Text S1. Mixing model

$\delta^{13}C\text{-}CO_2$ was proportional to inverse of CO_2 concentration in the headspace, as expected for stable isotopic mixing models (cf. Kendall and Caldwell, 1998). In our case, the labeled bicarbonate amendment could contribute to a total CO_2 concentration ($[CO_2]$) of ~ 0.3 %, which mixed with CO_2 released from respiration of the substrates during the incubation; this assumption neglects the known fractionation of CO_2 exchange between the headspace and medium, which we assume to be minimal given the high label (target AT% ^{13}C = 10%). Data from all incubations plotted along a hyperbolic curve, which was modeled as a simple inverse relationship:

$$\delta^{13}C_{CO2} = (929 \times 1/[CO_2]\ ) + \delta^{13}C_{subs} \qquad \textit{Equation S1}$$

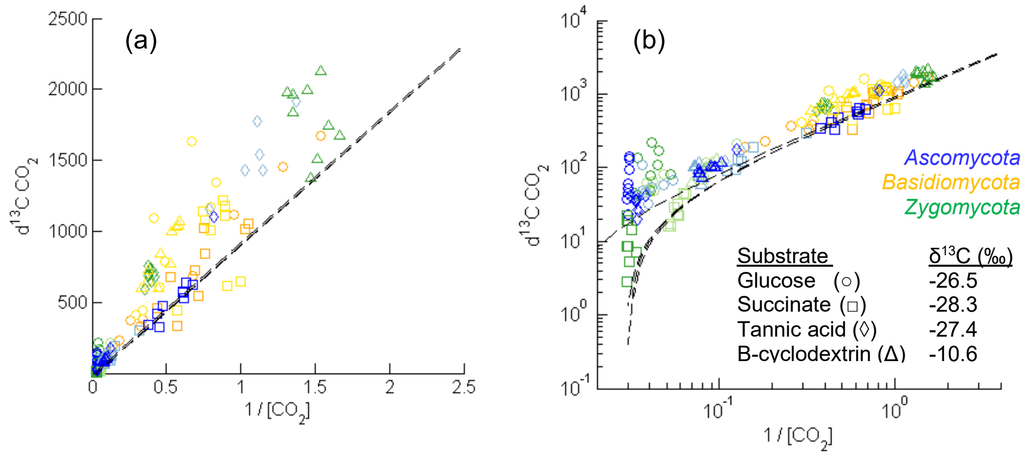

Figure S1. Hyperbolic evolution of $\delta^{13}C\text{-}CO_2$ with increasing $[CO_2]$ released from respiration, for incubations of *Ascomycota* (blue), *Basidiomycota* (orange/yellow), or *Zygomycota* (green) with glucose (circles), succinate (squares), tannic acid (diamonds), or β-cyclodextrin (triangles). Panel B is the log-log plot of panel A, indicating the expected mixing lines for respiration of each substrate.

The fit gave an R^2 of 0.8047 and RMSE of 251. These values improved when considering only incubations in which CO_2 levels increased above 1% (i.e., those that yielded sufficient biomass).

Because fungal lipid production occurred during the hyperbolic evolution of $\delta^{13}C\text{-}CO_2$, the accuracy of the %IC calculation (Eq. 1, main text) can be improved by estimating the weighted average $\delta^{13}C$ of inorganic C that could have been incorporated into lipids during the incubation as respired CO_2 accumulated. The weighted average stable C isotope composition of CO_2 that fungi were exposed to during the incubation can be approximated by the integral of the hyperbolic curve, normalized by $[CO_2]$ at harvest. The resulting integral is a logarithmic function of $[CO_2]$. Because we are considering incubations in which $[CO_2]$ approached 1%, where the integral function approaches zero (i.e., LN 1 = 0), the data were rather fitted by a hyperbolic curve described by the function:

$$\delta^{13}C_{CO2} = (a\ /\ (1 + [CO_2]) + b$$

Furthermore, the trend was more accurately approximated by plotting against $^{13}F\text{-}CO_2$ rather than $\delta^{13}C\text{-}CO_2$, with improved R^2 of 0.8472 and RMSE of 0.0016, and coefficients a = 0.02478 ± 0.00167 and b = 0.01054 ± 0.00037 (Fig. S2). Thus, the weighted average equation becomes:

$$\text{Weighted average } ^{13}F_{CO2} = (0.02478 \times LN(1+[CO_2]) + (0.01054 \times [CO_2]) \qquad \textit{Equation S2}$$

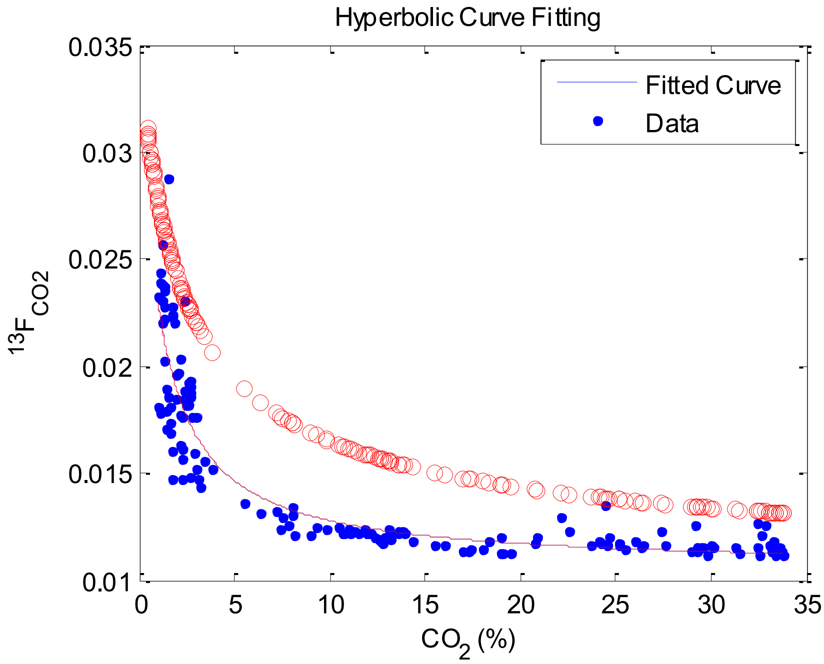

Figure S2. Hyperbolic curve of decreasing $^{13}F_{CO2}$ with increasing CO_2 in headspace during fungal respiration of substrates (filled blue circles). The fitted curve representing Eq. S2 is shown in pink. The weighted average $^{13}F_{CO2}$ during fungal production in each incubation (open red circles) was estimated as the integral of the fitted curve normalized by the CO_2 concentration at harvest.

Finally, the estimated weighted average $^{13}F_{CO2}$ was used to calculate the heterotrophic incorporation of inorganic C into lipids (%IC) via Eq. 1 of the main text, which amounted to < 3% for all experimental incubations. Sensitivity analysis of the fitted coefficients (i.e., 95% confidence intervals) indicated a coefficient of variation of the reported %IC values of up to 30%, with the highest reported fungal %IC values (i.e., *Penicillium jancewskii* grown on tannic acid) ranging from 1.8 to 2.8%.

Literature cited

Kendall, C., Caldwell, E. A. (1998). Fundamentals of isotope geochemistry. In Isotope tracers in catchment hydrology (pp. 51-86). Elsevier.