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1 The missing mushrooms: searching for fungi in ancient human dietary analysis

2 Abstract

3 Fungi are a common part of modern human diets, but are rarely discussed in an archaeological
4 context. Power et al. (2015) published data on bolete spores in human tooth calculus, suggesting
5 that Upper Palaeolithic peoples ate mushrooms. Here we briefly consider the likelihood of
6 mushroom consumption in the past, and examine whether or not stable isotopes may provide a way
7 of seeing this in archaeological populations. We also consider the complexities of fungal stable
8 isotopes using our own data and that from the literature. We conclude that fungi are highly variable
9 isotopically, and are an additional dietary factor that should be considered when trying to interpret
10 'terrestrial' carbon isotope signatures combined with relatively high nitrogen isotope values in
11 humans and other animals. Substantial mushroom ingestion could, in some cases, result in isotope
12 values that may be interpreted as considerable meat consumption.

13

14 1. Introduction

15 In April 2015 Power et al. (2015) published a paper on microremains in Palaeolithic human tooth
16 calculus from El Mirón cave, Spain. The press release that accompanied the paper emphasised the
17 finding of bolete mushroom spores, and postulated that Palaeolithic hunter-gatherers could have
18 been eating fungi under the title 'the oldest evidence for mushrooms used as a food source' (Anon,
19 2015). Fungal fruitbodies (sporocarps) are the macro-structure of a fungus that produces the
20 reproductive structures (Spooner and Roberts, 2005), and are here referred to as mushrooms. They
21 are a common food item in many modern human diets, yet they are rarely included when
22 archaeological foodstuffs are being discussed. Here we highlight that mushrooms should be included
23 in such discussions and examine another potential line of evidence for mushroom eating – that of
24 stable isotope analysis of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ from bone collagen in archaeological skeletons. Anomalous
25 bone collagen stable isotope values with apparently terrestrial $\delta^{13}\text{C}$ and relatively high $\delta^{15}\text{N}$ have
26 been reported from a number of sites and species, and we suggest that fungus may play a part in
27 explaining these results.

28 1.1 The potential importance of mushrooms

29 Mushrooms are consumed by modern *Homo sapiens* throughout the world. Different cultures favour
30 different species, and the quantity of mushrooms eaten can vary enormously, e.g. in 2007 estimated
31 consumption of mushrooms in China was 1,226,551 metric tons, while in Belarus it was 6,800 tons

32 (McCarty, 2010), equating to 0.93 kg and 0.71 kg per person respectively (population data from
33 Worldbank.org). The quantity of fresh and processed mushrooms consumed by any single individual
34 will vary according to taste, but in America it has been estimated to be 1.36 kg per person per year
35 (Hoyle, 2014) and in Germany 3.2 kg per person (Lelley, 2014). Mushrooms are proteinaceous, low in
36 fat and ergosterol (the functional equivalent of cholesterol), and contain useful dietary nutrients
37 (McCarty, 2010), such as sulphur (see supplementary information). Ancient texts mention
38 mushrooms (e.g. Theophrastus c.371-c.287 BC (Sharples and Minter, 1983)) and their hallucinogenic
39 and poisonous properties are also widely known from ethnographic studies (Stephenson, 2010). As
40 soft-bodied organisms mushrooms are very rarely found on archaeological sites and those taxa that
41 have been recovered are often woodier and may or may not have been collected to be eaten (e.g.
42 bracket fungi from the Neolithic Italian village of 'La Marmotta' (Bernicchia et al., 2006)). However, a
43 few examples do suggest consumption, in addition to the spores identified as those from bolete and
44 agaric mushrooms by Power et al. (2015). Oetzi the Copper Age 'iceman' from the European Alps
45 was carrying the birch polypore *Piptoporus betulinus* (Peintner and Pöder, 2000), which could have
46 been ingested as a vermifuge (Capasso, 1998). Puffballs *Bovista nigrescens* and *Calvatia utriformis*
47 have been found on UK archaeological sites and may have been used for culinary or medicinal
48 purposes (Watling and Seaward, 1976). These are rare exceptions to the archaeological invisibility of
49 mushrooms and there is little tangible evidence of the edible mushrooms that people are much
50 more likely to have encountered and eaten. In the temperate zone mushrooms are often available
51 from early summer through into the winter, although peak occurrence of fungal fruiting bodies is
52 during the autumn and some animals may become mushroom specialists at this time of year (e.g.
53 Avila et al., 1999) - however the extent of this 'fungi season' is in part controlled by changes in
54 climate, and this season is currently lengthening in Europe (Kausrud et al., 2012). Indeed in Europe
55 some species 'fruit' all year round (such as truffles and many bracket fungi). Mushrooms can yield
56 between 160-250g protein from a dried kg of fruiting bodies (de Román et al., 2006), and dried
57 mushrooms can last for several seasons, potentially extending their dietary impact over a much
58 longer period. The drying of mushrooms is not exclusive to humans, for example several North
59 American squirrel species are known to dry and cache fungi for later consumption (Stephenson,
60 2010). Mushrooms are likely to have been a frequent component in past human diets, but as yet
61 they are not often included in such discussions. Stable isotope analysis provides one way of
62 investigating the role of such invisible foods, although in the case of fungi their potential impact on
63 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values may be highly complex.

