Water sources and controls on water-loss rates of epigeous ectomycorrhizal fungal sporocarps during summer drought

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Summary

• Access to deeper soil water and water-conserving traits should reduce water stress for ectomycorrhizal fungi, permitting function during drought. Here, we explored whether epigeous fruiting of ectomycorrhizal fungi during drought was facilitated by access to deep soil water, how much water was lost from sporocarps, and how sporocarp surface to volume ratios affected water-loss rates.

• We used oxygen stable isotope analysis of water combined with modeling of water sources used by ectomycorrhizal fungi; measured sporocarp water loss using a transient porometer, and related water loss to vapor pressure deficit (VPD) and sporocarp morphology.

• In drier soils sporocarps likely derived a significant portion (25–80%) of their water from deep (> 30 cm) or hydraulically lifted water. *Amanita muscaria* had water-loss rates over twice those of *Suillus* sp., *Boletus edulis*, *Tricholoma* spp. and *Russula albonigra*. Vapor pressure deficit was an excellent predictor of water-loss rates for individual mushrooms. Sporocarp surface to volume ratios explained much of the variation among mushrooms in the slope of VPD–water loss relationships.

• Access to deeper soil water might be a significant driver of ectomycorrhizal symbiotic function, sporocarp distribution, fruiting habit and morphology. Sporocarp morphology can affect water-loss rates and hence influences fungal ability to fruit during summer drought.

Introduction

Summer drought in forested ecosystems presents numerous challenges to trees and their mycorrhizal symbionts. Low soil water potential, which reduces water availability, and high air temperatures and low humidity, which together drive high evaporative demand from tissues, can lead to severe water stress. Mycorrhizal fungi may have a role in supplying water to hosts under these conditions (Smith & Read, 1997) and alternatively can be recipients of water from their hosts via hydraulic lift or redistribution of water (Querejeta *et al.*, 2003; Egerton-Warburton *et al.*, 2007; Warren *et al.*, 2008).

One window into the water relations of ectomycorrhizal fungi is via their sporocarps. Sporocarps are directly integrated with both tree roots and soil via the fungal mycelium, receiving water from these sources through the creation of water potential gradients. As in plants these gradients are at least in part established by diurnal variation in evaporative demand at the surface of the sporocarp. Unlike plants, sporocarps do not possess structures analogous to stomata, so regulation of water loss must be via other mechanisms such as seasonality, macromorphology and microhabitat.

Sporulation is a key event in the life history of fungi, and how fungi solve the problem of sporulation in water-limited conditions affects fungal reproduction, population structure and forest food webs. In mesic ecosystems most macrofungi produce epigeous (above-ground) sporocarps, which facilitate advection of spores out of the boundary layer, resulting in long-distance dispersal. However, this habit exposes sporocarps to high near-surface air temperatures, low humidity and winds, leading to potentially rapid water loss and desiccation. In summer, in drought-prone ecosystems such as the forests of

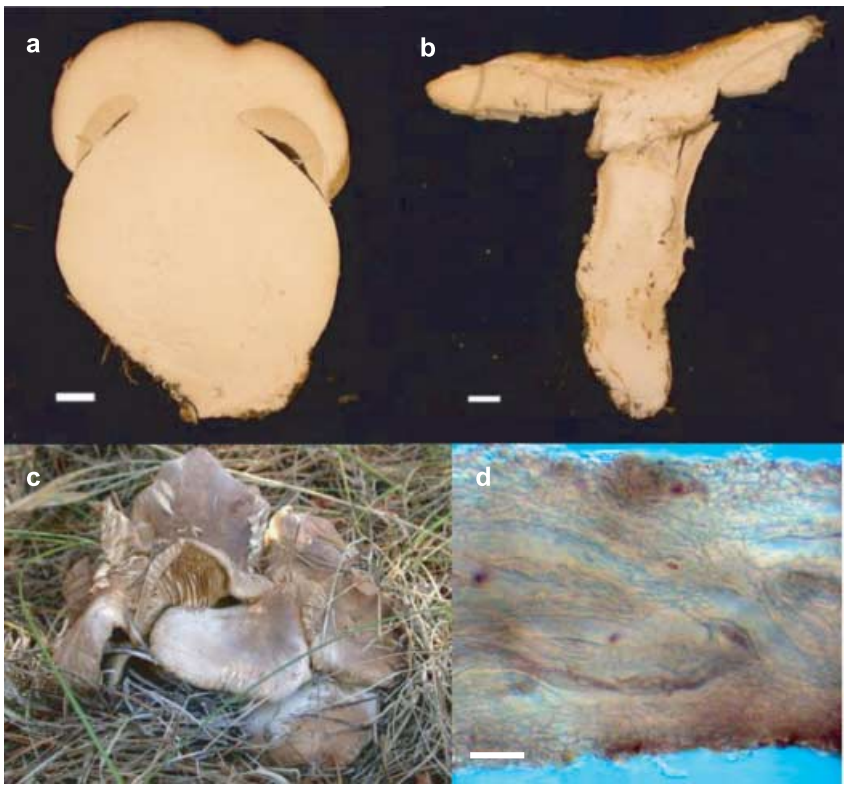

Fig. 1 Sporocarps and rhizomorphs of fungi investigated in the present study. (a) *Boletus edulis*, cross section of sporocarp (bar, 1 cm); (b) *Amanita muscaria*, cross-section of sporocarps (bar, 1 cm); (c) *Tricholoma* sp. exhibiting caespitose sporocarp morphology that effectively reduces sporocarp surface area; (d) *B. edulis* rhizomorph (bar, 25 μm).

the Sierra Nevada Mountains of California, USA, fungi have evolved a range of strategies that minimize the loss of water by sporocarps. Many fungal lineages appear to have independently evolved gastroid (closed) or hypogeous (below-ground) sporocarps, presumably in part to avoid the extreme water stress of the epigeous habit (Hibbett *et al.*, 1997; O'Donnell *et al.*, 1997). Some other fungi sporulate in the springtime, when water availability is rather high. However, C availability is likely low at this time, as host growth sinks are also quite active and therefore provide a competing sink for the products of photosynthesis.

A few Sierran fungi fruit epigeously during the late summer drought, but how they manage to do this is unclear. Of particular interest is *Boletus edulis*, an economically important edible fungus (Hall *et al.*, 1998) that can produce large quantities of sporocarp biomass under summer drought conditions. We observed *B. edulis* fruiting heavily along hillslopes adjacent to floodplains in the Sierra and hypothesized that *B. edulis* sporocarps access groundwater in these habitats. This access could occur via capillary water transport to surface soils, via hyphal water uptake from deeper soil or roots and vertical transport, or via hydraulic lift of groundwater by the host plant and subsequent transfer to fungi (Querejeta *et al.*, 2003). Hydraulic lift involves a diurnal cycle in which the direction of water potential gradients reverses between day and night, with gradients from soil into roots during the day, and from roots into soil at night after transpiration declines (Dawson, 1993; Caldwell *et al.*, 1998 for a review). Host plants conducting hydraulic lift can release groundwater to their fungal symbionts either directly into hyphae connected to their roots or indirectly via the soil (Querejeta *et al.*, 2003). Thus mycorrhizal fungi associated with hosts performing hydraulic lift can receive water from their host tree, and transfer this water to mycelium and sporocarps.

