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Functional complementarity of ancient plant-fungal mutualisms: contrasting nitrogen, phosphorus and carbon exchanges between Mucoromycotina and Glomeromycotina fungal symbionts of liverworts.

Running title: Complementarity of ancient plant-fungal mutualisms

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KJF, JRL, DDC, DJB, MIB, JGD and SP conceived and designed the research. KJF conducted the isotope tracer work and GAH conducted P analyses. MIB and WRR conducted the molecular analyses. SP and JGD conducted the cytological analyses. SP, KJF, MIB and JRL led the writing; all authors provided comments on the manuscript.

Data accessibility statement:

Should this manuscript be accepted, all data will be archived in the appropriate public repository and the data DOI will be included at the end of the article.

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Summary

- Liverworts, which are amongst the earliest-divergent plant lineages and important ecosystem pioneers, often form nutritional mutualisms with arbuscular mycorrhiza-forming Glomeromycotina and fine root endophyte Mucoromycotina fungi, both of which co-evolved with early land plants. Some liverworts, in common with many later-divergent plants, harbour both fungal groups, suggesting these fungi may complementarily improve plant access to different soil nutrients.

- We tested this hypothesis by growing liverworts in single and dual fungal partnerships under a modern atmosphere and under 1500 ppm [CO_2], as experienced by early land plants. Access to soil nutrients via fungal partners was investigated with ^{15}N-labelled algal necromass and ^{33}P orthophosphate. Photosynthate allocation to fungi was traced using $^{14}CO_2$.

- Only Mucoromycotina fungal partners provided liverworts with substantial access to algal ^{15}N, irrespective of atmospheric CO_2 concentration. Both symbionts increased ^{33}P uptake, but Glomeromycotina were often more effective. Dual partnerships showed complementarity of nutrient pool use and greatest photosynthate allocation to symbiotic fungi.

- We show there are important functional differences between the plant-fungal symbioses tested, providing new insights into the functional biology of Glomeromycotina and Mucoromycotina fungal groups that form symbioses with plants. This may explain the persistence of both fungal lineages in symbioses across the evolution of land plants.

Introduction

Mycorrhizal associations (and mycorrhizal-like associations in plants that lack roots, henceforth also referred to as mycorrhizas) are near-ubiquitous mutualisms between soil fungi and most land plant phyla (Wang & Qiu, 2006; Wang *et al.,* 2010) with key roles in plant nutrition and health (Smith & Read, 2008). The earliest-divergent extant branches of the land plant phylogeny supporting fungal symbionts, i.e. liverworts (Haplomitriopsida and thalloids), hornworts and lycophytes (Bidartondo *et al.,* 2011; Desirò *et al.,* 2013; Rimington *et al.,* 2015; Field *et al.,* 2015a), have recently been shown to form symbioses with members of two ancient fungal lineages, arbuscular mycorrhizal (AM) fungi falling within the Glomeromycotina, and the much less studied, and poorly understood, symbiotic fungi of the Mucoromycotina.

These findings, together with fossil evidence of Mucoromycotina-like and Glomeromycotina-like fungal associations in some of the first vascular plants of the early Devonian (Strullu-Derrien *et al.,* 2014), have led to the novel hypothesis that engagement with both fungal partners might have been a common strategy during early land plant evolution (Field *et al.,* 2015a). Furthermore, new molecular evidence reveals the same Mucoromycotina fungal symbionts are shared by liverworts, early-divergent vascular plants and angiosperms (Hoysted *et al.,* 2019). This is consistent with the recent finding that the globally widespread fine root endophytes (FRE), originally classified as *Glomus tenue* (more recently *Planticonsortium tenue* (Walker *et al.,* 2018)*,* are not members of the Glomeromycotina as hitherto assumed, but instead fall within the Mucoromycotina (Orchard *et al.,* 2017a; 2017b). In angiosperms Mucoromycotina-FRE, when present, seem to always co-occur with Glomeromycotina in the same host (Orchard *et al.,* 2017a; 2017b; Hoysted *et al.,* 2019), while exclusive Mucoromycotina-FRE symbioses have been reported so far in only one lycophyte (Rimington *et al.,* 2015; 2016; Pressel *et al.,* 2016) and in the earliest-divergent Haplomitriopsida liverworts *Haplomitrium* and *Treubia*. The

majority of early-divergent mycorrhizal plant clades, lycophytes, hornworts and (thalloid) liverworts (e.g. the complex thalloid *Neohodgsonia mirabilis*) engage in partnerships with both fungi, sometimes simultaneously in so-called 'dual' symbiosis (Desirò *et al.,* 2013; Field *et al.,* 2015a; 2016; Rimington *et al.,* 2015; 2016; Pressel *et al.,* 2016), paralleling the situation recently established for flowering plants. Only a few complex thalloid liverworts, such as species in the genus *Marchantia* appear to associate exclusively with Glomeromycotina fungi (Field *et al.,* 2015a; Rimington *et al.,* 2018). Taken together, these discoveries suggest that the ability of plants to form symbioses with both Mucoromycotina and Glomeromycotina has persisted from the ancient past (Strullu-Derrien *et al.,* 2014; Field *et al.,* 2015a; 2015b; Field & Pressel, 2018) to the present day, being now common in both natural and agricultural ecosystems (Orchard *et al.,* 2017a; 2017b). The apparent persistence of symbiosis with both fungal groups through the 500 my-long evolutionary history of land plants (Morris *et al.,* 2018) and the widespread occurrence of them simultaneously in diverse plant lineages today suggests the ability to form associations with both Glomeromycotina and Mucoromycotina fungi likely provides functional advantages over associations with only one or other of these fungal groups. However, to date there is limited evidence to test this hypothesis.

Studies on liverwort-Glomeromycotina (Humphreys *et al.,* 2010), liverwort-Mucoromycotina (Field *et al.,* 2015a; 2015b) and dual symbioses with both fungal groups (Field *et al.,* 2016) have confirmed that all three partnerships are mycorrhiza-like mutualisms with the fungi receiving plant photosynthate in exchange for mineral nutrients they have acquired from soil. Significant functional differences between these three types of symbiotic partnership were revealed in response to experimental simulations of the large changes in atmospheric CO_2 concentrations experienced during the long co-evolutionary history of plants and mycorrhizal fungi. Liverworts associating only with Glomeromycotina fungi gained considerably more ^{33}P orthophosphate tracer

136 via their fungal partners per unit of photosynthate C received at CO_2 concentrations
137 simulating the atmosphere of the early-mid Paleozoic under which land plants first
138 evolved (1,500 ppm a[CO_2]; Berner 2006; Lenton *et al.,* 2018) than under modern-day
139 a[CO_2] of 440 ppm (Field *et al.,* 2016). In contrast, Mucoromycotina fungal symbionts,
140 maintained or increased ^{33}P transfer to their hosts under the modern a[CO_2] compared
141 to the simulated Paleozoic a[CO_2]. These liverwort P gains under modern a[CO_2] were
142 further enhanced by the co-occurrence of Glomeromycotina fungi in dual symbiosis but
143 at a greater carbon cost (Field *et al.,* 2016).
144 Whilst the importance of Glomeromycotina AM symbioses in plant P nutrition is
145 well established (Smith *et al.,* 2015), their role in host plant N nutrition is much less clear
146 (Hodge & Fitter, 2010; Bücking & Kafle, 2015; Hodge & Storer, 2015; Thirkell *et al.,*
147 2016). This ambiguity has increased with the recent evidence of widespread dual
148 symbioses between FRE and Glomeromycotina fungi (Rimington *et al.,* 2015, Orchard *et*
149 *al.,* 2017a). Nutritional effects previously ascribed to Glomeromycotina fungi may have
150 been due to undetected Mucoromycotina as most primers used to characterize DNA
151 from arbuscular mycorrhizal symbionts do not amplify Mucoromycotina DNA (Bidartondo
152 *et al*., 2011). This major uncertainty increases the urgency of the need to resolve
153 whether Mucoromycotina and Glomeromycotina fungi are functionally distinct with
154 respect to their provisioning of host plants of both P and N, both of which are major
155 macronutrients that are often plant growth limiting.
156 Mucoromycotina symbionts may be facultative saprotrophs, i.e. able to access
157 and assimilate nutrients from organic matter for use in metabolism in a similar way to
158 free-living saprotrophs (Lindhal & Tunlid, 2015), as it has sometimes been possible to
159 achieve *in vitro* isolation and axenic culturing of these fungi (Field *et al.,* 2015b). In
160 contrast, Glomeromycotina fungi are strict obligate biotrophs, entirely reliant on
161 symbiosis with a living plant for organic carbon and thus considered unable to assimilate

nutrients by saprotrophic extracellular digestion of organic matter (Smith & Read, 2008); it is possible therefore that the Mucoromycotina may be better able to access organic N than the Glomeromycotina. If this were the case, then we might expect there to be differences in the ability of liverworts associated with the two groups of fungi, either singly or in dual symbiosis, to utilise soil nutrients with dual symbioses providing complementarity through increasing access both to inorganic P and to organic N compared to associations with only one type of fungus.

We set out to test this hypothesis by growing wild-collected liverworts, that naturally support single (*Haplomitrium gibbsiae* -Mucoromycotina; *Marchantia paleacea* - Glomeromycotina) or dual fungal (*Neohodgsonia mirabilis* – Mucoromycotina and Glomeromycotina) partnerships, in experimental microcosms on non-sterile soil containing native mycorrhizosphere microbial communities. Access to soil nutrients via the fungal partners was investigated with phosphorus provided as ^{33}P orthophosphate and ^{15}N in labelled necromass of the unicellular green alga *Chlorella* (Trebouxiophyceae). We selected *Chlorella* as a substrate that is likely to have co-existed with land plants from when they first transitioned from aquatic to terrestrial habitats (Edwards *et al.,* 2015). Both N and P sources were added to rhizoid-excluding soil compartments that could be accessed by fungal hyphae growing from their plant partners.