64

65 **2.0 Mushrooms and stable isotopes**

66 As mushrooms are highly proteinaceous (e.g. crude protein ranging from 16.5-59.4% dry matter
67 (Kalač, 2009)) they have considerable potential to affect body $\delta^{15}\text{N}$ values in their consumers. Recent
68 work has demonstrated that dietary $\delta^{15}\text{N}$ systems are complex with many possible contributors to
69 the results seen in archaeological material (e.g. Müldner and Richards, 2007; Szpak, 2014). Here we
70 encourage researchers to consider mushrooms as another factor within this complexity. Mushrooms
71 have a wide range of isotope values as illustrated by nearly 1000 stable isotope values for worldwide
72 fungi plotted in Figure 1. This shows that worldwide nitrogen values range from $\delta^{15}\text{N}$ -7.1‰ to
73 +21.8‰ and $\delta^{13}\text{C}$ values range from -31.7‰ to -19.0‰. However, not all species will be present in a
74 single region (although many taxa have a very wide geographic distribution) and more importantly,
75 not all taxa are edible, although only a small minority of mushrooms are really poisonous to humans
76 (Ramsbottom, 1953). Few studies of fungal stable isotopes have been undertaken in Europe, with
77 the exception of work in the Scandinavian forests (e.g. Taylor et al. 1997), in France (e.g. Zeller et al.
78 2007) and on UK waxcaps (*Hygrocybe* spp., Griffith, 2004). Almost no studies, with the exception of
79 the truffle analyses of Zeller et al. (2008), have focussed on taxa that are edible to humans. To
80 illustrate this, Figure 2 plots data for some common European edible mushrooms. These data are
81 from the same sources as Figure 1 but also include our own data from North West England – mainly
82 sampled from Mere Sands Wood nature reserve during October 2013 (see supplementary
83 information for full details of these previously unpublished analyses). Figure 2 demonstrates that
84 there is very wide variation, with $\delta^{15}\text{N}$ values ranging from -1.1‰ to 12.5‰ and $\delta^{13}\text{C}$ from -28.6‰ to
85 -21.1‰. Six species have values $\delta^{15}\text{N}$ >8‰, ceps, wood hedgehog, horse mushroom and the truffles.
86 There are replicate data for several species: notably the chanterelle has a very narrow range of
87 carbon values, but nitrogen values that differ by 7‰ ($\delta^{15}\text{N}$ 0.7‰ to 7.7‰, and $\delta^{13}\text{C}$ from -26.6‰ to -
88 25.2‰ n = 5), while the wood hedgehog has only a 0.6‰ difference in nitrogen, but a 3.2‰
89 difference in carbon values ($\delta^{15}\text{N}$ 8.6‰ to 9.2 ‰ and $\delta^{13}\text{C}$ from -28.6‰ to -24.5‰, n = 3).

