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RESEARCH

Can cardiolipins be used as a biomarker for arbuscular mycorrhizal fungi?

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Abstract

Specific biomarker molecules are increasingly being used for detection and quantification in plant and soil samples of arbuscular mycorrhizal (AM) fungi, an important and widespread microbial guild heavily implicated in transfers of nutrients and carbon between plants and soils and in the maintenance of soil physico-chemical properties. Yet, concerns have previously been raised as to the validity of a range of previously used approaches (e.g., microscopy, AM-specific fatty acids, sterols, glomalin-like molecules, ribosomal DNA sequences), justifying further research into novel biomarkers for AM fungal abundance and/or functioning. Here, we focused on complex polar lipids contained in pure biomass of *Rhizophagus irregularis* and in nonmycorrhizal and mycorrhizal roots of chicory (*Cichorium intybus*), leek (*Allium porrum*), and big bluestem (*Andropogon gerardii*). The lipids were analyzed by shotgun lipidomics using a high-resolution hybrid mass spectrometer. Size range between 1350 and 1550 Da was chosen for the detection of potential biomarkers among cardiolipins (1,3-bis(*sn*-3′-phosphatidyl)-*sn*-glycerols), a specific class of phospholipids. The analysis revealed a variety of molecular species, including cardiolipins containing one or two polyunsaturated fatty acids with 20 carbon atoms each, i.e., arachidonic and/or eicosapentaenoic acids, some of them apparently specific for the mycorrhizal samples. Although further verification using a greater variety of AM fungal species and samples from various soils/ecosystems/environmental conditions is needed, current results suggest the possibility to identify novel biochemical signatures specific for AM fungi within mycorrhizal roots. Whether they could be used for quantification of both root and soil colonization by the AM fungi merits further scrutiny.

Keywords Biomarker · Cardiolipins · Extraradical mycelium · Root colonization · Quantification · Shotgun lipidomics

Introduction

Arbuscular mycorrhizal (AM) fungi are widespread symbionts of plants, recruiting from Glomeromycotina and Mucoromycotina clades, and colonizing roots/rhizoids/thalli of a majority of plant species on Earth (Field and Pressel 2018; Rimington et al. 2019). These fungi are heavily implicated in bidirectional transfers of mineral nutrients and carbon between plants and soil, in soil aggregate stabilization, as well as in the maintenance/promotion of soil and ecosystem diversity and stability upon fluctuating/changing environmental conditions (van der Heijden et al. 2008; Bardgett and van der Putten 2014; Powell and Rillig 2018). The fungi colonize and functionally interconnect roots with the surrounding soil (Kuyper and Jansa 2023). Given the importance of AM symbioses in soil and ecosystem processes, quantification of AM fungal development both in the roots and in the soil has been under scrutiny for a long time (Vierheilig et al. 2005; Thonar et al. 2012; Janoušková et al. 2015; Arruda et al. 2022).

There are many methods to determine the biomass of AM fungi inside and to a lesser extent, also outside roots. These methods are either microscopic (Hanssen et al. 1974; Sylvia 1992; Vierheilig et al. 2005) or reliant on quantification of biochemical (Schmitz et al. 1991; Balser et al. 2005; Olsson and Lekberg 2022) or molecular signatures (Redecker 2000; Schwarzott and Schüβler 2001). Those biochemical signature compounds that have been tested in the past are, for example, chitin, ergosterol, or specific fatty acids. Unfortunately, chitin persists even after the fungus dies, its analysis is technically demanding, and it also is present in

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other (non-AM) fungi, insects, and arthropods (Sylvia 1992; Olsson 1999). The AM fungi (at least the Glomeromycotina clade) contain little or no detectable ergosterol (Frey et al. 1992, 1994; Fontaine et al. 2001; Olsson et al. 2003), which otherwise is frequently used for quantification of (non-AM) fungal biomass in various samples (van Groenigen et al. 2010; Heidarianpour et al. 2020). Therefore, in most cases, phospholipids or neutral lipids, specifically, the fatty acids contained in them, have been used as proxies for quantification of AM fungal biomass. Palmitvaccenic acid (16:1Δ11 or 16:1ω5) is the most used biomarker (or signature) compound found specifically in AM fungi from the Glomeromycotina clade and not in most plant roots. Hundreds of articles have been published to date using this biochemical signature; see Olsson and Lekberg (2022) for details. Those previous papers establish the use of 16:1ω5 fatty acid to quantify the biomass of various AM fungi in various sample types, sometimes combined with compound-specific stable isotopic analyses to measure the transfer of carbon from plant to AM fungus (e.g., Konvalinková et al. 2017). In the published papers, great discussion has arisen about the appropriateness of using the 16:1ω5 fatty acid as a biochemical signature specifically for the AM fungi, and which chemical forms (neutral- or phospho-lipids) would be most appropriate to use and why (Lekberg et al. 2022).

Besides the AM fungi, where the 16:1ω5 fatty acid is often present in large amounts (Vořísková et al. 2017), the same fatty acid also is found in some bacteria (Walker 1969; Nichols et al. 1986; Frostegård et al. 2011; Joung et al. 2015; Chen et al. 2020; Islam et al. 2020), although that often is considered to cause no major interference with quantification of AM fungal biomass (Joergensen (2022). Despite previous claims that plants do not produce phospholipids with 16:1ω5 fatty acid bound in them (Brands et al. 2020), some species within the Proteaceae family are obviously metabolically capable of synthesizing this fatty acid (Vickery 1971). A public database tool (https://plantfadb.org/tree; Ohlrogge et al. 2018) based on seed oil fatty acid profiles (Matthäus 2012) lists 93 occurrences of the 16:1ω5 fatty acid in 60 among approximately 8000 species of plants, which are included in the database, although most of the analyses were done with seeds or leaves, not roots. Therefore, plant interferences with the estimation of AM fungal biomass cannot be completely excluded.

Another technical issue in fatty acids profiling/identification is the similarity of mass spectra (MS) of positional and geometric (*cis–trans*) isomers (Christie 2023). This is because positional and geometric isomers are formed by distinct organisms, employing different metabolic pathways. For example, only the palmitvaccenic acid in *cis* configuration is present in significant amounts in AM fungal biomass, whereas palmitoleic acid (16:1ω7) is abundant in bacteria (Cronan and Thomas 2009; Brands et al. 2020; Olsson and Lekberg 2022). Therefore, separation of the different fatty acids using chromatography columns with high resolution and unequivocal identification of the position(s) of double bonds in the molecule by using chemical derivatization (e.g., 3-pyridylcarbinols, 4,4-dimethyloxazolines, pyrrolidides, or dimethyl disulfide) is necessary. Whereas separation of different fatty acids by gas chromatography (GC) often precedes MS analyses, precise structural analyses of double bond positions rarely have been done in AM research (but see Brands et al 2020 and Rezanka et al 2022 for notable exceptions).

