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Nitrogen content, amino acid composition and digestibility of fungi from a nutritional perspective in animal mycophagy

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ABSTRACT

Fungi comprise a major part of the diet of many animals. Even so, the nutritional value of fungi has been much debated, with some arguing that fungi are nutritionally poor. However, the chemical composition of fungi and of the biology of the animals that eat them are not well understood, particularly in reference to amino acid (AA) composition of fungi and digestibility of fungal protein. We analysed fibre, total nitrogen (N), available N, and AA contents and measured *in vitro* digestibility of a wide range of epigeous and hypogeous fungi collected in Australia and the USA to test three hypotheses: (i) fungi are nutritionally poor because they contain few nutrients or are otherwise of low digestibility, (ii) fungi vary substantially in their nutritional composition; and (iii) animals can counter this variable quality by eating diverse taxa. Resultant data indicate many fungi are a reasonable source of AAs and digestible nitrogen. However, they vary highly between species in AA content, and the protein has a poor balance of digestible AAs. This helps explain why many mycophagous animals eat a wide array of fungi and often have digestive strategies to cope with fungi, such as foregut fermentation. Another common strategy is to supplement the diet with high quality protein, such as insect protein. Accordingly, evaluating nutritional value of fungi requires consideration of physiology of the animal species and their whole diet.

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Introduction

A tremendous diversity of animals eat fungi, including beetles (e.g., Hochberg *et al.* 2003; Newton 1984; Sutherland & Parrella 2009), ants (Witte & Maschwitz 2008), arthropods (Buller 1909; McGraw *et al.* 2002), reptiles (Cooper & Vernes 2011; Dodd

2002), birds (Simpson 2000) and a wide range of terrestrial and partially arboreal mammals (e.g., Hanson *et al.* 2006; Maser *et al.* 1978; Vernes & Dunn 2009). Likewise, this feeding behaviour is widespread, reported from Europe (Blaschke & Baumler 1989), North America (Maser *et al.* 1978), South America (Hanson *et al.* 2006; Perez Calvo *et al.* 1989), Asia (Hanya

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2004), and Australia (Vernes & Dunn 2009). Mycophagy particularly prevails among mammals and is best documented among flying squirrels, rats, voles and a wide variety of marsupials; often they target sporocarps of hypogeous fungi. Some animals eat mainly fungi throughout the year (Bennett & Baxter 1989; Claridge & May 1994; Maser *et al.* 1978), whereas others rely on them when other foodstuffs are scarce in the environment (Claridge & Trappe 2005; Currah *et al.* 2000). Red squirrels even cache fungi when plentiful for later consumption when adequate food is scarce (Hardy 1949; Vernes & Poirier 2007).

Claridge & Trappe (2005) categorised animals by their relative dependence on fungi as food: (1) obligate, entirely or almost entirely dependent on fungal sporocarps; (2) preferential, prefer fungi over other dietary items but regularly or seasonally eat other types of food as well; (3) casual or opportunistic, occasionally feed on fungi as available and attractive; and (4) accidental, ingest fungal sporocarps or spores accidentally in the course of eating other foods.

Nutritional values of fungi are much debated in the scientific literature, due to a seeming mismatch between high nutrient concentrations but apparent low availability of these nutrients (Claridge & May 1994). This led Cork & Kenagy (1989) to ask, at the end of their paper that showed poor availability of nutrients from fungi, 'Why mycophagy?' Part of the answer came a few years later when Claridge & Cork (1994) showed that a foregut fermenting marsupial, the long-nosed potoroo (*Potorous tridactylus*), digests a high proportion of the nutrients in fungi. This aside, three lines of evidence suggest that fungi are nutritionally valuable. First, in Australian eucalypt forests, 40–80 % of herbivorous and omnivorous animals weighing less than 5 kg have diets with at least 10 % fungi (Johnson 1996). A similar situation prevails among mammals in the coniferous forests of the Pacific Northwest United States (Maser *et al.* 1978). Second, diets of some animals, such as potorine (rat-kangaroos) marsupials (Seebeck *et al.* 1990) are almost all fungi. Finally, flying squirrels, which can ill afford to fill their relatively simple gastrointestinal tracts with diets of poor digestibility, specialise in mycophagy during most times of the year (Currah *et al.* 2000; Flaherty *et al.* 2010; Lehmkuhl *et al.* 2004; Maser *et al.* 1985; Mitchell 2001; Pyare & Longland 2001).

Even so, evidence suggests that relative to a lot of plant material, such as young leaves or seeds, fungi typically contain high concentrations of protein but much lower concentrations of energy. Thus, if the protein leverage hypothesis (Sørensen *et al.* 2008) holds for mammalian fungivores, we might expect them to eat excess protein to meet their essential amino acid (EAA) and energy needs. Alternatively, they may find a rich source of energy-yielding nutrients elsewhere, or mycophagy may involve matching body size with appropriate gut morphology and feeding habits to capitalise on what has been considered a poor source of nutrition (e.g. Cork & Kenagy 1989; D'Alva *et al.* 2007). Digestive strategies differ between mammalian mycophagist species: species with foregut fermentation, such as the Australian potorine and macropodid marsupials (Vernes & Dunn 2009), can improve the nutritional value of ingested proteins through microbial fermentation. In contrast, rodents potentially obtain microbial AAs through coprophagy or caecotrophy (Soave & Brand 1991). Also, small

animals require more nutrients per unit of body mass than do larger animals but require fewer in absolute terms and thus can feed more selectively (Clauss *et al.* 2007).

An additional role of AAs in mycophagy is as precursors of the volatiles by which animals detect the mature fruiting bodies of hypogeous fungi. For example, methionine is a precursor of several sulphur compounds such as dimethyl sulphide and dimethyl disulphide, common to many truffle species and acting as attractants to mycophagists (Gioacchini *et al.* 2005; Karlovsky 2008; Splivallo *et al.* 2010).

To better understand relationships between mammalian fungivores and fungi, we analysed various fungal taxa with emphasis on AAs to test three hypotheses: (i) fungi are nutritionally poor because they contain few nutrients or are otherwise of low digestibility, (ii) fungi vary substantially in their nutritional composition; and (iii) animals can counter this variable quality by eating diverse taxa.