It is likely that epigeous fungi differ in the relative amounts of water they use for sporocarp production. Fungal surface to volume relationships could have a large impact on water loss. In the Sierra Nevada Mountains, *B. edulis* produces sporocarps with a very rounded cap and expanded stipe morphology with a high potential for water storage and low water loss (Fig. 1a). By contrast, other taxa that fruit above ground less frequently under these conditions, such as *Amanita muscaria* (Fig. 1b) and *Tricholoma* spp., produce sporocarps with much higher surface to volume ratios. We hypothesized that surface to volume ratios would be good predictors of water-loss rates from sporocarps.

To test our hypothesis about access to groundwater, we used natural abundance stable isotope methods that take advantage of the divergent oxygen stable isotope composition of groundwater and precipitation-derived surface water (Ehleringer & Dawson, 1992; Dawson *et al.*, 2002). To test our hypotheses about water loss and sporocarp morphology we characterized sporocarp water loss using a transient porometer designed to measure sporocarp water loss *in situ*, and by measuring evaporative mass loss under *in situ* field conditions; we then

characterized sporocarp morphology and related water loss to sporocarp morphology.

Materials and Methods

Study site

Our study site was located in the Sierra Nevada Mountains along Trout Creek, in South Lake Tahoe, CA, USA, elevation 1890 m, latitude 38°55′N, longitude 119°58′W. Trout Creek is a perennial stream fed by snowmelt. The floodplain of Trout Creek is open meadow with clumps of willow (*Salix* spp.) and lodgepole pine (*Pinus contorta* Dougl. ex. Loud.), with the adjacent hillslope dominated by Jeffrey pine (*Pinus jeffreyi* Grev. & Balf.). Sporocarps were concentrated along the edge of the floodplain and the lower few meters of the hillslope in association with both *Pinus* species. Sporocarp production was dominated by *B. edulis*, with rare occurrences of *A. muscaria*, *Tricholoma saponaceum*, *Tricholoma* sp., *Suillus* sp. and *Russula albonigra*. The floodplain soils are loamy alluvial soils, whereas the hillslope is formed of deep, unconsolidated glacial outwash soils classified as Elmira-Gefo loamy coarse sands (http://tahoe.usgs.gov/soil.html). Mean June, July, August and September temperatures are 12, 16, 16 and 12°C, respectively. Approximately 81 cm of precipitation falls annually, mostly as winter snowfall, with an average of only 8 cm falling from May to September (http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?cataho+nca).

Sporocarp water sources

Isotopic ratios of O in water can vary, with higher proportions of the heavier isotopes described as enriched and having a more positive δ value, and lower proportions of the heavier isotopes described as depleted and having a more negative δ value. The O stable isotopes of water can be used to trace water sources because groundwater and surface soil water differ in isotopic values (Ehleringer & Dawson, 1992). Thus surface soils will reflect rainwater inputs plus evaporative enrichment of surface soil water, and deep soils will reflect either groundwater values or deep soil water inputs and storage, which are typically depleted in the heavier O isotopes relative to rainwater because they derive largely from snowmelt that has much colder condensation temperatures than summer or spring rainfall (Gat, 1996; Dawson & Ehleringer, 1998).

We established groundwater wells in summer, 2000, located at the contact between the base of the hillslope and the floodplain, within a few meters of our sporocarp sampling locations. Wells were sealed with insulating plugs to prevent evaporative water loss. At the time of sampling, we determined depth to and sampled groundwater. We also located sporocarps of *B. edulis* and *A. muscaria*, sampled both stipe base and cap, and sealed samples in 15 ml falcon tubes. We took soil cores directly under sporocarps, sampling soil at 10 cm intervals to a depth of up to 80 cm. These samples were stored in 50 ml falcon tubes. Other soil subsamples were collected for determination of gravimetric water content after drying at 105°C. In order to determine the isotopic signature of the water taken up by the trees, we collected root samples from the soil cores. We also collected tree cores with phloem removed to limit sampling to the xylem water.

Isotopic composition of the samples was determined by standard methods at the Center for Stable Isotope Biogeochemistry at the University of California, Berkeley. For liquid samples, 200 μl of water for both standards and samples were pipetted into 10 ml Exetainers (Labco, High Wycombe, UK) and quickly sealed. For soil and plant samples, water was first obtained by cryogenic vacuum distillation (Ehleringer *et al.*, 2000) and then run the same way as our liquid samples as follows. Exetainers containing water samples were first purged with 0.2% CO_2 in helium and allowed to equilibrate at room temperature for at least 48 h. The ^{18}O in the CO_2 was then analyzed on a Finnigan Delta Plus XL isotope ratio mass spectrometer (Thermo Corp., Waltham, MA, USA), using a Gas Bench II interface. The long-term external precision for this analysis is ±0.17‰.

Metabolic water (i.e. water derived from respiration of organic compounds) production is usually considered to be a small portion of total water and is often ignored in assigning sources to water. However, given the potential high flux of sugars in water transferred from hosts to fungi, metabolic water might alter isotopic signatures of sporocarp water. We therefore examined the potential impact of metabolic water production on the isotopic signature of sporocarps. We used the results from our analysis of the potential proportion of metabolic water production, combined with fractionations determined from the literature, to determine the potential impact that metabolic water could have on the assignment of water to different sources. The results indicated that this effect is likely to be < 0.5‰, and the sign of this change depends on a variety of assumptions, so we did not include any correction for metabolic water in our analysis (see the Supporting Information, Notes S1 and Table S1 for detailed methods and results).

