., Malcolm, J.R., Smith, S.M., Bellocq, M.I., 2018. Response of saproxylic insect communities to logging history, tree species, stage of decay, and wood posture in the central Nearctic boreal forest. *J. For. Res.* 29, 1365–1377.
- Digel, C., Curtsdotter, A., Riede, J., Klarner, B., Brose, U., 2014. Unravelling the complex structure of forest soil food webs: higher omnivory and more trophic levels. *Oikos* 123, 1157–1172.
- Dobson, A., Lodge, D., Alder, J., Cumming, G.S., Keymer, J., McGlade, J., Mooney, H., Rusak, J.A., Sala, O., Wolters, V., Wall, D., Winfree, R., Xenopoulos, M.A., 2006. Habitat loss, trophic collapse, and the decline of ecosystem services. *Ecology* 87, 1915–1924.
- Epps, M.J., Penick, C.A., 2018. Facultative mushroom feeding by common woodland ants (Formicidae, *Aphaenogaster* spp.). *Food Webs* 14, 9–13.
- Etzestebe, I., Lencina, J.L., Pajares, J., 2013. Saproxylic community, guild and species responses to varying pheromone components of a pine bark beetle. *Bull. Entomol. Res.* 103, 497–510.
- Evans, A.V., 2014. *Beetles of Eastern North America*. Princeton University Press, New Jersey, USA.
- Fayt, P., Dufrière, M., Branquart, E., Hastir, P., Pontégnie, C., Henin, J.-M., Versteirt, V., 2006. Contrasting responses of saproxylic insects to focal habitat resources: the example of longhorn beetles and hoverflies in Belgian deciduous forests. *J. Insect Conserv.* 10, 129–150.
- Ferro, M.L., 2018. It's the end of the wood as we know it: Insects in veteris (highly-decomposed) wood. In: Ulyshen, M.D. (Ed.), *Saproxylic Insects: Diversity, Ecology and Conservation*. Springer, pp. 729–795.
- Ferro, M.L., Gimmel, M.L., Harms, K.E., Carlton, C.E., 2012a. Comparison of the Coleoptera communities in leaf litter and rotten wood in Great Smoky Mountains National Park, USA. *Insecta Mundi* 259, 1–58.
- Ferro, M.L., Gimmel, M.L., Harms, K.E., Carlton, C.E., 2012b. Comparison of Coleoptera emergent from various decay classes of downed coarse woody debris in Great Smoky Mountains National Park, USA. *Insecta Mundi* 260, 1–80.
- Fierro, A., Grez, A.A., Vergara, P.M., Ramírez-Hernández, A., Micó, E., 2017. How does the replacement of native forest by exotic forest plantations affect the diversity, abundance and trophic structure of saproxylic beetle assemblages? *For. Ecol. Manag.* 405, 246–256.
- Filipiak, M., 2018. Nutrient dynamics in decomposing dead wood in the context of wood eater requirements: The ecological stoichiometry of saproxylophagous insects. In: Ulyshen, M.D. (Ed.), *Saproxylic Insects: Diversity, Ecology and Conservation*. Springer, pp. 429–469.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3, 294–299.
- García-López, A., Martínez-Falcón, A.P., Micó, E., Estrada, P., Grez, A.A., 2016. Diversity distribution of saproxylic beetles in Chilean Mediterranean forests: influence of spatio-temporal heterogeneity and perturbation. *J. Insect Conserv.* 20, 723–736.
- Garrick, R.C., 2011. Montane refuges and topographic complexity generate and maintain invertebrate biodiversity: recurring themes across space and time. *J. Insect Conserv.* 15, 469–478.
- Garrick, R.C., Bouget, C., 2018. Molecular tools for assessing saproxylic insect diversity. In: Ulyshen, M.D. (Ed.), *Saproxylic Insects: Diversity, Ecology and Conservation*. Springer, pp. 849–884.
- Garrick, R.C., Sands, C.J., Rowell, D.M., Tait, N.N., Greenslade, P., Sunnucks, P., 2004. Phylogeography recapitulates topography: very fine-scale local endemism of a saproxylic 'giant' springtail at Tallaganda in the Great Dividing Range of south-east Australia. *Mol. Ecol.* 13, 3329–3344.