Materials and methods

Source, collection and preparation of fungi

Fungal sporocarps were collected opportunistically in Australia and the USA, often during surveys for hypogeous species which were found by raking (Claridge *et al.* 2000). Collections were mostly annotated and photographed at the end of the day of collection, then dried overnight on a portable electric food drier at 40 °C. To get enough dry biomass for herbarium voucher collections as well as the analyses, most samples required combining several collections; consequently, the data should reasonably represent the taxon. Because hypogeous fungi fruit belowground and thus cannot be detected until exposed by raking, sample sizes differed enormously; this dictated the type and number of analyses possible for each sample (Table 1). For example, we composited samples for *Protoglossum* and *Arcangeliella*, respectively, to measure *in vitro* available nitrogen (AvailN), but this excluded them from the AA score because we had too little material in the same sample to analyse AAs. We partitioned protein before developing the AvailN method, so we have the information from both analyses for some samples and one or no measure for others. For samples with both measures, we have two independent N values from different analyses as described later. Such limitations did not alter our overall aim of collecting as much information as possible on each species with a view to a fresh appraisal of the nutritional value of both epigeous and hypogeous taxa. For comparative purposes we included also two commercial species – dried Shiitake mushrooms (*Lentinula edodes*) and fresh button mushrooms (*Agaricus bisporus*) that we dried, as described above.

Analyses

Dried samples were divided into two replicates, then ground in a twin-blade coffee grinder and stored for later analysis in glass vials in a dark room. Prior to nutritional assay, we measured residual moisture in these samples by drying 1.0000 ± 0.01 g to constant mass at 60 °C, in order to express results as a proportion of dry matter (DM). If enough sample

Table 1 – A checklist of the species and the analyses we did on each. Measuring both the AA (AA) composition and available nitrogen (AvailN) allowed us to calculate an AA score for a sample.

Species	N Partition	Leco N	AvailN	Cell wall	AA	AA score	Ash
Hypogeous							
Arcangeliella					x		
Arcangeliella					x		
Arcangeliella					x		
Arcangeliella					x		
Arcangeliella composite sample		x	x				
Cortinarius globuliformis	x			x	x		x
Cortinarius globuliformis	x	x	x	x	x	x	x
Cortinarius globuliformis	x	x	x	x	x	x	x
Gautieria sp					x		
Gautieria monticola (immature)	x			x	x		x
Gautieria monticola (mature)	x			x	x		x
Gautieria monticola (mature)	x	x	x	x	x	x	x
Gautieria monticola (mature)	x	x	x	x	x	x	x
Gymnomyces sp					x		
Hysterangium coriaceum	x	x	x	x	x	x	
Hysterangium sp	x	x	x	x	x		
Hysterangium sp	x	x	x	x	x	x	
Mesophellia glauca	x			x	x		
Protoglossum sp					x		
Protoglossum sp					x		
Protoglossum sp					x		
Protoglossum sp composite sample		x	x				
Rhizopogon hawkeriae (immature)	x	x	x	x	x	x	
Rhizopogon hawkeriae (mature)	x	x	x	x	x	x	
Rhizopogon villosulus (immature)	x			x	x		
Rhizopogon villosulus (immature)	x	x	x	x	x	x	
Rhizopogon villosulus (immature)	x	x	x	x	x	x	
Rhizopogon villosulus (immature)	x	x	x	x	x	x	
Rhizopogon villosulus (mature)	x	x	x	x	x	x	
Rhizopogon villosulus (mature)	x	x	x	x	x	x	
Rhizopogon vinicolor (mature)	x			x	x		
Setchellogaster sp					x		
Truncocolumella citrina (immature)	x	x	x	x	x	x	
Truncocolumella citrina (mature)	x	x	x	x	x	x	
Epigeous							
Agaricus bisporus		x	x		x		
Agaricus xanthodermus		x	x		x		
Boletus edulis	x	x	x	x	x	x	x
Boletus edulis					x		
Boletus mirabilis	x			x	x		x
Boletus mirabilis					x		
Cantharellus formosus					x		
Cantharellus formosus					x		
Cantharellus formosus	x	x	x	x	x	x	x
Cantharellus formosus	x	x	x	x	x	x	x
Cantharellus formosus	x	x	x	x	x	x	x
Cantharellus subalbidus	x	x	x	x	x	x	x
Cantharellus subalbidus	x			x	x		x
Lentinula edodes		x	x		x		
Morchella elata					x		
Morchella elata					x		
Morchella elata (immature)	x	x	x	x	x		x
Morchella elata	x			x	x		x
Morchella elata	x	x	x	x	x		
Morchella elata	x			x	x		x
Tricholoma magnivelare		x	x		x		
Tricholoma magnivelare					x		
Tricholoma magnivelare					x		
Tricholoma magnivelare					x		
Tricholoma magnivelare	x	x	x	x	x	x	x
Tricholoma magnivelare	x	x	x	x	x	x	x

Table 1 – (continued)

Species	N Partition	Leco N	AvailN	Cell wall	AA	AA score	Ash
<i>Tricholoma magnivelare</i>	x	x	x	x	x	x	x
<i>Tricholoma magnivelare</i>	x	x	x	x	x	x	x
<i>Tricholoma magnivelare</i>	x	x	x	x	x	x	x
<i>Tricholoma magnivelare</i>	x	x	x	x	x	x	x

remained we then determined ash (mineral) content by weighing after ignition at 600 °C for 12 h.

Cell-wall material

Concentrations of cell-wall material (fibre) were extracted with pepsin in citrate buffer at 4 °C for 24 h (Faichney & White 1983) to remove protein and then with neutral detergent solution (Goering & Van Soest 1970), without sodium sulfite, for 3 h at 98 °C in tubes immersed in a boiling water bath to remove remaining cell contents.

Nitrogen

Total N content of fungal samples was determined in two ways. To partition N we used a micro-Kjeldahl digestion followed by distillation and auto-analysis of the resulting ammonia (Williams & Twine 1967). Values for total N from these samples are likely underestimated, because the digestion technique does not measure nitrate-N. Total N in sporocarps was partitioned as follows. (1) Cell-wall N was analysed on the residue after extraction with pepsin and neutral detergent; (2) N from protein and cell wall was analysed by extracting samples with 5 % (m/v) trichloroacetic acid (to remove non-protein N, which, unlike protein, is not precipitated by TCA), centrifugation and removal of the supernatant liquid, and analysis of the residue by micro-Kjeldahl digestion (Faichney & White 1983).

To measure AvailN described later, we used a Leco Truspec Carbon/Nitrogen Determinator (St. Joseph, U.S.A.) calibrated with EDTA and corn flour using 200 ± 10 mg of sample to determine N content of the dried fungus and 100 ± 10 mg to determine the N in the residue at the end of the *in vitro* digestion.

Amino acids (AAs)

Concentrations of AAs in fungi were determined by first hydrolysing samples (500 ± 1 mg) according to Llamas & Fontaine (1994) and then separating AAs by HPLC by the method of Cohen & Michaud (1993), essentially the AccQ.Tag™ method (Waters, Milford, MA, USA). We used α -amino-butyric acid as an internal standard and separated AAs using an AccQ.Tag™ column (150×3.9 mm Nova-Pak™ C₁₈, 4 μ m particle size; Waters, Milford, Massachusetts). The column was held at 37 °C on a Waters HPLC consisting of a 600 controller, a 717 autosampler, and a 474 fluorescence detector set to an excitation wavelength of 250 nm, emission wavelength of 250 nm, filter of 0.5, and gain of 100. AAs were quantified by determining the response factor for each based on running six independently prepared AA standards (AA-S-18; Sigma, NSW, Australia) to which we added internal standard. Acid hydrolysis destroys methionine, so we do not report results for that AA.

