1   **Carbon for nutrient exchange between the lycophyte, *Lycopodiella inundata* and**

2   **Mucoromycotina 'fine root endophytes' is unresponsive to high atmospheric CO$_2$**

3   **concentration.**

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14   Running Title: Lycopod-Mucoromycotina FRE interactions unaffected by high atmospheric

15   CO$_2$

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1 **Background and Aims**

2 Non-vascular plants associating with arbuscular mycorrhizal (AMF) and Mucoromycotina

3 'fine root endophyte' (MFRE) fungi derive greater benefits from their fungal associates under

4 higher atmospheric $[CO_2]$ than ambient, however nothing is known about how changes in

5 $[CO_2]$ affects MFRE function in vascular plants.

6 **Methods**

7 We measured movement of phosphorus (P), nitrogen (N) and carbon (C) between the

8 lycophyte, *Lycopodiella inundata* and Mucoromycotina fine root endophyte fungi using ^{33}P-

9 orthophosphate, ^{15}N-ammonium chloride and $^{14}CO_2$ isotope tracers under ambient and

10 elevated atmospheric $[CO_2]$ concentrations of 440 and 800 ppm, respectively.

11 **Key Results**

12 Transfer of ^{33}P and ^{15}N from MFRE to plant were unaffected by changes in $[CO_2]$. There was

13 a slight increase in C transfer from plant to MFRE under elevated $[CO_2]$.

14 **Conclusions**

15 Our results demonstrate that the exchange of C-for-nutrients between a vascular plant and

16 Mucoromycotina FRE is largely unaffected by changes in atmospheric $[CO_2]$. Unravelling

17 the role of MFRE in host plant nutrition and potential C-for-N trade changes between

18 symbionts under varying abiotic conditions is imperative to further our understanding of the

19 past, present and future roles of diverse plant-fungal symbioses in global ecosystems.

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21 Key words: Lycophyte, *Lycopodiella inundata,* Mucoromycotina, Fine root endophytes,

22 Vascular plants, Atmospheric CO_2

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1 Introduction

Changes in atmospheric CO_2 concentration ($[CO_2]$) have been a prominent feature throughout Earth's environmental history (Leaky and Lau, 2012). Geochemical models support fossil and stable isotope evidence that the global environment underwent major changes throughout the Palaeozoic Era (541-250 Ma) (Berner et al., 2006; Bergman et al., 2004; Lenton et al., 2016), consisting of a step-wise increase of the Earth's atmospheric oxygen ($[O_2]$), and a simultaneous decline in atmospheric $[CO_2]$. Today, Earth faces environmental changes on a similar scale, but with atmospheric $[CO_2]$ now rising at an unprecedented rate (Meinshausen et al., 2011).

Long before plants migrated onto land, Earth's terrestrial surfaces were colonised by a diverse array of microbes, including filamentous fungi (Blair, 2009; Berbee et al., 2017). Around 500 Mya, plants made the transition from an aquatic to a terrestrial existence (Morris et al., 2018), facilitated by symbiotic fungi (Nicolson, 1967; Pirosynski and Malloch, 1975). These ancient fungal symbionts are thought to have played an important role in helping the earliest land plants access scarce nutrients from the substrate onto which they had emerged, in much the same way as modern-day mycorrhizal fungi form nutritional mutualisms with plant roots (Pirozynski and Malloch, 1975; Krings et al., 2012). It is highly likely that ancient mycorrhiza-like fungi were closely related to, and subsequently evolved into, modern arbuscular mycorrhizal fungi (AMF) belonging to the fungal subphylum Glomeromycotina (also referred to as phylum Glomeromycota) (Redecker et al.. 2000; Wijayawardene et al., 2018; Radhakrishnan et al., 2020).

Recently, it was discovered that extant non-vascular plants, including the earliest divergent clade of liverworts, associate with a greater diversity of fungi than was previously thought, notably forming endophytic associations with members of the Mucoromycotina (Bidartondo et al., 2011; Desiró et al., 2014; Rimington et al., 2015; Field et al., 2015a). The

1   Mucoromycotina is a partially-saprotrophic fungal lineage (Bidartondo et al., 2011; Field et

2   al., 2015b; 2016) sister to, or pre-dating, the Glomeromycotina AMF, both within

3   Mucoromycota (Spatafora et al., 2017). This discovery, together with the finding that

4   liverwort-Mucoromycotina fungal associations are nutritionally mutualistic (Field et al.,

5   2015a; 2019), often co-occurring with AMF (Field et al., 2016), and emerging fossil evidence

6   (Strullu-Derrien et al., 2014) suggest that the earliest land plants had greater symbiotic

7   options available to them than was previously thought (Field et al., 2015b). Studies now show

8   that symbioses with Mucoromycotina fungi are not limited to early non-vascular plants, but

9   also span almost the entire extant land plant kingdom (Rimington et al., 2015; Orchard et al.,

10   2017a; Hoysted et al., 2018, 2019; Rimington et al., 2020), suggesting that this ancient

11   association may have key roles in modern terrestrial ecosystems.

12   Latest research into the functional significance of plant-Mucoromycotina fine root

13   endophyte (MFRE) associations indicates that MFRE play a complementary role to AMF by

14   facilitating plant nitrogen (N) assimilation alongside AMF-facilitated plant phosphorus (P)

15   acquisition through co-colonisation of the same plant host (Field et al., 2019). Such

16   functional complementarity is further supported by the observation that MFRE transfer

17   significant amounts of ^{15}N but relatively little ^{33}P tracers to a host lycophyte *Lycopodiella*

18   *inundata* in the first experimental demonstration of MFRE nutritional mutualism in a

19   vascular plant (Hoysted et al., 2019). These results contrast with the majority of studies on

20   MFRE and FRE which have, to date, focussed on the role of the fine root endophytes in

21   mediating plant phosphorus (P) acquisition (Orchard et al., 2017b, and literature within).

22   Today, plant symbiotic fungi play critical roles in ecosystem structure and function.

23   The bidirectional exchange of plant-fixed carbon (C) for fungal-acquired nutrients that is

24   characteristic of most mycorrhizal symbioses (Field and Pressel, 2018) holds huge

25   significance for carbon and nutrient flows and storage across ecosystems (Leake et al., 2004;

1   Rillig, 2004). By forming mutualistic symbioses with the vast majority of plants, including

2   economically-important crops, mycorrhizal fungi have great potential for exploitation within

3   a variety of sustainable management strategies in agriculture, conservation and restoration.

4   Application of diverse mycorrhiza-forming fungi, including both AMF and MFRE, to

5   promote sustainability in agricultural systems is particularly relevant in the context of global

6   climate change and depletion of natural resources (Field et al., 2020). MFRE in particular

7   hold potential for applications to reduce use of chemical fertilisers within sustainable arable

8   systems where routine over-application of N-based mineral fertilisers causes detrimental

9   environmental and down-stream economic impacts (Thirkell et al., 2017). Changes in abiotic

10   factors such as atmospheric [CO_2] (Cotton, 2018), which is predicted to continue rising in the

11   future (Meinshausen et al., 2011), has been shown to affect the rate and quantities of carbon

12   and nutrients exchanged between mycorrhizal partners (Field et al., 2012, 2015a, 2016;

13   Zheng et al., 2015; Thirkell et al., 2019). As such, insights into the impact of environmental

14   factors relevant to future climate change on carbon for nutrient exchange between symbiotic

15   fungi and plants must be a critical future research goal.

