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Stable isotopes of saproxylic beetles reveal low differences among trophic guilds and suggest a high dependence on fungi

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Abstract

Deadwood stores about 8% of global carbon stock, and its decomposition is a key factor in forest ecosystems. Deadwood-associated (saproxylic) organisms constitute a food web that sustains a substantial part of biodiversity globally. After fungi, saproxylic beetles are the most prominent agents of structural deadwood decomposition in forests. They are often classified according to their presumed link to the deadwood decomposition gradient, generally as feeding on fresh wood, decayed wood, fungi, or predators. These classifications are, however, based on ecomorphological characters (e.g., trophic morphology, habitat use) while information on their diet is globally limited. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios represent potential useful tracers to improve knowledge on the trophic ecology of this model group and the whole decomposition food web. We performed stable isotope analysis on 121 beetle species (530 samples) from a mixed-deciduous forest in Central Europe in order to (1) characterize drivers of saproxylic beetles' isotopic variability with respect to potential food sources along the wood decomposition gradient and in relation to the potentially key intrinsic factors such as phylogeny and body size and (2) to assess how isotope information matches with two trophic guild classifications based on ecomorphological characters which are commonly used in ecological studies. The analysis revealed a clear pattern of $\delta^{13}C$ increase and simultaneous C:N ratio decrease across potential food sources along the gradient from fresh to decayed deadwood and fungi. Beetle phylogeny and body size explained a significant part of their isotope variability, with values of $\delta^{13}C$ being lower in smaller species. After filtering out these effects, the $\delta^{13}C$ values reflected the position of beetle species on the decomposition gradient only loosely. Fungi-feeding guilds had higher $\delta^{13}C$ values than the guilds dependent on fresher deadwood, but otherwise the guilds were indistinguishable. Deadwood consumers did not differ from predators. The isotopic niches of different feeding guilds largely overlapped, and the large observed variation

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suggests that not only fungi feeders but species from most guilds may depend considerably on fungi and that mixed trophic strategies may be more common in the decomposition food web than currently acknowledged.

KEYWORDS
deadwood, decomposition gradient, feeding guilds, forest ecosystem, functional traits, fungi-insect interactions, insect nutrition, isotopic niche, mycetophagy, trophic ecology

INTRODUCTION

The majority of global primary productivity in aquatic and terrestrial ecosystems is not consumed as living tissue, but rather as nonliving, that is, through detritivory (Moore et al., 2004; Polis & Strong, 1996). The resulting "brown" food web (i.e., dead-tissue-based; as opposed to the "green" food web, in which consumers are supported by living autotrophic biomass) therefore channels most of an ecosystem's biomass (Hagen et al., 2012; Hyodo et al., 2015). In terrestrial ecosystems, much attention has been given to soil decomposer communities (e.g., Pollierer et al., 2019), while knowledge on the trophic ecology of organisms that depend on deadwood (i.e., saproxylic organisms) which constitute a major component of brown food webs, is still limited.

Wood constitutes the majority of terrestrial biomass and is a key factor for forest ecosystems (Fridman & Walheim, 2000; Jonsson & Kruys, 2001). Deadwood stores about 8% of global carbon stocks (Pan et al., 2011) and is a primary resource supporting a large diversity of consumers driving its decomposition (Grove, 2002), affecting nutrient cycles and soil development (Kupferschmid Albisetti et al., 2003; Pichler et al., 2012). While fungi and bacteria are the principal deadwood decomposers (Johnston et al., 2016; Lustenhouwer et al., 2020; Seibold et al., 2022), various invertebrates, especially insects, take a significant part in decomposition processes, notably by tunneling in wood, producing frass or accelerating decomposition by introducing their symbionts (fungi, bacteria) (Jacobsen et al., 2017; Ulyshen, 2015). These insects constitute important links between decomposed wood and higher trophic levels in terrestrial food webs. Termites are the main insect group driving wood decomposition in tropical forests (Seibold et al., 2021; Ulyshen, 2018), whereas beetles dominate the process in temperate and boreal forests (Stokland et al., 2012). Saproxylic beetles are among the most important taxonomic and functional groups in woodlands (Ulyshen, 2018). They may comprise several hundreds of species locally, and due to their wide range of life strategies and habitat requirements, and owing to their relatively easy identification, saproxylic beetles serve as a principal model group in forest ecology and biodiversity research (Ulyshen, 2018). Yet, their precise trophic ecology has been seldom investigated (Gimmel & Ferro, 2018).

Currently, the trophic ecology of saproxylic beetles is mostly based on trophic guild classifications which have been constructed on the basis of ecomorphological trait analysis. As in other taxa, there has been an increasing interest in the study of functional traits in saproxylic beetles recently (Burner et al., 2021, 2022; Drag et al., 2023; Micó et al., 2020; Pérez-Sánchez et al., 2020). Various morphological and ecological traits have been defined for saproxylic beetles such as body parts measurements (Hagge et al., 2021), preferred decay stage and diameter of deadwood, or deadwood microhabitats (Bouget et al., 2019; Seibold et al., 2015). Different trophic guild classifications have been proposed for larval stages of saproxylic beetles (Bouget et al., 2019; Köhler, 2000; Seibold et al., 2015; Stokland et al., 2012; Ulyshen, 2018). Trophic guilds are largely associated with the deadwood decomposition process, generally defining groups of species exploiting: (1) hard fresh or recently dead wood (referred to as xylophages), (2) decayed deadwood (saproxylophages, or saprophages if in advanced stages of decomposition), and (3) deadwood-decomposing fungi or their fruiting bodies (myco- or mycetophages) (Bouget et al., 2005; Seibold et al., 2015; Ulyshen, 2018) (Figure 1). In addition, many species are also defined as obligate or facultative predators (zoophages). Contrary to larval trophic guilds, adult feeding habits are rarely considered, because the lifespan of an adult saproxylic beetle is short in comparison with the larval development (Gallardo & Cárdenas, 2016; Stokland et al., 2012).

One potentially important caveat associated with trait-based trophic guilds is that neither habitat preference nor morphological attributes can be directly or specifically linked to trophic resource use. To the best of our knowledge, direct assessment (i.e., gut content analysis) or analysis of trophic biomarkers (e.g., stable isotopes) have never been used to determine the diet of saproxylic beetles (except for single species; Přikryl et al., 2012) and thus functional traits cannot be generally confronted with actual diet data. Further, many traits were established on the basis of experts' knowledge on a given

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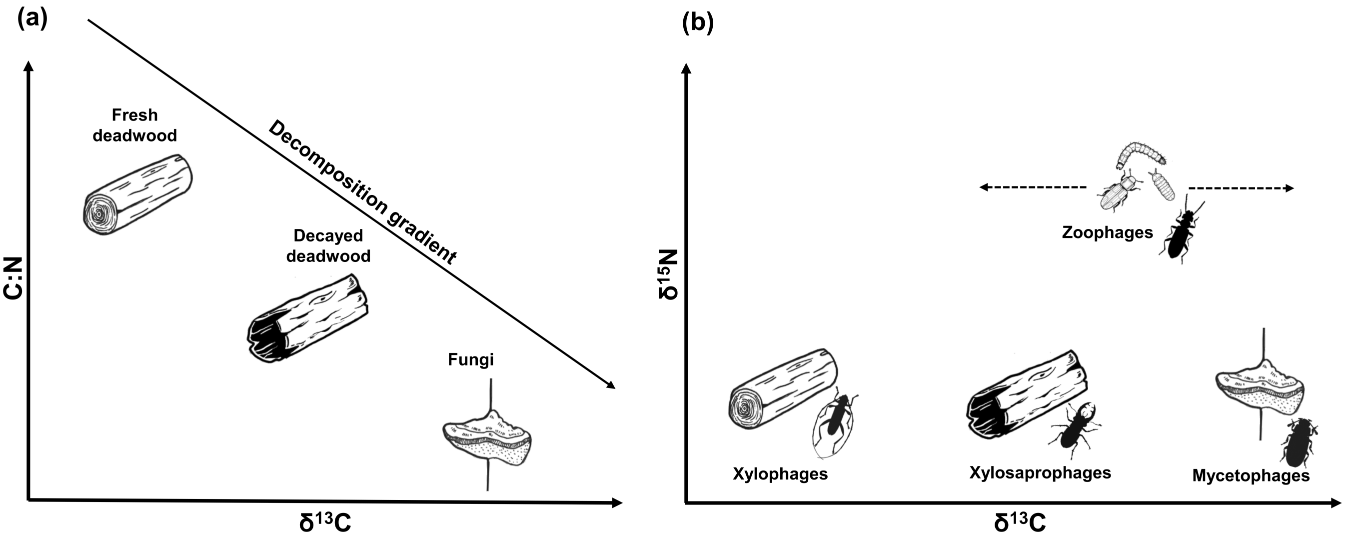

FIGURE 1 Prediction of the relationship between δ^{13}C and C:N ratio across potential food sources (a) illustrating the increase in the δ^{13}C during deadwood decomposition (measured by decreasing C:N ratio due to increase in nitrogen in the food sources). We expect that the stable isotope composition of measured saproxylic beetles would reflect the same gradient as their food sources (b) in δ^{13}C for xylophages, xylosaprophages, and mycetophages and in δ^{15}N for zoophages (indicating transition between trophic levels). The positions of the feeding guilds on the isotope ratio gradients probably overlap, especially in zoophages which may reflect food source (other saproxylic organisms) of different feeding guilds. Drawings credit: Michaela Helclová.

