New Phytologist

Letters

Discreet heterotrophs: green plants that receive fungal carbon through *Paris*-type arbuscular mycorrhiza

Arbuscular mycorrhiza (AM) represents a symbiosis between plants and Glomeromycotina fungi, and it is distributed throughout the plant kingdom and all terrestrial ecosystems. Colonization in plant roots usually takes structural form of either *Paris*- or *Arum*-type, distinguished by intracellular hyphal coils and arbuscules and exemplified by *Paris quadrifolia* and *Arum maculatum* (Gallaud, 1905; Smith & Smith, 1997; Dickson *et al.*, 2007), respectively, with a near 1 : 1 distribution among plant species (Dickson *et al.*, 2007). Extensive physiological research on the *Arum*-morphotype shows a mutualistic relationship, but the *Paris*-type has received much less attention with regard to its function (Dickson *et al.*, 2007). In this study, we show that green leaves of *P. quadrifolia* contain nearly 50% carbon of fungal origin, in striking contrast to *A. maculatum* in which carbon is entirely derived from photoassimilation. The evidence is based on stable isotope composition in the two species compared with cohabitant plant species with various types of colonization. This identifies *P. quadrifolia* as a partial mycoheterotroph on fungi, and one of the reference species, *Anemone nemorosa*, also with *Paris*-type colonization, shows evidence of similar but less pronounced carbon acquisition from fungi. Partial mycoheterotrophy could thus potentially be widespread among the *c.* 100 000 plant species that are known to develop *Paris*-type AM, with far-reaching implications for our understanding of plant community functioning.

In the *Arum*-morphotype, greatly branched hyphal structures (arbuscules) develop within root cortical cells while sparsely branched hyphae run in the intercellular spaces along cell files (Gallaud, 1905; Dickson *et al.*, 2007). Arbuscules presumably are the site of mineral nutrient fluxes from fungus to plant, and together with intercellular hyphae are also involved in photosynthate transfer from plant to fungus (Smith & Smith, 1997; Dickson *et al.*, 2007; Wipf *et al.*, 2019). This morphotype is frequent among agricultural plants (Dickson *et al.*, 2007). By contrast, the *Paris*-morphotype is characterized by a dense infection with intracellular hyphal coils and few, if any, intercellular hyphae (Gallaud, 1905; Dickson *et al.*, 2007). *Paris*-type colonization is typical of forest floor herbaceous plants, and long-lived, woody and evergreen plants (Dickson *et al.*, 2007). Furthermore, many of the 880 plant species with obvious chlorophyll (Chl)-deficiency and AM (Leake, 1994; Merckx *et al.*, 2013) show *Paris*-type endomycorrhiza (Imhof *et al.*, 2013). The mycoheterotrophy, that is, parasitism on fungi, of these achlorophyllous plants is well documented, primarily by stable isotope natural abundance approaches (Hynson *et al.*, 2013). The uptake of fungal carbon in such AM plants is revealed by a significant ^{13}C enrichment, compared with photoautotrophic plants. This enrichment, however, is not as pronounced as in mycoheterotrophic plants associated with ectomycorrhizal fungi (Merckx *et al.*, 2010; Courty *et al.*, 2011).

In many types of mycorrhiza, stable isotope signatures are essential in clarifying the plant–fungus relationships, particularly in species that obviously have low or no photosynthetic capacity due to low amounts of Chl. Thus, in orchid mycorrhiza, the achlorophyllous species that associate with fungi that simultaneously form ectomycorrhizas with forest trees have been found to mirror not only carbon, but also nitrogen and hydrogen stable isotope signatures of their ^{13}C-, ^{15}N- and ^{2}H-enriched fungi (Gebauer & Meyer, 2003; Trudell *et al.*, 2003; Hynson *et al.*, 2013; Gebauer *et al.*, 2016). Furthermore, nonphotosynthetic orchid species that associate with wood- or litter-decomposing fungi are significantly enriched in ^{13}C and ^{15}N compared with autotrophic surrounding plants (Ogura-Tsujita *et al.*, 2009, 2018; Lee *et al.*, 2015), and the same applies to Chl-deficient members of Ericaceae with ericaceous mycorrhizal connection to ectomycorrhizal fungi (Tedersoo *et al.*, 2007; Zimmer *et al.*, 2007).

