Tap into non-symbiotic carbon? Exogenous myristate fuels the growth of symbiotic arbuscular mycorrhizal fungi but disrupts their carbon–phosphorus exchange with host plants

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Abstract

Arbuscular mycorrhizal fungi (AMF) facilitate plant uptake of mineral nutrients, particularly phosphorus, and draw organic carbon from the plant. The ability of symbiotic AMF to utilize external non-symbiotic carbon sources remains unclear, complicating our comprehension of their ecosystem functions. Here we examine the direct absorption of exogenous $^{13}C_1$-labeled myristate by symbiotic AMF and their growth responses using an in-vitro dual culture system. We also investigated the impact of exogenous myristate on the carbon–phosphorus exchange between AMF and two different host plants in a greenhouse experiment, employing both stable isotope labeling ($^{13}CO_2$) and profiling of P transporter genes. Our results indicate that the extraradical hyphae of symbiotic AMF are capable of absorbing external myristate and transporting it (or its metabolic products) to intraradical structures. Myristate serves a dual function as a carbon source and signaling molecule, leading to increased intraradical and extraradical fungal biomasses, with RNA-Seq data indicating a suppressed mycorrhizal defense response as a potential mechanism. Intriguingly, exogenous myristate generally reduced the mycorrhizal phosphorus benefits for both alfalfa and rice, and decreased their carbon allocation to symbiotic AMF, likely by interfering with their normal trading mechanisms. These findings provide novel insights into the ecosystem functions and ecological applications of AMF.

Keywords： arbuscular mycorrhizal fungi (AMF), alfalfa (*Medicago sativa*), exogenous myristate, rice (*Oryza sativa*), carbon–phosphorus exchange, carbon source, signaling molecule

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are among the most widely distributed soil microorganisms, forming symbiotic relationships with the roots of 70–90% of terrestrial vascular plants (Shi *et al.*, 2023). In this symbiotic association, known as arbuscular mycorrhiza (AM), AMF develop an extensive extraradical hyphal network in the soil, facilitating nutrient uptake for host plants, particularly phosphorus (P) acquisition, and enhancing plant resilience to both biotic and abiotic stressors (Smith & Read, 2008). In return, host plants allocate 4−20% of their photosynthetically fixed carbon (C) to their fungal partners in the form of sugars and, more importantly, fatty acids (Helber *et al*., 2011; Jiang *et al.*, 2017). This C−P nutrient exchange, which serves as the cornerstone of the AM symbiosis (Smith & Read, 2008), not only plays a pivotal role in the biogeochemical cycling of C and P elements but also acts as a key mechanism through which AMF influences plant community structure and productivity (Johnson, 2010; Wipf *et al.*, 2019).

As one of the most ancient symbiotic relationships, AM have undergone extensive evolutionary processes (Foster *et al.*, 2006; Rich *et al.*, 2021), with their establishment and development intricately regulated by a set of symbiotic genes and signaling molecules based on biotic and abiotic conditions (Maclean *et al.*, 2017; Shi *et al.*, 2021). For instance, in AM plants, the two pathways for P uptake (i.e., the direct P pathway (via root hair and epidermis) and mycorrhizal P pathway (via AMF hyphae)) are coordinately regulated by the same phosphate sensing-centered pathway in plants (Shi *et al.*, 2021; Das *et al.*, 2022). Under P deficiency conditions, host plants synthesize and release strigolactones into the soil, activating the mycorrhizal P pathway by stimulating the elongation and branching of AMF hyphae (Mori *et al.*, 2016; Yu *et al.*, 2022). Additionally, the extent of AMF

65 colonization and AM benefits also largely rely on the host plant species, with some being
66 "responsive plants" that exhibit positive growth responses (e.g., alfalfa, maize) and others
67 being "nonresponsive plants" that show minimal or no growth enhancement (like rice,
68 wheat) (Hata *et al.*, 2010, Cope *et al.*, 2022).

69 AMF are thought to be obligate symbionts that depend on living host plants to
70 complete their life cycle by producing daughter spores (Smith & Read, 2008), which
71 hinders basic research on AM and their applications in agriculture and other fields. Thus
72 far, achieving a pure (plant-independent) culture of asymbiotic AMF has not been
73 successful, largely due to their dependence on plant-derived C (Bravo *et al.*, 2017;
74 Luginbuehl *et al.*, 2017; Kameoka *et al.*, 2019). Previous genomic and transcriptomic
75 analyses have revealed that AMF lack genes for their own biosynthesis of long-chain fatty
76 acids, and thereafter several research groups have demonstrated that fatty lipids are
77 transferred from host plants to AMF as the primary C source (Bravo *et al.*, 2017; Jiang *et*
78 *al.*, 2017; Keymer *et al.*, 2017; Luginbuehl *et al.*, 2017). Nevertheless, the molecular
79 mechanism underlying the transportation and absorption of plant-derived fatty acids to
80 fungi in AM symbiosis remains unclear. In plants, AMF colonization triggers the
81 expression of lipid biosynthesis and transport genes, including *DIS*, *FatM*, *RAM2*, and
82 *STR2* (Gutjahr *et al.*, 2012; Bravo *et al.*, 2017; Keymer *et al.*, 2017; Luginbuehl *et al.*,
83 2017), with lipid transport to the AM symbiotic interface relying on the periarbuscular
84 membrane-located transporters STR and STR2 (Zhang *et al.*, 2010; Gutjahr *et al.*, 2012)
85 and fungal uptake of plant-derived lipids via RiFAT1 in the model AMF species
86 *Rhizophagus irregularis* (Brands & Dörmann, 2022).

87 Fatty acids and their derivatives, crucial structural elements and energy materials of

88 plant, animal, and microbial cells, are commonly found in soil, especially in the rhizosphere,
89 where plant-derived fatty acids are accessible through root exudates and rhizodeposition
90 (Swenson *et al.*, 2018; Dai *et al.*, 2022; Liu *et al.*, 2024). Building on the discovery that
91 AMF receive fatty acids from their hosts during symbiosis, the utilization of fatty acids in
92 the cultivation of asymbiotic AMF has received considerable attention. Kameoka *et al.*
93 (2019) illustrated that under asymbiotic conditions exogenous palmitoleic acid (C16:1 fatty
94 acid) and several other fatty acids stimulated the elongation and branching of *R. irregularis*
95 hyphae, leading to the formation of morphologically and functionally incomplete spores.
96 Moreover, Sugiura *et al.* (2020) showed that exogenous myristate (tetradecanoic acid,
97 C14:0 fatty acid), a prevalent organic acid in various plant root exudates (Fernández-Piñar
98 *et al.*, 2012; Luo *et al.*, 2014; Li *et al.*, 2017; Liu *et al.*, 2024) and in soil substrates
99 (Swenson *et al.*, 2018; Dai *et al.*, 2022), can be used as a C and energy source for the
100 asymbiotic growth of *R. irregularis,* promoting spore germination, hyphae growth,
101 formation of branched absorbing structures, and the production of morphologically
102 incomplete fertile spores. Subsequent studies revealed that the combined use of plant
103 hormones (strigolactone and methyl jasmonate) with myristate improved the efficiency of
104 myristate as a C and energy source in asymbiotic *R. clarus*, leading to the generation of
105 numerous fertile spores that were smaller than those generated under symbiotic conditions
106 (Sugiura *et al.*, 2020; Tanaka *et al.*, 2022). These discoveries have significantly progressed
107 the pure culture of AMF and, equally importantly, identified myristate as the first external
108 C source accessible to asymbiotic AMF, offering outstanding opportunities to understand
109 and leverage the ecological functions of AMF (Rillig *et al.*, 2020).
110 However, the extent to which the physiological and ecological responses of AMF to

111 external myristate during the asymbiotic phase mirror those in symbiotic growth, the
112 typical state of AMF in nature, remains unclear. A recent investigation reported that
113 although exogenous myristate does not affect the extraradical biomass of symbiotic AMF,
114 it enhances AMF colonization by stimulating the growth and development of extraradical
115 hyphae (Liu *et al.*, 2023). To date, the effects of exogenous fatty acid on AM symbiosis
116 and their underlying mechanisms are still largely unexplored. Specifically, the ability of
117 symbiotic AMF to use myristate as an external non-symbiotic C source in the presence of
118 plant-derived C is yet to be determined. Given its prevalent presence in root exudates and
119 soil, this is a question vital for understanding the ecosystem roles and functions of AMF
120 (Rillig *et al.*, 2020). For sugars, the other host-derived C source for AM, symbiotic AMF
121 seems unable to additionally absorb them through extraradical hyphae (Bücking *et al.*,
122 2008; Helber *et al.*, 2011; Sugiura *et al.*, 2020; but see Pfeffer *et al.*, 1999).

