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Partial mycoheterotrophy is common among chlorophyllous plants with *Paris*-type arbuscular mycorrhiza

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- **Background and Aims** An arbuscular mycorrhiza is a mutualistic symbiosis with plants as carbon providers for fungi. However, achlorophyllous arbuscular mycorrhizal species are known to obtain carbon from fungi, i.e. they are mycoheterotrophic. These species all have the *Paris* type of arbuscular mycorrhiza. Recently, two chlorophyllous *Paris*-type species proved to be partially mycoheterotrophic. In this study, we explore the frequency of this condition and its association with *Paris*-type arbuscular mycorrhiza.
- **Methods** We searched for evidence of mycoheterotrophy in all currently published ^{13}C, ^{2}H and ^{15}N stable isotope abundance patterns suited for calculations of enrichment factors, i.e. isotopic differences between neighbouring *Paris*- and *Arum*-type species. We found suitable data for 135 plant species classified into the two arbuscular mycorrhizal morphotypes.
- **Key Results** About half of the chlorophyllous *Paris*-type species tested were significantly enriched in ^{13}C and often also enriched in ^{2}H and ^{15}N, compared with co-occurring *Arum*-type species. Based on a two-source linear mixing model, the carbon gain from the fungal source ranged between 7 and 93 % with ferns > horsetails > seed plants. The seed plants represented 13 families, many without a previous record of mycoheterotrophy. The ^{13}C-enriched chlorophyllous *Paris*-type species were exclusively herbaceous perennials, with a majority of them thriving on shady forest ground.
- **Conclusions** Significant carbon acquisition from fungi appears quite common and widespread among *Paris*-type species, this arbuscular mycorrhizal morphotype probably being a pre-condition for developing varying degrees of mycoheterotrophy.

Key words: Arbuscular mycorrhiza, *Arum*-type, Ellenberg values, ferns, horsetails, mycoheterotrophy, mycorrhizal networks, *Paris*-type, seed plants, stable isotopes, ^{13}C, ^{2}H.

INTRODUCTION

A number of plant species are known to cover their energy consumption entirely or partly by parasitism. Some parasitic species transfer photosynthates from another plant through haustoria (Těšitel *et al.*, 2010; Westwood *et al.*, 2010), but many parasitize endophytic fungi ('mycoheterotrophy'; Leake, 1994; Merckx, 2013; Waterman *et al.*, 2013). Ultimately, mycoheterotrophy must be based on hyphal connection to photo-assimilating plants or to photosynthetic products in organic debris. The physiology of parasitic plants holds a considerable fascination. Access to photosynthates either directly from another plant or through hyphae can be associated with a dramatic reduction of chlorophyll and thus of photosynthetic capacity (Westwood *et al.*, 2010; Merckx, 2013), but often some photosynthetic activity is retained ('hemiparasites' or 'partial mycoheterotrophs') (Těšitel *et al.*, 2010; Hynson *et al.*, 2013).

The role that parasitic plants play in the natural habitats where they occur is generally understudied and probably underestimated (Quested *et al.*, 2003; Quested, 2008). The vast majority of parasitic plants are herbaceous, often small in stature, and the condition is thought to be confined to certain specialized plant families (e.g. Burmanniaceae, Ericaceae, Gentianaceae, Orchidaceae, Orobanchaceae, Polygalaceae, Santalaceae, Thismiaceae and Triuridaceae; Westwood *et al.*, 2010; Merckx *et al.*, 2013). In a recent study, however, we demonstrated partial mycoheterotrophy in two plant species with *Paris*-type arbuscular mycorrhiza (AM): *Paris quadrifolia* (Melanthiaceae) and *Anemone nemorosa* (Ranunculaceae) (Giesemann *et al.*, 2020*b*). Nevertheless, both plant species have the outer appearance of typical photo-assimilating plants and no close relationship with previously known mycoheterotrophic species (Giesemann *et al.*, 2020*b*). This supports the suggestion that a capacity towards mycoheterotrophy may coincide with the *Paris* type of AM, as suggested by Imhof (1999), and be more widespread than previously recognized. The impact on plant communities could thus be quite considerable, if it amounts to regular and substantial transfers of photosynthates between common plant species.

In this study, we explore the prevalence of partial mycoheterotrophy and its suspected link to the *Paris* type of

AM by surveying published information. Evidence of carbon (C) gain from fungi may be found in the stable isotope composition in leaves of the species in question, where species with mycoheterotrophy deviate characteristically in composition from the purely photo-assimilating species (Gebauer and Meyer, 2003). Thus, we extracted information on carbon (^{13}C), nitrogen (^{15}N) and hydrogen (^{2}H) stable isotope composition in plant species with AM and sorted them into three groups: chlorophyllous *Arum*-type species, chlorophyllous *Paris*-type species and achlorophyllous *Paris*-type species. The distinction into morphotypes (*Arum* vs. *Paris*) was developed by Gallaud (1905) and is based on the infection pattern of hyphae within the roots. Since then the morphotyping has been applied to a wide range of plant species. About 80 % of higher plant species are estimated to form AM, and published records suggest that the morphotypes occur with about equal frequency, with few intermediate or variable forms. Furthermore, the morphotype is mostly consistent across the species of the same genus (Dickson *et al.*, 2007). AM is based on ubiquitous fungi belonging to Glomeromycotina.