taxon (e.g., microhabitat use and morphological variation in characteristics involved in feeding, such as mouth parts of larvae; Stokland et al., 2012) and may lack a general application or suffer potential biases. For instance, stout mandibles are a trait used to classify saproxylic beetles as xylophagous (i.e., consuming hard/fresh deadwood) (Gimmel & Ferro, 2018), but having stout mandibles does not exclude other trophic behaviors (e.g., predation). Such example points toward a risk of oversimplification of saproxylic beetles' trophic ecology, which may hamper our understanding of saproxylic food webs structure and functioning. Along these lines, it has been suggested that many species might not be specialists but likely have a multi-compound diet (like omnivores). The omnivory hypothesis emerges from studies pointing to the stoichiometric mismatch between xylophagous species and hard wood elemental compositions (Filipiak & Weiner, 2014). Fresh deadwood's elemental ratio and content in essential nutritional elements (most importantly nitrogen and phosphorus) are likely too low to allow for beetle larvae to develop solely on plant matter (Elser et al., 2000; Filipiak & Weiner, 2014; Six & Elser, 2020). This suggests that these beetles cannot derive all nutrients essential to their growth from wood alone. Recent studies have identified various mechanisms by which different saproxylic species may acquire these nutrients. Beetles currently classified as feeding on fresh deadwood (xylophages) may in fact be fungivores (mycetophages) or have mixed diets (xylomycetophages), because their true assimilated diet may be supplemented with essential elements originating from fungi and bacteria infecting the decaying wood (Filipiak, 2018; Filipiak & Weiner, 2014; Tanahashi et al., 2009). Some primary colonizers (therefore associated with fresh deadwood) such as ambrosia beetles (Scolytinae, Platypodinae, Lymexylidae) actually actively cultivate nutritional fungi as their main food source (Birkemoe et al., 2018; Mayers et al., 2022). Another mechanism of potential acquisition of essential elements such as nitrogen is through symbiotic relationships with gut microorganisms. Digestion of the substrate occurs in different parts of the gut with diverse anatomical structures and microbial communities which enable lignocellulose deconstruction and survival on an extremely nutrient-poor diet, as demonstrated, for example, in *Odontotaenius disjunctus* (Passalidae) (Ceja-Navarro et al., 2019). The majority of saproxylic beetles may largely depend on these microorganisms, but recent studies of beetle genomes also revealed that some species have acquired plant-degrading and detoxifying enzymes horizontally from microbes (e.g., Cerambycidae: *Anoplophora glabripennis*, Buprestidae: *Agrilus planipennis*, Scolytinae: *Dendroctonus ponderosae*) which may play a role in these digestive processes (Keeling et al., 2013; McKenna et al., 2016). The actual diet of species associated with later stages of deadwood decomposition (xylomycetophages or saprophages) may be even more difficult to assess, as they colonize deadwood that has become increasingly interwoven with fungal mycelia.

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For such species, studies suggest that the fungal mycelium likely constitutes an important component or even a principal food source (Filipiak & Weiner, 2017; Six & Elser, 2020; Stokland et al., 2012). For xylophages, xylomycetophages, or saprophages, trait-based approaches, that is, inferring species diet solely from morphological structures (e.g., shape and size of mandibles) may be problematic if habitat use of the species is not considered. There is thus a growing need to go beyond the determination of trophic ecology based on proxies such as morphology or microhabitat use and use techniques allowing the investigation of the actual assimilated diet of organisms, such as stable isotope analysis.

Natural abundance stable isotope analysis of consumers' tissues is a widely applied and established technique in trophic ecology, as stable isotopes are time- and space-integrative tracers of food source assimilation (Hyodo, 2015; Potapov, Tiunov, et al., 2019). Stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$), conventionally expressed as $\delta^{13}C$ and $\delta^{15}N$ relative to international standards, have been widely used to study trophic niches, as they respectively capture both horizontal (basal source of dietary carbon) and vertical (trophic level) dimensions of food webs in aquatic and terrestrial ecosystems (Layman et al., 2007; Newsome et al., 2007). They are especially useful to study food webs in contexts where individual interactions are often difficult to investigate through direct observation, such as in those involving arthropod communities. Stable isotope analyses have already been applied in various terrestrial arthropod groups, such as in termites (Bourguignon et al., 2009, 2011; Tayasu et al., 2002), ants (Blüthgen et al., 2003), and moths (Adams et al., 2016), including soil decomposer communities (Bluhm et al., 2015; Pollierer et al., 2019; Steffan et al., 2017), but never in beetles living in the deadwood system (but see Tuo et al., 2024, for the potential effect of saproxylic beetles on other invertebrates).

Yet, the data suggest an enrichment of organic matter in ^{13}C and ^{15}N isotopes along a decomposition/humification gradient; that is, an increase in $\delta^{13}C$ and $\delta^{15}N$ values from fresh wood to heavily mineralized organic matter (Tiunov, 2007; but see Steffan et al., 2017, where no pattern in nitrogen was found). Carbon and nitrogen isotope variation in saproxylic beetles should thus reflect assimilation of food resources along the wood decomposition gradient (Hyodo, 2015; Potapov, Tiunov, et al., 2019), allowing a critical insight into their actual trophic ecology which could be used to improve and interrogate the potential limitations of commonly used trait-based trophic guilds classifications.

By analyzing C and N stable isotope ratios of a large diversity of saproxylic (121 species) and non-saproxylic (10 species) beetles in a European forest, the general aims of the present study were to characterize drivers of saproxylic beetles' isotopic variability with respect to potential food sources along the wood decomposition gradient and in relation to the potentially key intrinsic factors such as phylogeny and body size and to assess how isotope information matches with two trophic guild classifications commonly used in ecological studies. Specifically, we aim to (1a) test whether saproxylic beetles can be distinguished from non-saproxylic ones based on their isotopic composition and whether $\delta^{15}N$ variation could be linked to predatory behavior of some taxa established from the literature; (1b) describe the isotopic and elemental (C, N) variability of potential food sources along the gradient from fresh to decayed deadwood and fungi and how the saproxylic beetle community fits along this gradient; (1c) test for the effects of key intrinsic, and potentially confounding factors of trophic variability, the phylogenetic relatedness and body size, on the isotopic composition of saproxylic beetles across the community; and (2) compare the observed isotopic variability across the saproxylic beetles community with trait-based guild classifications which are commonly used to describe their trophic ecology in the literature (Bouget et al., 2019; Köhler, 2000) in order to assess their potential limitations and future directions for a more integrative classification. We predict that (1a) saproxylic beetles depict globally higher $\delta^{13}C$ values than non-saproxylic species (Gessler et al., 2014), whereas in both groups, we expect species that are recognized as predators to depict higher $\delta^{15}N$ values than those classified as primary consumers (within the expected range of ~2‰–3‰ increase; Post, 2002). (1b) We expect saproxylic beetles to depict a range of isotope values corresponding to that of their potential food sources, which we expect to increase in both $\delta^{13}C$ and $\delta^{15}N$ values following a gradient from fresh to increasingly decomposed deadwood (as measured by decreasing C:N ratio) and fungi (Potapov, Tiunov, et al., 2019; Tiunov, 2007) (Figure 1). (1c) We expect $\delta^{13}C$ values of saproxylic beetles to decrease with increasing body size because digestion of fresh deadwood (high C:N ratio, low $\delta^{13}C$ values) should require a longer digestive tract (Birkemoe et al., 2018). We expect that body size may not be a good predictor of $\delta^{15}N$ values (although usually a proxy for trophic level; Post, 2002) because $\delta^{15}N$ increase with predation may be confounded with $\delta^{15}N$ variation along the decomposition gradient of food resources (Steffan et al., 2017). We expect phylogenetic relatedness to be a significant predictor of $\delta^{13}C$ (basal resource use) variation in saproxylic beetles because closely related species may share closer trophic strategies inherited from their common ancestor (Hellemans et al., 2022; Hunt et al., 2007). (2) We expect saproxylic beetles belonging to the different trophic guilds established on the basis of ecomorphological traits to show considerable overlap in their isotopic composition, reflecting a globally higher degree of omnivory (mixed mycetophagy and/or predatory behavior) than currently acknowledged and suggesting a principal role of these omnivory pathways, notably for the acquisition of a sufficient amount of nitrogen (Filipiak & Weiner, 2017; Stokland et al., 2012).

MATERIALS AND METHODS

Study site and beetle sampling

We collected beetles at three sites in the Stara obora game reserve and its vicinity in Hluboka nad Vltavou, South Bohemia, Czech Republic, in particular at Kamenik (49°5′52″ N, 14°26′31″ E), Zlatesovice (49°4′50″ N, 14°26′46″ E), and Hluboka (49°2′47″ N, 14°26′35″ E). The first two sites are located in a forested area with heterogeneous stands, characterized by mixed forests dominated by beech (*Fagus sylvatica*) with an admixture of sessile oak (*Quercus petraea*), hornbeam (*Carpinus betulus*), spruce (*Picea abies*), fir (*Abies* sp.), and elm (*Ulmus* sp.). Thanks to great amounts of deadwood and a variety of both open and closed canopy stands, the area supports a rich community of saproxylic beetles with many threatened or rare species. The third site is a former fishpond dam where many old open-grown veteran oaks, poplars, and willows can be found.

We collected adult beetles during the spring and summer of 2018 and 2019. Beetles were searched directly by thorough inspection of accessible substrates; on trees, logs, stumps, and other types of deadwood; in fruiting bodies of wood-decaying fungi; under bark; or on nearby vegetation with flowers. The sampling was designed in order to obtain a set of saproxylic beetle species that covered the major trophic guilds currently described in the literature (see below). In addition, we also sampled several species of non-saproxylic beetles by sweeping the vegetation at the sampling sites in order to obtain species that can be classified either as primary consumers of leaf matter (phytophages) or as predators (zoophages). The beetles were stored in 95% ethanol immediately after their collection. In addition, several beetle specimens were collected by window flight-interception traps (FIT) exposed at the same sites in 2018. These beetles were conserved in a saline solution for a maximum of 14 days before being transferred to 95% ethanol.

For stable isotope analysis, we used beetles' elytra as a tissue that should reflect resources used by beetles during their larval stage. Indeed, elytra mostly consist of chitin with a maximum 1% of fat, reflecting delayed metabolic response (Gratton & Forbes, 2006), and therefore, their isotope compositions are not affected by ethanol conservation (Blüthgen et al., 2003; Schallhart et al., 2009; Tallamy & Pesek, 1996). The amount of tissue varied according to the size of the beetles. For most specimens, tissue was taken from one individual (either both elytra or a piece of one elytron to ensure >1 mg of dry mass), while for smaller specimens, one sample was composed of elytra of several individuals. In total, we analyzed 530 samples from 1352 beetles altogether.

Additionally, we collected 79 samples of potential food substrates for stable isotope and C:N ratio analysis in order to study isotopic and elemental variation along the decomposition gradient of deadwood. We collected these substrates from the Kamenik and Zlatesovice sites (two sites out of three) since the majority of beetle samples were collected there. The substrates were fresh deadwood of beech (four samples), decayed deadwood (five samples from beech, five from oak), samples of inner bark (25 from beech, 10 from oak), samples of fruiting bodies of perennial polyporous fungi (*Fomes fomentarius*, *Daedaleopsis confragosa*, and *Trametes* sp.) (15), and samples of fruiting bodies of ephemeral fungi (*Laetiporus sulphureus*) (15). The substrate samples were primarily used to display general patterns in the decomposition gradient, not to directly infer substrate-beetle associations for which more thorough sampling from all sites would be necessary.