90

91 **2.1 Archaeological examples**

92 Typically, the trophic level effect for $\delta^{15}\text{N}$ is expected to be between +3 and +5‰ (Bocherens and
93 Drucker, 2003). The highest $\delta^{15}\text{N}$ values recorded from human bone collagen are typically around
94 the +20‰ range but values over +15‰ are usually interpreted as relatively high and evidence for
95 significant marine mammal intake. Such consumption would also result in relatively high $\delta^{13}\text{C}$ values,
96 but interpretation of diet is more difficult when relatively high $\delta^{15}\text{N}$ values are accompanied by

97 relatively low $\delta^{13}\text{C}$ values. Müldner and Richards (2007) examined a number of reasons for
98 unexpectedly high $\delta^{15}\text{N}$ values (but relatively low $\delta^{13}\text{C}$) in human bone collagen from Roman and
99 Medieval York, concluding that omnivore meat, bird eggs, marine molluscs, freshwater fish and/or
100 manuring could have contributed to this profile. However, mushrooms, a food source that may be
101 ^{15}N enriched but with a 'terrestrial' (i.e. relatively low) $\delta^{13}\text{C}$ signal were not considered, yet Figures 1
102 and 2 demonstrate that mushrooms can also fall into this isotopic range. In addition to humans,
103 individuals of several herbivore taxa such as red deer, *Cervus elaphus* (Stevens et al., 2006) and
104 woolly mammoths, *Mammuthus primigenius* (Fox-Dobbs et al. 2008) have been found to have
105 higher than predicted $\delta^{15}\text{N}$ values when compared to their assumed diet of vegetation, and
106 mushrooms may also have a role here.

107

108 A rare example of fungal stable isotopes being considered in an archaeological context is work by
109 Hamilton et al. (2009) which attempted to model the potential input of mushrooms into pig diets in
110 the Neolithic - but the evidence base for the fungal data was very limited. While the work focussed
111 on the contribution of mushrooms to $\delta^{13}\text{C}$, the model also included $\delta^{15}\text{N}$. This was based on
112 mushrooms being 1‰ to 3‰ higher in $\delta^{15}\text{N}$ than plant foods, which may be realistic if animals do
113 not discriminate between fungal taxa. However humans and other animals will target mushrooms
114 that are palatable, including some taxa that have particularly high $\delta^{15}\text{N}$ (e.g. truffles), and the means
115 of the edible fungi shown in Figure 2 are 7.9‰ for $\delta^{15}\text{N}$ (n=43) and -25.4‰ for $\delta^{13}\text{C}$ (n=43). Later
116 work (Hamilton and Thomas, 2012; Millard et al. 2013) has also focussed on the effect of fungi on
117 $\delta^{13}\text{C}$ values rather than $\delta^{15}\text{N}$ in pigs. Here we emphasise that $\delta^{15}\text{N}$ values may also be influenced by
118 mushrooms, and indeed this may lead to a trophic effect if people are consuming animals such as
119 pigs and deer which eat large quantities of mushrooms at certain times of year (Hohmann and
120 Huckschlag, 2005; Pokorny et al., 2004). Overall, the data shown in Figure 2 suggests that nitrogen
121 isotope values in edible mushrooms vary between those expected of legumes up to those present in
122 freshwater fish (Schoeninger and deNiro, 1984).

123

124 **2.2 Isotopic complexity in Fungi**

125 In parallel to science-based archaeology, there has been a significant increase in the application of
126 stable isotopes within fungal ecology over the last few decades (Griffith, 2004). This has focussed
127 largely, but not exclusively, around the fields of ecosystem ecology and food web studies. Stable
128 isotopes have the potential to quantify nutrient transfers in fungi, but the complex nature of isotope
129 pathways has meant that there are still considerable gaps in knowledge. This isotopic complexity in

