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

Abstract

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

1. Introduction

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

1.1 The potential importance of mushrooms

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

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

2.0 Mushrooms and stable isotopes

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

2.1 Archaeological examples

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

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

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

2.2 Isotopic complexity in Fungi

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

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

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

3.0 Conclusion

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

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Figure Captions

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

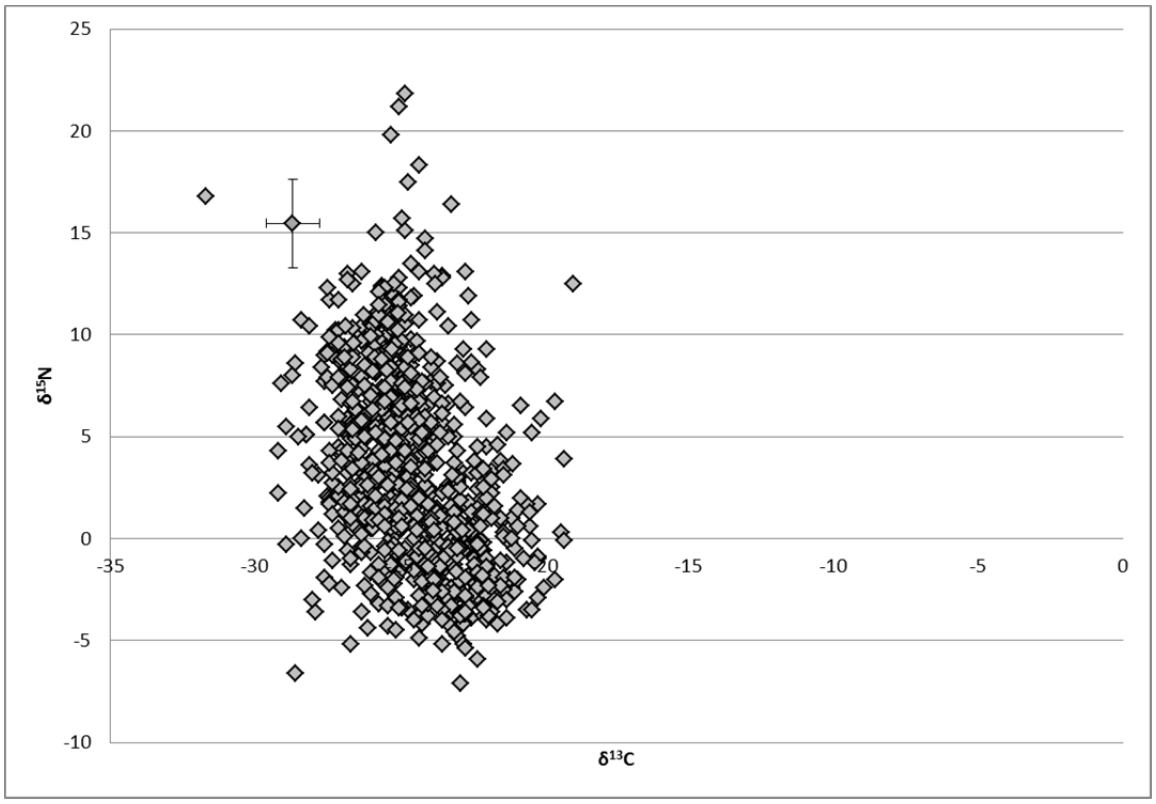
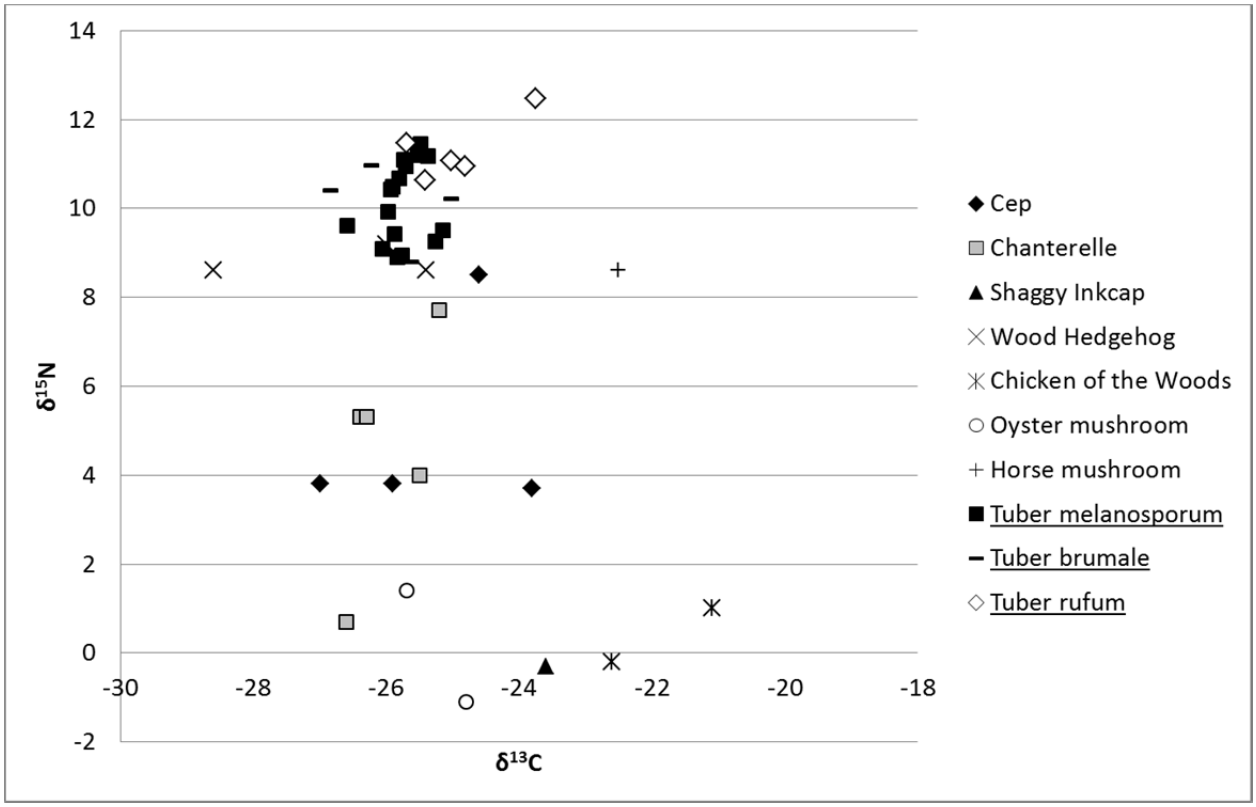