Stable isotope analysis

Elytra were oven-dried at 60°C for 72 h (Binder, Tubingen, Germany), they were further weighed in tin capsules and stored in a desiccator until being analyzed. Carbon and nitrogen stable isotope ratios were measured using an IRMS (Isoprime 100; Isoprime, UK) coupled to an elemental analyzer (Vario MICRO cube; Elementar, Langensbold, Germany) and conventionally expressed as δ values in per-mille. The certified reference materials from the International Atomic Energy Agency (IAEA, Vienna, Austria) used were ammonium sulphate (IAEA-N2; $\delta^{15}N = 20.3 \pm 0.2‰$) and sucrose (IAEA C-6; $\delta^{13}C = -10.8 \pm 0.5‰$). Both these reference materials are calibrated against the Vienna PeeDee Belemnite international references for carbon samples and atmospheric air for nitrogen. Internal standards (glycine) were inserted into all runs at regular intervals to assess potential drift over time. Repetitive measurements of glycine ($\delta^{15}N = 2.3 \pm 0.3‰$; $\delta^{13}C = -47.5 \pm 0.3‰$) were also used to calibrate isotopic data and as an elemental standard. Experimental precision, based on the SD of one beetle sample replicate, was ±0.10‰ and ±0.56‰ for carbon and nitrogen, respectively. Elemental data of feeding substrates ($n = 79$), expressed in % of dry weight, are used to calculate C:N ratios (w:w) and to display the

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relationship between the C:N and $\delta^{13}C$ ($\delta^{15}N$ could not be obtained for some samples due to too low N content).

Trophic guild classification

All collected species of saproxylic beetles were classified into trophic guilds based on two published concepts of trophic guilds widely used for European species. First, we used the classification list of Bouget et al. (2019), which is an extended version of the former Frisbee database (Bouget et al., 2008; hereinafter, "Frisbee list"). The list contains information on 2663 beetle species recorded in France and classifies them into five categories of primary larval trophic regimes (Table 1). Second, we used the list of Köhler (2000) (hereinafter, "Köhler list"), which contains information on 1374 beetle species recorded in Germany. This second list classifies species into nine categories of feeding types. The list was later simplified by Seibold et al. (2015), who merged several categories together, but for the purpose of this study, we used the original, more detailed list (Table 1).

Moreover, we classified the non-saproxylic species collected during sampling as either phytophages (herbivores) or zoophages (predators), based on a knowledge of their biology and reference literature (Hůrka, 2017). These two trophic guilds were used as analogues to saproxylic guilds of xylophages and zoophages, respectively (Table 1).

Data analysis

Isotopic composition of saproxylic and non-saproxylic beetles

We compared C and N stable isotope ratios from selected species corresponding to primary consumers and predators

TABLE 1 Trophic guild classification and number of species and samples collected in our study per particular category.

Classification	Abbreviation	Definition	No. species/ no. samples
Frisbee list (Bouget et al., 2019)	Xyl	Xylophages—Feeding on lignin component of dying or alive trees or on fresh deadwood	23/75
	Sxy	Saproxylophages—Feeding on partially or substantially decomposed and rotten wood	26/115
	Sap	Saprophages—Feeding on diverse organic remains: deadwood fragments, remains of animal bodies and exuviae, fungal hyphae or microorganisms	3/3
	Myc	Xylomycetophages—Feeding on hyphae or fruiting bodies of wood-decaying fungi	26/105
	Zoo	Zoophages—Predators and carnivores feeding on deadwood-associated invertebrates	39/181
Köhler list (Köhler, 2000)[a]	X	Xylophages—Feeding on wood	39/137
	XM[b]	Xylomycetophages—Feeding on wood infested with wood-decaying fungi	11/46
	XS[b]	Mixed diet of xylophages and saprophages (feeding on rotten plant matter)	5/26
	M	Mycetophages—Feeding on fungi	20/79
	XZ[b]	Xylo- and zoophages (mixed diet)	5/15
	Z	Zoophages—Predators of invertebrates	29/162
Non-saproxylic species	Phyto	Herbivores feeding on leaves or stems of nonwoody plant tissues	4/10
	Zoo	Generalist predators not associated with deadwood habitats (cf. Zoo and XZ, Z guilds above)	5/14

Note: The table provides a short definition (and abbreviation) for each trophic guild category based on the published lists of saproxylic beetles: The Frisbee list (Bouget et al., 2019) and the Köhler list (Köhler, 2000). Moreover, two additional categories were created for non-saproxylic species that were used as control specimens. Numbers of species collected in particular trophic guild category and numbers of samples created for stable isotope analysis are given in the right-most column.

[a] In addition to the six stated categories, the list further defines a category of *necrophages* (feeding on rotten animal matter), a category of *mycetophages feeding on molds*, and a category of *saprophages* (feeding on rotten plant matter). Species belonging to these three categories were not recorded among the sampled specimens in our study. The saprophages and necrophages defined in the Köhler list are merged together into a category called "detritivores" in the simplified list of Seibold et al. (2015).

[b] Categories XM, XS, and XZ are merged with xylophages (X) into a single category in the simplified list of Seibold et al. (2015).

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within both non-saproxylic and saproxylic groups to assess whether these two groups depict different δ^{13}C values and whether the general rule of ^{15}N enrichment with trophic level applied to both groups. The compared groups corresponded to: (1) non-saproxylic phytophages, (2) non-saproxylic zoophages, (3) saproxylic xylophages, and (4) saproxylic zoophages. For saproxylic species, we differentiated xylophages and zoophages using trophic guilds from the Frisbee list. We compared isotopic ratios of the four guilds by fitting linear mixed-effects models with Gaussian distribution using the "nlme" package (Pinheiro et al., 2020) in R 4.1.1. (R Core Team, 2021) and using samples (one individual measurement) as observation units. The δ^{13}C and δ^{15}N values were used as response variables in separate models. Site affiliation was first added as a covariate to the model to filter out the effect of spatial variability; trophic guild category was subsequently used as a fixed factor variable; and species identity as a random effect factor. Tukey honestly significant difference (HSD) post hoc tests were used for determination of differences among particular trophic guilds using the package "multcomp" (Hothorn et al., 2016) in R.

Patterns of isotopic and elemental variation among sources on the deadwood decomposition gradient

We described isotopic variation across different types of substrates (fresh deadwood, decayed deadwood, inner bark, perennial fungi, and ephemeral fungi) in order to assess whether δ^{13}C values and δ^{15}N values increase from fresh deadwood to decayed deadwood and fruiting body of fungi. Further, we tested whether δ^{13}C values increase with decreasing C:N (w:w) (proxy for increasing degradation/colonization of substrate by fungi) in multiple substrates using Spearman rank correlation.

Phylogenetic signal and body size effect on the isotopic composition of saproxylic beetles

We tested the effect of phylogeny and body size of saproxylic beetles on their C and N isotopic composition. We used the phylogeny of saproxylic beetles derived from Hagge et al. (2021). Separately for each element, we first fitted a null model with Gaussian distribution, including only the isotopic ratio of each element (δ^{13}C and δ^{15}N species mean values) as a response variable and the intercept. We then fitted a model containing phylogenetic autocorrelation structure of the studied saproxylic beetles; that is, a model assuming that species may have similar δ^{13}C or δ^{15}N values based on their close phylogenetic relationships. The phylogeny was imported to the model by the *read.tree()* function using the "ape" package (Paradis & Schliep, 2019) with corr = corPagel as a setting for correlation structure. We compared Akaike information criterion (AIC) values of these models to select the more parsimonious model in each case. We also subsequently used these as a starting model for analysis of body size effect and correspondence with trophic guilds classifications as controlling the effect of phylogeny is required for more comprehensive studies including large community datasets (Ikeda et al., 2006; Potapov et al., 2016). In models testing the effect of body size on δ^{13}C and δ^{15}N values of saproxylic beetles, ln-transformed body size was used as an explanatory variable. We finally used Tukey HSD post hoc tests in the package "multcomp" to reveal differences among trophic guilds by multiple comparisons.

Correspondence between isotopic variability and trophic guild classifications

Using the "SIBER" package (Jackson et al., 2011), we generated bivariate standard ellipses representing core isotopic niches of saproxylic beetles (calculated based on averaged isotope values for each species) while grouping them into trophic guilds according to each of the two classification lists (Frisbee and Köhler). These isotopic niches were used to test the concordance between established concepts of trophic guilds and observed isotopic variation among saproxylic beetles by describing patterns of niche sizes, overlaps, and location along the deadwood decomposition gradient. Two metrics were calculated, based on posterior distributions of standard ellipses of the trophic guilds: (1) areas of the ellipses associated with each trophic guild (Bayesian estimate of standard ellipse area; SEA_B) computed using Bayesian inference (MCMC parameters: two chains, 200,000 iterations, 10,000 burn-ins, thins = 50, and using an inverse Wishart prior; Jackson et al., 2011) and (2) pairwise ellipses' overlaps (the percentage of shared niche space between two trophic guilds; Lejeune et al., 2018). Species belonging to the "saprophagous" category in the Frisbee list were excluded from analyses because their number was too low to calculate an ellipse (<5 species). Finally, we used linear models to test whether affiliation of saproxylic beetle species to the different trophic guilds of the two lists was a significant predictor of their δ^{13}C or δ^{15}N values. To this end, we started from the more parsimonious models of phylogeny effect on δ^{13}C and δ^{15}N values. If body size was a significant predictor, it was included in the model as well as a covariate before the inclusion of the trophic guild affiliation as an explanatory

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factor (in two separate sets of models for Frisbee and Köhler lists). We finally used Tukey HSD post hoc tests in the package "multcomp" to reveal differences among trophic guilds by multiple comparisons.

RESULTS

Isotopic composition of saproxylic and non-saproxylic beetles

In total, we analyzed 530 samples, 503 samples belonging to 121 saproxylic species and 27 samples belonging to 10 non-saproxylic species. δ^{13}C values of the saproxylic species assemblage ranged from −28.0‰ to −19.0‰ and δ^{15}N ranged from −12.5‰ to 15.9‰. δ^{13}C values of non-saproxylic species collected ranged from −30.6‰ to −24.4‰ and δ^{15}N ranged from −6.7‰ to 8.7‰. The comparison of primary consumers and predators in both non-saproxylic and saproxylic beetles revealed that there were no differences between primary and secondary consumers within the same trophic web (non-saproxylic or saproxylic) (Figure 2). Xylophages had higher δ^{13}C values than phytophages, and saproxylic zoophages had higher δ^{13}C values than non-saproxylic zoophages ($F_{3,50} =$ 9.311, $p < 0.001$) (Figure 2). Phytophages had the lowest δ^{13}C values (mean = −27.9‰) and together with non-saproxylic zoophages they differed from saproxylic zoophages, but not from xylophages. In terms of nitrogen, predators appeared on average more enriched in ^{15}N compared with primary producers in both trophic webs, but there was considerable variation. The only significant difference in terms of δ^{15}N was found between non-saproxylic zoophages and saproxylic xylophages, with non-saproxylic zoophages having the highest values (mean δ^{15}N = 2.8‰) ($F_{3,50} = 3.188$, $p = 0.032$) (Figure 2).