We intended to test the following hypotheses: (i) that chlorophyllous *Paris*-type AM plant species would be enriched in ^{13}C, ^{15}N and ^{2}H stable isotopes compared with chlorophyllous *Arum*-type species, but less so than the achlorophyllous *Paris*-type AM species (cf. Gomes *et al.*, 2020; Giesemann *et al.*, 2020*b*); (ii) that the proportion of C gained by photosynthesis and through fungi, respectively, would vary within the group of chlorophyllous *Paris*-type species, reflecting different degrees of reliance on mycoheterotrophy, in the same way as was found in partial mycoheterotrophs with other mycorrhizal relationships (associated with either ectomycorrhizal or saprotrophic basidio- or ascomycetes; cf. Gebauer and Meyer, 2003; Zimmer *et al.*, 2007; Hynson *et al.*, 2009; Gebauer *et al.*, 2016; Schiebold *et al.*, 2018); and (iii) that shade-adapted *Paris*-type AM plant species would show a greater reliance on fungi as their C source, and thus a higher ^{13}C and ^{2}H enrichment, than *Paris*-morphotype AM plant species adapted to full sunlight. A similar light dependence is known in partial mycoheterotrophs on ectomycorrhizal fungi (Preiss *et al.*, 2010) and on saprotrophic rhizoctonia fungi (Schweiger *et al.*, 2019).

MATERIALS AND METHODS

Distinction of species to AM morphotype

The morphotype of each chlorophyllous plant genus/species was obtained from Dickson *et al.* (2007). This information was supplemented with data from Diallo *et al.* (2001), Becerra *et al.* (2007), Menoyo *et al.* (2007), Turnau *et al.* (2008), Zubek *et al.* (2008, 2011*a*, *b*), Shah *et al.* (2009), Zubek and Błaszkowski (2009), Druva-Lusite and Ievinsh (2010), Velázquez *et al.* (2010), Burni and Hussain (2011), Kołaczek *et al.* (2013), Shi *et al.* (2013) and Nobis *et al.* (2015) (see details in Supplementary data Table S1). All achlorophyllous plant species on AM fungi were assumed to possess *Paris*-type AM, as no contradictory evidence is present so far (Dickson *et al.*, 2007; Imhof *et al.*, 2013).

Stable isotope data for plant species with known AM morphotype, nitrogen concentration and light requirement

The literature was surveyed for C, N, H and oxygen (O) stable isotope natural abundance data suitable for the calculation of the stable isotope enrichment factors, ε (see below) (Supplementary data Table S1). These factors average the effect of habitat conditions, such as microclimate, soil respiration and soil conditions, on the stable isotope composition in leaves of autotrophic C_3 plants growing together in the respective habitats. Those drivers for variations in the stable isotope composition of plants collected from different habitats may thus be disregarded (Farquhar *et al.*, 1982, 1989; Sternberg *et al.*, 1984; Ziegler, 1988; Dawson *et al.*, 2002; Cernusak *et al.*, 2004; Gebauer *et al.*, 2016). We obtained raw data of 1300 stable isotope records on ε^{13}C/^{15}N and 225 on ε^{2}H/^{18}O from the following publications: Gebauer and Meyer (2003), Bidartondo *et al.* (2004), Zimmer *et al.* (2007, 2008), Hynson *et al.* (2009, 2015), Cameron and Bolin (2010), Liebel *et al.* (2010, 2015), Preiss *et al.* (2010), Girlanda *et al.* (2011), Ercole *et al.* (2015), Lee *et al.* (2015), Hynson (2016), Shutoh *et al.* (2016), Schiebold *et al.* (2017, 2018), Suetsugu *et al.* (2017), Ogura-Tsujita *et al.* (2018), Giesemann *et al.* (2020*a*, *b*) and Gomes *et al.* (2020). Additionally, unpublished data from the BayCEER Laboratory of Isotope Biogeochemistry Bayreuth were kindly provided. From this data set, 155 plant individuals (22 species) forming either intermediate morphotypes or establishing both *Arum* and *Paris* type were excluded (Dickson, 2004). Raw data from Courty *et al.* (2011) not made available are the only currently published data with AM reference plants missing in our survey. Thus, 1145 stable isotope records on ε^{13}C/^{15}N and 218 on ε^{2}H/^{18}O were analysed (135 plant species).

The isotope data were sorted into three functional groups: 13 species of achlorophyllous, full mycoheterotrophs on *Paris*-type AM (Merckx *et al.*, 2010; Gomes *et al.*, 2020), 63 species of chlorophyllous *Paris*- and 59 species of chlorophyllous *Arum*-type AM. From this data pool, leaf N concentrations were also available for 107 plant species ($n = 890$, 12 full mycoheterotrophs, 50 chlorophyllous *Paris*-type and 45 chlorophyllous *Arum*-type). Most belonged to the temperate climate zone in Europe (e.g. Austria, Germany and Italy) but there were a few records from other continents (e.g. Australia, Japan and the USA) (Supplementary data Table S1).

We compared the *Paris*-type enrichment factor ε (target plant, TP) with neighbouring *Arum*-type species (reference plant, RP) as $\varepsilon x = \delta x_{TP} - \mathrm{mean}(\delta x_{RP})$ ($x = {}^{13}$C, ^{15}N, ^{18}O, ^{2}H) (Preiss and Gebauer, 2008; Hynson *et al.*, 2013) [see hypotheses (i) and (ii)].

We classified the species with respect to light and temperature preferences by means of Ellenberg indicator values (Ellenberg *et al.*, 2001) which is a system of ordinally/quasi-cardinally scaled classification of plants in Central Europe, finding information on 96 *Paris*- and 149 *Arum*-type species. For a sub-set of plant species from Central Europe (81 species, $n = 787$), we had both Ellenberg indicator values and ε^{13}C enrichment factors. The 41 *Paris*-type species of this sub-set ($n = 315$) were analysed for a correlation between light availability and ε^{13}C enrichment factors, reflecting the degree of mycoheterotrophy [see hypothesis (iii)].

Statistical analysis

The degree of mycoheterotrophy was approximated based on the two-source linear mixing model on ^{13}C (Gebauer and Meyer, 2003; Hynson *et al.*, 2013). Plant species considered fully autotrophic (chlorophyllous *Arum*-type plant species) and plant species considered fully mycoheterotrophic (achlorophyllous *Paris*-type plant species; Merckx *et al.*, 2010; Gomes *et al.*, 2020) represent the two end members between which the partially mycoheterotrophic *Paris*-type species are expected to fall (Giesemann *et al.*, 2020*b*).