123 This study aims to investigate the ability of symbiotic AMF to utilize external non-
124 symbiotic myristate and the impact of exogenous myristate on the symbiotic relationships.
125 Specifically, we sought to determine: (i) whether symbiotic AMF can utilize myristate as
126 an external non-symbiotic C source, and if so, (ii) how this additional non-symbiotic C
127 source influences the symbiotic C allocation from plants to AMF, and (iii) the P transfer
128 from AMF to host plants. To address these questions, we performed a $^{13}C_1$-labeled
129 myristate tracing experiment to examine the direct uptake of exogenous myristate by AMF
130 in symbiosis with plant roots using a vitro dual culture system. Additionally, we assessed
131 the impact of various myristate concentrations on spore germination and the intra- and
132 extraradical growth of AMF associated with carrot hairy roots. Furthermore, a greenhouse
133 experiment, coupled with ^{13}C-Phospholipid fatty acid- isotope ratio mass spectrometry

134 (^{13}C-PLFA-IRM) technology, and physiological and transcriptome analyses, was
135 conducted further investigate how exogenous myristate influences the symbiotic
136 interaction between AMF and two host plants with different mycorrhizal responsiveness
137 (*Medicago sativa* and *Oryza sativa*). Our study provides, to our knowledge, the first solid
138 evidence showing that symbiotic AMF can tap into non-symbiotic C sources beyond those
139 allocated directly by their host plant. Additionally, our results suggest that under symbiotic
140 conditions, exogenous myristate functions as both a C source and a signaling molecule for
141 AMF, and, unexpectedly, disrupts C–P exchange, the cornerstone of AM symbiosis.
142

143 **MATERIALS AND METHODS**

144 **Experimental design**

145 Two interconnected and complementary experiments were conducted. In Exp. 1, a
146 $^{13}C_1$-labeled myristate ($^{13}C_1$-Myr) tracing experiment was conducted to verify if the
147 exogenous myristate can be directly taken up by AMF extraradical hyphae using a two-
148 compartment AMF-carrot hairy root co-cultivation system. Additionally, the effects of
149 exogenous myristate at various application concentrations (0.1, 0.5, 1, 5, 10 mM) on the
150 spore germination and growth of AMF intraradical and extraradical structures associated
151 with carrot hairy roots were assessed in an *in vitro* dual culture system.

152 In Exp. 2, a pot experiment in soil substrate was conducted to investigate the
153 mechanisms of the effect of exogenous myristate on the symbiotic relationship between
154 AMF and two typical host species (*Medicago sativa* and *Oryza sativa*) under two P levels.
155 The C delivery from plants to AMF was evaluated using a ^{13}C-Phospholipid fatty acid-
156 isotope ratio mass spectrometry technology. The P transfer from AMF to plants was

157 evaluated by analyzing the expression levels of the mycorrhizal P pathway marker genes
158 and plant P concentration. Additionally, the root transcriptome was analyzed to assess the
159 general effects of exogenous myristate on AMF-induced transcriptional reprogramming.

160 **AMF-hairy root co-cultivation system and quantification of AMF parameters**

161 In Exp. 1, 4 cm-long sterile carrot hairy root segments were cultivated in Modified
162 Strullu-Romand (MSR) medium with or without *R. irregularis* inoculations (200 sterilized
163 spores per plate). Potassium myristate solution (0, 0.1, 0.5, 1, 5, 10 mM, 200 μl) was
164 applied to the plates weekly. The AMF spore germination rate, hyphal branching, and
165 hyphal length were measured 4 days post AMF inoculation using ImageJ (Schneider *et al.*,
166 2012). The spore density was dynamically monitored every 2 weeks since 8 weeks post
167 AMF inoculation. The carrot hairy roots and culture medium were harvested at 14 weeks
168 post AMF inoculation. The roots and solid culture medium were separated using sterile
169 tweezers, and the biomass of carrot hairy roots was measured. The medium was dissolved
170 in 10 mM sodium citrate, and extraradical hyphae and spores were collected through
171 filtration using a 250 μm and 37 μm nylon mesh, respectively, assisted by manual selection
172 using tweezers under a stereoscopic microscope. The extraradical biomass of AMF was
173 then quantified after oven-dried at 80 °C for 48 h.

174 For assessment of AMF colonization intensity, 100 root segments (approximately 1
175 cm in length) were treated with a 10% KOH solution at 95°C for 10 min, decolorized in a
176 30% H_2O_2 solution at 25°C for 3 min, softened in a 1% HCl solution at 25°C for 3 min,
177 and then stained with 0.05% Trypan Blue at 95°C for 15 min. The evenly arranged root
178 segments were scored under a 400× magnification with a light microscope (Nikon Eclipse
179 80i, Japan). The colonization rate was determined using the gridline intersect method

180 (Giovannetti *et al.*, 1980).

181 **$^{13}C_1$-labeled myristate ($^{13}C_1$-Myr) tracing experiment**

182 A two-compartment AMF-hairy root co-cultivation system was utilized, with 25 ml
183 of solid MSR added in one compartment (hyphal compartment, HC) and 10 ml of solid
184 MSR media in the other (root compartment, RC), situated near the physical root barrier to
185 form a slope. This arrangement allowed fungal hyphae (but not roots) to traverse the
186 physical barrier from the RC to grow into the HC. A 4 cm-long sterile carrot hairy root
187 segments were co-cultivated with *R. irregularis* spores (200 spores per plate) in the RC for
188 ten weeks, after which dense AMF hyphae were noted in the HC. Subsequently, 200 µl of
189 0.1 mM Myr with 98% $^{13}C_1$ labeling or Myr without ^{13}C labeling was added weekly to the
190 HC. Four weeks later, AMF extraradical hyphae from both the HC and RC were collected
191 separately by dissolving the media in 10 mM sodium citrate and filtering through a 37 µm
192 nylon mesh. The carrot hairy roots in the root compartment were also harvested.

193 To determine ^{13}C, freeze-dried root and AMF hyphal materials (1.0 mg) were sealed
194 in tin capsules and analyzed using continuous-flow isotope ratio mass spectrometry
195 (IRMS). The ^{13}C:^{12}C ratio of CO_2 from the combusted samples was determined, and
196 expressed as atom % of ^{13}C, using a sucrose standard calibrated against the Pee Dee
197 Belemnite standard. The ^{13}C enrichments in plant root and fungal materials were calculated
198 by subtracting the background ^{13}C levels, which were determined from five non-labeled
199 controls (Bao et al., 2019).

200 **Establishment and maintenance of pot experiment (Exp. 2) and measurement of plant**
201 **physiological parameters**

202 In the greenhouse experiment (Exp. 2), *Medicago sativa* and *Oryza sativa* seeds were

203 germinated for 14 days in sterilized sand, and then transplanted into the corresponding
204 substrate consist of vermiculite, perlite, and sand (3:1:1, v/v/v). To mitigate potential
205 biomass differences between the two plant species, 3 *Medicago* seedlings or 1 *Oryza*
206 seedling were planted in each pot, containing either 300 or 1000 mL of substrate,
207 respectively, with or without *R. irregularis* spores (10 spores/mL of substrate). The plants
208 were grown in a greenhouse under a long-day photoperiod (16 h of light/8 h of dark), with
209 a temperature ranging from 20–30°C. For P treatments, 10 ml Hoagland nutrient solution
210 with either relatively low or high P level (*Medicago*: 25 (low) or 100 μM (high) KH_2PO_4,
211 *Oryza*: 100 (low) or 400 μM (high) KH_2PO_4) was supplied twice a week (Liu *et al.*, 2020;
212 Shi *et al.*, 2021). For myristate application, 5 ml of potassium myristate solution (0.1 mM)
213 or ddH_2O (Mock) was applied near the plant roots twice a week.

214 The plants were harvested at eight weeks post inoculation. Plants shoot and root parts
215 were washed, oven dried (60°C for 7 d) and weighted. Plant P concentration was
216 determined using a molybdenum antimony colorimetric method.

217 **RNA extraction and Illumina sequencing**

218 In Exp. 2, alfalfa and rice roots inoculated with AMF, both with and without myristate
219 application at two P levels, were sequenced, using three biological replicates (24 samples
220 in total). Total RNA was extracted from 100 mg of root tissue using the TRIzol R Plus RNA
221 purification kit (Takara, Dalian, China) following the manufacturer's instructions. Library
222 preparation was carried out with the NebNext Ultra RNA Library Prep Kit (New England
223 Biolabs, Ipswich, MA, USA). Qualified libraries were sequenced on the Illumina HiSeq
224 4000 platform with 150-bp paired-end reads. Fastp was used for basic statistics and quality
225 filtering of raw data. Hisat2 was utilized to map the sequencing results to the rice reference

226 genome (IRGSP-1.0) or alfalfa reference genome (zhongmuNO.1, Shen *et al.*, 2020).
227 Salmon quant was used for gene expression quantification analysis. The RNA-seq data
228 have been deposited in the Sequence Read Archive at the National Center for
229 Biotechnology Information under the BioProject ID numbers PRJNA1083641.

230 **Quantitative real-time PCR (qRT-PCR)**

231 The RNA was reverse-transcripted to cDNA using the QuantiTect Reverse
232 Transcription Kit (Qiagen, Valencia, CA). The qRT-PCR was performed in a
233 QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, CA, USA) with the SYBR
234 ROX Mastermix Kit (Qiagen, Valencia, CA) following the manufacturer's instructions. All
235 reactions were performed with three technical replicates. The relative expression levels of
236 genes involved in C delivery from plants to AMF (*MsSTR2*, *OsSTR2* and *RiFAT*) and in the
237 mycorrhizal P pathway (*MsPT8*, *OsPT11* and *RiPT*) were analyzed, using the alfalfa *actin*
238 *2* gene (*MsACT2*, Li *et al.*, 2018), rice cycle 2 gene (*Oscyc2*, Yang *et al.*, 2012) and *R.*
239 *irregularis* elongation factor-1 beta gene (*RiEF-1β*, Corrêa *et al.*, 2014) as reference genes.
240 Details of all the qRT-PCR primers used are available in the Supporting Information
241 (Table S1).

