New Phytologist

Commentary

The radiocarbon age of mycoheterotrophic plants

Mycorrhizal symbiosis, a widespread, > 400 million-year-old mutualism, consists of soil fungi associated with plant roots. In this symbiosis, soil mineral nutrients are exchanged for photosynthetic products (Smith & Read, 2008; van der Heijden *et al.*, 2015). In plant evolution, the fungi involved in this association diversified, and the exchange was modified in some cases (van der Heijden *et al.*, 2015; Strullu-Derrien *et al.*, 2018). Several groups of achlorophyllous plants associated with fungi independently emerged, which reversed the ancestral carbon flow. The so-called mycoheterotrophic plants exploit carbon substrates from their fungal hosts (Merckx, 2013; Figs 1a, 2). These fungi obtain their own carbon from other sources: some are saprotrophic and exploit dead organic matter, whereas others are mycorrhizal on autotrophic plants that provide the carbon and energy for the whole consortium (Fig. 1a). In this issue of *New Phytologist*, Suetsugu *et al.* (2020; pp. 1519–1529) elegantly use radiocarbon (carbon-14 (^{14}C)) to assess the age of carbon in diverse mycoheterotrophic plants.

'. . . *Suetsugu* et al. *(2020) have now pushed* ^{14}*C methods into the area of carbon transfers by mycelia and mycorrhizal networks* in situ.'

The authors further support the mycoheterotrophic use of different carbon sources depending on the fungal partners, which results in different transit times from initial photosynthesis to final use by mycoheterotrophic plants (Fig. 1a). Suetsugu *et al.* (2020) argue that mycorrhizal fungi allow a short transit time, while the photosynthesized carbon used by wood decayers indicates a longer transit time, congruently with some new results we present here to discuss the relevance of these findings (Fig. 2).

^{14}C dating for very recent samples

Radiocarbon (^{14}C) is widely used as a clock for archaeological samples (Taylor *et al.*, 1996). This method, based on natural ^{14}C abundance, can be confidently used for samples older than 100–150 yr and younger than 50 000 yr. More recent samples can be dated by using the huge temporary increase of atmospheric ^{14}C concentration caused by nuclear weapon tests in the late 1950s and early 1960s (the 'bomb peak' period; Fig. 1b). These tests doubled the atmospheric ^{14}C abundance that has since decreased slowly, due to the rapid exchanges between atmospheric carbon dioxide (CO_2) and the Earth's large carbon reservoirs (ocean and biosphere), as well as combustion of ^{14}C-free fossil fuel (Nydal, 1968; Levin & Kromer, 1997). The global atmospheric ^{14}C concentration is regularly monitored at numerous locations, and the last official global estimates were by Hua *et al.* in 2013. These estimates account for differences between the Tropics, the Northern Hemisphere, and the Southern Hemisphere and provide different regional curves covering the period until 2009. The most recent global review of atmospheric $^{14}CO_2$ was assembled by Graven *et al.* (2017) but an official extension of the global reconstruction is not expected before mid-2020. The ^{14}C dating using the bomb peak is based on ^{14}C abundance in the sample and provides highly precise results for events close to the peak maximum in 1963: it loses its accuracy when moving away from the peak as shown by the two examples in orange and green in Fig. 1(b). However, due to the curve shape, a given ^{14}C abundance matches two intervals of dates, and independent information is required to eliminate one of these intervals (Fig. 1b; Suetsugu *et al.* (2020) and we used the most recent interval).

In the past, this method was successfully applied to the dynamics of carbon turnover in soil and in subterranean trophic chains (Balesdent & Guillet, 1982; Hyodo *et al.*, 2008). Considering fungi, ^{14}C abundance of fungal fruitbodies revealed that mycorrhizal fungi consisted of 0- to 2-yr-old carbon (Hobbie *et al.*, 2002), while saprotrophic fungi are composed of carbon from 1 to > 30 yr in age, due to more or less long storage in dead organic matter (Hobbie *et al.*, 2020). In the food chain represented by the autotrophic plants, the fungi mycorrhizal on roots or saprotrophic on dead plant remains, and the mycoheterotrophic plant (Fig. 1a), carbon age had never been estimated before, although the ecology of fungal associates can be expected to entail differences between species.