The driver for the enrichment in heavy isotopes of mycoheterotrophic plants appears to be the simultaneous enrichment in their mycorrhizal fungi. Ectomycorrhizal fungi become enriched in ^{13}C, ^{15}N and ^{2}H, because they gain ^{13}C-enriched carbohydrates from their tree partners (Gleixner *et al.*, 1993), have access to soil organic matter enriched in ^{15}N due to their ability to release exoenzymes (Gebauer & Dietrich, 1993; Schiebold *et al.*, 2017), and are composed of secondary organic compounds that are enriched in ^{2}H in comparison to primary photosynthetic organic compounds produced by autotrophic plants (Yakir, 1992; Gebauer *et al.*, 2016; Cormier *et al.*, 2018, 2019). Wood- or litter-decomposing fungi are enriched in ^{13}C and ^{15}N, because they use ^{13}C-enriched cellulose as a carbon source (Gleixner *et al.*, 1993) and like other fungi they access recalcitrant soil organic matter to obtain nitrogen rich in ^{15}N (Gebauer & Taylor, 1999). As a logical consequence, although not yet investigated, AM fungi should also be enriched in ^{13}C, because they gain ^{13}C-enriched carbohydrates from their green plant partners. Finding rather low ^{13}C enrichment and no detectable ^{15}N enrichment in fully mycoheterotrophic AM plants may be due to the fact that the fungal partners (Glomeromycotina) lack the ability to synthesize lipids (Jiang *et al.*, 2017; Keymer *et al.*, 2017; Luginbuehl *et al.*, 2017; Rich *et al.*, 2017) and probably exoenzymes. Thus, AM fungi are considered to gain ^{13}C-depleted lipids (Gleixner *et al.*, 1993; Cormier *et al.*, 2019) in addition to ^{13}C-enriched carbohydrates, and to use mostly isotopically inconspicuous nitrate and

ammonium as nitrogen sources. This combination of factors is expected in the end to mirror the isotopic composition of AM mycoheterotrophic plants.

Apart from obviously achlorophyllous plant species, stable isotope signatures have also shed new light on green-leaved plant species previously thought to be simply photoautotrophic (Gebauer & Meyer, 2003). In recent years a steadily increasing number of such species have been identified as 'partial mycoheterotrophs', because their stable isotope composition values lie between nonmycoheterotrophic neighboring plants and full mycoheterotrophs (Hynson *et al.*, 2013, 2016; Gebauer *et al.*, 2016). This condition is frequent within Orchidaceae (Gebauer & Meyer, 2003; Hynson *et al.*, 2013, 2016; Gebauer *et al.*, 2016) and Ericaceae (Zimmer *et al.*, 2007; Hynson *et al.*, 2009, 2013, 2016) with their particular kinds of mycorrhiza, but has only been recorded for very few species with AM (Cameron & Bolin, 2010; Bolin *et al.*, 2017).

The fact that all AM mycoheterotrophs so far investigated develop the *Paris*-morphotype (Imhof *et al.*, 2013) led us to ask whether hyphal coils are required for a fungus-to-plant carbon transmission, and whether indeed plant species with *Paris*-type mycorrhiza might potentially obtain carbon from their fungal source, having green leaves or not. This could also explain why photosynthetic rates of *P. quadrifolia* and some other *Paris*-type AM plants appear to be low compared with *Arum*-type AM plants (Dalke *et al.*, 2018). To shed light on this question, we applied stable isotope abundance analysis to the two species that once provided the very definition for the AM morphotypes, namely *A. maculatum* and *P. quadrifolia* (Gallaud, 1905). Both species are fairly common in Eurasian forest habitats, and we selected two localities where they occur together (Fig. 1a–c). Their mycorrhizal morphotypes were confirmed by microscopy of roots (Fig. 1d,e). Green leaves were collected, simultaneously with reference samples of neighboring forest ground species: *Alliaria petiolata* (nonmycorrhizal), *Allium ursinum* (*Arum*-type AM), *A. nemorosa* (*Paris*-type AM), *Fraxinus excelsior* (*Arum*-type AM), *Galium odoratum* (various) and *Hedera helix* (*Arum*-type AM) (Supporting Information Table S1).

- We hypothesized that *P. quadrifolia* would show significant enrichment in stable isotopes, ^{13}C and ^{2}H, compared with *A. maculatum* and reference plants presumed to be fully photoautotrophic, while the latter would not be distinguishable.
- We hypothesized that any difference in ^{13}C and ^{2}H isotope abundances between *P. quadrifolia* and *A. maculatum* growing under identical microclimate conditions should not be explained by differences in stomatal regulation and transpiration. To test that, we also analyzed leaf tissue for oxygen isotope abundance.