Another factor altering sporocarp water isotopes is evaporative enrichment. We examined the data to determine whether sporocarps were likely to reflect source water or were evaporatively enriched by determining isotopic differences between cap and stipe, by calculating water loss from the different parts of the sporocarp (see below), and by comparing the relative signature of stipe and surface soil under varying soil moisture conditions. Under conditions where surface soils had higher moisture content, surface water would be more available to sporocarps, and so sporocarp isotopic signatures would be more likely to reflect surface soil isotopic signatures plus additional evaporative enrichment within the tissues. We used the enrichment of sporocarps relative to surface soils to correct sporocarp isotopic signatures to reflect their source pool. The corrected signatures were then compared with water across

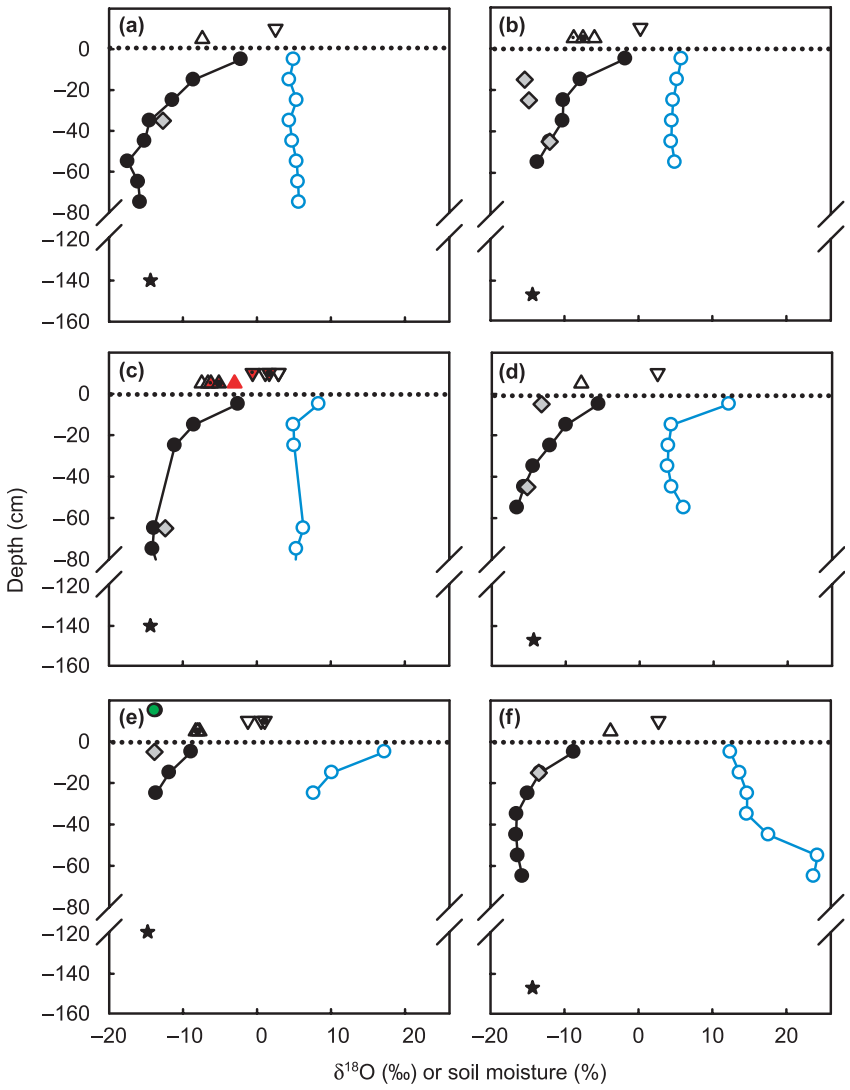

Fig. 2 Per cent soil moisture (open blue circles) and oxygen stable isotope ratios for groundwater (stars), soils (black closed circles), roots (grey diamonds), xylem (green circles) and sporocarp stipes (upward triangles) and caps (downward triangles) in a floodplain margin ecotone in South Lake Tahoe, CA, USA. *Boletus edulis* sporocarps are unfilled; *Amanita muscaria* sporocarps are filled red; individual sporocarps are differentiated by the fill pattern. Other than inversion, symbol is identical for cap and stipe from same sporocarp. Each panel represents one or more sporocarps associated with a specific soil core, root and xylem samples and groundwater well. Panels are ordered from very dry to progressively greater soil moisture.

depth profiles to determine likely depth of water sources using multiple source partitioning models. To reduce uncertainty in source partitioning, we first combined sources with similar isotopic signatures (deeper (> 30 cm) soil horizons, roots and groundwater; see Fig. 2) into one pool (Phillips *et al.*, 2005), expressed as the average of these pools. Other pools were 20–30 cm, 10–20 cm and 0–10 cm soil. We next used a source partitioning approach to model and constrain the possible sources (Phillips & Gregg, 2003). We used the ISOSOURCE program (http://www.epa.gov/wed/pages/models/stableIsotopes/isosource/isosource.htm) to make our mixing calculations and define the resulting constrained solutions. We plotted model output against soil moisture to determine whether water availability affected patterns of water source utilization.

Sporocarp water loss patterns

To examine water loss from different sporocarps, we used two approaches: we designed a transient porometer for *in situ* nondestructive mushroom water loss measurement and calculated mass loss from excised mushrooms left *in situ*.

Transient porometer

Transient porometers are used to measure water loss via change in water content of air in a closed container over a short measurement interval (Pearcy *et al.*, 1989b). The porometer was made from an 18.9 l white, high-density polyethylene bucket (Leaktite, Leominster, MA, USA). In order to insert intact sporocarps, the center of the lid was cut out, and replaced with a flexible plastic low-density polyethylene sheet, with a central opening fitted with an elastic pull cord that could be gently tightened around the base of a sporocarp. To ensure adequate mixing fans (model A6025M12D 12 V DC brushless fans; Mechatronics, Inc., Preston, WA, USA) were mounted on the sidewall (6 cm above the opening, lateral to the intact mushroom) and the top (26 cm above the opening) of the porometer, and were powered by a portable 12 V battery. Also

mounted on the sidewall 18 cm above the opening was a HOBO H8 Pro relative humidity and temperature logger (Onset Computer Corporation, Bourne, MA, USA) set to log temperature and humidity at 2-s intervals. Calibration check of HOBOs was carried out using saturated NaCl solutions in closed chambers. Sporocarps were sealed into the container by inverting the body of the container onto the lid, which was fitted with an air-tight o-ring. Temperature and humidity were logged, and at the end of the recording period (5–10 min), the container was opened and allowed to return to ambient humidity. To determine the diurnal time-course of water loss and the relationship of that water loss to vapor pressure deficit this was repeated over the course of the day by cycling through multiple sporocarps fitted with lids for repeated measurements. Temperature change during individual measurements was minimal (0.15 ± 0.13°C (SE)). At the end of the sampling period, sporocarps were excised and sealed in doubled heavy-duty Ziploc bags, placed on ice and returned to the lab.

Mass loss *in situ*

To determine the relationship between our porometer results and water loss under ambient conditions, a subset of the sporocarps measured with porometers were excised, weighed in the field using a portable balance, then replaced in their original location and orientation, and reweighed over a range of intervals to determine water-loss rates. To determine whether water was lost primarily from caps or stipes, several of these sporocarps were divided into cap and stipe and these were weighed separately. When this was done, the cut surfaces were coated with petroleum jelly to stop water loss from those surfaces, then cap and stipe were reassembled in the original orientation.