- Garrick, R.C., Sands, C.J., Rowell, D.M., Hillis, D.M., Sunnucks, P., 2007. Catches catch all: long-term population history of a giant springtail from the southeast Australian highlands – a multigene approach. *Mol. Ecol.* 16, 1865–1882.
- Garrick, R.C., Rowell, D.M., Simmons, C.S., Hillis, D.M., Sunnucks, P., 2008. Fine-scale phylogeographic congruence despite demographic incongruence in two low-mobility saproxylic springtails. *Evolution* 62, 1103–1118.
- Garrick, R.C., Rowell, D.M., Sunnucks, P., 2012. Phylogeography of saproxylic and forest floor invertebrates from Tallaganda, South-Eastern Australia. *Insects* 3, 270–294.
- Garrick, R.C., Sabree, Z.L., Jahnes, B.C., Oliver, J.C., 2017. Strong spatial-genetic congruence between a wood-feeding cockroach and its bacterial endosymbiont, across a topographically complex landscape. *J. Biogeogr.* 44, 1500–1511.
- Garrick, R.C., Newton, K.E., Worthington, R.J., 2018. Cryptic diversity in the southern Appalachian Mountains: genetic data reveal that the red centipede, *Scolopocryptops sexspinosus*, is a species complex. *J. Insect Conserv.* 22, 799–805.
- Gray, I.E., 1946. Observations on the life history of the Horned Passalus. *Am. Midl. Nat.* 35, 728–746.
- Grove, S.J., Forster, L., 2011. A decade of change in the saproxylic beetle fauna of eucalypt logs in the Warra long-term log-decay experiment, Tasmania. 1. Description of the fauna and seasonality patterns. *Biodivers. Conserv.* 20, 2149–2165.
- Harmon, M.E., Franklin, J.F., Swanson, F.J., Sollins, P., Gregory, S.V., Lattin, J.D., Anderson, N.H., Cline, S.P., Aumen, N.G., Sedell, J.R., Lienkaemper, G.W., Cromack Jr., K., Cummins, K.W., 1986. Ecology of coarse woody debris in temperate ecosystems. *Adv. Ecol. Res.* 15, 133–302.
- Hebert, P.D.N., Gregory, T.R., 2005. The promise of DNA barcoding for taxonomy. *Syst. Biol.* 54, 852–859.
- Hickerson, C.M., Anthony, C.D., Walton, B.M., 2005. Edge effects and intraguild predation in native and introduced centipedes: evidence from the field and from laboratory microcosms. *Oecologia* 146, 110–119.
- Hickerson, M.J., Meyer, C.P., Moritz, C., 2006. DNA barcoding will often fail to discover new animal species over broad parameter space. *Syst. Biol.* 55, 729–739.
- Hilszczański, J., 2018. Ecology, diversity and conservation of saproxylic hymenopteran parasitoids. In: Ulyshen, M.D. (Ed.), *Saproxylic Insects: Diversity, Ecology and Conservation*. Springer, pp. 193–216.
- Howden, H.F., Vogt, G.B., 1951. Insect communities of standing dead pine (*Pinus virginiana* Mill.). *Ann. Entomol. Soc. Am.* 44, 581–595.
- Hyodo, F., 2015. Use of stable carbon and nitrogen isotopes in insect trophic ecology. *Entomol. Sci.* 18, 295–312.
- Jackson, H.B., Baum, K.A., Robert, T., Cronin, J.T., 2009. Habitat-specific movement and edge-mediated behavior of the saproxylic insect *Odontotaenius disjunctus* (Coleoptera: Passalidae). *Environ. Entomol.* 38, 1411–1422.
- Jaworski, T., Plewa, R., Hilszczański, J., Szczepkowski, A., Horak, J., 2016. Saproxylic moths reveal complex within-group and group-environment patterns. *J. Insect Conserv.* 20, 677–690.
- Johansson, T., Olsson, J., Hjältén, J., Jonsson, B.G., Ericson, L., 2006. Beetle attraction to sporocarps and wood infected with mycelia of decay fungi in old-growth spruce forests of northern Sweden. *For. Ecol. Manag.* 237, 335–341.