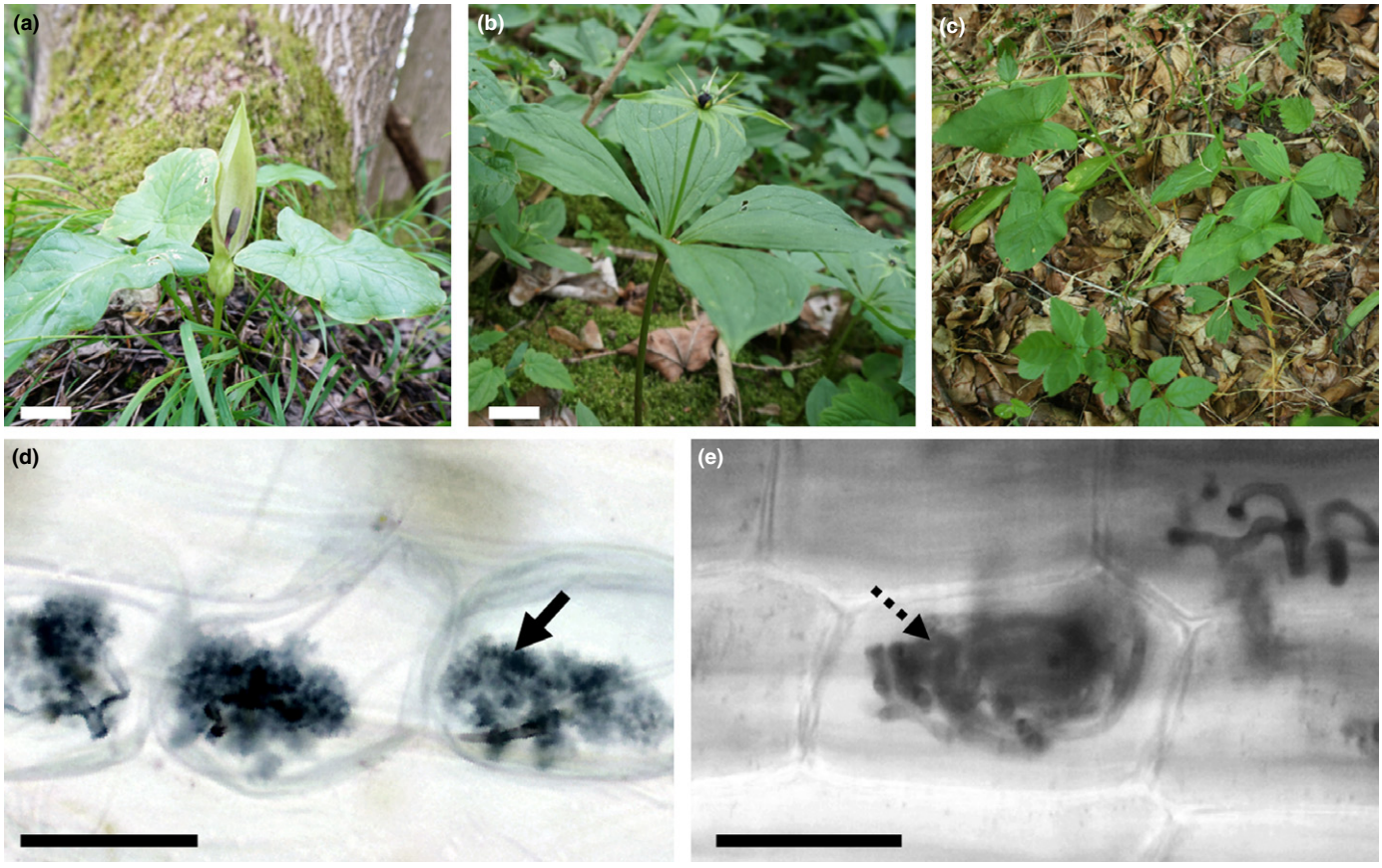

Fig. 1 *Arum*- and *Paris*-type arbuscular mycorrhiza. Habits of *Arum maculatum* (a), *Paris quadrifolia* (b) and both species growing in close proximity (c). (d) Ramified intracellular arbuscules in root cortical cells of *A. maculatum* (*Arum*-morphotype, solid arrow). (e) Dense intracellular hyphal coils in *P. quadrifolia* (*Paris*-morphotype, dashed arrow). The contrast in (d, e) is enhanced with Fiji ImageJ 1.51n. White bars, 2 cm; black bars, 50 μm.