Patterns of isotopic and elemental variation among sources on the deadwood decomposition gradient

We analyzed 79 samples belonging to five potential food substrate categories for saproxylic beetles (inner bark, fresh deadwood, decayed deadwood, perennial fungi, and ephemeral fungi). δ^{13}C values of potential substrates ranged from −27.5‰ to −20.3‰. δ^{13}C increased along the deadwood decomposition gradient as follows (mean ± SD): fresh deadwood (−26.5 ± 0.3‰), decayed deadwood (−26 ± 0.8‰), inner bark (−25.1 ± 1.4‰), ephemeral fungi (−23.6 ± 1.7‰), and perennial fungi (−22.9 ± 1.9‰) (Figure 3a). δ^{15}N values of potential substrates could only be obtained for 59 samples and ranged from −6.9‰ to 9.5‰. They showed large variation without clear pattern across substrates. Fresh and decayed deadwood were indiscernible based on δ^{15}N (−4.1 ± 1.7‰ both). Inner bark δ^{15}N values were on average higher than wood samples but largely overlapping (−2.4 ± 2.7‰). Perennial fungi showed the largest δ^{15}N

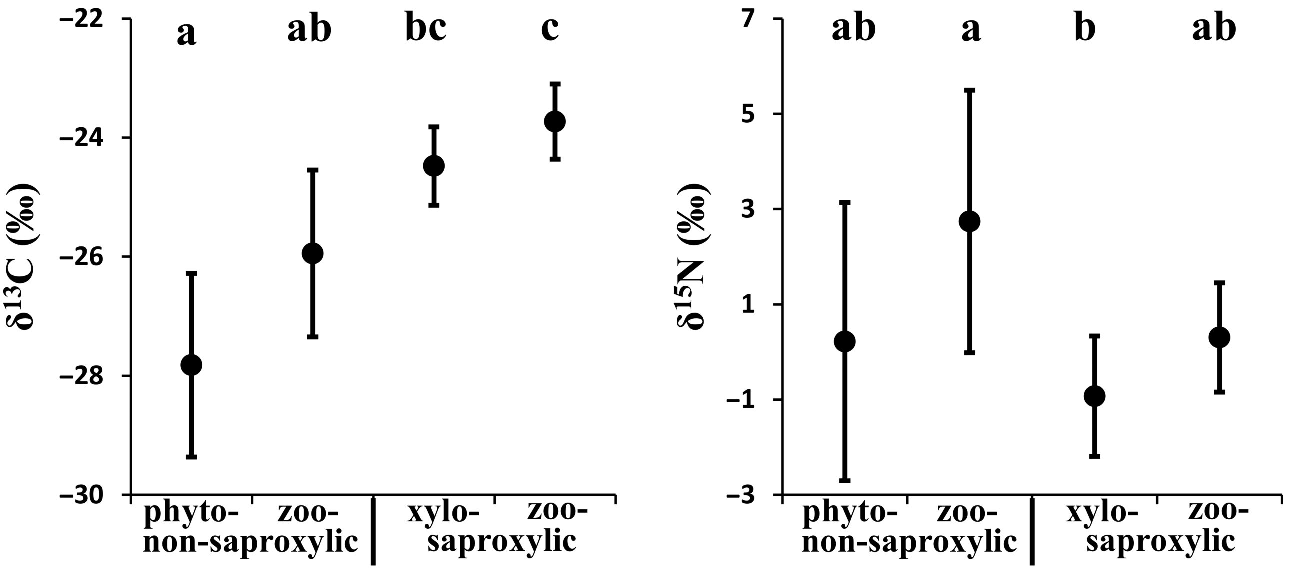

FIGURE 2 Comparison of δ^{13}C and δ^{15}N values between primary and secondary consumers of non-saproxylic and saproxylic beetles: phyto, phytophages; zoo, zoophages/predators (for both non-saproxylic and saproxylic beetles), xylo, xylophages. Mean values (full circles) and 95% CIs (error bars) are displayed. The different letters above the bars represent significant differences among the guilds according to Tukey honestly significant difference post hoc test.

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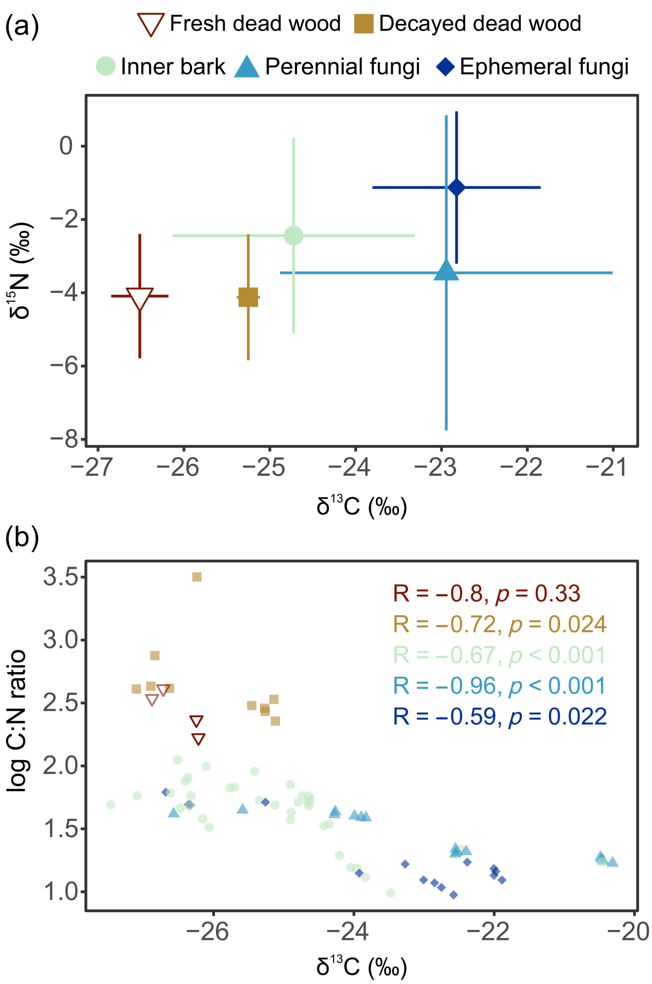

FIGURE 3 Stable isotope composition of potential food sources of saproxylic beetles sampled across the study site (a) and relation between δ^{13}C and C:N ratio across food sources (b). Data are presented as mean ± SD in (a) and are based on 59 samples for which both δ^{13}C and δ^{15}N values could be obtained. Data points are individual samples in (b) and are based on 79 samples. C:N ratios are log-transformed. *p*, *p*-value of the test; R, Spearman correlation coefficient between δ^{13}C and C:N ratio of each food source. Fresh deadwood = red hollow triangles, decayed deadwood = yellow squares, inner bark = turquoise dots, perennial fungi = light blue triangles, ephemeral fungi = dark blue diamonds.

variation (−3.5 ± 4.3‰), overlapping to a great extent with all other substrates, whereas ephemeral fungi had the highest δ^{15}N values on average, overlapping greatly with perennial fungi and inner bark samples (−1.1 ± 2.1‰) (Figure 3a). δ^{13}C values globally increased with decreasing C:N ratios across substrate types, from fresh deadwood to fruiting bodies of fungi (Figure 3b). δ^{13}C and C:N ratios (log-transformed) were negatively correlated in all substrates except fresh deadwood (but $n = 4$) ($R = -0.72$, $p = 0.024$ for decayed deadwood; $R = -0.67$, $p < 0.001$ for inner bark; $R = -0.96$, $p < 0.001$ for perennial fungi; $R = -0.59$, $p = 0.022$ for ephemeral fungi).

Phylogenetic signal and body size effect on the isotopic composition of saproxylic beetles

The phylogeny of saproxylic beetles had a significant global effect on δ^{13}C ($\text{AIC}_{\text{null}} = 474.5$, $\text{AIC}_{\text{phylo}} = 468.4$, $\chi^2_{(1)} = 8.10$, $p = 0.004$, Pagel's $\lambda = 0.66$), but not on δ^{15}N values ($\text{AIC}_{\text{null}} = 632.4$, $\text{AIC}_{\text{phylo}} = 632.3$, $\chi^2_{(1)} = 2.09$, $p = 0.15$, Pagel's $\lambda = 0.71$); therefore, the model including phylogeny was used for analysis of δ^{13}C but not for δ^{15}N (Figure 4). Further, after accounting for phylogeny, body size had a significant negative global effect on δ^{13}C ($F_{1,119} = 8.37$, $p = 0.005$), but no effect on δ^{15}N values ($F_{1,119} = 3.43$, $p = 0.066$), and was thus included as a covariate in the analysis of δ^{13}C (Figure 5).

Correspondence between isotopic variability and trophic guild classifications

After accounting for phylogeny and body size effects, we found significant δ^{13}C differences among trophic guilds from the Frisbee list ($F_{4,111} = 4.13$, $p = 0.004$) (Figure 6a). The species belonging to the category of xylomycetophages (Myc) had higher δ^{13}C values than xylophages (Xyl) and saproxylophages (Sxy), but not higher than saprophages (Sap) or zoophages (Zoo); the four latter groups were indistinguishable based on their δ^{13}C values. The analysis of the Köhler list showed significant differences among guilds for δ^{13}C ($F_{5,111} = 3.91$, $p = 0.003$), with the category of mycetophages (M) showing the highest δ^{13}C values, and different from xylophages (X) and mixed xylosaprophages (XS), but not from other guild categories defined by the list (Figure 4b). There were also no differences among other guilds. There were no differences in δ^{15}N values among the guilds for both the Frisbee and Köhler guild classification ($F_{4,112} = 0.69$, $p = 0.60$ and $F_{5,103} = 0.66$, $p = 0.65$, respectively) (Figure 6c,d).