RStudio 1.1 (R Core Team, 2019) and SigmaPlot 11.0 (Systat Software, San Jose, CA, USA) were applied for data analyses. The platform from Lenhard and Lenhard (2016) was used for estimations of effect sizes (Cohen's *d*). An effect size of $d > 0.8$ is usually counted as relevant (Cohen, 1992). Shapiro–Wilk tests for normal distribution ('stats' R package by R Core Team, 2019) and Levene tests for variance homogeneity ('car' R package by Fox and Weisberg, 2019) recommended a conservative, non-parametric test procedure for the comparison between *Arum*- and *Paris*-morphotype species. The one-tailed Mann–Whitney *U*-test using the 'stats' R package was performed to evaluate differences in Ellenberg indicator values between *Arum*- and *Paris*-morphotype species. One-tailed Kruskal–Wallis *H*-tests using the 'stats' R package were applied for a comparison of full mycoheterotrophs, chlorophyllous *Paris*- and *Arum*-morphotype species as well as for chlorophyllous *Arum*- and *Paris*-morphotype species separated into ferns, horsetails and seed plants, respectively. Dunn's post-hoc (*Z*) test for multiple comparison was performed after a significant *H*-test using the 'dunn.test' R package (Dinno, 2017). *P*-values were corrected according to the sequential Holm–Bonferroni method. A linear regression (Pearson correlation, r^2_{adj}) was performed for light availability and $\varepsilon^{13}C$ enrichment of *Paris*-morphotype plant species. The critical level of significance was set to $\alpha \leq 0.05$. The data are expressed as their mean value with their s.d. ($\bar{x}$ ± s.d.).

RESULTS

For *Arum*-morphotype AM species, as reference plants, the mean enrichment factor ε was zero by definition. Thus with standard deviation it was for $^{13}C = 0.0 \pm 0.6$ ‰, $^{15}N = 0.0 \pm 0.9$ ‰ and $^{2}H = 0.0 \pm 5.0$ ‰, while enrichments in achlorophyllous *Paris*-morphotype species were pronounced: $^{13}C = 5.0 \pm 2.3$ ‰, $^{15}N = 4.0 \pm 3.4$ ‰ and $^{2}H = 33.3 \pm 17.0$ ‰ (Fig. 1). Chlorophyllous *Paris*-type species were located between chlorophyllous *Arum*-type and achlorophyllous fully mycoheterotrophic plants, with intermediate enrichment values: $^{13}C = 0.9 \pm 1.6$ ‰, $^{15}N = 0.4 \pm 2.3$ ‰ and $^{2}H = 8.0 \pm 11.1$ ‰. (Fig. 1). These average figures cover a large variation among chlorophyllous *Paris*-type species, ranging from –2.7 ± 0.8 ‰ for *Asarum europaeum* to 4.5 ± 1.4 ‰ for *Athyrium filix-femina* in ^{13}C, from –3.9 ± 1.1 ‰ for *Asplenium ruta-muraria* to 7.7 ± 3.2 ‰ for *Equisetum palustre* in ^{15}N and from –15.5 ± 7.8 ‰ for *Gentiana bavarica* to 21.4 ± 6.4 ‰ for *Mercurialis perennis* in ^{2}H (Supplementary data Table S1).

Kruskal–Wallis tests indicated significant effects for the comparisons of *Arum*-type AM, achlorophyllous *Paris*-type AM and chlorophyllous *Paris*-type AM plants in $\varepsilon^{13}C$ [$H(2) = 330.523$, $P < 0.001$, Cohen's $d = 1.276$], $\varepsilon^{15}N$ [$H(2) = 139.422$, $P < 0.001$, Cohen's $d = 0.742$] and $\varepsilon^{2}H$ [$H(2) = 71.985$, $P < 0.001$, Cohen's $d = 1.389$]. Pairwise comparisons by Dunn's post-hoc test showed significant differences in $\varepsilon^{13}C$, $\varepsilon^{15}N$ and $\varepsilon^{2}H$ between each of the three functional groups (Table 1). Although Kruskal–Wallis test detected a significant effect between groups in ^{18}O enrichment [$H(2) = 6.001$, $P = 0.05$, Cohen's $d = 0.275$], the low Cohen's *d* value suggested that the effect was not relevant. Furthermore, no significant effect between groups was detected for leaf total N concentrations [$H(2) = 4.856$, $P = 0.09$, Cohen's $d = 0.113$]. The chlorophyllous *Paris*-type species were also consistently ^{13}C and ^{2}H enriched when *Paris*- and *Arum*-type species were separated into groups of horsetails (*Equisetum*), ferns and seed plants (Supplementary data Table S2). The ^{13}C enrichment follows the sequence ferns > horsetails > seed plants, while the limited data on ^{2}H enrichment suggest horsetails > seed plants (see below).

A total of 63 chlorophyllous *Paris*-type AM species were compared with their respective chlorophyllous *Arum*-type AM reference plants in stable isotope enrichment factors $\varepsilon^{13}C$, $\varepsilon^{15}N$ and $\varepsilon^{2}H$ (Supplementary data Table S3). Of these, 31 were found to be significantly ^{13}C enriched (Fig. 1, brownish symbols) while 32 remained inconspicuously enriched or depleted in their ^{13}C patterns (Fig. 1, white). For the group that was significantly ^{13}C enriched, a mean proportional C gain from the fungal source was calculated based on the linear two-source mixing model (Table 2). This mean proportional C gain from the fungal source ranged from 7 to 93 % (38±19 %) and follows the sequence ferns > horsetails > seed plants (Table 2).