Arum maculatum and *P. quadrifolia* (Fig. 1) were sampled in late May and early June 2017 at two sites, one in northern (49.6397°N, 11.2472°S, decimal WGS84) and one in southern Bavaria (47.6330°N, 11.1603°S), Germany. Both sites are carbonate-rich mixed temperate forests, the northern site receiving 850 mm annual precipitation, the southern site >1300 mm (Deutscher Wetterdienst, 2019). Sampling design for plant leaf material followed the approach of Gebauer & Meyer (2003), which included foliage leaf samples of *A. maculatum*, *P. quadrifolia* and three reference plant species within 1-m^2 plots each with five replicates. The reference plants cover a range of both herbaceous and woody, and deciduous and evergreen species forming AM with a range of AM-morphotypes or being nonmycorrhizal plants (Gallaud, 1905; Wang & Qiu, 2006; Dickson *et al.*, 2007; Fracchia *et al.*, 2009; Shah *et al.*, 2009; Brundrett & Tedersoo, 2019; Table S1). This set of reference plants reflects a natural variance in stable isotope abundance of chlorophyllous C_3 plants growing on shady forest floors and so far considered as completely photoautotrophic. Ten *A. maculatum* and 10 *P. quadrifolia* individuals were compared to 30 co-occurring reference plant individuals.

Plant leaf material was washed, species by species, with deionized water, oven-dried overnight, and ground to homogenous powder in a ball mill followed by storage in desiccators over silica gel until further processing (Table S2). Elemental analyzer isotope ratio mass spectrometry (EA-IRMS) was used to analyze natural relative abundances of carbon ($^{13}C : {}^{12}C$) and nitrogen ($^{15}N : {}^{14}N$) while a thermal conversion device (TC-IRMS) was used to analyze natural relative abundances of hydrogen ($^{2}H : {}^{1}H$) and oxygen isotopes ($^{18}O : {}^{16}O$) in leaves of every sampled species separately (Table S2). A memory bias was avoided by analyzing H isotope samples four times. All samples were plot-wise analyzed in identical batches to minimize an atmospheric bias by H atom exchange within the samples with water vapor in ambient air. The resulting relative isotope abundances follow the rules of the δ-notation: $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$ or $\delta^{18}O = (R_{sample}/R_{standard} - 1) \times 1000$ (‰), whereby R is the ratio of the heavy to the respective light isotope. The site-specific δ-values were normalized to enrichment factors ε according to Preiss & Gebauer (2008) by plot-wise calculating the difference between δ values of the target plants *A. maculatum* and *P. quadrifolia* (δ_T) and the mean values of their respective neighboring reference plants (δ_{Ref}) as $\varepsilon = \delta_T - \delta_{Ref}$. The relative amount of carbon that *P. quadrifolia* received from a fungal source was quantified applying the two-source linear mixing model (Gebauer & Meyer, 2003; Hynson *et al.*, 2013). This model requires an end-member exclusively obtaining carbon through photosynthesis (our reference plants) and an end-member solely covering its carbon demand from an AM fungal source (fully mycoheterotrophic plants). For this we used the enrichment factors of the fully mycoheterotrophic AM plant species *Voyria aphylla* and *Dictyostega orobanchoides* (Merckx *et al.*, 2010). Statistical test procedures can be retraced from Table S3. All values are given as mean and standard deviation (SD).

Samples of target (*A. maculatum* and *P. quadrifolia*) and reference plant roots for microscopy were washed with deionized water and stored at 4°C in 70% ethanol. Staining was performed according to Phillips & Hayman (1970) and Vierheilig *et al.* (2005) (Table S4).