242 **^{13}C labeling of plants, lipid extraction and measurement of signature fatty acids**

243 The ^{13}C labeling procedure were conducted following our previous study with minor
244 modifications (Bao *et al.*, 2019). Five days prior to harvest, alfalfa and rice plants
245 (randomly selected five replicates) were placed in a sealed acrylic chamber with $^{13}CO_2$ and
246 incubated for 4 hours each.

247 Extraction and analysis of signature fatty acids followed the method described by
248 Olsson *et al.* (2005), with slight modifications according to Bao et al. (2019). The

249 identification and quantification of fatty acid methyl esters were performed relative to an
250 added internal standard (fatty acid methyl ester 19:0). NLFA 16:1ω5 was used as a marker
251 for AMF in roots (Olsson & Lekberg, 2022).

252 **Quantification of ^{13}C enrichment and excess ^{13}C in fatty acids**

253 The ^{13}C enrichment in root and fatty acid methyl esters was assessed using IRMS
254 coupled to a Trace GC Ultra gas chromatograph via the ConFlow IV interface (Thermo
255 Fisher Scientific, Bremen, Germany), following our previous study (Bao *et al.*, 2019). The
256 chromatographic conditions were identical to those specified for lipid analysis, using
257 helium (He) as the carrier gas. The effluent from the capillary column was directed through
258 an Al tube filled with CuO wires at 860°C. The $\delta^{13}C$ values were calibrated against the
259 Vienna Pee Dee Belemnite (V-PDB) with reference to the CO_2 standard (RM 8542 (IAEA-
260 CH-6, sucrose)) and adjusted for the additional non-labeled methyl group introduced
261 during methanolysis. The ^{13}C enrichment was calculated by subtracting the background
262 ^{13}C levels determined from five non-labeled controls.

263 **Statistical analysis**

264 Mycorrhizal response (MR) was calculated for each treatment according to Corrêa *et*
265 *al.* (2014): MR(%) = (AM–NM) / NM * 100%, where NM represents the mean of the non-
266 mycorrhizal plants, and AM represents the sample values of the mycorrhizal plants.

267 Multiple comparisons among different treatments were conducted using one-way
268 analysis of variance (ANOVA) followed by post hoc Tukey's tests (equal variances). A
269 univariate two-way ANOVA, followed by a post hoc Tukey test, was utilized to examine
270 the effects of myristate, P level, and their interactions on AMF colonization intensity and
271 plant P concentration. The significant differences between AM and NM groups were

272 compared using Student's t test (for normally distributed data). For RNA-seq data, the
273 DESeq2 R package was employed to perform differentially expressed gene (DEG) analysis
274 between plants with (Myr) and without (Mock) myristate application (Love *et al.*, 2014).
275 The *p* values obtained were adjusted using the Benjamini-Hochberg correction to control
276 false discovery rate. Genes (transcripts) with an adjusted *p* value < 0.05 and $|\log_2(\text{fold}$
277 $\text{change})| > 1$ identified by DESeq2 were considered as differentially expressed. The
278 clusterProfiler method was applied for Gene Ontology (GO) enrichment analysis of DEGs
279 (Wu *et al.*, 2021), with GO terms showing an adjusted *p* value < 0.05 deemed significantly
280 enriched.
281
282 **RESULTS**
283 **Tracing of ^{13}C in AMF extraradical hyphae and carrot hairy root in a two-**
284 **compartment AMF-carrot hairy root co-cultivation system**
285 In the two-compartment AMF-carrot hairy root co-cultivation system, ^{13}C levels in
286 AMF hyphae from the HC (1.1635 ± 0.0329, n = 5) and the RC (1.1026 ± 0.0039, n = 3)
287 in the $^{13}C_1$-Myr treatment were significantly elevated compared to those in AMF hyphae
288 (HC: 1.0923 ± 0.0001, n = 5; RC: 1.0923 ± 0.0001, n = 3) from the non-labeled systems
289 ($P < 0.05$), demonstrating that under symbiotic conditions AMF extraradical hyphae are
290 capable of directly absorbing exogenous myristate Additionally, there was a significant
291 enrichment of ^{13}C in hairy root in the $^{13}C_1$-Myr treatment (1.0927 ± 0.0006, n = 5) after
292 subtracting the natural background (non-labeled) ^{13}C level in roots (1.0910 ± 0.0001, n=5)
293 ($P < 0.01$, Fig. 2a), and this enrichment was significantly lower than that observed in AMF
294 hyphae ($P < 0.01$). These results suggested that the extraradical hyphae absorbed myristate

295 and transported it (or its metabolic derivatives) into AMF intraradical structures during
296 symbiosis.

297 **Effects of exogenous myristate on AMF colonization, mycorrhizal growth response**
298 **(MGR) and extraradical structure development in AMF-carrot hairy root co-**
299 **cultivation systems**

300 In Exp. 1, exogenous myristate significantly promoted AMF spore germination ($P <$
301 0.05, Fig. 1c, g), and enhanced hyphal branching and length ($P < 0.05$, Fig. 1d, e) before
302 physical contact between AMF hyphae and hairy roots at 4-days post AMF inoculation.
303 When no exogenous myristate was applied, the extraradical hyphae tips oriented towards
304 the host roots (tropism growth); however, in myristate treated medium, extraradical hyphae
305 of AMF showed no tropism growth (Fig. 1h, i). Notably, discernible fungal branched
306 absorbing structures was exclusively observed in myristate-treated medium from 14 days
307 post-AMF inoculation (Fig. 1i). These observations indicate that myristate acts as signaling
308 molecule to AMF.

309 Morphological examinations showed that exogenous myristate significantly enhanced
310 the colonization intensities of hyphae, vesicles and arbuscules in carrot hairy roots across
311 various application levels ($P < 0.05$, Fig. 2a), with its promotional effects generally
312 increasing at higher myristate concentrations. Exogenous myristate also significantly
313 increased the extraradical biomass of AMF (hyphae and spore) at various application
314 concentrations ($P < 0.05$, Fig. 1b). Conversely, the mycorrhizal growth response (MGR)
315 of myristate-treated carrot hairy roots was significantly lower than that of the control
316 (mock), and it typically declined with rising myristate concentrations ($P < 0.05$, Fig. 1c).

317 Meanwhile, exogenous myristate significantly increased the density of AMF spores

318 from eight weeks post-AMF inoculation ($P < 0.05$, Fig. 1d), and the diameter of newly
319 produced AMF spores in mediums with exogenous myristate were identical to those in
320 mediums without myristate (Fig. 1e).

321 **Effects of exogenous myristate on AMF colonization and biomass during symbiosis**
322 **with alfalfa and rice in pot experiments**

323 In AMF-inoculated groups (AM), all typical AMF structures (hyphae, arbuscules, and
324 vesicles) were observed in the roots of both alfalfa and rice, while no AMF colonization
325 was detected in the non-inoculated (NM) groups. Under low-P conditions, exogenous
326 myristate significantly increased the arbuscule colonization in alfalfa roots ($P < 0.05$), but
327 did not significantly affect hyphae and vesicle colonization ($P > 0.05$, Fig. 3a). Similarly,
328 exogenous myristate significantly enhanced arbuscular colonization and vesicle
329 colonization in rice roots ($P < 0.05$, Fig. 3b). However, under high-P conditions, exogenous
330 myristate had no significant impact on AMF colonization in both alfalfa and rice roots.

331 The content of the AMF signature fatty acid (NLFA 16:1ω5) in AMF-inoculated roots
332 (alfalfa: 143.2 ± 13.8 nmol g^{-1}; rice: 197.3 ± 11.9 nmol g^{-1}) was 10–20 times higher than
333 their corresponding non-inoculated roots (alfalfa: 6.1 ± 0.5 nmol g^{-1}; rice: 17.3 ± 1.2 nmol
334 g^{-1}), suggesting that NLFA 16:1ω5 serves as a distinctive fatty acid marker for AMF in this
335 study. The levels of NLFA 16:1ω5 in plant roots correspond with the patterns observed in
336 AMF colonization. Under low-P conditions, the addition of exogenous myristate
337 significantly increased the NLFA 16:1ω5 levels in both alfalfa and rice roots ($P < 0.05$, Fig.
338 3c, d); however, it had no significant effect under high-P conditions. Similar patterns were
339 observed in the rhizosphere soils: under low-P conditions, exogenous myristate increased
340 the NLFA 16:1ω5 levels in the rhizosphere soils of alfalfa ($P < 0.05$) and rice ($P = 0.14$)

341 (Fig. 3c, d), but it had no significant impact on the NLFA 16:1ω5 levels in the rhizosphere
342 soil of either plant under high-P conditions.

343 **Effects of exogenous myristate on the activity of mycorrhizal P pathway and the MGR**

344 In pot experiments, AMF inoculation significantly increased P concentrations in both
345 alfalfa and rice plants ($P < 0.05$, Fig. 4a, b), indicating the improvement in P acquisition
346 through AM symbiosis. Exogenous myristate significantly influenced the mycorrhizal P
347 responses (MPR) in both alfalfa and rice ($P < 0.01$, Fig. 4c, d). Specifically, under low-P
348 conditions, exogenous myristate significantly decreased the MPR in both alfalfa and rice
349 ($P < 0.01$). Under high-P conditions, while exogenous myristate significantly decreased the
350 MPR in alfalfa ($P < 0.01$), it had no significant effect on rice.