Isotopic niche modeling of trophic guilds following Frisbee and Köhler classification lists revealed that in general, xylophages and mycetophages occupied niches on opposite sides of the gradient represented by δ^{13}C (Figure 7 and Appendix S1: Table S1). This pattern was roughly concordant with that of their putative food sources, with δ^{13}C values increasing from fresh deadwood to fruiting bodies of fungi (Figure 7a), and δ^{13}C decreasing with increasing C:N ratios (Figure 7b). However, the lowest mean values on the δ^{13}C gradient were not identified for xylophages (which are supposed to feed on freshly dead wood) but for saproxylophages (Sxy) and mixed xylo- and saprophages (XS) in the Frisbee and Köhler lists, respectively (Appendix S1: Table S1). As for

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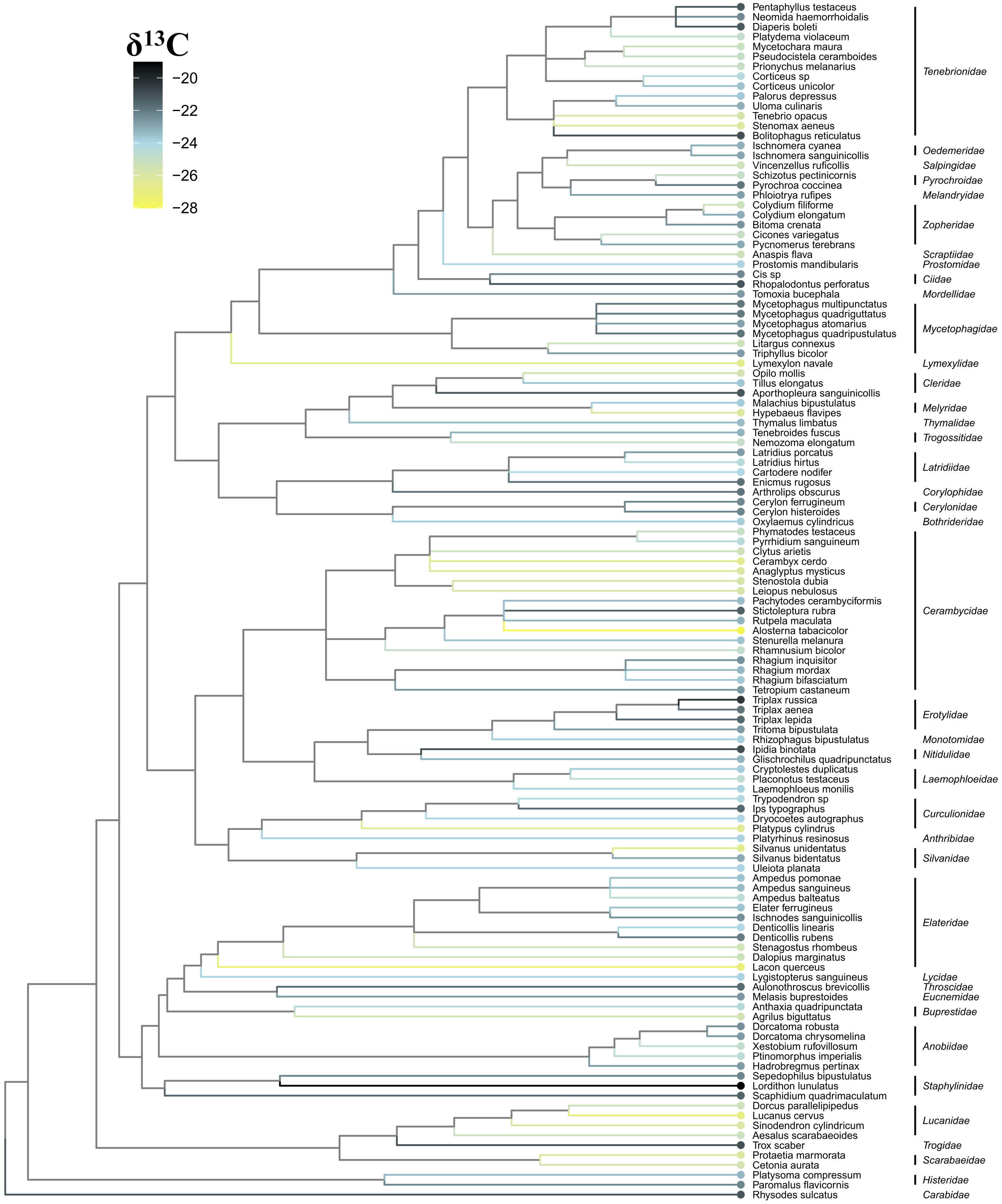

FIGURE 4 The effect of phylogeny on δ^{13}C values. The color gradient of the branches represents mean values of the δ^{13}C values measured for 121 species of saproxylic beetles (black color represents the highest values, blue intermediate values, and yellow the lowest values). The effect of phylogeny was significant for δ^{13}C but not for δ^{15}N (see *Results*). Phylogeny of saproxylic beetles was derived from Hagge et al. (2021); however, species names of *Anobium pertinax*, *Corymbia rubra*, *Dermestoides sanguinicollis*, *Hedobia imperialis*, *Leptura maculata*, *Mycetochara linearis*, *Nemosoma elongatum*, *Protaetia lugubris*, and *Ropalodontus perforatus* were renamed following the Frisbee list (Bouget et al., 2019).

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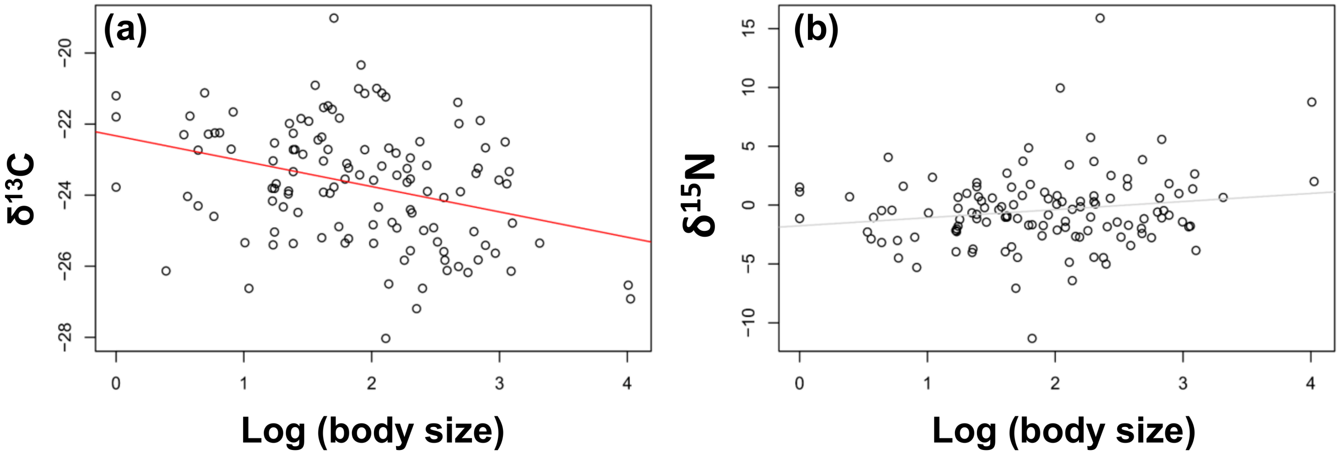

FIGURE 5 The relationship between ln-transformed body size (in millimeters) and values of $\delta^{13}C$ (a) and of $\delta^{15}N$ (b) based on averaged values of the isotopic ratios measured for 121 species of saproxylic beetles. The effect of body size on the isotopic values was significantly negative for $\delta^{13}C$; there was no significant relationship for $\delta^{15}N$ (see *Results*).

the analysis of differences in $\delta^{15}N$ values among trophic guilds (Figure 6), standard ellipses areas of the different trophic guilds encompassed a very similar range of $\delta^{15}N$ values, with no pattern of increasing niche position with either zoophagy or mycetophagy informed by the two lists (Figure 7). In both cases, xylophages (Xyl, X) showed larger isotopic niches ($SEA_B = 21.39$, $CI_{95} = 16.92–27.00$ for the Frisbee list, and $SEA_B = 21.20$, $CI_{95} = 17.90–25.20$ for the Köhler list; Appendix S1: Table S1), with direct probabilities of differences compared with the other guilds being >92% and >95%, respectively (see detailed tests in Appendix S1: Table S2). The smallest isotopic niches were represented by xylomycetophages (Myc) from the Frisbee list ($SEA_B = 10.89$, $CI_{95} = 8.92–13.35$; direct probabilities of differences = 100%) and xylomycetophages (XM) from the Köhler list ($SEA_B = 7.90$, $CI_{95} = 6.00–10.80$; direct probabilities of differences >88%) (Figure 7).

Analysis of isotopic niche overlaps revealed patterns incompatible with the inferred trophic ecology of most guilds in both classifications. In the case of the Frisbee list, the xylomycetophages (Myc) shared the least isotopic space with the other guilds (20% with zoophages, 6% shared niche space with both xylophages and saproxylophages). Other trophic guilds shared a considerably larger area of niche space (46% on average) (Appendix S1: Table S2). In the case of the Köhler list, xylosaprophages (XS) and mycetophages (M) were relatively well discriminated and both shared on average 11% of isotopic area with other guilds. At the same time, xylosaprophages and mycetophages are well separated from each other. Other trophic guilds shared a considerably larger area of niche space (33% on average) (see Appendix S1: Table S2 for pairwise comparisons of shared niche space). We displayed the mean values of $\delta^{13}C$ and $\delta^{15}N$ for each particular species based on the Frisbee classification in Appendix S1: Figure S1 and summarized the number of sampled specimens and elytra for each species in Assessment of species to trophic guilds.docx in Figshare repository.