For the target plants our microscopic observations confirmed the presence of AM fungi based on aseptate hyphae, vesicles and, with respect to the *Arum*-morphotype, ramified arbuscules (Fig. 1d); likewise, in the *Paris*-morphotype dense hyphal coils were seen (Fig. 1e). For the reference plants, we found that their mycorrhizal status conformed with previous published records of the same species (Table S1). We found enrichment in ^{13}C (2.6 ± 0.7‰), ^{15}N (0.6 ± 0.6‰) and ^{2}H (8.8 ± 5.3‰) in *P. quadrifolia* compared with both *A. maculatum* and the group of cohabitant reference plants (Fig. 2). By definition, the sum of reference plants had a mean enrichment factor ε of zero and SD of ±1.1‰ for $\varepsilon^{13}C$, ±0.6‰ for ^{15}N and ±7.3‰ for $\varepsilon^{2}H$ (Fig. 2). Falling within the range of reference plants, *A. maculatum* individuals were inconspicuous in stable isotope enrichment, scattering in $\varepsilon^{13}C$ by 0.6 ± 0.2‰, in $\varepsilon^{15}N$ by -0.2 ± 0.9‰ and in $\varepsilon^{2}H$ by -0.7 ± 5.6‰.

Kruskal–Wallis tests found significant differences among the groups in $\varepsilon^{13}C$ ($H(2) = 24.608$, $P < 0.001$), in $\varepsilon^{15}N$ ($H(2) = 6.890$, $P = 0.03$) and in $\varepsilon^{2}H$ ($H(2) = 13.215$, $P = 0.001$). Pairwise comparisons of groups by Dunn's *post hoc* test are shown in Table 1. We detected no significant differences in ^{18}O enrichment ($H(2) = 0.402$, $P = 0.82$) or leaf total nitrogen concentrations ($H(2) = 4.608$, $P = 0.10$) between the three groups. It is notable that the only *Paris*-type species among the reference plants, *A. nemorosa*, with respect to its $\varepsilon^{13}C$ and $\varepsilon^{2}H$ pattern was closer to *P. quadrifolia* than to *A. maculatum* and all other reference plants (Fig. 2). Stable isotope patterns (δ-values) and leaf total nitrogen concentrations for each of the respective sites and all investigated plant species are given in Table S5.

In principle, relative enrichment in ^{13}C and ^{2}H can arise simultaneously by (1) different photosynthetic pathways (Sternberg *et al.*, 1984; Farquhar *et al.*, 1989); (2) differing isotopic composition in the CO_2 and H_2O sources for photosynthesis (Farquhar *et al.*, 1982, 1989); (3) different light and microclimate conditions (Dawson *et al.*, 2002); (4) different transpiration rates (Farquhar *et al.*, 1982, 1989; Cernusak *et al.*, 2004); and (5) C and H gains from sources alternative or complementary to photosynthesis (Press *et al.*, 1987; Gebauer & Meyer, 2003; Těšitel *et al.*, 2010; Hynson *et al.*, 2013; Gebauer *et al.*, 2016). However, all the plant species we investigated are known to follow the C_3 pathway of photosynthesis, and (2) and (3) are unlikely because our plant material was growing under identical light and microclimatic conditions and was collected during the same time. Because increased transpiration, as known for many hemiparasitic plants (Cernusak *et al.*, 2004), changes the oxygen isotope abundance towards depletion of ^{18}O, we tested explanation (4) by analyzing leaf tissue oxygen isotope abundances but, as stated above, found no differences. Thus, all plants investigated had similar transpiration regulation. Explanation (5) remains the most likely reason for the stable isotope pattern seen in *P. quadrifolia*.

Natural ^{13}C, ^{15}N, ^{2}H and ^{18}O isotope abundance patterns in *A. maculatum* and *P. quadrifolia* are here shown for the first time. For more than 100 years these two species have served as models for

