1 SMALL MAMMAL INSECTIVORE STABLE CARBON ISOTOPE COMPOSITIONS AS HABITAT
2 PROXIES IN A SOUTH AFRICAN SAVANNA ECOSYSTEM
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29 ABSTRACT

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31 Carbon isotope analysis of fossil micromammal insectivores holds promise for
32 resolving questions about past environments because these animals have restricted home
33 ranges and are generalist feeders, thus their diets likely integrate ecological information
34 about local habitats.

35 In this study, we assessed the degree to which carbon isotope compositions of
36 three sympatric shrew species record spatial and temporal changes in habitat conditions
37 in a mosaic southern African savanna environment. Sampling sites were located within 2
38 km of one another, and microhabitat conditions ranged from very open (< 5% canopy
39 cover) to wooded (~60% canopy cover). We compared shrew hair $\delta^{13}\text{C}$ values between
40 microhabitat types, and across taxa, in order to test whether these data follow predictable
41 patterns based on local vegetation.

42 Shrew carbon isotope values varied with habitat in a predictable manner within
43 our study area. While taxonomy also influenced $\delta^{13}\text{C}$ values, this was largely due to
44 differences in habitat preferences of individual taxa and resultant variation in their
45 relative abundance within each environment. Isotopic differences between habitat types
46 were preserved within individual taxa where taxa occurred in multiple habitats. To
47 complement this modern study, we performed isotopic analysis of the enamel of insect-
48 eating fossil micromammals from the hominin sites Gladysvale and Sterkfontein in the
49 Cradle of Humankind, South Africa. This subset of fossil micromammals consumed
50 primarily C_4 -derived carbon.

1. INTRODUCTION

Understanding the environmental context of hominin evolution is a primary focus of paleoanthropological research (e.g. Dart, 1925; Vrba, 1975, 1985, 1988; Reed, 1997; Potts 1998; 2013; Bobe et al., 2002; Bonnefille et al., 2004; Kingston, 2007; Maslin et al., 2015). Though complicated and not unidirectional, there is strong evidence that during the Plio-Pleistocene, large-scale tectonic and climatic shifts influenced African ecosystems, driving the expansion of open environments at the expense of forested habitats (Vrba, 1975; deMenocal, 1995, 2004; Reed, 1997; Potts, 1998; Trauth et al., 2003, 2005, 2007, 2009; Kingston, 2007; Maslin and Christensen, 2007; Maslin and Trauth, 2009; Reed et al., 2014; Maslin et al., 2015). Against this larger backdrop of continental change, a wide diversity of mosaic habitats emerged that many believe played an important role in hominin evolution (Dominguez-Rodrigo, 2014; Reynolds et al., 2015).

Carbon isotopic analysis of paleosol carbonates is a well-established tool in paleoenvironmental reconstruction (Cerling, 1992, 1999; Cerling and Hay, 1986; Cerling et al., 1989, 1991, 1997, 2011; Levin et al., 2008, 2011; Segalen et al., 2007; Sikes, 1994, 1995; Wynn et al., 2000, 2004). Such analyses are based upon the different photosynthetic pathways used by most trees, shrubs and forbs (C_3 plants) and tropical grasses and sedges (C_4 plants). Plants using C_3 photosynthesis discriminate against ^{13}C resulting in a lower ratio of ^{13}C to ^{12}C in plant tissue relative to that of atmospheric CO_2 . C_4 plants discriminate less against ^{13}C , and therefore exhibit a relatively higher ratio of ^{13}C to ^{12}C . This differential discrimination results in distinct, non-overlapping carbon isotope distributions across the two types of plants (Smith and Epstein, 1971). Carbon isotopes are of particular utility in the savannas of Africa, where the landscape consists of a mix of C_3 and C_4 vegetation, distributed across the two major functional vegetation types in savanna environments (C_3 trees and shrubs; C_4 grasses). The carbon isotopic composition of paleosol carbonates reflects that of vegetation growing in soil during the time of soil formation (Cerling, 1984, 1999; Cerling et al., 1989, 1991; Quade et al., 1989; Cerling and Quade, 1993).