We conducted these experiments under both a modern 440 ppm a[CO_2], and the 1500 ppm a[CO_2] experienced by early land plants from the Ordovician/early Devonian periods as indicated by geochemical carbon cycle models (Berner, 2006; Lenton *et al.,* 2018). This allowed us to test the second hypothesis that mycorrhizal functioning in terms of fungal nutrient uptake from soil, and liverwort photosynthate allocation to external mycelium of fungal symbionts, is influenced by the changes in a[CO_2] that have

187 occurred through Palaeozoic (Berner, 2006), as we have shown previously in relation to
188 P assimilation (Field *et al.,* 2012; 2015b).
189
190 **Materials and Methods**
191 *Plant material and growth conditions*
192 *Haplomitrium gibbsiae* (Steph.) R.M. Schust. (known to exclusively associate with
193 Mucoromycotina fungal symbionts (Bidartondo et al., 2011) and *Neohodgsonia mirabilis*
194 Perss. (known to associate with both Mucoromycotina and Glomeromycotina symbionts
195 (Field *et al.,* 2016) were collected from the South Island of New Zealand in December
196 2013 (for location details please see SI), vouchers for which are deposited in the Natural
197 History Museum, London. *Marchantia paleacea* Bertol. plants (that exclusively associate
198 with Glomeromycotina fungal symbionts, Humphreys *et al.,* 2010) were originally
199 collected from cool temperate cloud forests in Veracruz, Mexico and symbiotic gemmae
200 propagated from thalli maintained in controlled environment growth chambers (Conviron
201 BDR16, Conviron, Canada) using the following chamber settings: half light-saturating
202 conditions for bryophytes of 50 μmol m^{-2} s^{-1} irradiance (Nobel, 1999; Fletcher *et al.,*
203 2006), 70% relative humidity, 12 h, 15 °C day, 12 °C night.
204 Shortly after collection, the liverworts were planted directly into microcosms (120
205 mm diameter, 100 mm depth pots) containing 95% pot volume acid-washed silica sand
206 and 5% pot volume Irish moss-peat to aid water retention of the substrate. Native soil
207 surrounding liverwort rhizoids was left in place to prevent damage and to act as a natural
208 inoculum, including mycorrhizal fungi, associated microorganisms and saprotrophs.
209 Based on the methods of Field *et al.* (2012), we constructed cylindrical plastic
210 cores (85 mm length, 15 mm diameter) with two 20 mm x 50 mm windows that, together
211 with the base, were covered with nylon mesh of 10 μm pore size to prevent ingrowth of
212 liverwort rhizoids while allowing penetration by fungal hyphae (Fig. 1a). Three cores

were inserted into each of the microcosms, two of these having a perforated, fine-bore capillary tube (100 mm length, 1.02 mm internal diameter; Portex, UK) placed vertically in the centre and secured to the basal mesh fast-setting cement. Both cores were filled with a mixture of acid-washed silica sand (89% core volume), finely ground tertiary basalt mineral grains (1% core volume) to encourage growth of fungal hyphae into the core (Quirk *et al.,* 2012) and native soil gathered from around rhizoids (*Marchantia* and *Neohodgsonia*) and underground axes (*Haplomitrium*) of wild plants (10% core volume) to act as a natural microbial inoculum containing fungi, hyphal-associated and saprotrophic microorganisms. The third mesh-covered windowed core was inserted and filled with glass wool to allow below-ground gas sampling during ^{14}C labelling.

A total of 40 microcosms were prepared for each of the liverwort species tested, 20 to be maintained under ambient atmospheric CO_2 concentrations (440 ppm) and 20 under an elevated CO_2 atmosphere of 1,500 ppm. Microcosms were regularly weeded to remove any seedlings or mosses, and maintained for 12 weeks to allow plant acclimation to cabinets and fungal mycelial networks to develop within the pots. Microcosms were rotated regularly within cabinets to avoid positional effects, and the chamber settings and contents swapped over every two weeks to avoid cabinet effects. Across the three plant species, microcosms were arranged to share a common drip tray in each cabinet, thereby providing a common pool of saprotrophic and hyphal-associated microorganisms to all the plant species.

After the acclimation period, microcosms were moved to individual drip-trays immediately prior to isotope labelling to avoid label-cross contamination. Upon commencement of isotope labelling, one core in each microcosm was left static ("static core"), ensuring hyphal connections between liverworts, fungi and core contents remained intact. A second core in each pot was rotated ("rotated core"), thereby severing the fungal hyphal connections between the liverwort and the core contents (Fig.

1b, c). This acts as a control wherein fungal symbionts were prevented from forming continuous hyphal connections between the liverwort host and the contents of the core.

Radio- and stable isotope tracer additions to cores in microcosms (Fig. 1)

In half of the microcosms for each CO_2 treatment (n = 10 in each CO_2 condition), an internal plastic core was inserted into one of the soil-filled cores with the space between the outer and inner cores filled with the sand/basalt/soil substrate. The inner core was filled with a ^{15}N-labelled algal necromass-sand mixture (1.25% algae; 0.05 g algae and 2800 μg ^{15}N per pot). To prepare the necromass-sand mixture, cultures of *Chlorella vulgaris* var. *viridis* Chodat (CCAP 211/12) were obtained from CCAP (Argyll, UK) and cultured in 3N-BBM +V medium (see SI), where $NaNO_3$ was supplied as $Na^{15}NO_3$ (≥ 98 atom % ^{15}N; Sigma Aldrich, UK). After growth in the labelled medium, the liquid cultures were filtered, rinsed with water, re-suspended and mixed well with 200 g acid-washed sand before drying in an oven (80 °C) for three hours. This generated algae-covered dry sand particles, 4 g of which were then introduced into the inner core (see Fig. 1b) using a small funnel. The solid-walled plastic inner core remained *in situ* until immediately before ^{33}P labelling, ensuring the algal necromass was only in contact with the rest of the core contents, and therefore accessible to the symbiotic fungi, for the same time period as the fungi had access to the ^{33}P tracer (see Fig. 1a).

Molecular identification of fungal associates

DNA analyses were conducted on five samples (thalli of *Marchantia* and *Neohodgsonia*, underground axes of *Haplomitrium*) to confirm that the three liverworts maintained their previously identified fungal associates (Bidartondo *et al.,* 2011; Field *et al.,* 2016; Humphreys *et al.,* 2010) throughout the experimental periods. Wild *Haplomitrium* and *Neohodgsonia* plants (please refer to SI for details of collections and sampling) were

prepared for DNA analyses within one day of collection to establish their native fungal partners. Similarly, we took samples of laboratory-grown *Marchantia* plants that have previously been confirmed to be colonised by Glomeromycotina fungi (Humphreys *et al.,* 2010), immediately before the experiments started. Additional representative samples of five individual plants across each of the three liverwort species used in our experiments were also taken immediately following our isotope labelling experiments. Sequencing of the fungal symbionts was performed using universal fungal primers and molecular cloning (Rimington *et al.,* 2018). Firstly, a full genomic DNA extraction was performed on a section of liverwort thallus using chloroform extraction (Gardes & Bruns, 1993) and the GeneClean II kit (QBioGene). The fungal DNA in the extract was then amplified using the Sigma JumpStart kit and the universal fungal primers NS1 (White *et al.,* 1990) and EF3 (Smit *et al.,* 1999). PCR products from all samples were cloned using the TOPO TA cloning kit (Invitrogen) and reamplification of fungal DNA was performed on four colonies per sample using the same primers and the Sigma JumpStart kit. The fungal DNA from reamplification was sequenced using the primers NS1, NS3 and NS5 (White *et al.,* 1990) and BigDye v. 3.1 (Applied Biosystems). Sequences were assembled into contigs using Geneious v. 7 (Kearse *et al.,* 2012) and identified to the level of fungal subphylum using BLAST searches of GenBank (Altschul *et al.,* 1997). Further detail of the fungal sequencing method can be found in Rimington *et al.* (2018). This method found Mucoromycotina colonisation in *Haplomitrium,* Glomeromycotina in *Marchantia,* and both fungal lineages in *Neohodgsonia* – in line with previous molecular and microscopy investigations (Field et al., 2015, 2016). New sequences for *Haplomitrium gibbsiae* (accession number MF621059) and *Neohodgsonia mirabilis* (MF621060 and MF621061) have been uploaded to GenBank while Glomeromycotina sequences from *Marchantia paleacea* were identical to those

290 previously found in these plants by Humphreys *et al.* (2010) (accession number
291 FR690120).
292
293 *Microscopy and cytology to confirm mycorrhizal status of the plants*
294 Wild-collected plants were processed for scanning electron microscopy (SEM) as
295 described previously (Duckett *et al.,* 2006). Briefly, underground axes of *Haplomitrium*
296 *gibbsiae* and thalli of *Marchantia paleacea* and *Neohodgsonia mirabilis* (10 samples of
297 each plant species) were fixed in 3% glutaraldehyde, dehydrated through an ethanol
298 series, critical-point dried using CO_2 as a transfusion fluid, sputter coated with 390 nm
299 palladium-gold and viewed under a FEI Quanta scanning electron microscope at 10 Kv.
300
301 *Quantification of carbon-for-nutrient exchange between liverworts and fungi*
302 After 12 weeks of growth, we introduced 100 µl, carrier-free ^{33}P-labelled H_2PO_4
303 orthophosphate solution (^{33}P specific activity 148 GBq mmol^{-1}, total 223 ng ^{33}P; Perkin
304 Elmer, Beaconsfield, UK) into one soil core in each microcosm (Fig. 1b, c). At the same
305 time as introducing the ^{33}P tracer, the plastic inner core separating the ^{15}N-labelled algal
306 necromass in pots with this treatment was removed to allow the symbiotic fungi to grow
307 into this part of the soil-filled core. Half of the isotope-labelled cores and half of the non-
308 labelled cores were then rotated to sever the symbiotic fungal hyphal connections
309 between host liverwort and the soil in the cores to serve as a control for the effects of
310 microbial nutrient cycling and saprotrophic fungal action (Fig. 1c). For each liverwort
311 species there were 5 replicates pots for each treatment combination at ambient (440
312 ppm a[CO_2]) and simulated Paleozoic (1,500 ppm a[CO_2]): **1**. Fungal access to ^{33}P and
313 ^{15}N-labelled algae; **2.** Fungal access to ^{33}P and no algae; **3.** No fungal access to ^{33}P or
314 ^{15}N-labelled algae; **4**. No fungal access to ^{33}P and no algae.

315 Sub-samples of cores from 18 pots where algal necromass was included
316 (Treatments 1 and 3) and 18 pots where algae were not included (Treatments 2 and 4)
317 were digested using concentrated sulphuric acid to determine total phosphorus pools
318 and potential for pool dilution effects. Total phosphorus was determined using a
319 colorimetric method adapted from Leake, (1988) (see SI for details). There were no
320 significant differences in total P content between cores where algae were included
321 compared to cores where algal necromass was not included (Fig. S1), therefore P pool
322 dilution effects were not included in the calculations for ^{33}P tracer uptake.
323 Twenty-one days after introducing the ^{33}P and exposing the algal necromass
324 patches to mycorrhizal fungal colonization, the tops of all the sand and soil filled cores in
325 all microcosms were sealed with anhydrous lanolin and plastic caps. Glass wool-filled
326 cores were sealed with a rubber septum (SubaSeal, Sigma) to allow regular below-
327 ground gas samples to be taken. We then placed each pot into a three-litre gas-tight
328 chamber and added 2 ml 10% lactic acid to 15 µl of $Na^{14}CO_3$ (specific activity 2.04 GBq
329 $mmol^{-1}$) in a cuvette within the chamber. This resulted in 1.1 MBq $^{14}CO_2$ being released
330 within the chambers (Fig. 1d). To monitor below-ground respiration of $^{14}CO_2$, 1 ml of soil
331 air was sampled via the glass wool-filled core after 1 hour and then every two hours
332 thereafter for ca. 17 hours. Gas samples were injected into gas-evacuated scintillation
333 vials containing 10 ml Carbosorb (Perkin Elmer, Beaconsfield, UK) and then mixed with
334 10 ml Permafluor (Perkin Elmer, Beaconsfield, UK) before radioactivity was measured
335 via scintillation counting (Packard Tri-Carb 3100TR, Isotech, Chesterfield, UK). At the
336 point at which the ^{14}C flux detected in below-ground gas samples stopped increasing, 2
337 ml 2M KOH was introduced into vials within the labelling chambers to trap any remaining
338 $^{14}CO_2$ over a subsequent period of 6 hours. An aliquot (1 ml) of the KOH was then
339 transferred to 10 ml Ultima Gold (Perkin Elmer, Beaconsfield, UK) and the radioactivity