Among the ^{13}C-enriched chlorophyllous *Paris*-type species were seven out of ten forest ferns ($\varepsilon^{13}C$ mean: 3.4 ± 1.1 ‰), four out of five forest horsetails ($\varepsilon^{13}C$ mean: 2.2 ± 1.3 ‰) and nine out of 24 forest-floor seed plants ($\varepsilon^{13}C$ mean: 1.4 ± 0.8 ‰). These species were all herbaceous perennials. In addition to ^{13}C enrichment, two species were also ^{2}H enriched (8.4 ± 3.9 ‰; *Anemone nemorosa* and *Oxalis acetosella*; Fig. 1, light brown) and four were ^{15}N enriched (2.8 ± 1.2 ‰; *Equisetum arvense*, *E. fluviatile*, *E. telmateia* and *Tamus communis*; Fig. 1, dark brown). The herbaceous forest species *Paris quadrifolia*, *Mercurialis perennis* and *Equisetum sylvaticum* turned out to be simultaneously ^{13}C (mean: 2.3 ± 0.8 ‰), ^{15}N (mean: 3.1 ± 3.8 ‰) and ^{2}H enriched (mean: 14.7 ± 6.4 ‰) (Fig. 1, dark gold).

Among the chlorophyllous *Paris*-type species that did not show significant ^{13}C enrichment, the forest perennial *Asarum europaeum* was nevertheless significantly ^{15}N and ^{2}H enriched (Supplementary data Table S3). Tree saplings of *Acer campestre* tended towards both ^{13}C and ^{15}N enrichment, and saplings of *Cornus controversa* tended towards a ^{13}C enrichment that was uncertain because of the small sample size. Saplings of *Acer pseudoplatanus* were not conspicuously ^{13}C enriched while they were significantly ^{2}H enriched.

In addition to the frequently ^{13}C-enriched chlorophyllous *Paris*-type species from forest sites, ^{13}C enrichments were also observed for 11 out of 23 herbaceous open-land species ($\varepsilon^{13}C$ mean: 1.2 ± 1.0 ‰), such as *Astrantia major*, *Gentiana lutea* and *Ligusticum mutellina*. Their conspicuousness was