The carbon isotopic composition of foods is incorporated into the tissues (e.g. bone, hair, enamel) of the animals that eat them (DeNiro and Epstein, 1978; Vogel, 1978; Cerling and Harris, 1999). Thus, the ratio of ^{13}C / ^{12}C in body tissues reveals information about the relative contribution of C_3 and C_4 foods to diet. For example, isotopic differences in tissue composition have been used to discriminate grazing (C_4 consuming), browsing (C_3 consuming), and mixed-feeding (mixed C_3 / C_4 consuming) herbivores in both modern and fossil assemblages (Ambrose and DeNiro, 1986, Koch et al., 1994, 1998; Cerling and Harris, 1999; Zazzo et al., 2000; Luyt and Lee-Thorp, 2003; Sponheimer and Lee-Thorp, 2003; Cerling et al., 2003; Kingston and Harrison, 2007; Lee-Thorp et al., 2007; Levin et al., 2008; Bedaso et al., 2010, 2013). Diet, in turn, reveals information about habitat since an animal can only consume what is available in its environment. For example, if C_4 grasses heavily dominate a landscape, and C_3 resources are rare, it is likely that many animals foraging in that environment will consume C_4 foods given that they are the most abundant and readily available resource. Thus, by using carbon isotope analysis to reconstruct the diets of communities of herbivores, one can make inferences about the dominant vegetation on a given landscape.

Carbon isotope analyses of tooth enamel have been widely employed by paleoanthropologists to understand past habitats (Luyt and Lee-Thorp, 2003; Sponheimer and Lee-Thorp, 2003; Kingston and Harrison, 2007; Lee-Thorp et al., 2007; Levin et al., 2008; Bedaso, 2010, 2013). Such studies use a variety of analytical approaches based on both intra-specific comparisons (e.g., within Bovidae) (Sponheimer, 1999; Luyt and Lee-Thorp, 2003; Sponheimer and Lee-Thorp, 2003) and comparisons across multiple taxonomic groups, specific dietary guilds, and whole ecological communities (Zazzo et al., 2000; Kingston and Harrison, 2007; Levin et al., 2008; Sponheimer and Lee-Thorp, 2009; Bedaso et al., 2010, 2013). These studies use $\delta^{13}\text{C}$ values to determine percentages of dietary categories (e.g., C_3 consumers, C_4 consumers) within assemblages (Luyt and Lee-Thorp, 2003; Sponheimer and Lee-Thorp 2003; Lee-Thorp et al., 2007), or use $\delta^{13}\text{C}$ distribution or summary data for a site to compare with other modern or fossil datasets (Kingston and Harrison, 2007; Levin et al., 2008; Sponheimer and Lee-Thorp, 2009), while some incorporate relative abundance data or biomass calculations (Luyt and Lee-

Thorp, 2003; Sponheimer and Lee-Thorp, 2003, 2009; Lee-Thorp et al., 2007; Bedaso et al., 2010, 2013).

In practice, it is usually not difficult to distinguish C₃ forests from environments where C₄ grasses are present (Ambrose and DeNiro, 1986; Cerling et al., 2003; MacFadden and Higgins, 2004). Such environments range from woodland with some grass to treeless grassland, but using carbon isotopes to reconstruct specific habitats within this broad range of environments is complex (Sponheimer and Lee-Thorp, 2009). Such reconstructions are limited by a poor understanding of how carbon isotopes are distributed in modern foodwebs, both at the level of the individual species and at the community level (Gannes et al., 1997; Codron et al., 2007b). For instance, how do we quantify the difference between “wooded grassland” and “grassland” (*sensu* Reed et al., 2013)? What differences should be manifest isotopically in environments with 10%, 25%, or 50% tree cover? These distinctions are important because all African ecosystems associated with fossil hominins contain a mix of C₃ and C₄ vegetation, and are often generalized as mosaics, although the relative abundance of C₃ and C₄ plants as well as the nature and distribution of plant types at many sites are heavily debated (Reynolds et al., 2015). Consensus in the field is that mosaic habitats require better characterization and more detailed study in modern context to evaluate their role in hominin evolution (Reynolds et al., 2015).

So how do we improve our understanding of how carbon isotopes are distributed in modern African ecosystems, and how they reflect local vegetation? Since it is often impractical to sample an entire ecological community, much less incorporate data on the relative abundance of each taxon (especially given taphonomic considerations in fossil assemblages), an ideal approach would be to identify a reliable isotopic ecological “integrator” – an organism whose isotopic signature accurately and consistently reflects the actual proportion of C₃ and C₄ vegetation in its habitat. This could be achieved by looking for taxa that are highly opportunistic, eclectic feeders that shift their diets in response to their ecological setting. Isotopically, most individual species are relatively poor indicators of environmental characteristics. The vast majority of African bovids are C₃ and C₄ specialists and maintain their specialized diets in heterogeneous environments (Sponheimer et al., 2003a; Cerling et al., 2003). Even flexible, generalist herbivores do

not seem to reliably record environmental characteristics isotopically, instead tending to browse or graze preferentially. For instance, both nyala (*Tragelaphus angasii*) and impala (*Aepyceros melampus*) are classified as “mixed-feeders”, yet the former prefers browse and the latter grass (Codron et al., 2007b). Similarly, the diets of mixed-feeding African elephants (*Loxodonta africana*) do not always accurately reflect regional differences in grass biomass, tree density, or canopy cover (Koch et al., 1995; Codron et al., 2006, 2011). Even most rodents, which are widely considered generalist feeders, were not found to change their diets across different habitats within a savanna environment (Codron et al., 2015).