AvailN and dry matter digestibility (DMD)

We determined *in vitro* AvailN and DMD in duplicate by the method of DeGabriel *et al.* (2008) that mimics digestion in a model herbivore. The purpose of the assay was to rank fungi with regard to AvailN and DMD rather than trying to give precise *in vivo* values for these constituents. The assay involved duplicate digestions of the samples (0.8050 ± 0.0050 g) in porous bags (Ankom F57, Ankom Technology, Macedon, New York) at 35 °C, first with pepsin (24 h) and then with cellulase (48 h). Knowing the mass of DM in the bag at the end of the assay enabled us to estimate DMD, while analysing this residue for N gave us values for the digestibility of N and AvailN.

Although we cannot find records of fungi producing tannins, we tested this possibility by incubating two bags per sample for 24 h either with the tannin-binding agent, polyethylene glycol MW 4000 (33.3 g L^{-1} in 0.05 M Tris–BASE buffer), or with buffer alone (both 25 mL sample⁻¹) at the start of the digestion. We then thoroughly washed the bags before drying them to constant mass at 50 °C and weighing them. We call the loss of mass, in this initial step, ‘solubles’.

EAA score

To assess fungal protein quality we calculated an AA score as follows:

$$\text{EAA score} = \text{in vitro N digestibility} \\ * (\text{EAA mg g}^{-1} \text{ protein in fungus} / \\ \text{EAA mg g}^{-1} \text{ protein in standard})$$

We included two non-essential AAs in the calculation, arginine because of potential antagonism with lysine (e.g., Jones *et al.* 1966) and tyrosine, because animals require aromatic AAs – tyrosine plus phenylalanine (e.g., Benevenga *et al.* 1994). The logic behind the calculation is that most animals need the same relative proportions of AAs for protein synthesis, while the absolute amount of AAs they need depends on their requirement for protein and digestibility of AAs they ingest (Boisen *et al.* 2000). We used the EAA requirement for rat growth (NRC 1995) to gauge quality of the fungal protein. As this calculation depended on a measure of N digestibility, we calculated the EAA score only for the 30 samples analysed also for AvailN. This calculation assumes that all AAs in the sample are of similar digestibility, as determined by digestibility of N in the sample.

Statistical analysis

We used GenStat Release 10.2, Lawes Agricultural Trust (Rothamsted Experimental station) for all statistical analyses,

which were largely descriptive. We used the unbalanced analysis of variance command to test differences in nutritional composition between hypogeous and epigeous taxa, transforming data where necessary to meet requirements of parametric statistics. Mean values were used for taxa represented by several samples. We tabulated results for each sample unless there were two or more samples for a species, whereby we show the mean, standard deviation (sd), and the number (N) of samples.

Results

Cell walls, soluble material, in vitro DMD, and ash

Epigeous and hypogeous fungi were similarly variable (coefficient of variation, CV = 24 % and 17 %) in their concentrations of cell wall material but epigeous fungi had significantly lower mean concentrations than did hypogeous taxa (27.2 versus 51.2 % DM; $P < 0.001$; Table 2). This was reflected in epigeous fungi having much higher mean concentrations of soluble material (58 versus 39 % DM) and much higher mean DMD (mean 77.3 versus 62.0 % DM) (Table 3). The concentration of soluble material was closely related to that of DMD across all fungal taxa ($r^2 = 87$, $F_{1,30} = 206$, $P < 0.0001$). We had too little material from hypogeous fungi to statistically compare ash contents. That said, three samples of *Cortinarius globuliformis* all contained a high proportion of ash (mean 27 %). The low CV (3.0 %) of this mean and care when collecting samples suggests a real value rather than contamination with soil. In contrast, mean ash content across seven epigeous species was around one-third that of *C. globuliformis* (mean 9.1 %) (Table 2).

Nitrogen, AvailN, and nitrogen partitioning

N values agreed well whether obtained by Kjeldahl analysis or combustion analysis ($r^2 = 0.92$, $F_{1,24} = 271$, $P < 0.0001$; Intercept = -0.107 , $P = 0.49$; Slope = 1.20 , 95 % CI = 1.05 – 1.35 ; $P < 0.0001$). Kjeldahl N does not measure nitrate and nitrite ions unless they are reduced before acid digestion, so we expected the slope to exceed 1.

Nitrogen values (% DM) ranged from 1.98 (10.6 % crude protein, CP) for *Gautieria monticola* to 8.39 (52.5 % CP) for *Agaricus xanthodermus* (Table 3), with an overall mean of 3.75 % N (23.4 % CP). Epigeous fungi contained significantly more N than did the hypogeous fungi both in the samples used to measure AvailN (4.59 versus 2.70 % N DM; $P = 0.016$) (Table 3) and in those used to partition N (3.44 versus 2.36 % N DM; $P = 0.015$) (Table 2). In the former data, *Agaricus bisporus* (6.3 % N DM), *A. xanthodermus* (8.39 % N DM) and *Boletus edulis* (6.21 %) inflated the mean. The cell contents from hypogeous and epigeous fungi had similar concentrations of N (mean = 4.04 and 4.47 % N DM; $P = 0.54$), although values varied more among the latter (CV = 40 versus 23 %) (Table 2). These concentrations were much higher than that of N in the cell walls, which differed significantly ($P = 0.037$) between hypogeous (0.61 % N DM; CV = 50 %) and epigeous taxa (0.34 % N DM; CV = 44 %). Because hypogeous fungi contained more cell wall material rich in N, a larger proportion of the N in these fungi occurred in the cell walls than in epigeous fungi

(4.1 versus 14.4 %; $F_{1,19} = 11.4$, $P = 0.003$). Even so, overall digestibility of N did not differ between hypogeous and epigeous taxa (73.9 % versus 71.3 %; $P = 0.65$) (Table 3). Except for *Hysterangium* sp., where N digestibility was only 39 %, more than half of the N in all other fungal species was digestible (55.1–84.1 %) (Table 3). The similar digestibility of N in the two groups meant that concentration of AvailN (Total N*digestibility of N) depended on total N concentration and so was higher in epigeous (4.57 % DM) than in hypogeous (2.07 % DM) taxa (data from AvailN assay only). If mean N digestibility was the same for taxa used in the N partition assay, then the corresponding AvailN values for those data would be 1.7 and 2.5 % N DM for hypogeous and epigeous taxa, respectively. In contrast to expectations, the digestibility of N was not related to either DMD or the concentration of soluble material.