16   The direct quantification of exchange of plant C and fungal-acquired mineral

17   nutrients, using isotope tracers, has shed important new light on the impact of varying

18   atmospheric [CO_2] on C-for-nutrient exchanges between plants and their symbiotic fungi

19   (Field et al., 2012, 2015a, 2016; Zheng et al., 2015; Thirkell et al., 2019). Experiments with

20   liverworts associating with MFRE fungi, either in exclusive or in dual symbioses alongside

21   AMF, suggest that these plants derive less benefit in terms of nutrient assimilation from their

22   fungal associates under a high atmospheric [CO_2] (1,500 ppm) than under a lower [CO_2] (440

23   ppm) (Field et al., 2015a; 2016), with the opposite being the case for liverworts associated

24   only with AMF (Field et al., 2012). However, when vascular plant-AMF associations were

25   exposed to high [CO_2] in the same experimental conditions, there were no changes in

1   mycorrhizal-acquired plant P assimilation (Field et al., 2012). Whether vascular plant-MFRE

2   symbioses respond to changing atmospheric [CO_2] is completely unknown.

3   Here, using stable and radioisotope tracers, we investigate how MFRE function in

4   *Lycopodiella inundata*, a homosporous perennial lycophyte widely distributed in the northern

5   hemisphere (Rasmussen and Lawesson, 2002) that associates only with MFRE fungal

6   partners, regarding climate change-relevant shifts in atmospheric [CO_2]. Specifically, we test

7   the hypotheses that (a) MFRE acquire greater amounts of plant-fixed C under high

8   atmospheric [CO_2] of 800 ppm as a result of there being larger amounts of photosynthate

9   available for transfer because of greater rates of C fixation by the plant via photosynthesis,

10   and (b), increased C allocation from plant to fungus increases transfer and assimilation of ^{15}N

11   and ^{33}P tracers from MFRE to plants to feed growing plant demand for nutrients to promote

12   growth.

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14   **Methods**

15   **Plant Material and growth conditions**

16   Mature *Lycopodiella inundata* (L.) plants were collected from Thursley National

17   Nature Reserve, Surrey, UK (SU 90081 39754) in June 2017. The *L. inundata* plants were

18   planted directly into pots (90 mm diameter x 85 mm depth) containing a homogeneous

19   mixture of acid-washed silica sand and 5% pot volume compost (No.2; Petersfield) to aid

20   retention properties of the substrate and to provide minimal nutrients. Soil surrounding plant

21   roots was left intact to prevent damage to the roots and to act as a natural inoculum, including

22   symbiotic fungi and associated microorganisms. Pots were weeded regularly to remove other

23   plant species. All *L. inundata* plants used during this study were collected from the wild.

24   Based on the methods of Field et al., (2012), three windowed cylindrical plastic cores

25   covered in 10 μm nylon mesh were inserted into the substrate within each experimental pot

1　(Figure 1a-d). Two of the cores were filled with the same substrate as the bulk soil within the

2　pots, comprising a homogeneous mixture of acid-washed silica sand and compost (No. 2;

3　Petersfield), together making up 95% of the core volume, native soil gathered from around

4　the roots of wild plants to ensure cores contained the same microbial communities as in the

5　bulk soil (4% core volume), and fine-ground tertiary basalt (1% core volume) to act as fungal

6　bait (Field et al. 2015a). The third core was filled with glass wool to allow below-ground gas

7　sampling throughout the ^{14}C-labelling period to monitor soil community respiration. Plants

8　were watered every other day with distilled water with no other application of nutrient

9　solutions. Microcosms shared a common drip-tray within each cabinet through the

10　acclimation period that ensured a common pool of rhizospheric microorganisms in each

11　microcosm.

12　　　　A total of 48 *L. inundata* microcosms were maintained in controlled environment

13　chambers (Model no. Micro Clima 1200; Snijders Labs) with a light cycle of 16-h daytime

14　(20°C and 70% humidity) and 8-h night-time (at 15°C and 70% humidity). Day-time

15　photosynthetically active radiation (PAR), supplied by LED lighting, was 225 µmol photons

16　$m^{-2} s^{-1}$. Plants were grown at two contrasting CO_2 atmospheres; 440 ppm [CO_2] (24 plants) to

17　represent a modern-day atmosphere, or at 800 ppm [CO_2] (24 plants) to simulate Palaeozoic

18　atmospheric conditions on Earth at the time vascular plants are thought to have diverged

19　(Berner, 2006) as well as predicted atmospheric [CO_2] for 2100 (Meinshausen et al., 2011).

20　Atmospheric [CO_2] was monitored using a Vaisala sensor system (Vaisala, Birmingham,

21　UK), maintained through addition of gaseous CO_2. All pots were rotated within cabinets to

22　control for possible cabinet and block effects. Plants were acclimated to chamber/growth

23　regimes for four weeks to allow establishment of mycelial networks within pots and

24　confirmed by hyphal extraction from soil and staining with trypan blue (Brundrett et al.,

25　1996). Additionally, roots were stained with acidified ink for the presence of fungi, based on

7

1   the methods of Brundrett et al., (1996). All plants were processed for molecular analyses of

2   fungal symbionts within one week of collection using the protocol in Hoysted et al., (2019).

3   **Cytological analyses**

4   Roots of experimental *L. inundata* plants were stained with trypan blue (Brundrett et

5   al., 1996), which is common for identifying MFRE (Orchard et al., 2017b), and photographed

6   under a Zeiss Axioscope (Zeiss, Oberkochen, Germany) equipped with a MRc digital camera.

7   To quantify root colonisation by MFRE, five plants were randomly selected per treatment,

8   and from these two intact, healthy roots (per plant) were excised and sectioned transversally

9   in up to six segments (depending on root length) before being processed for SEM according

10   to Duckett et al., (2006). Percentage root colonisation was then calculated by scoring each

11   segment (from a total of 56 and 58 segments respectively for the elevated and ambient [CO_2]

12   treatments) as colonised or non-colonised under the SEM (Figure 4).

13

14   **Quantification of C, ^{33}P and ^{15}N fluxes between lycophytes and fungi**

15   After the four-week acclimation period, microcosms were moved to individual drip-

16   trays immediately before isotope labelling to avoid cross-contamination of the isotope

17   tracers. A total of 100 µl of an aqueous mixture of ^{33}P-labelled orthophosphate (specific

18   activity 111 TBq mmol^{-1}, 0.3 ng ^{33}P added; Hartmann analytics) and ^{15}N-ammonium chloride

19   (1mg ml^{-1}; 0.1 mg ^{15}N added; Sigma-Aldrich) was introduced into one of the soil-filled mesh

20   cores in each pot through the installed capillary tube. In half (n = 12) of the pots, cores

21   containing isotope tracers were left static to preserve direct hyphal connections with the

22   lycophytes (Fig. 1a). Fungal access to isotope tracers was limited in the remaining half (12)

23   of the pots by rotating isotope tracer-containing cores through 90°, thereby severing the

24   hyphal connections between the plants and core soil (Fig. 1b). These were rotated every

25   second day thereafter, thus providing a control treatment that allows us to distinguish

1   between fungal and microbial contributions to tracer uptake by plants. Assimilation of ^{33}P

2   tracer into above-ground plant material was monitored using a hand-held Geiger counter held

3   over the plant material daily.

4   At detection of peak activity in above-ground plant tissues (21 days after the addition

5   of the ^{33}P and ^{15}N tracers), the tops of ^{33}P and ^{15}N-labelled cores were sealed with plastic

6   caps and anhydrous lanolin and the glass wool cores were sealed with rubber septa

7   (SubaSeal, Sigma-Aldrich). Before lights were switched on at 8 am, each pot was sealed into

8   a 3.5 L, gas-tight labelling chamber and 2 ml 10% (w/v) lactic acid was added to 30 μl

9   NaH^{14}CO$_3$ (specific activity 1.621 GBq/mmol^{-1}; Hartmann Analytics), releasing a 1.1-MBq

10   pulse of ^{14}CO$_2$ gas into the headspace of the labelling chamber (Fig. 1c). Pots were

11   maintained under growth chamber conditions, and 1 ml of headspace gas was sampled after 1

12   hour and every 1.5 hours thereafter. Below-ground respiration was monitored via gas

13   sampling from within the glass-wool filled core after 1 hour and every 1.5 hours thereafter

14   for ~16 h.