Traditionally, organisms at lower trophic levels, including primary consumers such as herbivores, have been used to make inferences about habitat under the assumption that their direct interaction with vegetation reflects the distribution of C₃ and C₄ resources on a landscape. However, it has been suggested that organisms at higher trophic levels might actually better reflect overall environmental characteristics by acting as ecological integrators, concentrating signals from multiple variable sources and averaging inputs from lower trophic levels through space and time (de Ruiter et al., 2005). Many carnivores are opportunistic dietary generalists, and select their prey primarily on the basis of body size, abundance and availability (Vezina, 1985; Murray et al., 1994; Radloff and du Toit, 2004; Hayward and Kerley, 2008), all variables that are intrinsically linked to habitat. Isotopic sampling of predators increases effective “sample size”, because predators integrate the tissues of multiple herbivore species which themselves consume many different primary producers (Bump et al., 2007).

The argument that predators can serve as isotopic integrators has been tentatively supported by data from fossil carnivores (Lee-Thorp et al., 2000, 2007; Bump et al., 2007; Hopley and Maslin, 2010). The idea has not been well tested in the context of modern isotopic ecology, but some support for its validity does exist. Bump et al. (2007) determined that environmental isotope patterns were better represented by higher trophic levels in both experimental and observational contexts. For example, using carbon isotope data they determined that wolves record historical trends in atmospheric CO₂ more accurately than tree rings. Similarly, data from Codron et al. (2007a) suggests that South African savanna-dwelling carnivores differ in $\delta^{13}\text{C}$ based on feeding behavior and

associated habitat preferences of different prey types. In particular, lions foraging in open grasslands were found to have higher $\delta^{13}\text{C}$ values than lions associated with more wooded habitats.

However, large to medium bodied carnivores are capable of ranging significant distances in search of prey and likely sample from a variety of habitat types (Gittleman and Harvey, 1982). While demonstrably useful in recording large-scale spatial and temporal patterns of carbon isotopes in the palaeontological record (e.g. changes in atmospheric CO_2 levels) (Kohn et al., 2005; Bump et al., 2007; Hopley and Maslin, 2010), they may offer poorer resolution at smaller spatial scales. In a paleoecological context, there is the added complication that predators are somewhat rare in the fossil record relative to other organisms (Brain, 1981; Werdelin and Lewis, 2005; Patterson et al., 2014).

Smaller bodied, insectivorous mammals such as Soricids (shrews) and Macroscelids (sengis or elephant shrews) are ideal potential candidates for carbon isotopic studies. Unlike larger predators, micromammals have restricted home ranges and are therefore closely tied to local habitat (Skinners and Chimimba, 2005). Shrews, in particular, are common elements of almost all African ecosystems and live in a diverse range of habitat types (Churchfield, 1990; Dickman, 1988, 1995; Churchfield et al., 1997a, 2004; Dudu et al., 2005; Skinner and Chimimba, 2005; Happold and Happold, 2013). They feed almost exclusively upon invertebrate prey, eat a wide variety of insect taxa, and are highly opportunistic, switching prey according to availability (Churchfield, 1982a, 1990, 1995; Churchfield et al., 1991, 1997b). Their high metabolic rates necessitate almost constant foraging and they are voracious feeders (Churchfield, 1982a, b, 1990, 2002; Churchfield et al., 1991, 1997b). Churchfield and Brown (1987) estimated that the daily consumption rate of invertebrates by shrews in grassland amounted to 6,800 prey per hectare. In a meta-study of the relationships between predator and prey, Vezina (1985) determined that insectivores generally take a wider range of prey size and type than do larger carnivores. This agrees well with data on the feeding ecology of sympatric African shrews. Churchfield et al. (2004) and Dudu et al. (2005) found no evidence of prey size selection or dietary specialization in multi-species communities of west African forest shrews. Therefore, micromammalian insectivores have the potential to act as

ecological integrators capable of providing environmental resolution at much smaller spatial scales than larger carnivores.

Micromammalian insectivores are rarely the focus of isotopic studies (but see Baugh et al. 2004), however some data do exist for African taxa (Hopley et al., 2006; Dammhann et al., 2013; Symes et al., 2013; Van den Heuvel and Midgley, 2014). Of these studies only Symes et al. (2013) includes data from modern insectivores collected in mixed C₃/C₄ habitats. That study examined resource partitioning in sympatric small mammals (including two shrew species) in an African forest-grassland mosaic and determined that specimens trapped in Afromontane grassland exhibited higher $\delta^{13}\text{C}$ values than those captured in Afromontane forest. In all studies, sample sizes for insectivorous taxa were very small. Given the dearth of available data on the topic, the degree to which the $\delta^{13}\text{C}$ values of insectivorous micromammals reflect habitat, particularly in mosaic savanna environments, remains unclear.