Because of various concerns, some of which are indicated above, several recent articles (Holátko et al. 2021; Lekberg et al. 2022) scrutinizing the use of 16:1ω5 and other compounds as biomarkers for AM fungi proposed searching for and using alternative compounds with greater complexity than fatty acids that are specific for AM fungi. One such possibility would be phosphatidylcholine (PC) with polyunsaturated fatty acids such as 20:3, 20:4, or 20:5 (20:5/20:5-PC, 20:5/20:4-PC or 20:5/20:3-PC (Wewer et al. 2014)). Unfortunately, there are still concerns about the variation in the amount of such compounds per unit of biomass between different species/families of AM fungi—similar to other biomarkers (Vořísková et al. 2017). In the research described here, we delved into screening for complex phospholipids as potentially novel biomarkers for AM fungi, capitalizing on knowledge and experience acquired previously (Rezanka 1993; Rezanka et al. 2016, 2020). The use of high-resolution electrospray ionization mass spectrometry (HR–ESI–MS), an instrument increasingly available in various labs around the globe, enabled the detection and identification of different compounds in picomolar concentrations by using fast-throughput shotgun lipidomics without the need to separate complex lipid mixtures by chromatography prior to the MS analyses.

Materials and methods

Biological materials

Pure biomass of *Rhizophagus irregularis* LPA9, devoid of plant roots and free of microbes, was produced in vitro, along with the root organ culture of cichory (*Cichorium intybus*) as described previously (Rezanka et al. 2022). The cultivation system is shown in supplementary Fig. S1. Non-mycorrhizal roots of chicory were produced using the same system and for the same length of time as the mycorrhizal in vitro cultures. Additionally, mycorrhizal and nonmycorrhizal roots of leek (*Allium porrum*) and big bluestem (*Andropogon gerardii*) were produced in pots filled with a potting substrate comprising previously sterilized soil, sand, and granular zeolite as described previously (Püschel

et al. 2016) and inoculated or not with an in vitro-produced inoculum of *Rhizophagus irregularis* LPA9. The pot cultures (four individual pots per plant and mycorrhizal treatment combination) were harvested after 4 months of growth in the glasshouse, whereas the in vitro cultures (five in vitro containers for each mycorrhizal treatment) were harvested after 7 months of growth in a darkroom at 24 °C. Upon harvest, the roots and the pure AM fungal biomass were briefly washed with deionized water, pooled per sample type and mycorrhizal treatment, frozen at − 80 °C, and subsequently lyophilized, yielding at least 350 mg dry mass for each of the seven samples. The samples were then milled using a ball mill MM200 (Retsch, Haan, Germany). Aliquots of the samples were used for the determination of the extent of root colonization by the AM fungus, employing Trypan blue staining (Koske and Gemma 1989) followed by microscopy (McGonigle et al. 1990), for the determination of the 16:1ω5 fatty acid concentration in the total lipid extracts (Welc et al. 2012), and for molecular quantification of the AM fungus targeting the mitochondrial large ribosomal subunit of *Rhizophagus* sp. (Couillerot et al. 2013)—see supplementary Table S1 for details.

Lipid extraction and analyses

The extraction procedure was based on the method published previously (Bligh and Dyer 1959) with a few modifications introduced later (Rezanka 1993). Briefly, lyophilized and pulverized materials were suspended in a dichloromethane:methanol mixture (2:1, v:v) for 30 min with stirring, after which dichloromethane and water were added, and the dichloromethane (i.e., lower) phase was evaporated to dryness under reduced pressure. Total lipids were dissolved in the mobile phase (acetonitrile:2-propanol (99:1, v:v)) for further analysis by shotgun lipidomics, employing HR–ESI–MS, with no further fractionation or derivatization. Additional technical details are provided in the supplementary material.

Limits of detection and quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) of the shotgun method were determined based on the analysis of a commercially available standard, i.e., cardiolipin (CL) containing oleic (18:1) and stearic (18:0) fatty acids (18:1/18:0/18:1/18:0-CL) with final concentrations of 10 fM, 100 fM, 1 pM, 10 pM, 100 pM, and 1 nM. The LOD and LOQ were determined as the concentrations with signal-to-noise ratios of 3 and 10, respectively. The reproducibility of the peak area was determined from six consecutive measurements of 18:1/18:0/18:1/18:0-CL for low (100 fM) and high (1 nM) concentrations. The LOD of 18:1/18:0/18:1/18:0-CL was 147 fM, well matching with the previously published value of LOD = 105.2 fM for $(14:0)_4$-CL by Lee et al. (2019). LOD and LOQ of a newly identified CL from the AM fungus were determined by serial dilution of the lipid extracts containing the compound and monitoring a characteristic ion signal height vs. noise ratio.

Statistical analyses

Relative abundances of the different molecular species of CLs determined in the total lipid profiles (see Table 1 for an overview) were compared between 3 mycorrhizal and 3 nonmycorrhizal root samples (one pair for each plant species) included in this study by Kruskal–Wallis nonparametric test, using Statgraphics Plus for Windows v. 3.1.

Results

The MS analyses were performed in the mass interval of 1350–1550 Da. This region was chosen because CLs appeared in this range, unlike other neutral, phospho- and glyco-lipids which all had a molecular weight well below 1200 Da and thus did not interfere with the analyses. For example, a triacylglycerol containing one eicosapentaenoic (20:5) and two arachidonic (20:4) acids has a molecular weight of 948 Da, 20:5/20:4-phosphatidylcholine has 827 Da, 20:5/20:4-phosphatidylglycerol has 816 Da, 20:5/20:4-monogalactosyldiacylglycerol has 824 Da, and 20:5/20:4-digalactosyldiacylglycerol has 986 Da. Most CLs, particularly the molecular species from *m/z* 1370 to *m/z* 1458, corresponding to 66:3-CL to 72:3-CL (with the number before the colon indicating the sum of carbon atoms in the fatty acids, whereas the number after the colon indicates the sum of double bonds in the fatty acids) had a similar abundance in all samples or, occasionally, the relative abundance in nonmycorrhizal samples was greater than in the mycorrhizal samples (Fig. 1a, Table 1). In contrast, the CLs with 74 and 76 carbon atoms often were relatively much more abundant in mycorrhizal than nonmycorrhizal samples (Fig. 1a, Table 1). Particularly, the nonmycorrhizal root samples did not contain any CLs with at least one polyunsaturated fatty acid (PUFA) such as the 20:4 and/or 20:5 acids present in the molecule. On the contrary, both the AM fungus alone and the mycorrhizal roots contained CLs with such acids. Therefore, we selected 76:12-CL, containing four different fatty acids including two PUFAs in the molecule (Fig. 1b and the supplementary Fig. S2) as the most promising biomarker among the different CLs for AM fungal biomass. This compound also had the highest relative abundance among CLs with 74 and 76 carbon atoms in the fatty acids (Table 1), although its absolute concentrations in the samples were rather low (see supplementary Table S1 for details). The amount of biomass needed for reliable