340 determined using liquid scintillation (Packard Tri-Carb 3100TR, Isotech, Chesterfield,
341 UK).
342
343 *Plant harvest and tissue analyses*
344 *Fungus-to-plant ^{15}N and ^{33}P transfer*
345 All plant tissues and soils were separated, freeze-dried and weighed. Plant tissues were
346 homogenised in a Yellowline A10 Analytical Grinder (IKA, Germany). The ^{15}N
347 abundance (ng) in plant tissues was determined using isotope ratio mass spectrometry.
348 Between 2 and 5 mg of homogenised, freeze-dried plant tissue was weighed out into 6 x
349 4 mm ultra-clean tin capsules (Sercon, Ltd., UK) and analysed using a continuous flow
350 IRMS (PDZ 2020, Sercon Ltd., UK). Air was used as the reference standard and the
351 detector was regularly calibrated to commercially available reference gases. To
352 calculate ^{15}N content of samples, previously published equations were used (see SI)
353 (Cameron *et al.,* 2006).
354 To determine the ^{33}P content of liverworts and the effect of the algal additions on
355 ^{33}P uptake via the fungal partners, between 10-30 mg of liverwort biomass was charred
356 in 1 ml conc. H_2SO_4 for two hours. Charred samples were then heated to 365 °C in a
357 block heater (Grant Instruments, BT5D-26L, Cambridge UK) for 15 minutes before
358 cooling and clearing with H_2O_2 to give complete digestion. Clear digests were diluted up
359 to 10 ml total volume using distilled H_2O. A 2 ml aliquot of this solution was then added
360 to 10 ml of Emulsify Safe (Perkin Elmer, Beaconsfield, UK) to measure the radioactivity
361 via liquid scintillation counting (Packard Tri-Carb 3100TR, Isotech, Chesterfield, UK).
362 Total ^{33}P transferred from fungi within the cores to the liverworts was determined using
363 previously published equations (see SI) (Cameron *et al.,* 2006). To determine total
364 symbiotic fungal-acquired ^{33}P in plant tissue where algal necromass was not added the
365 ^{33}P values in plant tissues in algae-free microcosms where fungal access to the isotope

was restricted by core rotation (Treatment 4) were subtracted from the ^{33}P values in plant tissues where fungal hyphal connections between liverworts and core contents remained intact (Treatment 2). This controls for diffusion of isotopes and microbial nutrient cycling in pots, ensuring only ^{33}P gained by the plant via intact fungal hyphal connections is accounted for. The equivalent analysis when algae was present were obtained by subtracting the ^{33}P values calculated from plant tissues in algae-containing microcosms where fungal access to the isotope was restricted by core rotation (Treatment 3) from the values from plant tissues where fungal hyphal connections between liverworts and core contents remained intact (Treatment 1).

Plant-to-fungus carbon transfer into mesh cores

The activity of the ^{14}C fixed by the liverworts and transferred via the external fungal hyphae into the mesh core substrate and remaining in this substrate was determined through sample oxidation (Packard 307 Sample Oxidiser, Isotech, UK) and liquid scintillation counting of $^{14}CO_2$ trapped in 10 ml Carbosorb and 10 ml Permafluor as described previously. Total carbon was then calculated as a function of the volume and CO_2 content of the labelling chamber and the proportion of the supplied $^{14}CO_2$ label that was fixed by the liverworts during the labelling period using previously published equations (Cameron *et al.,* 2008). To determine total plant-fixed carbon due to symbiotic fungi within soil cores in each pot, the values calculated from cores where symbiotic fungal hyphae were severed from connections to plants by core rotation were subtracted from the values in cores where fungal hyphal connections between liverworts and core contents remained intact.

Statistics

All data were checked for homogeneity of variance and normality prior to analysis of variance. Analysis was conducted with three-way ANOVA, General Linear Model (GLM), where core rotation, a[CO_2], and symbiont identity were the variables for plant ^{15}N uptake; presence of algae, a[CO_2], and symbiont identity were variables for plant ^{33}P uptake; and presence of algae, a[CO_2], and fungal symbiont identity were the variables for fungal C allocation. Where required, data were log10 transformed to meet the assumptions for ANOVA. Where there were significant interaction terms within the 3-way ANOVA's, additional 2-way ANOVAs were conducted to understand the nature of these interactions. All statistics were calculated using Minitab v.17 (Minitab, PA, USA).

Results

Confirmation of fungal partner identity using molecular and cytological methods (Fig. 2)

Microscopy analyses confirmed the DNA-based results and showed colonisation patterns of the underground axes of *H. gibbsiae* by Mucoromycotina fungi consistent with previous observations (Carafa *et al*., 2003), including intracellular fungal coils with terminal lumps (Fig. 2b, c). Fungal colonisation of *M. paleacea* was typical of plant-Glomeromycotina associations, comprising relatively coarse intracellular hyphae, arbuscules and vesicles (Fig. 2e, f). Dual fungal colonisations in *N. mirabilis* had features of both fungal symbionts, particularly in the presence of coarse (>3 μm in diameter) and finer (ca. 1.5 μm in diameter) intracellular hyphae forming arbuscules and coils (Fig. 2h, i).

Biomass (Fig S2)

Most of the plant biomass was developed before the experiments commenced, and it was not feasible to measure initial biomass in each microcosm at the commencement of the experiments. Unsurprisingly therefore, we did not detect significant effects of access

to algal necromass on final biomass ($F_{1, 48}$ = 1.18, P = 0.283; three-way ANOVA; Table S1), for any of the liverwort-fungal partnerships studied; Fig. S2, Table S1). The liverwort associated only with Mucoromycotina (*Neohodgsonia*) had significantly greater biomass than the Glomeromycotina-and dual symbiont liverworts ($F_{2, 48}$ = 8.20, P=0.01, three-way ANOVA; Table S1). *Neohodgsonia* also showed a significant interaction effect with lower final biomass for the elevated compared to ambient CO_2, treatments ($F_{2, 48}$ = 4.41, P = 0.018, three-way ANOVA; Table S1) but there was no overall effect of $_a[CO_2]$ across all the plant species ($F_{1, 48}$ = 2.83, P = 0.099, three-way ANOVA; Table S1).

Nitrogen uptake in plants via symbiotic fungi (Fig. 3)

Significant fungus uptake and transfer to plants from algal necromass determined by total ^{15}N in the liverworts, was limited to plants whose fungal partners had access to the algae within static cores (Fig. 3 and Table 1; effect of static versus rotated cores: $F_{1,48}$ = 23.10, P<0.001, three-way ANOVA). This resulted in significantly higher ^{15}N concentrations in liverwort thalli grown with static cores (Table 1: $F_{1,48}$ = 49.26, P<0.001, three-way ANOVA). The uptake of ^{15}N differed significantly by symbiont type (Table 1: $F_{1,48}$ = 4.79, P=0.018). Single and dual partnerships with Mucoromycotina fungi (i.e. *Neohodgsonia* and *Haplomitrium*) had mean ^{15}N contents more than 2.4 times those of the Glomeromycotina-only colonised *M. paleacea* at 440 ppm a[CO_2] (Fig. 3a), rising to >90-fold times more at 1,500 ppm a[CO_2], however there were no significant effects of a[CO_2], and no significant two or three-way interaction effects of core rotation, a[CO_2] or symbiont type (Table 1). The concentration of ^{15}N in the liverwort thalli (Fig. 3c,d), was 35-90-fold higher in plants associated with Mucoromycotina fungi compared to those associated only with Glomeromycotina fungi, this effect of symbionts being significant ($F_{2, 48}$ = 4.37, P = 0.027; three-way ANOVA; Table 1).

443

444 *Phosphorus uptake by plants via symbiotic fungi (Fig. 4)*

445 Total ^{33}P uptake by the liverworts grown at 1500 ppm a[CO_2] was nearly twice as high in
446 the Glomeromycotina-only *M. paleacea* than in the liverworts in single or dual
447 association with Mucoromycotina, (Fig. 4a- note the log scale), but for the liverworts
448 grown at 440 ppm a[CO_2] there was no significant difference between the symbionts
449 (Fig. 4b; Table S2). This resulted in a significant interaction between symbionts and
450 CO_2 treatment on total ^{33}P uptake by the liverworts ($F_{2, 48}$ = 5.39, P = 0.008, three-way
451 ANOVA; Table 2), but there were no other significant main or interactive effects of
452 symbiont identity, a[CO_2] and availability of algae (Table 2).

453 The ^{33}P concentrations in liverwort biomass was significantly affected by symbiont
454 identity (Table 2, $F_{2,48}$ = 8.99, P = 0.001), being highest in the Glomeromycotina-only *M.*
455 *paleacea.* In addition, there was a significant symbiont x a[CO_2] interaction (Table 2, $F_{2,}$
456 $_{48}$ = 5.24, P = 0.009), as well as a symbiont x a[CO_2] x algae interaction (Table 2, $F_{2, 48}$ =
457 4.60, P = 0.015) but no other significant effects. The significant interactions arose from
458 the dual-symbiotic liverwort showing no effects of algal additions (Fig. 4c,d), whereas the
459 Glomeromycotina-only and Mucoromycotina-only associated liverworts showed
460 responses to algae that differed in magnitude or direction depending on the a[CO_2], but
461 there was no overall effect of algal additions on ^{33}P concentrations (Table 2).

462 Algal additions to cores also did not have a significant effect on total (non-
463 radioactive) P content or P concentrations of cores (Fig. S1). Phosphorus pool dilution
464 effects in treatments where the ^{15}N labelled algal necromass was included would appear
465 to be negligible, and therefore unlikely to affect plant ^{33}P tracer uptake.

466

467 *Liverwort carbon allocation to external mycelium of symbiotic fungi (Fig. 5)*

468 The proportion of the liverwort photosynthate that was detected by ^{14}C tracing into the
469 unrotated mesh cores was significantly affected by the type of symbiosis (Table 2), and
470 was <2% of the total C fixed (Fig. 5a,b- note that the data are on a log scale in Fig. 5b).
471 External mycelium of dual partnerships in the mesh cores received a greater proportion
472 of photosynthate than mycelia of liverworts associating with Glomeromycotina only or
473 Mucoromycotina only (Table 2; $F_{2, 48} = 4.96$, $P = 0.011$, three-way ANOVA), but there
474 was no significant effect of a[CO_2], algae, or interactions between the symbionts or other
475 variables.