Radiocarbon dating of mycoheterotrophic plants

Suetsugu *et al.* (2020) used a large sampling of Japanese mycoheterotrophic orchids and Ericaceae plants, which associate with Basidiomycota fungi that are either ectomycorrhizal (i.e. associated with tree roots, such as Russulaceae or Thelephoraceae) or wood decayers (such as *Armillaria* or *Psathyrella* spp.). They revealed that the radiocarbon (^{14}C) age of carbon in mycoheterotrophic plants receiving resources from ectomycorrhizal fungi is similar to that of surrounding autotrophic plants, despite different nutrition types. Our own analyses reveal a similar pattern in a European forest for mycoheterotrophic orchids and Ericaceae

This article is a Commentary on Suetsugu *et al.* (2020), **227**: 1519–1529.

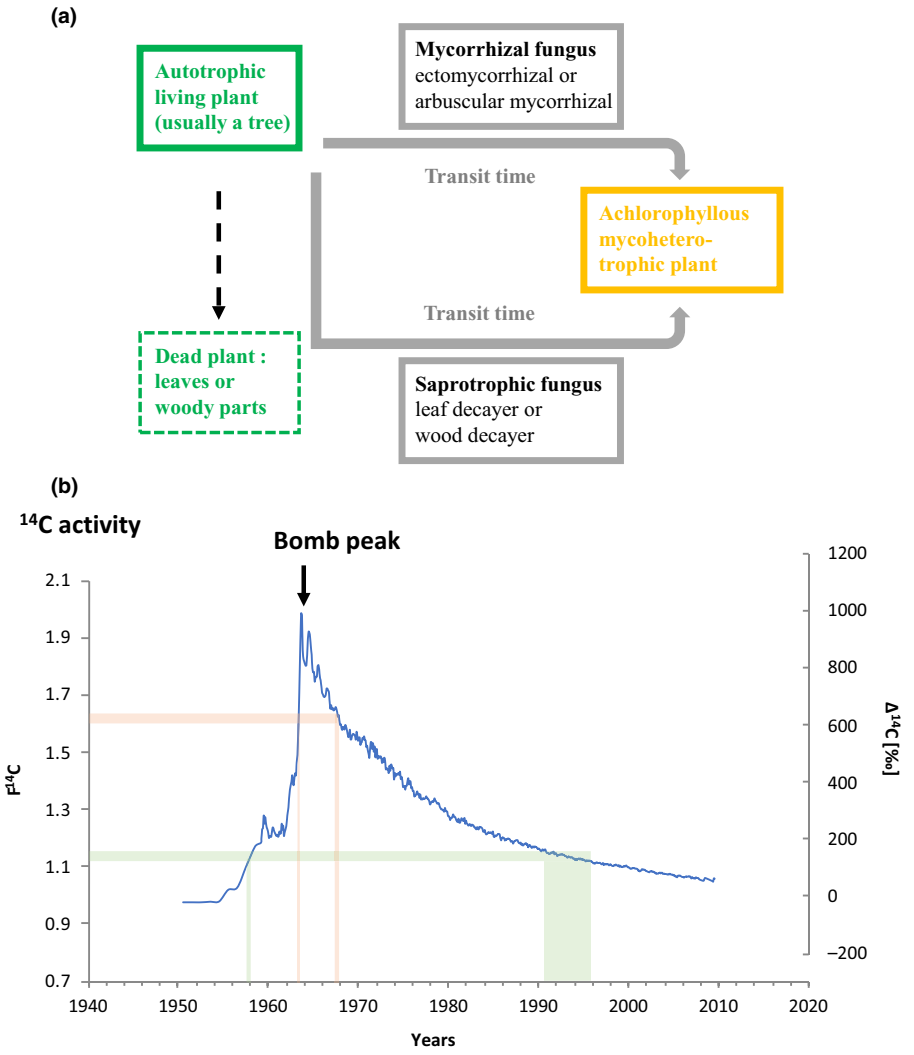

Fig. 1 The biological models and methods used by Suetsugu *et al*. (2020; pp. 1519–1529) in this issue of *New Phytologist*. (a) Biological model: the various ecologies of mycorrhizal fungi associated with mycoheterotrophic plants, which usually use a single partner fungal species that is ectomycorrhizal, or arbuscular mycorrhizal, or a wood or leaf decayer. (b) Methods of ^{14}C dating for recent samples: formal atmospheric ^{14}C record from Hua *et al*. (2013) for the Northern Hemisphere zone 1. Note the two commonly used units: $F^{14}C$, which shows ^{14}C activity samples relative to the ^{14}C international standard, and $\Delta^{14}C$, which shows deviation from the international standard. Orange and green lines exemplify the dating approach, and show that for a given uncertainty range in ^{14}C abundance (represented by the thickness of the horizontal line) the measurement projection on the curve (vertical lines) yields two calendar age intervals, one before and one after the maximum (here, only the latest is biologically relevant), and narrower age interval when close to the peak (orange lines) than remote from the 1960s (green lines). In their *New Phytologist* article in this issue, Suetsugu *et al*. (2020; pp. 1519–1529) use ^{14}C dating to estimate a transit time, that is the time from initial photosynthesis to final carbon utilization by mycoheterotrophic or mixotrophic plants.