- Johansson, T., Hjältén, J., Gibb, H., Hilszczański, J., Stenlid, J., Ball, J.P., Alinvi, O., Danell, K., 2007. Variable response of different functional groups of saproxylic beetles to substrate manipulation and forest management: implications for conservation strategies. *For. Ecol. Manag.* 242, 496–510.
- Kurczewski, F.E., Edwards, G.B., Pitts, J.P., 2017. Hosts, nesting behavior, and ecology of some north American spider wasps (Hymenoptera: Pompilidae), II. *Southeast. Nat.* 16, 1–82.
- Kuuluvainen, T., Siitonen, J., 2014. Fennoscandian boreal forests as complex adaptive systems. In: Messier, C., Puettmann, K.J., Coates, K.D. (Eds.), *Managing Forests as Complex Adaptive Systems: Building Resilience to the Challenge of Global Change*. The Earthscan Forest Library, Swales and Willis Ltd., pp. 244–268.
- Leather, S.R., Baumgart, E.A., Evans, H.F., Quicke, D.L.J., 2014. Seeing the trees for the wood – beech (*Fagus sylvatica*) decay fungal volatiles influence the structure of saproxylic beetle communities. *Insect Conserv. Divers.* 7, 314–326.
- Lee, S.-I., Spence, J.R., Langor, D.W., Pinzon, J., 2015. Retention patch size and conservation of saproxylic beetles in boreal white spruce stands. *For. Ecol. Manag.* 358, 98–107.
- Lubertazzi, D., 2012. The biology and natural history of *Aphaenogaster rudis*. *Psyche* 752815.
- Marek, P.E., 2010. A revision of the Appalachian millipede genus *Brachoria* Chamberlin, 1939 (Polydesmida: Xystodesmidae: Apheloriini). *Zool. J. Linnean Soc.* 159, 817–889.
- Mestre, L., Jansson, N., Ranius, T., 2018. Saproxylic biodiversity and decomposition rate decrease with small-scale isolation of tree hollows. *Biol. Conserv.* 227, 226–232.
- Mourant, A., Lecomte, N., Moreau, G., 2018. Indirect effects of an ecosystem engineer: how the Canadian beaver can drive the reproduction of saproxylic beetles. *J. Zool.* 304, 90–97.
- Müller, J., Bußler, H., Goßner, M., Rettelbach, T., Duelli, P., 2008. The European spruce bark beetle *Ips typographus* in a national park: from pest to keystone species. *Biodivers. Conserv.* 17, 2979–3001.
- Müller, J., Brustel, H., Brin, A., Bussler, H., Bouget, C., Obermaier, E., Heidinger, I.M.M., Lachat, T., Förster, B., Horak, J., Procházka, J., Köhler, F., Larrieu, L., Bense, U., Isaccson, G., Zapponi, L., Gossner, M.M., 2015. Increasing temperature may compensate for lower amounts of dead wood in driving richness of saproxylic beetles. *Ecography* 38, 499–509.
- National Park Service (NPS) US Department of the Interior, 2006. Foundation Document, Great Smoky Mountains National Park, North Carolina and Tennessee (GRSM 133/134152). Available at www.nps.gov/grsm.
- Oberle, B., Milo, A.M., Myers, J.A., Walton, M.L., Young, D.F., Zanne, A.E., 2016. Direct estimates of downslope deadwood movement over 30 years in a temperate forest illustrate impacts of treefall on forest ecosystem dynamics. *Can. J. For. Res.* 46, 351–361.
- Parisi, F., Lombardi, F., Sclarretta, A., Tognetti, R., Campanaro, A., Marchetti, M., Trematerra, P., 2016. Spatial patterns of saproxylic beetles in a relic silver fir forest (Central Italy), relationships with forest structure and biodiversity indicators. *For. Ecol. Manag.* 381, 217–234.
- Pearse, A.S., Patterson, M.T., Rankin, J.S., Wharton, G.W., 1936. The ecology of *Passalus cornutus* Fabricius, a beetle which lives in rotting logs. *Ecol. Monogr.* 6, 455–490.