476 The total ^{14}C traced into static cores via fungal partners was highly significantly
477 affected by the symbiosis (Fig. 5 c,d), with the liverworts with dual partnerships receiving
478 on average more than 5 times that of the single-fungal type liverworts (Table 2; $F_{2, 48} =$
479 49.84, $P < 0.001$; three-way ANOVA). In addition, there was a significant effect of
480 a[CO_2], with an average 4-fold increase in carbon allocation to the fungi across
481 symbioses and algal treatments at 1,500 ppm compared to 440 ppm a[CO_2] (Compare
482 Fig. 5c and d; Table 2: effect of CO_2 $F_{1, 48} = 6.87$, $P = 0.012$, three-way ANOVA). The
483 addition of algae significantly increased total photosynthate allocated into the mesh-
484 walled cores (Table 2: effect of algae $F_{1, 48} = 10.57$, $P = 0.002$, three-way ANOVA), and
485 there were no significant interactions between the symbiont types and the effects of
486 algae, or between algae and a[CO_2] treatments, and no three-way interactions.
487

488 **Discussion**

489 Our results show that there are important differences in functionality between plant-
490 fungal symbioses involving Mucoromycotina, Glomeromycotina, and dual symbioses
491 with both groups of fungi in the effectiveness of N transfer from organic matter and
492 inorganic P to host plants in non-sterile soil. The data from our microcosm experiments
493 suggest that variation in atmospheric carbon dioxide concentration and the form in which

494 nutrients are available affects the nutrient exchange dynamics between the liverwort
495 hosts tested and their fungal symbiont(s) and that these responses are distinct
496 according to the type of fungal partner present.
497
498 *Mucoromycotina fungi enhance transfer of N from algal necromass to liverwort partners.*
499 Although limited in the number of plant species tested and quantification of fungal
500 biomass not performed, our experiments demonstrated that Mucoromycotina fungal
501 partners, both when occurring singly and in dual partnerships with Glomeromycotina
502 assimilated and transferred significant amounts of ^{15}N supplied as algal necromass in
503 non-sterile soil to their liverwort hosts. In contrast, we detected negligible fungal ^{15}N
504 transfer to the plant in the *Marchantia*-Glomeromycotina symbiosis. The detection of
505 small amounts of ^{15}N in liverworts where the hyphae of symbiotic fungi growing into the
506 mesh cores were severed by rotation are likely to be due to mass flow and diffusion of
507 ^{15}N-containing molecules resulting from mineralization by soil microorganisms (Thirkell
508 *et al.,* 2016). Our data support the hypothesis that Mucoromycotina fungi are more
509 successful in competing with soil microbes for organic N resources than
510 Glomeromycotina fungi. The higher rates of N uptake via the Mucoromycotina fungi
511 compared to only Glomeromycotina might be due to these fungi (a) competing better
512 with soil saprotrophs for organic N from the algal necromass, (b) competing better for N
513 mineralized by the saprotrophs, or (c) supporting a mycorrhizosphere-microbial
514 community that better accelerates depolymerisation and / or mineralization of N. It is
515 well-established that the role of mycorrhizal fungi in nutrient uptake arises from their
516 interactions with other soil microorganisms, as most of the N in soil is naturally present
517 in organic forms originating from plant and microbial litter that require microbial
518 depolymerisation and potentially mineralization before it can be utilized (Vitousek &
519 Howarth, 1991). For example, AM fungi develop specific “mycorrhizosphere” microbial

populations that may be involved in increasing mobilization and mineralization of nutrients, as plant-growth promoting and P-solubilizing bacteria *Pseudomonas* and *Burkholderia* can be supported by organic C supplied by AM fungal mycelium (Zhang *et al.,* 2014, 2016). Consequently, the overall functioning of mycorrhizas in N uptake by plants is the result of complex interactions between the symbiotic fungi and other soil organisms in mutualistic and competitive/antagonistic activities in the processes of depolymerisation, mineralization and immobilization. Given this complexity for interpreting the role of mycorrhizal fungi in nutrient uptake, further studies are now required to resolve the mechanistic basis of the overall functioning of the mycorrhizal partnerships in interacting with soil microorganisms. It remains unclear whether the Mucoromycotina fungi are facultative saprotrophs and able to depolymerise the main constituents of algal necromass N such as proteins, lipids and nucleic acids, and structural carbohydrates such as cellulose cell walls.

Nonetheless, our findings provide new insights into the functional biology and ecology of these two fungal groups in symbiosis with extant members of early-divergent land plant groups in non-sterile soil. This is particularly pertinent to pioneer plants like liverworts, given the low N status of disturbed, primary successional habitats (Ollivier *et al.,* 2011) in which soil crusts containing fast-turning over green algae and cyanobacteria are likely to provide important N rich microsites, a situation not dissimilar to that assumed to have been experienced by early land plants colonising poorly-developed soils as part of cryptogamic ground covers (Edwards *et al.,* 2015). Where organic N is available in such systems, there is likely strong competition between plants and soil microbes (Hodge *et al.,* 2000; Kuzyakov & Xu, 2013).

In the Glomeromycotina association of *Marchantia,* the limited fungal transfer of ^{15}N to the host liverwort and corresponding plant-to-fungus C allocation patterns observed here are consistent with previous studies that have found especially under

546 elevated a[CO_2] and higher C fixation by plants, AM fungi become major sinks of plant
547 photosynthate (Hodge, 1996; Johnson *et al.,* 2002; Herman *et al.,* 2012). Increased C
548 efflux from mycorrhizal fungal mycelium into soil may help the priming of microbial
549 decomposers – a common soil-biotic response to increased organic matter availability
550 (de Graaff *et al.,* 2010; Drigo *et al.,* 2010; Verbruggen *et al.,* 2013). Thus, AM fungi may
551 also rely on members of the saprotrophic microbial community to decompose complex
552 organic sources and release N in inorganic form (Govindarajulu *et al.,* 2005; Leigh *et al.,*
553 2011) for hyphal capture. While several studies (Leigh *et al.,* 2009; Hodge & Fitter,
554 2010) have demonstrated that AM fungi transfer ^{15}N from ^{15}N-labelled organic patches
555 to their host angiosperm plants, and a plant ammonium transporter that is mycorrhiza-
556 specific and preferentially activated in cells containing arbuscules has been discovered
557 (Guether *et al.,* 2009a; 2009b), others have reported no or negligible transport of ^{15}N
558 from fungus to host (Hodge & Fitter, 2010). Hodge and Fitter (2010) showed that whilst
559 AM fungal hyphae proliferated on and acquired N from a ^{15}N-labelled organic patch the
560 fungus retained most of the ^{15}N rather than transferring it to the host plant. Our results
561 point toward similar N dynamics in the *Marchantia*-Glomeromycotina symbiosis as for
562 angiosperm-Glomeromycotina AM, given the major increase in plant C allocation to the
563 fungus with access to an organic patch at elevated a[CO_2] (Fig. 5c) for little to no ^{15}N
564 return (Fig. 3c). Thus, it is plausible that under elevated a[CO_2] the Glomeromycotina
565 symbiont contributes to soil N immobilization, limiting ^{15}N transfer to the liverwort host
566 rather than contributing to liverwort host N nutrition. It should also be noted that in
567 previous studies using non-sterile soil or root pieces from soil as fungal inocula that both
568 Glomeromycotina and FRE may have colonized test plant roots, so that nutritional
569 effects that have been attributed in the past exclusively to effects of Glomeromycotina
570 AM fungi, may sometimes have unknowingly involved both groups.
571

572 Whether the Mucoromycotina fungal symbionts use the organic nutrient patch as
573 a source of C or remains entirely dependent on the host liverwort as its main source of
574 metabolic C is yet to be elucidated. In this regard, it is interesting to note that the
575 general idea that putatively facultative saprotrophic ectomycorrhizal fungi behave like
576 free-living saprotrophs in gaining C compounds from organic matter decomposition
577 (Buée *et al.,* 2005; Courty *et al.,* 2007) has recently been challenged. Lindahl and
578 Tunlid (2015) proposed that ectomycorrhizal fungi perform co-metabolic oxidation of
579 organic matter with the main benefit being not the acquisition of metabolic C but the
580 mobilization of N locked up in non-hydrolysable, recalcitrant organic matter complexes
581 (Lindahl *et al.,* 2007). Thus, most ectomycorrhizal fungi remain dependent on their host
582 plants as their principal source of metabolic C, behaving as decomposers rather than
583 facultative saprotrophs (Lindahl & Tunlid, 2015). Our results, showing stable or
584 increased host C allocation to Mucoromycotina symbionts with access to an organic
585 nutrient patch (Fig. 5), point to similar C dynamics in the liverwort-Mucoromycotina
586 partnership.
587
588 *Fundamental functional differences among symbioses*
589 Overall, our results indicate functional differences among the three types of plant-
590 fungal symbioses investigated (Table 3):
591 1) Associations involving Mucoromycotina-only partners provide substantial
592 access to N supplied as algal necromass, and increase P returns to the liverwort host in
593 exchange for relatively little plant-fixed C, especially under modern a[CO_2]. However,
594 the amount of ^{33}P gained by a plant host per unit of C invested in the fungal partner was
595 reduced in patches containing algal necromass at 440 ppm but not at 1500 ppm a[CO_2],
596 possibly as a result of less effective P uptake or retaining more P in its own biomass and
597 passing a smaller proportion on to the host plant compared to Glomeromycotina fungi.

598 2) In Glomeromycotina-only associations, the fungal symbionts of *M. paleacea* do
599 not appear to facilitate significant plant N acquisition from an organic N source. This
600 symbiosis is very effective in ^{33}P transfer to the host plant and this is largely independent
601 of whether fresh algal necromass is added to soil – consistent with the obligately
602 biotrophic nature of these fungi and their highly evolved role in supplying P from soil to
603 plants.
604 3) Dual symbioses involving simultaneous Mucoromycotina and
605 Glomeromycotina fungal partners combine the nutritional benefits afforded to the host
606 plants by each of the single-fungus partnerships i.e. ^{15}N transfer from available organic
607 nutrient sources by the Mucoromycotina symbiosis together with the more constant ^{33}P
608 returns from the Glomeromycotina symbiosis, whether or not organic nutrient sources
609 are available. However, these nutritional benefits come at a considerably higher carbon
610 'cost' to the host than single-fungus symbioses (Table 3). The relative host carbon
611 allocation to, and nutrient gains from each fungal partner within dual colonisations
612 remain to be established.
613 Our findings support our hypothesis that the ability to engage with both
614 Mucoromycotina and Glomeromycotina fungal partners, whether singly or in
615 simultaneous dual symbiosis, offers additional plasticity in terms of nutrient uptake to
616 liverworts and other early-divergent land plant clades known to engage in dual
617 symbioses (Field *et al.,* 2015a; Desirò *et al.,* 2013; Rimington *et al.,* 2015). Our results
618 indicate that the outcome from these multiple symbiotic options is influenced by a
619 complex interplay of biotic and abiotic factors, including a[CO_2] and hence plant
620 photosynthetic capacity, fungal symbiont type, localized resource-patches (such as
621 provided by our ^{15}N algal necromass), as well as interactions with other soil microbiota
622 (which we have not specifically investigated). The apparent superior ability of
623 Mucoromycotina fungi over the Glomeromycotina fungi tested in supplying liverwort

partners with organically-derived N may provide a competitive advantage in habitats where N is limiting and/or where the distribution of organic nutrients is patchy, whether spatially or temporally. The apparent complementarity of the fungal partners with respect to provisioning of host plants with N and P may help to explain the apparent presence and persistence of both fungal lineages in partnerships with plants from the early stages of terrestrial ecosystems (Strullu-Derrien *et al.,* 2014) through to contemporary agro-ecosystems (Orchard *et al.,* 2017a; 2017b).