(Fig. 2a; see Supporting Information, Methods S1; Table S1), where transit time from canopy leaves to the recipient mycoheterotrophic plants was evaluated as between 0 and 1.4 yr (median, 0.3–0.7 yr; see Table S1). Such a fast transit time is congruent with (1) the young age of carbon in fruitbodies of ectomycorrhizal fungi (as mentioned earlier; Hobbie *et al*., 2002) and (2) labelling experiments showing that photosynthates move quickly to ectomycorrhizal fungi, within days (Högberg *et al*., 2008; Teramoto *et al*., 2012) or months (Le Tacon *et al*., 2013), or to mycoheterotrophic plants (Björkman, 1960).

By contrast, for mycoheterotrophic plants associated with wood decayers, Suetsugu *et al*. (2020) demonstrated access to much older carbon (at least 10–40 yr). The mean age of the woody tissues digested and their slow decomposition, for example due to the presence of recalcitrant tannins and lignins, likely explain this age, which does not necessarily represent a slower transit in the fungal hyphae. For example, the transit time to *Armillaria*-associated orchids recorded by Suetsugu *et al*. (2020) ranges from 12 to 26 yr, a variability that may reflect that of the mean age of the woody organs themselves. For mycoheterotrophic species that associate with litter-decaying fungi, which were not investigated by Suetsugu *et al*. (2020), transit times should be shorter. We analysed *Wullschlaegelia calcrata*, a tropical mycoheterotrophic orchid from Guadeloupe (Fig. 2b; see Fig. S1; Methods S1; Table S1), whose roots are linked by conspicuous rhizomorphs of litter-decaying *Mycena* spp. to dead leaves (Martos *et al*., 2009; inset of Fig. 2b). As expected, dead leaves fallen from the canopy had older carbon than living leaves (Fig. 2b); the fungus had older carbon age; and the orchid had, in turn, even older carbon, perhaps due to admixture of fungal carbon and carbon stored from previous years in its starch-rich tuberous roots (Martos *et al*., 2009). In this case, the transit time was evaluated as between 6.7 and 9.9 yr (median, 8.4 yr); surprisingly long, but shorter than with wood-decaying fungi.

