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

Hanwen Chen[1, #], Tian Xiong[1, #], Baoxing Guan[1, #], Jiaqi Huang[1, #], Danrui Zhao[1], Yao Chen[1], Haoran Liang[1], Yingwei Li[1], Jingwen Wu[1], Shaoping Ye[2], Ting Li[2], Wensheng Shu[1], Jin-tian Li[1], Yutao Wang[1, *]

[1]Guangdong Provincial Key Laboratory of Biotechnology for Plant Development, Guangzhou Key Laboratory of Subtropical Biodiversity and Biomonitoring, School of Life Sciences, South China Normal University, Guangzhou, 510631, China

[2]Guangzhou Institute of Forestry and Landscape Architecture, Guangzhou, 510405, China

*Correspondence: School of Life Sciences, South China Normal University, Guangzhou, 510631, China; Email: wangyutao@scnu.edu.cn (Yutao Wang); Tel./Fax: +86 20 85211850

[#]: These authors contributed equally to this work.

ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs that rely on symbiotic carbohydrates and, in particular, lipids derived from their host plants. However, it remains unclear whether symbiotic AMF can access exogenous non-symbiotic lipids in the presence of plant-derived carbon, complicating our understanding of their relationship with host plants. Here, we investigated the direct uptake of exogenous $^{13}C_1$-labeled myristate by three symbiotic AMF species (*Rhizophagus irregularis*, *R. intraradices*, and *R. diaphanous*) and assessed their growth responses using AMF-carrot hairy root co-culture systems. Furthermore, we explored the environmental distribution of myristate, and evaluated the impact of exogenous myristate on the carbon-phosphorus exchange between *R. irregularis* and alfalfa or rice in a greenhouse experiment. Our results showed that symbiotic AMF can absorb exogenous myristate, as evidenced by ^{13}C enrichment and transcriptional activation of fatty acid transport and metabolism genes in AMF extraradical hyphae. Myristate is commonly present in various soil and plant environments, and its application increased both intraradical and extraradical fungal biomass, possibly linked to suppressed mycorrhizal-activated defense responses in host roots. Unexpectedly, exogenous myristate reduced the mycorrhizal phosphorus benefits for both alfalfa and rice and decreased their symbiotic carbon allocation to root-colonizing AMF, suggesting that the application of exogenous myristate may not be a promising strategy to enhance AM symbiosis. These findings provide new insights into understanding and manipulating the nutritional interactions between AMF and host plants.

Keywords： arbuscular mycorrhizal fungi (AMF); carbon-phosphorus exchange; exogenous non-symbiotic carbon; myristate; stable isotope labeling

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are among the most widely distributed soil microorganisms, forming symbiotic relationships with approximately 70% of terrestrial vascular plants (Brundrett and Tedersoo, 2018). In this symbiosis, referred to as arbuscular mycorrhiza (AM), AMF develop an extensive extraradical hyphal network in the soil, which facilitates nutrient uptake for host plants—particularly phosphorus (P)—and enhances plant resilience to both biotic and abiotic stressors (Smith and 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 fatty acids (Helber et al., 2011; Jiang et al., 2017). This symbiotic C-P exchange, the cornerstone of AM symbiosis (Smith and 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 influence plant community structure and productivity (Johnson, 2010; Wipf et al., 2019).

As one of the most ancient symbiotic relationships, AM has undergone extensive evolutionary processes (Foster and Kokko, 2006; Rich et al., 2021), with its establishment and development intricately regulated by a set of symbiotic genes and signaling molecules influenced by both biotic and abiotic factors (Maclean et al., 2017; Shi et al., 2021). For instance, in AM plants, the two pathways for P uptake—the direct P pathway via root hairs and epidermis and the mycorrhizal P pathway via AMF hyphae—are coordinately regulated by the same phosphate-sensing pathway (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 root colonization and the benefits conferred by AMF largely depend on the host plant species and even their genotypes (Watts-Williams et al., 2019;

68 Huang et al., 2020). Some species are classified as "responsive plants", which exhibit
69 positive growth responses across most genotypes (e.g., alfalfa and maize), while others
70 are referred to "nonresponsive plants", exhibiting minimal or no growth enhancement
71 but showing increased AM-activated defense responses in many genotypes (e.g., rice
72 and wheat) (Hata et al., 2010; Li et al., 2024).

73 AMF are considered obligate symbionts that rely on living host plants to complete
74 their life cycle by producing daughter spores (Smith and Read, 2008), which presents
75 challenges for basic research on AM and their applications in agriculture and other
76 fields. Despite recent progresses (Tanaka et al., 2022), achieving a completely
77 asymbiotic culture of AMF—specifically, a host-free culture of daughter spores that
78 retains the same physiological and genetic traits as their mother spore—has not been
79 successful, partially due to their dependence on plant-derived symbiotic fatty acids
80 (Bravo et al., 2017; Luginbuehl et al., 2017; Kameoka et al., 2019). Genomic and
81 transcriptomic analyses have shown that AMF lack the genes required for the
82 biosynthesis of long-chain fatty acids, and subsequent studies have demonstrated that
83 fatty acids are transferred from host plants to AMF as their primary C source (Bravo et
84 al., 2017; Jiang et al., 2017; Keymer et al., 2017; Luginbuehl et al., 2017). However,
85 the mechanism underlying the allocation of plant-derived fatty acids to fungi in AM
86 symbiosis remains unclear. In host plants, AMF colonization triggers the expression of
87 lipid biosynthesis and transport genes (Bravo et al., 2017), with lipid transport to the
88 AM symbiotic interface relying on the periarbuscular membrane-localized transporters
89 stunted arbuscule (STR) and STR2 (Jiang et al., 2017; Keymer et al., 2017), and fungal
90 uptake of plant-derived lipids mediated by fatty acid transporter 1 (RiFAT1) and
91 RiFAT2 in the model AMF species *Rhizophagus irregularis* (Brands and Dörmann,
92 2022). This symbiotic C allocation is functionally linked to fungal P delivery within a

93 framework of mutual nutritional rewards (Kiers et al., 2011), which have been disrupted
94 during AMF-nonhost interactions due to the strong AM-activated defense responses in
95 non-host roots (Wang et al., 2023).

96 Fatty acids and their derivatives, essential structural components and energy
97 sources for plant, animal, and microbial cells, are commonly found in soil, particularly
98 in the rhizosphere, where plant-derived fatty acids are made available through root
99 exudates and rhizodeposition (Swenson et al., 2018; Dai et al., 2022; Liu et al., 2024).
100 Following the discovery that AMF acquire fatty acids from their hosts during symbiosis,
101 the use of fatty acids in cultivating asymbiotic AMF has attracted considerable attention.
102 Kameoka et al. (2019) demonstrated that under asymbiotic conditions, exogenous
103 palmitoleic acid and several other fatty acids stimulated the hyphal elongation and
104 branching in *Rhisophagus irregularis* and *R. clarus,* but not in *Gigaspora margarita*,
105 resulting in the formation of infection-competent but morphologically and functionally
106 incomplete spores with reduced size, lower germination rates, and decreased infection
107 capacity compared to symbiotic spores. Additionally, Sugiura et al. (2020) showed that
108 exogenous myristate (tetradecanoic acid)—a common organic acid in various plant root
109 exudates (Li et al., 2017; Liu et al., 2024) and soil substrates (Swenson et al., 2018; Dai
110 et al., 2022)—can serve as a C source for the asymbiotic growth of *R. irregularis* and
111 *R. clarus,* promoting hyphal growth, the formation of branched absorbing structures,
112 and the production of morphologically incomplete, infection-competent spores.
113 Subsequent studies revealed that combining plant hormones with myristate enhanced
114 the efficiency of myristate as a C source in asymbiotic *R. clarus*, leading to the
115 generation of numerous infection-competent spores that were smaller than those
116 generated under symbiotic conditions (Sugiura et al., 2020; Tanaka et al., 2022). These
117 discoveries have significantly advanced the pure culture of AMF and, importantly,

118 identified myristate as the first external C source accessible to AMF under asymbiotic
119 conditions, providing exceptional opportunities for understanding and utilizing the
120 ecological functions of AM symbiosis in plant community regulation and global C
121 cycling (Rillig et al., 2020).

122 However, the effects of external myristate on the growth and development of
123 symbiotic AMF—the typical state of functioning AMF in nature—remain unclear.
124 Regarding sugars, another type of host-derived C source, while evidence suggests that
125 AMF extraradical hyphae in symbiosis with a carrot hairy root may absorb exogenous
126 monosaccharides (Helber et al., 2011), the impact of such uptake on the development
127 of symbiotic AMF remains uncertain. Moreover, no nutritional roles were observed in
128 supporting asymbiotic spore growth following the exogenous application of sugars
129 (Sugiura et al., 2020). A recent study reported that while exogenous myristate does not
130 affect the extraradical biomass of symbiotic *R. irregularis* and *R. intraradices*, it
131 enhances their colonization rates by stimulating the growth and development of
132 extraradical hyphae. Based on this, the authors inferred that symbiotic AMF may
133 acquire exogenous myristate (Liu et al., 2023). To date, the effects of exogenous
134 myristate on AM symbiosis and their underlying mechanisms remain limited.
135 Specifically, whether symbiotic AMF can uptake exogenous myristate via extraradical
136 hyphae in the presence of plant-derived symbiotic C is yet to be determined. This
137 question is crucial for understanding the symbiotic mechanisms and ecological roles of
138 AM symbiosis (Rillig et al., 2020).

139 To address this significant knowledge gap, this study investigated the ability of
140 symbiotic AMF to absorb external non-symbiotic myristate and examined the effect of
141 exogenous myristate on symbiotic C-P exchanges in AM. Specifically, we aimed to
142 determine: (i) whether symbiotic AMF can take up exogenous non-symbiotic myristate,

143 (ii) how this additional non-symbiotic C source influences the symbiotic C allocation
144 from plants to AMF, and (iii) if and how fungal P transfer to the host plant is affected
145 by exogenous myristate. This work provides, to our knowledge, the strongest evidence
146 to date that symbiotic AMF can absorb non-symbiotic fatty acids like myristate in the
147 presence of plant-derived symbiotic carbohydrates and lipids. Furthermore, our
148 findings reveal that exogenous myristate may, unexpectedly, disrupt the C-P exchange
149 in AM symbiosis.
150

151 **RESULTS**

152 **Tracing of ^{13}C in AMF extraradical hyphae (Exp. 1)**

153 To investigate the direct uptake of exogenous myristate by symbiotic AMF, we
154 conducted an isotopic tracing experiment using $^{13}C_1$-labeled myristate within the two-
155 compartment AMF-carrot hairy root co-cultivation system (Exp. 1, Fig. 1a). Exogenous
156 myristate specifically induced the formation of branched absorbing structures in *R.*
157 *irregularis*, *R. intraradices*, and *R. diaphanus* in the hyphal compartment (HC)
158 approximately two weeks after Myr^+ treatment (Fig. 1b). For *R. irregularis*, the $^{13}C/^{12}C$
159 ratio of extraradical hyphae in HC (trial 1: 1.1504 ± 0.0575%, trial 2: 1.0919 ± 0.0001%)
160 and root compartment (RC, trial 1: 1.1026 ± 0.0055%, trial 2: 1.0918 ± 0.0002%) under
161 ^{13}C-Myr treatment was slightly but significantly higher than that in the corresponding
162 HC (trial 1: 1.0922 ± 0.0003%, trial 2: 1.0908 ± 0.0003%) and RC (trial 1: 1.0923 ±
163 0.0002%, trial 2: 1.0908 ± 0.0002%) from the non-labeled control (^{12}C-Myr),
164 respectively ($P < 0.05$, Fig. 1c). Similarly, for *R. intraradices* and *R. diaphanus*, the
165 $^{13}C/^{12}C$ ratio of extraradical hyphae in HC (*R. intraradices*: 1.0933 ± 0.0002%, *R.*
166 *diaphanus*: 1.0922 ± 0.0005%) and RC (*R. intraradices*: 1.0927 ± 0.0005%, *R.*
167 *diaphanus*: 1.0930 ± 0.0004%) under ^{13}C-Myr treatment was slightly but significantly

168 higher than those in HC (*R. intraradices*: 1.0917 ± 0.0001%, *R. diaphanus*: 1.0908 ±
169 0.00003%) and RC (*R. intraradices*: 1.0918 ± 0.00004%, *R. diaphanus*: 1.0908 ±
170 0.00003%) from the non-labeled control ($P < 0.01$, Fig. 1c). Additionally, the calculated
171 ^{13}C enrichment in extraradical hyphae between HC and RC showed no significant
172 difference for *R. irregularis* and *R. diaphanus* (Fig. 1d, e), although it was higher in HC
173 than in RC for *R. intraradices* ($P < 0.05$), excluding the possibility that the ^{13}C
174 enrichment in AMF extraradical hyphae originated from ^{13}C-myristate retention on the
175 hyphal surface from the HC. Collectively, these results suggest that these three AMF
176 species can take up a small amount of exogenous myristate through their extraradical
177 hyphae when associated with host roots.

178 The ^{13}C enrichment levels among the three AMF species were calculated to
179 compare their capacity for myristate absorption. In HC, the calculated ^{13}C enrichments
180 in extraradical hyphae of *R. irregularis* (trial 1: 0.5823 ± 0.4111‰, trial 2: 0.0107 ±
181 0.0014‰), *R. intraradices* (0.0163 ± 0.0031‰), and *R. diaphanus* (0.0136 ± 0.0067‰)
182 showed no significant differences in the ^{13}C-Myr treatment (Fig. 1d). In RC, the ^{13}C
183 enrichment in extraradical hyphae also showed no significant differences (Fig. 1e),
184 except for a slightly but significantly higher ^{13}C enrichment in *R. diaphanus* (0.0217 ±
185 0.0031‰) compared to *R. intraradices* (0.0082 ± 0.0037‰) and *R. irregularis* in trial
186 2 (0.0093 ± 0.0017‰, $P < 0.05$). These results suggest a generally similar myristate
187 absorption capacity among these AMF species under the tested conditions.