Latest evidence indicates that fine root endophyte fungi (FRE), which are widespread ecologically and across vascular plants, are members of the Mucoromycotina and closely related to liverwort and lycophyte Mucoromycotina symbionts (Bidartondo *et al.,* 2011; Rimington *et al.,* 2015; 2016; Field *et al.,* 2015b; Orchard *et al.,* 2017a). Furthermore, the same Mucoromycotina fungi can enter into symbiosis with liverworts, early vascular plants and angiosperms (Hoysted *et al.,* 2019). This finding greatly expands the potential significance of Mucoromycotina fungi in modern ecosystems (Field *et al.,* 2015a; Hoysted *et al.,* 2018; Field and Pressel, 2018). Thus, it is now critical to explore the functioning of Mucoromycotina fungal symbionts in later-divergent groups of land plants such as lycophytes, ferns and angiosperms. Such investigations together with a better appreciation of the roles of Glomeromycotina fungi in plant host N nutrition, are key to understanding the consequences of diverse nutritional mutualisms among plants and fungi for biogeochemistry and ecosystems in the past, present and future.

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655

656 **Competing Interests**

657 The authors declare no competing interests.

658

659 **References**

660 Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ. 1997.
661 Gapped BLAST and PSI-BLAST: a new generation of protein database search
662 programs. *Nucleic Acids Research* **25**: 3389-3402

663 Ames RN, Reid CPP, Porter LK, Cambardella C. 1983. Hyphal uptake and transport of
664 nitrogen from two ^{15}N-labelled sources of *Glomus mosseae*, a vesicular-arbuscular
665 mycorrhizal fungus. *New Phytologist* **95**: 381–396.

666 Azcón R, Ambrosano E, Charest C. 2003. Nutrient acquisition in mycorrhizal lettuce
667 plants under different phosphorous and nitrogen concentration. *Plant Science* **165**:
668 1137–1145.

669 Berner, R.A., 2006. GEOCARBSULF: a combined model for Phanerozoic atmospheric
670 O_2 and CO_2 *Geochimica et Cosmochimica Acta* **70**(23): 5653-5664.

671 Bidartondo MI, Read DJ, Trappe JM, Merckx V, Ligrone R, Duckett JG. 2011. The dawn
672 of symbiosis between plants and fungi. *Biology Letters* **7**: 574–577.

673 Bücking H, Kafle A. 2015. Role of arbuscular mycorrhizal fungi in nitrogen uptake of plants:
674 current knowledge and research gaps. *Agronomy* **5**: 587-612.

675 Buee M, Vairelles D, Garbaye J. 2005. Year-round monitoring of diversity and potential
676 metabolic activity of the ectomycorrhizal community in a beech (*Fagus sylvatica*) forest
677 subjected to two thinning regimes. *Mycorrhiza* **15**: 235–245.

678 Cameron DD, Leake JR, Read DJ. 2006. Mutualistic mycorrhiza in orchids: evidence
679 from plant-fungus carbon and nitrogen transfers in the green-leaved terrestrial orchid
680 *Goodyera repens*. *New Phytologist* **171**: 405–416.

681 Cameron DD, Johnson I, Read DJ, Leake JR. 2008. Giving and receiving: measuring
682 the carbon cost of mycorrhizas in the green orchid *Goodyera repens*. *New Phytologist*
683 **180**: 176–184.

684 Carafa A, Duckett JG, Ligrone R. 2003. Subterranean gametophytic axes in the primitive
685 liverwort Haplomitrium harbour a unique type of endophytic association with aseptate
686 fungi. *New Phytologist* **160**: 185-197.

687 Courty PE, Breda N, Garbaye J. 2007. Relation between oak tree phenology and the
688 secretion of organic matter degrading enzymes by *Lactarius quietus* ectomycorrhizas
689 before and during bud break. *Soil Biology and Biochem*istry **39**: 1655–1663.

690 de Graaff MA, Classen AT, Castro HF, Schadt CW. 2010. Labile soil carbon inputs
691 mediate the soil microbial community composition and plant residue decomposition
692 rates. *New Phytologist* **188**: 1055–1064

693 Desirò A, Duckett JG, Pressel S, Villarreal JC, Bidartondo, MI. 2013. Fungal symbioses
694 in hornworts: a chequered history. *Proceedings of the Royal Society: B* **280:** 20130207.

695 Drigo B, Pijl AS, Duyts H, Kielak AM, Gamper HA, Houtekamer MJ, Boschker HTS,
696 Bodelier PLE, Whiteley AS, van Veen JA, Kowalchuk GA. 2010. Shifting carbon flow
697 from roots into associated microbial communities in response to elevated atmospheric
698 CO_2. *Proceedings of the National Academy of Sciences of the United States of America*
699 **107**: 10938–10942.

700 Duckett, Carafa A, Ligrone R. 2006. A highly differentiated glomeromycotean
701 association with the mucilage-secreting, primitive antipodean liverwort *Treubia*
702 (Treubiaceae): clues to the origins of mycorrhizas. *American Journal of Botany* **93**: 797–
703 813.

704 Edwards E, Cherns L, Raven JA. 2015 Could land-based early photosynthesizing
705 ecosystems have bioengineered the planet in mid-Palaeozoic times? *Palaeontology* **58**:
706 803–837.

707 Field KJ, Cameron DD, Leake JR, Tille S, Bidartondo MI, Beerling DJ. 2012. Contrasting
708 arbuscular mycorrhizal responses of vascular and non-vascular plants to a simulated
709 Palaeozoic CO_2 decline. *Nature Communications* **3**: 835.

710 Field KJ, Pressel S, Duckett JG, Rimington WR, Bidartondo MI. 2015a. Symbiotic
711 options for the conquest of land. *Trends in Ecology and Evolution* **30**: 477–486

712 Field KJ, Rimington WR, Bidartondo MI, Allison KE, Beerling DJ, Cameron DD, Duckett
713 JG, Leake JR, Pressel S. 2015b. First evidence of mutualism between ancient plant
714 lineages (Haplomitriopsida liverworts) and Mucoromycotina fungi and its response to
715 simulated Palaeozoic changes in atmospheric CO_2. *New Phytologist* **154**: 743–756.

716 Field KJ, Rimington WR, Bidartondo MI, Allison KE, Beerling DJ, Cameron DD, Duckett
717 JG, Leake JR, Pressel S. 2016. Functional analysis of liverworts in dual symbiosis with
718 Glomeromycota and Mucoromycotina fungi under a simulated Palaeozoic CO_2 decline.
719 *The ISME Journal* **10:** 1514–1526.

720 Field KJ**,** Pressel S. 2018. Tansley Review - Unity in diversity: structural and functional
721 insights into an ancient partnership between plants and fungi. *New Phytologist* doi:
722 10.1111/nph.15158

723 Fletcher BJ, Brentnall SJ, Quick WP, Beerling DJ. 2006. BRYOCARB: a process-based
724 model of thallose liverwort isotope fractionation in response to CO_2, O_2, light and
725 temperature. *Geochimica et Cosmochimica Acta* **70**: 5676–5691.

726 Gardes M, Bruns TD. (1993). ITS primers with enhanced specificity for Basidiomycetes -
727 application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113-118

728 Govindarajulu M, Pfeffer PE, Jin HR, Abubaker J, Douds DD, Allen JW, Bücking H,
729 Lammers PJ, Shachar-Hill Y. 2005. Nitrogen transfer in the arbuscular mycorrhizal
730 symbiosis. *Nature* **435**: 819–823.

731 Guether M, Neuhaüser B, Balestrini R, Dynowski M, Ludewig U, Bonfante P. 2009a. A
732 mycorrhizal-specific ammonium transporter rom *Lotus japonicus* acquires nitrogen
733 released by arbuscular mycorrhizal fungi. *Plant Physiology* **150**: 73–83.

734 Guether M, Balestrini R, Hannah M, He J, Udvardi MK, Bonfante P. 2009b. Genome-
735 wide reprogramming of regulatory networks, transport, cell wall and membrane
736 biogenesis during arbuscular mycorrhizal symbiosis in *Lotus japonicus*. *New Phytologist*
737 **182**: 200–212.

738 Herman DJ, Firestone MK, Nuccio E, Hodge A. 2012. Interactions between an
739 arbuscular mycorrhizal fungus and a soil microbial community mediating litter
740 decomposition. *FEMS Microbiology Ecol*ogy **80**: 236–247.

741 Hodge A. 1996. Impact of elevated CO_2 on mycorrhizal associations and implications for
742 plant growth*. Biology and Fertility of Soils* **23**, 388–398.

743 Hodge A, Robinson D, Fitter A. 2000. Are microorganisms more effective than plants at
744 competing for nitrogen? *Trends in Plant Science* **5**: 304-308

745 Hodge A, Fitter AH. 2010. Substantial nitrogen acquisition by arbuscular mycorrhizal
746 fungi from organic material has implications for N cycling. *Proceedings of the National*
747 *Academy of Science of the United States of America* **107**: 13754–13759.

748 Hodge A, Storer K. 2015. Arbuscular mycorrhiza and nitrogen: implications from individual plants
749 through to ecosystems. *Plant and Soil* **386**: 1-19.

750 Hoysted GA**,** Kowal J, Jacob A, Rimington WR, Duckett JG, Pressel S, Orchard S, Ryan
751 M, Field KJ, Bidartondo MI. 2018. A mycorrhizal revolution. *Current Opinions in Plant*
752 *Biology* **44**: 1-6.

753 Hoysted GA, Jacob AS, Kowal J, Giesemann P, Bidartondo MI, Duckett JG, Gebauer G,
754 Rimington WR, Schornack S, Pressel S, Field KJ. 2019. Mucoromycotina fine root
755 endophyte fungi form nutritional mutualisms with vascular plants. *bioRxiv*,
756 https://doi.org/10.1101/531103

757 Humphreys CP, Franks PJ, Rees M, Bidartondo MI, Leake JR, Beerling DJ. 2010.
758 Mutualistic mycorrhiza-like symbiosis in the most ancient group of land plants. *Nature*
759 *Communications* **1**: 7.

760 Johnson D, Leake JR, Ostle N, Ineson P, Read DJ. 2002. In situ CO_2-C^{13} pulse
761 labelling of upland grassland demonstrates a rapid pathway of carbon flux from
762 arbuscular mycorrhizal mycelia to the soil. *New Phytol*ogist **153**: 327–334.

763 Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper
764 A, Markowitz S, Duran C, Thierer T. 2012. Geneious Basic: an integrated and
765 extendable desktop software platform for the organization and analysis of sequence
766 data. *Bioinformatics* **28**: 1647-1649.

767 Kuzyakov Y, Xu X. 2013. Competition between roots and microorganisms for nitrogen:
768 mechanisms and ecological reference. *New Phytologist* **198**: 656-669

769 Leake, JR. 1988. *Causes and Effects of Soil Acidification by Calluna vulgaris (L.) Hull:*
770 *With Special Reference to the Role of Mycorrhizas* (Doctoral dissertation, University of
771 Sheffield, Department of Plant Sciences).

772 Leigh J, Hodge A, Fitter AH. 2009. Arbuscular mycorrhizal fungi can transfer substantial
773 amounts of nitrogen to their host plant from organic material. *New Phytologist* **181**: 199–
774 207.

775 Leigh J, Fitter AH, Hodge A. 2011. Growth and symbiotic effectiveness of an arbuscular
776 mycorrhizal fungus in organic matter in competition with soil bacteria. *FEMS*
777 *Microbiology Ecology* **76**: 428–438.