The variable transit time thus depends on the fungal ecology and, for saprotrophic fungi, on the decayed substrate. Suetsugu *et al*. (2020) did not access mycoheterotrophic species feeding on the most widespread mycorrhizal symbiosis. The arbuscular mycorrhizal (AM) symbiosis involves > 80% of land plants (van der Heijden *et al*., 2015) with Glomeromycotina, fungi that do not form fruitbodies and thus were not submitted to age estimation based on ^{14}C dating. The Guadeloupe site where *W. calcrata* was

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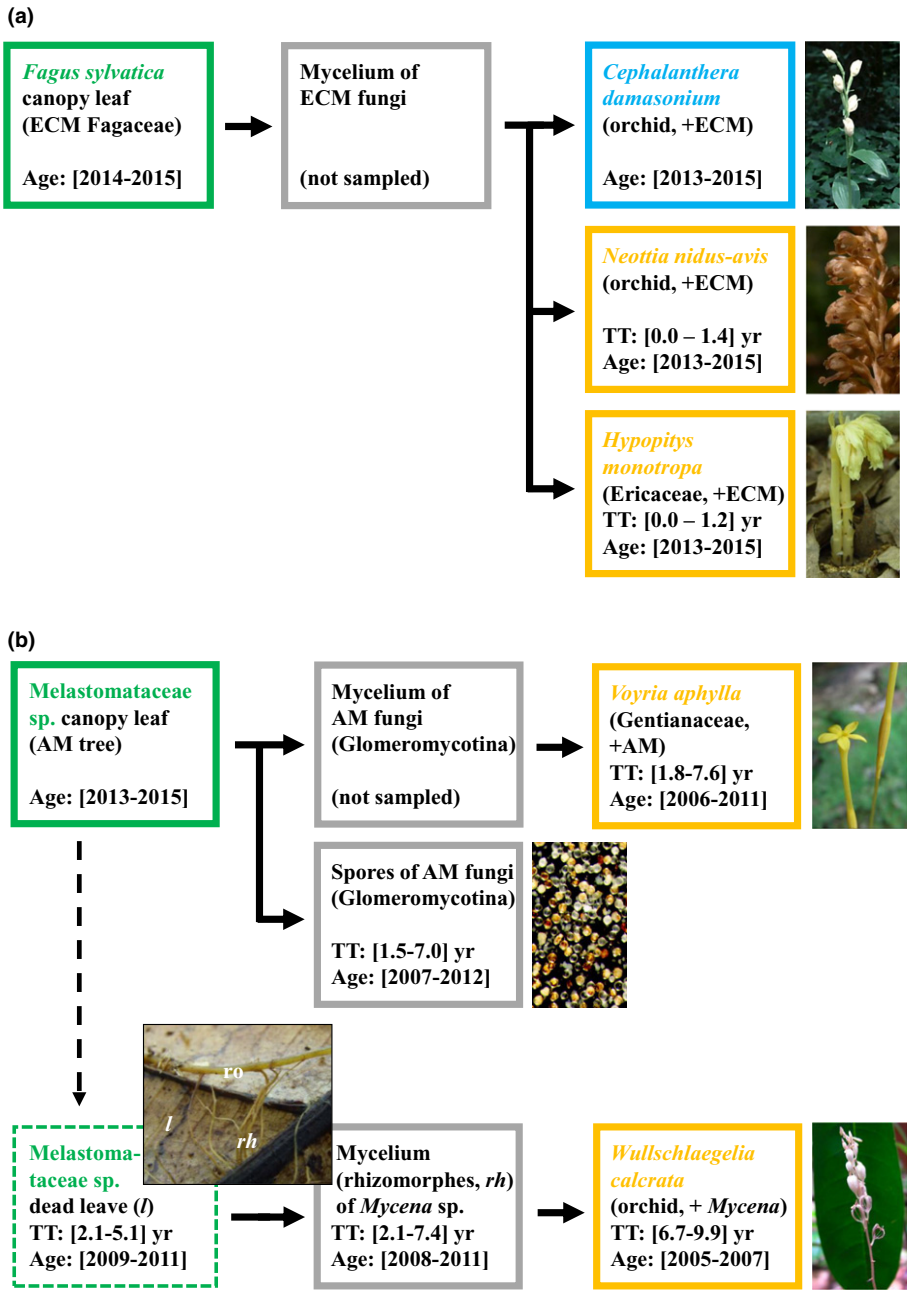