In this study, we attempt to address this gap by assessing the degree to which micromammalian insectivores record habitat signals in a mosaic environment under spatially and seasonally constrained conditions. We sampled three sympatric shrew taxa in three microhabitat types within a southern African savanna (see Codron et al., 2015 for isotopic composition of rodents in the area). Sampling sites were all located within 2 km of one another, but range from relatively open (less than 5% tree cover) to moderately closed (~ 50% tree cover). We compared insectivore $\delta^{13}\text{C}$ values between microhabitat types, and across taxa, in order to test whether these data follow predictable patterns reflecting local vegetation. We expect that insectivorous taxa occurring in more closed habitats will exhibit lower $\delta^{13}\text{C}$ values, reflecting the higher proportion of C₃ resources present in such habitats. Conversely, those occurring in more open habitats are expected to exhibit higher $\delta^{13}\text{C}$ values, reflecting a greater availability of C₄ resources. In addition, we conducted a pilot stable isotope study of fossil micromammals from the hominin-bearing sites Gladysvale and Sterkfontein to explore the feasibility of using such data to investigate past habitats.

2. MATERIALS AND METHODS

2.1 Field Collection Methods

This study was conducted in the Cradle Nature Reserve, located in the Cradle of Humankind World Heritage Site, South Africa (see Figure 1). The reserve is located in the Sterkfontein Valley, approximately 50 km northwest of Johannesburg, Gauteng Province at an elevation of about 1550m. The Sterkfontein Valley is well known for its Plio-Pleistocene aged hominin-bearing fossil cave deposits.

Figure 1: Location of modern and fossil sampling sites in the Cradle of Humankind World Heritage Site, South Africa. Map data: Google, DigitalGlobe.

The reserve lies within the Mesic Highveld Grassland vegetation type of the Southern African Grassland Biome (Acocks, 1988; Mucina and Rutherford, 2006) and includes a mix of high inland plateau grassland and low inland plateau “bushveld” vegetation types. The landscape is a heterogeneous mix of open grasslands, wooded areas, and vleis (marshes). The vegetation is regularly exposed to fires and winter frosts. Annual temperatures range from -12°C to $+39^{\circ}\text{C}$ with a marked wet summer rainfall period (October to March) and a dry winter period (April to September). Mean annual rainfall (1990-2009) is approximately 450mm (Climate Research Unit, University of East Anglia; GPS 26.02 S, 27.88 E).

A significant sampling effort was undertaken within the reserve (12 separate microhabitats sampled, ~ 30,000 total trap nights for the dry season, where one trap night is equivalent to one trap set for a 24 hour period). Shrews were trapped at three of the 12

microhabitats. These sites varied from open grassland to riverine woodland and yielded enough insectivores for hypothesis testing. Pieter's Vlei (PV) represents the most open site and consists of a spring-fed vlei completely surrounded by grassland. Little Marsh (LM) is also a spring-fed vlei, but is located within a mixed woodland environment. Tick River (TR) is a riverine woodland and represents the most closed microhabitat sampled. Characteristics of local vegetation were evaluated using step point line transects at each site. Transects were 250m in length and vegetation was recorded at 5m intervals along each transect. A 1x1m grid was placed on the ground every 5m and percent vegetative cover (including percent overall canopy cover, trees, forbs, grasses, sedges/reeds, and succulents) and ground cover (including proportions of plant cover, bare ground, rock, and detritus) were estimated visually. Additionally all plant species within a 3m radius of each step point were identified (Mentis, 1981) (see Table 1).

Insectivores were trapped with the permissions of the Cradle of Humankind World Heritage Site Management Authority, the Gauteng Provincial Government and the Cradle Nature Reserve during May 2011 in collaboration the National Museum, Bloemfontein, South Africa. Trapping was timed to correspond with the first seasonal frost to optimize trapping success rates following the methods of Avenant (2011). Both ventilated Sherman-type metal box traps and wooden snap traps were used to sample specimens. Fifty of each trap type were used with a 5m spacing between traps along two 250m transects at each site. These 100 traps per site were in place for a 19-day trap period, totaling an early-dry season sampling effort of 5,700 trap nights at the three sites.

Each trap was baited with a mixture of peanut butter, oats, sunflower oil, and a yeast-based spread (Bovril®). Traps were checked, re-baited, and re-set at dawn and dusk each day within the sampling period. Upon capture, species identifications were made and basic biological data, including body mass, were recorded (Avenant, 2011). A small amount of hair was clipped from each specimen for isotopic analyses and these markings were used to ensure that the same individuals were not re-sampled within transects. For specimens sampled using snap traps, species identifications were independently confirmed using cranial morphology after processing and specimens were accessioned to the Bloemfontein National Museum Mammal Collection.