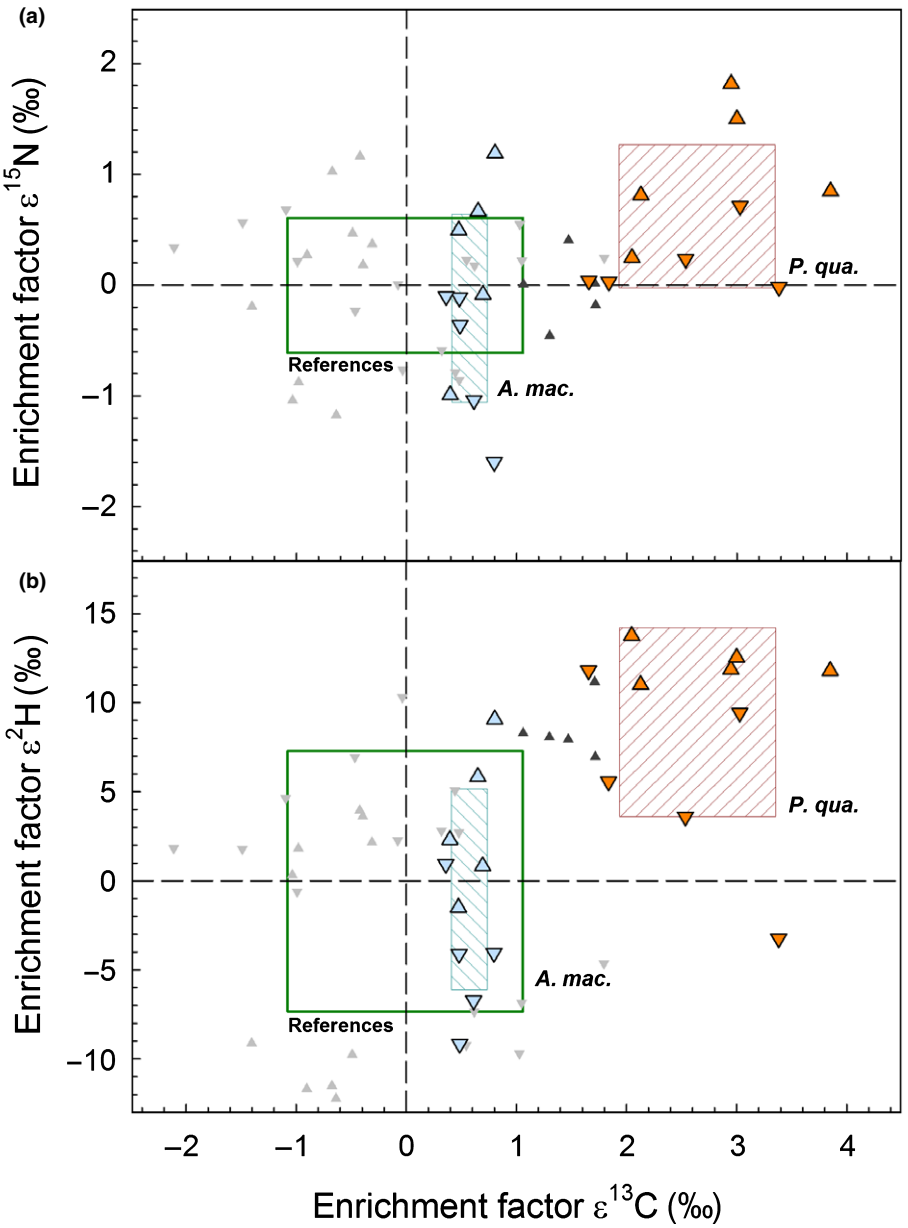

Fig. 2 Carbon and nitrogen (a) and carbon and hydrogen (b) stable isotope enrichment factors ε for *Arum maculatum* (blue triangles), *Paris quadrifolia* (red triangles). The shaded boxes indicate SD for all investigated *A. maculatum* ($n = 10$) and *P. quadrifolia* ($n = 10$). By definition, mean ε-values of the reference plants are zero, and SD is shown by green frames ($n = 30$). Upwards-triangles represent the North Bavarian site; downwards-triangles, the South Bavarian site. Dark gray symbols illustrate the reference plants forming *Paris*-type (*Anemone nemorosa*) and light gray symbols all other types (*Alliaria petiolata*, *Allium ursinum*, *Fraxinus excelsior*, *Galium odoratum*, *Hedera helix*).

Table 1 Test for differences between *Paris quadrifolia* (*P. qua.*, $n = 10$), *Arum maculatum* (*A. mac.*, $n = 10$) and neighboring plant species as references ($n = 30$) in enrichment factors ε of ^{13}C, ^{15}N and ^{2}H.

	$\varepsilon^{13}C$		$\varepsilon^{15}N$		$\varepsilon^{2}H$	
	Test statistics	*P*	Test statistics	*P*	Test statistics	*P*
P. qua. vs *A. mac.*	$Z = 2.960$	**0.003**	$Z = 2.439$	**0.022**	$Z = 3.099$	**0.002**
P. qua. vs references	$Z = 4.960$	**<0.001**	$Z = 2.261$	**0.024**	$Z = 3.400$	**0.001**
A. mac. vs references	$Z = 1.334$	0.091	$Z = 1.136$	0.233	$Z = 0.395$	0.347