15

16   **Plant harvest and sample analyses**

17   Upon detection of maximum below-ground flux of ^{14}C, ~16h after the release of the

18   ^{14}CO$_2$ pulse, each microcosm compartment (i.e. plant material and soil) was separated,

19   freeze-dried, weighed and homogenised. The ^{33}P activity in plant and soil samples was

20   quantified by digesting in concentrated H$_2$SO$_4$ and liquid scintillation (Tricarb 3100TR liquid

21   scintillation analyser, Isotech). The quantity of ^{33}P tracer that was transferred to plant by its

22   fungal partner was then calculated using previously published equations (Cameron et al.

23   2007). To determine total symbiotic fungal-acquired ^{33}P transferred to *L. inundata*, the mean

24   ^{33}P content of plants that did not have access to the tracer because cores into which the ^{33}P

25   was introduced were rotated, was subtracted from the total ^{33}P in each plant that did have

9

1   access to the isotopes within the core via intact fungal hyphal connections (i.e. static cores).

2   This calculation controls for diffusion of isotopes and microbial nutrient cycling in pots,

3   ensuring only ^{33}P gained by the plant via intact fungal hyphal connections is accounted for

4   and therefore serves as a conservative measure of the minimum fungal transfer of tracer to

5   the plant.

6   Between two and four mg of freeze-dried, homogenised plant tissue was weighed into

7   6 x 4 mm^2 tin capsules (Sercon) and ^{15}N abundance was determined using a continuous flow

8   IRMS (PDZ 2020 IRMS, Sercon). Air was used as the reference standard, and the IRMS

9   detector was regularly calibrated to commercially available reference gases. The ^{15}N

10   transferred from fungus to plant was then calculated using equations published previously in

11   Field et al., (2016). In a similar manner as for the ^{33}P, the mean of the total ^{15}N in plants

12   without access to the isotope because of broken hyphal connections between plant and core

13   contents was subtracted from total ^{15}N in each plant with intact hyphal connections to the

14   mesh-covered core to give fungal acquired ^{15}N. Again, this provides a conservative measure

15   of ^{15}N transfer from fungus to plant as it ensures only ^{15}N gained by the plant via intact fungal

16   hyphal connections is accounted for.

17   The ^{14}C activity of plant and soil samples was quantified through sample oxidation

18   (307 Packard Sample Oxidiser, Isotech) followed by liquid scintillation. Total C (^{12}C + ^{14}C)

19   fixed by the plant and transferred to the fungal network was calculated as a function of the

20   total volume and CO_2 content of the labelling chamber and the proportion of the supplied

21   $^{14}CO_2$ label fixed by plants. The difference in total C between the values obtained for static

22   and rotated core contents in each pot is considered equivalent to the total C transferred from

23   plant to symbiotic fungus within the soil core for that microcosm, noting that a small

24   proportion will be lost through soil microbial respiration. The total C budget for each

25   experimental pot was calculated using equations from Cameron et al., (2006). Total percent

10

1   allocation of plant-fixed C to extra-radical symbiotic fungal hyphae was calculated by

2   subtracting the activity (in Becquerels (Bq)) of rotated core samples from that detected in

3   static core samples in each pot, dividing this by the sum of activity detected in all

4   components of each microcosm, then multiplying it by 100.

5

6   **Statistics**

7   Effects of plant species, $[CO_2]$ and the interaction between these factors on the C, ^{33}P

8   and ^{15}N fluxes between plants and Mucoromycotina FRE fungi were tested using analysis of

9   variance (ANOVA) or Mann-Whitney U where indicated. Data were checked for

10 homogeneity and normality. Where assumptions for ANOVA were not met, data were

11 transformed using $\log_{10}$. If assumptions for ANOVA were still not met, a Mann-Whitney U

12 as well as a Kruskal-Wallis statistical test was performed. All statistics were carried out using

13 the statistical software package SPSS Version 24 (IBM Analytics).

14

15 **Results**

16 **Molecular identification of fungal symbionts**

17 Analysis of experimental *Lycopodiella inundata* plants grown under ambient and

18 elevated $[CO_2]$ confirmed that they were colonised by Mucoromycotina fine root endophyte

19 fungi. Glomeromycotina fungal sequences were not detected. Mucoromycotina OTUs that

20 have previously been identified in wild-collected lycophytes from diverse locations

21 (Rimington et al., 2015; Hoysted et al., 2019), were detected before and after the

22 experiments. (GenBank/EMBL accession numbers: MK673773-MK673803).

23

24 **Cytology of fungal colonisation in plants**

1   Trypan blue staining and SEM of *L. inundata* roots grown under ambient and elevated

2   atmospheric $[CO_2]$ revealed the same fungal symbiont morphology consistent with that

3   previously observed for *L. inundata*-Mucoromycotina FRE (Hoysted et al., 2019) and MFRE

4   colonisation in other vascular plants (Orchard et al., 2017a) including fine branching, aseptate

5   hyphae (<2 μm diameter) with small intercalary and terminal swellings/vesicles (usually 5-10

6   but up to 15 μm diameter) but, differently from those in flowering plants, no arbuscules.

7   While no differences were detected in the cytology of colonisation between the roots of

8   plants grown under contrasting atmospheric $[CO_2]$, those grown under 800 ppm $[CO_2]$

9   showed higher percentage colonisation (79%) than those grown under 440 ppm $[CO_2]$ (53%).

10

11   C transfer from *L. inundata* to MFRE symbionts

12   The amount of carbon allocated from *L. inundata* to Mucoromycotina FRE fungi

13   under elevated atmospheric $[CO_2]$ concentrations compared to that when plants were grown

14   under atmospheric $[CO_2]$ of 440 ppm was greater, with *L. inundata* allocating 2.8 times more

15   recent photosynthate to MFRE fungi in elevated atmospheric $[CO_2]$ compared with ambient

16   atmospheric $[CO_2]$, although this was not statistically significant (Figure 2a; Mann-Whitney

17   U = 194, *P* = 0.864, n = 24). In terms of total C transferred from plants to MFRE, a similar

18   trend was observed with *L. inundata* transferring ca. 2.7 times more C to MFRE fungal

19   partners at elevated atmospheric $[CO_2]$ concentrations of 800 ppm compared to those

20   maintained under atmospheric $[CO_2]$ of 440 ppm (Figure 2b; Mann-Whitney U = 197.5, *P* =

21   0.942, n = 24).

22

23   Fungus-to-lycophyte ^{33}P and ^{15}N transfer

24   Mucoromycotina FRE transferred both ^{33}P and ^{15}N to *L. inundata* in both atmospheric

25   CO_2 treatments (Figure 3). There were no significant differences in the amounts of either [33]P

12

1   or ^{15}N tracer acquired by MFRE in *L. inundata* plant tissue when grown under elevated $[CO_2]$

2   of 800 ppm compared to plants grown under $[CO_2]$ conditions of 440 ppm, either in terms of

3   absolute quantities (Figure 3a; ANOVA $[F_{1,\ 23} = 0.009, P = 0.924, N = 10]$; Figure 3c;

4   ANOVA $[F_{1,\ 22} = 0.126, P = 0.726, n = 10]$) or when normalised to plant biomass (Figure 3b;

5   ANOVA $[F_{1,\ 23} = 0.085, P = 0.774, n = 10]$; Figure 3d; ANOVA $[F_{1,\ 22} = 0.770, P = 0.390, n$

6    $= 10]$). Although not significantly different, there was a trend of more nitrogen transferred

7   between MFRE under ambient $[CO_2]$ compared to elevated $[CO_2]$ (Figure 3d). Within the

8   experimental microcosms, MFRE transferred 6.65% ($\pm$ 2.55) ^{33}P and 0.07% ($\pm$0.02) ^{15}N

9   tracer under ambient $[CO_2]$ and 6.9% ($\pm$4.75) ^{33}P and 0.03% ($\pm$0.18) ^{15}N tracer under

10   elevated $[CO_2]$.