2.2 Stable Isotope Analyses

In total, hair samples from three soricid taxa and 143 individuals were collected for carbon and nitrogen isotope analyses. General ecological information for each taxon (*Crocidura cyanea*, *Crocidura mariquensis*, and *Myosorex varius*) is presented in Table 2. All three taxa are strictly insectivorous, feeding predominantly on terrestrial, soft-bodied invertebrate prey. They differ primarily in body mass, and to a lesser degree in habitat preference, but are sympatric in all habitats sampled in this study (Skinner and Chimimba, 2005; Happold and Happold, 2013).

Hair was first cleaned with ethanol and approximately 1 mg of sample was weighed into Costech 3.5 X 5 mm pressed tin capsules. Samples were analyzed for $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ isotope ratios at the University of California Davis, using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope mass spectrometer. Samples were combusted at 1000° C in a reactor packed with chromium oxide and silvered copper oxide. Following combustion, oxides were removed in a reduction reactor (reduced copper at 650° C), and the resultant CO₂ and N₂ gases were separated using a Carbosieve GC column (65° C, 65mL/min) before entering the isotope ratio mass spectrometer. Stable isotope ratios are presented in delta (δ) notation in parts per thousand (‰) relative to the VPDB (Vienna PeeDee Belemnite) standard for carbon and AIR standard for nitrogen. Analytical precision, based on repeated measurements of laboratory standards (approximately one of each standard for every ten samples), was less than 0.2‰ and 0.3‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. Only data for $\delta^{13}\text{C}$ values are discussed here, but all $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and body mass data are available on Figshare (<http://figshare.com/s/49f14ce0897c11e594a006ec4b8d1f61>)

Data were tested for normality and measures of central tendency are reported for habitats, taxa, and body mass. Median values, IQR and n are listed in parentheses and can also be found in Tables 3 and 4. The effects of habitat, taxon, and body mass on isotopic composition, as well as the interactions between these variables were analyzed using nested ANOVA and 2-way ANOVA (least squares method) in JMP Pro12 statistical software (SAS Institute Inc., 2015). Omega-squared (ω^2) effect sizes were calculated in MS-Excel.

3. RESULTS

Insectivore $\delta^{13}\text{C}$ values vary from a minimum value of -23.6‰ to a maximum value of -11.4‰. $\delta^{13}\text{C}$ values are highest at PV (-14.4‰, -15.7 to -13.4‰, $n=104$), lowest at TR (-20.3‰, -21.4 to -19.9‰, $n=11$), and intermediate at LM (median = -17.9‰, -18.5 to -16.7‰, $n=28$) (Figure 2 and Table 3).

Figure 2: Box plot of insectivore $\delta^{13}\text{C}_{\text{hair}}$ values by site.

Taxa differ in mean body mass. *Crocidura cyanea* is the smallest taxon (mean = $7.2\text{g} \pm \text{SD } 1.0$, $n=13$), *Myosorex varius* (mean = $10.8\text{g} \pm \text{SD } 1.5$, $n=76$) is the largest taxon and *Crocidura mariquensis* is intermediate between the two (mean = $8.7\text{g} \pm \text{SD } 1.4$, $n=49$) and (Figure 3). There is no correlation between $\delta^{13}\text{C}_{\text{hair}}$ and body mass (see Figure 4).

Figure 3: Box plot body mass by taxon.

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332 Taxa also differ in median $\delta^{13}\text{C}$ values although there is overlap between species

(see Figure 4 and Table 4). *Crocidura cyanea* has the lowest median $\delta^{13}\text{C}$ value (-20.3‰,

333 -21.5 to -19.9‰, $n=14$), followed by *Crocidura mariquensis* (-16.0‰, -18.1 to -14.5‰,

334 $n=51$) and *Myosorex varius* (-14.1‰, -15.6 to -13.4‰, $n=77$).

Figure 4: Regressions of $\delta^{13}\text{C}_{\text{hair}}$ values against body mass for each taxon and site. There is no correlation between $\delta^{13}\text{C}_{\text{hair}}$ values and body mass.