188 **Effects of myristate on root colonization and expression of fatty acid uptake and**
189 **metabolism marker genes (Exp. 1)**

190 The expression of fatty acid uptake and metabolism marker genes in *R. irregularis*
191 extraradical hyphae were analyzed to investigate their uptake of myristate from HC at
192 the transcriptional level. The results showed that exogenous myristate significantly

193 increased the expression levels of the fungal fatty acid transporter gene *RiFAT2* ($P <$
194 0.05), while had no significant effect on *RiFAT1* (Fig. 2a). Additionally, exogenous
195 myristate significantly or marginally significantly increased the expression levels of the
196 fungal genes involved in fatty acid β-oxidation (*RiFAD1*, $P < 0.05$), palmitvaccenic acid
197 synthesis (*RiOLE1*, $P = 0.057$), and TCA cycle activity (*RiCIT1*, $P < 0.05$) (Fig. 2b).
198 These results suggest that exogenous myristate activates the expression of fungal genes
199 related to fatty acid uptake and metabolism in the extraradical hyphae of *R. irregularis*.
200 For AMF colonization intensity, exogenous myristate significantly increased the
201 hyphal and arbuscular colonization rates of *R. irregularis, R. intraradice*, and *R.*
202 *diaphanus* in carrot hairy roots ($P < 0.05$, Fig. 2c−e) but had no effect on vesicle
203 colonization.

204 **Effects of exogenous myristate on AMF spore germination, root colonization, and**
205 **extraradical structure development (Exp. 2)**

206 The impact of exogenous myristate on the growth and development of symbiotic
207 AMF was evaluated in a single-compartment *R. irregularis*-carrot hairy root co-
208 cultivation system (Exp. 2, Fig. 3a). Exogenous myristate significantly promoted spore
209 germination ($P < 0.001$, Fig. 3b), and enhanced hyphal branching and length ($P < 0.001$,
210 Fig. 3c, d) before visible physical contact between hyphae and hairy roots at four days
211 after AMF inoculation. Morphological examinations showed that exogenous myristate
212 significantly enhanced the colonization intensities of hyphae and arbuscules, but not
213 vesicles, in carrot hairy roots across various application levels (Fig. 3e). Exogenous
214 myristate also significantly increased the extraradical biomass of AMF (hyphae and
215 spore) at different application concentrations ($P < 0.05$, Fig. 3f).

216 Meanwhile, exogenous myristate significantly increased the density of AMF
217 spores from eight weeks post-AMF inoculation ($P < 0.05$, Fig. 3g), and the diameter of

218 newly produced AMF spores in media with exogenous myristate was identical to those
219 in media without myristate (Fig. 3h).

220 **Environmental myristate levels and the effects of exogenous myristate on AMF**
221 **intraradical and extraradical biomass during symbiosis with alfalfa and rice (Exp.**
222 **3)**

223 In Exp. 3 (Fig. 4a), our field survey on the environmental myristate distributions
224 revealed that myristate was extensively detected in soil and plant samples from the
225 paddy field, grassland, and woodland at a 2.87–14.68 mg kg^{-1} level (Fig. 4b). On
226 average, the myristate level in plant (fresh leaf or root) is mostly higher than that in soil
227 (leaf litter, rhizosphere soil, or bulk soil) ($P < 0.05$, Fig. 4b). Additionally, the myristate
228 levels in leaf litter and rhizosphere soil were usually greater than in bulk soil ($P < 0.05$),
229 suggesting that plants are significant source of myristate in the soil. The myristate
230 contents from the same sample type typically varied across different habitats ($P < 0.05$).

231 A pot experiment was further conducted to assess the effect of exogenous
232 myristate on C-P nutritional exchanges in AM symbiosis (Exp. 3). All typical AMF
233 intraradical structures (hyphae, arbuscules, and vesicles) were observed in the roots of
234 both alfalfa and rice in the *R. irregularis*-inoculated groups (AM), while no AMF
235 colonization was detected in the non-inoculated (NM) groups. Under low-P conditions,
236 exogenous myristate significantly increased arbuscule abundance in alfalfa roots ($P <$
237 0.05, Fig. 4c) but did not significantly affect hyphae or vesicle abundance (Supporting
238 Information Fig. S1a, c). Exogenous myristate significantly enhanced arbuscule,
239 hyphae, and vesicle abundance in rice roots ($P < 0.05$, Fig. 4e, Supporting Information
240 Fig. S1b, d). However, under high-P conditions, exogenous myristate had no effect on
241 AMF abundance in either alfalfa or rice roots (Fig. 4, Supporting Information Fig. S1).

242 AMF signature fatty acid (NLFA 16:1ω5) and the relative expression of the AMF

243 marker gene elongation factor-1 beta (*RiEF-1β*) were analyzed as indicators of AMF
244 biomass. For intraradical AMF biomass, exogenous myristate significantly increased
245 the levels of the NLFA 16:1ω5 and the *RiEF-1β* expression in both alfalfa and rice roots
246 under low-P conditions ($P < 0.05$, Fig. 4d, f, g, h); however, it had no significant impact
247 under high-P conditions. In alfalfa roots, NLFA 16:1ω5 levels were higher under high-
248 P than low-P conditions ($P < 0.05$). For extraradical AMF biomass, exogenous
249 myristate increased NLFA 16:1ω5 levels in the rhizosphere soils of alfalfa ($P < 0.05$)
250 but not in rice ($P = 0.14$) under low-P conditions (Fig. 4g, h). Under high-P conditions,
251 myristate had no effect on NLFA 16:1ω5 levels in the rhizosphere soil of either plant.

252 **Effects of exogenous myristate on the transfer of symbiotic C to AMF (Exp. 3)**

253 The analysis of ^{13}C-NLFA-isotope ratio mass spectrometry was performed to
254 investigate the impact of exogenous myristate on the allocation of symbiotic C from
255 plants to AMF. In AM plants, there was a notable enrichment of ^{13}C (δ^{13}C = 1.75 ±
256 0.6‰) in the AMF signature fatty acid (NLFA 16:1ω5) compared to NM plants (δ^{13}C =
257 −24.8 ± 2.3‰), demonstrating the transfer of newly fixed ^{13}C from plants to AMF. The
258 calculated ^{13}C flow from alfalfa to root-colonizing AMF (0.3400–1.2642 μg) was over
259 ten times higher than that from rice (0.0261–0.0596 μg), highlighting a substantially
260 greater efficiency of plant-derived symbiotic C delivery to AMF in AM-responsive
261 alfalfa compared to non-responsive rice. In alfalfa, exogenous myristate reduced the
262 symbiotic C transfer to AMF (Myr effect: $P < 0.001$, Fig. 5a), although this effect was
263 not significant under high-P conditions. In rice, myristate also significantly influenced
264 the symbiotic C flow to AMF, with its effect significantly interacting with soil P level
265 (Myr × P: $P = 0.004$, Fig. 5b): myristate significantly increased the allocation of ^{13}C to
266 AMF under low-P conditions ($P < 0.05$) but decreased it under high-P conditions ($P <$
267 0.01).

268 At the transcriptional level, the qRT-PCR analysis revealed that the expressions of
269 the symbiotic lipid transfer marker genes *STR2* (in alfalfa and rice) and the fungal genes
270 responsible for symbiotic fatty acid uptake *(RiFAT1* and *RiFAT2)* or monosaccharide
271 uptake (*Monosaccharide Transporter 2* (*RiMST2*)) were exclusively detected in AM
272 roots, except for a low expression level of alfalfa *MsSTR2* occasionally observed in NM
273 roots (Fig. 5c–h). In AMF-inoculated alfalfa roots, exogenous myristate significantly
274 downregulated the expressions of the symbiotic C transfer genes *MsSTR2* in the plant
275 and *RiMST2* in AMF ($P < 0.05$), while having no effect on *RiFAT1* and *RiFAT2* in AMF
276 (Fig. 5c, g, e). In AMF-inoculated rice roots, the effects of myristate on the tested
277 symbiotic C transfer genes were largely influenced by soil P levels ($P < 0.05$). Under
278 low-P conditions, myristate significantly increased the expressions of plant *OsSTR2* and
279 fungal *RiFAT1* and *RiFAT2*, while decreasing the expression of fungal *RiMST2* ($P <$
280 0.05, Fig. 5d, f, h). Under high-P conditions, myristate significantly increased the
281 expression of *RiMST2* ($P < 0.05$) but had no effects on *OsSTR2*, *RiFAT1*, and *RiFAT2*.
282 In both plant species with AMF inoculation, soil P level showed significant effects on
283 *STR*, *RiFAT1*, and *MST2* genes ($P < 0.05$), with generally lower expression levels of
284 these genes under high-P compared to low-P conditions.

285 **Effects of exogenous myristate on the mycorrhizal P pathway and mycorrhizal**
286 **growth response (Exp. 3)**

287 We further analyzed the mycorrhizal P pathway and mycorrhizal growth response
288 in the pot experiment to evaluate the effects of exogenous myristate on AM symbiosis
289 (Exp. 3). AMF inoculation significantly increased P concentrations in both alfalfa and
290 rice plants ($P < 0.001$, Fig. 6a, b). Myristate generally exhibited no effects on plant P
291 concentrations for AM and NM plants, except that it significantly decreased P
292 concentration in AMF-colonized alfalfa under high-P conditions ($P < 0.05$). Under low-

293 P conditions, exogenous myristate significantly decreased the mycorrhizal P response
294 in both alfalfa and rice ($P < 0.05$, Fig. 6c, d). Under high-P conditions, while exogenous
295 myristate significantly decreased the mycorrhizal P response in alfalfa ($P < 0.05$), it had
296 no significant effect on rice.

297 At the transcriptional level, the expression of mycorrhizal P pathway marker genes
298 in plants (*Phosphate Transporter*, *MsPT8* or *OsPT11*) and AMF (*RiPT*) was detected
299 exclusively in the AM roots of rice and alfalfa, except for a low expression level of rice
300 *OsPT11* observed in the NM root under low-P and Myr^{-} conditions. This finding
301 confirmed that AM symbiosis activated the mycorrhizal P pathway at the transcriptional
302 level in both plant species (Fig. 6e, f). Exogenous myristate significantly influenced the
303 transcriptional responses of the mycorrhizal P pathway in both alfalfa and rice ($P <$
304 0.01). In alfalfa, exogenous myristate downregulated the expressions of *MsPT8* and
305 *RiPT* under both low- and high-P conditions ($P < 0.05$, Fig. 6e). In rice, exogenous
306 myristate decreased the expression levels of *OsPT11* and *RiPT* under low-P conditions
307 ($P < 0.05$), but not under high-P conditions (Fig. 6f).

308 In alfalfa, AMF inoculation significantly increased plant biomass under both low-
309 and high-P conditions ($P < 0.05$, Supporting Information Fig. S2a), with mycorrhizal
310 growth responses significantly higher under high-P than low-P conditions ($P < 0.05$,
311 Supporting Information Fig. S2c). In rice, AMF inoculation had no effect on plant
312 biomass under low-P conditions, but reduced rice biomass under high-P conditions (P
313 < 0.05); mycorrhizal growth responses were significantly lower under high-P than low-
314 P conditions ($P < 0.05$, Supporting Information Fig. S2b, d). Exogenous myristate had
315 no effect on plant biomass or mycorrhizal growth response in either alfalfa or rice, while
316 soil P levels significantly influenced mycorrhizal growth response: high-P treatment

317 increased mycorrhizal growth response in alfalfa but decreased it in rice compared to
318 low-P conditions ($P < 0.01$, Supporting Information Fig. S2c, d).

319 **Effects of exogenous myristate on the transcriptome of AM roots (Exp. 3)**

320 Transcriptome analysis was performed to preliminarily explore the effects of
321 exogenous myristate on mycorrhizal roots at the transcriptional level. The RNA-seq
322 data identified 39,944 and 33,056 genes (transcripts) in alfalfa and rice, respectively.
323 Principal component analysis of gene expression profiles in mycorrhizal roots of alfalfa
324 and rice showed that the first and second principal components together explained 32.4%
325 and 44.1% of the variation, respectively (Supporting Information Fig. S3a, b). In both
326 alfalfa and rice roots, gene expression profiles with or without myristate application
327 could be partially separated under low-P conditions, but not under high-P conditions,
328 suggesting the importance of P status in myristate's effect.

329 Exogenous myristate induced a variety of differentially expressed genes (DEGs,
330 Myr^+ *vs.* Myr^-) in mycorrhizal alfalfa (LP: 308, HP: 137) and rice (LP: 590, HP: 855)
331 roots (Supporting Information Fig. S3c, d). Gene Ontology (GO) analysis of these
332 DEGs enriched several GO terms related to plant stress or defense, most of which were
333 downregulated by exogenous myristate (Fig. 7a, b). These included "defense response",
334 "response to salicylic acid", "peroxidase activity", etc., in rice, and "glycosyltransferase
335 activity" and "alternative oxidase activity" in alfalfa, suggesting that myristate may
336 modulate the normal plant defense response in AM symbiosis.

337 To further characterize this response, we subsequently analyzed the expression
338 profiles of the component genes associated with enriched GO terms that are related to
339 defense or stress, using the RNA-seq dataset (Fig. S4a, b). In alfalfa, exogenous
340 myristate significantly downregulated several key genes involved in reactive oxygen
341 species balance (*alternative oxidase 2* (*AOX2*), *AOX3*) and stress tolerance (*UDP-*

342 *glycose flavonoid glycosyltransferase* (*UFGT*), *UDP-glycosyltransferase* (*UGT*) *73C3*,
343 *UGT73C6*) under low-P conditions, as well as typical stress-responsive genes
344 (*peroxidase* (*PRX*) *P7*, *cytochromes P450* (*CYP*) *83B1*), and gene families such as
345 *geraniol 8-hydroxylase-like* (*G8HL*), *CYP71D10*, and *CYP736A12* under high-P
346 conditions (Supplementary Fig. S4a). In rice, exogenous myristate downregulated
347 several typical defense-associated transcriptional factors (*TGA2*, *TGA4*, *WRKY45*,
348 *WRKY62*, *WRKY76*, and *bZIP79*) and marker genes (*pathogenesis-related 5-like*
349 (*PR5L*), *protein phosphatase 2C 35* (*OsPP2C35*), *1-aminocyclopropane-1-carboxylate*
350 *synthase 2* (*ACS2*), *DWARF4*, *pyricularia oryzae resistance-ta* (*PITA*), *mildew*
351 *resistance locus O2* (*MLO2*), *NRR repressor homologue 1* (*RH1*), and *chitinase 1*
352 (*CHT1*)) under low-P conditions (Supplementary Fig. S4b). However, most of these
353 genes were upregulated following myristate application under high-P conditions.
354 Collectively, these results indicate that exogenous myristate modified AM-activated
355 plant defense response in plants, although this effect is influenced by host identity and
356 soil P levels.