Table 1 Relative abundance of different molecular species (mol spec) of cardiolipins in seven biological samples included in this study, representing roots of plants (*Allium*, *Andropogon* or *Cichorium*) without mycorrhizal fungus (NM) or colonized by the mycorrhizal fungus *Rhizophagus irregularis* (M), and the pure biomass of *Rhizophagus irregularis* (genotype LPA9) produced in a monoxenic in vitro system

mol spec	*Rhizophagus* pure biomass	*Allium* (NM)	*Allium* + *Rhizophagus* (M)	*Andropogon* (NM)	*Andropogon* + *Rhizophagus* (M)	*Cichorium* (NM)	*Cichorium* + *Rhizophagus* (M)	Difference
66:3[a]	0.2	0.2	0.1	0.1	0.2	0.1	0.2	
66:2	0.1	0.2	0.2	0.2	0.3	0.3	0.4	
68:6	0.5	0.8	0.4	1.0	0.7	0.5	0.4	
68:5	0.8	1.0	0.6	0.9	0.6	0.8	0.5	NM > M
68:4	0.6	1.1	0.7	0.8	0.5	0.7	0.6	
68:3	0.1	0.0	0.1	0.1	0.1	0.2	0.2	
68:2	0.0	0.1	0.1	0.2	0.1	0.1	0.1	
68:1	0.0	0.2	0.1	0.2	0.1	0.0	0.1	
70:10	0.9	1.5	1.5	1.4	1.1	0.7	0.9	
70:9	1.3	0.5	0.1	0.7	0.2	0.1	0.8	
70:8	1.4	1.8	1.3	1.3	0.5	0.6	1.4	
70:7	1.8	1.1	0.7	1.1	0.5	0.5	0.9	
70:6	0.5	1.1	0.6	1.2	0.9	0.5	1.1	
70:5	0.6	0.6	0.4	0.9	0.4	0.8	0.6	NM > M
70:4	0.5	0.6	0.5	0.6	0.5	0.8	0.7	
70:3	0.3	0.2	0.2	0.4	0.2	0.3	0.4	
70:2	0.1	0.4	0.1	0.4	0.3	0.1	0.3	
72:12	2.2	5.8	1.9	5.8	2.6	2.4	4.9	
72:11	8.5	8.3	12.3	9.2	10.6	9.3	7.5	
72:10	10.5	10.5	14.7	10.1	12.2	14.5	15.3	
72:9	14.6	9.8	11.8	18.8	15.1	15.4	9.8	
72:8	12.1	17.6	12.6	11.4	8.3	16.7	13.3	
72:7	10.2	19.6	11.3	13.2	8.9	11.0	11.8	
72:6	13.9	9.5	11.4	12.3	16.6	14.2	7.7	
72:5	6.3	7.1	8.4	7.4	8.2	9.0	10.6	
72:4	0.1	0.2	0.1	0.1	0.1	0.2	0.1	
72:3	**0.5**	**0.2**	**0.4**	**0.2**	**0.4**	**0.2**	**0.3**	**M > NM**
74:13	**0.9**	**0.0**	**0.4**	**0.0**	**0.3**	**0.0**	**0.3**	**M > NM**
74:12	**1.1**	**0.0**	**0.3**	**0.0**	**0.6**	**0.0**	**0.5**	**M > NM**
74:11	**0.9**	**0.0**	**0.5**	**0.0**	**0.8**	**0.0**	**0.8**	**M > NM**
74:10	**0.6**	**0.0**	**0.5**	**0.0**	**0.7**	**0.0**	**0.6**	**M > NM**
74:9	**0.4**	**0.0**	**0.3**	**0.0**	**0.9**	**0.0**	**0.9**	**M > NM**
74:8	**0.5**	**0.0**	**0.4**	**0.0**	**0.5**	**0.0**	**0.7**	**M > NM**
74:7	**0.3**	**0.0**	**0.2**	**0.0**	**0.6**	**0.0**	**0.6**	**M > NM**
74:6	0.1	0.0	0.0	0.0	0.0	0.0	0.0	
74:5	0.1	0.0	0.0	0.0	0.0	0.0	0.1	
74:4	**0.2**	**0.0**	**0.1**	**0.0**	**0.1**	**0.0**	**0.1**	**M > NM**
76:15	**0.8**	**0.0**	**0.4**	**0.0**	**0.8**	**0.0**	**0.6**	**M > NM**
76:14	**1.1**	**0.0**	**0.6**	**0.0**	**0.7**	**0.0**	**0.6**	**M > NM**
76:13	**0.9**	**0.0**	**0.9**	**0.0**	**0.7**	**0.0**	**0.9**	**M > NM**
76:12	**1.2**	**0.0**	**1.1**	**0.0**	**1.1**	**0.0**	**1.1**	**M > NM**
76:11	**1.1**	**0.0**	**0.9**	**0.0**	**0.9**	**0.0**	**0.7**	**M > NM**
76:10	**0.9**	**0.0**	**0.6**	**0.0**	**1.0**	**0.0**	**0.5**	**M > NM**
76:9	0.1	0.0	0.1	0.0	0.0	0.0	0.0	
76:8	**0.2**	**0.0**	**0.1**	**0.0**	**0.1**	**0.0**	**0.1**	**M > NM**

The difference between NM root samples ($n = 3$) and M root samples ($n = 3$) was tested for each molecular species by a Kruskal-Wallis nonparametric test (only results with $p < 0.05$ are shown). Potential biomarkers for the mycorrhizal samples are shown in bold

[a]The number before the colon is the sum of carbon atoms in the four acyls (i.e., fatty acids), the number after the colon is the number of double bonds in the acyls

quantification of this compound was estimated between 18 µg for the pure *Rhizophagus* biomass and 49–66 µg of the mycorrhizal roots (supplementary Table S1 and Fig. S3).