11

12   **Discussion**

13   Our results demonstrate for the first time, that the exchange of C-for-nutrients

14   between a vascular plant and MFRE symbionts is largely unaffected by changes in

15   atmospheric $[CO_2]$, with MFRE maintaining ^{33}P and ^{15}N assimilation and transfer to the plant

16   host across $[CO_2]$ treatments (Fig. 3a, b), despite MFRE colonisation being more abundant

17   within the roots of plants grown under the elevated $[CO_2]$ atmosphere. In our experiments,

18   *Lycopodiella inundata* allocated ca. 2.8 times more photosynthate to MFRE under elevated

19   a$[CO_2]$ compared with plants that were grown under ambient a$[CO_2]$ (Fig. 2a, b), but without

20   a reciprocal increase in fungal-acquired ^{33}P or ^{15}N tracer assimilation. Although the

21   difference in C transfer between plants under different $[CO_2]$ atmospheres was not

22   statistically significant, the trend observed is in line with previous studies where

23   Mucoromycotina fungi (and both Mucoromycotina and AMF partners co-colonising the same

24   host in 'dual' symbiosis) fungi gained a greater proportion of recently fixed photosynthates

25   from their non-vascular plant partners, but did not deliver greater amounts of ^{33}P or ^{15}N

13

1   tracers when grown under an elevated a[CO_2] compared to current ambient conditions (Field

2   et al., 2012, 2015a, 2016). This contrasts with patterns of carbon-for-nutrient exchange

3   between other vascular plants and AMF symbionts where increased allocation of carbon to

4   fungal symbionts is usually associated with increases in nutrient delivery from AMF to the

5   host plant (Kiers et al., 2011; Wipf et al., 2019). Such variances may be partly explained by

6   the different lifestyles of the partially saprotrophic Mucoromycotina and the strictly

7   biotrophic Glomeromycotina (Field et al., 2015b, 2016; Field and Pressel, 2018) therefore

8   conventional 'rules' governing AMF-plant symbioses may not necessarily apply to

9   Mucoromycotina-plant symbioses. It is possible that when atmospheric [CO_2] is high,

10   liverworts and relatively simple vascular plants such as *L. inundata* produce photosynthates

11   that they are unable to utilise effectively for growth or reproduction as they possess no or

12   limited vasculature and specialised storage organs to provide transport and storage of excess

13   carbohydrates (Kenrick and Crane, 1997). As a consequence, surplus photosynthates may be

14   either stored as insoluble starch granules, transferred directly to mycobionts (Field et al.,

15   2016), or released into surrounding soil as exudates (Galloway et al., 2017). By moving

16   excess photosynthates into mycobionts, competition for soil resources, space and potential

17   pathogenicity from surrounding saprotrophic organisms such as bacteria and fungi may be

18   reduced (Field et al., 2016).

19   Additionally, biomass of mycorrhizal fungi may increase in response to elevated

20   a[CO_2] but this increase does not necessarily result in greater nutrient transfer to the host

21   plant (Alberton et al., 2005), instead inducing a negative feedback through enhanced

22   competition for nutrients between symbiotic partners (Fransson et al., 2007). Studies on

23   ectomycorrhizal fungi, another group of widespread symbiotic fungi with plants that may act

24   as facultative saprotrophs, showed that despite an increase in the amount of extraradical

25   hyphae under elevated [CO_2], there was no corresponding enhanced transfer of N to the host,

14

1   suggesting that the fungus had become a larger sink of nutrients (Fransson et al., 2005).

2   While we did not measure fungal biomass in this study, our observation of greater percentage

3   colonisation in the roots of *Lycopodiella* plants grown under an elevated $[CO_2]$ of 800 ppm

4   compared to those grown under ambient concentrations but with no corresponding increase in

5   fungus-to-plant N and P transfer may suggest a similar possible scenario.

6   In Field et al. (2015a; 2016), the liverworts *Haplomitrium gibbsiae* and *Treubia*

7   *lacunosa* transferred approximately 56 and 189 times less photosynthate, respectively, to

8   their MFRE partners under elevated $a[CO_2]$ than the *L. inundata* in our experiments did –

9   although Field et al.'s (2016) experiments were carried out at higher $a[CO_2]$ (1,500 ppm) than

10   the present study (800 ppm). Given that *L. inundata* can regulate and maintain CO_2

11   assimilation and thus C fixation through stomata and vasculature (Kenrick and Crane, 1997),

12   it is possible that $[CO_2]$ would affect rates of C fixation and subsequent transfer of plant C to

13   symbiotic fungi less than it might do in poikilohydric liverworts. Our results are consistent

14   with previous studies of AMF symbiosis showing that vascular plants associating with AMF

15   transferred more C to their fungal partners compared to non-vascular species (Field et al.,

16   2012). As such, our findings add to mounting evidence that C transfer from plant to fungus

17   responds differently to changing atmospheric $[CO_2]$ according to both plant and fungal

18   identity.

19   We observed no difference in the amount of fungal-acquired ^{33}P tracer transferred to

20   *L. inundata* sporophytes between atmospheric $[CO_2]$ treatments. This aligns with the

21   responses of MFRE symbionts in non-vascular liverworts which also transferred the same

22   amount (or more in the case of *Treubia* mycobionts) of ^{33}P and more ^{15}N to plant hosts under

23   ambient atmospheric $[CO_2]$ compared to elevated $[CO_2]$ (Field et al., 2015a; 2016). The

24   amount of ^{33}P transferred to *L. inundata* was much less than has previously being recorded

25   for Mucoromycotina in liverworts (Field et al., 2016) and for Glomeromycotina-associated

1   ferns and angiosperms (Field et al., 2012), despite the same amount of ^{33}P being made

2   available, indicating that MFRE fungi do not play a critical role in lycophyte P nutrition.

3   MFRE transferred considerable amounts of ^{15}N to their host (see also Hoysted et al., 2019),

4   under both [CO_2] treatments. This observation together with previous findings that MFRE

5   fungi facilitate the transfer of both organic and inorganic ^{15}N to non-vascular liverworts

6   suggest that MFRE may play a complementary role to AMF in plant nutrition, with a more

7   prominent role in N assimilation (Field et al., 2019; Hoysted et al., 2019). While it has been

8   shown that AMF can transfer N to their associated host (Ames et al., 1983; Hodge et al.,

9   2001) significant doubts remain as to the ecological relevance of an AMF-N uptake pathway

10   (see Read 1991; Smith and Smith, 2011). In particular, regarding the exact mechanism of N

11   transfer and, more importantly, the amounts of N transferred via the AMF compared to the N

12   requirements of the plant remain highly equivocal (Smith and Smith, 2011). This murky view

13   of AMF in N transfer, coupled with recent molecular re-identification of fine root endophytes

14   as belonging within Mucoromycotina and not Glomeromycotina (Orchard et al., 2017a) and

15   evidence pointing to a significant role of MFRE in ^{15}N transfer to both non-vascular (Field et

16   al., 2015a; 2016; 2019) and vascular plants (Hoysted et al., 2019) suggest that effects in plant

17   N nutrition previously ascribed to AMF may instead be attributed to co-occurring MFRE

18   (Field et al., 2019).

19   Given that symbioses involving Mucoromycotina FRE fungi are much more

20   widespread than were initially thought, covering a wide variety of habitats (Bidartondo et al.,

21   2011; Rimington et al., 2015, 2020; Orchard et al., 2017a, b, c; Hoysted et al., 2018; 2019),

22   their role in plant N nutrition and responses to high [CO_2] may have much broader ecological

23   significance than previously assumed. It remains critical that we test how mycorrhizal

24   plasticity (both AMF and FRE) translates into function in order to understand how climate

25   change may affect nutrient fluxes between symbionts in the past, present and, importantly,

16

1   the future (Field and Pressel, 2018). With the growing interest in the application of

2   mycorrhizal fungi to improve vascular plant community diversity, increasing MFRE in soils

3   alongside AMF may improve host nutrient capture and ecosystem robustness, including

4   conservation of endangered species, such as *L. inundata*, in the face of global climate change

5   (van der Heijden et al., 1998; 2015).