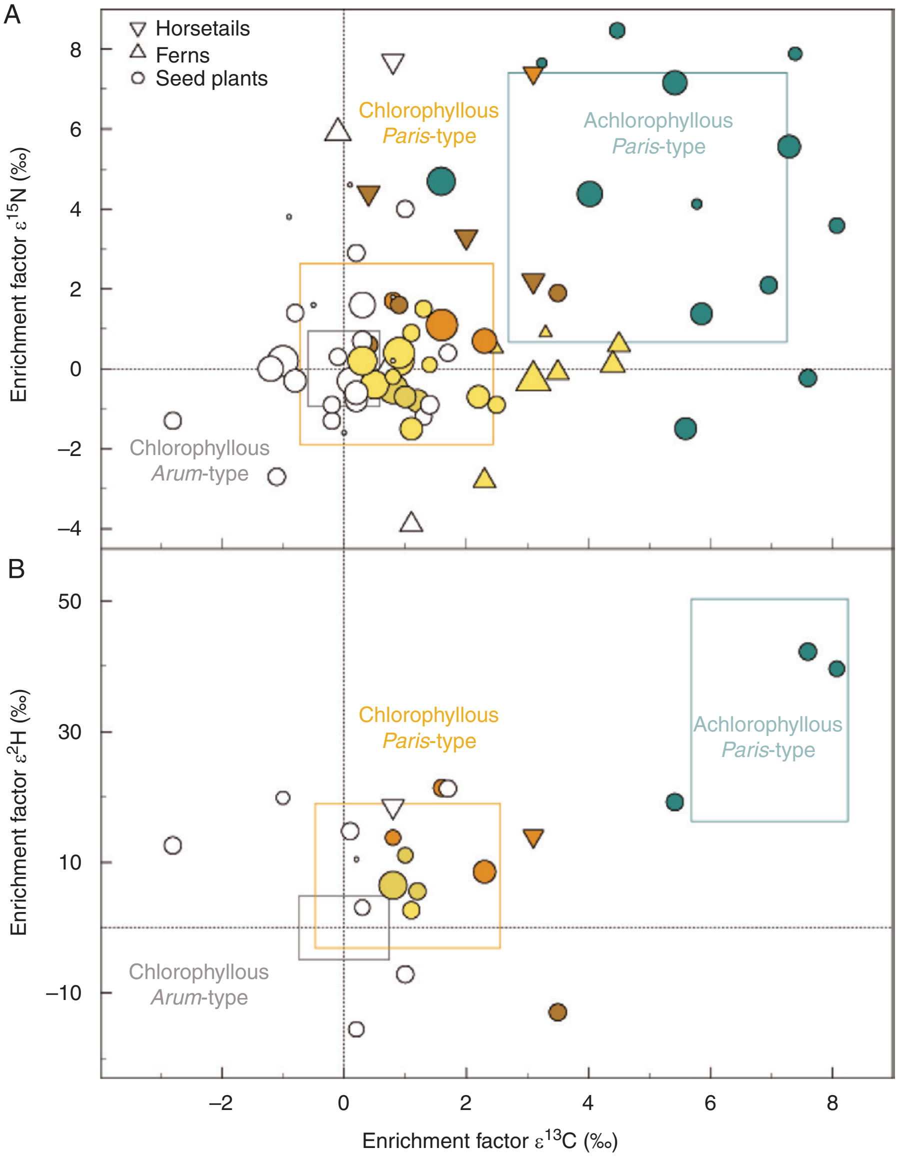

Fig. 1. (A) Carbon and nitrogen enrichment factors ($\varepsilon^{13}C$ and $\varepsilon^{15}N$) and (B) carbon and hydrogen enrichment factors ($\varepsilon^{13}C$ and $\varepsilon^{2}H$) for chlorophyllous *Arum*-type arbuscular mycorrhizal (AM) plant species (grey frame, s.d.), chlorophyllous *Paris*-type AM plant species (brownish tones, brown frame, s.d.) and achlorophyllous, full mycoheterotrophs on AM fungi (blue, blue frame, s.d.). AM morphotype assignment was obtained from the literature (see the Materials and Methods). Each species is represented by mean values and the s.d. is omitted for clarity. Symbol size reflects the sample size of the *Paris*-type species (n = 1–31, see Supplementary data Table S3). Each chlorophyllous *Paris*-type AM plant species was tested for significance of differences in $\varepsilon^{13}C$, $\varepsilon^{15}N$ and $\varepsilon^{2}H$ from co-occurring chlorophyllous *Arum*-type AM plant species (see Supplementary data Table S3). Chlorophyllous *Paris*-type AM plant species shown in coloured symbols are significant in at least one trait (^{13}C enrichment, light gold; ^{13}C + ^{2}H enrichment, light brown; ^{13}C + ^{15}N enrichment, dark brown, ^{13}C + ^{2}H + ^{15}N enrichment, dark gold; no significant enrichment, white). Achlorophyllous plant species were not included in the test procedure (see Gomes *et al.*, 2020). The data comprise for $^{13}C/^{15}N$: 13 achlorophyllous *Paris*-type species (n = 99), 63 chlorophyllous *Paris*-type species (n = 520) and 59 chlorophyllous *Arum*-type species (n = 530). The data comprise for ^{2}H: three achlorophyllous species (n = 14), 18 chlorophyllous *Paris*-type species (n = 100) and 15 chlorophyllous *Arum*-type species (n = 104).

supported by either an ^{15}N enrichment (1.3 ± 0.9 ‰, *Gentiana lutea* and *Alchemilla* sp.; Fig. 1, dark brown), an ^{2}H enrichment (6.5 ± 6.0 ‰, *Astrantia major*; Fig. 1, light brown) or both (1.7 ± 1.1 ‰ and 13.8 ± 4.4 ‰, *Ligusticum mutellina*, Fig. 1, dark gold; Table 2). The open-land species *Aquilegia atrata* and *Trollius europaeus* were found to be ^{13}C inconspicuous (0.9 ± 0.9 ‰) while a significant ^{2}H enrichment was disclosed (14.8 ± 6.3 ‰ and 21.3 ± 5.6 ‰, respectively; Supplementary data Table S3). Species that were ^{13}C enriched but where data on ^{2}H are not available could be suspected of partial mycoheterotrophic nutrition and should be further investigated (2.0 ± 1.3 ‰, e.g. *Asphodelus aestivus*, *Geranium sylvaticum*, *Meum athamanticum*, *Scandix pecten-veneris* and ferns). *Botrychium lunaria*, *Brachypodium sylvaticum*, *Molinia caerulea* and *Pimpinella saxifraga* were found to be only ^{15}N enriched, while data on ^{2}H enrichment are still missing for these species.

Ellenberg indicator values found chlorophyllous *Paris*-type species to be significantly distinguished from chlorophyllous *Arum*-type species in their preferences for light availability

Table 1. *Pairwise Dunn's post-hoc test (Z) for significance of differences between the three functional groups of achlorophyllous species on* Paris-*type AM (full mycoheterotrophs), chlorophyllous potentially partial mycoheterotrophs on* Paris-*type AM and chlorophyllous* Arum-*type AM plant species as references in enrichment factors* $\varepsilon^{13}C$, $\varepsilon^{15}N$ *and* $\varepsilon^{2}H$

	ε^{13}C		ε^{15}N		ε^{2}H	
	Test statistics	*P*	Test statistics	*P*	Test statistics	*P*
Achlorophyllous *Paris*-type vs. chlorophyllous *Paris*-type	$Z = 11.193$	**<0.001**	$Z = 10.384$	**<0.001**	$Z = 3.885$	**<0.001**
Achlorophyllous *Paris*-type vs. chlorophyllous *Arum*-type	$Z = 17.176$	**<0.001**	$Z = 11.772$	**<0.001**	$Z = 7.009$	**<0.001**
Chlorophyllous *Paris*-type vs. chlorophyllous *Arum*-type	$Z = 10.570$	**<0.001**	$Z = 2.451$	**0.007**	$Z = 6.331$	**<0.001**

Significances are highlighted in bold.

For ^{13}C/^{15}N, the data comprise 13 achlorophyllous *Paris*-type species ($n = 99$), 63 chlorophyllous *Paris*-type species ($n = 520$) and 59 chlorophyllous *Arum*-type species ($n = 530$). For ^{2}H, the data comprise three achlorophyllous species ($n = 14$), 18 chlorophyllous *Paris*-type species ($n = 100$) and 15 chlorophyllous *Arum*-type species ($n = 104$).

Table 2. *Proportional C gain (%) of all 31 chlorophyllous* Paris-*type species that were identified as significantly different in stable isotope enrichment factors* $\varepsilon^{13}C$ *from neighbouring* Arum-*type species*