Surface area and volume estimation

In the laboratory sporocarps were weighed and external dimensions were measured for use in surface area and volume calculations. Sporocarp volume was also determined by displacement of millet seed. Sporocarps were then dried and reweighed to determine water weight and dry weight.

For calculation of surface area and volume, we measured cap diameter, cap thickness at the stipe apex, stipe length, stipe apical diameter, stipe maximum diameter, stipe basal diameter, and height above stipe base of the stipe maximal diameter. From this information we calculated the surface area and volume of sporocarps. We assumed that the top of the cap of the sporocarp was half of an oblate spheroid and the pore surface was a circle with the area of the stipe apex (also a circle) subtracted. For *B. edulis* and *Tricholoma* sp. the stipe was approximated by a prolate spheroid (i.e. elongated sphere), for *A. muscaria* and *T. saponaceum*, by a frustum (truncated cone) and for *R. albonigra*, by a cylinder. We used standard equations for calculation of the surface and volume of these idealized shapes (Beyer, 1987). From the this information we calculated surface to volume ratios for each sporocarp, and compared measured and mathematically calculated volumes.

Porometer water loss calculations

Absolute humidity within the porometer was plotted as a function of time. The curves from the time of closure to the end of the measurement period had a sigmoid form. We interpreted the lower, concave part of this curve as the period of post-closure equilibration of the porometer. We interpreted the upper, convex part of this curve as meaningful data on a well-mixed air volume, with the decline in rate of increase in humidity driven by the effect of increasing absolute humidity in the porometer on sporocarp water-loss rates. We fitted this part of each curve with a quadratic equation to represent the water vapor accumulation as absolute humidity increased. Fits typically had an $r^2 > 0.99$. To determine the instantaneous rate of water loss at ambient absolute humidity outside of the porometer, we took the derivative of the quadratic equation and determined its value at the point at which the extrapolated fitted line for the quadratic equation matched the ambient absolute humidity at time of chamber closure.

Calculation of diurnal water loss

To scale individual measurements up to a diurnal water-loss rate, we fitted linear relationships for individual mushrooms between VPD and water loss. The VPD was calculated from the HOBO ambient relative humidity (rh) and temperature data, using data from tables for saturation vapor pressure–temperature relationships (Pearcy *et al.*, 1989a). We used logged relative humidity and temperature data to calculate the diurnal time-course of VPD, and combined this information with the fitted relationships of water loss vs VPD (see later) to model the cumulative water loss over the course of 24 h for individual mushrooms, assuming the same VPD conditions for all mushrooms. Overnight VPD data were obtained from a HOBO left running the night before sampling. Daytime VPD data were obtained by taking the daytime ambient humidity data and generating a smoothed time–VPD relationship in Sigmaplot (negative exponential fit).

Comparisons of water loss by species

To compare water-loss rates by different species we performed linear regressions of per cent water loss against VPD and compared slopes of those relationships. These relationships were well-described by a linear model ($r^2 > 0.90$). For this reason, for sporocarps with only a single point estimate of water loss and VPD, we assumed a linear slope from the origin, and used this line as our estimate of the VPD–water loss relationship for that sporocarp.

Comparison of water loss estimates from porometer and mass loss approaches

We fitted linear relationships between VPD and per cent water loss for data from both the porometer and mass loss approaches for four *B. edulis* sporocarps for which both methods were used, and compared the slopes of these relationships using regression. In addition, to determine whether there was greater evidence of declining slopes of the water loss–VPD relationship for cut sporocarps, we compared the slope of the ascending curve with that of the descending curve. To do this, we selected a measurement as near to the midpoint of the ascending curve (before maximum diurnal VPD) as was available, and then found the nearest point to that on the descending curve (after maximum diurnal VPD). If the latter was not available (because of shorter course of measurement) we compared the midpoint measurement to the last measurement in the series made near peak VPD. This makes the test conservative regarding the decline in water loss in cut mushrooms, as we had longer time-courses for the uncut than the cut mushrooms. We then determined the slope to the point from the origin, and calculated the per cent change in the slope from the ascending to the descending part of the curve. Given lack of homogeneity of variance, we used the nonparametric Mann–Whitney U-test to determine whether cut mushrooms (mass loss method) had a significantly greater likelihood of a decrease in slope compared with intact mushrooms (porometer method).

Results

Isotopic determination of water sources

Soil $\delta^{18}O$ varied with depth, with isotopic enrichment increasing toward the surface (Fig. 2). In dry soils, roots near the surface were less enriched than surface soils and were isotopically indistinguishable from groundwater and deeper soil water (Fig. 2b,d,e), consistent with a model of hydraulic lift. Within sporocarps of both *B. edulis* and *A. muscaria* caps were consistently enriched in ^{18}O relative to stipes. This enrichment was greater in *B. edulis* than *A. muscaria* at a given location (Fig. 2). The relationship between stipe and surface soil $\delta^{18}O$ varied with surface soil moisture. When soils had higher water content, stipe bases were enriched relative to surface (0–10 cm) soils. However, in drier soils, stipe bases were depleted relative to surface soils. Assuming that stipe water will always be enriched through evaporation relative to its source pool, this pattern is consistent with a model of uptake of some deeper (> 10 cm deep) soil water under dry conditions with an increasing use of soil surface water in wetter soil conditions.

To determine the likely depth of the water source we need to estimate the magnitude of evaporative enrichment of stipes relative to source water. The estimated importance of deeper water sources increases with increasing corrections for evaporative enrichment. Evaporative enrichment of the interior of the stipe is expected if there is any back-diffusion of evaporatively enriched water from the surface of the stipe (the Péclet effect; Farquhar & Lloyd, 1993).

We do not have all information necessary to calculate the magnitude of this effect. However, we used three other approaches to estimate the magnitude of evaporative enrichment. First, we determined how our results would be affected if stipe tissue is as enriched relative to its source water as cap tissue is to stipe tissue. The average *B. edulis* cap isotopic enrichment relative to stipes is 8.39‰, and the minimum is 6.2‰. As another constraint on minimum amount of stipe isotopic enrichment, under conditions of high water availability we would expect sporocarp isotopes to reflect input of the readily available surface soil water rather than deeper water. We observed that the stipe tissue in the sporocarp from the wettest surface soil is enriched 4.95‰ relative to surface soil, which is in turn the most enriched water source available (Fig. 2f). Thus, if we make the conservative assumption that surface water is the sole source of stipe water for that sporocarp, then evaporative enrichment is 4.95‰. To test more formally the assumption that more surface water was used in wetter soils we regressed the relative enrichment of stipes relative to surface soil against average soil moisture in the top 0–10, 0–20 and 0–30 cm of soil. We found significant positive relationships for all, with the strongest relationship for 0–30 cm (Fig. 3). This is consistent with a model of increased use of surface soil water as soil water availability increases. The fitted value for the regression at the highest soil water availability is 3.45‰ (Fig. 3c, right end of regression line). This is the most conservative correction used and is most likely overly conservative, as it does not permit modeling of sources for the sporocarp from the wettest soil because corrected sporocarps are still enriched relative to all source pools.