778 Lenton TM, Daines SJ, Mills BJW. 2018. COPSE reloaded: An improved model of
779 biogeochemical cycling over Phanerozoic time. Earth Science Reviews **178**: 1-28

780 Lindahl BD, Ihrmark K, Boberg J, Trumbore SE, Högberg P, Stenlid J, Finlay RD. 2007.
781 Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal
782 forest. *New Phytologist* **173**(3): 611-620.

783 Lindahl BD, Tunlid A. 2015. Ectomycorrhizal fungi – potential organic matter
784 decomposers, yet not saprotrophs. *New Phytologist* **205**: 1443–1447.

785 Morris JL, Puttick MN, Clark JW, Edwards D, Kenrick P, Pressel S, Wellman CH, Yang
786 Z, Schneider H, Donoghue PC. 2018. The timescale of early land plant
787 evolution. *Proceedings of the National Academy of Sciences* **115**(10):.E2274-E2283.

788 Nobel PS. 1999. *Physicochemical and environmental plant physiology*, 2nd edn.
789 Amsterdam, the Netherlands: Academic Press.

790 Ollivier J, Toewe S, Bannert A, Hai B, Kastl EM, Meyer A, Su MX, Kleineidam K,
791 Schloter M. 2011. Nitrogen turnover in soil and global change. *FEMS Microbiology*
792 *Ecology* **7**8: 3–16

793 Orchard, S, Hilton S, Bending GD, Dickie IA, Standish RJ, Gleesons DB, Jeffery RP,
794 Powell JR, Walker C, Bass D, Monk J, Simonin A, Ryan MH. 2017a. Fine endophytes
795 (*Glomus tenue*) are related to Mucoromycotina not Glomeromycota. *New Phytologist*
796 **213**: 481–486.

797 Orchard S, Standish RJ, Dickie IA, Renton M, Walker C, Moot D, Ryan MH. 2017b. Fine
798 root endophytes under scrutiny: a review of the literature on arbuscule-producing fungi
799 recently suggested to belong to the Mucoromycotina. *Mycorrhiza* **27**: 619-638

800 Pressel S, Bidartondo MI, Field KJ, Rimington WR, Duckett JG. 2016. Pteridophyte
801 fungal associations: Current knowledge and future perspectives. *Journal of Systematics*
802 *and Evolution* **54**: 666–678.

803 Quirk J, Beerling DJ, Banwart SA, Kakonyi G, Romero-Gonzalez ME, Leake JR. 2012.
804 Evolution of trees and mycorrhizal fungi intensifies silicate mineral weathering. *Biology*
805 *letters* **8**: 1006-1011.

806 Rimington WR, Pressel S, Duckett JG, Bidartondo MI. 2015. Fungal diversity in early
807 vascular plants: Reopening a closed book? *New Phytologist* **205**: 1394–1398.

808 Rimington WR, Pressel S, Field KJ, Strullu-Derrien C, Duckett JG, Bidartondo MI. 2016.
809 Reappraising the origin of mycorrhizas. In *Molecular Mycorrhizal Symbiosis* (ed. F
810 Martin). pp. 31-32 Hoboken, NJ, USA: John Wiley & Sons Inc.

811 Rimington WR, Pressel S, Duckett JG, Field KJ, Read DJ, Bidartondo MI (2018).
812 Ancient plants with ancient fungi: liverworts associate with early-diverging arbuscular
813 mycorrhizal fungi. *Proceedings of the Royal Society B: Biological Sciences* **285:**
814 20181600.

815 Smit E, Leeflang P, Glandorf B, van Elsas JD, Wernars K. 1999. Analysis of fungal
816 diversity in the wheat rhizosphere by sequencing of cloned PCR- amplified genes
817 encoding 18S rRNA and temperature gradient gel electrophoresis. *Applied and*
818 *Environmental Microbiology* **65**: 2614-2621.

819 Smith SE, Read DJ. 2008. *Mycorrhizal Symbiosis*. Cambridge, UK: Academic Press.

820 Smith SE, Anderson IC, Smith FA. 2015. Mycorrhizal associations and phosphorus
821 acquisition: from cells to ecosystems. *Annual Plant Reviews* **48**: 409-440.

822 Strullu-Derrien C, Kenrick P, Pressel S, Duckett JG, Rioult JP, Strullu DG. 2014. Fungal
823 associations in *Horneophyton ligneri* from the Rhynie Chert (c. 407 million year old)
824 closely resemble those in extant lower land plants: novel insights into ancestral plant–
825 fungus symbioses. *New Phytologist* **203**: 964-979.

826 Thirkell T, Cameron DD, Hodge A. 2016. Resolving the 'nitrogen paradox' of arbuscular
827 mycorrhizas: fertilization with organic matter brings considerable benefits for plant
828 nutrition and growth. *Plant Cell Environment* **39**: 1683–1690.

829 Verbruggen E, Veresoglou SD, Anderson IC, Caruso T, Hammer EC, Kohler J, Rillig
830 MC. 2013. Arbuscular mycorrhizal fungi – short-term liability but long-term benefits for
831 soil carbon storage? *New Phytologist* **197**: 366–368.

832 Vitousek PM, Howarth RW. 1991. Nitrogen limitation on land and in the sea; how can it
833 occur? *Biogeochemistry* **13**: 87–115.

834 Walker C, Gollotte A, Redecker D (2018). A new genus, *Planticonsortium*
835 (Mucoromycotina), and new combination *(P. tenue),* for the fine root endophyte*, Glomus*
836 *tenue (basionym Rhizophagus tenuis). Mycorrhiza* **28**: 213-219.

837 Wang B, Qui Y-L. 2006. Phylogenetic distribution and evolution of mycorrhizas.
838 *Mycorrhiza* **16**: 299–363.

839 Wang B, Yeun L H, Xue J-Y, Liu Y, Ané J-M, Qiu Y-L. 2010. Presence of three
840 mycorrhizal genes in the common ancestor of land plants suggests a key role of
841 mycorrhizas in the colonization of land by plants. *New Phytologist* **186**: 514–525.
842 White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of
843 fungal ribosomal RNA genes for phylogenetics. In PCR Protocols: a Guide to Methods
844 and Applications, M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White, eds. (Orlando:
845 Academic Press), pp. 315-322.

Zhang L, Fan J, Ding X, He X, Zhang F, Feng G, 2014. Hyphosphere interactions between an arbuscular mycorrhizal fungus and a phosphate solubilizing bacterium promote phytate mineralization in soil. *Soil Biology & Biochemistry* **74**: 177–183.

Zhang L, Xu M, Liu Y, Zhang F, Hodge A, Feng G, 2016. Carbon and phosphorus exchange may enable cooperation between an arbuscular mycorrhizal fungus and a phosphate-solubilizing bacterium. *New Phytologist* **210**: 1022–1032.

Supporting Information

Figure S1. Mean total phosphorus content and concentration in core substrate where algal necromass was absent (- algae) and where algal necromass was included (+ algae).

Figure S2. Total mean biomass of liverworts in plants grown under ambient atmospheric [CO_2] (440 ppm) and elevated atmospheric [CO_2] (1,500 ppm) with (and without fungal access to algal necromassa.

Notes S1 - Supplementary materials and methods (analysis of total P, biomass, fungus-to-plant ^{15}N and ^{33}P transfer and plant-to-fungus C transfer).

Notes S2 – Supplementary results (total phosphorus content of mesh-walled cores, plant biomass).

Table S1. Three-way ANOVA (GLM) showing effects of fungal symbiont, inclusion of algae, and a[CO_2] on liverwort biomass at end of experiment.

Table S2. Two-way ANOVA (GLM) showing effects of fungal symbiont and access to algae within static core (S) treatments. Data are log10 transformed to conform to ANOVA assumptions.

Figure Legends

Fig. 1. Diagrams of experimental microcosms showing (a) contents of cores containing ^{15}N-labelled algal necromass (right) and cores without algal addition (left). Location of algal necromass indicated by green shading running length of the soil column within the core; (b) experimental microcosm where liverworts have access to ^{15}N-labelled algal necromass and ^{33}P-orthophosphate via intact fungal hyphal connections between host liverwort and core contents (static core treatment); (c) experimental microcosm where liverworts do not have access to ^{15}N-labelled nutrient patch and ^{33}P-orthophosphate via severed fungal hyphal connections between core contents and host plant achieved by rotating the core (blue arrow; rotated core treatment) prior to isotope introduction (red arrow), and (d) ^{14}C labelling experimental set-up showing fixation of $^{14}CO_2$ by liverworts and transfer of labelled carbon compounds to fungi within static cores.

Fig. 2. Liverworts and fungal symbionts studied in the present investigation; wild whole plants (a, d, g) and scanning electron micrographs of respective fungal endophytes (b, c, e, f, h, i). (a-c) The earliest-divergent Haplomitriopsida liverwort *Haplomitrium gibbsiae* (a) associates exclusively with Mucoromycotina-FRE fungi. Mucoromycotina colonise *Haplomitrium* underground axes where their fine hyphae (0.5-1.5 µm) form coils with swellings (arrowed in b and enlarged in c). (d-f) *Marchantia paleacea* harbouring in its thallus only Glomeromycotina fungal symbionts with cytology consisting of coarse hyphae (> 3 µm), arbuscules (e) and large vesicles (arrowed in f),

as typical of AM symbioses. (g-i) *Neohodgsonia mirabilis* in dual symbiosis with Glomeromycotina and Mucoromycotina fungi showing in its thallus structures typical of both fungi: arbuscules on trunk hyphae (Glomeromycotina, h) and fine hyphae with arbuscule-like structures (Mucoromycotina, i). Scale bars: (e) 200 μm; (b) 100 μm; (f, h, i) 50 μm; (c) 20 μm.

Fig. 3. Fungus-to-plant nitrogen transfer between liverworts and fungi at simulated Palaeozoic and modern ambient atmospheric [CO_2]. Mean total fungal-acquired ^{15}N (a, b) and concentration of fungal-acquired ^{15}N detected in plants (c, d) at simulated Palaeozoic a[CO_2] of 1,500 ppm (a, c) and modern ambient a[CO_2] of 440 ppm (b, d) where ^{15}N labelled algal necromass was ("Static"; black bars) or was not ("Rotated"; white bars) accessible to fungal symbionts. Error bars indicate ± S.E., *n* = 5 (see Table 1 for statistics).

Fig. 4. Fungus-to-plant phosphorus transfer between liverworts and fungi at simulated Palaeozoic and modern ambient atmospheric [CO_2]. Mean total fungal-acquired ^{33}P (a, b) and concentration of fungal-acquired ^{33}P in plants (c, d) at simulated Palaeozoic a[CO_2] of 1,500 ppm (a, c) and modern ambient a[CO_2] of 440 ppm (b, d). "+ algae" values represent fungal-acquired ^{33}P where algal necromass was present, "- algae" representing fungal-acquired ^{33}P where algal necromass was not present. Error bars indicate ± S.E., *n* = 5 (see Table 2 for statistics).

Fig. 5. Plant-to-fungus carbon transfer between liverworts and fungi at simulated Palaeozoic and modern ambient atmospheric [CO_2]. Percentage allocation (a, b) and total (c, d) liverwort-fixed carbon transferred to fungal partners within soil cores with (black bars) and without (white bars) presence of organic nutrient patch at (a, c) 1,500

924 ppm [CO_2] and (b, d) 440 ppm [CO_2]. Note difference in scale of Y axis in figures (c) and

925 (d). Error bars indicate ± S.E., *n* = 5 (see Table 2 for statistics).