Fig 2. Stable isotope data for commonly edible fungi. Data from Mayor et al. (2009), Zeller et al. (2008 and pers. comm) and this study (see supplementary information). Scientific names: Cep *Boletus edulis,* Chanterelle *Cantharellus cibarius*, Shaggy ink cap *Coprinus comatus,* Wood hedgehog *Hydnum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



Supplementary Information

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

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

Methods

Samples

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

Drying and analysis

All samples were dried at between 50-100 ^{o}C, initially in a domestic fan oven and later a standard drying oven. Work by Taylor et al. (1997) demonstrated that there were no significant differences in δ^{15}N when samples were dried at temperatures between 40-105^{o}C. Samples were weighed into tin capsules for analysis with additional V_2O_5 as a combustion aid for the sulphur analysis. δ^{13}C analyses were performed by combustion in a Costech ECS4010 Elemental Analyser (EA) on-line to a VG TripleTrap (plus secondary cryogenic trap) and Optima dual-inlet mass spectrometer, with δ^{13}C values calculated to the VPDB scale using a within-run laboratory standard (BROC2) with expected delta values of –27.48‰ (calibrated against CH7, IAEA). Replicate analysis of well-mixed samples indicated a precision of $\pm$ <0.1‰ (1 SD). %C analyses were calibrated against an Acetanilide standard. δ^{15}N and δ^{34}S analyses were performed by Continuous Flow Isotope Ratio Mass Spectrometry (CFIRMS). The instrumentation is comprised of an Elemental analyser (Flash/EA) coupled to a Thermo Finnigan Delta^{Plus} XL isotope ratio mass spectrometer via a ConFlo III interface. δ^{15}N and δ^{34}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 ($\delta^{13}C$,
344 $\delta^{15}N$ and $\delta^{34}S$) are reported in per mil (‰) relative to VPDB, AIR and VCDT standards respectively.
345 The 1σ reproducibility for mass spectrometry controls for these analyses were $\delta^{15}N$ = ± 0.06‰, $\delta^{13}C$
346 = ± 0.10‰ and $\delta^{34}S$ = ± 0.20‰ respectively.