We modeled sources using three corrections for stipe isotopic enrichment: 6.2‰ (minimum cap – stipe enrichment), 4.95‰ (observed wet soil stipe-surface soil enrichment) and 3.45‰ (fitted stipe enrichment relative to soil). For the ISOSOURCE model using the intermediate 4.95‰ correction, surface (0–10 cm) soils accounted for a maximum of 20% of sporocarp water in all but the wettest sites (Fig. 4a). Sporocarps from the driest soils exhibited the highest minimum percentage (25%) of deep water (> 30 cm or root water), whereas the two wettest soils yielded minimum estimates of zero deep water (Fig. 4d). Sporocarps of *A. muscaria* were not constrained by the model to use as much deep water or as little surface water as *B. edulis* (Fig. 4a,d).

The result is sensitive to the value of the correction used for evaporative enrichment. A larger correction increases the minimum proportion of deep water and decreases the maximum proportion of surface water, whereas a lower correction has the reverse effect, reducing the minimum proportion of deep water and increasing the maximum proportion of surface water. For example, the extremely conservative correction of

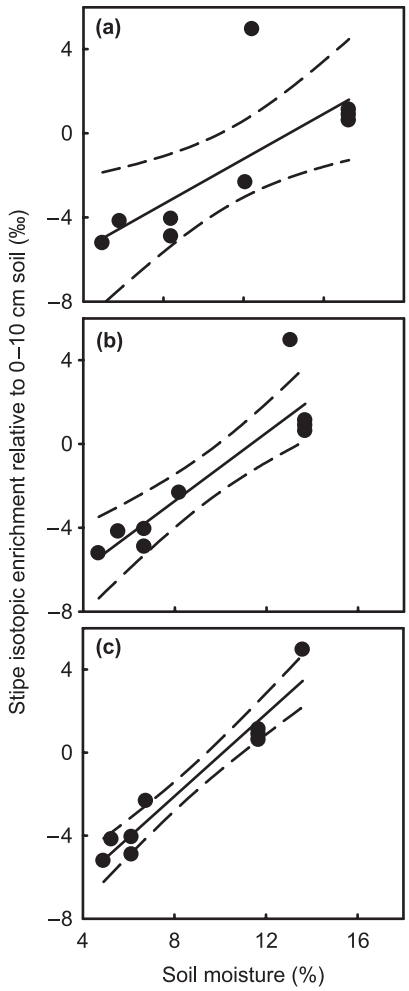

Fig. 3 Regression relationships between *Boletus edulis* stipe $\delta^{18}O$ enrichment relative to surface (0–10 cm) soils and soil moisture at various depths: (a) 0–10 cm soil moisture, $r^2 = 0.57$, $P = 0.02$, maximum fitted enrichment = 1.60‰; (b) 0–20 cm soil moisture, $r^2 = 0.82$, $P = 0.0008$, maximum fitted enrichment = 1.91‰; (c) 0–30 cm soil moisture, $r^2 = 0.94$, $P < 0.0001$, maximum fitted enrichment = 3.45‰.

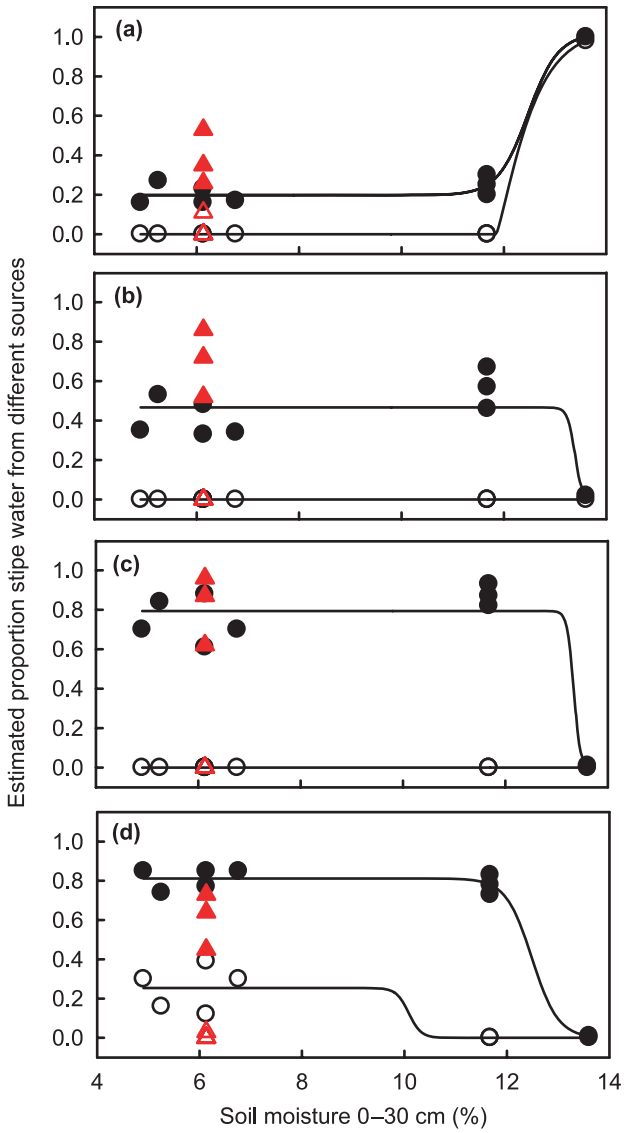

Fig. 4 The relationship between per cent soil moisture (0–30 cm) and the estimated minimum and maximum proportion of *Boletus edulis* stipe water from four different source pools, based on an isotopic source partitioning model, using a 4.95‰ correction for stipe $\delta^{18}O$ isotopic enrichment. Different panels represent the proportion of stipe water derived from a specific pool: (a) 0–10 cm soil water, (b) 10–20 cm soil water, (c) 20–30 cm soil water, (d) roots+ > 30 cm soil water/groundwater). *Boletus edulis* data were fitted with sigmoid curves; lower and upper lines are fits to the model data for minimum and maximum proportion water derived from that pool, respectively. Circles, *B. edulis*; Triangles, *Amanita muscaria*. Open symbols, model minimum estimates; closed symbols, model maximum estimates.