357 **qRT-PCR verification of the effects of exogenous myristate on AM-activated**
358 **defense genes in mycorrhizal roots (Exp. 3)**

359 To verify the impact of exogenous myristate on the AM-activated defense response,
360 qRT-PCR was performed to analyze the expression of defense marker genes in both
361 plant species. In alfalfa roots, exogenous myristate significantly decreased the
362 expression of *alternative oxidase 3 (MsAOX3)* and *UDP-glycosyltransferase 85A*
363 (*MsUGT85A*) ($P < 0.05$), while its effect on *peroxidase P7* (*MsPRXP7*) depended on
364 soil P level (Myr × P: $P < 0.05$): exogenous myristate significantly decreased *MsPRXP7*
365 expression under high-P conditions ($P < 0.05$) but had no effect under low-P conditions
366 (Fig. 7c). In rice roots, the effect of exogenous myristate on *chitinase 1* (*OsCHT1*) was

367 also dependent on soil P level (Myr × P: $P < 0.05$): it marginally decreased *OsCHT1*
368 expression under low-P conditions ($P = 0.053$) but increased it under high-P conditions
369 ($P = 0.050$). Exogenous myristate significantly suppressed the expression of defense-
370 related transcriptional factor *OsTGA2* and *peroxidase 39* (*OsPRX39*) under low-P and
371 high-P conditions, respectively ($P < 0.05$, Fig. 7d). Overall, the qRT-PCR results largely
372 confirmed the inhibitory effects of myristate on AM-activated defense responses in
373 alfalfa and low-P rice.
374
375 **DISCUSSION**
376 AMF are obligate biotrophs that rely on host-derived symbiotic lipids for
377 reproduction (Bravo et al., 2017; Jiang et al., 2017; Keymer et al., 2017; Luginbuehl et
378 al., 2017). However, whether symbiotic AMF can uptake external non-symbiotic lipids
379 in the presence of plant-derived C remains poorly understood (Rillig et al., 2020; Liu
380 et al., 2023). Under asymbiotic conditions, Sugiura et al. (2020) found that among eight
381 saturated or unsaturated fatty acids (C12 to C18) and two β-monoacylglycerols supplied,
382 only myristate led to an increase in the biomass of *R. irregularis*. In our study, we
383 observed slight yet statistically significant ^{13}C enrichments in the extraradical hyphae
384 of three AMF species from both hyphal and root compartments during the $^{13}C_1$-
385 myristate tracing experiment (Fig. 1c), indicating that the extraradical hyphae of the
386 tested AMF species can absorb external non-symbiotic myristate in the presence of
387 plant-derived symbiotic carbohydrates and lipids. Several additional observations
388 further supported this finding. First, exogenous myristate specifically induced the
389 formation of branched absorbing structures in AMF extraradical hyphae (Fig. 1b),
390 which may serve as preferential sites for myristate uptake (Bago et al., 1998; Sugiura
391 et al., 2020). Second, exogenous myristate activated the transcription of marker genes

392 associated with fatty acid uptake and metabolism in extraradical hyphae (Fig. 2). Third,
393 myristate application frequently enhanced both intraradical and extraradical AMF
394 biomass (Figs. 2, 3, and 4), even when the symbiotic C supply from the plant was
395 reduced (Figs. 4 and 5), accompanied by decreased mycorrhizal P responses (Fig. 6).
396 In a priority study, myristate application also increased colonization intensity, but not
397 the extraradical biomass of AMF in tomato roots (Liu et al., 2023). Overall, our findings
398 indicate that the extraradical hyphae of symbiotic AMF can uptake external non-
399 symbiotic fatty acids like myristate beyond those directly delivered by the host, at least
400 under the conditions studied.

401 Notably, myristate has been frequently detected in root exudates and soil substrates
402 from non-targeted metabolome analyses (Li et al., 2017; Swenson et al., 2018; Dai et
403 al., 2022; Liu et al., 2024), although its specific levels in various soil environments
404 remain largely unknown. Our field survey demonstrates that myristate is widely present
405 in soil, litter, and plant (Fig. 4b), with its environmental level (2.87−14.68 mg kg^{-1})
406 encompassing the myristate treatments applied in our greenhouse experiment (Exp. 3,
407 totaling 1.83 mg per 1000 ml or 300 ml soil substrate in rice and alfalfa, respectively).
408 Therefore, it is likely that the widespread AMF extraradical hyphae network may
409 absorb myristate from soil environments such as root exudates, decaying plants, or litter
410 in realistic environments. Further research is still needed to validate this hypothesis;
411 however, it effectively explains a long-observed but poorly understood phenomenon—
412 AMF often colonize and thrive in leaf litter and undecomposed plant material despite
413 their limitations in assimilating organic forms of nutrients (Went and Stark, 1968;
414 Zhang et al., 2018; Bunn et al., 2019)—as the myristate concentration in plant and leaf
415 litter is higher than that in soil (Fig. 4b).

416 During AM establishment, plant roots typically activate low-level defense

417 responses to limit excessive C consumption caused by over-colonization (Blilou et al.,
418 2000; Feng et al., 2019). In our study, myristate increased both intraradical and
419 extraradical biomass of the symbiotic AMF and promoted the formation of normal-
420 sized secondary spores (Figs. 2 and 3), unlike the small spores induced by myristate
421 under asymbiotic conditions (Sugiura et al., 2020). This suggests that plant roots supply
422 additional substances to AMF beyond myristate, the identification of which is essential
423 for the pure culture of AMF. Meanwhile, exogenous myristate transcriptionally
424 downregulated plant genes associated with AM-activated defenses under low-P
425 conditions (Fig. 7), suggesting that myristate may interfere with normal defense
426 mechanisms in host roots to facilitate AMF colonization. This effect contrast with AM
427 signals like strigolactones and quercetin, which enhance plant defenses (Sudheeran et
428 al., 2020; Kusajima et al., 2022). Further research is required to clarify myristate's role
429 in regulating AMF colonization and development.

430 Despite increased arbuscule abundance (Fig. 4)—where symbiotic nutritional
431 exchange occurs (Smith and Read, 2008)—exogenous myristate unexpectedly reduced
432 the mycorrhizal P response and downregulated plant and fungal mycorrhizal P
433 transporter gene expression in both alfalfa and rice roots (Fig. 6), indicating a
434 diminished mycorrhizal P benefit through inactivation of mycorrhizal P pathway
435 transcription. Meanwhile, exogenous myristate also reduced C supply to AMF from
436 both alfalfa and rice, except in low-P rice (Fig. 5). Together, these results suggest that
437 exogenous myristate disrupts the symbiotic C-P exchange, which is functionally
438 coupled in AM symbiosis (Kiers et al., 2011). This disruption could be attributed to
439 several factors. First, it may result from AMF partially shifting from utilizing symbiotic
440 C to absorbing external C (myristate, Fig. 1), thus reducing their competition for
441 symbiotic C at the AM interaction interface (Bitterlich et al., 2014). Correspondingly,

442 this decline in symbiotic C supply to AMF may decrease mycorrhizal P benefit, since
443 AMF can modulate P transport based on the rate at which plants supply C (Kiers et al.,
444 2011; Argüello et al., 2016). Second, exogenous myristate may interfere with AM
445 signaling, as implied by the interference in normal AM-activated defenses (Fig. 7),
446 thereby limiting nutrient exchange in the symbiosis. Third, AMF P transport proteins
447 expressed in arbuscular cells can re-uptake P from the symbiotic interface, creating P
448 competition between the plant and AMF (Xie et al., 2022). In our study, the increased
449 fungal biomass with exogenous myristate suggests a higher P demand by AMF,
450 reducing the feasibility of nutritional trade.

451 In contrast to its effect under high-P treatment, myristate increased, rather than
452 decreased, the allocation of rice-derived C to AMF under low-P conditions,
453 accompanied by enhanced expression of marker genes for fatty acid (but not sugar)
454 allocation from rice to AMF (Fig. 5b, f, g). These results highlight that myristate's
455 impact on AM symbiosis is influenced by soil P status, a significant regulatory factor
456 of the symbiosis by coordinating the direct P pathway and mycorrhizal P pathway in
457 AM plants (Shi et al., 2021; Das et al., 2022). However, the significant effects of soil P
458 status on myristate's impact on C-P exchange were not observed in alfalfa (Figs. 5 and
459 6), where the unexpectedly higher AMF intraradical biomass (Fig. 4) and greater
460 mycorrhizal growth response (Fig. S2) in the high-P compared to the low-P treatment
461 are likely due to the inhibitory effects of extremely low-P environments on AMF
462 colonization and functioning (Janos, 2007; Wang et al., 2020). The varying responses
463 of myristate's effect to soil P treatment between rice and alfalfa may arise not only from
464 the different status of plant P nutrition but also from their varying symbiotic strategies:
465 AM-responsive alfalfa demonstrated significantly higher efficiency in C-P nutritional
466 exchange compared to AM-nonresponsive rice (Figs. 5 and 6). Collectively, our

467 findings regarding the disruptive effects of myristate on symbiotic C-P exchange, along
468 with the broad yet varied presence of myristate in different soil components (Fig. 4),
469 imply that myristate may be a critical influencing factor in AM symbiosis. Interestingly,
470 myristate also exhibited inhibitory effects on rhizospheric *Devosia*, which is
471 responsible for promoting growth and suppressing disease in *Arabidopsis* (Liu et al.,
472 2024). Further research is required to clarify the mechanisms underlying the disruptive
473 effect of exogenous myristate on symbiotic C-P exchange. Nevertheless, our findings
474 advise caution against the application of myristate, and possibly other non-symbiotic C
475 sources of AMF, to enhance AM symbiosis, as it disrupts the symbiotic nutritional
476 equilibrium and may potentially weaken plant immunity.

477 Despite these encouraging findings, our work has several limitations. First, the
478 absorption of myristate by symbiotic AMF was observed only after exogenous
479 application under artificial conditions, which may not accurately reflect natural
480 environments. Second, our study focused exclusively on myristate and AMF species
481 from the genus *Rhizophagus*, although different AMF groups may respond to myristate
482 or other fatty acids in various ways, at least under asymbiotic conditions (Kameoka et
483 al., 2019; Sugiura et al., 2020). Third, our investigation into the mechanism by which
484 myristate disrupts C-P exchange in AM symbiosis remains preliminary, and the limited
485 P supply along with varied growth conditions between alfalfa and rice further
486 complicate the mechanistic analysis. Therefore, further research is needed to verify and
487 quantitatively assess the absorption of external myristate and other C sources by
488 symbiotic AMF under natural conditions, as well as to elucidate their regulatory
489 mechanisms and ecological significance in AM symbiosis.

490

491 **CONCLUSIONS**

492 In summary (Fig. 8), our study demonstrates that symbiotic AMF can uptake
493 external non-symbiotic fatty acids such as myristate, which is commonly present in soil
494 components. Exogenous myristate enhanced both intraradical and extraradical biomass
495 of symbiotic AMF, possibly linked to the suppression of AM-activated defense
496 responses in host roots. Unexpectedly, exogenous myristate disrupted the C-P exchange
497 in AM symbiosis, resulting in reduced mycorrhizal P benefits for plants and decreased
498 allocation of symbiotic C to AMF, indicating that the application of exogenous
499 myristate may not be a promising strategy to enhance AM symbiosis. These findings
500 provide new insights into the nutritional interactions in AM symbiosis, which are
501 crucial for understanding and managing this ancient relationship in a changing world.
502

503 **MATERIALS AND METHODS**

504 **Biological materials and experimental design**

505 *In vitro* cultures of AMF species *Rhizophagus irregularis* (strain: MUCL 41833),
506 *R. intraradices* (strain: MUCL 49410), and *R. diaphanus* (strain: MUCL 49416)
507 associated with carrot (*Daucus carota*) hairy roots were generously supplied by the
508 Glomeromycota *In vitro* Collection (GINCO).

509 Our study consisted of three interconnected and complementary experiments. In
510 brief, we investigated whether exogenous myristate could be directly absorbed by *R.*
511 *irregularis*, *R. intraradices*, and *R. diaphanus* (Exp. 1), and examined its effect on the
512 growth and development of *R. irregularis* (Exp. 2), using Ri T-DNA-transformed carrot
513 roots as the AMF host. In Exp. 3, a field survey was conducted to evaluate the
514 environmental distribution of myristate in soil and plants, along with a pot experiment
515 evaluating the effect of exogenous myristate on C-P nutritional exchanges in AM
516 symbiosis between *R. irregularis* and two host species.

517 **Exp. 1: $^{13}C_1$-labeled myristate ($^{13}C_1$-Myr) tracing in two-compartment AMF-hairy**
518 **root co-culture systems**

519 In Exp. 1, $^{13}C_1$-Myr tracing experiments were performed to determine whether
520 exogenous myristate can be directly absorbed by the extraradical hyphae of
521 *Rhizophagus irregularis* (two independent trials), *R. intraradices*, and *R. diaphanus*
522 associated with carrot hairy root cultivated in solid modified Strullu–Romand (MSR)
523 medium, utilizing a two-compartment Petri dish system (90 × 15 mm, see Fig. 1a) as
524 described by St-Arnaud et al. (1996), with minor modifications (see Supporting
525 Information Methods S1).