130 mushrooms is not surprising given that the fungi are usually considered to comprise an independent
131 Kingdom, with the main edible fungi being found in two different fungal Phyla (Margulis and
132 Chapman, 2009). Part of this complexity may arise because different fungal species can feed at
133 different trophic levels within a food web and this will impact isotope fractionation (Steffan et al.,
134 2015). The majority of work has focussed on nutrient cycling and has examined isotope fractionation
135 in fungal sporocarps in the context of their ecosystem. Natural abundance stable isotope studies
136 utilise the fact that the majority of biogeochemical processes fractionate against the heavy isotopes
137 resulting in measurable differences in stable isotope ratios. Trophic strategies have been examined
138 using carbon and nitrogen isotopes, with apparent differences between saprophytic and mycorrhizal
139 fungi (edible fungi can be found in both groups). Hobbie et al. (2012) demonstrated mycorrhizal
140 fungi to be relatively enriched in ^{15}N but depleted in ^{13}C compared to saprotrophic taxa in the same
141 habitat which they attributed to variations in elemental exchange processes. However, this
142 difference is highly dependent both on the substrate and the species. This makes it complex to
143 separate isotopic effects due to fungal processing from those caused by variations in the substrate.
144 The isotope effects of decomposition, for example, have had comparably less attention and are less
145 well understood as a result (Henn and Chapela, 2000). Even within the same fruiting body there can
146 be appreciable differences ($\pm 2\text{‰}$) in ^{15}N enrichment. For example, Taylor et al. (1997)
147 demonstrated higher $\delta^{15}\text{N}$ values in caps vs. the stem (stipes) in four different taxa including the fly
148 agaric *Amanita muscaria* (Taylor et al., 1997; see also the supplementary data in this study). Handley
149 et al. (1996) also found caps had higher $\delta^{15}\text{N}$ values compared with stems on specimens from
150 Scotland, and they also observed differences in enrichment after rain, in which N values were
151 lowered, but the enrichment of cap vs. stem remained. Isotope values may differ between the same
152 species from the same site, although they may also be very similar across sites. For example, in our
153 data from North West England (see SI) the birch polypore, despite being from two different
154 localities, had very similar values, while the common bonnet results from the same locality differed
155 in $\delta^{15}\text{N}$ by 3.3‰ ($\delta^{13}\text{C} -21.1\text{‰}$, $\delta^{15}\text{N} 3.7\text{‰}$; $\delta^{13}\text{C} -22.8\text{‰}$, $\delta^{15}\text{N} 0.4\text{‰}$). Sulphur isotope values on the
156 same samples range from 3.2‰ to 7.9‰ and appear to have a negative relationship with $\delta^{13}\text{C}$
157 values, suggesting they reflect local habitat substrate conditions (see SI). While these differences will
158 affect the overall isotope composition of a particular fruiting body, they are unlikely to affect the
159 dietary choices of a vertebrate forager. Therefore, understanding the role of mushrooms in human
160 diets will be highly complex, but they should at least be considered, particularly for those sites
161 where groups or individuals appear to have anomalous dietary values. For example, UK waxcap data
162 ($n=112$) illustrate that some edible fungi can be very highly enriched in ^{15}N (mean = 15.4‰) and
163 depleted in ^{13}C (mean = -28.6‰) (Griffith, 2004 and pers. comm).

164 As the cell walls of mushrooms are chitinous, there is a question about the bioavailability of the
165 protein (and therefore the ^{15}N) that they contain. The widely eaten mycoprotein *Fusarium*
166 *venetatum* (the main ingredient in Quorn™) is not a mushroom, but is reported to be higher in
167 digestible protein than beef (www.mycoprotein.org), indicating that at least some types of fungal
168 proteins are digestible by humans. Unrelated studies on rats demonstrated that animals fed purely
169 on mushrooms resulted in little or no weight gain, but that protein was absorbed from the fungi
170 (Longvah and Deosthale, 1998), while stable isotope analyses of small marsupials (bettongs and
171 bandicoots) demonstrated that $\delta^{15}\text{N}$ in faecal samples was derived from the consumption of fungi
172 (McIlwee and Johnson, 1998). A further isotope example is the increase in caesium-137 in both deer
173 and wild boar flesh following Chernobyl, an increase that resulted from the animals consuming fungi
174 that bioaccumulated the radioactive isotopes (Hohmann and Huckschlag, 2005; Avila et al., 1999),
175 clearly showing that nutrients within fungi can be utilized by mammals.