	***Paris*-type species**	**Family**	***n***	**Proportional C gain (%)**	
				Range	Mean s.d.
H,o	*Equisetum arvense*	Equisetaceae	5	53–81	63 ± 10
H,f	*Equisetum fluviatile*	Equisetaceae	5	6–12	9 ± 3
H,f	*Equisetum sylvaticum*	Equisetaceae	4	48–81	63 ± 14
H,f	*Equisetum telmateia*	Equisetaceae	5	12–73	41 ± 24
F,f	*Pteridium* sp.	Dennstaedtiaceae	2	64–72	68
F,f	*Blechnum* sp.	Blechnaceae	2	25–79	52
F,f	*Athyrium filix-femina*	Athyriaceae	5	69–139	93 ± 28
F,f	*Diplazium sandwichianum*	Athyriaceae	6	50–122	91 ± 30
F,f	*Dryopteris filix-mas*	Dryopteridaceae	5	33–99	73 ± 25
F,f	*Polystichum* sp.	Dryopteridaceae	16	3–113	64 ± 31
F,f	*Polypodium vulgare*	Polypodiaceae	5	30–70	48 ± 16
S,f	*Tamus communis*	Dioscoreaceae	5	–13 to 41	19 ± 22
S,f	*Paris quadrifolia*	Melanthiaceae	13	24–79	47 ± 16
S,f	*Smilax aspera*	Smilacaceae	5	5–48	26 ± 19
S,o	*Asphodelus aestivus*	Asphodelaceae	25	–16 to 91	18 ± 26
S,o	*Bromus erectus*	Poaceae	29	–7 to 54	18 ± 15
S,o	*Bromus* sp.	Poaceae	21	–42 to 50	11 ± 22
S,f	*Melica nutans*	Poaceae	4	5–42	16 ± 17
S,f	*Anemone nemorosa*	Ranunculaceae	10	–5 to 53	25 ± 17
S,o	*Ranunculus* sp.	Ranunculaceae	23	–41 to 38	7 ± 20
S,o	*Alchemilla vulgaris*	Rosaceae	4	18–36	29 ± 8
S,o	*Alchemilla* sp.	Rosaceae	6	–81 to 64	8 ± 49
S,f	*Oxalis acetosella*	Oxalidaceae	9	–9 to 59	20 ± 21
S,f	*Mercurialis perennis*	Euphorbiaceae	31	–3 to 85	33 ± 22
S,f	*Geranium sylvaticum*	Geraniaceae	5	42–66	52 ± 10
S,f	*Lysimachia nummularia*	Primulaceae	5	5–32	23 ± 11
S,o	*Gentiana lutea*	Gentianaceae	5	55–108	73 ± 21
S,o	*Astrantia major*	Apiaceae	25	–31 to 50	17 ± 19
S,o	*Ligusticum mutellina*	Apiaceae	5	13–25	16 ± 5
S,o	*Meum athamanticum*	Apiaceae	10	23–76	46 ± 17
S,o	*Scandix pecten-veneris*	Apiaceae	10	–4 to 44	22 ± 17

In total, 63 chlorophyllous *Paris*-type plant species were tested, thus 32 species were insignificantly enriched in ^{13}C (see Supplementary data Table S3 for the complete list). Nomenclature follows the sources APG IV (2016) and PPG I (2016). Family sequences are according to PPG I (2016) (pteridophytes), Haston *et al.* (2009) and APG IV (2016) (angiosperms).

F, fern; H, horsetail; S, seed plant; f, forest; o, open-land.

[$U(94,148) = 5416.5$, $P = 0.003$, Cohen's $d = 0.4$] and temperature [$H(66,111) = 2605.5$, $P < 0.001$, Cohen's $d = 0.5$]. Although the Cohen's *d* index probably penalizes the imbalance in sample size distribution, these differences tendentially indicate that *Paris*-type species occur preferentially in habitats of lower light availability and lower temperature (Supplementary data Fig. S1). Among the chlorophyllous *Paris*-type ferns and horsetails for which both Ellenberg light availability values and

ε^{13}C enrichment factors of *Paris*-type species were available, significant relationships were found to ^{13}C enrichments. This means proportionally higher C gains from the fungal source under conditions of lower light availability (Fig. 2). There was no significant correlation between Ellenberg light availability values and ε^{13}C in seed plants, which could be due to the existence of a few highly ^{13}C-enriched open-land plant species (*Gentiana lutea* and *Meum athamanticum*). Chlorophyllous *Paris*-type ferns, horsetails and seed plants from forests together were more ^{13}C enriched than open-land *Paris*-type plants [$U(148,163) = 6272.6$, $P < 0.001$, Cohen's $d = 0.9$]. This relationship also holds when only comparing seed plants that were significantly ^{13}C enriched from forests and open lands [$U(73,84) = 2368$, $P = 0.014$, Cohen's $d = 0.4$; Fig. 2].

DISCUSSION

We found that partial mycoheterotrophy is common in plants with AM. In plants with *Paris*-type AM, we found significant ^{13}C enrichment in about half of the species under study (31 out of 63 *Paris*-type species). Two of these, *Paris quadrifolia* and *Anemone nemorosa*, coincide with our previous report (Giesemann *et al.*, 2020*b*). Among some of the remaining species with *Paris*-type AM, the enrichment in ^{15}N, ^{2}H or both may suggest that some mycoheterotrophy did occur. Plant species with *Paris*-type AM might thus present a continuous range between full autotrophy and full mycoheterotrophy. Our results are in support of Imhof (1999) who suggested that hyphal growth within root cells, such as the intracellular hyphal coils of the *Paris* morphotype, is an important prerequisite for the evolution of mycoheterotrophy.

Paris-type partial mycoheterotrophy seemed to be common in the group of pteridophytes, and among seed plants we found it in 13 families, both mono- and dicotyledons. This suggests that the capacity to parasitize Glomeromycotina fungi in a *Paris*-type AM relationship is widespread in the plant kingdom and, important to note, exists among plants with fully developed leaves which up to now have been thought to be fully photo-assimilating.

Our 31 species with partial mycoheterotrophy on *Paris*-type AM add to the steadily increasing list of plant species with this kind of nutrition; currently 124 plant species are known. Most of these belong in Orchidaceae (orchid mycorrhiza) and Ericales (ericoid mycorrhiza) (Hynson *et al.*, 2013, 2016; Gebauer *et al.*, 2016; Schiebold *et al.*, 2018).