Tannins are unknown from fungi and thus values for N digestibility from samples incubated with buffer were generally similar to those for corresponding samples incubated with buffer plus PEG. There was, however, one exception. The digestibility of N (81.2 %) and thus available N (1.805 % DM) was much higher in *Hysterangium coriaceum* incubated with PEG than without PEG (67.2 %, 1.496 % DM). Reanalysis confirmed the result.

Between 67 % and 93 % of the N in hypogeous species occurred as protein, whereas the range was much greater for epigeous taxa (18–81 %) (Table 2). The mean proportion of protein N was lower for epigeous species (55 versus 72 %; $P = 0.016$), largely due to low values for the two *Boletus* species – *B. edulis* and *B. mirabilis* (18 and 32 % N as protein, respectively).

AA concentrations

For all taxa for which we had the concentrations of both AAs and N, there was a significant relationship between the traits for all AAs ($P < 0.0001$). In keeping with their higher concentrations of N, species of epigeous fungi tended to have higher concentrations of most AAs than hypogeous taxa (P values ranging from 0.05 to 0.01) (Table 4). The exceptions were aspartic acid and histidine, the concentrations of which did not differ significantly between the fungal forms, although the P values (0.10 and 0.13) suggested a trend. In contrast, there was no relationship ($P > 0.5$) between the concentration of protein-N and that of individual AAs for the 18 taxa for which we had paired data. This was largely due to low concentrations of protein-N but high concentrations of AAs in the *Boletus* species, suggesting that a substantial quantity of AAs in these taxa existed in the free form. Removing these values improved the relationship, but taxa still had much lower AA concentrations than expected, with mature *Rhizopogon hawkeriae* a prime example (Table 4). A repeat analysis confirmed our original concentrations.

AA balance

The concentrations of AAs corrected for in vitro digestibility of N suggest that fungal protein is of poor quality for rats. Expressed relative to that of ideal protein, most fungi supply about 50 % of the rat's requirements for each EAA (Table 5). The most commonly limiting EAA is lysine, of which epigeous

Table 2 – The concentrations of cell walls, total nitrogen (N), N in cell walls and cell contents (% DM) and the partition of N between protein and non-protein.

Species		Ash %	Cell Wall %	N %	Cell Wall N %	Cell cont. N % ^a	Prot. N % ^b	Prot. N %/N % ^c
Hypogeous								
<i>Cortinarius globuliformis</i> (N=3)	Mean	27.9	49.8	1.63	0.39	2.98	1.20	0.74
	sd	1.2	3.28	0.17	0.05	0.31	0.14	0.02
<i>Gautieria monticola</i> (immat)		6.3	57.0	2.38	0.36	5.27	1.61	0.67
<i>Gautieria monticola</i> (mat) (N=3)	Mean	5.5	69.0	2.00	0.53	5.79	1.40	0.72
	sd	0.78	5.9	0.41	0.26	1.11	0.27	0.04
<i>Hysterangium coriaceum</i>			59.0	2.07	0.82	4.41	1.48	0.71
<i>Hysterangium</i> sp (N=2)	Mean		49.6	3.01	1.06	5.32	2.03	0.68
	sd		6.4	0.02	0.14	0.63	0.02	0.00
<i>Mesophellia glauca</i>			53.4	2.18	1.23	3.83	1.76	0.81
<i>Rhizopogon hawkeriae</i> (immat)			43.8	3.07	0.37	5.25	2.38	0.78
<i>Rhizopogon hawkeriae</i> (mat)			49.9	1.85	0.59	3.31	1.72	0.93
<i>Rhizopogon villosulus</i> (immat) (N=4)	Mean		47.7	2.62	0.42	4.75	1.72	0.67
	sd		4.8	0.61	0.06	0.99	0.33	0.06
	CV %		10	23	14	21	19	9
<i>Rhizopogon villosulus</i> (mat) (N=2)	Mean		48.3	1.94	0.51	3.43	1.29	0.67
	sd		3.8	0.20	0.00	0.13	0.17	0.02
<i>Rhizopogon vinicolor</i> (mat)			62.2	1.81	0.59	4.29	1.23	0.68
<i>Truncocolumella citrina</i> (immat)			43.4	2.23	0.32	3.76	1.53	0.69
<i>Truncocolumella citrina</i> (mat)			34.8	1.94	0.34	2.75	1.28	0.673
Epigeous								
<i>Boletus edulis</i>		7.1	18.7	5.78	0.22	7.02	1.06	0.18
<i>Boletus mirabilis</i>		7.8	24.6	4.67	0.27	6.08	1.51	0.32
<i>Cantharellus formosus</i> (N=3)	Mean	10.8	32.0	2.21	0.53	3.02	1.71	0.77
	sd	0.47	1.41	0.19	0.03	0.26	0.10	0.06
<i>Cantharellus subalbidus</i> (N=2)	Mean	10.4	36.0	1.69	0.55	2.37	1.37	0.81
	sd	0.16	1.41	0.11	0.09	0.19	0.02	0.04
<i>Morchella elata</i> (immat)		9.6	24.0	4.15	0.28	5.34	2.26	0.54
<i>Morchella elata</i> (N=3)	Mean	10.2	21.9	3.63	0.27	4.54	2.17	0.60
	sd	0.95	7.98	0.10	0.11	0.48	0.12	0.03
<i>Tricholoma magnivelare</i> (N=6)	Mean	8.1	33.0	1.96	0.24	2.82	1.18	0.60
	sd	0.77	3.30	0.21	0.05	0.31	0.13	0.02
	CV %	10	10	11	21	11	13	3
Mean (hypogeous)		13.2	51.2	2.36	0.61	4.04	1.62	0.72
Standard deviation		12.7	8.7	0.48	0.31	0.92	0.35	0.07
CV %		96	17	20	51	23	22	10
Mean (epigeous)		9.1	27.2	3.44	0.34	4.47	1.61	0.55
Standard deviation		1.5	6.5	1.54	0.14	1.81	0.46	0.23
CV %		16	24	45	41	40	29	42
Epigeous versus hypogeous		ns	41.3	7.2	5.03	ns	ns	7.05
$F_{1,19}$ ($F_{1,9}$ for ash)								
P		0.39	<0.001	0.015	0.037	0.54	0.95	0.016
Least significant difference (5 %)			7.84	0.925	0.259			0.098

a The N % in the cell contents calculated from total N, cell wall %, and cell wall N %.

b The N % that exists as protein.

c The protein N expressed as a proportion of total N.

and hypogeous fungi supply on average 35 % and 43 % of the rat's requirements. Other AAs, especially arginine (76 % and 86 %) and, in the case of epigeous taxa, the sum of phenylalanine and tyrosine (95 %), provide much higher proportions of the requirements, suggesting imbalanced protein.