A nested ANOVA was used to evaluate the influence of habitat, taxon, and body mass on $\delta^{13}\text{C}$ values. Species were nested within site and body mass was included as a co-variable (Figure 4). The whole-model ANOVA is significant ($F=32.1620$, $p<0.0001$) and effect tests show that site ($F=20.2958$, $p<0.0001$) and species nested within site ($F=8.3416$, $p<0.0001$) are both significant, while body mass has no effect upon $\delta^{13}\text{C}$ values ($F=0.3238$, $p=0.5703$). Post-hoc analyses (Tukey's HSD) imply that PV and LM do not differ and that species do not differ significantly between sites in several instances. However, the lack of difference between sites is driven by small sample sizes for some comparisons. When the underrepresented taxa at each site (five specimens total; two *C. cyanea* each at PV and LM and one *C. mariquensis* at TR; Table 4) were excluded from analyses, post-hoc tests indicate that all sites clearly differ from one another.

Taxa are distributed differently across the three habitats. In equal trapping effort, *C. cyanea* was the only species found in relatively high numbers at TR; low numbers of this species were found at the more open sites PV and LM (see Table 4 for trapping frequencies). *C. mariquensis* and *M. varius* occur in sufficient numbers for comparison at two sites, the more open PV and the intermediately closed LM. To further explore the interaction between habitat and taxon we ran a two-way ANOVA (least squares method) and included interaction terms. Both site ($F= 53.8436$, <0.0001) and species ($F= 10.1518$, $p=0.018$) were found to be significant, however, there was no significant interaction between site and species ($F=1.5600$, $p=0.2140$). (See Figure 5 for Least Squares Means Plot).

Figure 5: Least Squares Means Plot showing the lack of interaction between site and species.

After removing all non-significant interactions, both site ($F=54.8845$, $p<0.0001$) and species ($F=22.2934$, $p<0.0001$) remain highly significant. Effect sizes (ω^2) were calculated to determine the degree to which habitat and taxon influence the variance in $\delta^{13}\text{C}$ values. Habitat accounts for a greater percentage of the observed variance (habitat: $\omega^2= 0.25$; taxon: $\omega^2= 0.10$).

4. DISCUSSION

This study examined the carbon isotope values of three sympatric insectivorous micromammalian species from three different habitats in a South African savanna.

In all comparisons, insectivore $\delta^{13}\text{C}$ values differ in the manner expected (i.e. habitats with a greater proportion of C_4 vegetation have higher $\delta^{13}\text{C}$ values). The relatively high median $\delta^{13}\text{C}$ value characterizing insectivores at PV reflects the high proportion of C_4 plant biomass surrounding the vle. TR, the riverine woodland habitat, has the lowest median $\delta^{13}\text{C}$ value, the greatest degree of tree cover, and the highest proportion of C_3 plant biomass. Insectivores at LM, characterized by mixed woodland, exhibit an intermediate median $\delta^{13}\text{C}$ value.

$\delta^{13}\text{C}$ values also differ taxonomically, but this is at least partly because taxa are differentially distributed across the three habitat types. *Myosorex varius* and *Crocidura mariquensis* occur in roughly equal numbers at PV and LM, but are rare at TR. In contrast, *Crocidura cyanea* is most common at TR and poorly represented or absent at the other two sites. This unequal distribution of taxa across sites is not unexpected given the different microhabitat preferences reported for each species (see Table 2). Regardless, median $\delta^{13}\text{C}$ values *within* individual taxa also differ in the manner expected across habitat types (see Figure 6).

Figure 6: $\delta^{13}\text{C}_{\text{hair}}$ values by species and site showing the directional shift in *C. mariquensis* and *M. varius* $\delta^{13}\text{C}_{\text{hair}}$ values from site to site.

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386 Having established that insectivore $\delta^{13}\text{C}$ values reflect habitat at a general level in
 387 our study area, we can now begin to investigate the degree to which insectivore values
 388 reflect the actual proportions of C_3 and C_4 vegetation in these habitats. For this study four
 389 metrics, percent tree cover, percent canopy cover, percent trees + forbs, and percent
 390 canopy cover + forbs were evaluated as proxies for total C_3 vegetation. Forbs were
 391 included in two of our metrics in an effort to more accurately approximate the total
 392 percentage of C_3 vegetation at a scale appropriate for small fauna. Though the inclusion
 393 of forbs is uncommon in studies focused on larger animals, forbs represent a legitimate
 394 food source for small mammals and insects and may contribute significantly to the
 395 carbon isotope signal (Scholtz and Holm, 1985; Skinners and Chimimba, 2005). To
 396 simulate an idealized micromammalian “ecological integrator”, we used a linear mixing
 397 model with end member values for C_4 and C_3 vegetation of -12.4‰ and -27.0‰
 398 respectively (data subset from Codron et al., 2013). A diet to hair fractionation of $+1.0\text{‰}$

399 for a small mammalian faunivore (see DeNiro and Epstein, 1978; Tieszen et al., 1983;
 400 Sponheimer et al., 2003b; Sare et al., 2005; Fox-Dobbs et al., 2007; Miller et al., 2008)
 401 was then applied resulting in an approximate C₄ endmember of -11.4‰ and C₃
 402 endmember of -26.0‰. We generated regressions using vegetation proxy data (% tree
 403 cover, % canopy cover, % trees + forbs, % canopy cover + forbs) and insectivore mean
 404 $\delta^{13}\text{C}$ values for each transect (Figure 7).