526 When dense AMF extraradical hyphae were observed in the hyphae compartment
527 (HC), 12–14 weeks later, 200 μL of 0.1 mM myristate (ZZBIO, China,
528 https://www.zzsrm.cn/product/289111) with 98% $^{13}C_1$ labeling ($^{13}C_1$-Myr) or unlabeled
529 (^{12}C-Myr, control) myristate (Sigma-Aldrich, China,
530 https://www.sigmaaldrich.cn/CN/zh/product/sigma/m3128) was added weekly to the
531 HC until harvest, two or three weeks later. At harvest, AMF extraradical hyphae from
532 the HC and root compartment (RC) were collected separately by dissolving the medium
533 in 10 mM sodium citrate, filtering through a 37 μm nylon mesh, and thoroughly rinsing
534 five times with sodium citrate followed by ultrapure water. To check for possible
535 contamination of ^{13}C-myristate from the HC to the RC, a small portion of the MSR
536 medium from the RC was also collected randomly from three $^{13}C_1$-Myr and three ^{12}C-
537 Myr (control) treatments, with roots and AMF hyphae removed using tweezers under a
538 stereomicroscope (M250FA, Leica, Germany), and then used for determination of ^{13}C
539 enrichment. During the experiment, the MSR medium in the RC was not contaminated
540 by ^{13}C-myristate from the HC, as evidenced by the identical $^{13}C/^{12}C$ ratios of the MSR
541 medium collected in the RC in $^{13}C_1$-Myr (1.0966 ± 0.0012) and ^{12}C-Myr (1.0967 ±

542 0.0032) treatments.

543 The determination of ^{13}C enrichment in AMF extraradical hyphae and solid MSR
544 medium was performed as previously described (Bao et al., 2019), with the details
545 provided in the Supporting Information Methods S2.

546 **Quantitative real-time PCR (qRT-PCR) targeting fungal marker genes and**
547 **quantification of AMF colonization intensity (Exp. 1)**

548 In Exp. 1, the expression of fungal marker genes related to fatty acid uptake
549 (*RiFAT1* and *RiFAT2*, Brands and Dörmann, 2022) and metabolism—β-oxidation (*fatty*
550 *acid degradation 1* (*RiFAD1*)), palmitvaccenic acid synthesis (*oleate desaturase-like*
551 *enzyme 1* (*RiOLE1*), Brands et al., 2020), and the TCA cycle (*citrate synthase 1*
552 (*RiCIT1*))—in extraradical hyphae of *R. irregularis* from the HC, with or without
553 myristate addition, was assessed, using the elongation factor-1 beta gene (*RiEF-1β*) as
554 the reference gene (Das et al., 2022). Relative gene expression levels were calculated
555 using the comparative $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), with four
556 independent biological replicates and three technical replicates per biological replicate.
557 The collection of extraradical hyphae from the HC and qRT-PCR procedures is detailed
558 in the Supporting Information (Method S3, Table S1).

559 Carrot hairy roots in the RC from the two-compartment AMF-hairy root co-culture
560 system (Exp. 1) were also harvested using sterile tweezers and used to assess AMF
561 colonization intensity (see Supporting Information Methods S4).

562 **Exp. 2: Effects of exogenous myristate on AMF development in single-**
563 **compartment AMF-hairy root co-culture systems**

564 In Exp. 2, we assessed the effects of exogenous myristate at varying concentrations
565 on spore germination and the development of *R. irregularis* intraradical and extraradical
566 structures associated with carrot hairy roots in a single-compartment co-culture system.

567 Briefly, three 4-cm-long sterile carrot hairy root segments were cultivated in MSR
568 medium with or without *R. irregularis* inoculation (200 sterilized spores per Petri dish).
569 Potassium myristate solution (0, 0.1, 0.5, 1, 5, or 10 mM, 200 μL) was applied to the
570 plates weekly until harvest, with five biological replicates (plates) per treatment. The
571 germination rate of AMF spores, as well as extraradical hyphal branching and length,
572 were monitored under a stereomicroscope (M250FA, Leica, Germany) and calculated
573 using ImageJ (version 2.0.0) at 4 days post-inoculation (Schneider et al., 2012). Spore
574 density was monitored every 2 weeks starting 8 weeks after AMF inoculation.

575 Carrot hairy roots and MSR medium were harvested at 14 weeks post-AMF
576 inoculation. The roots and solid culture medium were carefully separated using sterile
577 tweezers. The medium was weighed and dissolved in 10 mM sodium citrate, and the
578 AMF collection was preliminarily separated into hyphae and spores using a blender,
579 followed by filtration through a 250 μm and 37 μm nylon meshes, respectively, and
580 manual selection with tweezers under a stereomicroscope (M250FA, Leica, Germany).
581 Biomasses of the extraradical hyphae and spores were oven-dried at 80°C for 48 hours
582 and quantified separately. To evaluate the development of AMF intraradical structures
583 in the carrot hairy roots, hyphal, vesicle, and arbuscular colonization intensities were
584 assessed (Supporting Information Methods S4).

585 **Exp. 3: Environmental distribution of myristate and the effect of exogenous**
586 **myristate on AM symbiosis**

587 In Exp. 3, a field survey and a pot experiment were conducted. The field survey
588 aimed to evaluate the environmental distribution of myristate in soil and plants. We
589 analyzed myristate concentrations in bulk soil (0-10 cm) and in plant leaves, roots, leaf
590 litter, and rhizosphere soil from the dominant species *O. sativa*, *Axonopus compressus*,
591 and *Morus alba* in a paddy field, grassland, and woodland, respectively. The procedures

592 regarding sample collection, pretreatment, and analysis of myristate were detailed in
593 Supporting Information Methods S5.

594 The pot experiment in Exp. 3 was conducted to assess the effect of exogenous
595 myristate on C-P nutritional exchanges in AM symbiosis between *R. irregularis* and
596 two phylogenetically distant plant species—the AM-responsive plant *Medicago sativa*
597 L. cv. Sanditi (alfalfa, dicot) and the AM-nonresponsive plant *Oryza sativa* L. cv.
598 Zhonghua 11 (rice, monocot) (Hata et al., 2010; Li et al., 2024). This was achieved
599 using ^{13}C-neutral lipid fatty acid (NLFA)-isotope ratio mass spectrometry technology,
600 along with physiological and transcriptome analyses. The pot experiment employed a
601 full factorial design with four treatment factors: myristate addition (with or without 0.1
602 mM myristate), AMF inoculation (with or without *R. irregularis*), host species (alfalfa
603 or rice), and soil P level (relatively low or high). Ten biological replicates were included
604 per treatment, totaling 160 pots. The experimental procedures for plant cultivation, P or
605 myristate treatment, plant harvest, and measurement of plant P concentration are
606 detailed in Supporting Information Methods S6.

607 **RNA sequencing (RNA-seq) on alfalfa and rice roots and bioinformatics (Exp. 3)**

608 In Exp. 3, alfalfa and rice roots inoculated with AMF, with or without myristate
609 application at two P levels, were subjected to RNA sequencing (RNA-seq). Three
610 biological replicates (roots from a single pot per replicate) were randomly selected from
611 ten replicates per treatment, resulting in a total of 24 samples. The procedures for RNA-
612 seq and bioinformatics followed our previous study (Wang et al., 2023), with details
613 provided in Supporting Information Methods S7.

614 **Quantitative real-time PCR (qRT-PCR, Exp. 3)**

615 In Exp. 3, RNA extracted from alfalfa or rice roots was reverse-transcribed into
616 cDNA using the QuantiTect Reverse Transcription Kit (Qiagen, Valencia, CA). qRT-

PCR was performed, with three independent biological replicates (randomly selected from ten replicates) for each treatment and three technical replicates per biological replicate. The relative expression levels of plant and fungal genes involved in C delivery from plants to AMF (*MsSTR2*, *OsSTR2*, *RiFAT1*, *RiFAT2*, and *RiMST2*) (Brands and Dörmann, 2022; Das et al., 2022), the mycorrhizal P pathway (*MsPT8*, *OsPT11*, and *RiPT*) (Yang et al., 2012; Fiorilli et al., 2013), and AM-activated plant defense—specifically, reactive oxygen species balance (*MsAOX3*), stress responses (*MsUGT85A*, *MsPRXP7*, and *OsPRX39*), and typical defense marker (*OsCHT1* and *OsTGA2*) (Rehman et al., 2018; Suleman et al., 2021; Zhang et al., 2021)—were analyzed to characterize the impact of exogenous myristate on C-P nutritional exchange in AM symbiosis and the underlying mechanisms at the transcriptional level. The alfalfa *actin 2* (*MsACT2*), rice *cyclin 2* (*OsCyc2*), or *R. irregularis elongation factor-1 beta* (*RiEF-1β*) genes were used as reference genes (Yang et al., 2012; Das et al., 2022), with the procedures and primers provided in the Supporting Information (Method S3, Table S1).

^{13}C labeling of plants, lipid extraction, measurement of AMF signature fatty acids, and quantification of ^{13}C enrichment and ^{13}C flow (Exp. 3)

In Exp. 3, ^{13}C labeling of plants, lipid extraction, measurement of AMF signature fatty acids (NLFA 16:1ω5, Olsson et al., 2005), and quantification of ^{13}C enrichment and ^{13}C flow to NLFA 16:1ω5 were performed as described in previous studies (Olsson et al., 2005; Bao et al., 2019), with details provided in Supporting Information Methods S8.

Statistical analysis

Mycorrhizal response was calculated based on plant biomass (mycorrhizal growth response) and plant P concentration (mycorrhizal P response), respectively, following Corrêa et al. (2014): Mycorrhizal response (%) = (AM − NM) / NM × 100%, where

642 AM denotes the observed value for the target parameter (biomass or P concentration)
643 in mycorrhizal plants, and NM represents the mean value of nonmycorrhizal plants.
644 Statistical analyses were performed using one-way ANOVA to compare ^{13}C enrichment
645 levels in ERH among different AMF species and to evaluate the effect of myristate on
646 AMF growth. Two-way ANOVA was used to evaluate the effects of myristate,
647 phosphorus level, and their interaction on AMF colonization intensity, the concentration
648 of AMF signature fatty acids, the expression levels of *RiEF-1β*, and metrics of plant
649 carbon-phosphorus exchange. A three-way ANOVA was applied to evaluate the effects
650 of AMF inoculation, myristate, phosphorus level, and their interactions on plant
651 phosphorus concentration. Post-hoc comparisons were conducted using Tukey's test
652 (equal variances) or Games-Howell's test (unequal variances). Significant differences
653 between AM and NM groups or between myristate-treated and untreated groups were
654 determined using Student's t test (for normally distributed data) or Mann-Whitney U-
655 test (for non-normally distributed data).
656

657 **Author Contributions**

658 **Hanwen Chen:** Investigation, Formal analysis, Methodology, Visualization. **Tian**
659 **Xiong:** Investigation, Formal analysis, Data curation, Methodology. **Baoxing Guan:**
660 Writing – original draft, Investigation, Formal analysis, Data curation, Visualization.
661 **Jiaqi Huang:** Investigation, Methodology, Resources. **Danrui Zhao:** Investigation,
662 Resources. **Yao Chen:** Investigation, Resources. **Haoran Liang:** Investigation,
663 Visualization. **Yingwei Li:** Investigation, Resources. **Jingwen Wu:** Investigation,
664 Resources. **Shaoping Ye:** Resources. **Ting Li:** Resources. **Wensheng Shu:** Supervision.
665 **Jin-tian Li:** Supervision. **Yutao Wang:** Conceptualization, Data curation, Writing –

original draft, Writing – review & editing, Funding acquisition, Supervision, Project administration.

Acknowledgments

We thank the financial support from the National Key R&D Program of China (Key special Project for Marine Environmental Security and Sustainable Development of Coral Reefs 2022-702), the National Natural Science Foundation of China (nos. 32272203 and 31772397), the Fujian Province Science and Technology Plan Project (no. 2024I1011), the Shenzhen Science and Technology Program (KQTD20190929172839621), the Agricultural and Social Development Project of Guangzhou Municipal Science and Technology Bureau (no. 202206010058), and the Guangdong Provincial Key Laboratory of Plant Resources (no. 2023PlantKFP03).

Data Availability

The RNA-seq data generated in this study have been deposited in the Sequence Read Archive at the National Center for Biotechnology Information under BioProject ID PRJNA1083641. The raw data are provided as a Source Data File.

Competing Interest

The authors declare that they have no competing interests.

References

Argüello A, O'Brien MJ, van der Heijden MGA, Wiemken A, Schmid B, Niklaus PA. 2016. Options of partners improve carbon for phosphorus trade in the arbuscular mycorrhizal mutualism. *Ecology Letters* **19**: 648–656.