- Pilskog, H.E., Sverdrup-Thygeson, A., Evju, M., Framstad, E., Birkemoe, T., 2018. Long-lasting effects of logging on beetles in hollow oaks. *Ecol. Evol.* 8, 10126–10137.
- Quinto, J., De Los Angeles Marcos-García, M., Díaz-Castelazo, C., Rico-Gray, V., Galante, E., Micó, E., 2015. Association patterns in saproxylic insect networks in three Iberian Mediterranean woodlands and their resistance to microhabitat loss. *PLoS One* 10, e0122141.

- Ranlund, Å., Victorsson, J., 2018. Stump extraction in the surrounding landscape: predatory saproxylic beetles are more negatively affected than lower trophic levels. *For. Ecol. Manag.* 408, 75–86.
- Rissler, L.J., Smith, W.H., 2010. Mapping amphibian contact zones and phylogeographical break hotspots across the United States. *Mol. Ecol.* 19, 5404–5416.
- Sánchez-Galván, I.R., Quinto, J., Micó, E., Galante, E., Marcos-García, M.A., 2014. Facilitation among saproxylic insects inhabiting tree hollows in a mediterranean forest: the case of cetoniids (Coleoptera: Cetoniidae) and syrphids (Diptera: Syrphidae). *Environ. Entomol.* 43, 336–343.
- Sánchez-Galván, I.R., Marcos-García, M.Á., Galante, E., Azeria, E.T., Micó, E., 2018. Unraveling saproxylic insect interactions in tree hollows from Iberian Mediterranean forest. *Environ. Entomol.* 47, 300–308.
- Savely, H.E., 1939. Ecological relations of certain animals in dead pine and oak logs. *Ecol. Monogr.* 9, 321–385.
- Schiegg, K., 2000. Effects of dead wood volume and connectivity on saproxylic insect species diversity. *Ecoscience* 7, 290–298.
- Schwartz, S.K., Wagner Jr., W.E., Hebets, E.A., 2014. Obligate male death and sexual cannibalism in dark fishing spiders. *Anim. Behav.* 93, 151–156.
- Seibold, S., Bässler, C., Brandl, R., Fahrig, L., Förster, B., Heurich, M., Hothorn, T., Scheipl, F., Thorn, S., Müller, J., 2017. An experimental test of the habitat-amount hypothesis for saproxylic beetles in a forested region. *Ecology* 98, 1613–1622.
- Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Am.* 87, 651–701.
- Speight, M.C.D., 1989. Saproxylic invertebrates and their conservation. *Nature and Environment Series No. 42*. Council of Europe, Strasbourg, France.
- Steffan, S.A., Chikaraishi, Y., Dharampal, P.S., Pauli, J.N., Guédot, C., Ohkouchi, N., 2017. Unpacking brown food-webs: animal trophic identity reflects rampant microbivory. *Ecol. Evol.* 7, 3532–3541.
- Stokland, J.N., 2012. The saproxylic food web. In: Stokland, J.N., Siitonen, J., Jonsson, B.G. (Eds.), *Biodiversity in Dead Wood: Ecology, Biodiversity and Conservation*. Cambridge University Press, pp. 29–57.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30, 2725–2729.