3.45‰ would require no minimum amount of > 30 cm/root water, but still would limit average maximum surface soil water to 39% of stipe water, with the remainder from > 10 cm or roots. The correction of 6.2‰ leads to estimates of an average minimum > 30 cm/root water contribution of 59%, and an average maximum surface soil water contribution of 15%. Thus, the most conservative conclusion regarding the hypothesis of the use of deep or root water is that under dry conditions surface soil (0–10 cm) water can account for no more than 40% of sporocarp water, on average, with the rest provided by some combination of deeper soil and roots.

Sporocarp water loss patterns

Water-loss rates were highest at midday and varied among species and individuals (Fig. 5), and over the course of a day individual sporocarp per cent water-loss rates were very well predicted by VPD (Fig. 6). The slope of the VPD–water loss relationship was much steeper for *A. muscaria* than for other species.

When we examined diurnal trends in the slope of the water loss–VPD relationships we found that the per cent change in the slope over the course of the day was relatively more negative

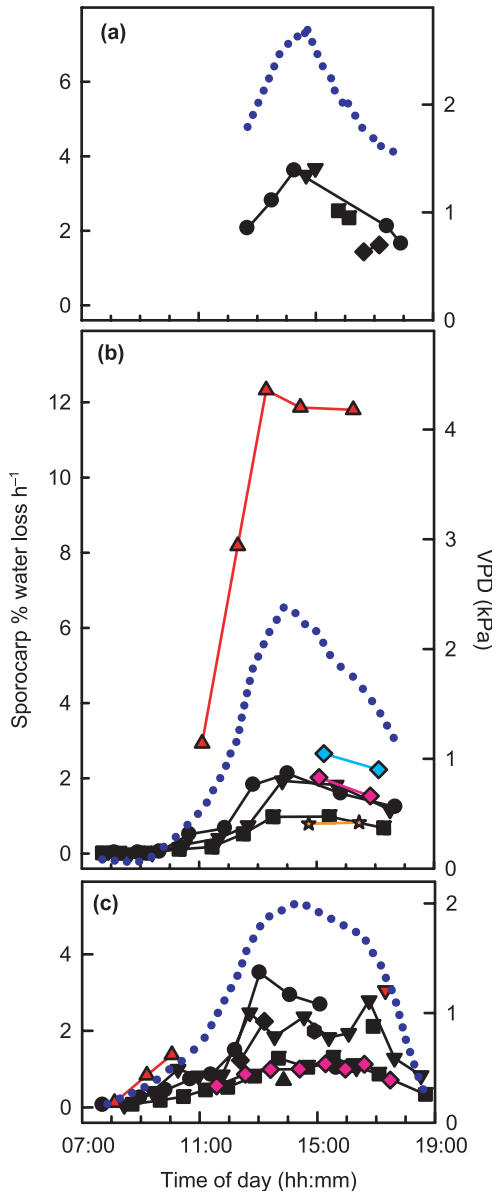

Fig. 5 Diurnal sporocarp water-loss rate in South Lake Tahoe, CA, USA. Measurements were taken on (a) 21 September 2000, (b) 28 September 2000, (c) 23 October 2001. Black closed symbols, different individual *Boletus edulis* sporocarps; red triangles, *Amanita muscaria*; blue diamonds, *Suillus* sp.; pink closed diamonds, *Tricholoma* spp.; orange stars, *Russula albonigra*. Blue dotted lines are fitted vapor pressure deficit (VPD).

for cut mushrooms than for intact mushrooms ($P = 0.042$). Over the course of the day, the slope of the water loss–VPD relationship actually increased in intact sporocarps (50 ± 35% SE), indicating that when *B. edulis* sporocarps remained attached to their water source there was, on average, no decline in the water loss–VPD relationship over the course of the day, suggesting that water transport into the sporocarps occurred over the course of the day, rather than being confined solely to nocturnal recharge. By contrast, the slope decreased

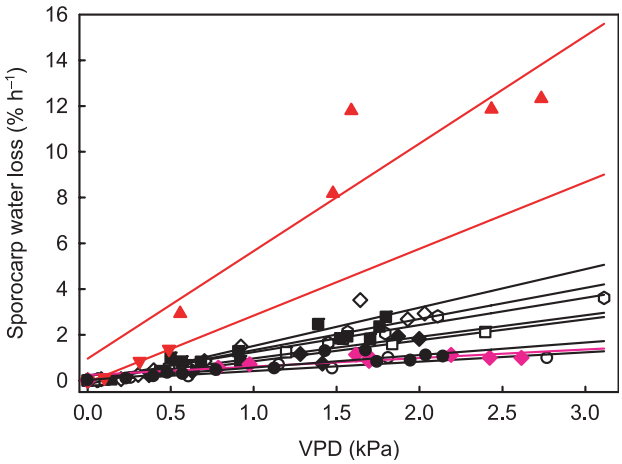

Fig. 6 Sporocarp per cent water-loss rate as a function of vapor pressure deficit (VPD) for individual sporocarps of three species. Each line represents is a least-squares regression for an individual sporocarp. Black and white closed symbols, *Boletus edulis*; red closed symbols, *Amanita muscaria*, with measurements from different sporocarps represented by different symbols; pink closed diamonds, *Tricholoma* sp. Average r^2 and P values for regressions: *B. edulis*, $r^2 = 0.91 \pm 0.02$, all $P < 0.0002$; *A. muscaria* $r^2 = 0.94 \pm 0.05$, all $P < 0.01$; *Tricholoma* sp., $r^2 = 0.76$, $P = 0.002$.

in cut sporocarps (–35 ± 16% SE), indicating that when *B. edulis* sporocarps were cut off from their water source, the slope of water loss–VPD relationships declined over time. Apparently, dehydration over the course of the day, in the absence of resupply of water from roots or soils, was sufficient to reduce water-loss rates.

Sporocarps varied greatly in the percentage of water they lost. Using data from transient porometers we estimated that over the course of a day *B. edulis* transpired 13.4 ± 1.3% of its water content. By contrast, *A. muscaria* transpired 50.7 ± 13.2%. Of the species sampled only once, water-loss rate for the erumpent *R. albonigra* (5.4%) was just below the range of *B. edulis*, *Tricholoma* sp. (8.6%) and *T. saponaceum* (11.6%) were within the range, and *Suillus* sp. (20.9%) was just above the range.

Porometer vs mass loss comparison

Comparison of slopes of water loss–VPD relationships estimated via both porometer and mass-loss methods indicated that slopes derived from the porometer method were 54 ± 1.8% of those derived from initial mass loss before dehydration. Thus, actual 24-h per cent water turnover rates under ambient conditions are probably closer to 25% for *B. edulis*, and 100% for *A. muscaria*.