691 Bago B, Azcón-Aguilar C, Goulet A, Piché Y. 1998. Branched absorbing structures
692 (BAS): A feature of the extraradical mycelium of symbiotic arbuscular
693 mycorrhizal fungi. *New Phytologist* **139**: 375–388.
694 Bao X, Wang Y, Olsson PA. 2019. Arbuscular mycorrhiza under water—Carbon–
695 phosphorus exchange between rice and arbuscular mycorrhizal fungi under
696 different flooding regimes. *Soil Biology and Biochemistry* **129**: 169–177.
697 Bitterlich M, Krügel U, Boldt-Burisch K, Franken P, Kühn C. 2014. The sucrose
698 transporter SlSUT2 from tomato interacts with brassinosteroid functioning and
699 affects arbuscular mycorrhiza formation. *The Plant Journal* **78**: 877–889.
700 Blilou I, Ocampo JA, García-Garrido JM. 2000. Induction of *Ltp* (lipid transfer protein)
701 and *Pal* (phenylalanine ammonia-lyase) gene expression in rice roots colonized by
702 the arbuscular mycorrhizal fungus *Glomus mosseae*. *Journal of Experimental*
703 *Botany* **51**: 1969–1977.
704 Brands M, Cahoon EB, Dörmann P. 2020. Palmitvaccenic Acid (Δ11-cis-hexadecenoic
705 acid) is synthesized by an OLE1-like desaturase in the arbuscular mycorrhiza
706 fungus *Rhizophagus irregularis*. *Biochemistry* **59**: 1163–1172.
707 Brands M, Dörmann P. 2022. Two AMP-binding domain proteins from *Rhizophagus*
708 *irregularis* involved in import of exogenous fatty acids. *Molecular Plant-Microbe*
709 *Interactions* **35**: 464–476.
710 Bravo A, Brands M, Wewer V, Dörmann P, Harrison MJ. 2017. Arbuscular mycorrhiza-
711 specific enzymes FatM and RAM2 fine-tune lipid biosynthesis to promote
712 development of arbuscular mycorrhiza. *New Phytologist* **214**: 1631–1645.
713 Brundrett MC, Tedersoo L. 2018. Evolutionary history of mycorrhizal symbioses and
714 global host plant diversity. *New Phytologist* **220**: 1108–1115.
715 Bunn RA, Simpson DT, Bullington LS, Lekberg Y, Janos DP. 2019. Revisiting the

716 “direct mineral cycling” hypothesis: arbuscular mycorrhizal fungi colonize leaf
717 litter, but why? *The ISME Journal* **13**: 1891–1898.
718 Corrêa A, Cruz C, Pérez-Tienda J, Ferrol N. 2014. Shedding light onto nutrient
719 responses of arbuscular mycorrhizal plants: nutrient interactions may lead to
720 unpredicted outcomes of the symbiosis. *Plant Science* **221–222**: 29–41.
721 Dai H, Zhang X, Zhao B, Shi J, Zhang C, Wang G, Yu N, Wang E. 2022. Colonization
722 of mutualistic mycorrhizal and parasitic blast fungi requires OsRAM2-regulated
723 fatty acid biosynthesis in rice. *Molecular Plant-Microbe Interactions* **35**: 178–186.
724 Das D, Paries M, Hobecker K, Gigl M, Dawid C, Lam HM, Zhang J, Chen M, Gutjahr
725 C. 2022. PHOSPHATE STARVATION RESPONSE transcription factors enable
726 arbuscular mycorrhiza symbiosis. *Nature Communications* **13**: 1–12.
727 Feng F, Sun J, Radhakrishnan GV, Lee T, Bozsóki Z, Fort S, Gavrin A, Gysel K,
728 Thygesen MB, Andersen KR, Radutoiu S, Stougaard J, Oldroyd GED. 2019. A
729 combination of chitooligosaccharide and lipochitooligosaccharide recognition
730 promotes arbuscular mycorrhizal associations in *Medicago truncatula*. *Nature*
731 *Communications* **10**: 5047.
732 Fiorilli V, Lanfranco L, Bonfante P. 2013. The expression of *GintPT*, the phosphate
733 transporter of *Rhizophagus irregularis*, depends on the symbiotic status and
734 phosphate availability. *Planta* **237**: 1267–1277.
735 Foster KR, Kokko H. 2006. Cheating can stabilize cooperation in mutualisms.
736 *Proceedings of the Royal Society B: Biological Sciences* **273**: 2233–2239.
737 Gutjahr C, Radovanovic D, Geoffroy J, Zhang Q, Siegler H, Chiapello M, Casieri L,
738 An K, An G, Guiderdoni E, Kumar CS, Sundaresan V, Harrison MJ, Paszkowski
739 U. 2012. The half-size ABC transporters STR1 and STR2 are indispensable for
740 mycorrhizal arbuscule formation in rice. *The Plant Journal* **69**: 906–920.

741 Hata S, Kobae Y, Banba M. 2010. Interactions between plants and arbuscular
742 mycorrhizal fungi. *International Review of Cell and Molecular Biology* **281**: 1–48.
743 Helber N, Wippel K, Sauer N, Schaarschmidt S, Hause B, Requena N. 2011. A versatile
744 monosaccharide transporter that operates in the arbuscular mycorrhizal fungus
745 *Glomus sp* is crucial for the symbiotic relationship with plants. *The Plant Cell* **23**:
746 3812–3823.
747 Huang R, Li Z, Mao C, Zhang H, Sun Z, Li H, Huang C, Feng Y, Shen X, Bucher M.
748 2020. Natural variation at OsCERK1 regulates arbuscular mycorrhizal symbiosis
749 in rice. *New Phytologist* **225**: 1762–1776.
750 Janos DP. 2007. Plant responsiveness to mycorrhizas differs from dependence upon
751 mycorrhizas. *Mycorrhiza* **17**: 75–91.
752 Jiang Y, Wang W, Xie Q, Liu N, Liu L, Wang D, Zhang X, Yang C, Chen X, Tang D,
753 Wang E. 2017. Plants transfer lipids to sustain colonization by mutualistic
754 mycorrhizal and parasitic fungi. *Science* **356**: 1172–1175.
755 Johnson NC. 2010. Resource stoichiometry elucidates the structure and function of
756 arbuscular mycorrhizas across scales. *New Phytologist* **185**: 631–647.
757 Kameoka H, Tsutsui I, Saito K, Kikuchi Y, Handa Y, Ezawa T, Hayashi H, Kawaguchi
758 M, Akiyama K. 2019. Stimulation of asymbiotic sporulation in arbuscular
759 mycorrhizal fungi by fatty acids. *Nature Microbiology* **4**: 1654–1660.
760 Keymer A, Pimprikar P, Wewer V, Huber C, Brands M, Bucerius SL, Delaux PM, Klingl
761 V, Röpenack-Lahaye EV, Wang TL, Eisenreich W, Dörmann P, Parniske M,
762 Gutjahr C. 2017. Lipid transfer from plants to arbuscular mycorrhiza fungi. *eLife*
763 **6**: e29107.
764 Kiers ET, Duhamel M, Beesetty Y, Mensah J, Franken O, Verbruggen E, Fellbaum CR,
765 Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoornhuyse P,

766 Jansa J, Bücking H. 2011. Reciprocal rewards stabilize cooperation in the
767 mycorrhizal symbiosis. *Science* **333**: 880–882.
768 Kusajima M, Fujita M, Soudthedlath K, Nakamura H, Yoneyama K, Nomura T,
769 Akiyama K, Maruyama-Nakashita A, Asami T, Nakashita H. 2022. Strigolactones
770 modulate salicylic acid-mediated disease resistance in *Arabidopsis thaliana*.
771 *International Journal of Molecular Sciences* **23**: 5246.
772 Lanfranco L, Fiorilli V, Gutjahr C. 2018. Partner communication and role of nutrients
773 in the arbuscular mycorrhizal symbiosis. *New Phytologist* **220**: 1–10.
774 Li S, Xu C, Wang J, Guo B, Yang L, Chen J, Ding W. 2017. Cinnamic, myristic and
775 fumaric acids in tobacco root exudates induce the infection of plants by *Ralstonia*
776 *solanacearum*. *Plant and Soil* **412**: 381–395.
777 Li Y, Chen H, Gu L, Wu J, Zheng X, Fan Z, Pan D, Li JT, Shu W, Rosendahl S, Wang
778 Y. 2024. Domestication of rice may have changed its arbuscular mycorrhizal
779 properties by modifying phosphorus nutrition-related traits and decreasing
780 symbiotic compatibility. *New Phytologist* **243**: 1554–1570.
781 Liu X, Feng Z, Zhang W, Yao Q, Zhu H. 2023. Exogenous myristate promotes the
782 colonization of arbuscular mycorrhizal fungi in tomato. *Frontiers in Plant Science*
783 **14**: 1250684.
784 Liu Y, Zhang H, Wang J, Gao W, Sun X, Xiong Q, Shu X, Miao Y, Shen Q, Xun W,
785 Zhang R. 2024. Nonpathogenic *Pseudomonas syringae* derivatives and its
786 metabolites trigger the plant "cry for help" response to assemble disease
787 suppressing and growth promoting rhizomicrobiome. *Nature Communications* **15**:
788 1907.
789 Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-
790 time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **25**: 402–408.

791 Luginbuehl LH, Menard GN, Kurup S, Van Erp H, Radhakrishnan GV, Breakspear A,
792 Oldroyd GED, Eastmond PJ. 2017. Fatty acids in arbuscular mycorrhizal fungi are
793 synthesized by the host plant. *Science* **356**: 1175–1178.
794 MacLean AM, Bravo A, Harrison MJ. 2017. Plant signaling and metabolic pathways
795 enabling arbuscular mycorrhizal symbiosis. *The Plant Cell* **29**: 2319–2335.
796 Mori N, Nishiuma K, Sugiyama T, Hayashi H, Akiyama K. 2016. Carlactone-type
797 strigolactones and their synthetic analogues as inducers of hyphal branching in
798 arbuscular mycorrhizal fungi. *Phytochemistry* **130**: 90–98.
799 Olsson PA, van Aarle IM, Gavito ME, Bengtson P, Bengtsson G. 2005. ^{13}C
800 incorporation into signature fatty acids as an assay for carbon allocation in
801 arbuscular mycorrhiza. *Applied and Environmental Microbiology* **71**: 2592–2599.
802 Rehman HM, Nawaz MA, Shah ZH, Ludwig-Müller J, Chung G, Ahmad MQ, Yang
803 SH, Lee SI. 2018. Comparative genomic and transcriptomic analyses of Family-1
804 UDP glycosyltransferase in three *Brassica* species and *Arabidopsis* indicates
805 stress-responsive regulation. *Scientific Reports* **8**: 1875.
806 Rich MK, Vigneron N, Libourel C, Keller J, Xue L, Hajheidari M, Radhakrishnan GV,
807 Le Ru A, Diop SI, Potente G, Conti E, Duijsings D, Batut A, Le Faouder P,
808 Kodama K, Kyozuka J, Sallet E, Bécard G, Rodriguez-Franco M, Ott T, Bertrand-
809 Michel J, Oldroyd GED, Szövényi P, Bucher M, Delaux PM. 2021. Lipid
810 exchanges drove the evolution of mutualism during plant terrestrialization.
811 *Science* **372**: 864–868.
812 Rillig MC, Aguilar-Trigueros CA, Anderson IC, Antonovics J, Ballhausen MB,
813 Bergmann JBM, Chaudhary VB, Deveautour C, Grünfeld L, Hempel S, Lakovic
814 M, Lammel DR, Lehmann A, Lehmann J, Leifheit EF, Liang Y, Li E, Lozano YM,
815 Manntschke A, Mansour I, Oviatt P, Pinek L, Powell JR, Roy J, Ryo M, Sosa-

816 Hernández MA, Veresoglou SD, Wang D, Yang G, Zhang H. 2020. Myristate and
817 the ecology of AM fungi: significance, opportunities, applications and challenges.
818 *New Phytologist* **227**: 1610–1614.
819 Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of
820 image analysis. *Nature Methods* **9**: 671–675.
821 Shi J, Zhao B, Zheng S, Zhang X, Wang X, Dong W, Xie Q, Wang G, Xiao Y, Chen F,
822 Yu N, Wang E. 2021. A phosphate starvation response-centered network regulates
823 mycorrhizal symbiosis. *Cell* **184**: 5527–5540.
824 Smith SE, Read DJ. 2008. *Mycorrhizal symbiosis*. Academic press, London.
825 St-Arnaud M, Hamel C, Vimard B, Caron M, Fortin JA. 1996. Enhanced hyphal growth
826 and spore production of the arbuscular mycorrhizal fungus *Glomus intraradices*
827 in an in vitro system in the absence of host roots. *Mycological Research* **100**: 328–
828 332.
829 Sudheeran PK, Ovadia R, Galsarker O, Maoz I, Sela N, Maurer D, Feygenberg O, Oren
830 Shamir M, Alkan N. 2020. Glycosylated flavonoids: fruit's concealed antifungal
831 arsenal. *New Phytologist* **225**: 1788–1798.
832 Sugiura Y, Akiyama R, Tanaka S, Yano K, Kameoka H, Marui S, Saito M, Kawaguchi
833 M, Akiyama K, Saito K. 2020. Myristate can be used as a carbon and energy source
834 for the asymbiotic growth of arbuscular mycorrhizal fungi. *Proceedings of the*
835 *National Academy of Sciences of the United States of America* **117**: 25779–25788.
836 Suleman M, Ma M, Ge G, Hua D, Li H. 2021. The role of alternative oxidase in plant
837 hypersensitive response. *Plant Biology* **23**: 415–419.
838 Swenson TL, Karaoz U, Swenson JM, Bowen BP, Northen TR. 2018. Linking soil
839 biology and chemistry in biological soil crust using isolate exometabolomics.
840 *Nature Communications* **9**: 19.