- Telfer, A.C., Young, M.R., Quinn, J., Perez, K., Sobel, C.N., Sones, J.E., Levesque-Beaudin, V., Derbyshire, R., Fernandez-Triana, J., Rougerie, R., Thevanayagam, A., Boskovic, A., Borisenko, A.V., Cadel, A., Brown, A., Pages, A., Castillo, A.H., Nicolai, A., Mockford, B.M.G., Bukowski, B., Wilson, B., Trojahn, B., Lacroix, C.A., Brimblecombe, C., Hay, C., Ho, C., Steinke, C., Warne, C.P., Cortes, C.G., Engelking, D., Wright, D., Lijtmaer, D.A., Gascoigne, D., Martich, D.H., Morningstar, D., Neumann, D., Steinke, D., DeBruin, D.D.M., Dobias, D., Sears, E., Richard, E., Damstra, E., Zakharov, E.V., Laberge, F., Collins, G.E., Blagoev, G.A., Grainge, G., Ansell, G., Meredith, G., Hogg, I., McKeown, J., Topan, J., Bracey, J., Guenther, J., Sills-Gilligan, J., Addesi, J., Persi, J., Layton, K.K.S., D'Souza, K., Dorji, K., Grundy, K., Nghidinwa, K., Ronnenberg, K., Lee, K.M., Xie, L., Lu, L., Penev, L., Gonzalez, M., Rosati, M.E., Kekkonen, M., Kuzmina, M., Iskandar, M., Mutanen, M., Fatahi, M., Pentinsaari, M., Bauman, M., Nikolova, N., Ivanova, N.V., Jones, N., Weerasuriya, N., Monkhouse, N., Lavinia, P.D., Jannetta, P., Hanisch, P.E., McMullin, R.T., Flores, R.O., Mouttet, R., Vender, R., Labbee, R.N., Forsyth, R., Lauder, R., Dickson, R., Kroft, R., Miller, S.E., MacDonald, S., Panthi, S., Pedersen, S., Sobek-Swant, S., Naik, S., Lipinskaya, T., Eagalle, T., Decaëns, T., Kosuth, T., Braukmann, T., Woodcock, T., Roslin, T., Zammit, T., Campbell, V., Dinca, V., Peneva, V., Hebert, P.D.N., deWaard, J.R., 2015. Biodiversity inventories in high gear: DNA barcoding facilitates a rapid biotic survey of a temperate nature reserve. *Biodivers. Data J.* 3, e6313.
- Tini, M., Bardiani, M., Chiari, S., Campanaro, A., Maurizi, E., Toni, I., Mason, F., Audisio, P.A., Carpaneto, G.M., 2018. Use of space and dispersal ability of a flagship saproxylic insect: a telemetric study of the stag beetle (*Lucanus cervus*) in a relict lowland forest. *Insect Conserv. Divers.* 11, 116–129.
- Ulyshen, M.D., Šobotník, J., 2018. An introduction to the diversity, ecology and conservation of saproxylic insects. In: Ulyshen, M.D. (Ed.), *Saproxylic Insects: Diversity, Ecology and Conservation*. Springer, pp. 1–47.
- Ulyshen, M.D., Zachos, L.G., Stireman III, J.O., Sheehan, T.N., Garrick, R.C., 2017. Insights into the ecology, genetics and distribution of *Lucanus elaphus* Fabricius (Coleoptera: Lucanidae), North America's giant stag beetle. *Insect Conserv. Divers.* 10, 331–340.
- Umphrey, G.J., 1996. Morphometric discrimination among sibling species in the *fulvuridis-texana* complex of the ant genus *Aphaenogaster* (Hymenoptera: Formicidae). *Can. J. Zool.* 74, 528–559.
- United States Department of Agriculture (USDA) Forest Service, 2003. Final Environmental Impact Statement: Forest Health Restoration Project, National Forests in Alabama, Bankhead National Forest. Available at: www.fs.usda.gov.
- Vargo, E.L., 2003. Hierarchical analysis of colony and population genetic structure of the eastern subterranean termite, *Reticulitermes flavipes*, using two classes of molecular markers. *Evolution* 57, 2805–2818.
- Walker, M.J., Stockman, A.K., Marek, P.E., Bond, J.E., 2009. Pleistocene glacial refugia across the Appalachian mountains and coastal plain in the millipede genus *Narceus*: evidence from population genetic, phylogeographic, and paleoclimatic data. *BMC Evol. Biol.* 9, 25.
- Wende, B., Gossner, M.M., Grass, I., Armstadt, T., Hofrichter, M., Floren, A., Linsenmair, K.E., Weisser, W.W., Steffan-Dewenter, I., 2017. Trophic level, successional age and trait matching determine specialization of deadwood-based interaction networks of saproxylic beetles. *Proc. R. Soc. B Biol. Sci.* 284, 20170198.
- Weslien, J., Djupström, L.B., Schroeder, M., Widenfalk, O., 2011. Long-term priority effects among insects and fungi colonizing decaying wood. *J. Anim. Ecol.* 80, 1155–1162.