351 The expression of marker genes in the mycorrhizal P pathway, including *MsPT8* in
352 alfalfa and *OsPT11* in rice, as well as the AMF gene *RiPT* responsible for P transfer to the
353 symbiotic interface, was detected in the roots of AMF-inoculated plants but not in non-
354 inoculated roots. This observation confirmed that AM symbiosis activated the mycorrhizal
355 P pathway at the transcriptional level in both species (Fig. 4e, f). Notably, exogenous
356 myristate significantly affected the transcriptional responses of the mycorrhizal P pathway
357 in both alfalfa and rice ($P < 0.01$). In alfalfa, the expression levels of *MsPT8* and *RiPT* were
358 diminished by exogenous myristate under both low- and high-P conditions ($P < 0.05$, Fig.
359 4e). In rice, exogenous myristate similarly decreased the expression levels of *OsPT11* and
360 *RiPT* under low-P conditions ($P < 0.05$, Fig. 4f), but not under high-P conditions.

361 AMF inoculation significantly increased the biomass of alfalfa plants under both low-
362 and high-P conditions ($P < 0.05$, Supplementary Fig. S1a), and the MGR was significantly
363 higher under high-P conditions compared to low-P conditions ($P < 0.05$, Supplementary

364 Fig. S1c). In rice, AMF inoculation had no effect on the biomass under low-P conditions
365 ($P > 0.05$, Supplementary Fig. S1b). However, it significantly reduced rice biomass under
366 high-P conditions ($P < 0.05$, Supplementary Fig. S1d). Generally, exogenous myristate had
367 no impact on the MGR in either alfalfa or rice, while P levels significantly influenced MGR
368 ($P < 0.05$, Supplementary Fig. S1c, d).

369 **Effects of exogenous myristate on plant transcriptional responses to AMF**

370 RNA-seq data encompassed the majority of plant genes (transcripts), with 39,944 and
371 33,056 genes in alfalfa and rice, respectively. Principal component analysis (PCA) of gene
372 expression profiles in mycorrhizal roots of alfalfa and rice showed that the first and second
373 principal components explained 32.4% and 45.8% of the variation (Fig. 5a, b). Under low-
374 P (but not high-P) conditions, the gene expression profiles with or without exogenous
375 myristate were distinctly separated in both alfalfa and rice, highlighting the significant
376 impact of exogenous myristate on their mycorrhizal transcriptional responses.

377 Exogenous myristate triggered a considerable number of differentially expressed gene
378 (DEG, Myr *vs.* Mock) in mycorrhizal alfalfa (LP: 308, HP: 137) and rice (LP: 590, HP:
379 855) roots, respectively (Fig. 5c, d), and its influence was markedly affected by soil P
380 conditions and plant species. In alfalfa, the number of downregulated DEGs exceeded that
381 of upregulated DEGs under both P conditions (Fig. 5c). In rice, the number of
382 downregulated DEGs was higher than that of upregulated DEGs under low P condition,
383 whereas the reverse was observed under high P condition (Fig. 5d).

384 Gene Ontology (GO) enrichment analysis for DEGs in response to exogenous
385 myristate (Myr *vs.* Mock) revealed that downregulated DEGs were primarily enriched in
386 defense-related GO terms in both alfalfa and rice (Fig. 5e,f). These include

387 glycosyltransferase activity, oxidoreductase activity, heme binding, sesquiterpene
388 biosynthesis, and methylation processes in alfalfa, along with defense response, salicylic
389 acid response, heme binding, and peroxide metabolism processes in rice (Fig. 5f). This
390 suggests that exogenous myristate attenuates the AMF-triggered defense responses in host
391 plants. Additionally, under high-P condition myristate also reduced the transcriptional
392 activities of processes involved in fatty acid biosynthesis and elongation in rice (marked in
393 green, Fig. 5f). In both alfalfa and rice, exogenous myristate upregulated GO terms
394 associated with signaling and transcription/translation-related processes (Fig. 5e, f).

395 This study specifically examined the response of symbiotic genes involved in
396 signaling recognition and regulation to exogenous myristate in plants, revealing differential
397 impacts of exogenous myristate on symbiotic signals in alfalfa (19 genes) and rice (25
398 genes) (Fig. 5g, h, Supplementary Table S2). In alfalfa, exogenous myristate significantly
399 upregulated symbiotic signal recognition genes (*MsNSP1*, *MsLysM-RLK3*), the SL
400 synthesis gene (*MsCullin-4*) under low-P condition, and the symbiotic signal recognition
401 gene (*MsLysM-RLK3*) under high-P conditions. Conversely, the expression of the
402 symbiotic signal negative regulatory gene (*MsSMAX1L*) was significantly downregulated
403 under high-phosphorus ($P < 0.05$, Fig. 5g). In rice, exogenous myristate significantly
404 downregulated symbiotic signal recognition genes (*OsPOLLUX*, *OsBCP1a*, *OsLYK1*) and
405 SL receptor gene (*OsD14L*) under low-P (but not high-P) conditions ($P < 0.05$, Fig. 5h).

406 **Effects of exogenous myristate on the transfer of plant-derived C to AMF**

407 The ^{13}C-NLFA-IRMS experiment aimed to investigate the effect of exogenous
408 myristate on C transfer from plants to AMF. In AMF-inoculated plants, a significant
409 enrichment of ^{13}C (δ^{13}C = 1.75 ± 0.6) in AMF signature fatty acid (NLFA 16:1ω5) was

410 observed compared to non-inoculated plants ($\delta^{13}C$ = -24.8 ± 2.3), demonstrating the
411 transfer of newly fixed ^{13}C from plants to AMF. Exogenous myristate significantly
412 influenced this C transfer to AMF ($P < 0.05$), with its impact varying based on P levels and
413 plant species. Under low-P conditions, exogenous myristate significantly decreased the
414 allocation of ^{13}C to AMF in alfalfa ($P < 0.05$, Fig. 6a), but significantly increased it in rice
415 ($P < 0.05$, Fig. 6b). Under high-P conditions, exogenous myristate did not significantly
416 affect the allocation of ^{13}C to AMF in either plant species ($P > 0.05$).

417 qRT-PCR results revealed that expressions of the symbiotic lipid transfer marker
418 genes *STR2* (in alfalfa and rice) and the gene responsible for fatty acid uptake at the
419 symbiotic interface *RiFAT* (in AMF), were exclusively detected in AMF-inoculated roots.
420 This indicates that AMF colonization triggers the activation of the plant symbiotic lipid
421 transfer pathway at the transcriptional level (Fig. 6c, d). Exogenous myristate significantly
422 altered the expression profiles of the symbiotic lipid transfer marker genes ($P < 0.05$), with
423 the effect being dependent on P levels and plant species ($P < 0.05$). Under low-P conditions,
424 exogenous myristate significantly downregulated the expression of *MsSTR2* in alfalfa roots
425 ($P < 0.05$, Fig. 6c), but significantly upregulated the expression of *OsSTR2* and *RiFAT* in
426 rice roots ($P < 0.05$, Fig. 6d). Under high-P conditions, exogenous myristate had no
427 significant impact on the expression of symbiotic lipid transfer pathway genes in both
428 alfalfa and rice roots ($P > 0.05$).

429 We further investigated the transcriptional profiles of plant glycolysis and FA
430 biosynthesis pathways in RNA-seq data, revealing generally stronger transcriptional
431 responses to exogenous myristate in rice compared to alfalfa (Fig. 6e). In alfalfa,
432 exogenous myristate significantly reduced the expression of a glycolysis gene (*MsPGK*)

433 and a fatty acid biosynthesis gene (*MsACCD*) under low-P conditions. Conversely, it
434 significantly increased the expression of glycolysis genes (*MsPFK*, *MsPK*, *MsAceE*) and
435 decreased fatty acid biosynthesis genes (*MsACCD*, *MsFabH*) under high-P conditions (P
436 < 0.05, Fig. 6e). In rice, exogenous myristate significantly elevated the expression of
437 glycolysis genes (*OsPGM*, *OsPFP*, *OsGAPDH*, *OsENO*, *OsPK*) and fatty acid biosynthesis
438 genes (*OsFabH*, *OsFabD*, *OsFabG*, *OsFabI*) under low-P conditions ($P < 0.05$, Fig. 6e).
439 However, it had no significant effect under high-P conditions.
440

441 **DISCUSSION**

442 Our results from $^{13}C_1$-myristate tracing experiment demonstrate that AMF, in
443 symbiosis with plants, can absorb external non-symbiotic myristate, and transport it (or its
444 metabolic products) toward intraradical structures. Myristate is a common component of
445 root exudates from various plants, including the non-host species of AMF (Fernández-Piñar
446 *et al.*, 2012; Guelette *et al.*, 2012; Li *et al.*, 2017; Liu *et al.*, 2024), and is widely present in
447 litter and soil substrates (Swenson *et al.*, 2018; Dai *et al.*, 2022). Therefore, our findings
448 suggest that in realistic environments extraradical hyphae of symbiotic AMF could uptake
449 myristate from sources such as root exudates, decaying plant/animal material, litter, or
450 other microbes, as C sources in addition to directly from the host plant. Supportively, in
451 our greenhouse experiment, myristate application generally resulted in a significant
452 increase in both intraradical and extraradical AMF biomasses, even when there was a
453 decline in symbiotic C supply from the plant to AMF. This study provides, to our
454 knowledge, the first solid evidence showing that symbiotic AMF can tap into organic C
455 sources beyond those allocated directly by their host plant. It has largely, but not universally,

456 been suggested that AMF are limited to assimilating inorganic forms of nutrients (Hodge
457 & Storer, 2015; Smith *et al.*, 2015), which makes the reason for AMF hyphae colonizing
458 and proliferating in leaf litter and not-yet-decomposed plant matter, a common
459 phenomenon observed since half a century ago (Went & Stark, 1968a, b; Bunn *et al.*, 2019),
460 still unresolved (see Bunn *et al.*, 2019). Our findings offer another possible explanation for
461 this phenomenon: AMF may assimilate organic nutrients such as myristate from dead
462 leaves as an additional C source for proliferation. Although further research is required to
463 understand how often symbiotic AMF encounter myristate (and other unidentified C
464 sources) and to determine the concentration of myristate in various microenvironments,
465 our study offers novel insights into the roles of AMF in ecosystems.