926

Tables

Table 1. Summary of three-way ANOVA (GLM) results testing the effects of core rotation on ^{15}N content and [^{15}N] of liverworts (Fig. 3). Data were log10 transformed to meet assumptions of ANOVA. Hyphal connections between liverworts and ^{15}N-labelled algal necromass are preserved in static core treatments whereas rotated core treatment causes hyphal connections between liverwort to be severed. Experiments carried out under 440 ppm a[CO_2] and 1,500 ppm a[CO_2]. Significant results (P<0.05) are indicated in **bold**, n.s. = P>0.05.

Variable	Symbiosis (S) *d.f. 2, 48*		CO_2 (C) *d.f. 1, 48*		Core rotation (R) *d.f. 1, 48*		S x C *d.f. 2, 48*		S x R *d.f. 2, 48*		C x R *d.f. 1, 48*		S x C x R *d.f. 2, 48*	
	F	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
Plant ^{15}N (ng) (See Fig. 3a,b)	4.79	**0.018**	0.30	n.s.	23.10	**<0.001**	2.45	n.s.	1.33	n.s.	1.31	n.s.	0.75	n.s.
Plant [^{15}N] (ng g^{-1}) (See Fig. 3 c,d)	4.37	**0.027**	0.52	n.s.	49.26	**<0.001**	11.34	n.s.	2.44	n.s.	0.93	n.s.	2.04	n.s.

Table 2. Summary of three-way ANOVA (GLM) results testing the effects of algae inclusion and fungal symbiont(s) on exchange of fungal-acquired phosphorus (Fig. 4) for plant carbon (Fig. 5) in experiments conducted under 440 ppm a[CO_2] and 1,500 ppm a[CO_2]. Data were log10 transformed to meet assumptions of ANOVA. Significant results (P<0.05) are indicated in **bold**, n.s. = P>0.05.

Variable	Symbiosis (S) *d.f. 2, 48*		CO_2 (C) *d.f. 1, 48*		Algae (A) *d.f. 1, 48*		S x C *d.f. 2, 48*		S x A *d.f. 2, 48*		C x A *d.f. 1, 48*		S x C x A *d.f. 2, 48*	
	F	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
Plant ^{33}P (ng)	0.96	n.s.	1.50	n.s.	0.50	n.s.	5.39	**0.008**	0.54	n.s.	1.84	n.s.	1.13	n.s.
Plant [^{33}P] (ng g^{-1})	8.99	**0.001**	4.42	n.s.	2.23	n.s.	5.24	**0.009**	1.97	n.s.	0.10	n.s.	4.60	**0.015**
% C allocation to cores	4.96	**0.011**	0.08	n.s.	0.04	n.s.	2.87	n.s.	1.39	n.s.	0.98	n.s.	1.53	n.s.
Plant allocation of C to fungi within cores (ng)	49.84	**<0.001**	6.87	**0.012**	10.57	**0.002**	0.59	n.s.	1.49	n.s.	0.15	n.s.	1.77	n.s.

944 **Table 3.** Summary of key findings of the costs and benefits of single and dual symbiosis
945 with Mucoromycotina and Glomeromycotina fungi in liverworts.
946

Functions	**Mycorrhiza type**		
	Mucoromycotina fungi	Glomeromycotina fungi	Both Mucoromycotina and Glomeromycotina
Relative carbon costs of external mycelium to host plant	Low	Low	High
^{15}N from algal necromass supplied to host plant	High	Low	High
^{33}P orthophosphate supplied to host plant	Low-High Reduced by algal necromass at ambient CO_2 but not at elevated CO_2.	High Unaffected by algal necromass	Moderate-High Unaffected by algal necromass

947

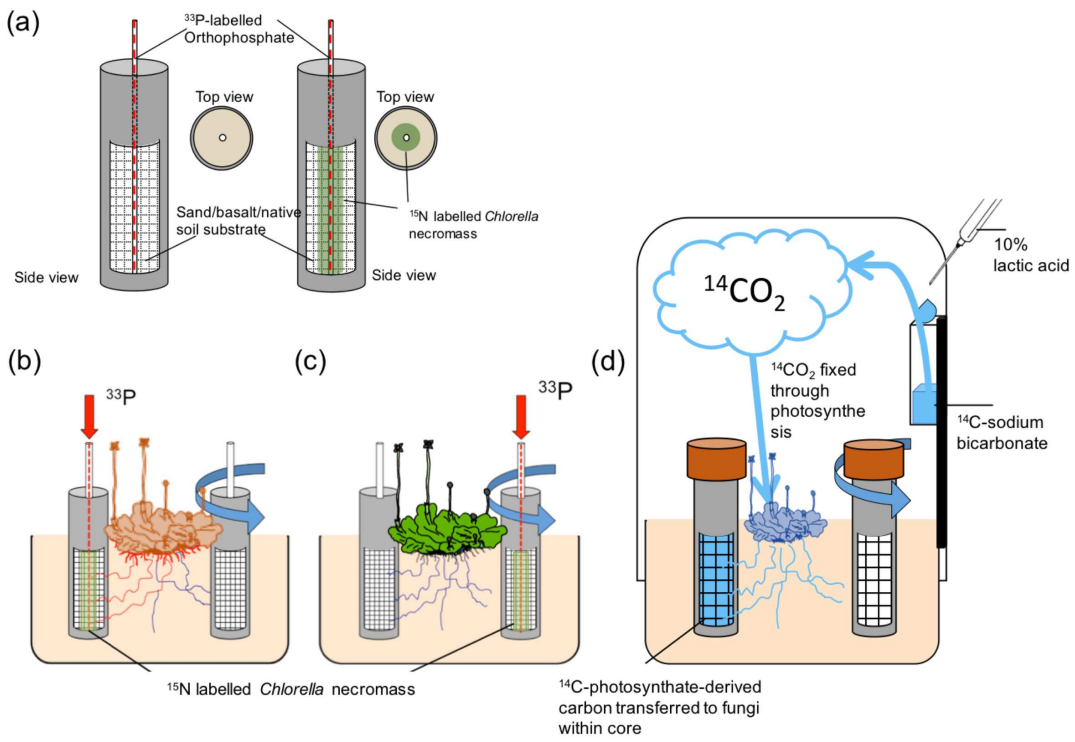
(a)
^{33}P-labelled Orthophosphate
Top view
Top view
Sand/basalt/native soil substrate
^{15}N labelled *Chlorella* necromass
Side view
Side view
(b)
(c)
(d)
^{33}P
^{33}P
$^{14}CO_2$
$^{14}CO_2$ fixed through photosynthesis
10% lactic acid
^{14}C-sodium bicarbonate
^{15}N labelled *Chlorella* necromass
^{14}C-photosynthate-derived carbon transferred to fungi within core

Figure 1.

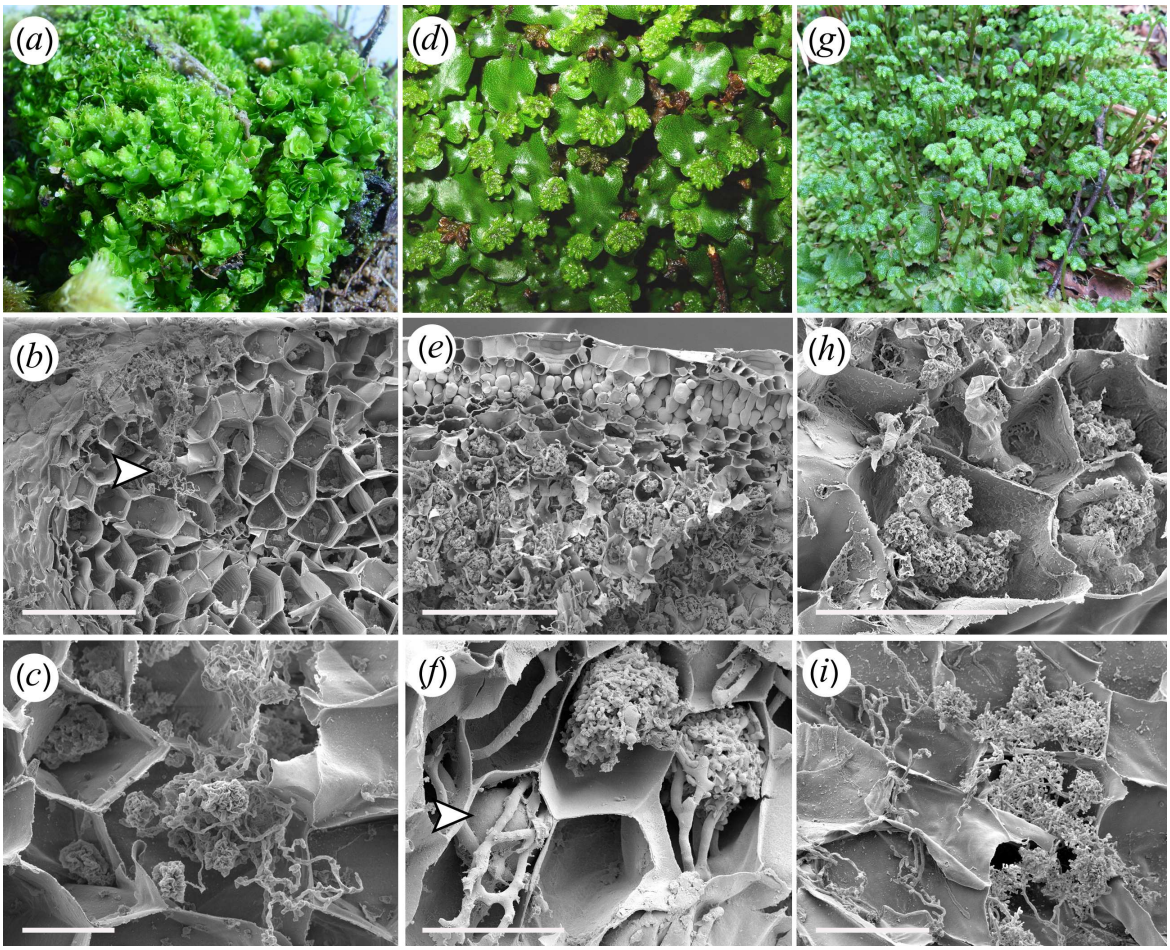

Figure 2.