Discussion

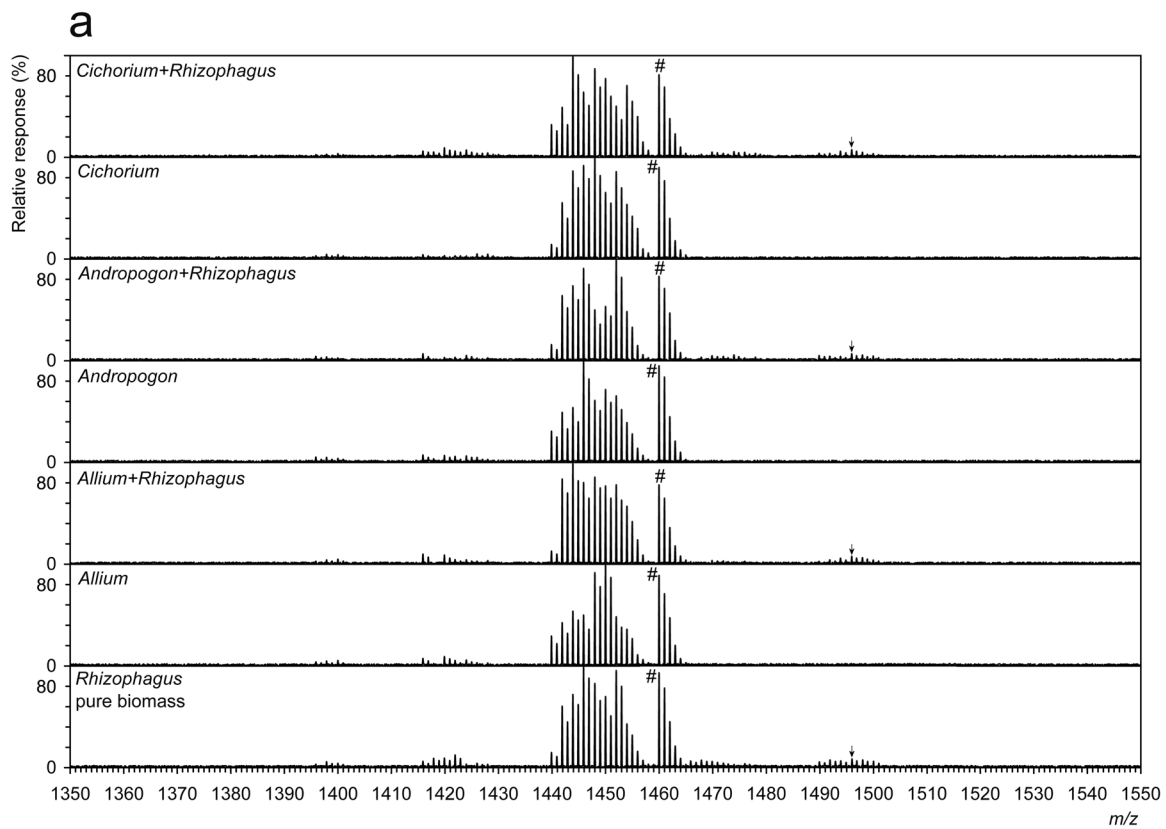
Cardiolipins occur widely in bacterial and mitochondrial membranes, which means the CLs also are supposed to be present in all eukaryotic cells wherever mitochondria occur; the occurrence of CLs in archaea is, however, still subject to debate (Schlame et al. 2000; Horvath and Daum 2013; Paradies et al. 2019; Sohlenkamp 2021). Thus, it is not quite surprising that CLs previously have been reported from a variety of plant (Zhou et al. 2016) and fungal species including the AM fungi (Beilby and Kidby 1980; Schlame et al. 1993; Gu et al. 2004; Fakas et al. 2006; Kotani et al. 2016) besides animals, from which tissues they first were isolated almost a century ago (Schlame et al. 2000). In this regard, it appears unusual that a recent detailed lipidomics analysis of *Mortierella alpina* contained no mention of any molecular species of CLs (Lu et al. 2019). Those results are particularly interesting in the context of our research because some members of the Mucoromycotina clade (where *Mortierella* belongs), the *Planticonsortium* spp., clearly form AM symbioses with some plants (Hoysted et al. 2018; Field et al. 2019), although they are phylogenetically distant from the Glomeromycotina (Walker et al. 2018).

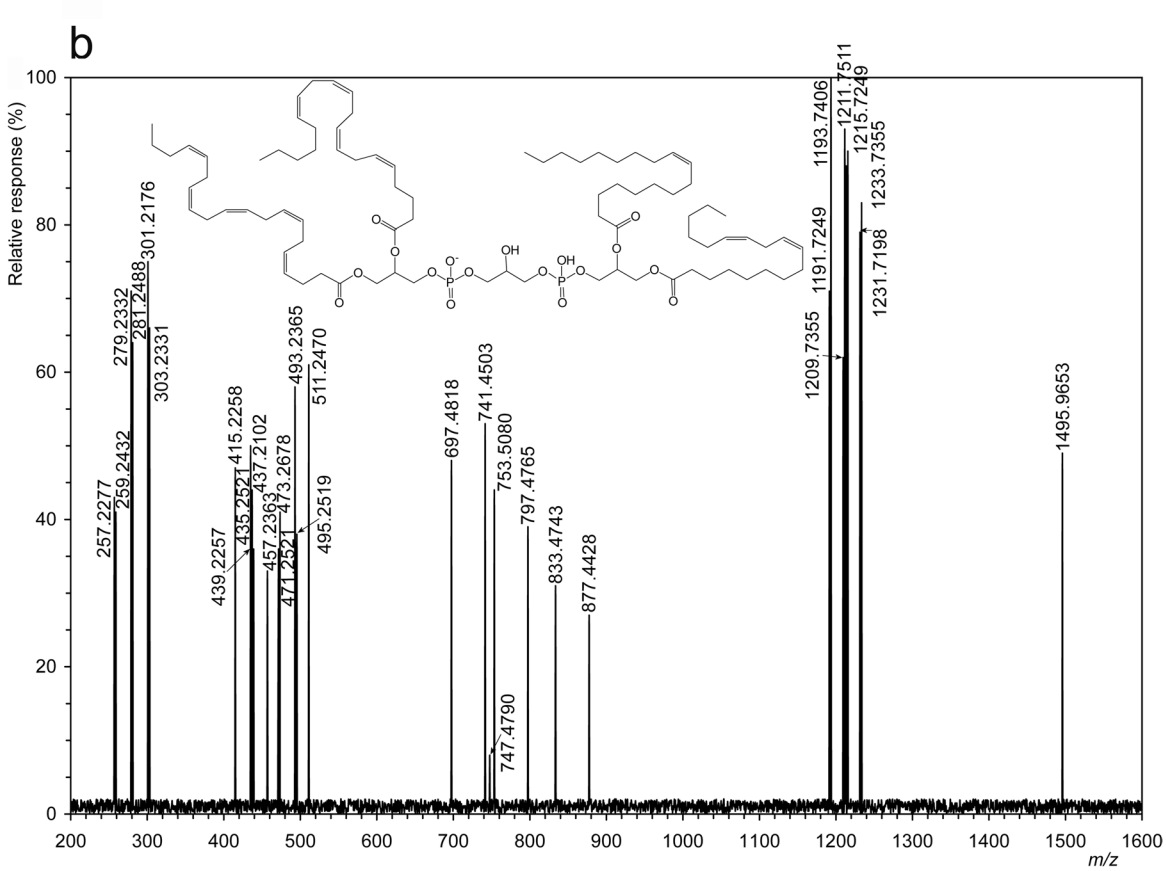
Cardiolipins are among the bulkiest phospholipid molecules and the composition of their fatty acids (four fatty acids per molecule) may differ among organisms. Previous screening of various plant species reported CLs containing mostly unsaturated fatty acids with 18 carbon atoms, the linoleic (18:2) and linolenic acid (18:3), resulting in summary formulas between 72:12 and 72:8 (Zhou et al. 2016). This agrees with our observation of small CLs prevailing in the nonmycorrhizal root samples (Table 1).

