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A laboratory experiment of carbon stable isotope fractionation by several fungal species

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Fungi are a critical component of the carbon cycle, having an important role in the decomposition of soil organic matter. During the decomposition process, CO_2 is released in the soil where it dissolves into infiltrating water, forming carbonic acid. In carbonate settings, the resulting carbonic acid is the main driver behind karst formation and speleothem deposition. The isotopic composition of carbon in CO_2 is one of the main factors affecting the $\delta^{13}C$ in speleothems, an important paleoenvironmental proxy.

In this study, we investigate the extent to which several fungi species are fractionating carbon isotopes during their development, as compared to the growth medium. For this experiment, we took four soil samples under grassland and forest cover in the Mehedinţi Mountains (SW Romania), where speleothem $\delta^{13}C$ records were published (Drăguşin *et al.*, 2014; Staubwasser *et al.*, 2018) and a cave monitoring program is ongoing (Drăguşin *et al.*, 2017, 2020). Soil samples were taken from 10 cm and 30 cm depths, aliquots of 1 g from each sample were mixed with 9 mL of sterile water solution, then three-fold dilutions were obtained and 100 µL aliquots of each dilution were spread on Sabouraud dextrose agar (this medium contains glucose, meat peptone, casein peptone, and agar). The fungal colonies were observed after 5–7 days after incubation at room temperature and colonies with different morphologies were randomly selected from each sampling point to be subcultured on the same media in order to obtain pure cultures. Examination of macroscopic characters of culture and colony, together with examination of mycelium structure under the microscope allowed a presumptive phenotypic identification, followed by the confirmation of fungal species / genera by MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization-Time of Flight) mass spectrometry.

All strains of isolated microfungi belong to the *Ascomycota* phylum: *Aspergillus*, *Fusarium*, *Penicillium*, *Trichoderma*, *Cladosporium*, and *Scopulariopsis* genera.

For this study, we have selected some of the filamentous fungal strains: *Fusarium oxysporum* isolated from both grassland and forest ecosystems, *Trichoderma* sp. that has been dominant in the grassland, *Fusarium proliferatum* isolated from grassland soil, and *Scopulariopsis brevicaulis* identified in forest soil. All the microfungi were individually cultured for 13 days on Sabouraud agar, as well as on maize (*Zea mays*), a C4 plant. After incubation, the next step consisted of sampling from maize together with the mycelium developed and separating the fungal biomass, respectively, followed by freezing at −20 °C and then, freeze-drying.

An amount of 0.5 mg–0.6 mg from each sample, as well as growth medium samples, were analyzed for $\delta^{13}C$ at the Stable Isotopes Laboratory of the Research Institute of the University of Bucharest

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(ICUB-SIL) using a Nu Instruments Horizon 2 Isotope Ratio Mass Spectrometer (IRMS) coupled to a EuroVector 3024 elemental analyzer. The samples were corrected using an in-house standard, a C4 plant (Blue Grama), and two international standards, IAEA-CH3 and IAEA-600. Within-run standard deviation values for these standards were 0.15‰ for Blue Grama, 0.08‰ for IAEA-CH3, and 0.06‰ for IAEA-600.

Initial growth medium values were −12.95‰ for maize and −20.00‰ for Sabouraud. After 4 days since cultivation, maize had average values of −12.66‰ (SD=0.04‰, n=6), while after 7 days the average δ^{13}C value was −12.52 (SD=0.3, n=6). Sabouraud medium was sampled only on the 13th day after cultivation and had an average value of −18.40‰ (SD=1.26, n=6).

Fungal biomass recovered from Sabouraud agar after 13 days of cultivation exhibited in all cases an enrichment in ^{13}C between 2.44‰ and 5.34‰ This is in accordance with other similar studies that found ^{13}C enrichment between fungi and their growth substrate (Henn *et al.*, 2002; Zeller *et al.*, 2007).

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References

Drăguşin, V., Staubwasser, M., Hoffmann, D.L., Ersek, V., Onac, B.P., Vereş, D., 2014. Constraining Holocene hydrological changes in the Carpathian-Balkan region using speleothem δ^{18}O and pollen-based temperature reconstructions. Clim. Past., 10, 1363–1380. 7

Drăguşin, V., Balan, S., Blamart, D., Forray, F.L., Marin, C., Mirea, I., Nagavciuc, V., Orăşeanu, I., Perşoiu, A., Tîrlă, L., Tudorache, A., Vlaicu, M., 2017. Transfer of environmental signals from the surface to the underground at Ascunsă Cave, Romania. Hydrol. Earth Syst. Sci., 21, 5357–5373. 7

Drăguşin, V., Mirea, I.C., Ersek, V., Tîrlă, L., 2020. Farmed calcite δ^{13}C at Ascunsă cave, Romania, and its relation with CO_2 outgassing and drip rate. Quaternaire, 31/2, 165–174. 7

Henn, M.R., Gleixner, G., Chapela, I.H., 2002. Growth-dependent stable carbon isotope fractionation by basidiomycete fungi: δ^{13}C pattern and physiological process. Appl. Environ. Microbiol., 68, 4956–4964. 8

Staubwasser, M., Drăguşin, V., Onac, B.P., Assonov, S., Ersek, V., Hoffmann, D.L., Vereş, D., 2018. Impact of climate change on the transition of Neanderthals to modern humans in Europe. Proc. Natl Acad. Sci. USA, 115, 9116–9121. 7

Zeller, B., Brechet, C., Maurice, J.P., Le Tacon, F., 2007. ^{13}C and ^{15}N isotopic fractionation in trees, soils and fungi in a natural forest stand and a Norway spruce plantation. Ann. For. Sci., 64, 419–429. 8