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) are obligate biotrophs that rely on host-derived symbiotic carbohydrates. However, it remains unclear whether symbiotic AMF can access exogenous non-symbiotic carbon sources, 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. 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, although these effects varied with soil phosphorus conditions. 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 AMF colonization and the benefits conferred by AMF largely depend on the host plant species (genotypes), with some being "responsive plants" that

exhibit positive growth responses (e.g., alfalfa, maize) and others being "nonresponsive plants" that show minimal or no growth enhancement while exhibiting increased AM-activated defense responses (e.g., rice, wheat; Hata et al., 2010; Li et al., 2024).

AMF are considered obligate symbionts that rely on living host plants to complete their life cycle by producing daughter spores (Smith and Read, 2008), which presents challenges for basic research on AM and their applications in agriculture and other fields. To date, achieving a pure (plant-independent) culture of asymbiotic AMF has not been successful, partially due to their dependence on plant-derived symbiotic C (Bravo et al., 2017; Luginbuehl et al., 2017; Kameoka et al., 2019). Genomic and transcriptomic analyses have shown that AMF lack the genes required for the biosynthesis of long-chain fatty acids, and subsequent studies have demonstrated that fatty acids are transferred from host plants to AMF as their primary C source (Bravo et al., 2017; Jiang et al., 2017; Keymer et al., 2017; Luginbuehl et al., 2017). However, the mechanism underlying the allocation of plant-derived fatty acids to fungi in AM symbiosis remains unclear. In host plants, AMF colonization triggers the expression of lipid biosynthesis and transport genes (Gutjahr et al., 2012; Bravo et al., 2017; Keymer et al., 2017; Luginbuehl et al., 2017), with lipid transport to the AM symbiotic interface relying on the periarbuscular membrane-localized transporters stunted arbuscule (STR) and STR2 (Zhang et al., 2010; Gutjahr et al., 2012), and fungal uptake of plant-derived lipids mediated by fatty acid transporter 1 (RiFAT1) and RiFAT2 in the model AMF species *Rhizophagus irregularis* (Brands and Dörmann, 2022). This symbiotic C allocation is functionally linked to fungal P delivery within a framework of mutual nutritional rewards (Kiers et al., 2011), which have been disrupted during AMF-nonhost interactions due to the strong AM-activated defense responses in non-host roots (Wang et al., 2023).

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

116 cycling (Rillig et al., 2020).

117 However, the effects of external myristate on the growth and development of
118 symbiotic AMF—the typical state of functioning AMF in nature—remain unclear.
119 Regarding sugars, another type of host-derived C source, symbiotic AMF may be
120 unable to absorb them additionally through extraradical hyphae (Bücking et al., 2008;
121 Helber et al., 2011; Sugiura et al., 2020). A recent study reported that while exogenous
122 myristate does not affect the extraradical biomass of symbiotic *R. irregularis* and *R.*
123 *intraradices*, it enhances their colonization rates by stimulating the growth and
124 development of extraradical hyphae. Based on this, the authors inferred that symbiotic
125 AMF may acquire exogenous myristate (Liu et al., 2023). To date, the effects of
126 exogenous myristate on AM symbiosis and their underlying mechanisms remain limited.
127 Specifically, whether symbiotic AMF can uptake exogenous myristate via extraradical
128 hyphae in the presence of plant-derived symbiotic C is yet to be determined. This
129 question is crucial for understanding the symbiotic mechanisms and ecological roles of
130 AM symbiosis (Rillig et al., 2020).

131 To address this significant knowledge gap, this study investigated the ability of
132 symbiotic AMF to absorb external non-symbiotic myristate and examined the effect of
133 exogenous myristate on symbiotic C-P exchanges in AM. Specifically, we aimed to
134 determine: (i) whether symbiotic AMF can take up exogenous non-symbiotic myristate,
135 (ii) how this additional non-symbiotic C source influences the symbiotic C allocation
136 from plants to AMF, and (iii) if and how fungal P transfer to the host plant is affected
137 by exogenous myristate. This work provides, to our knowledge, the strongest evidence
138 to date that symbiotic AMF can absorb non-symbiotic C sources like myristate in the
139 presence of plant-derived symbiotic carbohydrates. Furthermore, our findings reveal
140 that exogenous myristate may, unexpectedly, disrupt the C-P exchange in AM

141 symbiosis.