6   In this study, we provide a first assessment of the effects of varying [CO_2] on carbon-

7   for-nutrient exchanges between MFRE and a vascular plant. Our results point to important

8   differences in responses to changing [CO_2] between MFRE and AMF and between MFRE

9   symbioses in vascular vs. non-vascular plants, however they are restricted to one, early

10   diverging, vascular species, generally growing in severely N-limited habitats. It is now

11   critical that similar investigations are extended to a broader range of taxa, including

12   flowering plants known to engage in symbiosis with both MFRE and AMF. In doing so,

13   efforts towards the potential exploitation of these symbiotic fungi to help meet sustainability

14   targets of the future may be better informed and the likelihood of success vastly improved.

15

Acknowledgements

21

Literature Cited

23   Alberton O, Kuyper TW, Gorissen A. 2005. Taking mycocentrism seriously: mycorrhizal

24   fungal and plant responses to elevated CO_2. *New Phytologist*, 167: 859-868.

25

1   Ames RN, Reid CPP, Poter LK, Cambardella C. 1983. Hyphal uptake and transport of

2   nitrogen from two 15N-labelled sources by *Glomus mosseae*, a vesicular-arbuscular

3   mycorrhizal fungus. *New Phytologist*, 95(3), 381-396.

4

5   Berbee ML, James TY, Strullu-Derrien C. 2017. Early diverging fungi: diversity and impact

6   at the dawn of terrestrial life. *Annual Review of Microbiology*, 71.

7

8   Berner RA. 2006. Geocarbsulf: A combined model for Phanerozoic atmospheric O_2 and CO_2.

9   *Geochimica et Cosmochimica Acta*, 70: 5653-5664.

10

11   Bergman NM, Lenton TM, Watson AJ. 2004. COPSE: a new model of biogeochemical

12   cycling over Phanerozoic time. *American Journal of Science*, 304: 397-437.

13   Bidartondo MI, Read DJ, Trappe JM, Merckx V, Ligrone R, Duckett JG. 2011. The dawn of

14   symbiosis between plants and fungi. *Biology Letters*, 7(4): 574-577.

15

16   Blair JE. 2009. The fungi in the timetree of life. Hedges SB & Kumar S. eds.

17

18   Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N. 1996. Working with mycorrhizas in

19   forestry and agriculture. Monograph, Australian Centre for International Agricultural

20   Research. Pirie Printers, Canberra.

21

22   Cameron DD, Leake JR, Read DJ. 2006. Mutualistic mycorrhiza in orchids: evidence from

23   plant-fungus carbon and nitrogen transfers in the green-leaved terrestrial orchid *Goodyera*

24   *repens*. *New Phytologist*, 171: 405-416.

25

1   Cameron DD, Johnson I, Leake JR, Read DJ. 2007.Mycorrhizal acquisition of inorganic

2   phosphorus by the green-leaved terrestrial orchid *Goodyera repens*. *Annals of Botany*, 99(5):

3   831-834.

4

5   Cotton TA. 2018. Arbuscular mycorrhizal fungal communities and global change. An

6   uncertain future. *FEMS microbiology ecology*, 94(11): 179.

7

8   Desirò A, Duckett JG, Pressel S, Villarreal JC, Bidartondo MI. 2014. Fungal symbioses in

9   hornworts: a chequered history. *Proceedings of the Royal Society B: Biological Sciences*,

10   280(1759): 20130207.

11

12   Duckett JG, Carafa A, Ligrone R. 2006. A highly differentiated glomeromycotean association

13   with the mucilage-secreting, primitive antipodean liverwort *Treubia* (Treubiaceae): Clues to

14   the origins of mycorrhizas. *American Journal of Botany*, 93: 797-813.

15

16   Field KJ, Cameron DD, Leake JR, Tille S, Bidartondo MI, Beerling DJ. 2012. Contrasting

17   arbuscular mycorrhizal responses of vascular and non-vascular plants to a simulated

18   Palaeozoic CO_2 decline. *Nature Communications*, 3:1-8.

19

20   Field KJ, Rimington WR, Bidartondo MI, Allinson KE, Beerling DJ, Cameron DD, *et al*.

21   2015a. First evidence of mutualism between ancient plant lineages (Haplomitropsida

22   liverworts) and Mucoromycotina fungi and its response to simulated Palaeozoic changes in

23   atmospheric CO_2. *New Phytologist*, 205(2): 743-756.

24

1   Field KJ, Pressel S Duckett JG, Rimington WR, Bidartondo MI. 2015b. Symbiotic options

2   for the conquest of land. *Trends in Ecology and Evolution*, 30(8): 477-486.

3

4   Field KJ, Rimington WR, Bidartondo MI, Allinson KE, Beerling DJ, Cameron DD*, et al.*

5   2016. Functional analysis of liverworts in dual symbiosis with Glomeromycota and

6   Mucoromycotina fungi under simulated Palaeozoic CO_2 decline. *The ISME Journa*l, 10(6):

7   1514-1526.

8

9   Field KJ, Pressel S. 2018. Unity in diversity: structural and functional insights into the

10   ancient partnerships between plants and fungi. *New Phytologist*, 220(4): 996-1011.

11

12   Field KJ, Bidartondo MI, Rimington WR, Hoysted GA, Beerling D, Cameron DD, *et al*.

13   2019. Functional complementarity of ancient plant-fungal mutualisms: contrasting nitrogen,

14   phosphorus and carbon exchanges between Mucoromycotina and Glomeromycotina fungal

15   symbionts of liverworts. *New Phytologist*, 223(2): 908-921.

16

17   Field KJ, Daniell T, Johnson D, Helgason T. 2020. Mycorrhizas for a changing world:

18   sustainability, conservation, and society. *Plants, People, Planet*, 2(2): 98-103.

19

20   Fransson FMA, Taylor AFS, Finlay RD. 2005. Mycelial production, spread and root

21   colonisation by ectomycorrhizal fungi *Hebeloma crustuliniforme* and *Paxillus involutus*

22   under elevated atmospheric CO_2. *Mycorrhiza*, 15: 25-31.

23

24   Fransson FMA, Anderson IC, Alexander IJ. 2007. Ectomycorrhizal fungi in culture respond

25   differently to increased carbon availability. *FEMS Microbiology Ecology*, 61: 246-257.

1

2   Galloway AF, Pederson MJ, Merry B, Marcus SE, Blacker J, Benning LG, *et al.* 2018.

3   Xyloglucan is released by plants and promotes soil particle aggregation. *New Phytologist*,

4   217(3): 1128-1136.

5

6   Gavito ME, Schweiger P, Jakobsen I. 2003. P uptake by arbuscular mycorrhizal hyphae:

7   effect of soil temperature and atmospheric CO_2 enrichment. *Global Change Biology*, 9(1):

8   106-116.

9

10   Hodge, A, Campbell CD, Fitter AH. 2001. An arbuscular mycorrhizal fungus accelerates

11   decomposition and acquires nitrogen directly from organic material. *Nature*, 413(6853): 297-

12   299.

13

14   Hoysted GA, Kowal J, Jacob, A, Rimington WR, Duckett JG, Pressel S, *et al*. 2018. A

15   mycorrhizal revolution. *Current Opinion in Plant Biology*, 44: 1-6.

16

17   Hoysted GA, Jacob A, Kowal J, Giesemann P, Bidartondo MI, Duckett JG, *et al*. 2019.

18   Mucoromycotina fine root endophyte fungi form nutritional mutualisms with vascular plants.

19   *Plant Physiology*, 181(2): 565-577.