466 Our work revealed that in the presence of host plants, exogenous myristate acts as a
467 signaling (stimulating) molecule, enhancing AMF branching and sporulation. Intriguingly,
468 exogenous myristate caused a loss of directional growth in extraradical hyphae towards the
469 roots (tropism) in our study (Fig. 1) and in others' research (Liu *et al.*, 2023), suggesting
470 an interruption in the normal signal communication in AM. Notably, the signaling role of
471 myristate has also been found in several soil-borne bacteria (Fernández-Piñar *et al.*, 2012;
472 Liu *et al.*, 2024), implying its universal role in regulating the soil microbiome. AMF signal
473 molecules such as strigolactones and flavonoids increase AMF colonization by promoting
474 spore germination, and hyphal growth and branching (Mori *et al.*, 2016; Lidoy *et al.*, 2023;
475 Yu *et al.*, 2022); and palmitoleic acid even stimulates asymbiotic AMF to generate
476 infection-competent, albeit morphologically and functionally incomplete, secondary
477 spores, without increasing AMF biomass (Sugiura *et al.*, 2020). Unlike those signaling
478 molecules, myristate enhanced both intraradical and extraradical fungal biomasses in our

479 study, likely due to its dual role as a signaling molecule and a C source for AMF.
480 Remarkably, contrary to asymbiotic conditions, myristate triggered the formation of
481 morphologically complete secondary spores of the symbiotic AMF in our study, suggesting
482 that plant roots may provide additional substances to AMF beyond myristate. Our findings
483 suggest myristate as a potential strategy to enhance AMF biomass accumulation *in situ*, but
484 also emphasize the importance of identifying plant-derived substances other than myristate
485 for the successful pure culture of AMF.

486 During AM establishment, plant roots exhibit varying levels of mycorrhizal defense
487 responses to prevent excessive C consumption due to over-colonization (Blilou *et al.*, 2000;
488 Siciliano *et al.*, 2015; Feng *et al.*, 2019). In our study, exogenous myristate led to the
489 downregulation of various genes associated with mycorrhizal defense, including those
490 related to oxidative stress, salicylic acid defense response, and secondary metabolite
491 production, suggesting that exogenous myristate hinders the transcriptional activation of
492 normal mycorrhizal defense in host roots. This effect contrasts with some other AM
493 signaling molecules, such as strigolactones and quercetin, which have been reported to
494 enhance mycorrhizal defense responses in plants (Sudheeran *et al.*, 2020; Kusajima *et al.*,
495 2022). Our findings suggest that the suppression of mycorrhizal defense response is a
496 mechanism through which exogenous myristate facilitates AMF colonization, although the
497 precise regulatory mechanisms remain to be clarified.

498 In our study, exogenous myristate reduced MPR, and downregulated the expression
499 of mycorrhizal P transporter genes in both alfalfa and rice, indicating a decrease of
500 mycorrhizal P benefit. There are several possible explanations for this. Firstly, prior study
501 has shown that introducing *R. irregularis* into *F. mosseae*-plants interaction system

502 enhances *F. mosseae*'s cooperation, indicating a shift in resource value due to altered
503 supply-demand dynamics (Argüello *et al.*, 2016). Kiers *et al.* (2011) further revealed that
504 AMF can assess and adjust to the plants' C supply rate, thus regulating P transport. In our
505 study, myristate, serving as an external non-symbiotic C source, likely lowered the "price"
506 of symbiotic C supply from plants, leading to decreased P transfer. Secondly, exogenous
507 myristate interrupted the signal communication in AM, potentially limiting the extent of P
508 acquisition by fungal extraradical hyphae and its delivery to host. This might partly explain
509 why the impact of exogenous myristate on reducing MPR is only evident under low-P
510 conditions. Thirdly, AMF growth and development also require the consumption of
511 nutrients such as P and N. In low-P soil, AMF P transport proteins expressed in arbuscular
512 cells can re-uptake P from the symbiotic interface, leading to nutrient competition between
513 the plant and AMF (Xie *et al.*, 2022). In our study, exogenous myristate led to a significant
514 increase in fungal biomass, suggesting an increased demand for P consumption by AMF.

515 Along with the reduction of mycorrhizal P benefits for the host, external myristate
516 also decreased the C supply to AMF from both alfalfa and rice, with the exception of rice
517 under low-P conditions. This decrease could be attributed to several factors. First, in AM
518 symbiosis, there is a competition for C sources between plants and fungi (Bitterlich *et al.*,
519 2014). In our study, AMF could switch for symbiotically obtained C to external C
520 (myristate), thereby reducing its competition for C. Second, symbiotic signaling molecules
521 like strigolactones and flavonoids can modulate fatty acid biosynthesis and metabolism in
522 host plant (Xuan *et al.*, 2018; Richmond *et al.*, 2022). In our study, myristate significantly
523 reduced the expression of essential genes involved in symbiotic C allocation and various
524 genes from the common symbiosis signaling pathway (Figs. 5, 6), which are vital for

525 nutrient exchange (Gutjahr *et al.*, 2008; Gutjahr *et al.*, 2012; Shi *et al.*, 2021). These
526 findings suggest that myristate, acting as a signaling molecule, could interfere with the
527 essential signal exchange needed for symbiotic C allocation. Third, this effect could be
528 interrelated to the reduced fungal P delivery to the host plant, as in AM symbiosis, the C–
529 P exchange is functionally coupled (Hammer *et al.*, 2011; Kiers *et al.*, 2011). Collectively,
530 our research reveals that exogenous myristate disrupted the symbiotic C−P exchange,
531 which is fundamental to the functioning and maintenance of AM (Smith & Read, 2008).

532 The exception that exogenous myristate increased, rather than decreased, the
533 allocation of plant-derived C to AMF in rice under low-P conditions indicates the non-
534 ignorable impacts of host identity and soil P status on symbiotic nutritional exchanges and
535 their reaction to exogenous myristate. In AM-responsive alfalfa, both the ^{13}C transfer
536 efficiency and the expression level of the *RiFAT* gene are over tenfold higher than in AM-
537 non-responsive rice (Fig. 6), accompanied with approximately fivefold greater expression
538 levels of fungal P transporter genes. Notably, the boost in rice-derived C caused by
539 myristate under low-P conditions appears to be unrewarded, as it correlates with a
540 diminished mycorrhizal P benefit. AM symbiosis represents one of the most ancient
541 coevolving species interactions (Rich *et al.*, 2021), with its evolutionary and ecological
542 success depending on an efficient and multifactorial communication system for partner
543 recognition, and on a fine-tuned and reciprocal metabolic regulation of each symbiont to
544 reach an optimal functional integration (Lanfranco *et al.*, 2018). The observation of the
545 disruptive effect of myristate on AM symbiosis in our study emphasizes the necessity for
546 a thorough comprehension of this ancient relationship while developing strategies to
547 improve AM outcomes.

CONCLUSIONS

Our study revealed that extraradical hyphae of symbiotic AMF can absorb external non-symbiotic myristate and transport it (or its metabolic products) towards intraradical structures, offering the first solid evidence that symbiotic AMF may utilize organic C sources beyond those allocated directly from their host plant. Additionally, myristate serves as a signaling molecule, and its dual role leads to the enhanced proliferation of both intraradical and extraradical biomasses for symbiotic AMF. RNA-seq data suggested that the suppression of mycorrhizal defense response may be a mechanism through which exogenous myristate facilitates AMF colonization. Interestingly, exogenous myristate disrupts the C−P exchange in AM symbiosis, possibly by interfering with their normal trading mechanisms, resulting in diminished mycorrhizal P benefits for plants and reduced plant-derived C allocation to AMF, although these effects are influenced by host identity and soil P conditions. These findings offer novel insights into the ecosystem roles of AM and the modulation of this symbiosis.