Fig. 2 Age of carbon in samples from 2014 at two sites with mycoheterotrophic (orange) and mixotrophic (blue) plants, linked by various fungi (grey) and trophic chains to autotrophic plants (green), with 95% confidence intervals for transit time (TT) since initial photosynthesis and for carbon age [in brackets]. See Supporting Information Methods S1 and Table S1. (a) An ectomycorrhizal (ECM) network in a French temperate forest (Pagny-la-Blanche-Côte) links *Fagus sylvatica* to mixotrophic *Cephalanthera damasonium* and mycoheterotrophic *Neottia nidus-avis* and *Hypopitys monotropa monotropa* (the two carbon sources of *C. damasonium* make calculation of transit time irrelevant). (b) In a Caribbean tropical forest (Sofaïa, Guadeloupe), two chains link arbuscular mycorrhizal (AM) trees, such as the dominant Melastomataceae tree (unknown sp.), to AM mycoheterotrophic *Voyria aphylla* by way of AM fungi, whose subterranean spores were also sampled, and mycoheterotrophic *Wullschlaegelia calcrata* that are mycorrhizal with saprotrophic *Mycena* sp. Clusters of *Mycena* sp. hyphae (rhizomorphs, *rh* in inset picture) simultaneously colonize dead Melastomataceae sp. leaves (*l*) and *W. calcrata* roots (*ro*; Fig. S1).

collected also harbours the mycoheterotrophic *Voyria aphylla* associated with Glomeromycotina (Fig. 2b; Table S1; Methods S1), and we isolated subterranean spores of Glomeromycotina (i.e. reflecting the whole community, isolation as in Courty *et al.*, 2011). Surprisingly, the transit time from the atmosphere to *V. aphylla* turned out to be between 1.9 and 7.6 yr (median, 4.3 yr). Little is known of the storage abilities of *V. aphylla* (Merckx, 2013), which could retain old carbon, but the ^{14}C concentration difference between Melastomataceae sp. leaves and Glomeromycotina spores also tends to a high age difference, between 1.5 and 7.0 yr (median, 4.1 yr). On the one hand, spores may be older than AM hyphae, but on the other hand, carbon in AM fungi may be older than expected from its fast transfer from photosynthesis (Jiang *et al.*, 2017). Glomeromycotina may either access carbon that was stored in the tree roots before delivery to the fungus, or even store carbon themselves. Indeed, in mycorrhizal roots they form inflated hyphae called vesicles (Smith & Read, 2008), specialized in lipid storage with unknown turnover. Whether AM fungi handle older carbon than ectomycorrhizal fungi and display a longer transit time to mycoheterotrophic plants remains to be assessed by future studies.

Limits and prospects of ^{14}C dating in mycorrhizal networks

These discussions on radiocarbon age have two main limitations. First, the analysis provides a bulk age: the admixture of two or more sources cannot be ruled out, which would blur the interpretation if the ages of these sources differ. Moreover, due

to the slow decrease of the bomb peak curve in recent times (Fig. 1b), the further one moves away from the 1960s, the less precise is the age estimation (see the examples in orange and green in Fig. 1b). Suetsugu *et al.* (2020) were luckily working on environments where carbon was old, and therefore highly ^{14}C-enriched. This exceptional situation may not occur in many other locations. Last but not least, in today's environment it is necessary to wait before the data can be interpreted, because the newest official global atmospheric ^{14}C records, as stated earlier, must be assembled and validated by the research community before an extended post-2009 ^{14}C curve can be plotted. An interesting alternative is the use of herbaria samples harvested in the 1960s to the 1980s, a period when ^{14}C atmospheric abundance is recorded and offers better resolution (Fig. 1b).