DISCUSSION

This is the first study using natural abundance stable isotope analysis to describe the trophic ecology of saproxylic beetles, insects that play a key role in nutrients cycling and are classically used as a model group in forest ecology (Seibold et al., 2022; Tuo et al., 2024). Although regionally there are approximately 1800 species of saproxylic beetles in Central Europe (Bouget et al., 2019; Köhler, 2000), local studies typically record around 200–350 species over a year of sampling (Kozel et al., 2021). Our dataset of 121 European saproxylic beetle species thus theoretically represents one third of the local community and covers a substantial range of feeding strategies. Our results showed that both primary and secondary consumers among saproxylic species had higher $\delta^{13}C$ values than phytophages and that only $\delta^{13}C$ may be relevant for further discrimination between some trophic strategies within saproxylic beetles. Indeed, potential food sources could be well discriminated based on their C isotope composition. $\delta^{13}C$ values increased along the deadwood decomposition gradient up to fruiting bodies of fungi (Figure 3), and the range of saproxylic beetle values generally reflected that of their potential sources along the C axis. Yet, there were multiple important mismatches between trophic guilds from the literature (informed by ecomorphological traits) and the expected trophic ecology of saproxylic beetle species based on similarities between their isotopic composition

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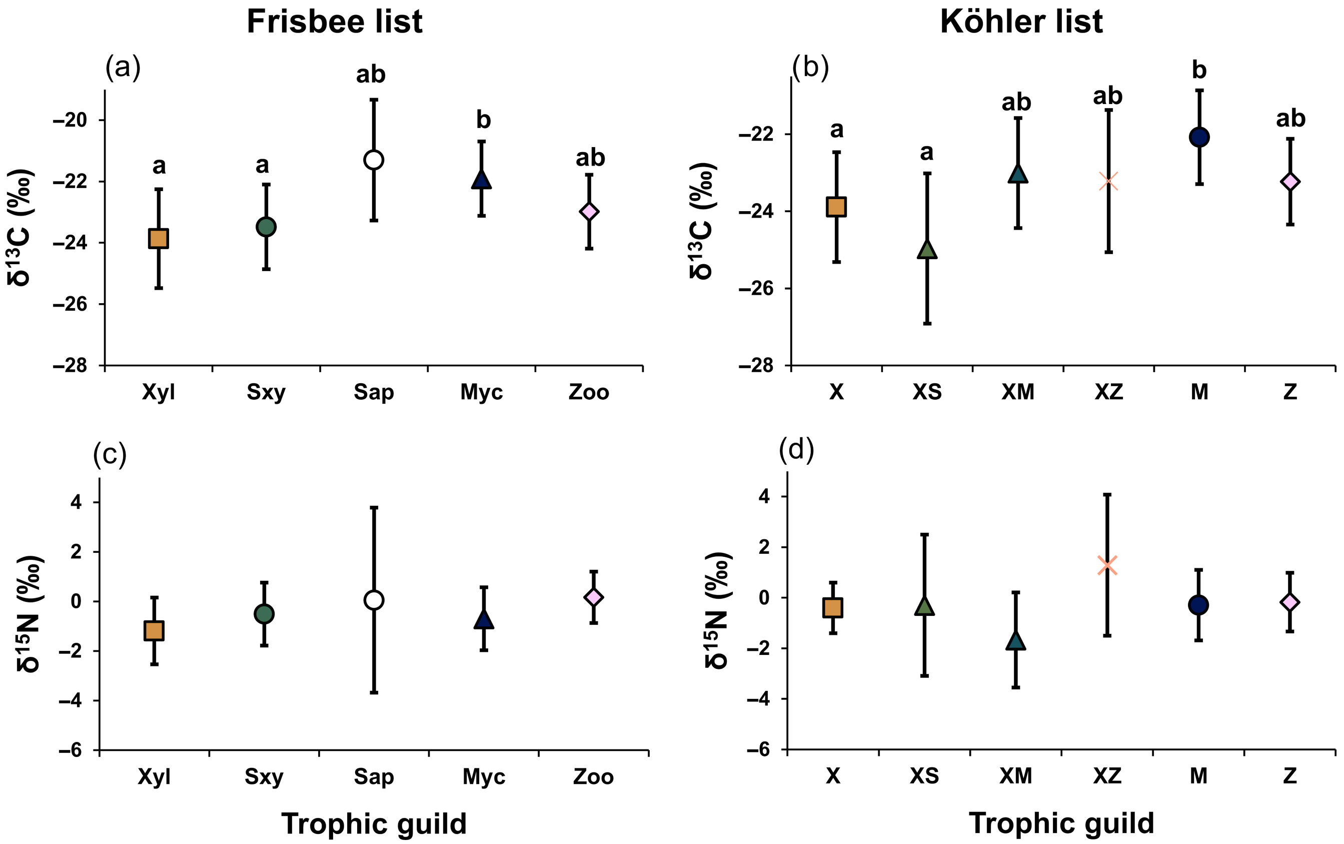

FIGURE 6 Comparison of $\delta^{13}C$ and $\delta^{15}N$ values among the trophic guilds based on Frisbee (a, c) and Köhler (b, d) classification lists. Mean values and 95% CIs (error bars) are displayed, letters above the bars represent significant differences among the guilds according to Tukey honestly significant difference post hoc test. No significant differences were found for values of $\delta^{15}N$ (c, d). The abbreviations of the guilds follow the classifications. Frisbee list (Bouget et al., 2019): Myc, xylomycetophages (dark blue triangles); Sap, saprophages (white dots); Sxy, saproxylophages (green dots); Xyl, xylophages (yellow square); Zoo, zoophages (pink diamonds). Köhler list (Köhler, 2000): M, mycetophages (dark blue dots); X, xylophages (yellow squares); XM, xylomycetophages (blue triangles); XS, mixed xylo- and saprophages (green triangles); XZ, mixed xylo- and zoophages (salmon crosses); Z, zoophages (pink diamonds).

and that of their potential food sources along the deadwood decomposition gradient. Only the species classified as mycetophages displayed an isotopic composition that was consistent with that of their putative food source and could be relatively well distinguished from species belonging to other trophic guilds. Other saproxylic guilds shared isotopic niche space to a great extent and could not be well discriminated based on their C and N stable isotope composition. Both isotopic niche location and important intra-guild variation suggest that most species may considerably depend on fungi or that mixed trophic strategies may be more common than currently acknowledged. Further, overwhelming variation both within and across trophic guilds, particularly concerning $\delta^{15}N$, suggests potential uncoupling of C and N assimilation pathways in saproxylic beetles that is currently largely unaccounted for. Variation in N isotope ratios may be linked to various N assimilation pathways in wood-decomposing fungi and bacteria, reinforcing the hypothesis that their importance as dietary sources of N for saproxylic beetles may currently be well underestimated. Body size and phylogenetic relationships were important factors of isotopic variability among saproxylic beetles, which might potentially make them more useful to predict trophic ecology than trophic guild classifications. However, a larger sample including several individuals of each species would be needed to confirm this pattern.

Primary consumers versus predators among non-saproxylic and saproxylic beetles

$\delta^{15}N$ variation between primary consumers and predators followed the expected general rule of fractionation (^{15}N enrichment with trophic level) in both non-saproxylic and saproxylic beetles, but the variation was too high to

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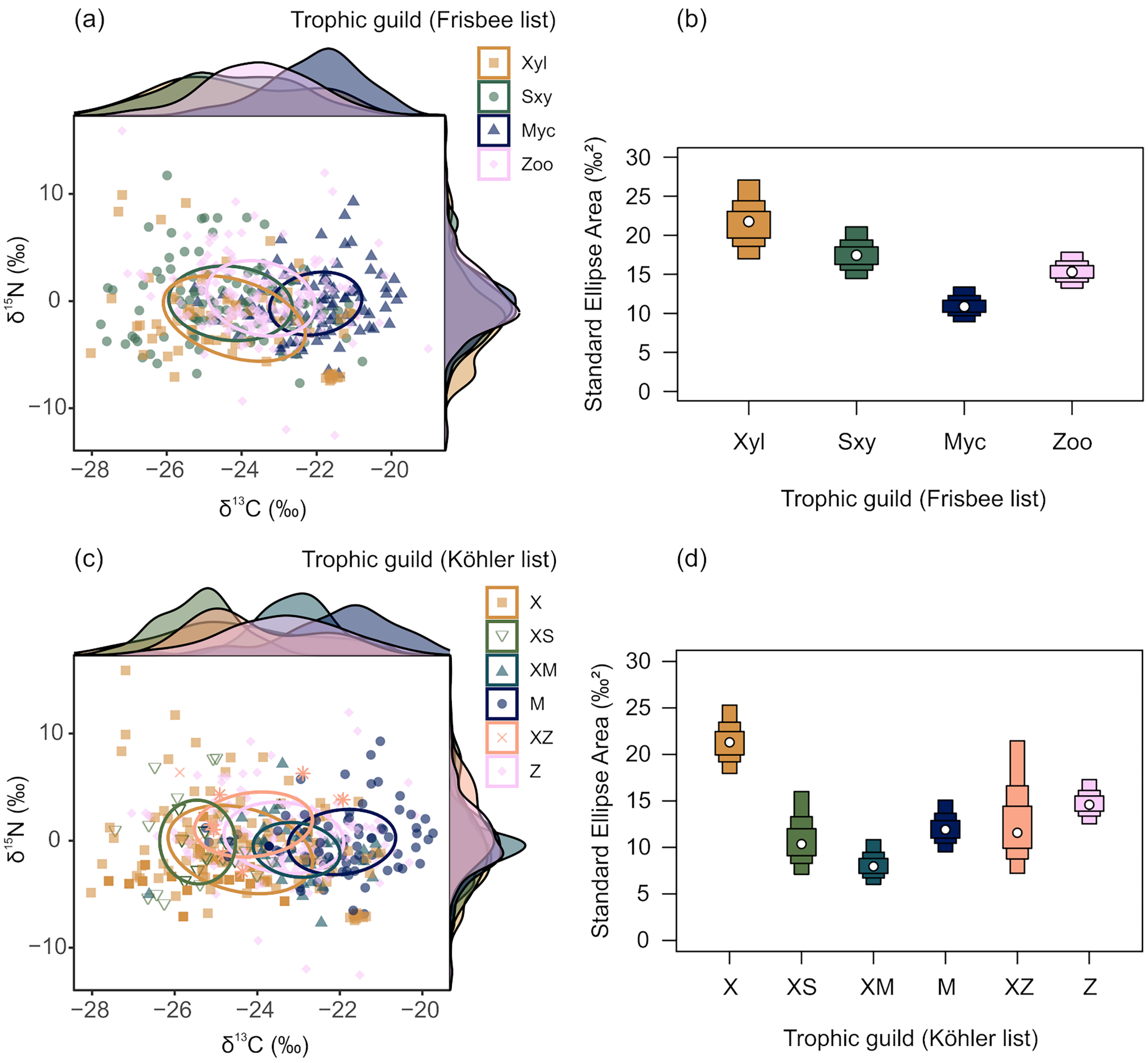

FIGURE 7 Isotopic niches of the different trophic guilds of saproxylic beetles, classified according to the Frisbee list (a, b) and Köhler list (c, d). Standard ellipses (proxies for core trophic niches) are presented for each trophic guild of the Frisbee and Köhler lists in panels a and c, respectively. Mode, 50%, 75%, and 95% credible intervals of $\mathrm{SEA_B}$ (Standard ellipses area estimated using Bayesian inference) of trophic guilds are presented in panels b and d for Frisbee and Köhler lists, respectively. Frisbee list (Bouget et al., 2019), Myc, xylomycetophages (dark blue triangles); Sxy, saproxylophages (green dots); Xyl, xylophages (yellow square); Zoo, zoophages (pink diamonds), Köhler list (Köhler, 2000), M, mycetophages (dark blue dots); X, xylophages (yellow squares); XM, xylomycetophages (blue triangles); XS, mixed xylo- and saprophages (green hollow triangles); XZ, mixed xylo- and zoophages (salmon stars); Z, zoophages (pink diamonds).

allow differentiation between a priori primary consumers and predators in both systems (Figure 2). In addition, while it likely fell within the most generally observed ~2‰–3‰ difference in non-saproxylic beetles (McCutchan et al., 2003), the average difference was lower than expected (~1‰) in saproxylic beetles, hinting at a potentially higher degree of omnivory (i.e., mixed diet) in saproxylic compared with non-saproxylic predators, or the misclassification of some species as predators in current classifications. Alternatively, the low difference between primary consumers and predators in the saproxylic system may be caused by the activity of microorganisms involved in early deadwood decomposition, meaning that this resource may actually already be relatively enriched in ^{15}N. As there is an enrichment in ^{15}N along both microbial and animal food chains (Steffan et al., 2015), the

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distinction between saproxylic predators and primary consumers may be further impeded. Non-saproxylic beetle guilds had lower $\delta^{13}C$ values than their analogues among saproxylic beetles; that is, phytophages had lower values than xylophages, and non-saproxylic zoophages had lower values than saproxylic zoophages. This is consistent with the fact that wood generally has higher $\delta^{13}C$ values than leaves (Cullen & MacFarlane, 2005; Gessler et al., 2014; Hope Jahren, 2004; Tedersoo et al., 2012) and confirms that saproxylic organisms (belonging to the brown chain; i.e., dead-tissue-based) can be differentiated from the green chain (living autotrophic tissue-based) using bulk stable isotope analysis of C and N.