Pairwise Dunn's *post hoc* tests (*Z*). Significant results are highlighted in bold.
Alliaria petiolata (nonmycorrhizal), *Allium ursinum* (*Arum*-type arbuscular mycorrhiza (AM)), *Anemone nemorosa* (*Paris*-type AM), *Fraxinus excelsior* saplings (*Arum*-type AM), *Galium odoratum* (various) and *Hedera helix* (*Arum*-type AM) served as reference plants.

the two morphotypes of AM without recognition of any functional differences between them. We found that *P. quadrifolia* was significantly enriched in heavy isotopes while *A. maculatum* resembled the reference plants. This finding is consistent with our first hypothesis and places *P. quadrifolia* as a partial mycoheterotroph because it clearly obtains carbon through mycobionts as well as by photosynthesis. By contrast, *A. maculatum* appears fully photoautotrophic and is probably engaged in a mutualistic AM relationship where it gives off carbon compounds to the mycobionts.

With respect to relative ^{13}C enrichment, *P. quadrifolia* resembles selected members of Gentianaceae (Cameron & Bolin, 2010) and Burmanniaceae (Bolin *et al.*, 2017) that are considered partial mycoheterotrophs. Their arbuscular morphotype is not known, however. Stable isotope patterns in *P. quadrifolia* also correspond to those found in several members of Orchidaceae (Gebauer & Meyer, 2003; Hynson *et al.*, 2016) and Ericaceae (Zimmer *et al.*, 2007; Hynson *et al.*, 2009, 2016) with other types of mycorrhizal association and are acknowledged as partial mycoheterotrophs.

It appears that *P. quadrifolia* obtains considerable amounts of carbon from its associated Glomeromycotina mycobionts. By applying the two-source linear mixing model using neighboring plants as fully autotrophic references receiving their C completely from photosynthesis as the lower end-members, and fully mycoheterotrophic AM plant species *V. aphylla* and *D. orobanchoides* (Merckx *et al.*, 2010) as the upper end-members, we estimate that *P. quadrifolia* received about half ($48 \pm 13\%$) of its carbon nutrients from a fungal source. This agrees with members of Gentianaceae (Cameron & Bolin, 2010) and Burmanniaceae (Bolin *et al.*, 2017) also forming AM ($44 \pm 27\%$), and is within the range found in partial mycoheterotrophic orchids and Ericaceae (Hynson *et al.*, 2013).

It was hypothesized by Imhof (1999) that intracellular hyphal growth is a prerequisite for the evolution of mycoheterotrophy (see *pelotons* in orchids, *hyphal pegs* in Ericaceae or *Paris*-type hyphal coils in achlorophyllous AM plants, Imhof *et al.*, 2013). It is known that different infection patterns may develop in different plant species by the same strain of Glomeromycotina, including the absence or presence of hyphal coils (Burleigh *et al.*, 2002). Thus, if a plant can trigger the fungus to develop intracellular coils, it may change carbon-loss into gain of fungal carbon. A selective advantage is suggested for plant control over morphotype establishment (Dickson, 2004; Dickson *et al.*, 2007). However, the process of carbon transfer from fungi to plants is yet not completely clear (Dickson *et al.*, 2007; Wipf *et al.*, 2019).

Interestingly, the ^{15}N enrichment of *P. quadrifolia*, partially mycoheterotrophic Gentianaceae (Cameron & Bolin, 2010) and Burmanniaceae (Bolin *et al.*, 2017) as well as fully mycoheterotrophic plants on AM fungi (Merckx *et al.*, 2010; Courty *et al.*, 2011; Hynson *et al.*, 2013) appears to be considerably lower than for mycoheterotrophic plants associated with fungi simultaneously forming ectomycorrhizas with neighboring forest trees (Hynson *et al.*, 2016). This suggests different nitrogen sources for mycoheterotrophs depending on whether the fungal connection is arbuscular or ectomycorrhizal. The majority of ectomycorrhizal fungi are known to access recalcitrant ^{15}N-enriched soil organic compounds through the release of exoenzymes. In this way, the hyphae become ^{15}N-enriched (Gebauer & Dietrich, 1993; Gebauer & Taylor, 1999; Mayor *et al.*, 2009) and may transfer this ^{15}N enrichment to tissues of their mycoheterotrophic plant partners. Weak or undetectable ^{15}N enrichment as in mycoheterotrophs on AM (Cameron & Bolin, 2010; Merckx *et al.*, 2010; Bolin *et al.*, 2017) – now including *P. quadrifolia* – suggests that their main nitrogen sources are ammonium and nitrate, which are less ^{15}N-enriched than organic nitrogen components in soils.

