

Changes in host-mycorrhiza relationships revealed by stable isotopes after naturally-induced thinning of the stand: case study on *Tuber aestivum*.

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Terrestrial plants overcome nutrients and water limitations by forming mutualistic associations with mycorrhizal fungi. Fungi, in return, take advantage from the carbohydrates supplied by the host. Some mycorrhizal fruit bodies, like that of *Tuber* spp., have a peculiar gastronomic value with many efforts being undertaken to predict and enhance their productivity. However, many issues of truffle-producing mycorrhizal ecology are still poorly understood, in particular optimal conditions favoring fruit formation, potential host plants and host-mycorrhiza relationships. In this study, we tested the applicability of stable isotope measurements under natural abundance to identify the plants which likely host the mycorrhiza of *Tuber aestivum* and to characterize host-mycorrhizal nutrient, water and carbohydrate exchange under plant natural growing conditions and with the change of the forest cover after naturally occurred thinning. For these purposes, sampling of the fruit bodies of *T. aestivum* was performed during the growing season 2011 in a mixed broadleaved-coniferous forest in central Italy (initially the site was a manmade pine plantation). Nine truffle-producing parcels were identified with five being composed of the original *Pinus pinaster* -dominated vegetation and four in which pine was replaced by broadleaf species after both wind-induced thinning and natural dieback of pine trees. Seasonal variation of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ were analyzed in the fungal material, in the surrounding soil and in the plant material of the potential host species (xylem water in the trunk, branches and leaves, recently assimilated carbohydrates in phloem and leaves). The results showed a possibility of the identification of the mycorrhizal host species applying isotope analyses, with mycorrhiza receiving most part of the carbohydrates from the pine in pine-dominated parcels. Interestingly, in thinned parcels, the truffle bodies maintained isotope composition similar to bodies gathered from parcels where pine was present. Change of the trophic strategy to saprotrophic nutrition with remaining pine stumps as a primary source of C explained the observed seasonal patterns of $\delta^{13}\text{C}$ in truffles in thinned parcels. Fractionation steps during the ^{15}N and H_2^{18}O uptake and upward transport and ^{13}C assimilation and its downward translocation were identified. The more N was available for mycorrhizal, the more enriched were the host pine trees in ^{15}N , suggesting that typical mycorrhizal fractionation against ^{14}N ceases with increasing N abundance. Change of the plant species composition has shortened the productive season of truffles, likely due to microclimatic variations induced by thinning. Our results also indicate that, although *T. aestivum* did not establish renewed mycorrhiza with new hosts within 9 years after the thinning, the fungus has a prolonged vitality. Such an evidence suggests that conifer thinning and conversion towards a broadleaved stand can be undertaken without immediate risk for *T. aestivum* persistence.