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574
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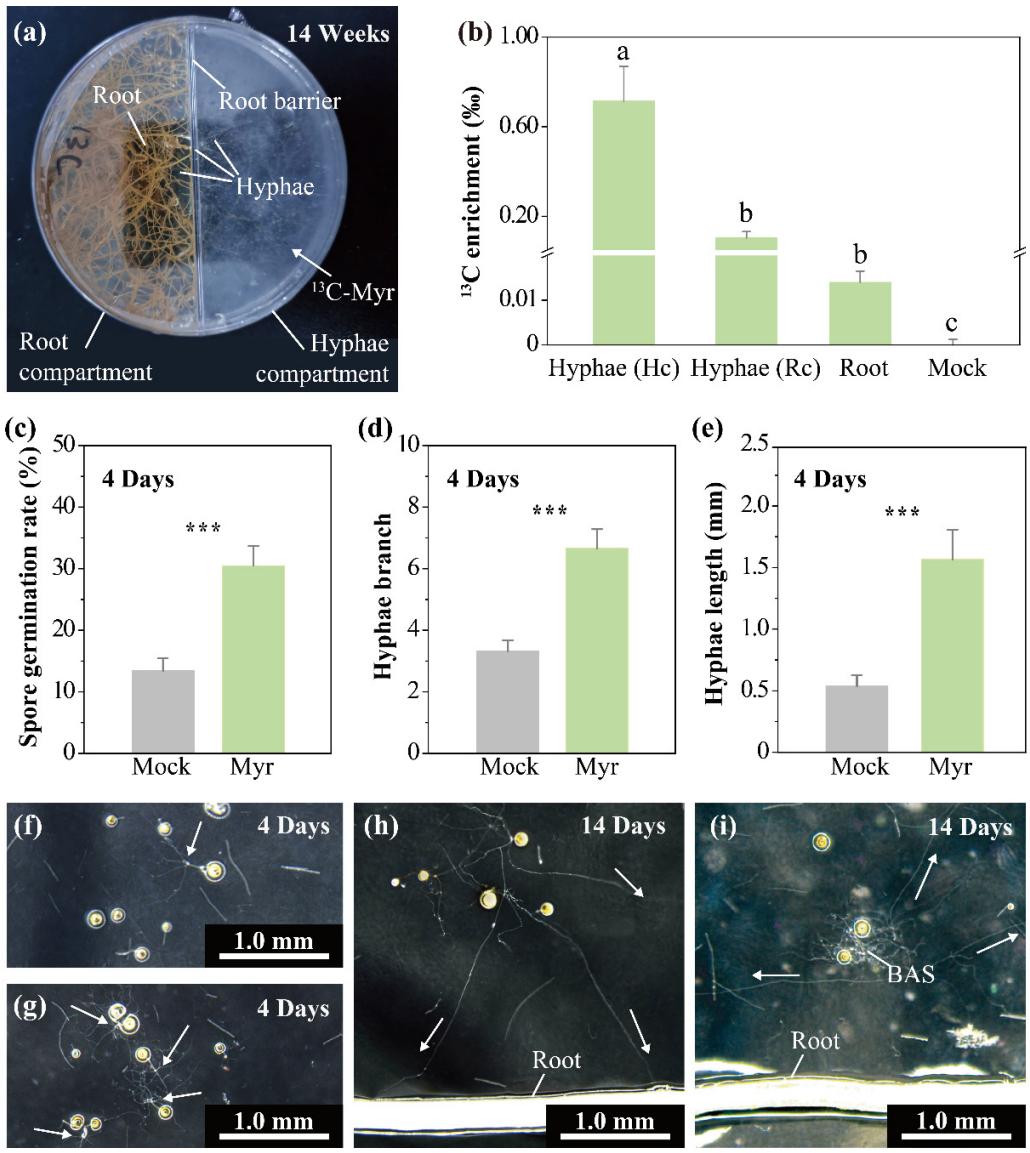


780

781 **Fig. 1** Effects of exogenous myristate on arbuscular mycorrhizal fungal (AMF) spore
782 germination and hyphae growth, and the uptake of exogenous $^{13}C_1$-labeled myristate by
783 AMF extraradical hyphae. **(a)** Carrot hairy root and AMF hyphae growth in two-
784 compartment AMF-carrot hairy root co-cultivation system; and **(b)** ^{13}C enrichment in AMF
785 hyphae and root. ^{13}C enrichment is the excess ^{13}C when the natural background, determined
786 in the hairy root (1.0910 ± 0.0001, n = 5) and extraradical hyphae (1.0923 ± 0.0001, n = 5)

787 from the non-labeled (Mock) systems, has been subtracted. **(c)** Spore germination rate; **(d)**
788 hyphae branch; and **(e)** hypha length in AMF-hairy roots dual culture systems with or
789 without 0.1 mM myristate (n = 6). **(f−i)** AMF spore germination, extraradical hyphal
790 growth and branched absorbing structures (BAS) in the solid medium without **(f, h)** or with
791 0.1 mM Myr **(g, i)**. Arrows show the spore in germination (d, e) and the direction of hyphal
792 elongation (f, g). Means ± SE, different letters indicate significant differences at $P < 0.05$
793 level based on the Tukey's test; *** represents statistically significant at the 0.001
794 probability levels.

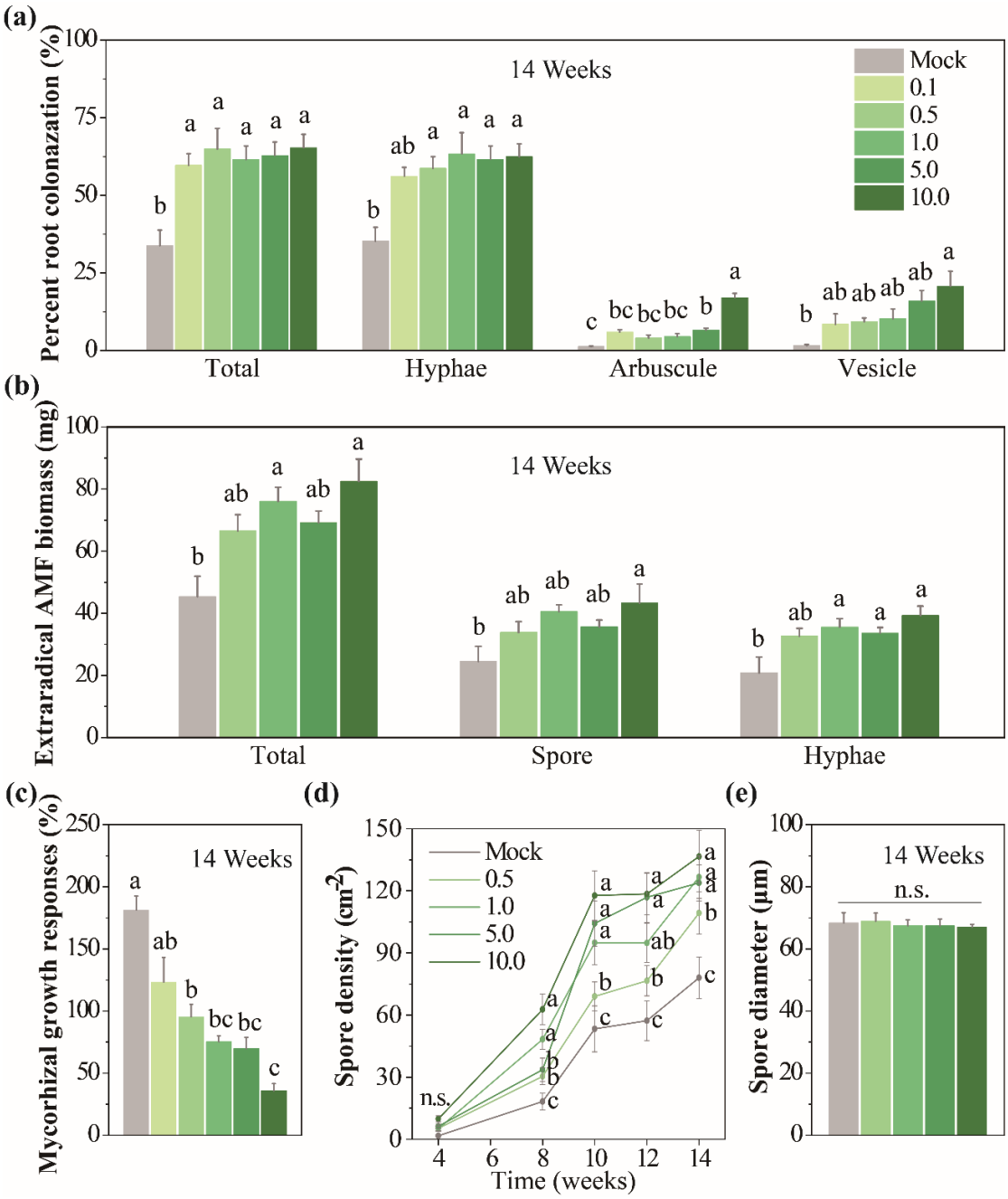


Fig. 2 Effects of exogenous myristate on arbuscular mycorrhizal fungal (AMF) development and mycorrhizal growth responses in the *in vitro* dual culture system. **(a)** AMF colonization intensities of hyphae, vesicles and arbuscules; **(b)** biomass of the AMF extraradical structures; **(c)** mycorrhizal growth responses (MGR) of the hairy root organ; **(d)** AMF spore density time curves; **(e)** AMF spore diameter with 0.1, 0.5, 1, 5, 10 mM

801 myristate (Myr) or ddH_2O (Mock) (n = 6). Means ± SE, different letters indicate significant

802 differences at $P < 0.05$ level based on the Tukey's test.