142

143 **RESULTS**

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

145 In the two-compartment AMF-carrot hairy root co-cultivation system (Exp. 1, Fig.
146 1a), exogenous myristate specifically induced the formation of branched absorbing
147 structures in *R. irregularis*, *R. intraradices*, and *R. diaphanus* in the HC approximately
148 two weeks after Myr^+ treatment (Fig. 1b). For *R. irregularis*, the $^{13}C/^{12}C$ ratio of
149 extraradical hyphae in HC (trial 1: 1.1504 ± 0.0575%, trial 2: 1.0919 ± 0.0001%) and
150 RC (trial 1: 1.1026 ± 0.0055%, trial 2: 1.0918 ± 0.0002%) under ^{13}C-Myr treatment
151 was slightly but significantly higher than that in the corresponding HC (trial 1: 1.0922
152 ± 0.0003%, trial 2: 1.0908 ± 0.0003%) and RC (trial 1: 1.0923 ± 0.0002%, trial 2:
153 1.0908 ± 0.0002%) from the non-labeled control (^{12}C-Myr), respectively ($P < 0.05$, Fig.
154 1c). Similarly, for *R. intraradices* and *R. diaphanus*, the $^{13}C/^{12}C$ ratio of extraradical
155 hyphae in HC (*R. intraradices*: 1.0933 ± 0.0002%, *R. diaphanus*: 1.0922 ± 0.0005%)
156 and RC (*R. intraradices*: 1.0927 ± 0.0005%, *R. diaphanus*: 1.0930 ± 0.0004%) under
157 ^{13}C-Myr treatment was slightly but significantly higher than those in HC (*R.*
158 *intraradices*: 1.0917 ± 0.0001%, *R. diaphanus*: 1.0908 ± 0.00003%) and RC (*R.*
159 *intraradices*: 1.0918 ± 0.00004%, *R. diaphanus*: 1.0908 ± 0.00003%) from the non-
160 labeled control ($P < 0.01$, Fig. 1c). Additionally, the calculated ^{13}C enrichment in
161 extraradical hyphae between HC and RC showed no significant difference for *R.*
162 *irregularis* and *R. diaphanus* (Fig. 1d, e), although it was higher in HC than in RC for
163 *R. intraradices* ($P < 0.05$), excluding the possibility that the ^{13}C enrichment in AMF
164 extraradical hyphae originated from ^{13}C-myristate retention on the hyphal surface from
165 the HC. Collectively, these results suggest that these three AMF species can take up a

166 small amount of exogenous myristate through their extraradical hyphae when
167 associated with host roots.

168 In HC, the calculated ^{13}C enrichments in extraradical hyphae of *R. irregularis* (trial
169 1: 0.5823 ± 0.4111‰, trial 2: 0.0107 ± 0.0014‰), *R. intraradices* (0.0163 ± 0.0031‰),
170 and *R. diaphanus* (0.0136 ± 0.0067‰) showed no significant differences in the ^{13}C-
171 Myr treatment (Fig. 1d). In RC, the ^{13}C enrichment in extraradical hyphae also showed
172 no significant differences (Fig. 1e), except for a slightly but significantly higher ^{13}C
173 enrichment in *R. diaphanus* (0.0217 ± 0.0031‰) compared to *R. intraradices* (0.0082
174 ± 0.0037‰) and *R. irregularis* in trial 2 (0.0093 ± 0.0017‰, $P < 0.05$). These results
175 suggest a generally similar myristate absorption capacity among these AMF species
176 under the tested conditions.

177 **Effects of myristate on AMF colonization and expression of fatty acid uptake and**
178 **metabolism marker genes (Exp. 1)**

179 The expression of fatty acid uptake and metabolism marker genes in *R. irregularis*
180 extraradical hyphae were analyzed to investigate their uptake of myristate from HC at
181 the transcriptional level. The results showed that exogenous myristate significantly
182 increased the expression levels of the fungal fatty acid transporter gene *RiFAT2* ($P <$
183 0.05), while had no significant effect on *RiFAT1* (Fig. 2a). Additionally, exogenous
184 myristate significantly or marginally significantly increased the expression levels of the
185 fungal genes involved in fatty acid β-oxidation (*RiFAD1*, $P < 0.05$), palmitvaccenic acid
186 synthesis (*RiOLE1*, $P = 0.057$), and TCA cycle activity (*RiCIT1*, $P < 0.05$) (Fig. 2b).
187 These results suggest that exogenous myristate activates the expression of fungal genes
188 related to fatty acid uptake and metabolism in the extraradical hyphae of *R. irregularis*.

189 For AMF colonization intensity, exogenous myristate significantly increased the
190 hyphal and arbuscular colonization rates of *R. irregularis, R. intraradice*, and *R.*

191 *diaphanus* in carrot hairy roots ($P < 0.05$, Fig. 2c−e) but had no effect on vesicle
192 colonization.