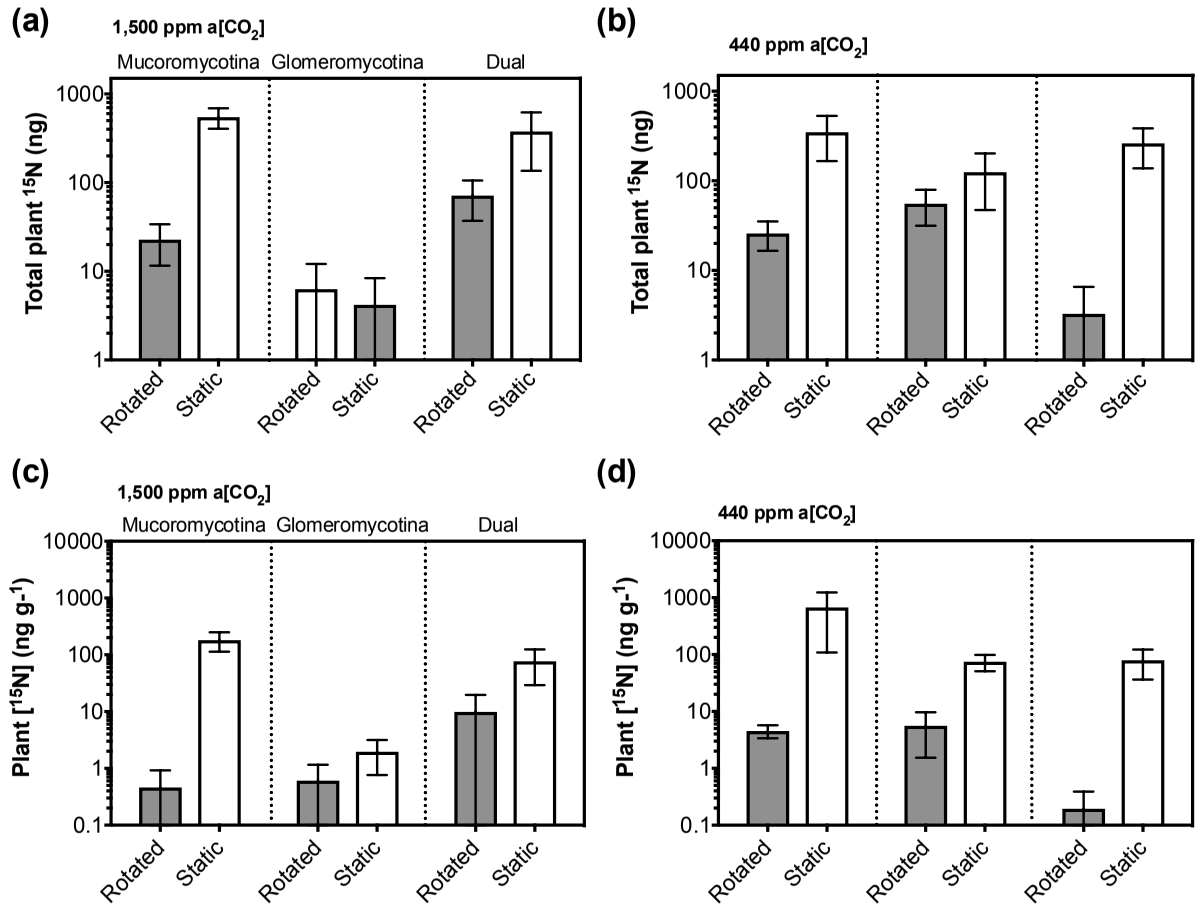
(a)
1,500 ppm a[CO2]
Mucoromycotina
Glomeromycotina
Dual
Total plant 15N (ng)
1000
100
10
1
Rotated
Static
(b)
440 ppm a[CO2]
Total plant 15N (ng)
(c)
1,500 ppm a[CO2]
Plant [15N] (ng g-1)
10000
0.1
(d)
440 ppm a[CO2]

Figure 3.

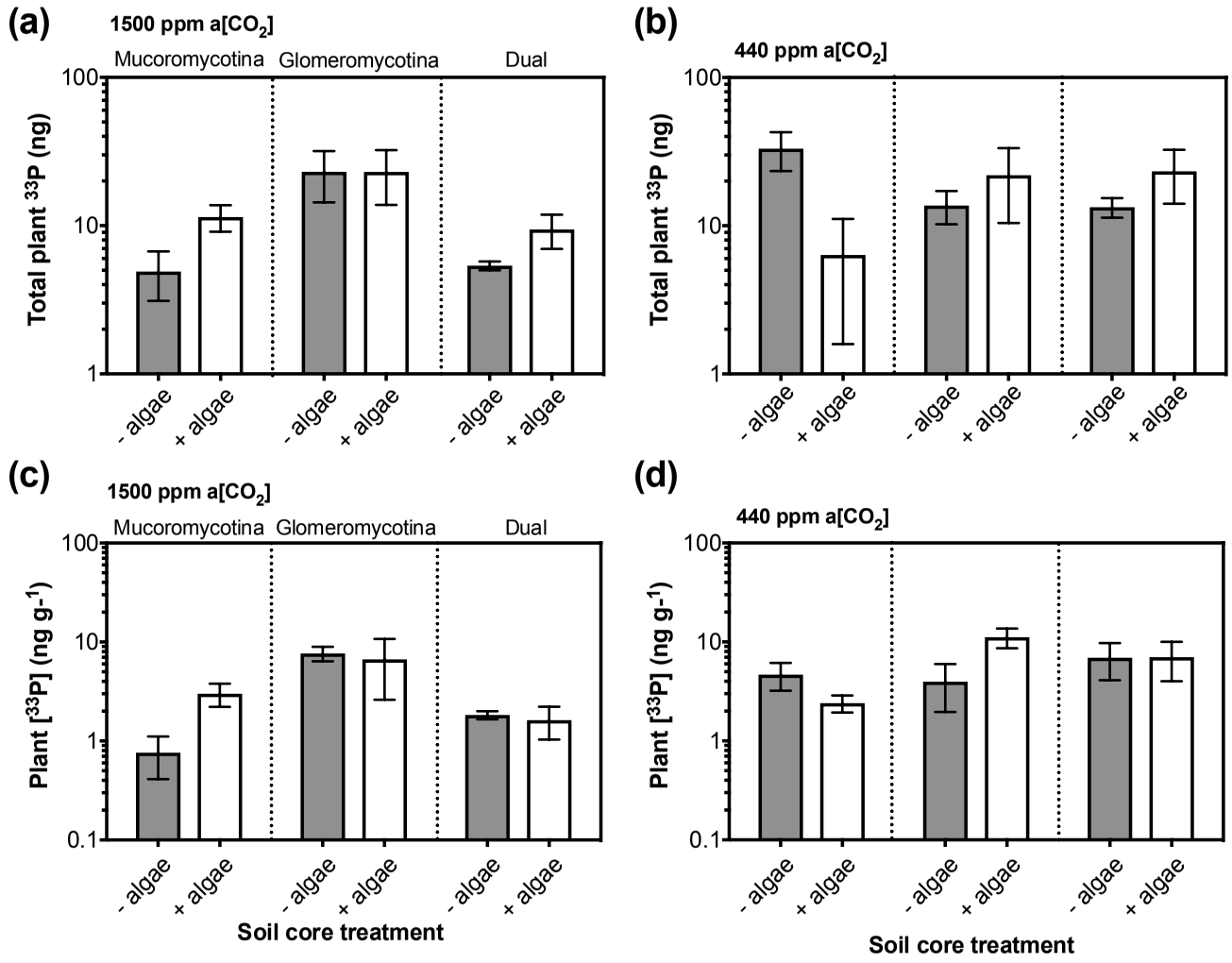
(a)
1500 ppm a[CO2]
Mucoromycotina
Glomeromycotina
Dual
Total plant 33P (ng)
100
10
1
- algae
+ algae
(b)
440 ppm a[CO2]
(c)
Plant [33P] (ng g-1)
0.1
Soil core treatment
(d)

Figure 4.

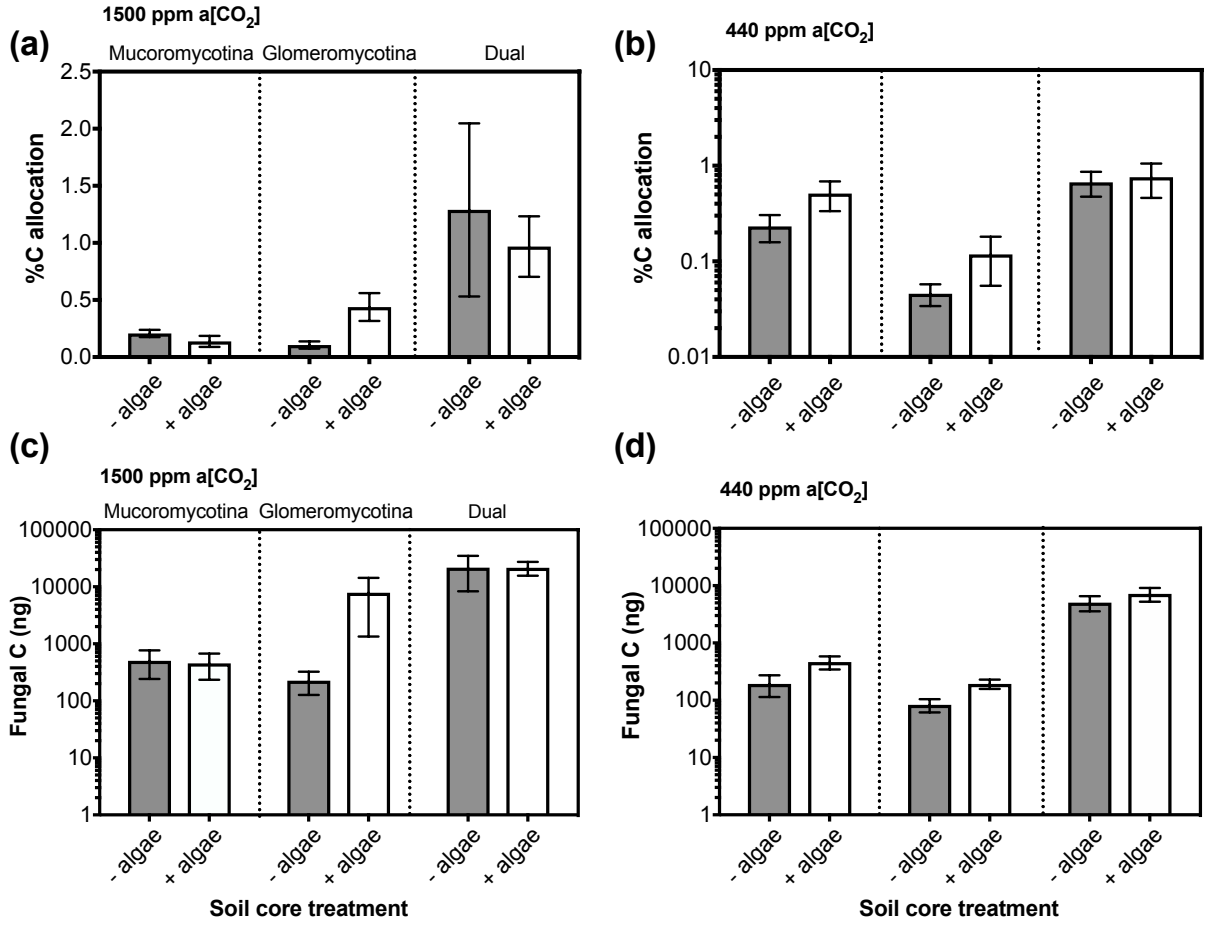
(a)
1500 ppm a[CO2]
Mucoromycotina
Glomeromycotina
Dual
%C allocation
2.5
2.0
1.5
1.0
0.5
0.0
- algae
+ algae
(b)
440 ppm a[CO2]
%C allocation
10
1
0.1
0.01
(c)
1500 ppm a[CO2]
Fungal C (ng)
100000
10000
1000
100
10
1
Soil core treatment
(d)
440 ppm a[CO2]
Soil core treatment

Figure 5.