A total of nine major CLs were identified in the fungus *Neurospora crassa* containing the following fatty acids: 18:3, 18:2, oleic (18:1), and palmitic (16:0) acid. The most abundant were two molecular species, namely 18:1/18:2/18:2/18:2-CL and 16:0/18:2/18:2/18:2-CL (Schlame et al. 1993). CLs containing 16:0, 18:0, 18:1, 18:2 and γ-linolenic (γ-18:3) acids were identified in the oleaginous fungus *Cunninghamella echinulata* (Fakas et al. 2006); unfortunately, the molecular species were not analyzed. Another fungus (*Aspergillus oryzae*) contained a number of molecular CL species, namely 72:5-CL, 72:6-CL, and 72:7-CL, having a combination of only 18:1 and 18:2 fatty acids (Kotani et al. 2016). The most studied fungi are yeasts, including the species *Saccharomyces cerevisiae* (wild-type), which have the majority of their CLs with a combination of palmitoleic (16:1) and 18:1 acids, e.g., 16:1/16:1/16:1/16:1-CL, 16:1/16:1/16:1/18:1-CL, and 16:1/16:1/18:1/18:1-CL. (Schlame et al. 1993; Gu et al. 2004). Unfortunately, the publication by Wewer et al. (2014), although reporting dozens of molecular species of lipids from *Rhizophagus irregularis* during colonization by AM fungi of the roots of *Lotus japonicus*, did not mention any CLs.

Unlike most of the CLs contained in plants and saprotrophic fungi (see above), the AM fungus studied here contained a remarkable share of CLs containing PUFAs with lengths above 18 carbon atoms. The PUFAs with 20 or more carbon atoms, such as 20:4 and 20:5, are classic essential fatty acids in animals. Such compounds have not yet been broadly characterized with analytical certainty in significant amounts in spermatophytes (Groenewald and van der Westhuizen 1997; Ezeagu et al. 1998; Özcan 2007; Vickery et al. 1984), with some notable exceptions such as the lipid profiles of Araucariaceae seeds (Wolff et al. 2000). On the contrary, plants from the basal phylogenetic clades (e.g., algae including microalgae, bryophytes, and pteridophytes) have the ability to synthesize both 20:4 and 20:5 acids (Kumari and Pabbi 2023; Lu et al. 2023).

Because we observed a strong contrast in CLs containing 74 or 76 carbon atoms, i.e., CLs containing either one or two PUFAs, between mycorrhizal and nonmycorrhizal samples, it seems that such compounds, and particularly the 76:12-CL could well serve as a novel biomarker for *Rhizophagus irregularis* and perhaps AM fungi generally. The specificity and correlation with other biomass proxies still needs to be confirmed for field soil samples and for other AM fungal genotypes/species/genera. Nevertheless, identification of this novel biomarker potentially has far reaching consequences because a shortage of suitable biomarkers for the obligatorily biotrophic AM fungi persists, and there are ongoing discussions concerning if and how the contents of specific signature compounds, such as specific fatty acids, microscopic quantification of both root and soil colonization by the AM fungi, or quantification of marker genes, can be converted to biomass and/or metabolic activity of the AM fungi (Thonar et al. 2012; Voříšková et al. 2017; Lekberg et al. 2022). The use of CLs might have an added value in that such compounds are nearly exclusively found in mitochondria (Paradies et al. 2019), so they might serve as indicators of metabolic activity rather than biomass, following the same arguments as in a previous study where taxon-specific motifs in mitochondrial rRNA genes have been used to measure differences in metabolic activity of closely related AM fungal species (Kiers et al. 2011). Correlation of CL profiles of root and soil samples with symbiotic functions such as transfers of carbon and phosphorus (e.g., assessed by isotopic labelling and tracing) between the symbiotic partners and/or expression of specific genes involved in these processes ought to be studied as well. Furthermore,


a
Relative response (%)
Cichorium+Rhizophagus
Cichorium
Andropogon+Rhizophagus
Andropogon
Allium+Rhizophagus
Allium
Rhizophagus
pure biomass
#
0
80
1350 1360 1370 1380 1390 1400 1410 1420 1430 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550
m/z


b
Relative response (%)
257.2277
259.2432
279.2332
281.2488
301.2176
303.2331
415.2258
435.2521
437.2102
439.2257
457.2363
471.2521
473.2678
493.2365
495.2519
511.2470
697.4818
741.4503
747.4790
753.5080
797.4765
833.4743
877.4428
1191.7249
1193.7406
1209.7355
1211.7511
1215.7249
1231.7198
1233.7355
1495.9653
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200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600
m/z

◂**Fig. 1** **a** Mass spectra (negative ion mode) of total lipids from seven samples (*Cichorium* + *Rhizophagus, Cichorium, Andropogon* + *Rhizophagus, Andropogon, Allium* + *Rhizophagus, Allium,* and *Rhizophagus* pure biomass) acquired by high resolution electrospray ionization mass spectrometry (mass interval of 1350–1550 Da). The ion at mass-to-charge ratio (*m/z*) 1496.9653 in the mass spectra marked with an arrow is the biomarker (76:12-cardiolipin), the ion at *m/z* 1460.0594 marked with # is an internal standard (cardiolipin containing two oleic and two stearic acids). Numbers correspond to the accurate *m/z* values. **b** Mass spectrum from the secondary (tandem) mass spectrometer of the 76:12-cardiolipin from the pure *Rhizophagus irregularis* biomass (i.e., the compound shown by an arrow in the above mass spectra), which was exposed to fragmentation after analysis in the primary mass spectrometer, including the proposed structure of this compound, containing one of each arachidonic, eicosapentaenoic, oleic, and linoleic fatty acids

CLs should be monitored throughout a time series of root and soil colonization development and the contribution of hyphae and spores/intraradical vesicles should be quantified. A particularly relevant analysis would also be to determine CLs in *Planticonsortium s*pp., another AM fungus only distantly related to Glomeromycotina (see above). It also would be advantageous to know the exact order of individual acyls in the 76:12-CL (which currently is not known). This would require several additional analytical steps such as separation of individual compounds, controlled cleavage with phospholipase D, and dedicated tandem MS analyses. Although that may not be necessary for routine monitoring of the CLs as AM fungal biomarkers, detailed structural analyses of the AM-specific CLs may allow further insights into the biosynthesis of such compounds and metabolic pathways involved; similarly, detailed analysis of the AM-specific fatty acid 16:1ω5 helped to uncover the metabolic co-operation in fatty acid transfer between the plant host and the AM fungus (Brands et al 2020).