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

195 In the single-compartment *R. irregularis*-carrot hairy root co-cultivation system
196 (Exp. 2, Fig. 3a), exogenous myristate significantly promoted spore germination ($P <$
197 0.001, Fig. 3b), and enhanced hyphal branching and length ($P < 0.001$, Fig. 3c, d) before
198 visible physical contact between hyphae and hairy roots at four days after AMF
199 inoculation. Morphological examinations showed that exogenous myristate
200 significantly enhanced the colonization intensities of hyphae and arbuscules, but not
201 vesicles, in carrot hairy roots across various application levels (Fig. 3e). Exogenous
202 myristate also significantly increased the extraradical biomass of AMF (hyphae and
203 spore) at different application concentrations ($P < 0.05$, Fig. 3f).

204 Meanwhile, exogenous myristate significantly increased the density of AMF
205 spores from eight weeks post-AMF inoculation ($P < 0.05$, Fig. 3g), and the diameter of
206 newly produced AMF spores in media with exogenous myristate was identical to those
207 in media without myristate (Fig. 3h).

208 **Environmental myristate levels and the effects of exogenous myristate on AMF**
209 **intraradical and extraradical biomass during symbiosis with alfalfa and rice (Exp.**
210 **3)**

211 In Exp. 3 (Fig. 4a), our field survey revealed that myristate was extensively
212 detected in soil and plant samples from the paddy field, grassland, and woodland at a
213 2.87–14.68 mg kg^{-1} level (Fig. 4b). On average, the myristate level in plant (fresh leaf
214 or root) is mostly higher than that in soil (leaf litter, rhizosphere soil, or bulk soil) ($P <$
215 0.05, Fig. 4b). Additionally, the myristate levels in leaf litter and rhizosphere soil were

216 usually greater than in bulk soil ($P < 0.05$), suggesting that plants are significant source
217 of myristate in the soil. The myristate contents from the same sample type typically
218 varied across different habitats ($P < 0.05$).

219 In the pot experiment (Exp. 3), all typical AMF intraradical structures (hyphae,
220 arbuscules, and vesicles) were observed in the roots of both alfalfa and rice in the *R.*
221 *irregularis*-inoculated groups (AM), while no AMF colonization was detected in the
222 non-inoculated (NM) groups. Under low-P conditions, exogenous myristate
223 significantly increased arbuscule abundance in alfalfa roots ($P < 0.05$, Fig. 4c) but did
224 not significantly affect hyphae or vesicle abundance (Supporting Information Fig. S1a,
225 c). Exogenous myristate significantly enhanced arbuscule, hyphae, and vesicle
226 abundance in rice roots ($P < 0.05$, Fig. 4d, Supporting Information Fig. S1b, d).
227 However, under high-P conditions, exogenous myristate had no effect on AMF
228 abundance in either alfalfa or rice roots (Fig. 4, Supporting Information Fig. S1).

229 For intraradical AMF biomass, exogenous myristate significantly increased the
230 levels of the AMF signature fatty acid (NLFA 16:1ω5) in both alfalfa and rice roots
231 under low-P conditions ($P < 0.05$, Fig. 4e, f); however, it had no significant impact
232 under high-P conditions. In rice roots, NLFA 16:1ω5 levels were higher under high-P
233 than low-P conditions ($P < 0.05$). For extraradical AMF biomass, exogenous myristate
234 increased NLFA 16:1ω5 levels in the rhizosphere soils of alfalfa ($P < 0.05$) but not in
235 rice ($P = 0.14$) under low-P conditions (Fig. 4e, f). Under high-P conditions, myristate
236 had no effect on NLFA 16:1ω5 levels in the rhizosphere soil of either plant.

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

238 The analysis of ^{13}C-NLFA-isotope ratio mass spectrometry was performed to
239 investigate the impact of exogenous myristate on the allocation of symbiotic C from
240 plants to AMF. In AM plants, there was a notable enrichment of ^{13}C (δ^{13}C = 1.75 ±

241 0.6‰) in the AMF signature fatty acid (NLFA 16:1ω5) compared to NM plants ($\delta^{13}C$ =
242 −24.8 ± 2.3‰), demonstrating the transfer of newly fixed ^{13}C from plants to AMF. The
243 calculated ^{13}C flow from alfalfa to root-colonizing AMF (0.3400–1.2642 μg) was over
244 ten times higher than that from rice (0.0261–0.0596 μg), highlighting a substantially
245 greater efficiency of plant-derived symbiotic C delivery to AMF in AM-responsive
246 alfalfa compared to non-responsive rice. In alfalfa, exogenous myristate reduced the
247 symbiotic C transfer to AMF (Myr effect: $P < 0.001$, Fig. 5a), although this effect was
248 not significant under high-P conditions. In rice, myristate also significantly influenced
249 the symbiotic C flow to AMF, with its effect significantly interacting with soil P level
250 (Myr × P: $P = 0.004$, Fig. 5b): myristate significantly increased the allocation of ^{13}C to
251 AMF under low-P conditions ($P < 0.05$) but decreased it under high-P conditions ($P <$
252 0.01).