Effect of surface to volume ratios of sporocarps on water loss

Measured and calculated volumes gave similar estimates of surface to volume ratios ($r^2 = 0.90$, slope = 0.89), suggesting

Table 1 Surface to volume ratio data used in estimation of relationship between water loss, vapor pressure deficit (VPD) and surface to volume ratios. Data are means ± standard error

Species	Surface area (cm^2)	Calculated volume (cm^3)	Volume measured/ volume calculated	Surface to calculated volume ratio (cm^2 : cm^3)	*n*
Boletus edulis	394 ± 44	551 ± 82	1.06 ± 0.05	0.65 ± 0.05	20
Tricholoma sp.	164 ± 31	127 ± 33	1.10 ± 0.16	1.37 ± 0.58	4
Tricholoma saponaceum	78 ± 8	29 ± 5	1.10 nd	2.88 ± 0.11	11
Amanita muscaria	231 ± 68	136 ± 66	0.98 ± 0.07	1.88 ± 0.24	3
Russula albonigra	477	416	1.02	1.14	1

nd, No data. Volume was measured for all mushrooms in a caespitose cluster of this species as a group, so no error was estimated.

that our idealized shapes were a reasonable approximation of sporocarp morphology. Species differed greatly in their surface : volume ratios (Fig. 1, Table 1). *Boletus edulis* had the lowest ratio, followed by *R. albonigra*, *Tricholoma* sp., *A. muscaria* and *T. saponaceum*. The slopes of sporocarp water loss–VPD relationships for *B. edulis* and *A. muscaria* were positively related to surface to volume ratio, and were both fitted by the same linear relationship (Fig. 7). However, *Tricholoma* species and *R. albonigra* deviated from this pattern, exhibiting lower slopes for a given surface : volume ratio.

Water loss from caps and stipes

Sporocarps lost water from both caps and stipes. Rates of water loss from stipes were 54 ± 0.08% of those from caps.

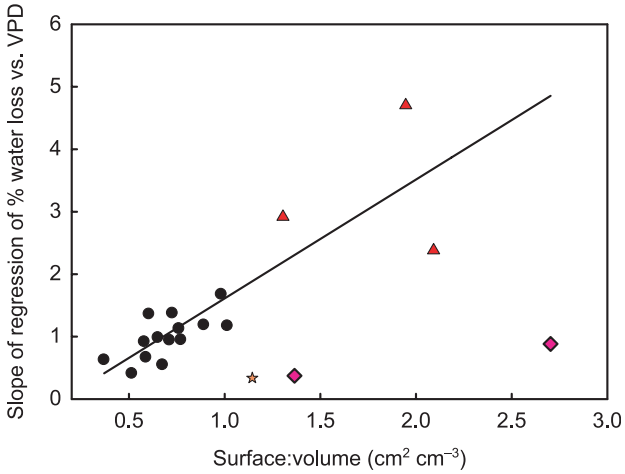

Fig. 7 Relationship of the slope of vapor pressure deficit (VPD)–water-loss relationships with sporocarp surface to volume ratios. *Boletus edulis* (circles) and *Amanita muscaria* (triangles) are fitted well by the same equation (solid line; $r^2 = 0.71$, $P < 0.0001$), whereas *Tricholoma* spp. (diamonds) and *Russula albonigra* (star) fell well below this line.

Discussion

Water sources

Our isotope results are consistent with a model of mycorrhizal access to deeper soil water under drought conditions, either via hydraulic lift from their plant associates or via direct hyphal transport of deeper soil water. A conservative value for the evaporative enrichment of stipes requires some water from deep sources. However, the estimated magnitude of deep water source use is sensitive to the correction chosen for stipe evaporative enrichment. We believe that the 4.95‰ correction is conservative for two reasons: it is the minimum correction needed to bring all stipe isotope ratios within the range of soil water sources; it is well below (60%) of the cap enrichment relative to stipes. The 50% lower rates of percent water loss from *B. edulis* stipes than from caps could actually lead to higher evaporative enrichment rates of stipes, as the Péclet effect depends on the balance of convection and diffusion – when diffusion dominates over convection (small Péclet number), the potential for enrichment at a distance from the source of evaporation is greater (Farquhar & Lloyd, 1993). For the same reason we would expect *A. muscaria*, which had higher mass-specific water flux rates and higher stipe surface to volume ratios, to have a lower stipe to cap enrichment. Indeed, enrichment of *A. muscaria* caps relative to stipes (6.2 ± 0.6‰) was less than that of *B. edulis* (9.1 ± 1.3‰) within the same plot.

Use of hydraulically lifted water by ectomycorrhizal fungi has been found in both mesocosms (Querejeta *et al.*, 2003) and field studies (Warren *et al.*, 2007, 2008; T. E. Dawson, unpublished data). Our pine root isotopic values indicate they are carrying out hydraulic lift, consistent with the results of other studies (Brooks *et al.*, 2002; Peñuelas & Filella, 2003; Espeleta *et al.*, 2004). The feasibility of transfer of hydraulically lifted water to ectomycorrhizas depends largely on the fungal conductance from root vs soil sources. Given the dry surface soil conditions at these sites and direct linkage of fungi to host roots via rhizomorphs, roots recharged with water overnight would provide a ready source of water to mycorrhizal fungi. How well those fungi would compete with aboveground water sinks during the day would depend on the water potential of the sporocarp compared with those in the plant canopy. The fact that we saw no decline in the VPD–water loss

relationships during the day in intact sporocarps suggests that water continues to be delivered to sporocarps over the course of the day; however, we do not know if this is root or soil water. The analysis of the isotope composition of water entering sporocarps might indicate whether ectomycorrhizal fungal water sources shift from host to soil water over the course of the day.

Effective movement of water from hosts to sporocarps requires efficient transport mechanisms for water, with rhizomorph anatomy likely influencing capacity for water transport. Rhizomorphs are organized mycelial secondary structures with varying degrees of size, hydrophobicity and hyphal differentiation (Agerer, 2001). *Boletus edulis* and *Suillus* sp. are well adapted to long-distance water transport, possessing complex rhizomorphs with enlarged, vessel-like internal hyphae providing low resistance to water transport within hydrophobic rhizomorphs (Fig. 1d). *Tricholoma* spp. tend to have morphologies that range from medium to long-distance exploration types (Agerer, 2001). We observed the medium distance types (25–150 μm diameter with relatively undifferentiated hyphae) associated with *Tricholoma* sp. sporocarps, and *Tricholoma saponaceum* has been described as possessing rhizomorphs of medium to long-distance exploration types with varying levels of differentiation (Yamada *et al.*, 2001). *Amanita muscaria* rhizomorph morphology is described as of the medium distance smooth type that is typically undifferentiated (Mohan *et al.*, 1993; Cripps & Miller, 1995; Agerer, 2001).