A final consideration is that the technical feasibility to analyze CLs in plant and soil samples should be weighed carefully against the potential benefits. Although sample preparation is easy and fast, compared to phospholipid fatty acids, for instance, because it includes no fractionation or derivatization steps, high-resolution shotgun lipidomics requires special equipment, one or two orders of magnitude more expensive than an ordinary GC instrument used for fatty acid profiling. Although such high-end instrumentation like the Orbitrap MS is becoming accessible to scientists in large research centers around the globe, running costs (including operation specialists' wages) may incur significant expense. On the other hand, the shotgun analyses are fast, taking only a few minutes each, compared to dozens of minutes per single fatty acid profile by GC, for example. The advantage of shotgun lipidomics is the sensitivity and reproducibility—although the CLs were not very abundant in our samples (see supplementary Table S1 for details), it actually was sufficient to enable us to use just a few milligrams of fungal/root biomass to obtain reliable results. Moreover, if the CLs amounts could indeed be related to AM fungal activity/function, such a biomarker would be of enormous value for both researchers and practitioners aiming at tracing/quantification of mycorrhizal development and benefits.

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Author contribution TŘ conceived the study and conducted the biochemical analyses. Together with JJ, he also wrote the first draft of the manuscript. HH and JJ provided biological materials and contributed to writing. LK contributed to data analysis and writing. All authors approved the final version of the manuscript before submission.

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Data availability All data generated during this study are included in this published article or its electronic supplements.

Declarations

Competing interests The authors declare that all relevant sources of funding have been acknowledged and that they are not aware of any circumstances that could be construed as constituting a financial or nonfinancial conflict of interest. Generative artificial intelligence tools have not been used during preparation of this manuscript.

References

Arruda B, Rodrigues YF, Herrera WFB, Robin A, Cotta SR, Andreote FD (2022) Experimental validation under controlled conditions of real time PCR to quantify arbuscular mycorrhizal colonization in root. J Microbiol Meth 192:106382. https://doi.org/10.1016/j.mimet.2021.106382

Balser TC, Treseder KK, Ekenler M (2005) Using lipid analysis and hyphal length to quantify AM and saprotrophic fungal abundance along a soil chronosequence. Soil Biol Biochem 37:601–604. https://doi.org/10.1016/j.soilbio.2004.08.019

Bardgett RD, van der Putten WH (2014) Belowground biodiversity and ecosystem functioning. Nature 515:505–511. https://doi.org/10.1038/nature13855

Beilby JP, Kidby DK (1980) Biochemistry of ungerminated and germinated spores of the vesicular-arbuscular mycorrhizal fungus, *Glomus caledonius*: changes in neutral and polar lipids. J Lipid Res 21:739–750. https://doi.org/10.1016/S0022-2275(20)34801-X

Bligh E, Dyer W (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917. https://doi.org/10.1139/o59-099

Brands M, Cahoon EB, Dörmann P (2020) Palmitvaccenic acid (Δ11-cis-hexadecenoic acid) is synthesized by an OLE1-like desaturase in the arbuscular mycorrhiza fungus *Rhizophagus irregularis*. Biochemistry 59:1163–1172. https://doi.org/10.1021/acs.biochem.0c00051

Chen W, Cai C, Sheu S (2020) *Sandaracinomonas limnophila* gen. nov., sp. nov., a new member of the family Cytophagaceae isolated from a freshwater mesocosm. Int J Syst Evol Microbiol 70:2178–2185. https://doi.org/10.1099/ijsem.0.003532

Christie WW (2023) The LipidWeb [WWW Document]. URL https://www.lipidmaps.org/resources/lipidweb/lipidweb_html/index.html

Couillerot O, Ramírez-Trujillo A, Walker V, von Felten A, Jansa J, Maurhofer M, Défago G, Prigent-Combaret C, Comte G, Caballero-Mellado J, Moënne-Loccoz Y (2013) Comparison of prominent *Azospirillum* strains in *Azospirillum–Pseudomonas–Glomus* consortia for promotion of maize growth. Appl Microbiol Biotech 97:4639–4649. https://doi.org/10.1007/s00253-012-4249-z

Cronan JE, Thomas J (2009) Bacterial fatty acid synthesis and its relationships with polyketide synthetic pathways. Meth Enzymol 459:395–433. https://doi.org/10.1016/S0076-6879(09)04617-5

Ezeagu IE, Petzke KJ, Lange E, Metges CC (1998) Fat content and fatty acid composition of oils extracted from selected wild-gathered tropical plant seeds from Nigeria. J Amer Oil Chem Soc 75:1031–1035. https://doi.org/10.1007/s11746-998-0282-6

Fakas S, Papanikolaou S, Galiotou-Panayotou M, Komaitis M, Aggelis G (2006) Lipids of *Cunninghamella echinulata* with emphasis to γ-linolenic acid distribution among lipid classes. Appl Microbiol Biotech 73:676–683. https://doi.org/10.1007/s00253-006-0506-3

Field KJ, Pressel S (2018) Unity in diversity: structural and functional insights into the ancient partnerships between plants and fungi. New Phytol 220:996–1011. https://doi.org/10.1111/nph.15158

Field KJ, Bidartondo MI, Rimington WR, Hoysted GA, Beerling DJ, Cameron DD, Duckett JG, Leake JR, Pressel S (2019) Functional complementarity of ancient plant–fungal mutualisms: contrasting nitrogen, phosphorus and carbon exchanges between Mucoromycotina and Glomeromycotina fungal symbionts of liverworts. New Phytol 223:908–921. https://doi.org/10.1111/nph.15819

Fontaine J, Grandmougin-Ferjani A, Hartmann M, Sancholle M (2001) Sterol biosynthesis by the arbuscular mycorrhizal fungus *Glomus intraradices*. Lipids 36:1357–1363. https://doi.org/10.1007/s11745-001-0852-z

Frey B, Buser H, Schuepp H (1992) Identification of ergosterol in vesicular-arbuscular mycorrhizae. Biol Fert Soils 13:229–234. https://doi.org/10.1007/BF00340581

Frey B, Vilarino A, Schuepp H, Arines J (1994) Chitin and ergosterol content of extraradical and intraradical mycelium of the vesicular-arbuscular mycorrhizal fungus *Glomus intraradices*. Soil Biol Biochem 26:711–717. https://doi.org/10.1016/0038-0717(94)90263-1

Frostegård A, Tunlid A, Bååth E (2011) Use and misuse of PLFA measurements in soils. Soil Biol Biochem 43:1621–1625. https://doi.org/10.1016/j.soilbio.2010.11.021

Groenewald EG, van der Westhuizen AJ (1997) Prostaglandins and related substances in plants. Bot Rev 63:199–220. https://doi.org/10.1007/BF02857948

Gu Z, Valianpour F, Chen S, Vaz FM, Hakkaart GA, Wanders RJA, Greenberg ML (2004) Aberrant cardiolipin metabolism in the yeast taz1 mutant: a model for Barth syndrome. Mol Microbiol 51:149–158. https://doi.org/10.1046/j.1365-2958.2003.03802.x

Hanssen J, Thingstad T, Goksoyr J (1974) Evaluation of hyphal lengths and fungal biomass in soil by a membrane-filter technique. Oikos 25:102–107. https://doi.org/10.2307/3543552