176

177 **3.0 Conclusion**

178 Overall, mushrooms are likely to have formed part of the diet of archaeological populations
179 (especially given the opportunity they provide to be dried and eaten year-round), but as they are
180 rarely preserved on sites they are often overlooked. Stable isotope analysis may provide some
181 insight into their consumption. Perhaps just as important are the possible effects of mushrooms on
182 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values when anomalous results are found in humans and in non-human taxa
183 (especially when those taxa are known fungivores). In cases with high $\delta^{15}\text{N}$ and low (terrestrial) $\delta^{13}\text{C}$
184 values in archaeological populations, we suggest that mushroom consumption should be considered,
185 alongside other more commonly invoked explanations as described by Müldner and Richards (2007).
186 Although stable isotope analysis has been successfully used to identify fungal food sources in some
187 mammals (e.g. McIlwee and Johnson, 1998) the complexity of human diets, combined with the
188 range of fungal isotopic compositions described above, means that it may be unrealistic to expect to
189 find an unambiguous 'fungal signal' in archaeological populations. However, further research on
190 edible taxa is required to help clarify these complexities.

191

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196 sharing their data and giving permission to plot them in Figures 1 and 2.

197

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291

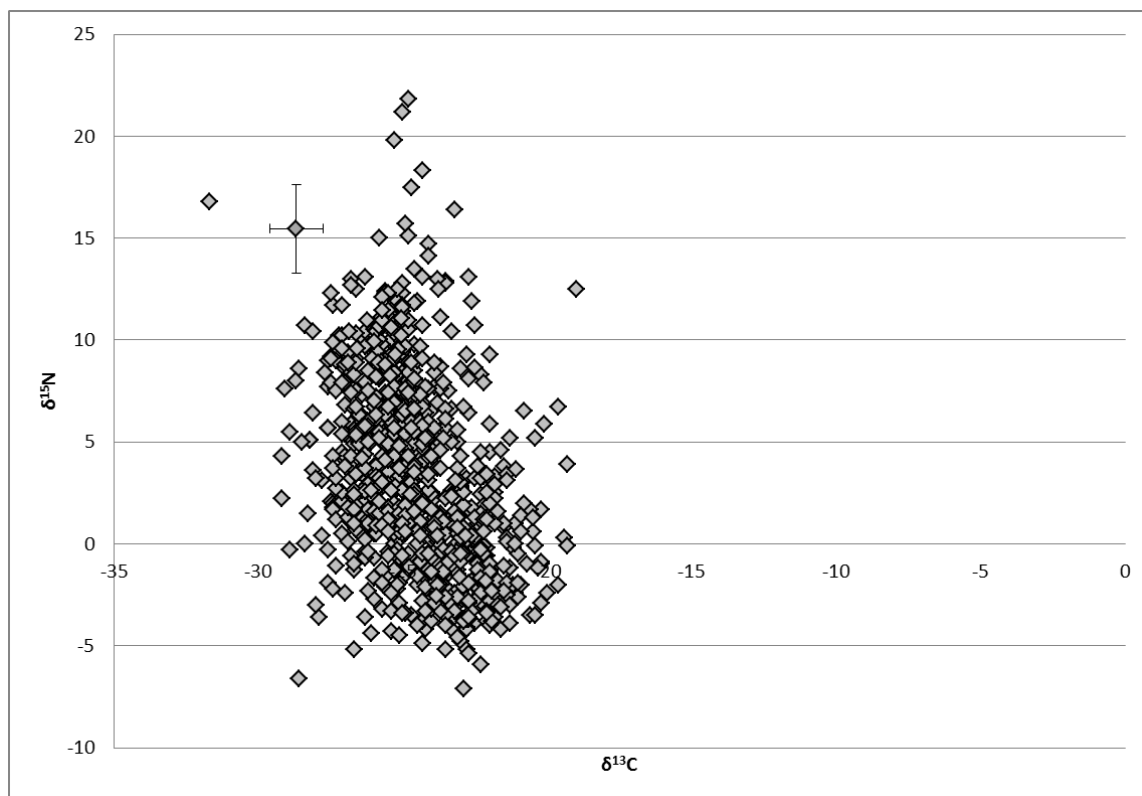
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293

294 **Figure Captions**

295 Fig 1. Published worldwide fungi $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values. Data from Mayor et al. (2009, n = 843), Zeller
296 et al. (2008 and pers. comm., n = 25), our data (n = 11, mean of fly agaric plotted (see supplementary
297 information)), and waxcap summary statistics from Griffiths et al. (2004 and pers. comm, mean + SD,
298 n = 112).