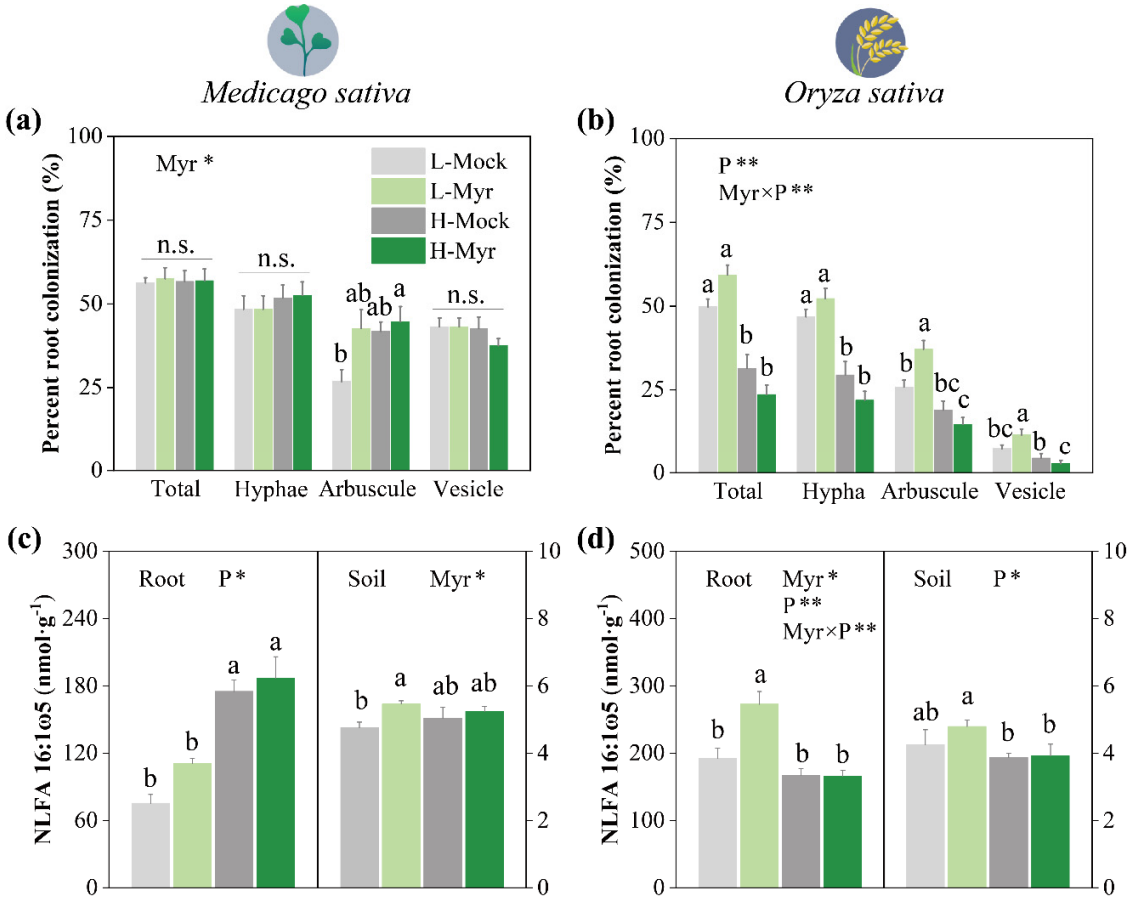


Fig. 3 Effects of exogenous myristate on arbuscular mycorrhizal fungal (AMF) colonization and biomass of intraradical and extraradical structures in alfalfa and rice under low- (L) or high-P (H) conditions in soil substrate. **(a, b)** AMF colonization intensities of hyphae, vesicles and arbuscules (n =10). **(c, d)** AMF signature fatty acid (NLFA 16:1ω5) in roots and rhizosphere soil of alfalfa and rice treated with 0.1 mM myristate (Myr) or ddH_2O (Mock) (n =5). Means ± SE denoted by the different letter are significantly different based on the Tukey's test ($P < 0.05$). *, ** represent statistically significant at the 0.05 and 0.01 probability levels, respectively.

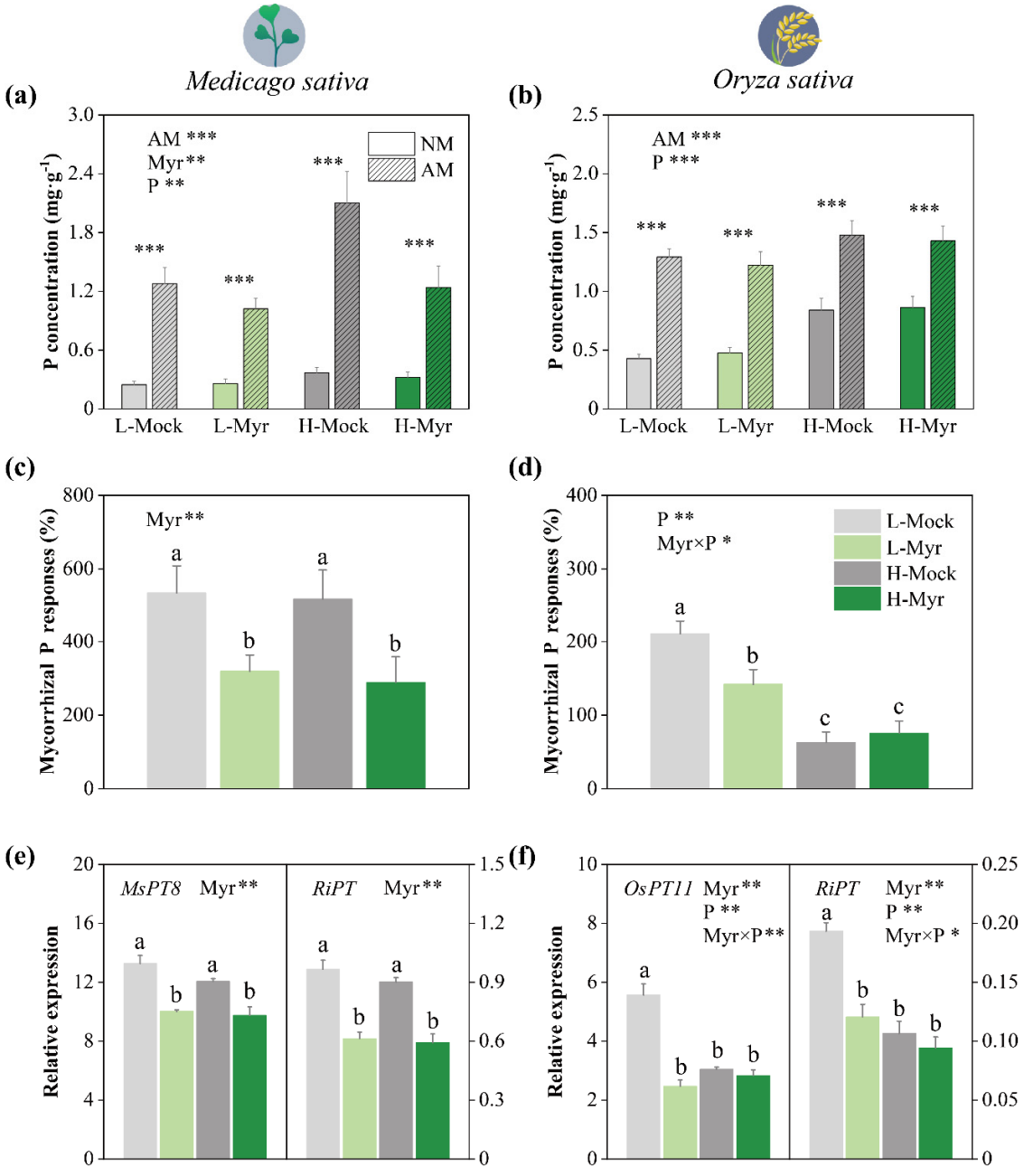
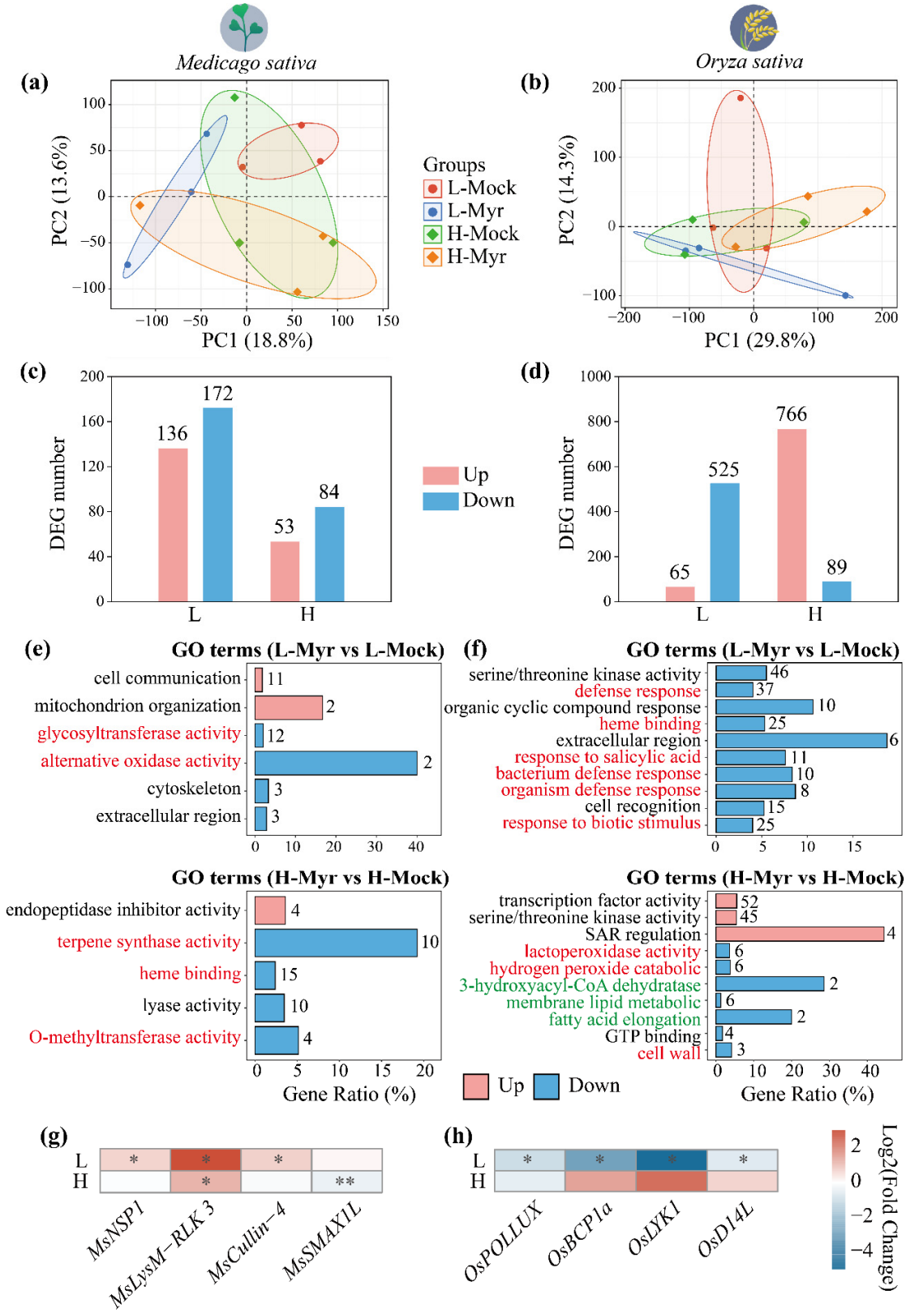