Heidarianpour MB, Aliasgharzad N, Olsson PA (2020) Positive effects of co-inoculation with *Rhizophagus irregularis* and *Serendipita indica* on tomato growth under saline conditions, and their individual colonization estimated by signature lipids. Mycorrhiza 30:455–466. https://doi.org/10.1007/s00572-020-00962-y

Holátko J, Brtnický M, Kučerík J, Kotianová M, Elbl J, Kintl A, Kynický J, Benada O, Datta R, Jansa J (2021) Glomalin – truths, myths, and the future of this elusive soil glycoprotein. Soil Biol Biochem 153:108116. https://doi.org/10.1016/j.soilbio.2020.108116

Horvath SE, Daum G (2013) Lipids of mitochondria. Prog Lipid Res 52:590–614. https://doi.org/10.1016/j.plipres.2013.07.002

Hoysted GA, Kowal J, Jacob A, Rimington WR, Duckett JG, Pressel S, Orchard S, Ryan MH, Field KJ, Bidartondo MI (2018) A mycorrhizal revolution. Curr Opin Plant Biol 44:1–6. https://doi.org/10.1016/j.pbi.2017.12.004

Islam T, Larsen O, Birkeland N (2020) A novel cold-adapted *Methylovulum* species, with a high C16:1ω5c content, isolated from an arctic thermal spring in Spitsbergen. Microbes Environ 35:ME20044. https://doi.org/10.1264/jsme2.ME20044

Janoušková M, Püschel D, Hujslová M, Slavíková R, Jansa J (2015) Quantification of arbuscular mycorrhizal fungal DNA in roots: how important is material preservation? Mycorrhiza 25:205–214. https://doi.org/10.1007/s00572-014-0602-7

Joergensen R (2022) Phospholipid fatty acids in soil-drawbacks and future prospects. Biol Fert Soils 58:1–6. https://doi.org/10.1007/s00374-021-01613-w

Joung Y, Seo M, Kang H, Kim H, Ahn T, Cho J, Joh K (2015) *Emticicia aquatica* sp nov., a species of the family Cytophagaceae isolated from fresh water. Int J Syst Evol Microbiol 65:4358–4362. https://doi.org/10.1099/ijsem.0.000577

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoornhuyse P, Jansa J, Bücking H (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science 333:880–882. https://doi.org/10.1126/science.1208473

Konvalinková T, Püschel D, Řezáčová V, Gryndlerová H, Jansa J (2017) Carbon flow from plant to arbuscular mycorrhizal fungi is reduced under phosphorus fertilization. Plant Soil 419:319–333. https://doi.org/10.1007/s11104-017-3350-6

Koske RE, Gemma JN (1989) A modified procedure for staining roots to detect VA mycorrhizas. Mycol Res 92:486–488. https://doi.org/10.1016/S0953-7562(89)80195-9

Kotani S, Izawa S, Komai N, Takayanagi A, Arioka M (2016) Mitochondria-localized phospholipase A2, AoPlaA, in *Aspergillus oryzae* displays phosphatidylethanolamine-specific activity and is involved in the maintenance of mitochondrial phospholipid composition. Fungal Genet Biol 96:1–11. https://doi.org/10.1016/j.fgb.2016.09.001

Kumari A, Pabbi S (2023) Tyagi A (2023) Recent advances in enhancing the production of long chain omega-3 fatty acids in microalgae. Crit Rev Food Sci Nutr 10(1080/10408398):2226720

Kuyper TW, Jansa J (2023) Arbuscular mycorrhiza: advances and retreats in our understanding of the ecological functioning of the mother of all root symbioses. Plant Soil 489:41–88. https://doi.org/10.1007/s11104-023-06045-z

Lee JC, Yang JS, Moon MH (2019) Simultaneous relative quantification of various polyglycerophospholipids with isotope-labeled methylation by nanoflow ultrahigh performance liquid chromatography-tandem mass spectrometry. Anal Chem 91:6716–6723. https://doi.org/10.1021/acs.analchem.9b00800

Lekberg Y, Bååth E, Frostegård Å, Hammer E, Hedlund K, Jansa J, Kaiser C, Ramsey PW, Řezanka T, Rousk J, Wallander H, Welc M, Olsson PA (2022) Fatty acid 16:1ω5 as a proxy for arbuscular mycorrhizal fungal biomass: current challenges and ways forward. Biol Fert Soils 58:835–842. https://doi.org/10.1007/s00374-022-01670-9

Lu H, Chen H, Tang X, Yang Q, Zhang H, Chen YQ, Chen W (2019) Ultra performance liquid chromatography–Q exactive orbitrap/mass spectrometry-based lipidomics reveals the influence of nitrogen sources on lipid biosynthesis of *Mortierella alpina*. J Agr Food Chem 67:10984–10993. https://doi.org/10.1021/acs.jafc.9b04455

Lu Y, Eiriksson FF, Thorsteinsdóttir M, Cronberg N, Simonsen HT (2023) Lipidomes of Icelandic bryophytes and screening of high contents of polyunsaturated fatty acids by using lipidomics approach. Phytochemistry 206:113560. https://doi.org/10.1016/j.phytochem.2022.113560

Matthäus B (2012) The database Seed Oil Fatty Acids (SOFA) is back on the Internet! Eur J Lipid Sci Tech 114:701–702. https://doi.org/10.1002/ejlt.201200160

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi. New Phytol 115:495–501. https://doi.org/10.1111/j.1469-8137.1990.tb00476.x

Nichols P, Stulp B, Jones J, White D (1986) Comparison of fatty-acid content and DNA homology of the filamentous gliding bacteria *Vitreoscilla, Flexibacter, Filibacter*. Arch Microbiol 146:1–6. https://doi.org/10.1007/BF00690149

Ohlrogge J, Thrower N, Mhaske V, Stymne S, Baxter M, Yang W, Liu J, Shaw K, Shorrosh B, Zhang M, Wilkerson C, Matthäus B (2018) PlantFAdb: a resource for exploring hundreds of plant fatty acid structures synthesized by thousands of plants and their phylogenetic relationships. Plant J 96:1299–1308. https://doi.org/10.1111/tpj.14102

Olsson P (1999) Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. FEMS Microbiol Ecol 29:303–310. https://doi.org/10.1016/S0168-6496(99)00021-5

Olsson PA, Lekberg Y (2022) A critical review of the use of lipid signature molecules for the quantification of arbuscular mycorrhiza fungi. Soil Biol Biochem 166:108574. https://doi.org/10.1016/j.soilbio.2022.108574

Olsson P, Larsson L, Bago B, Wallander H, van Aarle I (2003) Ergosterol and fatty acids for biomass estimation of mycorrhizal fungi. New Phytol 159:7–10. https://doi.org/10.1046/j.1469-8137.2003.00810.x

Özcan T (2007) Characterization of Turkish *Quercus* L. taxa based on fatty acid compositions of the acorns. J Am Oil Chem Soc 84:653–662. https://doi.org/10.1007/s11746-007-1087-8