Variation within and among fungi

Irrespective of whether fungi were epigeous or hypogeous, the chemical composition of their sporocarps varied widely (Tables 2–4). For example, across the different species of

hypogeous fungi CVs were high (>40 %) for ash, cell-wall N and most AAs. Among epigeous fungi, CVs were also similarly high for AAs, as well as N, cell-wall N, cell-contents N and AvailN.

The limited data show that variation for some nutritional properties is much lower within taxa than among taxa. For example, from Table 5 data it is evident that the CV for many AAs is lower from samples averaged within species than averaged across species. This is particularly so for epigeous species such as *Cantharellus formosus* and *Tricholoma magnivelare*, where multiple collections were available for analysis, compared to values averaged across all epigeous species.

Table 3 – The concentrations of nitrogen (N), solubles and available nitrogen (AvailN) along with the digestibilities of dry matter (DMD) and nitrogen (Ndig). All are % dry matter.

Species		N	Solubles	DMD	Ndig	AvailN
Hypogeous						
<i>Arcangeliella</i> (composite)		3.98	43	62.7	67.3	2.66
<i>Cortinarius globuliformis</i> (N=2)	Mean	1.78	40	58.1	77.1	1.36
	sd	0.04	4	7.3	2.0	0.06
<i>Gautieria monticola</i> (mat) (N=2)	Mean	1.98	18	41.4	76.2	1.50
<i>Gautieria monticola</i> (mat)	sd	0.38	3	5.6	3.0	0.22
<i>Hysterangium coriaceum</i>		2.23	24	50.3	81.2	1.80
<i>Hysterangium</i> sp		3.52	24	45.2	39.4	1.38
<i>Protoglossum</i> sp (composite)		2.99	47	62.5	64.2	1.90
<i>Rhizopogon hawkeri</i> (immature)		3.73	40	71.0	76.1	2.84
<i>Rhizopogon hawkeri</i> (mat)		2.08	47	75.2	81.0	1.67
<i>Rhizopogon villosulus</i> (immat) (N=3)	Mean	3.34	43	68.9	77.1	2.58
	sd	0.78	0.2	4.0	4.8	0.50
<i>Rhizopogon villosulus</i> (mat) (N=2)	Mean	2.15	45	68.8	84.1	1.79
	sd	0.23	5	3.58	1.2	0.16
<i>Truncocolumella citrina</i> (immat)		2.57	42	65.1	80.2	2.06
<i>Truncocolumella citrina</i> (mat)		2.15	55	75.2	85.1	1.83
Epigeous						
<i>Agaricus bisporus</i>		6.30	71	85.1	64.2	4.04
<i>Agaricus xanthodermus</i>		8.39	66	88.3	73.2	6.15
<i>Boletus edulis</i>		6.21	57	82.8	79.2	4.90
<i>Cantharellus formosus</i> (N=3)	Mean	2.57	51	67.7	55.1	1.41
	sd	0.225	4	2.57	1.51	0.109
<i>Cantharellus subalbidus</i>		1.92	51	66.3	62.1	1.18
<i>Lentinula edodes</i>		3.80	53	76.3	84.3	3.17
<i>Tricholoma magnivelare</i> (N=7)	Mean	2.36	56	74.7	82.2	1.93
	sd	0.256	2.3	3.16	1.32	0.213
	CV %	11	4	4	2	11
Mean (hypogeous)		2.70	39	62.0	73.9	1.95
Standard deviation (hypogeous)		0.77	11	11.2	12.6	0.50
CV %		29	28	18	17	26
Mean (epigeous)		4.59	58	77.3	71.3	3.26
Standard deviation (epigeous)		2.30	8	8.5	11.1	1.88
CV %		50	14	11	16	58
Epigeous versus hypogeous $F_{1,18}$		7.00	16.2	9.8	ns	5.39
P		0.016	<0.001	0.006	0.65	0.033
Least significant difference (5 %)		1.49	0.098	10.3		1.189

Discussion

Our nutritional analyses give resounding answers to the three hypotheses we posed: (i) fungi are nutritionally poor because they contain few nutrients or are otherwise of low digestibility, (ii) fungi vary substantially in their nutritional composition, and (iii) animals can counter this variable quality by eating diverse taxa. First, in agreement with other published literature (e.g., Claridge & Cork 1994; Fogel & Trappe 1978; Grönwall & Pehrson 1984; Kalac 2009; Singer 1961) we found that both epigeous and hypogeous fungi are a potentially rich source of nutrients, on average rejecting hypothesis (i). Our assays, which concentrated on nitrogenous components, indicated that most of the N in fungi occurs as protein and, in some cases, notably *Boletus* sp., a substantial amount occurs as free AAs. However, as pointed out by Cork & Kenagy (1989), the key interest is not total concentration of a nutrient but instead concentration of available nutrients in the food. To that extent, our *in vitro* digestibility analysis showed that the fungi contain much

material (ca 50 %) soluble in buffer, whereas about 70 % of the total DM and the N are capable of being digested. Considering they are from an *in vitro* assay, these results show reasonable agreement with some of the results from the few studies in which researchers have fed fungi to animals to measure digestibility or availability of nutrients. These include the findings of Claridge & Cork (1994) who fed two species of hypogeous fungi to the long-nosed potoroo, a foregut-fermenting marsupial; Claridge et al. (1999), who fed a single species of hypogeous fungus to red-backed voles and flying squirrels; and D'Alva et al. (2007), who fed epigeous *Russula* to deer mice and volcano mice. While our DMD values are similar to these published values, our N digestibility values are much higher than those of Cork & Kenagy (1989), who fed the hypogeous *Elaphomyces granulosus* to ground squirrels (N digestibility = 30 %) and Dubay et al. (2008) who fed hypogeous fungi to red-backed voles (N digestibility = 12.5 %) and flying squirrels (N digestibility = 24.9 %). Difficulties in obtaining sufficient fungi for feeding in this last study forced very short digestion trials, where endpoint errors are likely large. This makes it difficult

Table 4 – The concentration of amino acids (AAs) in fungi (% dry matter). The values are for individual samples or means and standard deviation (sd) of N samples when we analysed more than two different collections of a taxon.