20

21   Kenrick P, Crane PR. 1997. Origin and early diversification of land plants. Smithsonian

22   Institution Press.

23

1   Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, *et al.* 2011.

2   Reciprocal rewards stabilise cooperation in the mycorrhizal symbiosis. *Science*, 333(6044):

3   880-882.

4

5   Krings M, Taylor TN, Dotzler N. 2012. Fungal endophytes as a driving force in land plant

6   evolution: evidence from the fossil record. *Biocomplexity of plant-fungal interactions*: 5-28.

7

8   Leake J, Johnson D, Donnelly D, Muckle G, Boddy L, Read D. 2004. Networks of power and

9   influence: the role of mycorrhizal mycelium in controlling plant communities and

10 agroecosystem functioning. *Canadian Journal of Botany*, 82(8): 1016-1045.

11

12 Leaky AD, Lau JA. 2012. Evolutionary context for understanding and manipulating plant

13 responses to past, present and future atmospheric [CO_2]. *Philosophical Transactions of the*

14 *Royal Society B: Biological Sciences*, 367(1588): 613-629.

15

16 Lenton TM, Dahl TW, Daines SJ, Mills BJW, Ozaki K, Saltzman MR, *et al*. 2016. Earliest

17 land plants created modern levels of atmospheric oxygen. *Proceedings of the National*

18 *Academy of Sciences, USA*, 113: 9704-9709.

19

20 Meinshausen M, Smith SJ, Calvin K, Daniel JS, Kainuma MLT, Lamarque JF, *et al*. 2011.

21 The RCP greenhouse gas concentrations and their extensions from 1765 to 2300. *Climatic*

22 *Change*, 109(1-2): 213.

23

1 Morris JL, Puttick MN, Clark JW, Edwards D, Kenrick P, Pressel S, *et al*. 2018. The

2 timescale of early land plant evolution. *Proceedings of the National Academy of Sciences*,

3 115(10): 2274-2283.

4

5 Nicolson TH. 1967. Vesicular-arbuscular mycorrhiza – a universal plant symbiosis. *Science*

6 *Progress*, (1933-): 561-581.

7

8 Orchard S, Hilton S, Bending GD, Dickie IA, Standish RJ, Gleeson DB, *et al*. 2017a. Fine

9 endophytes (*Glomus tenue*) are related to Mucoromycotina, not Glomeromycota. *New*

10 *Phytologist*, 213(2): 481-486.

11

12 Orchard, S, Standish RJ, Dickie IA, Renton M, Walker C, Moot D, *et al.* 2017b. Fine root

13 endophytes under scrutiny: a review of the literature on arbuscular-producing fungi recently

14 suggested to belong to the Mucoromycotina. *Mycorrhiza*, 27(7): 619-638.

15

16 Orchard S, Standish RJ, Nicol D, Dickie IA, Ryan MH. 2017c. Sample storage conditions

17 alter colonisation structures of arbuscular mycorrhizal fungi and, particularly fine root

18 endophyte. *Plant and Soil*, 412(1-2): 35-42.

19

20 Pirosynski KA, Malloch DW. 1975. The origin of land plants: a matter of mycoheterotrophy.

21 *Biosystems*, 6: 153-164.

22

23 Radhakrishnan GV, Keller J, Rich MK, Vernié T, Mbadinga DLM, Vigneron N, *et al*. 2020.

24 An Ancestral signalling pathway is conserved in intracellular symbioses-forming plant

25 lineages. *Nature Plants*, 6(3): 280-289.

1

2 Rasmussen KK, Lawesson JE. 2002. *Lycopodiella inundata* in British plant communities and

3 reasons for its decline. *Watsonia*, 24(1): 45-56.

4

5 Read DJ. 1991. Mycorrhizas in ecosystems. *Experientia*, 47(4): 376-391.

6

7 Redecker D. 2000. Specific PCR primers to identify arbuscular mycorrhizal fungi with

8 colonised roots. *Mycorrhiza*, 10(2): 73-80.

9

10 Rillig MC. 2004. Arbuscular mycorrhizae and terrestrial ecosystem processes. *Ecology

11 Letters*, 7(8): 740-754.

12

13 Rimington WR, Pressel S, Duckett JG, Bidartondo MI. 2015. Fungal associations of basal

14 vascular plants: reopening a closed book? *The New Phytologist*, 205(4): 1394.

15

16 Rimington WR, Duckett JG, Field KJ, Bidartondo M, Pressel S. 2020. The distribution and

17 evolution of fungal symbioses in ancient lineages of land plants. *Mycorrhiza*, 1-27.

18

19 Smith SE, Smith FA. 2011. Roles of arbuscular mycorrhizas in plant nutrition and growth:

20 new paradigms from cellular to ecosystem scales. *Annual Review of Plant Biology*, 62: 227-

21 250.

22

23 Spatafora JW, Aime MC, Grigoriev IV, Martin F, Stajich JE, Blackwell M. The fungal tree of

24 life: from molecular systematics to genome-scale phylogenies. *Microbiology Spectrum*, 5(5):

25 1-32.

1

2 Strullu-Derrien C, Kenrick P, Pressel S, Duckett JG, Rioult JP, Strullu DG. 2014. Fungal

3 associaions in *Horneophyton ligneri* from the Rynie Chert (c. 407 million years old) closely

4 resemble those in extant lower land plants: novel insights into ancestral plant-fungus

5 symbioses. *New Phytologist*, 203(3): 964-979.

6

7 Thirkell TJ, Pastok D, Field KJ. 2019. Carbon for nutrient exchange between arbuscular

8 mycorrhizal fungi and wheat varies according to cultivar and changes in atmospheric carbon

9 dioxide concentration. *Global Change Biology*, 26(3), 1725-1738.

10

11 van der Heijden MG, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, *et*

12 *al*. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability

13 and productivity. *Nature*, 396(6706): 69-72.

14

15 Van der Heijden MG, Martin FM, Selosse MA, Sanders IR. 2015. mycorrhizal ecology and

16 evolution: the past, the present, and the future. *New Phytologist*, 205(4): 1406-1423.

17 Wijayawardene NN, Pawłowska J, Letcher PM, Kirk PM, Humber RA, Schüßler A, *et al*.

18 2018. Notes for genera: basal clades of Fungi (including Aphelidiomycota,

19 Basidiobolomycota, Blastocladiomycota, Calcarisporiellomycota, Caulochytriomycota,

20 Chytridiomycota, Entomophthoromycota, Glomeromycota, Kickxellomycota,

21 Monoblepharomycota, Mortierellomycota, Mucoromycota, Neocallimastigomycota,

22 Olpidiomycota, Rozellomycota and Zoopagomycota). *Fungal diversity*, 92(1): 43-129.

23

1   Wipf D, Krajinski F, van Tuinen D, Recorbet G, Courty PE. 2019. Trading on the arbuscular

2   mycorrhiza market: from arbuscules to common mycorrhizal networks. *New Phytologist*,

3   223(3): 1127-1142.

4

5   Zheng C, Ji B, Zhang J, Zhang F, Bever JD. 2015. Shading decreases plant carbon

6   preferential allocation towards the most beneficial mycorrhizal mutualist. *New Phytologist*,

7   205(1): 361-368.

8

9   **Figure Captions:**

10 **Figure 1**. Schematic diagram and photograph of radio and stable isotope tracing system in a

11 lycophyte. (a) Microcosm containing static core in which ^{33}P and ^{15}N was injected; (b)

12 Microcosm containing rotated core in which ^{33}P and ^{15}N was injected; (c) Microcosm set-up

13 enclosed in an air-tight container in which ^{14}C isotope tracing was conducted and (d)

14 Photograph of *Lycopodiella inundata*-MFRE radio- and stable isotope tracing experimental

15 system.