841 Tanaka S, Hashimoto K, Kobayashi Y, Yano K, Maeda T, Kameoka H, Ezawa T, Saito
842 K, Akiyama K, Kawaguchi M. 2022. Asymbiotic mass production of the
843 arbuscular mycorrhizal fungus *Rhizophagus clarus*. *Communications Biology* **5**:
844 43.
845 Wang XX, Li HB, Chu Q, Feng G, Kuyper TW, Rengel Z. 2020. Mycorrhizal impacts
846 on root trait plasticity of six maize varieties along a phosphorus supply gradient.
847 *Plant and Soil* **448**: 71–86.
848 Wang YT, Chen HW, Shao MS, Zhu T, Li SS, Olsson PA, Hammer EC. 2023.
849 Arbuscular mycorrhizal fungi trigger danger-associated peptide signaling and
850 inhibit carbon–phosphorus exchange with nonhost plants. *Plant Cell &*
851 *Environment* **46**: 2206–2221.
852 Watts-Williams SJ, Emmett BD, Levesque-Tremblay V, MacLean AM, Sun X, Satterlee
853 JW, Fei Z, Harrison MJ. 2019. Diverse Sorghum bicolor accessions show marked
854 variation in growth and transcriptional responses to arbuscular mycorrhizal fungi.
855 *Plant Cell and Environment* **42**: 1758–1774.
856 Went FW, Stark N. 1968. The biological and mechanical role of soil fungi. *Proceedings*
857 *of the National Academy of Sciences of the United States of America* **60**: 497–504.
858 Wipf D, Krajinski F, van Tuinen D, Recorbet G, Courty PE. 2019. Trading on the
859 arbuscular mycorrhiza market: from arbuscules to common mycorrhizal networks.
860 *New Phytologist* **223**: 1127–1142.
861 Xie X, Lai W, Che X, Wang S, Ren Y, Hu W, Chen H, Tang M. 2022. A SPX domain-
862 containing phosphate transporter from *Rhizophagus irregularis* handles phosphate
863 homeostasis at symbiotic interface of arbuscular mycorrhizas. *New Phytologist*
864 **234**: 650–671.
865 Yang S, Grønlund M, Jakobsen I, Grotemeyer MS, Rentsch D, Miyao A, Hirochika H,

866 Kumar CS, Sundaresan V, Salamin N, Catausan S, Mattes N, Heuer S, Paszkowski
867 U. 2012. Nonredundant regulation of rice arbuscular mycorrhizal symbiosis by
868 two members of the phosphate transporter1 gene family. *The Plant Cell* **24**: 4236–
869 4251.
870 Yu H, He Y, Zhang W, Chen L, Zhang J, Zhang X, Dawson W, Ding J. 2022. Greater
871 chemical signaling in root exudates enhances soil mutualistic associations in
872 invasive plants compared to natives. *New Phytologist* **236**: 1140–1153.
873 Zhang C, He J, Dai H, Wang G, Zhang X, Wang C, Shi J, Chen X, Wang D, Wang E.
874 2021. Discriminating symbiosis and immunity signals by receptor competition in
875 rice. *Proceedings of the National Academy of Sciences of the United States of*
876 *America* **118**: e2023738118.
877 Zhang L, Feng G, Declerk S. 2018. Signal beyond nutrient, fructose, exuded by an
878 arbuscular mycorrhizal fungus triggers phytate mineralization by a phosphate
879 solubilizing bacterium. *The ISME Journal* **12**: 2339–2351.
880 Zhang Q, Blaylock LA, Harrison MJ. 2010. Two *Medicago truncatula* half-ABC
881 transporters are essential for arbuscule development in arbuscular mycorrhizal
882 symbiosis. *The Plant Cell* **22**: 1483–1497.
883

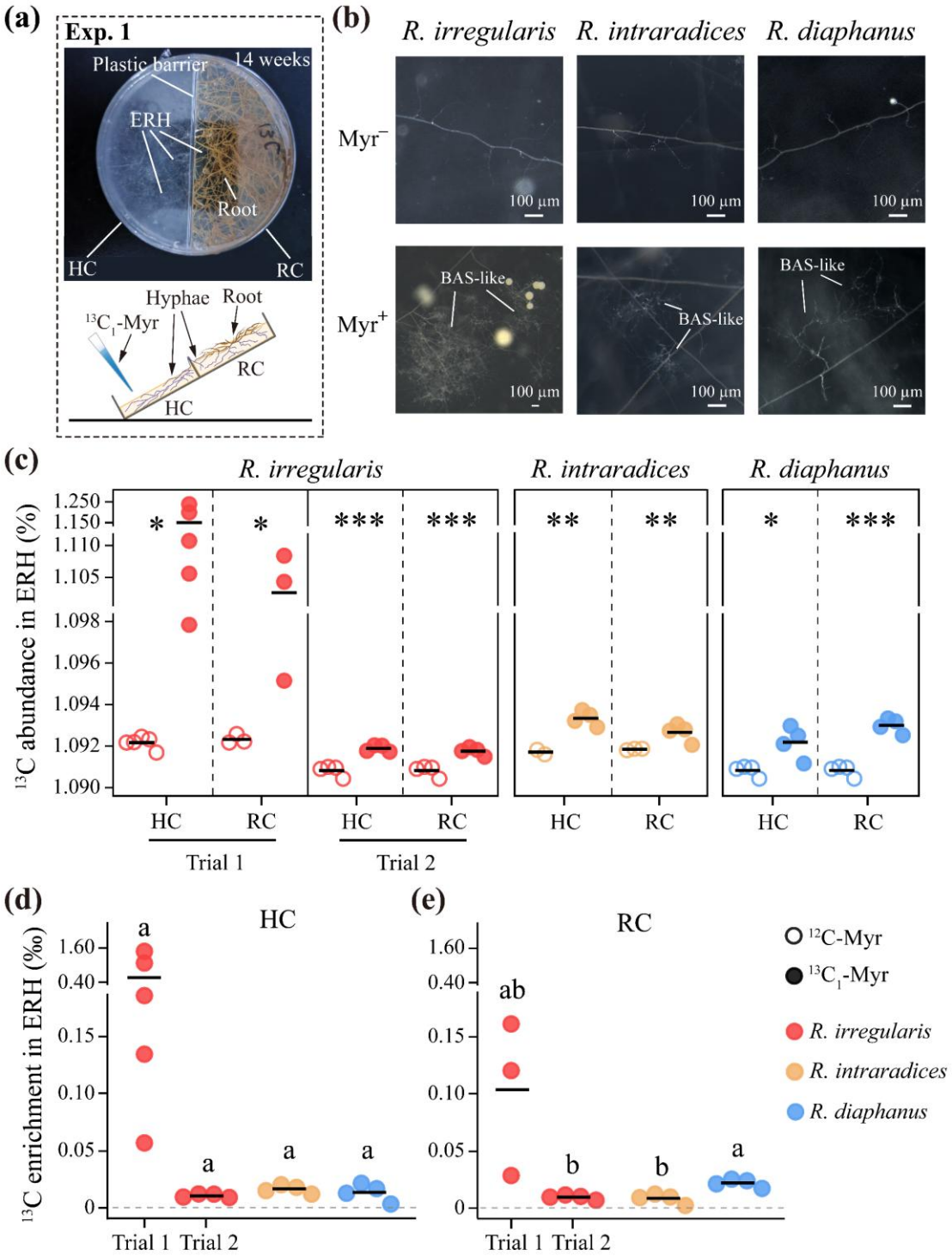


Fig. 1 Uptake of exogenous myristate by extraradical hyphae (ERH) of arbuscular mycorrhizal fungi (AMF) under symbiotic conditions in Exp. 1. **(a)** Schematic illustration of the two-compartment AMF–carrot hairy root co-cultivation system and the addition of myristate to the hyphae compartment (HC). Carrot hairy roots colonized by *Rhizophagus irregularis*, *R. intraradices*, or *R. diaphanus* were grown in the root

890 compartment (RC). Twelve to fourteen weeks later, 0.1 mM of $^{13}C_1$-labeled ($^{13}C_1$-Myr),
891 non-labeled (^{12}C-Myr) myristate, or ddH_2O (Myr^-, control) was added weekly to the
892 HC with well-developed AMF extraradical hyphae (ERH) until harvest. **(b)** Branched
893 absorbing structures (BAS) of *R. irregularis*, *R. intraradices*, and *R. diaphanus* were
894 observed in the HC only when treated with exogenous myristate (Myr^+). **(c)** $^{13}C/^{12}C$
895 ratio of the harvested ERH from HC or RC of *R. irregularis*, *R. intraradices*, and *R.*
896 *diaphanus* when $^{13}C_1$-labeled ($^{13}C_1$-Myr) or non-labeled (^{12}C-Myr, background)
897 myristate was added to HC. Two independent experimental trials were performed for
898 *R. irregularis.* **(d, e)** Calculated ^{13}C enrichment in ERH of *R. irregularis*, *R.*
899 *intraradices*, and *R. diaphanus* in the HC and RC under ^{13}C-Myr treatment. Black
900 horizontal lines represent mean value from 2–5 biological replicates. *, **, and ***
901 indicate statistical significance between $^{13}C_1$-labeled and non-labeled treatments at the
902 0.05, 0.01, and 0.001 probability levels (Mann-Whitney U-test), respectively. Different
903 letters indicate significant difference at the 0.05 probability level (one-way ANOVA
904 followed by Games-Howell's post hoc test). Source data are provided as a Source Data
905 file.

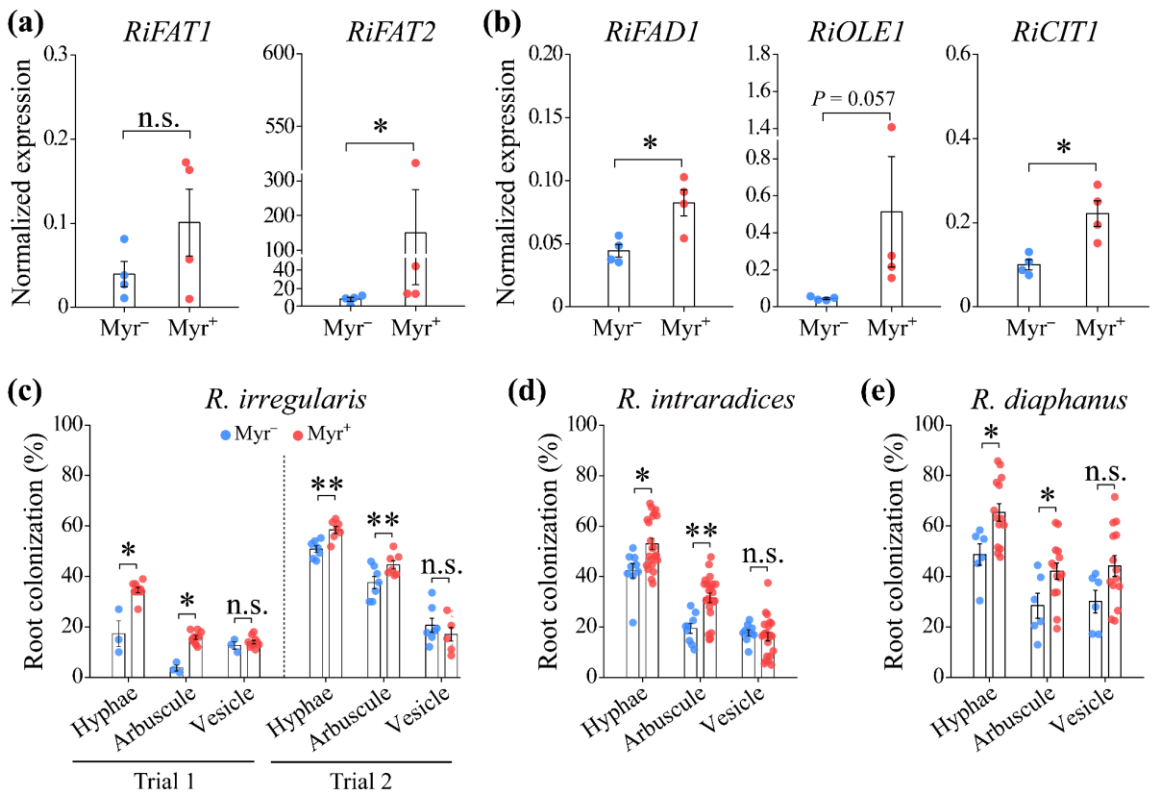
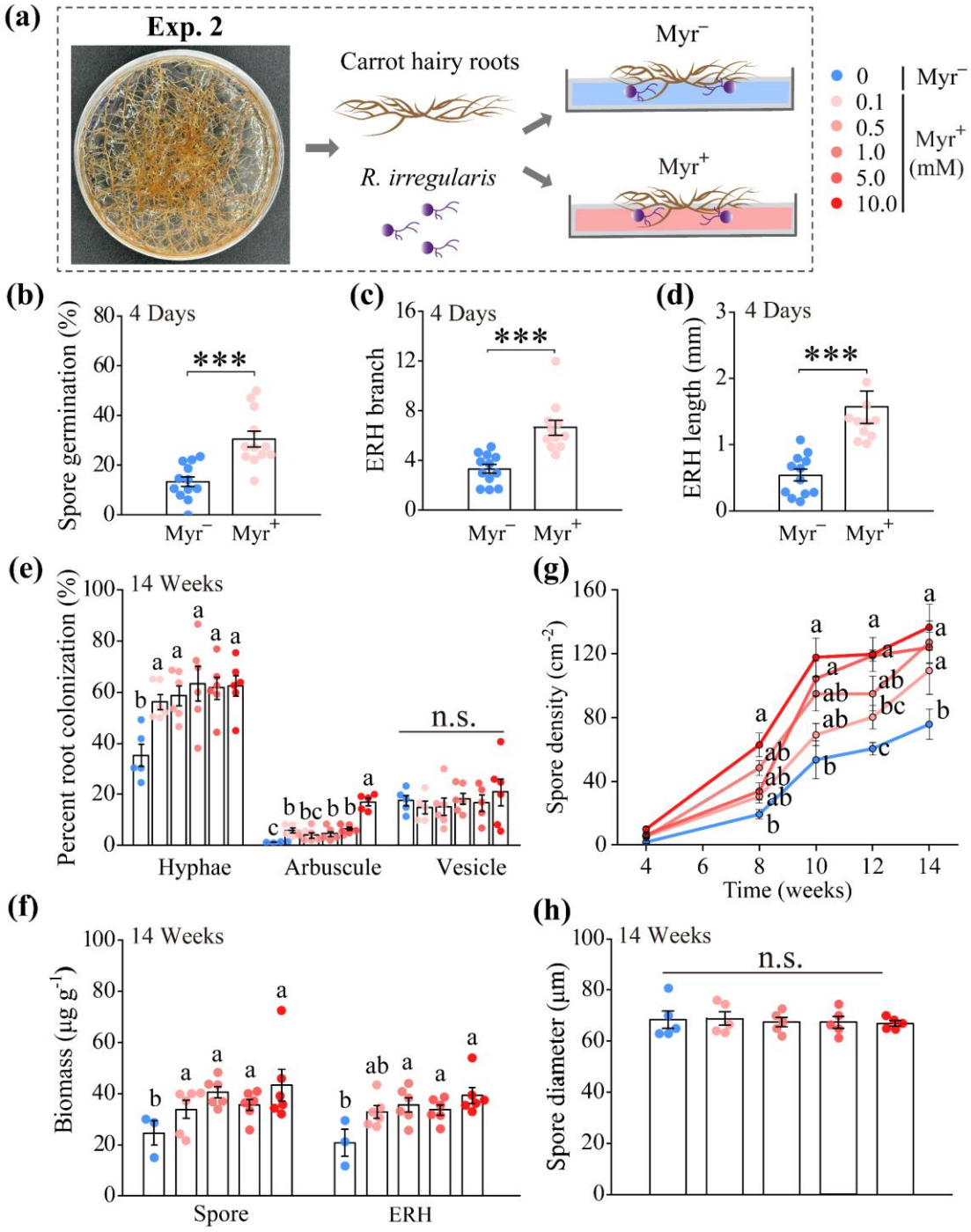


Fig. 2 Effects of exogenous myristate on the expression of fungal fatty acid uptake and metabolism genes in the extraradical hyphae (ERH) and on root colonization by arbuscular mycorrhizal fungi (AMF) in Exp. 1. (**a, b**) Normalized expression (relative to *RiEF-1β*) of AMF genes involved in **(a)** fatty acid uptake (*RiFAT1* and *RiFAT2*) and **(b)** metabolism—β-oxidation (*RiFAD1*), palmitvaccenic acid synthesis (*RiOLE1*), and TCA cycle (*RiCIT1*)—in the ERH of *R. irregularis* collected from the hyphae compartment (HC) under myristate (Myr^+) or ddH_2O (Myr^-) treatments (n = 4). **(c–e)** Percent root colonization by **(c)** *R. irregularis* (n = 3–10), **(d)** *R. intraradices* (n = 9–22), and **(e)** *R. diaphanus* (n = 6–14) in carrot hairy roots from the root compartment (RC) when HC was treated with myristate (Myr^+) or ddH_2O (Myr^-). Two independent experimental trials were performed for *R. irregularis*. Means ± SE, *, **, and *** indicate statistical significance between Myr^+ and Myr^- treatments at the 0.05, 0.01, and 0.001 probability levels, respectively. Source data are provided as a Source Data file.