Fig. 4 Effects of exogenous myristate on plant P concentration, mycorrhizal P response (MPR), and relative expression of mycorrhizal P pathway marker genes for alfalfa and rice during greenhouse experiment. **(a, b)** Phosphorus concentration, **(c, d)** MPR (n = 10). **(e, f)** Relative expression of *OsPT11/MsPT8* and *RiPT* (n = 3) of *Medicago sativa* and *Oryza sativa* at low (L) and high (H) phosphorus levels applied with 0.1 mM myristate (Myr) or

818 ddH_2O (Mock). Means ± SE denoted by the different letter are significantly different based

819 on the Tukey's test ($P < 0.05$). *, **, *** represent statistically significant at the 0.05, 0.01

820 and 0.001 probability levels, respectively.


Medicago sativa
Oryza sativa
(a)
(b)
PC2 (13.6%)
PC1 (18.8%)
PC2 (14.3%)
PC1 (29.8%)
Groups
L-Mock
L-Myr
H-Mock
H-Myr
(c)
(d)
DEG number
136
172
53
84
65
525
766
89
L
H
Up
Down
(e)
(f)
GO terms (L-Myr vs L-Mock)
cell communication
mitochondrion organization
glycosyltransferase activity
alternative oxidase activity
cytoskeleton
extracellular region
serine/threonine kinase activity
defense response
organic cyclic compound response
heme binding
extracellular region
response to salicylic acid
bacterium defense response
organism defense response
cell recognition
response to biotic stimulus
GO terms (H-Myr vs H-Mock)
endopeptidase inhibitor activity
terpene synthase activity
heme binding
lyase activity
O-methyltransferase activity
transcription factor activity
serine/threonine kinase activity
SAR regulation
lactoperoxidase activity
hydrogen peroxide catabolic
3-hydroxyacyl-CoA dehydratase
membrane lipid metabolic
fatty acid elongation
GTP binding
cell wall
Gene Ratio (%)
(g)
(h)
MsNSP1
MsLysM-RLK 3
MsCullin-4
MsSMAX1L
OsPOLLUX
OsBCP1a
OsLYK1
OsD14L
Log2(Fold Change)

821

822 **Fig. 5** Effects of exogenous myristate on transcriptomic responses *Medicago sativa* and
823 *Oryza sativa* roots to arbuscular mycorrhizal fungi (AMF). **(a, b)** Number of up- and down-
824 regulated differentially expressed genes (DEGs), **(c, d)** principal component analysis **(**PCA)
825 of gene expression Transcript per million (TPM) values, (**e, f)** enrich gene ontology (GO)
826 terms of the DEGs (adjust $P < 0.05$), **(g, h)** expression profiles of symbiotic signaling genes
827 (Gene list is available in Supplemental Information Table S2) in AMF-infected roots of
828 *Medicago sativa* and *Oryza sativa* at low (L) and high (H) phosphorus levels applied with
829 0.1 mM myristate (Myr) or ddH_2O (Mock). Gene Ratio represents the ratio of DEG
830 numbers (beside or in the bar) to the number of background genes in each GO category. In
831 red and green are defense-related and fatty acid biosynthesis GO terms, respectively. *
832 represents significant difference between Myr and Mock plants ($P < 0.05$).

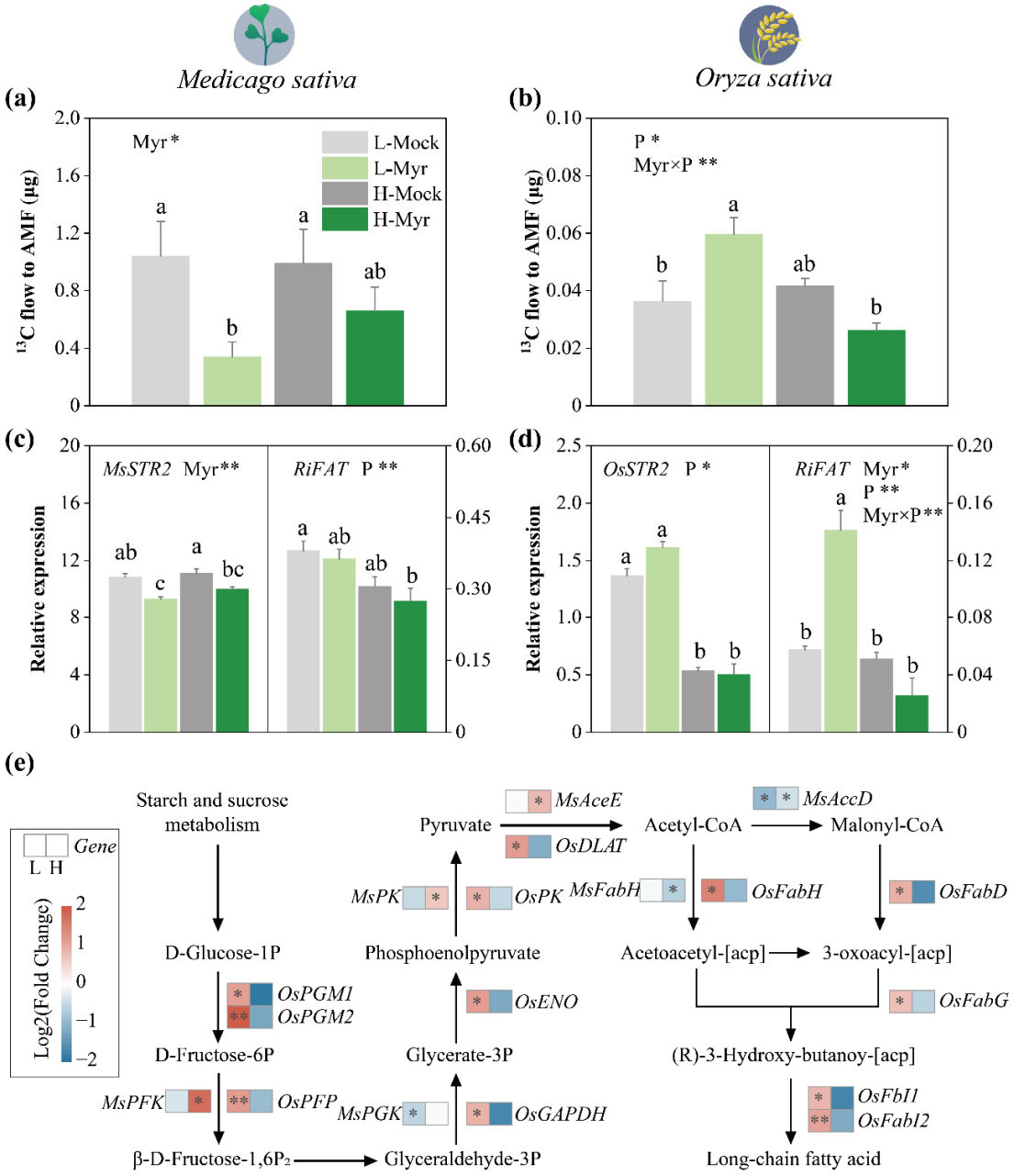


Fig. 6 Effects of exogenous myristate on the C allocation from plants to arbuscular mycorrhizal fungi (AMF), the relative expression of the marker genes for symbiotic carbon transportation, and the differentially expressed genes (DEGs) in glycolysis and fatty acid biosynthesis pathways in *Medicago sativa* and *Oryza sativa*. **(a, b)** ^{13}C flow to the AMF signature fatty acid NLFA 16:1ω5 (n = 5). **(c, d)** Relative expression of the symbiotic lipid

839 transfer marker genes *STR2* and *RiFAT* (qRT-PCR, n = 3). Means ± SE denoted by the
840 different letter are significantly different based on the Tukey's test ($P < 0.05$). **(e)**
841 Expression profiles of genes in glycolysis and fatty acid biosynthesis pathways (Gene list
842 is available in Supplemental Information Table S2) in mycorrhizal roots of *Medicago sativa*
843 and *Oryza sativa* treated with 0.1 mM myristate (Myr) or ddH_2O (Mock) under low- and
844 high-P conditions. * represents significantly different in plants treated with 0.1 mM
845 potassium myristate compared to the mock plants ($P < 0.05$).