Species		Asp ^a	Ser	Glu	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Lys	Ile	Leu	Phe	EEA ^b
Hypogeous species																	
<i>Arcangeliiella</i> (N=3)	Mean	1.78	0.93	3.36	0.87	0.58	1.32	0.89	1.29	1.11	0.60	0.99	1.03	0.75	1.37	0.83	7.04
	sd	0.11	0.07	0.18	0.04	0.05	0.4	0.05	0.13	0.08	0.02	0.07	0.09	0.05	0.10	0.02	0.45
<i>Cortinarius globuliformis</i> (N=3)	Mean	0.38	0.18	0.71	0.22	0.06	0.18	0.09	0.24	0.13	0.1	0.12	0.15	0.09	0.13	0.07	0.81
	sd	0.09	0.04	0.03	0.03	0	0.02	0.02	0.07	0.04	0.02	0.05	0.03	0.03	0.03	0.02	0.20
<i>Gautieria monticola</i> (immature) (N=2)	Mean	0.98	0.58	1.66	0.64	0.41	0.57	0.55	0.68	0.64	0.35	0.59	0.54	0.50	0.77	0.46	4.16
	sd	0.01	0.08	0.37	0.08	0.21	0.01	0.04	0.01	0.07	0.04	0.00	0.01	0.02	0.01	0.01	0.25
<i>Gautieria monticola</i> (mature) (N=3)	Mean	0.97	0.58	1.65	0.64	0.41	0.57	0.55	0.68	0.64	0.35	0.59	0.28	0.34	0.47	0.33	3.32
	sd	0.01	0.07	0.37	0.09	0.22	0.02	0.05	0	0.07	0.04	0.01	0.06	0.06	0.1	0.07	0.61
<i>Gymnomyces</i>		1.69	0.99	3.22	0.96	0.57	1.37	0.88	1.12	1.19	0.68	1.00	1.06	0.79	1.41	0.89	7.28
<i>Hysterangium coriaceum</i>		1.35	0.23	0.77	0.54	0.38	0.43	0.34	0.44	0.45	0.2	0.42	0.43	0.34	0.45	0.34	2.88
<i>Hysterangium</i> sp (N=2)	Mean	2.02	0.38	1.41	0.73	0.48	1.44	0.55	0.72	0.68	0.35	0.62	0.71	0.54	0.76	0.49	4.49
	sd	0.10	0.01	0.12	0.04	0.01	0.07	0.21	0.21	0.17	0.28	0.09	0.01	0.04	0.07	0.06	0.28
<i>Mesophellia glauca</i>		1.26	0.3	0.99	0.49	0.32	0.52	0.29	0.38	0.3	0.31	0.34	0.45	0.35	0.54	0.27	2.87
<i>Protoglossum</i> sp (N=3)	Mean	1.13	0.68	1.9	0.69	0.46	0.71	0.64	0.76	0.64	0.44	0.68	0.59	0.56	0.91	0.6	4.88
	sd	0.21	0.11	0.47	0.12	0.14	0.2	0.13	0.14	0.13	0.09	0.11	0.14	0.1	0.16	0.12	0.99
<i>Rhizopogon hawkeriae</i> (immature)		1.23	0.74	2.25	0.56	0.24	0.62	0.57	0.78	0.78	0.4	0.47	0.67	0.38	0.7	0.4	3.83
<i>Rhizopogon hawkeriae</i> (mature)		0.16	0.1	0.25	0.1	0.54	0.09	0.05	0.1	0.09	0.11	0.06	0.08	0.05	0.06	0.03	0.98
<i>Rhizopogon villosulus</i> (immature) (N=4)	Mean	1.15	0.58	1.87	0.5	0.22	0.53	0.52	0.69	0.75	0.32	0.53	0.62	0.43	0.7	0.39	3.73
	sd	0.26	0.14	0.74	0.12	0.07	0.24	0.17	0.17	0.16	0.09	0.2	0.15	0.17	0.25	0.13	1.23
	CV%	25	14	25	13	28	46	33	14	18	27	37	25	44	35	34	30
<i>Rhizopogon villosulus</i> (mature) (N=2)	Mean	1.07	0.40	1.08	0.37	0.15	0.38	0.42	0.49	0.56	0.25	0.44	0.53	0.35	0.53	0.30	2.96
	sd	0.05	0.04	0.11	0.03	0.01	0.03	0.02	0.02	0.08	0.03	0.04	0.04	0.03	0.02	0.17	0.38
<i>Rhizopogon vinicolor</i> (mature)		0.64	0.37	1.28	0.33	0.16	0.43	0.36	0.38	0.46	0.21	0.38	0.34	0.33	0.47	0.26	2.51
<i>Setchelliogaster</i> sp		1.71	0.94	2.4	1.11	0.51	1.24	0.92	1.1	0.95	0.64	1.03	0.72	0.82	1.33	0.94	6.91
<i>Truncocolumella citrina</i> (immature)		0.99	0.53	1.54	0.51	0.2	0.52	0.52	0.62	0.6	0.33	0.58	0.38	0.5	0.77	0.43	3.71
<i>Truncocolumella citrina</i> (mature)		0.83	0.45	1.21	0.43	0.15	0.31	0.45	0.49	0.44	0.25	0.45	0.25	0.37	0.61	0.36	2.89
Epigeous species																	
<i>Agaricus bisporus</i>		1.36	0.85	2.88	0.8	0.45	0.84	0.82	0.87	1.53	0.53	0.83	0.9	0.64	1.12	0.69	5.98
<i>Agaricus xanthodermus</i>		2.33	1.21	4.76	1.73	0.96	1.7	1.35	2.19	5.7	1.03	1.73	1.87	1.33	2.3	1.57	12.14
<i>Boletus edulis</i>		1.91	1.04	3.77	0.94	0.41	0.77	0.81	1.47	1.46	0.6	0.56	1.14	0.61	1.08	0.56	5.77
<i>Boletus edulis</i>		2.65	1.36	5.4	1.4	0.73	1.63	1.37	1.94	1.74	0.86	1.52	1.58	1.12	1.96	1.07	10.21
<i>Boletus mirabilis</i>		1.31	0.74	2.22	0.66	0.26	0.6	0.46	0.81	1.47	0.41	0.47	0.74	0.4	0.68	0.37	3.79
<i>Boletus mirabilis</i>		1.84	0.97	2.84	0.91	0.5	1.16	1.06	1.12	1.78	0.61	1.17	1.01	0.85	1.35	0.79	7.34
<i>Cantharellus formosus</i> (N=5)	Mean	0.93	0.54	1.37	0.56	0.25	0.67	0.56	0.64	0.76	0.34	0.59	0.47	0.55	0.81	0.51	4.08
	sd	0.13	0.05	0.2	0.1	0.05	0.14	0.05	0.07	0.2	0.07	0.07	0.06	0.09	0.09	0.09	0.57
	CV%	14	9	15	18	20	21	9	11	26	21	12	13	16	11	18	14
<i>Cantharellus subalbidus</i>	Mean	0.62	0.43	0.84	0.43	0.21	0.42	0.42	0.46	0.52	0.27	0.43	0.30	0.45	0.62	0.40	3.09
	sd	0.03	0.03	0.06	0.02	0.01	0.05	0.03	0.02	0.11	0.01	0.01	0.01	0.01	0.03	0.01	0.13

(continued on next page)

Table 4 – (continued)