920

921 **Fig. 3** Effects of exogenous myristate on the development of intraradical and
922 extraradical structures of AMF (*Rhizophagus irregularis*) in the single-compartment
923 AMF-carrot hairy root co-cultivation system (Exp. 2). **(a)** Schematic illustration of the
924 single-compartment *R. irregularis*-hairy root co-cultivation system and the addition
925 (Myr^+) or absence (Myr^-) of myristate. **(b)** Spore germination rate, **(c)** extraradical
926 hyphae (ERH) branching, and **(d)** ERH length in the *R. irregularis*-hairy root co-

927 cultivation system with (Myr^+) or without (Myr^-) myristate treatment at 4 days post-
928 inoculation (n = 11−12). **(e)** AMF percent root colonization in carrot hairy roots and **(f)**
929 biomass of the AMF extraradical spore and hypha (per gram of medium) at 14 weeks
930 post-inoculation (n = 3−6). **(g)** Dynamics of AMF spore density over time (per square
931 centimeter, n = 3−5); and **(h)** AMF spore diameter at harvest with 0, 0.1, 0.5, 1, 5, or
932 10 mM myristate treatment under symbiotic conditions (n = 5, calculation from > 900
933 spores in each replicate). Means ± SE; different letters indicate significant differences
934 at $P < 0.05$ level (one-way ANOVA followed by Tukey's post hoc test); *** indicates
935 statistical significance at the 0.001 probability level (Student's *t*-test). Source data are
936 provided as a Source Data file.

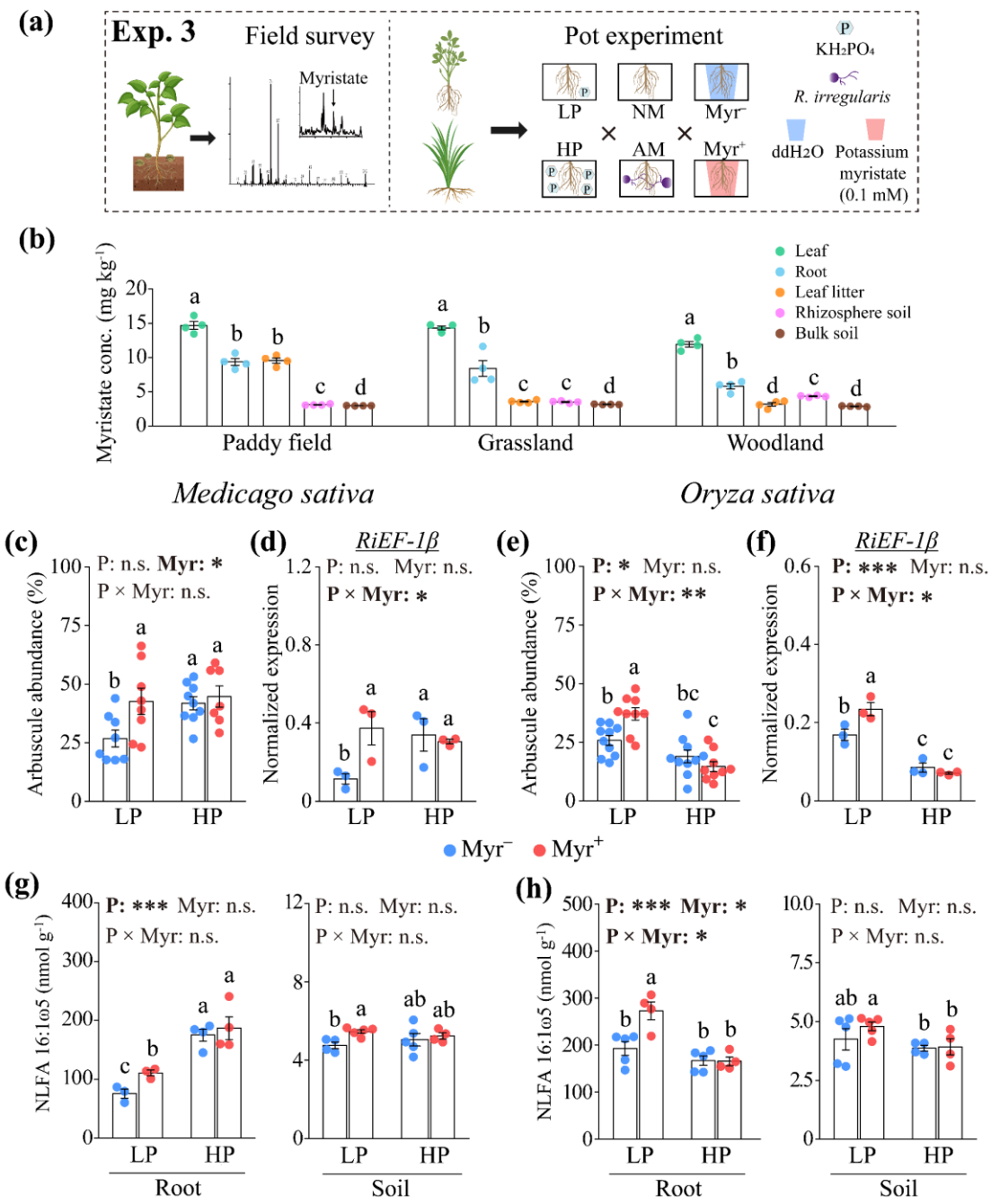


Fig. 4 Environmental myristate levels and the effects of exogenous myristate on arbuscule abundance, as well as the intraradical and extraradical biomass of arbuscular mycorrhizal fungi (AMF, *Rhizophagus irregularis*) associated with *Medicago sativa* or *Oryza sativa* under low (LP) or high (HP) phosphorus conditions (Exp. 3). **(a)** Schematic illustration of the field survey of environmental myristate levels and experimental design in the pot experiment (Exp. 3). **(b)** Myristate concentration in bulk soil, as well as in plant leaves, roots, leaf litter, and rhizosphere soil from the dominant

945 species *O. sativa*, *Axonopus compressus*, and *Morus alba* in the paddy field, grassland,
946 and woodland, respectively (n = 4). **(c, e)** Arbuscule abundance in *M. sativa* and *O.*
947 *sativa* roots treated with 0.1 mM myristate (Myr^+) or ddH_2O ($Myr^–$) (n = 10; vesicle
948 and hyphal abundance are shown in Supporting Information Fig. S1); **(d, f)** Normalized
949 expression of *RiEF-1β* (relative to *MsACT2* in *M. sativa* and *OsCyc2* in *O. sativa*) in
950 AMF-colonized roots with 0.1 mM myristate (Myr^+) or ddH_2O ($Myr^–$) treatments (n =
951 3). **(g, h)** AMF biomass, indicated by the AMF signature fatty acid (NLFA 16:1ω5), in
952 AMF-colonized roots and rhizosphere soil of *M. sativa* and *O. sativa* treated with
953 myristate (Myr^+) or ddH_2O ($Myr^–$) (n = 5). Means ± SE; different letters indicate
954 significant differences at $P < 0.05$ level (**b**: Student's *t*-test, **c**–**h**: two-way ANOVA
955 followed by Tukey's post hoc test). Source data are provided as a Source Data file.

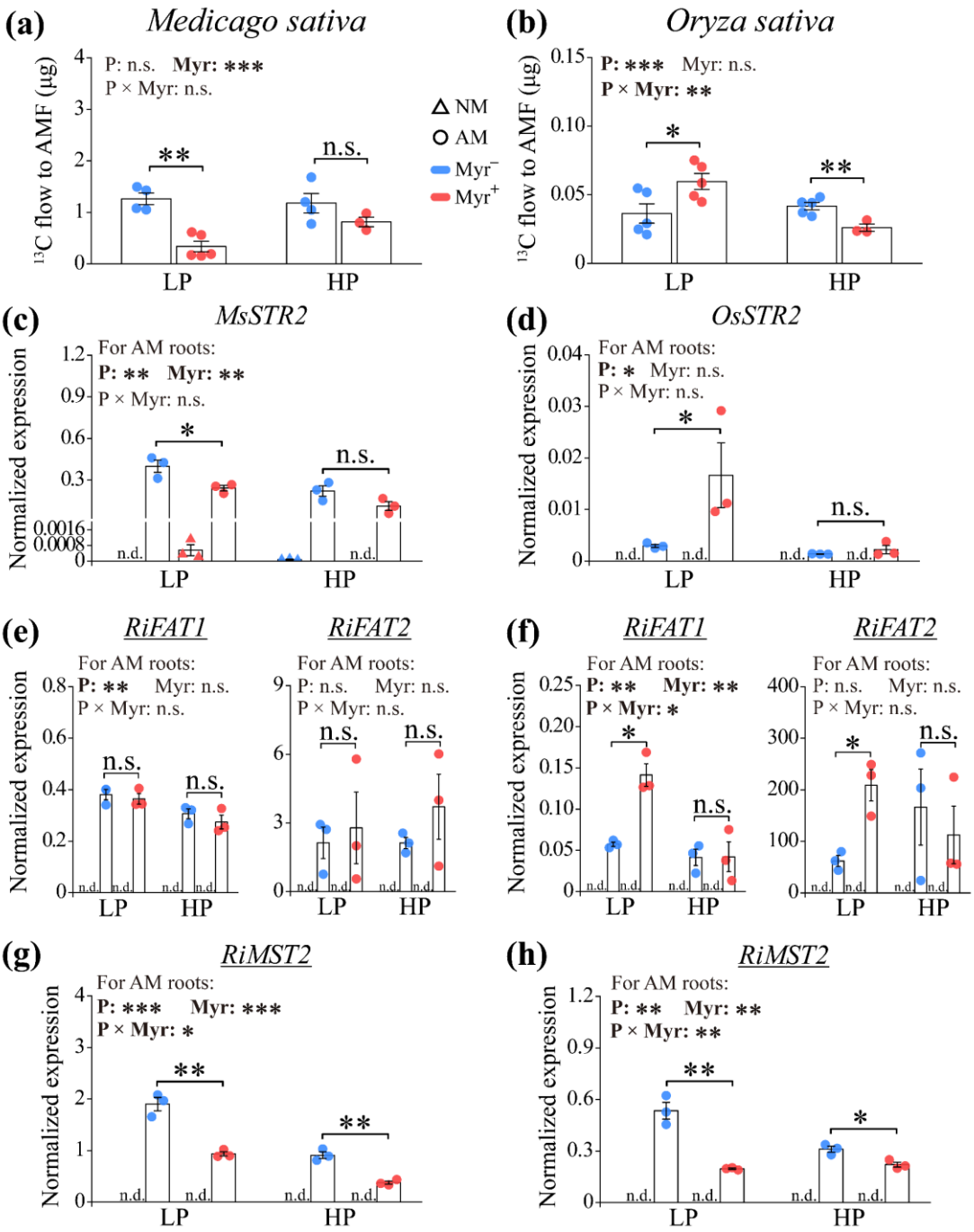


Fig. 5 Effects of exogenous myristate on symbiotic carbon (C) allocation from *Medicago sativa* and *Oryza sativa* to arbuscular mycorrhizal fungi (AMF, *Rhizophagus irregularis*) and on the expression profiles of mycorrhizal C transportation marker genes. **(a, b)** Plant-derived ^{13}C flow to the AMF signature fatty acid (NLFA 16:1ω5) in AMF-colonized roots of *M. sativa* and *O. sativa* treated with 0.1 mM myristate (Myr^+) or ddH_2O (Myr^-) under low (LP) and high (HP) phosphorus conditions (n = 5, two-way

963 ANOVA tested the effects of the treatments and their interaction). (**c**–**h**) Normalized
964 expression of plant and fungal (underlined) marker genes for mycorrhizal C transport
965 in AMF-colonized (AM) and non-colonized (NM) roots treated with 0.1 mM myristate
966 (Myr^+) or ddH_2O (Myr^-) (n = 3). The mycorrhizal C transportation genes include the
967 plant symbiotic fatty acid transporter *STR2* **(c, d)**, the fungal fatty acid transporters
968 (*RiFAT1, RiFAT2*; **e, f**), and the monosaccharide transporter (*RiMST2*; **g, h**). Normalized
969 expression of *M. sativa*, *O. sativa*, and AMF genes was calculated relative to *MsACT2*,
970 *OsCyc2*, and *RiEF-1β*, respectively; n.d., not detected. In c–h, the two-way ANOVA in
971 each histogram applies only to AM roots. Means ± SE; * and ** indicate statistical
972 significance at the 0.05 and 0.01 probability levels, respectively (Student's *t*-test).
973 Source data are provided as a Source Data file.