An extensive literature search showed that the significant ^{13}C and ^{15}N enrichment, which we found in *P. quadrifolia*, but not in *A. maculatum*, is confirmed by data deeply buried in two previous publications (Liebel *et al.*, 2010; Hynson *et al.*, 2015). In these cases, *P. quadrifolia* from Sweden and *Arum pictum* from Italy (closely related to *A. maculatum*) served as neighboring reference plants for orchid and Ericaceae mycoheterotrophs.

Our identification of *Paris*-morphotype AM as a partially mycoheterotrophic mode of nutrition may have far-reaching implications. Summarized data from 1895 to 2006 concerning 941 plant species from 147 families listed 59% as *Arum*-type only and 41% as *Paris*-type only (intermediate types excluded) (Dickson *et al.*, 2007). As the *Paris*-type was frequently ignored or once even classified as nonmycorrhizal, it is suggested that both morphotypes are almost equally frequent on a species level (Smith & Smith, 1997; Dickson *et al.*, 2007). This means that about half of the 200 000 AM plant species that presently are considered fully photoautotrophic could potentially gain carbon from fungi, perhaps under conditions limiting their own autotrophic carbon gain. In each of these cases, suitable sampling designs for analysis of stable isotope natural abundances may shed light on the extent to which they rely on the fungi as a carbon source. The *Paris*-type reference plant species *A. nemorosa* of the present study is a very first starting point and confirms similar enrichment in ^{13}C and ^{2}H as *P. quadrifolia*. Thus, *A. nemorosa* is apparently also partially mycoheterotrophic. Colonization patterns intermediate between the *Arum* and *Paris* morphotypes (Dickson, 2004), as well as plant species classified as nonmycorrhizal because of 'unusual *Paris*-type morphology' (Dickson *et al.*, 2007), should also be scrutinized for mycoheterotrophism. Partial mycoheterotrophy on AM fungi could be widely distributed within the plant kingdom, far beyond the currently known few members of Gentianaceae (Cameron & Bolin, 2010) and Burmanniaceae (Bolin *et al.*, 2017).

A further speculation on this finding concerns the plant species with *Arum*-type AM. Fungi belonging to Glomeromycotina are considered obligate symbionts with little capacity for saprotrophy (Lanfranco *et al.*, 2017; but see Hempel *et al.*, 2007). If so, the fungal carbon received by *P. quadrifolia* must have been acquired by the fungus via an *Arum*-type colonization within a living donor plant. A transfer of photosynthates from one green plant to another, from *Arum*- to *Paris*-type, potentially at a larger scale, would be an important mechanism for coherence in plant communities, the implications of which we have overlooked until now. By analogy to findings for partially mycoheterotrophic orchids (Preiss *et al.*, 2010), we suggest that partially mycoheterotrophic AM plants have two carbon sources, that is, from their own photosynthesis and from other plants via associated fungi. The latter source would be particularly relevant under low-light conditions. This is consistent

with the typical distribution of *Paris*-type AM (Dickson *et al.* 2007) in herbaceous plants of the forest floor and in long-lived woody plants with shaded early life stages.

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Author contributions

PG contributed to the research design, conducted major parts of the field survey, analyzed and treated the results and wrote the first manuscript draft. HNR essentially initiated the idea for this research on the basis of an unpublished literature review. HTL helped to identify sampling locations and performed some of the sample collection. GG coordinated the project, supervised the isotope abundance analyses and supported data treatment. All coauthors contributed to the manuscript.

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Supporting Information

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

Table S1 The mycorrhizal type and arbuscular mycorrhizal subtype of target plant species *A. maculatum*, *P. quadrifolia* and their respective reference plants separated by sampling location.

Table S2 Equipment and substances related to stable isotope measurements and their reproducibility.

Table S3 Statistical test procedure on stable isotope enrichment factors ε.

Table S4 Root staining procedure and microphoto-documentation of hyphal structures.

Table S5 Stable isotope natural abundances in δ-values (‰) and leaf total nitrogen concentrations (mmol g^{-1} $_{dry\ weight}$) of the target plant species *A. maculatum*, *P. quadrifolia* and their respective reference plants.

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Key words: arbuscular mycorrhiza (AM), *Arum maculatum*, *Arum*-type, myco-heterotrophy, *Paris quadrifolia*, *Paris*-type, stable isotope natural abundance.

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