Phylogenetic signal and body size effect on isotopic composition

When exploring the potential roles of intrinsic factors of trophic niche variation (phylogeny and body size), we found a significant phylogenetic signal in $\delta^{13}C$ variation across beetle species, but not in $\delta^{15}N$. Studies examining the effect of phylogeny on stable isotopes of terrestrial organisms are scarce, and mainly focused on fungi (Tedersoo et al., 2012) or vertebrates (Grundler et al., 2017; but see Hellemans et al., 2022). Instead, taxonomy is often used as a proxy of phylogeny. Such studies showed that the more related the species, the more similar their isotopic composition, because related taxa tend to share biological traits inherited from common ancestors (Bourguignon et al., 2011; Potapov et al., 2016). This pattern can also depend on the taxonomic level considered and on the analyzed elements (Potapov, Scheu, et al., 2019). In some cases, the phylogenetic effect has been shown to override that of environmental factors on the isotopic composition (and hence trophic ecology) of consumers (Allgeier et al., 2021). In our case, despite a significant global effect on $\delta^{13}C$ values, large variation still remained within some families, and further effects of body size and trophic guilds were revealed. For example, $\delta^{13}C$ values tended to be similar within some beetle families (e.g., Lucanidae, Staphylinidae, and Mycetophagidae) or subfamilies (e.g., Cerambycinae and Lamiinae in the Cerambycidae family). This is in line with feeding habits of the species of these families, which are usually very similar. For example, all studied members of the Mycetophagidae family feed on saproxylic fungi (Hůrka, 2017), which appears to be validated by their consistently high $\delta^{13}C$ values in our results, matching those of fungi. Similarly, species of the Lucanidae family feed on highly decomposed deadwood (Hůrka, 2017), which appears to be validated by their consistently low $\delta^{13}C$ values in our results. On the other hand, other families showed greater variability in $\delta^{13}C$ values among species (e.g., Tenebrionidae, Elateridae, or Cerambycidae as a whole). The feeding habits of species within these families may differ greatly. For example, within the Tenebrionidae family, species of the genus *Corticeus* are considered zoophages, species of the genera *Neomida* and *Platydema* are considered mycetophages, and species of the genera *Mycetochara* and *Prionychus* feed on rotting wood infested with molds and saproxylic fungi (Novák, 2014), all concordant with their $\delta^{13}C$ values.

We found a global significant effect of body size on $\delta^{13}C$: the smaller the saproxylic species, the greater their values of $\delta^{13}C$, but no global effect on $\delta^{15}N$ values. This result challenges the expectation that increased body size may be linked to higher trophic level (higher $\delta^{15}N$ values), and therefore also increasing $\delta^{13}C$, since there is usually a small fractionation in the C isotope ratio with trophic level (McCutchan et al., 2003). However, this expectation may be especially suited in situations when consumers are organized along a single trophic chain where increased body size may provide a competitive or predatory advantage (Brooks & Dodson, 1965). For example, in insects, $\delta^{15}N$ increase with body size has been previously shown in necrophagous beetles (Ikeda et al., 2006). In complex food webs, changes in body size along ontogeny or phylogeny can also promote access to underused or inaccessible resources from other trophic chains, sometimes resulting in the opposite pattern; that is, in some amphibians, smaller body sizes are associated with increased $\delta^{13}C$ and $\delta^{15}N$ values, and larger body sizes to more negative $\delta^{13}C$ and $\delta^{15}N$ values (Lejeune et al., 2021). In saproxylic beetles, decreasing $\delta^{13}C$ values with increasing body sizes may indicate that larger species tend to rely more on fresh deadwood which is more depleted in ^{13}C, while smaller species tend to rely more on fungi as a source of C. This is consistent with the findings of Birkemoe et al. (2018), who noted that the species feeding directly on deadwood are much larger (mean body length of 8.3 mm) than the mycetophages (mean 2.4 mm), whereas the species feeding on a mixture of wood and fungi have intermediate lengths (mean 5.3 mm). This pattern appears similar to that found in mammals, in which larger species can tolerate a lower quality diet than smaller species thanks to the fact that gut capacity remains a constant fraction of the body size, and the specific metabolic rate decreases with increasing body mass (Müller et al., 2013; Owen-Smith, 1988). In saproxylic beetles, a longer and more structured gut may enable hosting more fungi and bacteria able to feed on food sources that have a high carbon but low nitrogen content (like fresh deadwood) (Ceja-Navarro et al., 2019). Saproxylic beetles indeed harbor more diverse bacterial communities than other insects (Colman et al., 2012),

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emphasizing their importance for a wood-based diet. Larger beetles might therefore be getting nitrogen from their gut microbiota, instead of the deadwood as such (Falqueto et al., 2022). The absence of effect on δ^{15}N, together with its wide variation, again supports the hypothesis that saproxylic beetles, even those classified as xylophagous, may not derive N directly from wood, but more likely from other decomposers (e.g., fungi, bacteria) (Filipiak & Weiner, 2014).

Little correspondence between trait-based derived trophic guilds and isotopic composition of saproxylic beetles

The analysis of trophic guilds established on the basis of ecomorphological traits via regression models and isotopic niche modeling revealed that only pure mycetophages (Myc and M categories in the Frisbee and Köhler list, respectively) can be distinguished from the other guilds on the basis of their C and N isotope composition. Our results suggest that the species with δ^{13}C values higher than −23‰ are primarily those associated exclusively with fungi (see Appendix S1: Figure S1). However, isotopic niches of the other defined trophic guilds overlapped considerably with each other, making it impossible to distinguish among them. Together with important δ^{15}N variation across all guilds (Figure 7), stable isotope analysis suggests a generally more complex trophic ecology than anticipated, likely involving uncoupling of C and N assimilations with N potentially obtained through mixed mycetophagy or microbiota interaction (Filipiak & Weiner, 2014; Mooshammer et al., 2014; Six & Elser, 2020) and possibly opportunistic predatory behavior (Whitehead, 2003). Hereinafter, we discuss mismatches between trophic guild classification and stable isotope analysis with respect to (1) the principal role of fungi in the saproxylic network, (2) omnivory and opportunistic zoophagy of saproxylic beetles, and (3) misclassification of species to guilds.

The principal role of fungi in the saproxylic network

Surprisingly, the xylophages, that is, the primary consumers feeding on fresh deadwood, did not differ in isotopic composition from the saproxylophages or xylomycetophages, who consume rotten wood infested with fungi. All three occupied a position in the isospace likely reflecting mixed mycetophagy based on the correspondence with the gradient of food sources. This suggests a considerable importance of fungi or other microbial organisms (bacteria, protozoa) even for specialists of early-stage wood decomposition (e.g., Buprestidae, Cerambycidae, Scolytinae). Across potential food sources, δ^{13}C values globally increased and C:N ratios globally decreased from fresh deadwood to fruiting bodies of fungi (Figure 6), although it must be noted that in our analysis, C:N ratio of fresh and decayed deadwood was similar. C:N was further negatively correlated with δ^{13}C within deadwood and inner bark samples, which may be a marker of increased decomposition, as being increasingly interwoven with fungi is expected to decrease the C:N ratio of the source material (Zhang & Elser, 2017). Fresh deadwood is a highly suboptimal food resource for heterotrophs because of its poor digestibility and nutrient deficiency, causing a mismatch between elemental composition of the food resource and the requirements of the consumer (Filipiak, 2018). However, it has been found that this mismatch rapidly changes over 3–4 years of development in some xylophagous species (Filipiak & Weiner, 2017), probably due to the increased activity of fungi which enrich the resource in limiting elements like nitrogen, phosphorus, or potassium (Filipiak et al., 2016; Six & Elser, 2020). Even pure xylophages have long been suspected to depend upon nutrients provided by fungi during their development because without them, development would take disproportionally longer than observed (estimated to 10–20 times longer) (Filipiak & Weiner, 2014; Harvey & Gange, 2003). This is further supported by the fact that beetles, in general, do not possess enzymes to break down cellulose and lignin, the main components of fresh deadwood that should provide them with carbon, hydrogen, and oxygen (but see also studies where plant-degrading enzyme gene families were found in some xylophagous beetle genomes; Birkemoe et al., 2018; Keeling et al., 2013; McKenna et al., 2016). The cellulose and lignin breakdown is suspected to be mostly provided by the enzymatic activity of fungi and microbes (Biedermann & Vega, 2020; Vega & Dowd, 2005), either directly in the infested wood or in the beetles' gut by gut symbionts (Falqueto et al., 2022). The particular links between saproxylic beetles and wood-decomposing fungi are still largely understudied (Stokland et al., 2012; Ulyshen, 2018). But evidently, some saproxylic beetles, such as bark beetles (Scolytinae), pinhole borers (Platypodinae) and other ambrosia beetles, ship-timber beetles (Lymexylidae), or stag beetles (Lucanidae) transfer spores of wood-decomposing fungi and bacteria to their feeding sites purposely, sometimes using specialized morphological structures like mycetangia/mycangia (Birkemoe et al., 2018; Cognato et al., 2011; Harrington, 2005; Jacobsen et al., 2017; Malloch & Blackwell, 1993; Mayers et al., 2022; Tanahashi et al., 2010). In addition, fungal and