Figure 7: $\delta^{13}\text{C}$ values and percentage C₃ vegetation (where 100% C₃ = 0% C₄). The dashed line that crosses the graph at a diagonal represents an idealized micromammalian “ecological integrator” (Ideal Integrator). A linear mixing model using known regional end member values for C₄ and C₃ vegetation plus a diet to hair fractionation of +1.0‰ was used to generate a regression for an idealized integrator. Regressions were generated for each C₃ vegetation proxy (T=Trees, CC=Canopy Cover, T+F=Trees + Forbs, and CC+F=Canopy Cover + Forbs) using recorded data and insectivore mean $\delta^{13}\text{C}$ values for each habitat. $\delta^{13}\text{C}$ means for each site are indicated on the graph.

Insectivore mean $\delta^{13}\text{C}$ values are, on average, slightly lower than would be predicted if insectivores were acting as perfect ecological integrators given known vegetation data for each transect (see Figure 7). When either tree cover or canopy cover alone are used as proxies, insectivore carbon values slightly overestimate the proportion of C_3 vegetation and underestimate the proportion of C_4 vegetation. These results are not necessarily unexpected given the close proximity of all transects to water (where C_3 grasses often grow (Ellis et al., 1980)), and that shrews tend to forage under dense vegetation where they are concealed from predators and where invertebrate prey are plentiful (Dickman, 1988, 1991; Churchfield, 1990; Skinner and Chimimba, 2005). Intriguingly, canopy cover + forbs and tree cover + forbs yielded vegetation estimates that were much closer to those predicted if insectivores are acting as ideal ecological integrators in each habitat (see Figure 7). It is therefore likely that tree cover and canopy cover alone are somewhat poor indicators of available C_3 resources at the scale relevant to small fauna such as insects and shrews.

Percent tree cover most consistently reflects the magnitude of change in vegetation (% difference) from site to site (e.g. the slope of the tree cover regression is similar to that of our idealized model). However, this measure is furthest from the proportion C_3 predicted by an ideal integrator using *observed* $\delta^{13}\text{C}$ means for each site (see Figure 7). Alternatively, percentage trees + forbs yielded estimates of C_3 vegetation which were closest to those predicted given $\delta^{13}\text{C}$ values for each transect (see Figure 7), but the magnitude of change from site to site was not as consistent (e.g. the slope is dissimilar from that of the model) as it is for trees alone. In any case, $\delta^{13}\text{C}$ values always shift in the direction expected regardless of which proxy is used to estimate C_3 vegetation.

The fact that insectivores (individual species and also the insectivore community as a whole) clearly evince a habitat signal is promising, both for modern ecological studies and for application to the fossil record. Small mammals are plentiful in many fossil deposits and insectivorous taxa occur frequently (Andrews, 1990; Avery, 2001; Reed, 2007).

As part of a related project, tooth enamel from modern and fossil micromammalian insectivores (including members of both Soricidae and

437 Macrosceledidae) from the Cradle of Humankind World Heritage Site, was sampled to
 438 determine its carbon isotope composition using laser ablation (Cerling and Sharp, 1996;
 439 Sharp and Cerling, 1996; Passey and Cerling, 2006). Initial results from Sterkfontein
 440 (~2.4 Ma) and Gladysvaal (~700 Ka) indicate that the fossils have significantly higher
 441 $\delta^{13}\text{C}$ values ($p < 0.0001$) than modern specimens (after adjusting for the fossil fuel effect)
 442 (See Figure 8).

Figure 8: $\delta^{13}\text{C}_{\text{enamel}}$ (VDPB) values from fossil and modern small mammal insectivores. For comparison, values have been adjusted from original laser method values to conventional acid method based upon Passey and Cerling (2006). Fossil values were adjusted by +0.5‰ ($\epsilon^*_{\text{laser-conv}}$). Modern values were adjusted by +0.3‰ ($\epsilon^*_{\text{laser-conv}}$) and +1.5‰ (fossil fuel effect). Fossil $\delta^{13}\text{C}_{\text{enamel}}$ values are notably higher than modern enamel values.