Paradies G, Paradies V, Ruggiero FM, Petrosillo G (2019) Role of cardiolipin in mitochondrial function and dynamics in health and disease: molecular and pharmacological aspects. Cells 8:728. https://doi.org/10.3390/cells8070728

Powell JR, Rillig MC (2018) Biodiversity of arbuscular mycorrhizal fungi and ecosystem function. New Phytol 220:1059–1075. https://doi.org/10.1111/nph.15119

Püschel D, Janoušková M, Hujslová M, Slavíková R, Gryndlerová H, Jansa J (2016) Plant–fungus competition for nitrogen erases mycorrhizal growth benefits of *Andropogon gerardii* under limited nitrogen supply. Ecol Evol 6:4332–4346. https://doi.org/10.1002/ece3.2207

Redecker D (2000) Specific PCR primers to identify arbuscular mycorrhizal fungi within colonized roots. Mycorrhiza 10:73–80. https://doi.org/10.1007/s005720000061

Rezanka T (1993) Polyunsaturated and unusual fatty acids from slime moulds. Phytochemistry 33:1441–1444. https://doi.org/10.1016/0031-9422(93)85106-2

Rezanka T, Kolouchova I, Sigler K (2016) Lipidomic analysis of psychrophilic yeasts cultivated at different temperatures. Biochim Biophys Acta - Mol Cell Biol Lipids 1861:1634–1642. https://doi.org/10.1016/j.bbalip.2016.07.005

Rezanka T, Rezanka M, Mezricky D, Vítová M (2020) Lipidomic analysis of diatoms cultivated with silica nanoparticles. Phytochemistry 177:112452. https://doi.org/10.1016/j.phytochem.2020.112452

Rezanka T, Lukavský J, Rozmoš M, Nedbalová L, Jansa J (2022) Separation of triacylglycerols containing positional isomers of hexadecenoic acids by enantiomeric liquid chromatography-mass spectrometry. J Chrom B 1208:123401. https://doi.org/10.1016/j.jchromb.2022.123401

Rimington WR, Pressel S, Duckett JG, Field KJ, Bidartondo MI (2019) Evolution and networks in ancient and widespread symbioses between Mucoromycotina and liverworts. Mycorrhiza 29:551–565. https://doi.org/10.1007/s00572-019-00918-x

Schlame M, Brody S, Hostetler KY (1993) Mitochondrial cardiolipin in diverse eukaryotes. Eur J Biochem 212:727–733. https://doi.org/10.1111/j.1432-1033.1993.tb17711.x

Schlame M, Rua D, Greenberg ML (2000) The biosynthesis and functional role of cardiolipin. Prog Lipid Res 39:257–288. https://doi.org/10.1016/S0163-7827(00)00005-9

Schmitz O, Danneberg G, Hundeshagen B, Klingner A, Bothe H (1991) Quantification of vesicular-arbuscular mycorrhiza by biochemical parameters. J Plant Physiol 139:106–114. https://doi.org/10.1016/S0176-1617(11)80174-4

Schwarzott D, Schüßler A (2001) A simple and reliable method for SSU rRNA gene DNA extraction, amplification, and cloning from single AM fungal spores. Mycorrhiza 10:203–207. https://doi.org/10.1007/PL00009996

Sohlenkamp C (2021) Crossing the lipid divide. J Biol Chem 297:100859. https://doi.org/10.1016/j.jbc.2021.100859

Sylvia D (1992) Quantification of external hyphae of vesicular-arbuscular mycorrhizal fungi. Meth Microbiol 24:53–65. https://doi.org/10.1016/S0580-9517(08)70086-2

Thonar C, Erb A, Jansa J (2012) Real-time PCR to quantify composition of arbuscular mycorrhizal fungal communities—marker design, verification, calibration and field validation. Mol Ecol Res 12:219–232. https://doi.org/10.1111/j.1755-0998.2011.03086.x

van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310. https://doi.org/10.1111/j.1461-0248.2007.01139.x

van Groenigen K-J, Bloem J, Bååth E, Boeckx P, Rousk J, Bodé S, Forristal D, Jones MB (2010) Abundance, production and stabilization of microbial biomass under conventional and reduced tillage. Soil Biol Biochem 42:48–55. https://doi.org/10.1016/j.soilbio.2009.09.023

Vickery JR (1971) The fatty acid composition of the seed oils of proteaceae: a chemotaxonomic study. Phytochemistry 10:123–130. https://doi.org/10.1016/S0031-9422(00)90259-0

Vickery J, Whitfield F, Ford G, Kennett B (1984) The fatty-acid composition of gymnospermae seed and leaf oils. J Am Oil Chem Soc 61:573–575. https://doi.org/10.1007/BF02677035

Vierheilig H, Schweiger P, Brundrett M (2005) An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots. Phys Plant 125:393–404. https://doi.org/10.1111/j.1399-3054.2005.00564.x

Voříšková A, Jansa J, Püschel D, Krüger M, Cajthaml T, Vosátka M, Janoušková M (2017) Real-time PCR quantification of arbuscular mycorrhizal fungi: does the use of nuclear or mitochondrial markers make a difference? Mycorrhiza 27:577–585. https://doi.org/10.1007/s00572-017-0777-9

Walker R (1969) Cis-11-hexadecenoic acid from *Cytophaga hutchinsonii* lipids. Lipids 4:15–18. https://doi.org/10.1007/BF02531788

Walker C, Gollotte A, Redecker D (2018) A new genus, *Planticonsortium* (Mucoromycotina), and new combination (*P. tenue*), for the fine root endophyte, *Glomus tenue* (basionym *Rhizophagus tenuis*). Mycorrhiza 28:213–219. https://doi.org/10.1007/s00572-017-0815-7

Welc M, Bünemann EK, Fließbach A, Frossard E, Jansa J (2012) Soil bacterial and fungal communities along a soil chronosequence assessed by fatty acid profiling. Soil Biol Biochem 49:184–192. https://doi.org/10.1016/j.soilbio.2012.01.032

Wewer V, Brands M, Dörmann P (2014) Fatty acid synthesis and lipid metabolism in the obligate biotrophic fungus *Rhizophagus irregularis* during mycorrhization of *Lotus japonicus*. Plant J 79:398–412. https://doi.org/10.1111/tpj.12566

Wolff R, Christie W, Aitzetmuller K, Pasquier E, Pedrono F, Destaillats F, Marpeau A (2000) Arachidonic and eicosapentaenoic acids in

Araucariaceae, a unique feature among seed plants. Oléagineux, Corps Gras, Lipides 7:113–117. https://doi.org/10.1051/ocl.2000.0113

Zhou Y, Peisker H, Dörmann P (2016) Molecular species composition of plant cardiolipin determined by liquid chromatography mass spectrometry. J Lipid Res 57:1308–1321. https://doi.org/10.1194/jlr.D068429