Previous discoveries of partial mycoheterotrophy on *Paris*-type AM amount to a few species with apparent physiological and evolutionary prerequisites. *Ophioglossum kawamurae*, *O. parvum* and *O. thermale* (sporophyte, Ophioglossaceae), found by Suetsugu *et al.* (2020*b*) to be partially mycoheterotrophic, belong to a family where the gametophyte generation is known to be achlorophyllous and mycoheterotrophic. Other examples belong to families known to contain achlorophyllous members: *Bartonia virginica*, *Obolaria virginica* and *Pterygocalyx volubilis* in Gentianaceae (Cameron and Bolin, 2010; Suetsugu *et al.*, 2020*a*) and *Burmannia coelestis* in Burmanniaceae (Bolin *et al.*, 2017). In view of our new records, these first few examples appear to just graze the surface of something

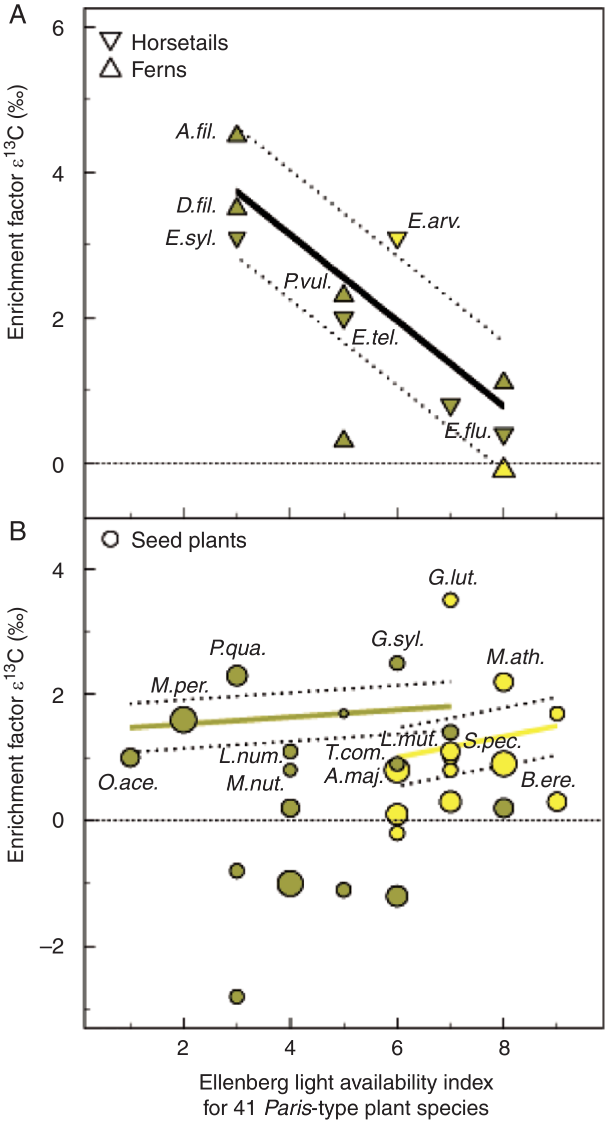

FIG. 2. Relationship between the Ellenberg light availability index and stable isotope enrichment factors ε^{13}C for 41 *Paris*-morphotype arbuscular mycorrhizal (AM) plant species. Significance of ^{13}C enrichment tested towards co-occurring *Arum*-type AM species is highlighted by species labels (see Supplementary data Table S3). (A) Pteridophytes (horsetails and ferns). A negative correlation between light and fungal carbon gain [black solid line, $t(34) = 12.8$, $r^2_{\text{adj}} = 0.7$, $P = 0.016$, dotted line: 95 % confidence intervals]. (B) Seed plant species. No clear correlation. The coloured solid lines illustrate a regression among seed plant species significantly ^{13}C enriched separated by forest habitats [dark khaki-coloured solid line and symbols; $t(73) = 0.1$, $r^2_{\text{adj}} = 0.0$, $P = 0.741$] and open lands [light khaki-coloured solid line and symbols; $t(84) = 0.1$, $r^2_{\text{adj}} = 0.0$, $P = 0.729$] (dotted 95 % confidence intervals). Ellenberg light availability index and AM morphotype were obtained from the literature (see the Materials and Methods). The single *Paris*-type AM plant species are represented by mean values. Standard deviations are omitted for clarity. Symbol size reflects the sample size of the *Paris*-type species ($n = 1$–31, see Supplementary data Table S3). The figure counts only *Paris*-type AM species with data on ε^{13}C and Ellenberg light availability values. *Paris*-type plant species that were significantly ^{13}C enriched: (A) A.fil. *Athyrium filix-femina*, D.fil. *Dryopteris filix-mas*, E. syl. *Equisetum sylvaticum*, P.vul. *Polypodium vulgare*, E.tel. *Equisetum telmateia*, E.arv. *Equisetum arvense*, E.flu. *Equisetum fluviatile*; (B) O.ace. *Oxalis acetosella*, M.per. *Mercurialis perennis*, P.qua. *Paris quadrifolia*, L.num. *Lysimachia nummularia*, M.nut. *Melica nutans*, G.syl. *Geranium sylvaticum*, T.com. *Tamus communis*, A.maj. *Astrantia major*, G.lut. *Gentiana lutea*, S.pec. *Scandix pecten-veneris*, L.mut. *Ligusticum mutellina*, M.ath. *Meum athamanticum*, B.ere. *Bromus erectus*.

much bigger. In surveying plants that have served as references in other studies, we were able to make an assessment without any pre-conceived expectations regarding certain species or groups. Thus, we could demonstrate significant mycoheterotrophy not only in most pteridophytes tested, but also in ten families of seed plants where it has not been recorded, or even suspected, previously (Apiaceae, Asphodelaceae, Dioscoreaceae, Euphorbiaceae, Geraniaceae, Oxalidaceae, Poaceae, Primulaceae, Rosaceae and Smilacaceae). To this list we may add two more families: Melanthiaceae and Ranunculaceae for *Paris quadrifolia* and *Anemone nemorosa* which we identified as partially mycoheterotrophic quite recently (Giesemann *et al.*, 2020*b*).