Lack of well-developed rhizomorphs may constrain epigeous fruiting under dry conditions. *Russula albonigra* was not observed fruiting above ground, but erumpent sporocarps were repeatedly observed. *Russula* species have not been found to produce rhizomorphs and have hydrophilic hyphae (Agerer, 2001), so this species may be constrained to this fruiting habit both by limited availability of water near the surface, and by inability to transport sufficient water through undifferentiated hydrophilic hyphae to produce an epigeous sporocarp even in the presence of hydraulically lifted water.

Combining well-developed rhizomorphs, low sporocarp surface-volume ratio and apparent access to relatively deep water, *B. edulis* has clearly developed a successful strategy for epigeous reproduction under desiccating conditions where groundwater is accessible. *Amanita muscaria* appears more prone to desiccation because of higher surface to volume ratio which drives extremely high water-loss rates, as well as less differentiated hyphal anatomy that likely constrains the range of habitats in which this species can fruit. This species drops spores very rapidly after expansion (Li, 2005), which may be an alternative strategy for dispersing spores effectively under these adverse conditions. A direct comparison of gross spore production per unit carbon or nitrogen used would enhance our understanding of the relative resource use efficiency of these two strategies.

An alternative hypothesis for the origin of the high rates of water loss by *A. muscaria* is that they parasitize *B. edulis*. *Boletus edulis* and *A. muscaria* co-occur often enough that the latter is often cited as an indicator for *B. edulis* (Arora, 1986). There is some evidence of a potential tripartite interaction between host plants, *Boletus edulis* and *A. muscaria*, as shown by direct observations of *B. edulis* hyphae merging with *A. muscaria* ectomycorrhizas (Yun & Hall, 2004). Given the profligate water use by *A. muscaria* combined with relatively less developed rhizomorphs, it seems possible that *A. muscaria* is a facultative parasite of the more drought-adapted *B. edulis*, deriving water and carbon from *B. edulis* mycelia. This type of relationship has been observed between Gomphidiaceae and suilloid ectomycorrhizal fungi (Olsson *et al.*, 2000). Furthermore, parasitism in mistletoes has been associated with less conservative water use by the parasite than the host (Ehleringer *et al.*, 1986). However, the morphology might also be indicative of the reverse, with *B. edulis* parasitizing *A. muscaria*.

Controls on water loss

For fungi to produce, release and disperse sufficient numbers of spores their sporocarp morphology must balance the constraints of sporocarp structure, spore release and boundary layer interactions with the constraints imposed by availability of water and carbon for sporocarp growth and maintenance. The strong control that surface to volume ratios exerted on water loss in the present study indicates the potential for low surface to volume ratios to be effective in buffering sporocarps against desiccation. This has also been found in a laboratory setting (Badham, 1985).

However, the carbon cost of the low surface to volume morphology is substantial, with most carbon going into nonreproductive tissue. Therefore, if low surface to volume ratio is selected for by high VPD we would expect to find that surface to volume ratios increase with increasing water availability and decreasing VPD during fruiting. Both genetic (McKnight, 1992) and environmental (Plunkett, 1956) factors might drive such variation. A biogeographic analysis of the variation in morphology of *B. edulis* from different climates would be enlightening in this regard.

A variety of other traits have the potential to reduce water loss. Although *Tricholoma* sp. also have relatively high surface to volume ratios, they deviated from the expected relationship of surface to volume vs water-loss rate exhibited by other species. One hypothesis is that this is caused by the caespitose (clumped sporocarps) fruiting morphology (Fig. 1c) exhibited by both species; this should reduce evaporative loss by increasing the thickness of the boundary layer around individual mushrooms. *Russula albonigra* also lost less water than expected based on its morphology. This is not surprising, as it exhibited an erumpent fruiting habit (i.e. the sporocarp was mostly subterranean, with only a small portion exposed to the air). This habit is transitional between the fully epigeous and full hypogeous habits, and indicates one advantage of hypogeous fruiting for sporocarp water balance.

Other factors, such as developmental stage (intactness of partial veil, openness of pores, spacing of gills) could also influence effective surface area. In addition, any factor that affects the resistance to water flux at the surface of the sporocarp, such as desiccation, size, surface roughness, osmotic potential of surface cells, hydrophobic coatings, etc., would also alter flux rates.

Links between water and carbon balance

If hydraulic lift is an important mechanism for provision of water to these sporocarps, this presents the intriguing possibility that loss of water facilitates the rapid delivery of host sugars from roots to sporocarps. Sporocarp mass gain is substantial over very short periods, requiring rapid mobilization of large quantities of fixed carbon. Mass flow of sugars and sugar alcohols in water directly transferred from roots to the ectomycorrhizal partner would provide a very efficient mechanism for such transfer.

Alternative models of sugar and water transport in fungal mycelium include diffusion, cytoplasmic streaming, and turgor-driven mass flow (Cairney, 1992, 2005). All of these mechanisms could well be functioning simultaneously. Cairney (2005) questioned the importance of mass loss of water via evaporation in driving water and solute movement in mycelium in soil under most circumstances. However, it is clear that when fungi fruit epigeously the evaporative gradient increases greatly, and thus mass flow through hyphae driven by evaporative water loss has the potential to be a driver of substantial water and carbon flux under these conditions.

Method comparison

We expect that the mass-loss method is likely to be a better estimator of actual water-loss rates, at least before sporocarp dessication, because sporocarps are in ambient conditions of temperature, humidity, insolation and wind speed. By contrast, the porometer method is nondestructive, providing a more accurate time-course of relative rates of water loss than the mass-loss method, and provides data under comparable conditions of insolation and windspeed. Thus, for comparison of the effects of morphology and growth habit, the porometer method is more useful.

Conclusions

Some ectomycorrhizal fungi appear to be able to access deep soil water sources, either via hydraulic lift via host plant roots or by mycelial translocation from deeper soil. Whatever the mechanism, access to this deeper water likely contributes to the ability of these fungi to survive, reproduce and function symbiotically under summer drought conditions. Furthermore, sporocarp surface to volume ratios appear to influence water loss–VPD relationships. The potential for evaporative water flux to facilitate carbon transfer to sporocarps is high, pointing to the potential for evolutionary optimization of traits that regulate water loss by fungal sporocarps, in order to balance between the stress associated with desiccation and the potential benefit of accelerated carbon and nutrient supply to support rapid sporocarp growth and spore dispersal. A better understanding of these trade-offs may elucidate some of the forces that have contributed to the high diversity of fungal reproductive strategies.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Notes S1 Calculation of potential influence of metabolic water on sporocarp oxygen isotopic signature

Table S1 Results of sensitivity analysis of metabolic water effect on stipe water $\delta^{18}O$ isotopic signature

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