Mixotrophic plants, which mix their own photosynthetic carbon and fungal carbon (Selosse & Roy, 2009; Selosse *et al.*, 2017; Suetsugu *et al.*, 2017), offer an example of a mixture of sources and of the limits of dating for recent periods. They may be predicted to have younger carbon than mycoheterotrophic plants due to input from their own photosynthesis (Hynson *et al.*, 2013). Suetsugu *et al.* (2020) and our analyses (on *Cephalanthera damasonium*, a mixotrophic orchid (Julou *et al.*, 2005); Fig. 2a) did not reveal a clear difference from autotrophs or mycoheterotrophs, but this may be because of large uncertainties in age estimates. A confounding factor for autotrophs and mixotrophs from the forest understorey is that photosynthesis could use a mixture of CO_2 from the free atmosphere and from soil respiration, which is some decades older and thus richer in ^{14}C. For post-bomb peak periods, such an admixture yields an older apparent age of photosynthates, which may explain why we do not observe the expected younger age of carbon in mixotrophs (Suetsugu *et al.*, 2020; Fig. 2a).

After the pilot studies by Hobbie *et al.* (2002) on carbon age in fungi of different ecologies, Suetsugu *et al.* (2020) have now pushed ^{14}C methods into the area of carbon transfers by mycelia and mycorrhizal networks *in situ*. They reveal expected trends in carbon ages but, as shown by the diversity of their results and our own studies (Fig. 2), transit times may be variable, with unexpected delays that call for a more precise understanding of the transit time of each step, and of potential admixtures. In the past, stable isotopes such as carbon-13 (^{13}C) and nitrogen-15 (^{15}N) (Gebauer & Meyer, 2003; Trudell *et al.*, 2003; Hynson *et al.*, 2013) provided decisive help to demonstrate the fungal nutrition of mycoheterotrophic and mixotrophic plants. Now, ^{14}C methods will certainly help to refine our view of the dynamics of carbon flow in these complex plant–fungal systems.

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ORCID

Christine Hatté https://orcid.org/0000-0002-7086-2672
Marc-André Selosse https://orcid.org/0000-0003-3471-9067
Antoine Zazzo https://orcid.org/0000-0001-7608-2207

Christine Hatté[1], Antoine Zazzo[2] and Marc-André Selosse[3,4]*

[1]Laboratoire des Sciences du Climat et de l'Environnement, UMR 8212 CEA CNRS UVSQ, Université Paris-Saclay, 91191 Gif-sur-Yvette, France;
[2]Archéozoologie, Archéobotanique: Sociétés, Pratiques et Environnements (AASPE), Muséum National d'Histoire Naturelle, CNRS, CP56, 55 rue Buffon, 75005 Paris, France;
[3]Institut de Systématique, Evolution, Biodiversité (ISYEB – UMR 7205 – CNRS, MNHN, SU, EPHE), Muséum National d'Histoire Naturelle, 57 rue Cuvier, 75005 Paris, France;
[4]Faculty of Biology, University of Gdańsk, ul. Wita Stwosza 59, 80-308 Gdańsk, Poland
(*Author for correspondence: tel +33 607123418; email ma.selosse@wanadoo.fr)

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Ages of components in the trophic chain leading to *Wullschlaegelia calcrata* at Sofaïa.

Methods S1 Sampling and ^{14}C measurement.

Table S1 Raw and processed data for the samples of the two investigated sites.

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Key words: ^{14}C dating, mixotrophy, mycorrhizal networks, mycorrhizas, partial mycoheterotrophy, transit time.