16

17 **Figure 2**. Carbon exchange between *Lycopdiella inundata* and Mucoromycotina fine root

18 endophyte fungi (MFRE). (a) % allocation of plant-fixed C to MFRE; (b) Total plant-fixed C

19 transferred to Mucorocomycotina FRE by *L. inundata*. All experiments conducted at a[CO_2]

20 of 800 p.p.m. (grey bars) and 440 p.p.m. (white bars). All bars in each panel represent the

21 difference in isotopes between the static and rotated cores inserted into each microcosm. In

22 all panels, error bars denote SEM. In panels (a)-(b) $n = 24$ for both 800 p.p.m and 440 p.p.m

23 a[CO_2].

24

1   **Figure 3.** Fungal acquired nutrients by Mucoromycotina fine root endophyte (MFRE) fungi

2   and total shoot nutrients in above ground plant tissue of *L. inundata*. (a) Total plant tissue ^{33}P

3   content (ng) and (b) total plant tissue ^{15}N content (ng) in *L. inundata* tissue; (c) tissue

4   concentration (ng g^{-1}) of fungal-acquired ^{33}P and (d) tissue concentration of ^{15}N (ng g^{-1}) in

5   shoot tissue of *L. inundata*; (e) Total shoot P content (mg) equating to both plant and fungal

6   acquired P and (f) total shoot N content (mg) equating to both plant and fungal acquired N in

7   shoots of *L. inundata*. All experiments conducted at a[CO_2] of 800 p.p.m. (grey bars) and 440

8   p.p.m. (white bars). All bars in each panel (a-d) represent the difference in isotopes between

9   the static and rotated cores inserted into each microcosm. In all panels, error bars denote

10   SEM. In panels (a)-(d) $n = 12$ and panels (e)-(f) $n = 24$ for both 800 p.p.m and 440 p.p.m

11   a[CO_2].

12

13   **Figure 4**. Experimental *Lycopodiella* roots colonised by MFRE. a) Light micrographs of

14   trypan blue stained roots showing fine branching hyphae with intercalary and terminal small

15   vesicles (see insert). b) Scanning electron micrograph of transverse section of root showing

16   abundant branching hyphae (*) and vesicles (arrowed). SEMs at this magnification (x150)

17   were used to quantify % colonisation of roots of experimental plants grown under the two

18   contrasting atmospheric [CO_2] regimes, shown here in a plant grown under the elevated

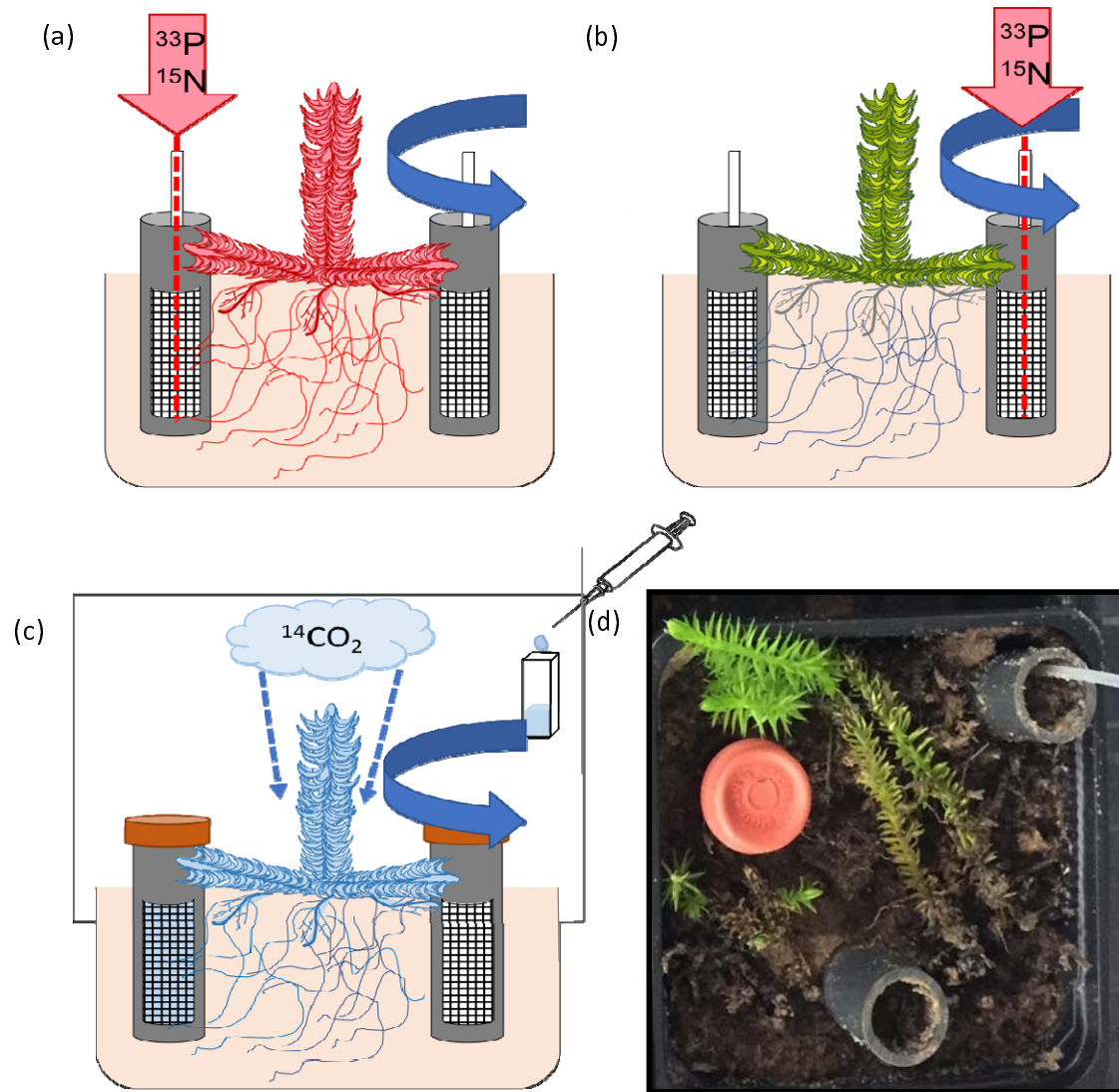19   a[CO_2] of 800 p.p.m. Scale bars: a) (and (a) insert) 50 µm; b) 100 µm.