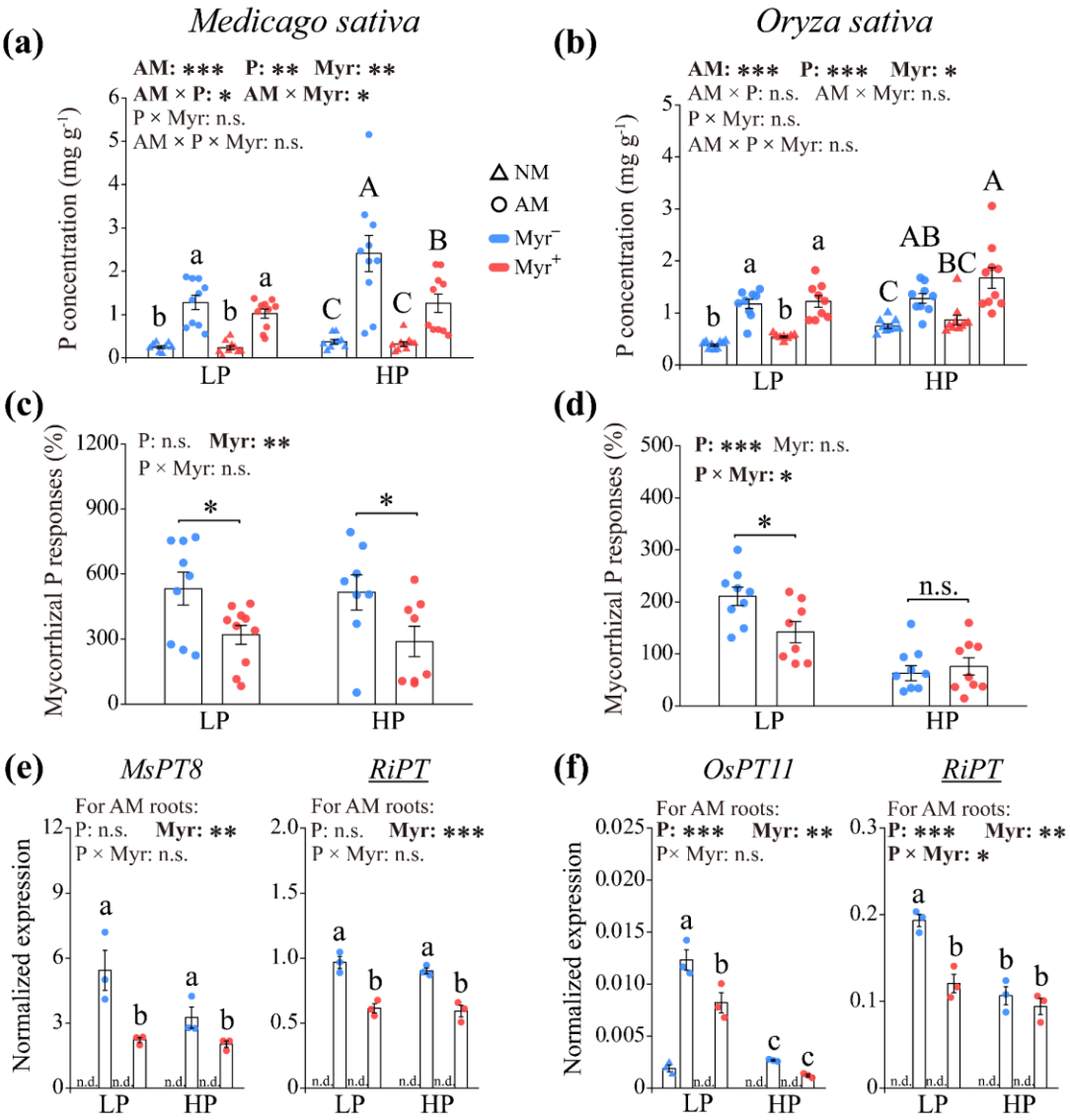


Fig. 6 Effects of exogenous myristate on plant phosphorus (P) concentration, mycorrhizal P response, and expression of plant and fungal (underlined) marker genes for the mycorrhizal P pathway in *Medicago sativa* and *Oryza sativa*. **(a, b)** Plant P concentration (n = 8−10); **(c, d)** mycorrhizal P response (n = 8−10); and **(e, f)** relative expression of *MsPT8*, *OsPT11*, and *RiPT* in *M. sativa* and *O. sativa* roots under low (LP) and high (HP) phosphorus conditions with 0.1 mM myristate (Myr^+) or ddH_2O (Myr^-) treatments (n = 3). AM, inoculated with *Rhizophagus irregularis*; NM, non-inoculated; n.d., not detected. Normalized expression of *M. sativa*, *O. sativa*, and AMF genes was calculated relative to *MsACT2*, *OsCyc2*, and *RiEF-1β*, respectively. In a−d,

two- or three-way ANOVA tested the effects of the treatments and their interaction; In e and f, two-way ANOVA in each histogram applies only to AM roots, followed by Tukey's post hoc test for multiple comparisons. Means ± SE; different letters indicate significant differences at $P < 0.05$ level. * and *** indicate statistical significance at the 0.05 and 0.001 probability levels (Student's *t*-test), respectively. Source data are provided as a Source Data file.

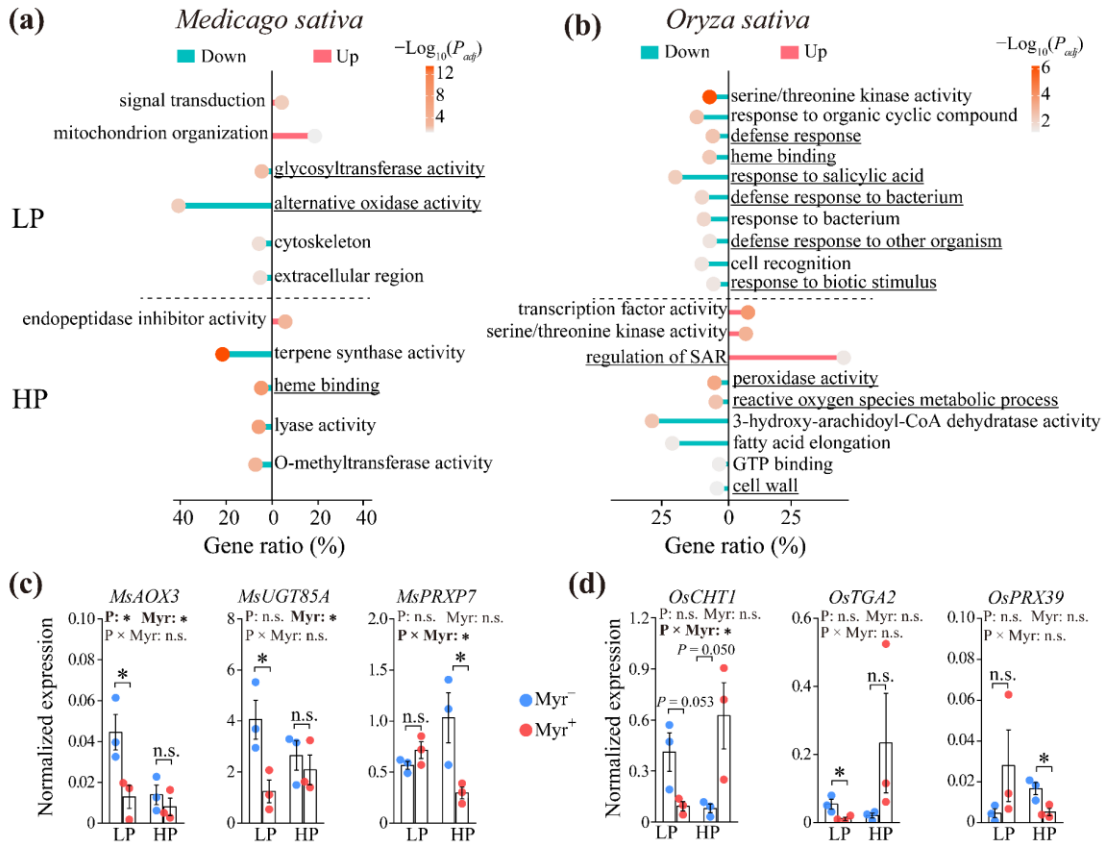


Fig. 7 Effects of exogenous myristate on the global transcriptome and putative AM-activated defense genes in *Medicago sativa* and *Oryza sativa* roots inoculated with arbuscular mycorrhizal fungi (AMF, *Rhizophagus irregularis*) under low (LP) and high (HP) phosphorus conditions. **(a, b)** Significantly enriched gene ontology (GO) terms ($P_{adj} < 0.05$) for differentially expressed genes (DEGs) between myristate (Myr^+) and ddH_2O (Myr^-) treatments in RNA-seq. Underlined terms denote stress- or defense-related GO categories, with the expression profile of their component genes provided in Supporting Information (Fig. S4). **(c, d)** Quantitative real-time PCR analysis of the AM-activated defense gene expression—reactive oxygen species (ROS) balance (*MsAOX3*), stress responses (*MsUGT85A*, *MsPRXP7*, and *OsPRX39*), and defense responses *(OsCHT1* and *OsTGA2*) — between Myr^+ and Myr^- treatments under LP and HP conditions (n = 3, two-way ANOVA tested the effects of the treatments and their interaction). Means ± SE; * indicates statistical significance between Myr^+ and Myr^-

1004 treatments at the 0.05 probability level (Student's *t*-test). Source data are provided as a

1005 Source Data file.

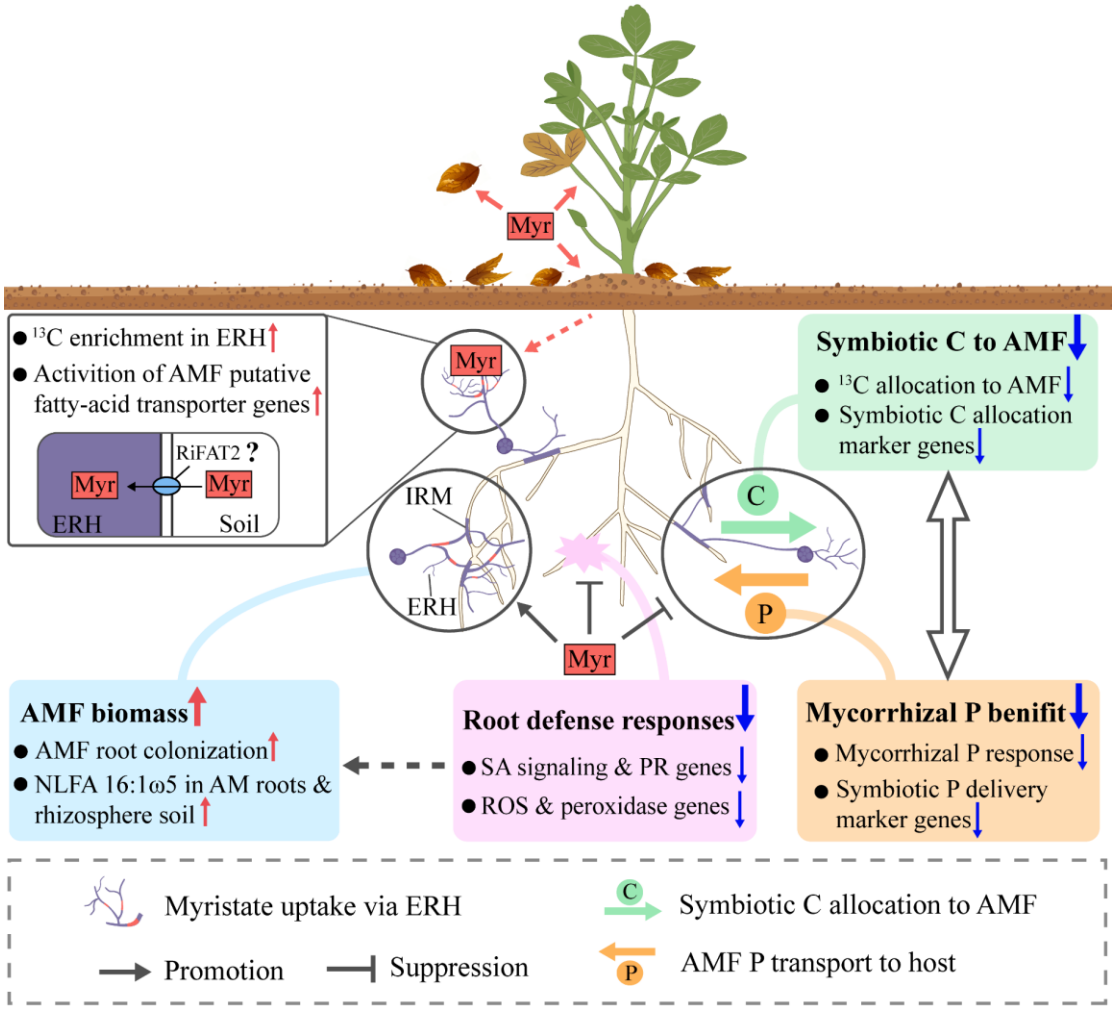


Fig. 8 A proposed model for direct uptake of exogenous myristate by symbiotic arbuscular mycorrhizal fungi (AMF) and its regulatory effects on the arbuscular mycorrhizal symbiosis. Symbiotic AMF can uptake exogenous myristate, which is frequently found in diverse soil and plant environments, possibly through the fungal fatty acid transporters RiFAT2 in the extraradical hyphae (ERH), as indicated by ^{13}C enrichment and transcriptional activation of fatty acid transport and metabolism genes in AMF ERH. Exogenous myristate increased both intraradical and extraradical fungal biomass, possibly linked to the suppression of AM-activated defense responses in host roots. Unexpectedly, as a non-symbiotic carbon source, exogenous myristate disrupted the carbon (C)-phosphorus (P) exchange in AM symbiosis, resulting in reduced mycorrhizal P benefits for plants and decreased allocation of symbiotic C to AMF.

1018 Question mark or dashed arrow indicates putative or unclear mechanisms. IRM,
1019 intraradical mycelium; SA, salicylic acid; ROS, reactive oxygen species; PR,
1020 pathogenesis-related; NLFA 16:1ω5, neutral lipid fatty acid 16:1ω5 (AMF marker).