443
 444 This demonstrates a greater contribution of C_4 vegetation to the dietary signal in
 445 the fossil taxa than in modern taxa from the same area. It is important to note, however,
 446 that all fossil specimens analyzed so far belong to Macroscelididae and all modern

specimens belong to Soricidae. Though both taxa are highly insectivorous and present in modern and fossil contexts, they differ somewhat both in their habitat tolerances and dietary behaviors (Skinner and Chimimba, 2005). More data are needed to properly interpret carbon isotope values from fossil insectivores, but this preliminary look at available data from the Plio-Pleistocene of South Africa suggests that further investigation is warranted. We also acknowledge that inferring habitat data from fossil specimens requires close attention to taphonomy and other sources of potential bias, but which are beyond the scope of the present study (Andrews, 1990; Reed et al., 2013).

5. CONCLUSION

The purpose of this study was to determine if the carbon isotope compositions of small mammal insectivores in a southern African savanna vary with habitat in a predictable manner. We determined that insectivore carbon isotope compositions track changes in the percentages of C₃ and C₄ vegetation in the local environment.

Future studies across a broader array of habitats, and which are explicit with regard to seasonality, taxonomy, and relative abundance, are needed to confirm the link between insectivores and environment. If properly developed, insectivore carbon isotope analyses may prove a valuable tool for resolving questions about ancient vegetation composition and structure at a finer scale than is possible with larger fauna. In so doing, it could contribute to our understanding of hominin landscape use and the impact of environmental change on hominin evolution.

468

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470

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Tables

Table 1: Characteristics of the three habitats sampled including vegetation type (in percentages), ground cover, and GPS coordinates for each site.

Transect	Vegetation Type (%)					Ground Cover				GPS Coordinates
	Grasses	Sedges/ Reeds	Forbs	Trees	Canopy Cover	Plant Cover	Bare Ground	Rock	Detritus	
Pieter's Vlei	55	30	10	5	5	90	10	0	0	Lat: 25°55'12" S Lon: 27°52'10" E
Little Marsh	45	20	15	20	30	70	15	15	0	Lat: 25°54'50" S Lon: 27°50'59" E
Tick River	30	5	25	40	55	85	5	0	10	Lat: 25°55'02" S Lon: 27°52'39" E

Table 2: Ecological information for each of the three species (*C. cyanea*, *C. mariquensis*, and *M. varius*) included in this study.

Species	Common Name	Body Mass (g)	Activity Pattern	Locomotor Pattern	Diet	Habitat Preference
<i>Crocidura cyanea</i>	Reddish-grey Musk Shrew	5-11 g	Active intermittently both at night and during the day	Terrestrial	Insectivorous and opportunistic, occasionally consuming vertebrate remains.	Wide tolerance – has been recorded in moist, dense grassy habitats, stream borders, wet vleis, dry bushveld, montane forest, karroid scrub, and fynbos. Often found amongst rocks, can tolerate relatively arid conditions (<500mm rainfall) and high altitudes.
<i>Crocidura mariquensis</i>	Swamp Musk Shrew	4-20 g	Active intermittently both at night and during the day	Terrestrial (Possible semi-aquatic adaptations)	Insectivorous	Occurs only in moist habitats including reed beds, swamps, and thick grass along river banks.
<i>Myosorex varius</i>	Forest Shrew	7-19 g	Predominantly nocturnal, but intermittently active throughout the day especially in midwinter.	Terrestrial	Insectivorous and highly opportunistic. Occasionally cannibalistic and will scavenge rodent carcasses.	Moist, dense grassland, especially on the banks of streams, rocky areas, forests.

Table 3: Stable carbon isotope compositions for each site sampled. $\delta^{13}\text{C}$ values reflect the entire small mammal insectivore community sampled at each site.

Habitat	$\delta^{13}\text{C}$ (‰)		
	Median	IQR	<i>n</i>
<i>Pieter's Vlei</i>	-14.4‰	-15.7 to -13.4‰	104
<i>Little Marsh</i>	-17.9‰	-18.5 to -16.7‰	28
<i>Tick River</i>	-20.3‰	-21.4 to -19.9‰	11

Table 4: Stable carbon isotope compositions of each species and for each species by site.

Taxon	Habitat	n	$\delta^{13}\text{C}$ (‰)	
			Median	IQR
<i>C. cyanea</i>	All	14	-20.3‰	-21.5 to -19.9‰
	Pieter's Vlei	2	--	--
	Little Marsh	2	--	--
	Tick River	10	-20.5‰	-21.5‰ to -20.1‰
<i>C. mariquensis</i>	All	51	-16.0‰	-18.1 to -14.5‰
	Pieter's Vlei	37	-15.5‰	-16.7 to -14.2‰
	Little Marsh	13	-18.1‰	-18.6 to -16.8‰
	Tick River	1	--	--
<i>M. varius</i>	All	77	-14.2‰	-15.6 to -13.4‰
	Pieter's Vlei	64	-13.9‰	-15.0 to -13.2‰
	Little Marsh	13	-17.1‰	-18.1 to -16.5‰
	Tick River	--	--	--