Since organic C within the fungi ultimately comes from photo-assimilating plants (either alive or as plant debris), partial mycoheterotrophy does not interact only with the fungi in question, but also with potential donor plants in the ecosystem. Since fungi belonging to the Glomeromycotina are considered to be obligate biotrophs, we should be expecting live donor plants. We found partial mycoheterotrophy only in herbaceous species with *Paris*-type AM. The woody species under study were either *Arum*-type plants, or *Paris*-type without any clear indication of mycoheterotrophy. Transfer of photosynthates from the roots of woody species could be a way for understorey AM species to compensate for a light-limited environment. The negative relationship in pteridophyte species between light and ^{13}C enrichment suggests such a mechanism and is consistent with our third hypothesis. Previous studies indicated a limited photosynthetic capacity of many ferns and horsetails (e.g. Ludlow and Wolf, 1975; Gago *et al.*, 2013; Nadal *et al.*, 2018), and photosynthetic rates lower than in corresponding *Arum*-type species were found in several *Paris*-type AM deciduous forest trees (Wright *et al.*, 2004) and *Paris*-type AM forest ground herbaceous species (Dalke *et al.*, 2018).

Some *Paris*-type AM tree saplings (*Acer campestre*, *A. pseudoplatanus* and *Cornus controversa*) did show indications towards partial mycoheterotrophy. Whether this condition only characterizes the juvenile trees or persists throughout life is a matter for future investigations.

Between herbaceous plant species, however, partial mycoheterotrophy could also be a competitive advantage in relation to species with *Arum*-type mycorrhizal connections. Partial mycoheterotrophy in some species might increase biodiversity by restricting development in other, more vigorously growing members, in analogy with the effect that hemiparasitic plant species may have on the surrounding vegetation and ecosystem (Quested *et al.*, 2003; Quested, 2008; Hartley *et al.*, 2015). Partial mycoheterotrophy may thus have profound effects with respect not only to C cycling but also to the cycling of nutrients such as N.

Interspecific and interfamilial variations in ^{15}N enrichments are known from other groups of mycoheterotrophic plants (Orchidaceae and Pyroloideae; Hynson *et al.*, 2016) and also from AM full mycoheterotrophs (Merckx *et al.*, 2010; Courty *et al.*, 2011; Gomes *et al.*, 2020). The various ^{15}N enrichments were attributed to different fungal partners that access different N nutrient sources (Schiebold *et al.*, 2017). The variations in this study of ^{15}N enrichments in the partially mycoheterotrophic species might indicate different forms of N utilized by Glomeromycotina fungi. Significant ^{15}N enrichments without any ^{13}C and ^{2}H enrichments might, however, also be attributed to a functional role in N acquisition by entirely different fungal endophytes (Hoysted *et al.*, 2019; Giesemann *et al.*, 2020*a*). In addition to stable isotope natural abundance, the total leaf N concentration has been used to differentiate between mycoheterotrophy on orchid mycorrhiza (high leaf N concentrations) and ericoid mycorrhiza (low leaf N concentrations) (Hynson *et al.*, 2016). This difference is thought to be due to different fungal matter uptake mechanisms by the various types of mycoheterotrophic plants: digestion of fungal ‘pelotons’ in addition to active membrane transport in orchid mycorrhiza and exclusively active membrane transport in ericoid mycorrhiza (Hynson *et al.*, 2016). Since partially mycoheterotrophic *Paris*-type AM species do not differ markedly in leaf total N concentration from fully autotrophic *Arum*-type AM species, a similar active membrane transport mechanism for the uptake of N is suggested for both. However, digestion of *Paris*-morphotype coils has also been documented (Imhof *et al.*, 2013) and life history strategies rather than fungal substrate and trophic strategies could contribute to leaf total N concentration patterns as well (cf. Hynson *et al.*, 2016).

The high diversity in partial mycoheterotrophs as found here is in agreement with the recent finding of low phylogenetic constraints for developing mycoheterotrophy (Perez-Lamarque *et al.*, 2020). A significant proportion of the AM plants, and thus also of all plant species globally, possess the required infection pattern (*Paris*-type AM) to obtain C from a fungal source. Based on these findings, partial mycoheterotrophy, indeed, is more widespread than recognized so far (Giesemann *et al.*, 2020*b*). Nonetheless, there still remain a lot of open questions to be resolved. Our current survey on the occurrence of *Paris*-type AM is mostly based on sometimes old lists from the literature that might require careful re-evaluation. A matter of further research has to be also the consideration of intermediate AM types, plant species that are able to form either *Arum*- or *Paris*-type AM and the influences of environmental conditions and AM fungal taxa on the formation of either *Arum*- or *Paris*-type AM.

We conclude that the fungal *Paris*-coiling type appears to be a necessary prerequisite for partial mycoheterotrophy in chlorophyllous AM plant species. However, not all chlorophyllous *Paris*-morphotype AM plant species turned out to be partially mycoheterotrophic.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Figure S1: Ellenberg indicator value for light availability and temperature between *Arum*- and *Paris*-type arbuscular mycorrhizal plant species. Table S1: stable isotope enrichment factors ε with s.d., leaf total N concentrations with s.d. and the arbuscular mycorrhizal type. Table S2: pairwise Dunn’s post-hoc test for significance of differences between chlorophyllous *Arum*- and *Paris*-type arbuscular mycorrhizal plant species separated by groups of horsetails, ferns and seed plants in enrichment factors ε^{13}C, ε^{15}N and ε^{2}H. Table S3: Mann–Whitney *U*-test for significance of differences between chlorophyllous *Paris*-type species and their respective chlorophyllous *Arum*-type reference plant species in stable isotope enrichment factors ε^{13}C, ε^{15}N and ε^{2}H.

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