Species		Asp ^a	Ser	Glu	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Lys	Ile	Leu	Phe	EAA ^b
<i>Lentinula edodes</i>		1.86	1.11	5.01	1.11	0.65	1.03	1.2	2.53	2.82	0.51	1.28	1.17	0.96	1.59	0.99	8.35
<i>Morchella elata</i> (N=6)	Mean	1.19	0.63	1.86	0.65	0.26	0.94	0.6	0.85	0.75	0.38	0.48	0.78	0.39	0.64	0.38	3.95
	sd	0.42	0.2	0.46	0.15	0.09	0.33	0.32	0.22	0.36	0.13	0.3	0.24	0.23	0.31	0.2	1.82
	CV%	35	32	25	23	35	35	53	26	48	34	63	31	59	48	53	47
<i>Tricholoma magnivelare</i> (N=11)	Mean	0.99	0.52	1.6	0.5	0.21	0.55	0.5	0.63	0.62	0.32	0.51	0.51	0.47	0.68	0.41	3.61
	sd	0.24	0.1	0.42	0.09	0.05	0.15	0.11	0.12	0.14	0.05	0.12	0.12	0.1	0.14	0.08	0.77
	CV%	24	19	26	18	24	27	22	19	23	16	24	24	21	21	20	21
Descriptive statistics																	
Hypogeous species	Mean	1.14	0.53	1.63	0.57	0.34	0.66	0.51	0.65	0.62	0.35	0.55	0.52	0.44	0.71	0.44	3.86
	sd	0.49	0.27	0.85	0.26	0.17	0.42	0.25	0.31	0.30	0.17	0.27	0.28	0.22	0.39	0.26	1.89
	CV	43	51	52	46	50	64	49	48	48	49	49	54	50	55	59	49
	Min	0.16	0.10	0.25	0.10	0.06	0.09	0.05	0.10	0.09	0.10	0.06	0.08	0.05	0.06	0.03	0.81
	Max	2.02	0.99	3.36	1.11	0.58	1.44	0.92	1.29	1.19	0.68	1.03	1.07	0.82	1.44	0.94	7.28
Epigeous species	Mean	1.58	0.82	3.03	0.93	0.48	1.02	0.89	1.29	1.85	0.54	0.99	1.00	0.80	1.28	0.78	6.77
	sd	0.72	0.29	1.66	0.45	0.27	0.47	0.39	0.76	1.64	0.26	0.53	0.54	0.37	0.65	0.40	3.36
	CV	46	35	55	48	56	46	44	59	89	48	54	54	46	51	51	50
	Min	0.62	0.43	0.84	0.43	0.20	0.42	0.42	0.46	0.52	0.27	0.43	0.30	0.39	0.62	0.38	3.09
	Max	2.57	1.21	5.01	1.73	0.96	1.70	1.36	2.53	5.70	1.03	1.73	1.87	1.33	2.30	1.57	12.14
	F _{1,24}	2.88	6.42	8.17	6.77	2.50	4.18	8.00	9.22	11.9	5.60	8.09	8.91	9.54	7.89	7.00	8.07
	P	0.10	0.02	0.01	0.02	0.13	0.05	0.01	0.01	0.01	0.03	0.01	0.01	0.01	0.01	0.01	0.01
	lsd (5 %)		0.24	1.01	0.28		0.37	0.26	0.43	0.83	0.17	0.32	0.33	0.24	0.42	0.27	2.11
	r ^{2c}	0.61	0.66	0.66	0.77	0.59	0.68	0.69	0.56	0.72	0.84	0.70	0.82	0.69	0.74	0.76	0.77
	F _{1,21} ^c	33	41	42	72	31	44	45	27	54	107	48	98	46	60	66	70

^b EAA is the sum of the essential AAs.

^a asp (aspartic acid, non-essential), ser (serine, NE), glu (glutamic acid, NE), gly (glycine, NE), his (histidine, E), arg (arginine, NE but see text), thr (threonine, E), ala (alanine, NE), pro (proline, NE), tyr (tyrosine, E but see text), val (valine, E), lys (lysine, E) ile (isoleucine, E), leu (leucine, E), phe (phenylalanine, E).

^c r² and F: the coefficient of determination between the concentration of an AA and total nitrogen and the variance ratio (F) for the relationship. All were highly significant (P < 0.0001).

Table 5 – The quality of fungal protein, calculated from the amino acid (AA) pattern corrected for nitrogen digestibility, and expressed relative to that of ideal protein for growing rats.

Species		Arg	His	Ile	Leu	Lys	Phe + Tyr	Thr	Val
Hypogeous									
<i>Cortinarius globuliformis</i> (N=2)	Mean	58	19^a	37	51	47	61	41	38
	sd	17	5	12	21	9	28	24	0
<i>Gautieria monticola</i> (mature) (N=2)	Mean	61	48	50	36	24	42	42	39
	sd	10	10	16	8	9	7	8	9
<i>Hysterangium coriaceum</i>		73	99	39	31	34	38	40	39
<i>Hysterangium</i> sp		87	48	25	20	21	23	25	24
<i>Rhizopogon hawkeri</i> (immature)		71	43	30	32	36	38	45	31
<i>Rhizopogon villosulus</i> (immat) (N=2)	Mean	94	55	51	47	44	48	60	53
	sd	5	4	1	3	5	1	1	2
<i>Rhizopogon villosulus</i> (mature) (N=3)	Mean	85	52	55	48	55	52	66	57
	sd	4	2	3	2	7	2	6	3
<i>Truncocolumella citrina</i> (immature)		90	56	61	54	31	56	63	59
<i>Truncocolumella citrina</i> (mature)		68	50	56	54	26	56	69	58
Epigeous									
<i>Agaricus bisporus</i>		58	57	38	36	31	110	47	42
<i>Agaricus xanthodermus</i>		82	73	45	45	42	190	46	49
<i>Boletus edulis</i>		112	74	64	57	55	53	61	67
<i>Cantharellus formosus</i> (N=3)	Mean	80	49	50	40	26	46	48	43
	sd	20	11	7	5	3	7	5	6
<i>Cantharellus subalbidus</i>		68	35	79	109	53	117	72	76
<i>Lentinula edodes</i>		103	87	55	55	52	90	70	60
<i>Tricholoma magnivelare</i> (N=7)	Mean	96	61	66	50	42	58	63	54
	sd	14	9	16	9	3	9	10	12
Mean (hypogeous)		76	52	45	41	35	46	50	44
sd		13	21	13	12	12	12	15	13
Mean (epigeous)		86	62	57	56	43	95	58	56
sd		19	17	14	24	11	51	11	13
F _{1,14}		ns	ns	ns	ns	ns	9.46	ns	ns
P		0.26	0.31	0.10	0.12	0.20	0.008	0.25	0.09
Least significant difference (5 %)							3.6		

A bold font indicates the limiting AA.

a Arg-Arginine; His-Histidine; Ile-Isoleucine; Leu-Leucine; Lys-Lysine; Phe+Tyr-Phenylalanine+Tyrosine.

to assess whether animals maintained weight on the diets and whether they were in positive or negative N balance. Our results also indicate that the DMDs of fungi are similar to those of various plants fed to a wide range of ruminant species (Van Soest 1982). On this basis fungi appear to be a good source of available nutrients, so it is not surprising that mycophagy is such a common feeding strategy among mammals (Claridge & May 1994; Maser et al. 1978).