299

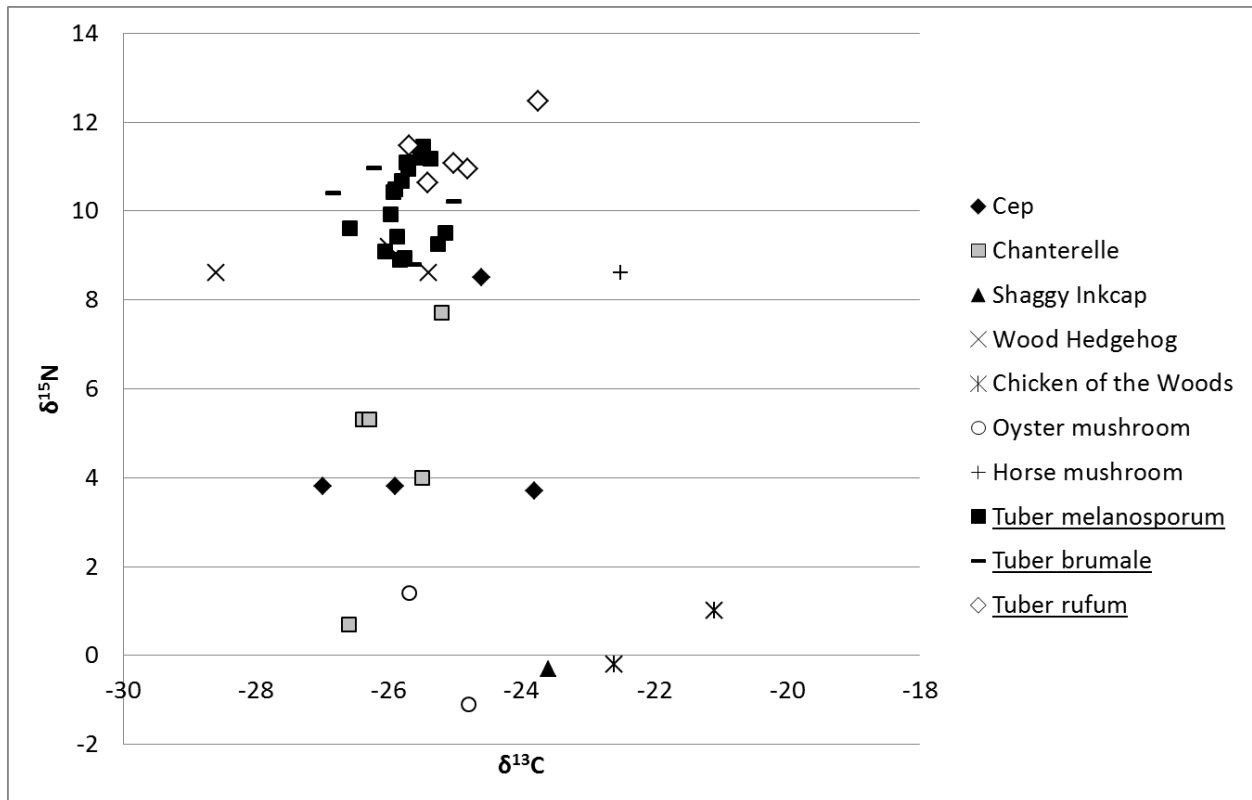


300

301

302 Fig 2. Stable isotope data for commonly edible fungi. Data from Mayor et al. (2009), Zeller et al.
 303 (2008 and pers. comm) and this study (see supplementary information). Scientific names: Cep
 304 *Boletus edulis*, Chanterelle *Cantharellus cibarius*, Shaggy ink cap *Coprinus comatus*, Wood hedgehog
 305 *Hydnum repandum*, Chicken of the woods *Laetiporus sulphurous*, Oyster mushroom *Pleurotus*
 306 *ostreatus*, Horse Mushroom *Agaricus arvensis*.