Figure 1. Schematic diagram and photograph of radio and stable isotope tracing system in a lycophyte. (a) Microcosm containing static core in which ^{33}P and ^{15}N was injected; (b) Microcosm containing rotated core in which ^{33}P and ^{15}N was injected; (c) Microcosm set-up enclosed in an air-tight container in which ^{14}C isotope tracing was conducted and (d) Photograph of *Lycopodiella inundata*-MFRE radio- and stable isotope tracing experimental
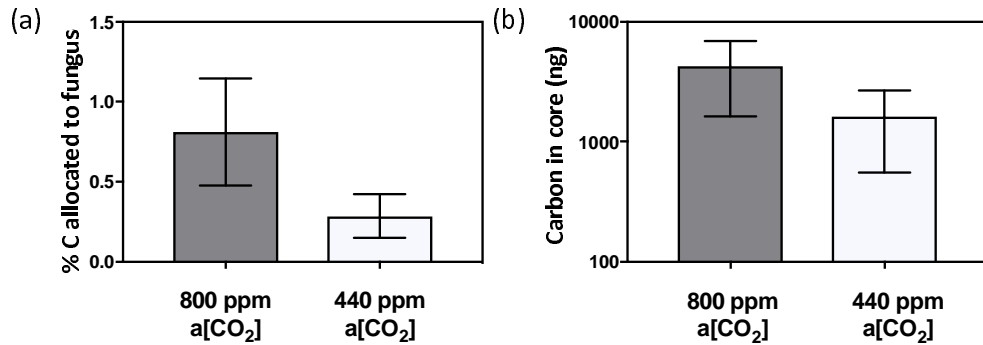
Figure 2. Carbon exchange between *Lycopdiella inundata* and Mucoromycotina fine root endophyte fungi (MFRE). (a) % allocation of plant-fixed C to MFRE; (b) Total plant-fixed C transferred to Mucorocomycotina FRE by *L. inundata*. All experiments conducted at a[CO_2] of 800 p.p.m. (grey bars) and 440 p.p.m. (white bars). All bars in each panel represent the difference in isotopes between the static and rotated cores inserted into each microcosm. In all panels, error bars denote SEM. In panels (a)-(b) n = 24 for both 800 p.p.m and 440 p.p.m a[CO_2].
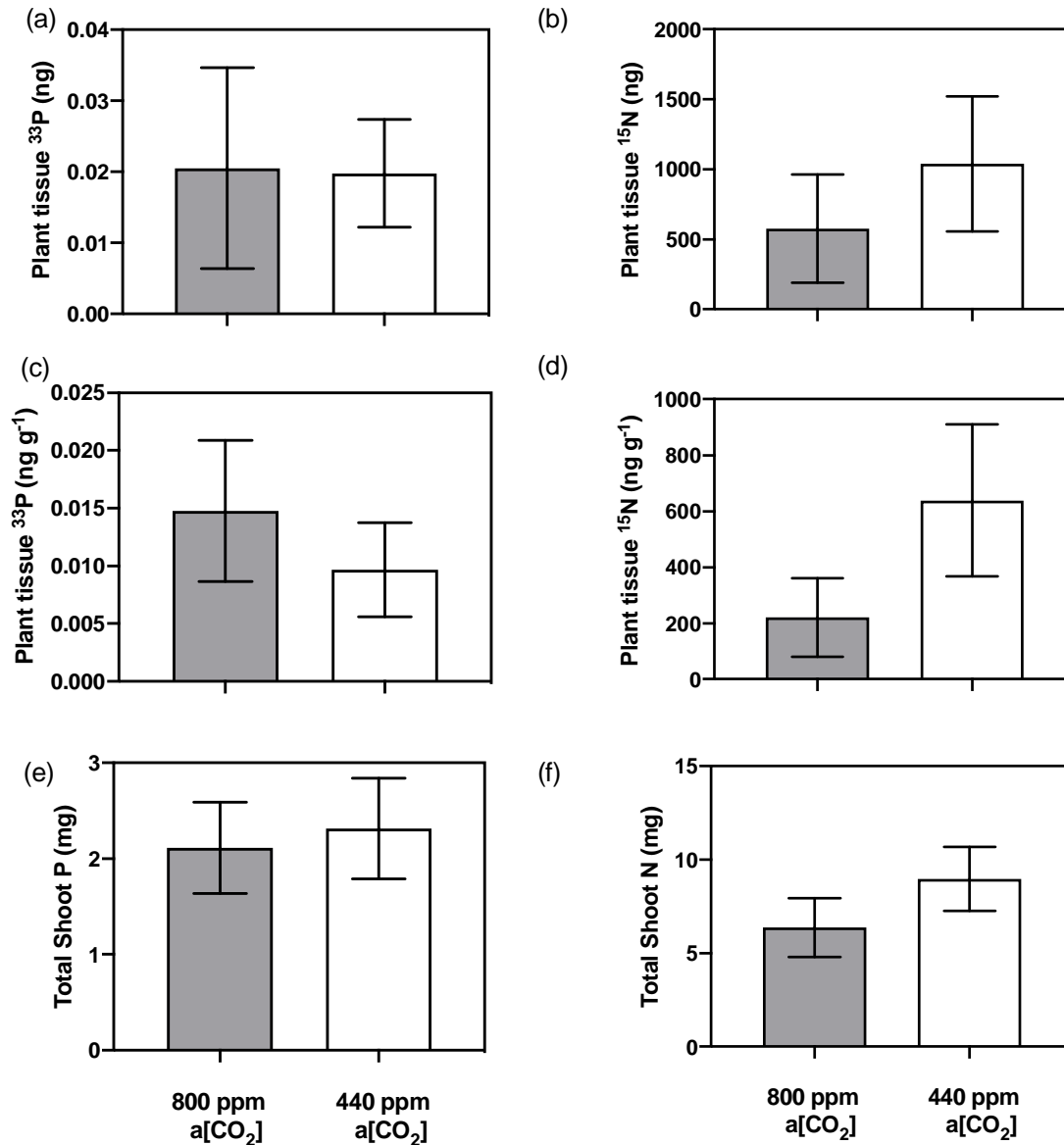
Figure 3. Fungal acquired nutrients by Mucoromycotina fine root endophyte (MFRE) fungi and total shoot nutrients in above ground plant tissue of *L. inundata*. (a) Total plant tissue ^{33}P content (ng) and (b) total plant tissue ^{15}N content (ng) in *L. inundata* tissue; (c) tissue concentration (ng g^{-1}) of fungal-acquired ^{33}P and (d) tissue concentration of ^{15}N (ng g^{-1}) in shoot tissue of *L. inundata*; (e) Total shoot P content (mg) equating to both plant and fungal acquired P and (f) total shoot N content (mg) equating to both plant and fungal acquired N in shoots of *L. inundata*. All experiments conducted at a[CO_2] of 800 p.p.m. (grey bars) and 440 p.p.m. (white bars). All bars in each panel (a-d) represent the difference in isotopes between the static and rotated cores inserted into each microcosm. In all panels, error bars denote SEM. In panels (a)-(d) *n* = 12 and panels (e)-(f) *n* = 24 for both 800 p.p.m and 440 p.p.m a[CO_2].
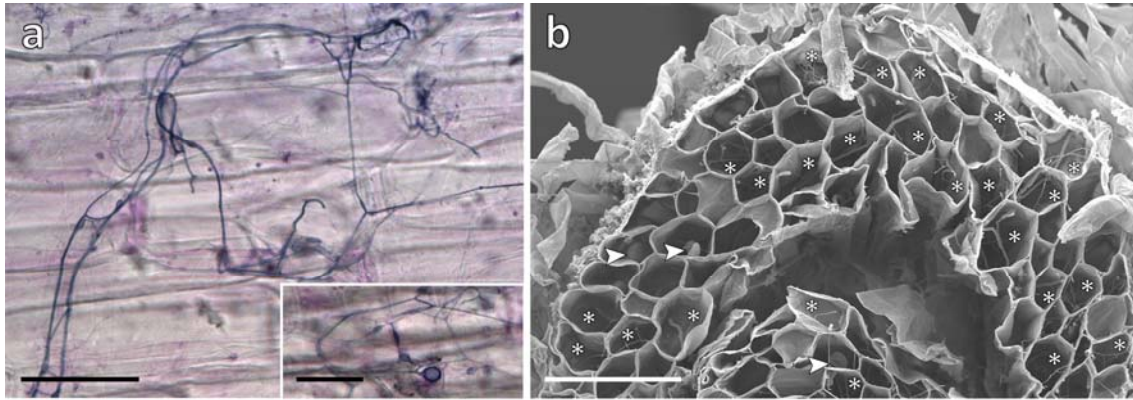
Figure 4. Experimental *Lycopodiella* roots colonised by MFRE. a) Light micrographs of trypan blue stained roots showing fine branching hyphae with intercalary and terminal small vesicles (see insert). b) Scanning electron micrograph of transverse section of root showing abundant branching hyphae (*) and vesicles (arrowed). SEMs at this magnification (x150) were used to quantify % colonisation of roots of experimental plants grown under the two contrasting atmospheric [CO_2] regimes, shown here in a plant grown under the elevated a[CO_2] of 800 p.p.m. Scale bars: a) (and (a) insert) 50 μm; b) 100 μm.