347 **Results**

348 The results of the $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ analyses are shown in Table S1. The results demonstrate
349 considerable variability with $\delta^{15}N$ ranging from -2.6‰ to 8.6‰, $\delta^{13}C$ from -26.7‰ to -21.1‰ and
350 $\delta^{34}S$ 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	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
F10	Birch Polypore	*Piptoporus betulinus*	Mere Sands Wood	-22.1	-1.8	3.9
F11	Birch Polypore	*Piptoporus betulinus*	Hadden Wood, Wirral	-23.0	-1.6	3.2
F2	Clouded Funnel	*Clitocybe nebularis*	Mere Sands Wood	-23.8	-2.6	5.2
F4	Common Bonnet	*Mycena galericulata*	Mere Sands Wood	-21.1	3.7	3.9
F6	Common Bonnet	*Mycena galericulata*	Mere Sands Wood	-22.8	0.4	5.4
F1	Common Funnel	*Clitocybe gibba*	Mere Sands Wood	-23.5	-2.6	3.6
F7	Conifer Tuft	*Hypholoma capnoides*	Hadden Wood, Wirral	-23.2	3.1	3.4
F8	Fly Argaric stem	*Amanita muscaria*	Mere Sands Wood	-26.7	2.1	7.9
F5	Fly Argaric cap	*Amanita muscaria*	Mere Sands Wood	-25.3	4.0	6.2
F3	Honey Fungus	*Armillaria mellea*	Mere Sands Wood	-24.7	2.4	5.2
F12	Horse Mushroom	*Agaricus arvensis*	Willaston Garden	-22.5	8.6	3.8
F9	Oyster Mushroom	*Pleurotus osteatus*	Mere Sands Wood	-24.8	-1.1	3.6

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

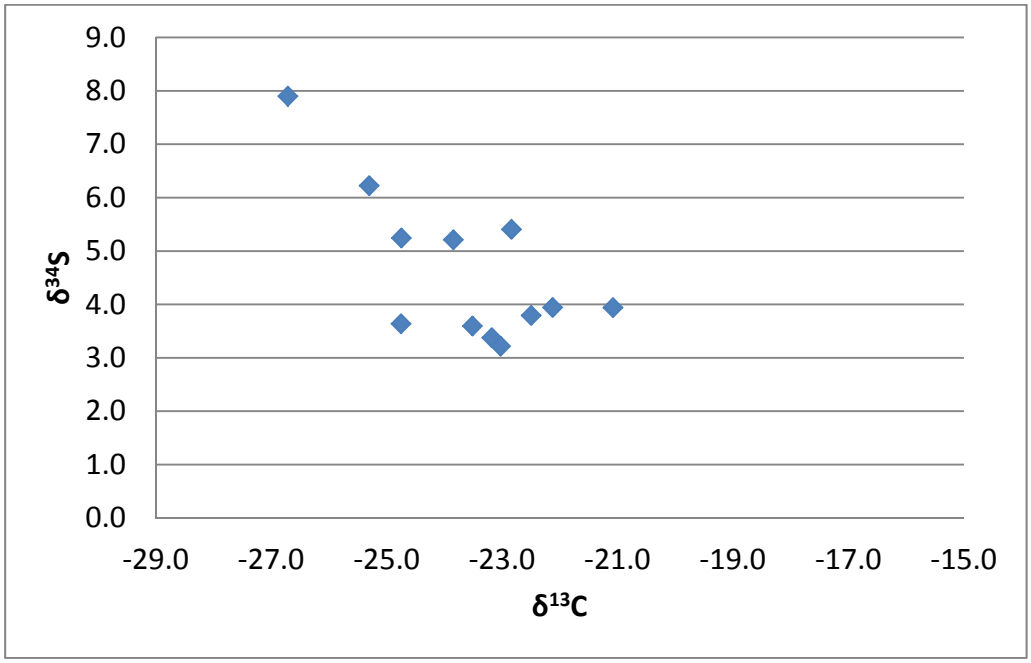

Figure S1. $\delta^{13}C$ plotted against $\delta^{34}S$ for the fungi in Table S1.