307
 308



311 **Supplementary Information**

312 Information on sample collection and analysis, plus results of stable isotope analysis for C, N and Sr
313 for fungi from North West England.

314

315 **O'Regan *et al.* Supplementary information: measurements of stable isotope chemistry from fungal**
316 **fruiting bodies collected in North West England.**

317 **Methods**

318 *Samples*

319 Samples were collected from three locations in Northwest England in October 2013. Mere Sands
320 Wood Nature Reserve is in Rufford, Lancashire (53.6355°N, 2.8371°W) and is a series of former sand
321 quarries surrounded by wood and heathland. Hadden Wood, Wirral, Cheshire is a plantation of
322 largely coniferous woodlands (53.2707°N, 3.0246°W), and Willaston Garden, Wirral, Cheshire is a
323 suburban garden with largely deciduous shrubs and trees (53.2922°N, 3.0067°W). Eleven fruiting
324 bodies were collected in total, eight from Mere Sands Wood, two from Hadden Wood and one from
325 Willaston Garden (table 1). In the case of taxa that are difficult to reliably identify on just fruiting
326 body morphology spore colour and microscopic examination of spore size and morphology was used
327 to confirm the identifications.

328 *Drying and analysis*

329 All samples were dried at between 50-100 °C, initially in a domestic fan oven and later a standard
330 drying oven. Work by Taylor *et al.* (1997) demonstrated that there were no significant differences in
331 $\delta^{15}\text{N}$ when samples were dried at temperatures between 40-105°C. Samples were weighed into tin
332 capsules for analysis with additional V_2O_5 as a combustion aid for the sulphur analysis. $\delta^{13}\text{C}$ analyses
333 were performed by combustion in a Costech ECS4010 Elemental Analyser (EA) on-line to a VG
334 TripleTrap (plus secondary cryogenic trap) and Optima dual-inlet mass spectrometer, with $\delta^{13}\text{C}$
335 values calculated to the VPDB scale using a within-run laboratory standard (BROC2) with expected
336 delta values of -27.48‰ (calibrated against CH7, IAEA). Replicate analysis of well-mixed samples
337 indicated a precision of $\pm <0.1\text{‰}$ (1 SD). %C analyses were calibrated against an Acetanilide
338 standard. $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ analyses were performed by Continuous Flow Isotope Ratio Mass
339 Spectrometry (CFIRMS). The instrumentation is comprised of an Elemental analyser (Flash/EA)
340 coupled to a Thermo Finnigan Delta^{plus} XL isotope ratio mass spectrometer via a ConFlo III interface.
341 $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values were calibrated using an in-house reference material BROC-2 with expected

342 delta values of +1.5‰ (calibrated against N-1 and N-2, IAEA) for N and expected delta values of
 343 11.7‰ (calibrated against S-1 and S-2, IAEA) for S. Carbon, nitrogen and sulphur isotope ratios ($\delta^{13}\text{C}$,
 344 $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$) are reported in per mil (‰) relative to VPDB, AIR and VCDT standards respectively.
 345 The 1 σ reproducibility for mass spectrometry controls for these analyses were $\delta^{15}\text{N} = \pm 0.06\text{‰}$, $\delta^{13}\text{C}$
 346 $= \pm 0.10\text{‰}$ and $\delta^{34}\text{S} = \pm 0.20\text{‰}$ respectively.

347 Results

348 The results of the $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ analyses are shown in Table S1. The results demonstrate
 349 considerable variability with $\delta^{15}\text{N}$ ranging from -2.6‰ to 8.6‰, $\delta^{13}\text{C}$ from -26.7‰ to -21.1‰ and
 350 $\delta^{34}\text{S}$ from 3.2‰ to 7.9‰.