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microbial symbionts may be potentially transmitted to the very fresh wood by ovipositors of females when laying eggs (Tanahashi & Hawes, 2016), a pattern also commonly known in wood-boring sawflies (Hymenoptera: Siricidae and Xiphydriidae) (Kukor & Martin, 1983; Thompson et al., 2014). The fact that arthropods may enhance their nutritional intake through the consumption of fungi can be assumed also from studies on other taxa. Enrichment of diet may be common even in the non-saproxylic trophic chain. For instance, caterpillars of the gypsy moth (*Lymantria dispar*) feeding on foliage of black poplar preferred leaves infected with the rust fungus (Eberl et al., 2020). In the decomposer food webs of beech and spruce forest floor, soil microarthropods (Collembola and oribatid mites) in litter were found to be largely dependent on saprotrophic fungi as a main resource of their diet rather than on plant litter itself (Pollierer & Scheu, 2021). The interaction with fungi may, however, be different among study systems. The diet of collembolans from arctic peatlands was found to contain only a low share of fungi in comparison with plant matter, and a partial role of symbiotic bacteria has been revealed (Larsen et al., 2016). In conclusion, the classification of many species exploiting fresh deadwood as xylophages may only relate to microhabitat preference, while omitting their very tight relationship with fungi and potential nutrient assimilation through mycetophagy. Their very similar isotopic composition to beetles usually associated with later stages of wood decomposition supports this hypothesis. On the other hand, an unexpected nutrient deficiency in decayed wood compared with fresh deadwood might also potentially explain the observed pattern. It has been hypothesized that the activity of fungi brought into the fresh deadwood by bark or ambrosia beetles in the initial phases of wood decomposition may lead to a translocation of N and other trace elements to fruiting bodies of fungi (which are then consumed by these beetles) (Lehenberger et al., 2021; Skelton et al., 2020). This may cause an unexpected deficiency in these elements in the deadwood itself after a few years despite the fact that the deadwood is getting increasingly interwoven with fungal mycelia, which could explain previous reports that decayed wood may have high C and N isotopic variability (e.g., Tuo et al., 2024), sometimes resulting in lower stable isotope values than fresh deadwood. Ultimately, such nutrient deficiency might introduce heterogeneity in the stable isotope composition of decaying wood which would be transferred to later arriving saproxylophagous species and could potentially contribute to limiting the ability to differentiate between xylophages and saproxylophages or xylomycetophages.

Omnivory and opportunistic zoophagy

Another factor potentially affecting the large overlap among most guilds' isotopic niches may be a large degree of omnivory and opportunistic feeding behavior. Niche modeling may fail when investigating communities with large proportions of omnivores (Jabot et al., 2017). Indeed, the true diet requirements of most saproxylic beetles, including many of the flagship species, are largely unknown. For instance, the larvae of *Limoniscus violaceus* (Elateridae), a specialist of basal tree hollows, are considered omnivorous and facultative carnivorous by some authors (Whitehead, 2003), while others consider the species to be sapronecrophagous, that is, depending on wood mold and carrion (Brustel, 2004). However, the beetle probably only needs organically rich wood mold while the presence of dead animal remains is not indispensable (Gouix et al., 2012). Another model species, *Cucujus cinnaberinus* (Cucujidae), a specialist of the subcortical zone of dead trees, is traditionally considered a scavenger or a facultative, alternatively obligate predator (Bílý, 1990; Bonacci et al., 2020; Hůrka, 2017), which is supported by the fact that its larvae can be reared on a purely animal diet (Bonacci et al., 2020). However, gut content analysis suggests that in nature, the species is rather an opportunistic omnivore, foraging on various types of wood and phloem debris, fungi, and invertebrates (Přikryl et al., 2012). Similarly, longhorn beetles (Cerambycidae) are generally considered xylophages or xylomycetophages, although evidence suggests that they are often facultatively preying on other larvae and are sometimes even cannibalistic (Dodds et al., 2001; Ware & Stephen, 2006). This reinforces the claim that many saproxylic beetles exhibit wide opportunistic feeding strategies, instead of the specialist feeding strategies that are classically described but generally only based on their microhabitat preferences.

Misclassification of species to guilds

Finally, the high degree of overlap among trophic guilds may potentially be caused by incorrect classification of species to predefined trophic guilds because they harbor similar traits. Both classification lists have been created based on the knowledge of expert entomologists who have long experience in the field, and through many detailed observations. There are, however, other literature sources with description of dietary information for particular species of beetles (Sláma, 1998) which might in some cases be in contrast with the classification lists (see Assessment of species to trophic guilds.

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docx in Figshare repository). We observe discrepancies between stable isotope analysis, classification lists, and dietary information for some studied species. In some cases, δ^{13}C composition proved to be in agreement with the guild classifications, but in contrast with the dietary information of the species. For instance, *Platypus cylindrus* (Platypodinae) belongs to ambrosia beetles and is thus supposed to feed on the ambrosia fungus (Leschen & Beutel, 2014). However, δ^{13}C values of this species are very low, which is in line with both classifications considering it a xylophage. By contrast, we observed cases where stable isotope analyses provided results consistent with available dietary information, but inconsistent with classification lists. For instance, δ^{13}C values of longhorn beetles like *Rhagium bifasciatum* or *Leptura maculata* (Cerambycidae: Lepturinae) were relatively high, likely corresponding to fungi influence, which would be in line with dietary information (Sláma, 1998), but both the Frisbee and Köhler lists classify them as xylophages. As a result, the established "trophic" guilds may sometimes represent more the microhabitat use (xylophages are the species associated with fresh deadwood, saproxylophages are associated with decomposed wood, etc.) than the resource the beetles actually feed on in this microhabitat. In this respect, the currently established guilds such as xylophages or saproxylophages are useful when referring to microhabitat use of species but care must be taken when referring to trophic relationships. Therefore, the concepts of trophic guilds should be revised and their information crosschecked. We present a comparison among our results of isotope analysis, dietary information based on literature sources, and Frisbee and Köhler classifications together with comments on potential misclassifications in Assessment of species to trophic guilds.docx in Figshare repository as a basis for reflection on the development of a unifying trophic guild classification.

Based on our stable isotope results we may assume that there is a value of −23‰ for δ^{13}C which can be roughly used as a threshold for discriminating between mycetophages and other trophic guilds (see Appendix S1: Figure S1; also partly visible from Figure 7a,c). This value is, however, applicable probably only to our data, as stable isotope values of beetles from other locations or regions may be influenced by baseline variations. In order to potentially distinguish between xylophages and saproxylophages in terms of their trophic position, further research should focus on a systematic sampling and stable isotope analysis of species from the two respective guilds coupled with an analysis of particular wood substrates they dwell in, that is, fresh deadwood and wood of different decay levels.

Zoophagy versus mycetophagy and the weak explanatory power of δ^{15}N

The published literature claims that high δ^{15}N values indicate a zoophage feeding regime (Heethoff & Scheu, 2016; Hyodo, 2015; Potapov, Tiunov, et al., 2019). For example, invertebrate predators showed higher values of δ^{15}N than saprophages and microphytophages from the soil of beech forest (Scheu & Falca, 2000). Contrary to these findings, our results reveal that the interpretation of the δ^{15}N signal as an indicator of zoophagy in saproxylic beetles would be doubtful. This is likely due to the fact that on average, fungi show elevated δ^{15}N values compared with wood, as they are in essence consumers subject to isotopic fractionation (Steffan et al., 2017). Thus, in saproxylic beetles, the potential zoophagy signal could be confounded with mycetophagy. This is consistent with the view that detritivory globally corresponds to omnivory, in the sense that detritivory may generally imply joint assimilation of bacteria or fungi themselves feeding on detritus; that is, analogue to feeding at multiple trophic levels (Steffan et al., 2017). Another possible confounding factor stems from the fact that the N source of trees mainly comes from the soil, which can show spatial variability in δ^{15}N even at small scale due to variability in local nutrient composition. This potential source of variation was not controlled in this study. However, sampled food sources revealed that δ^{15}N variation was higher in decomposed wood and fungi than in fresh wood, which suggest a potential role of fungi in generating this variation. During decomposition, wood is enriched by fungi mycelia and microbiota which may affect nitrogen isotope composition (Filipiak et al., 2016). Sampling in a wider area, including collection of material from several sites, may allow studying the effect of baseline δ^{15}N variation and the links with wood, fungi, and beetles' δ^{15}N variation with more precision. Rather low differences in the values of δ^{15}N among trophic guilds may be caused by important variability in substrates, and particularly fungi, as mentioned for carbon (see *Omnivory and opportunistic zoophagy*) (Steffan et al., 2015). Following the hypothesis that many saproxylic beetles are more dependent on fungi than generally acknowledged, and because fungi may occupy various positions along the δ^{15}N axis due to important variability in isotope fractionation or N sources, we can speculate that beetles might in some cases take apparently large steps on the δ^{15}N axis because they are feeding on fungi that have particularly high δ^{15}N values.

CONCLUSIONS

This is the first study attempting to reveal the trophic ecology of saproxylic beetles using stable isotope analysis

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and confront it against established trophic guilds. Results suggest that traditionally used trophic guilds (Bouget et al., 2019; Köhler, 2000) are not well supported by niche modeling based on trophic tracers such as $\delta^{13}C$ and $\delta^{15}N$. It seems that trophic guild classifications based on ecomorphological traits do not fit with the isotopic position of many saproxylic beetles on the deadwood decomposition gradient, with stable isotopes only allowing a relatively clear distinction between strict mycetophages and xylophages. Discrepancies between isotopic signal and trophic guild affiliation indicate the need for a better characterization of the trophic ecology of saproxylic species based on actual diet analysis and its inclusion as legitimate traits in classification lists. Further studies, combining experimental approaches using stable isotope analysis and barcoding of microbiota in the digestive tract of beetles (sensu Falqueto et al., 2022), may be necessary to precisely determine the role of fungi (including potential fungal symbionts). This may also be coupled with traditional gut content analysis, which is very rarely performed even on large beetle species (but see Přikryl et al., 2012), alternatively supplemented with information on mandibles' morphology. In the future, isotopic composition could represent a properly established functional trait for saproxylic beetles (Drag et al., 2023), describing their resource diet and its variation, which would be applicable in many functional trait-based studies. This, however, requires compiling a considerably larger dataset comprising hundreds of saproxylic species sampled systematically across several sites and/or larger spatial scales. Sampling of greater numbers of individuals for each species would be beneficial to minimize excessive variability among the specimens. In addition, a thorough and systematic sampling and stable isotope analysis of potential feeding substrates at various study sites, including sampling of local substrates from different tree species and from wood of different decay stages, is necessary in order to build a baseline for future interpretation of saproxylic beetle feeding habits.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Kozel et al., 2025) are available in Figshare at https://doi.org/10.6084/m9.figshare.28172060.v1.

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