Hypothesis (ii) that the AA content of fungi would vary highly was confirmed, as expected for various reasons. For instance, fungal sporocarps show tremendous morphological diversity and presumably different structures require different chemical building blocks and, in some cases, chemical defences. Epigeous fungi might use air currents to spread spores but they may also use animals. In contrast, many ectomycorrhizal fungi produce hypogeous sporocarps and rely on mycophagous animals to spread the spores (Claridge & May 1994; Maser et al. 1978). The close evolutionary association among fungi, plants, and animals suggests that fungi might provide nutritional reward to animals for spreading spores. If so, we would expect the within-taxa variation in composition we saw for *Tricholoma*, *Morchella*, *Cantharellus*, and *Rhizopogon*. We might also expect some fungi to protect their protein, as we saw with one species, *Hysterangium coriaceum*, that appeared to produce tannin-like

compounds that bind protein. Sporocarps also pass through stages of maturation and these too may affect nutrient concentrations. Finally, the nutritional composition of fungal sporocarps will also be partial reflection of the nutrient status of the soils they inhabit, and this can vary from one site to another. Regardless of the nutritional characteristic that we measured – for example N content, N digestibility, and the concentration of AAs, there was considerable variation among taxa, again in agreement with the findings of other researchers (e.g. Kalac 2009) and the in-vivo feeding studies mentioned previously (Cork & Kenagy 1989; Claridge & Cork 1994; Claridge et al. 1999; D'Alva et al. 2007; Dubay et al. 2008).

Hypothesis (iii) was that animals can counter the variability between fungal species in nutritional quality by eating a diversity of taxa (see Claridge & Cork 1994; Claridge & May 1994; Claridge et al. 1999; Maser et al. 1978). Analyses of faeces from mycophagous animals indicate this is the norm for obligate and preferential mycophagists and common with opportunistic mycophagists (Currah et al. 2000; Johnson 1994; Mangan & Adler 2000; Maser et al. 1978; Seebeck et al. 1990; Tory et al. 1997; Vernes & Dunn 2009). For example, the small obligate mycophagist, the California red-backed vole averaged five fungal species in its digestive tract, the result of foraging over the previous 24 h (Maser et al. 1978). A single sporocarp

of many of those fungal species would fill their stomach several times over. Instead, digestive tracts from these voles contained smaller fragments of sporocarps from multiple species, highlighting their diverse foraging strategy. Over the course of a season, other mammal species have been recorded as eating up to 50 different species of fungi (e.g. Claridge & May 1994). It is extremely difficult to replicate this strategy in feeding studies, which may explain why studies with one or two species of fungi sometimes indicate that fungi are of poor nutritional value (e.g. Cork & Kenagy 1989; Dubay et al. 2008).

Evidence is growing that animals forage to satisfy a protein requirement – the protein leverage hypothesis (Felton et al. 2009; Sørensen et al. 2008). Animals, however, require EAAs, not protein (DeGabriel et al. 2002). Our results suggest that although fungi contain reasonable amounts of protein, the balance of digestible EAAs is poor, with lysine and leucine the most commonly limiting. To counteract this, an animal can adopt several strategies to improve the quality of its dietary protein. One strategy is to seek other food types with differing AAs to supplement those in fungi, e.g. insects: mycophagous animals are reported to eat fungi infested by insects (Bozinovic & Muñoz-Pederos 1995) and otherwise are known to feed on a range of invertebrates (Claridge & May 1994).

Yet another approach to coping with imbalanced nutrients, particularly AAs, is to evolve appropriate metabolic and digestive strategies. Australia's potoroine (rat-kangaroo) marsupials are a prime example: they are among the smallest macropods (up to 3 kg with most less than 1 kg), have foregut fermentation and low N requirements (Wallis 1994; Wallis & Hume 1992). For these mycophagous animals, a key advantage is that microbes turn poor quality fungal protein into more readily available microbial protein. This protein turnover costs energy, but it is partly compensated by microbes also hydrolysing cell wall material and synthesising volatile fatty acids (Wallis 1994). Animals with hindgut fermentation can capture some of these advantages by practising caecotrophy or coprophagy (Liu et al. 2007; Soave & Brand 1991), which is common among rodents, including voles (Nicodemus et al. 1999).

The combination of mycophagy, foregut fermentation and coevolution between animals and fungi may explain some Australian phenomena. Potoroine marsupials, obligate or preferential mycophagists, frequently occur in areas prone to fire (Claridge & Trappe 2004). They often survive destructive fires and can maintain body mass on the limited selection of hypogeous fungi that remain immediately after disturbance (Johnson 1997; Vernes 2000; Vernes et al. 2004). These fungi are typically in the hypogeous family Mesophelliaceae, which survive when other, more fire-sensitive fungi temporarily stop fruiting (Claridge & Trappe 2004; Trappe et al. 2006). Nothing in our limited analyses of only one sample of Mesophelliaceae suggest an AA composition better than that of other fungi. In this situation, an animal would be unable to satisfy its EAA requirements simply by eating a broad range of taxa, but foregut fermentation would allow it to enhance protein quality. Wallabies, opportunistic mycophagists, are also common in fire affected sites and forage for members of the Mesophelliaceae (Claridge et al. 2001). As opportunists, however, they can vary their diet by browsing plants that escaped the fire or by foraging in adjacent unburned areas where other species of fungi and browse vegetation are available.

In terms of broader aspects of mycophagist nutrition, fungal sporocarps are excellent sources of minerals, fats, vitamins and water in addition to AAs. Claridge & Trappe (2005) and Maser et al. (2008) cover these nutritional aspects in detail. The data we present here round out the evidence for fungi as good, all-round nutritional sources. In conclusion, fungi are good source of nutrients that many animals use through possessing suitable metabolic and physiological adaptations. The co-evolutionary aspects are so strong that a tripartite relationship often exists between trees, animals, and ectomycorrhizal fungi (Claridge & Trappe 2005; Maser et al. 2008; Trappe & Claridge 2005). One outstanding question relating to the nutritional value of fungi is why so many mycophagous animals eat predominantly hypogeous fungi when so many epigeous species are often readily available in their habitats? Fungal toxins do not provide an adequate answer because one role of the foregut is microbial detoxification (Wallis 1994). One possible factor is that hypogeous fungi produce aromatic enticements that attract many kinds of animals (Claridge et al. 2010; Paull et al. 2011) whereas epigeous fungi may be primarily detected visually. Small mammals especially tend to forage by odours rather than by sight (Donaldson & Stoddart 1994).

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