351

352 Table S1. Stable isotope data for modern fungi from three localities in North West England.

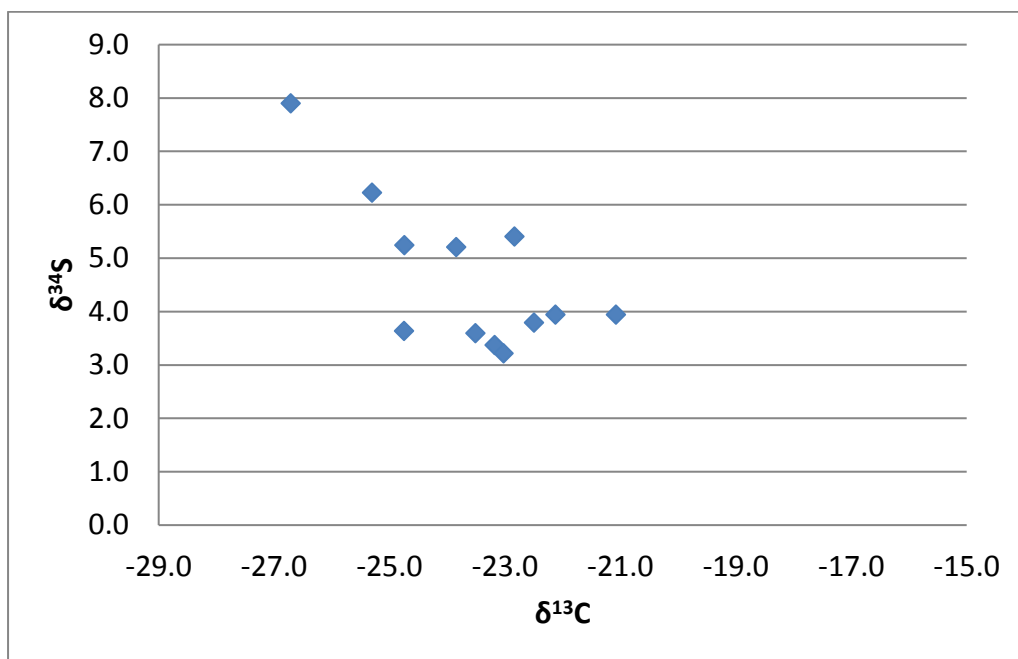
Sample	Common name	Species	location	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{34}\text{S}$
F10	Birch Polypore	<i>Piptoporus betulinus</i>	Mere Sands Wood	-22.1	-1.8	3.9
F11	Birch Polypore	<i>Piptoporus betulinus</i>	Hadden Wood, Wirral	-23.0	-1.6	3.2
F2	Clouded Funnel	<i>Clitocybe nebularis</i>	Mere Sands Wood	-23.8	-2.6	5.2
F4	Common Bonnet	<i>Mycena galericulata</i>	Mere Sands Wood	-21.1	3.7	3.9
F6	Common Bonnet	<i>Mycena galericulata</i>	Mere Sands Wood	-22.8	0.4	5.4
F1	Common Funnel	<i>Clitocybe gibba</i>	Mere Sands Wood	-23.5	-2.6	3.6
F7	Conifer Tuft	<i>Hypholoma capnoides</i>	Hadden Wood, Wirral	-23.2	3.1	3.4
F8	Fly Argaric stem	<i>Amanita muscaria</i>	Mere Sands Wood	-26.7	2.1	7.9
F5	Fly Argaric cap	<i>Amanita muscaria</i>	Mere Sands Wood	-25.3	4.0	6.2
F3	Honey Fungus	<i>Armillaria mellea</i>	Mere Sands Wood	-24.7	2.4	5.2
F12	Horse Mushroom	<i>Agaricus arvensis</i>	Willaston Garden	-22.5	8.6	3.8
F9	Oyster Mushroom	<i>Pleurotus ostreatus</i>	Mere Sands Wood	-24.8	-1.1	3.6

353

354

355 There is an indication of a negative relationship between $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ within the data, albeit non-
356 significant (Spearman's $r = -0.38$, $p=0.23$), which may indicate that sulphur is reflecting local habitat
357 conditions (see Fig. S1).

358



359

360 Figure S1. $\delta^{13}\text{C}$ plotted against $\delta^{34}\text{S}$ for the fungi in Table S1.

361