253 At the transcriptional level, the qRT-PCR analysis revealed that the expressions of
254 the symbiotic lipid transfer marker genes *STR2* (in alfalfa and rice) and the fungal genes
255 responsible for symbiotic fatty acid uptake *(RiFAT1* and *RiFAT2)* or monosaccharide
256 uptake (*Monosaccharide Transporter 2* (*RiMST2*)) were exclusively detected in AM
257 roots, except for a low expression level of alfalfa *MsSTR2* occasionally observed in NM
258 roots (Fig. 5c–h). In AMF-inoculated alfalfa roots, exogenous myristate significantly
259 downregulated the expressions of the symbiotic C transfer genes *MsSTR2* in the plant
260 and *RiMST2* in AMF ($P < 0.05$), while having no effect on *RiFAT1* and *RiFAT2* in AMF
261 (Fig. 5c, g, e). In AMF-inoculated rice roots, the effects of myristate on the tested
262 symbiotic C transfer genes were largely influenced by soil P levels ($P < 0.05$). Under
263 low-P conditions, myristate significantly increased the expressions of plant *OsSTR2* and
264 fungal *RiFAT1* and *RiFAT2*, while decreasing the expression of fungal *RiMST2* ($P <$
265 0.05, Fig. 5d, f, h). Under high-P conditions, myristate significantly increased the

266 expression of *RiMST2* ($P < 0.05$) but had no effects on *OsSTR2*, *RiFAT1*, and *RiFAT2*.
267 In both plant species with AMF inoculation, soil P level showed significant effects on
268 *STR*, *RiFAT1*, and *MST2* genes ($P < 0.05$), with generally lower expression levels of
269 these genes under high-P compared to low-P conditions.

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

272 In the pot experiments (Exp. 3), AMF inoculation significantly increased P
273 concentrations in both alfalfa and rice plants ($P < 0.001$, Fig. 6a, b). Under low-P
274 conditions, exogenous myristate significantly decreased the mycorrhizal P pathway in
275 both alfalfa and rice ($P < 0.05$, Fig. 6c, d). Under high-P conditions, while exogenous
276 myristate significantly decreased the mycorrhizal P response in alfalfa ($P < 0.05$), it had
277 no significant effect on rice.

278 At the transcriptional level, the expression of mycorrhizal P pathway marker genes
279 in plants (*Phosphate Transporter*, *MsPT8* or *OsPT11*) and AMF (*RiPT*) was detected
280 exclusively in the AM roots of rice and alfalfa, except for a low expression level of rice
281 *OsPT11* observed in the NM root under low-P and Myr^- conditions. This finding
282 confirmed that AM symbiosis activated the mycorrhizal P pathway at the transcriptional
283 level in both plant species (Fig. 6e, f). Exogenous myristate significantly influenced the
284 transcriptional responses of the mycorrhizal P pathway in both alfalfa and rice ($P <$
285 0.01). In alfalfa, exogenous myristate downregulated the expressions of *MsPT8* and
286 *RiPT* under both low- and high-P conditions ($P < 0.05$, Fig. 6e). In rice, exogenous
287 myristate decreased the expression levels of *OsPT11* and *RiPT* under low-P conditions
288 ($P < 0.05$), but not under high-P conditions (Fig. 6f).

289 In alfalfa, AMF inoculation significantly increased plant biomass under both low-
290 and high-P conditions ($P < 0.05$, Supporting Information Fig. S2a), with mycorrhizal

291 growth responses significantly higher under high-P than low-P conditions ($P < 0.05$,
292 Supporting Information Fig. S2c). In rice, AMF inoculation had no effect on plant
293 biomass under low-P conditions, but reduced rice biomass under high-P conditions (P
294 < 0.05); mycorrhizal growth responses were significantly lower under high-P than low-
295 P conditions ($P < 0.05$, Supporting Information Fig. S2b, d). Exogenous myristate had
296 no effect on plant biomass or mycorrhizal growth response in either alfalfa or rice, while
297 soil P levels significantly influenced mycorrhizal growth response: high-P treatment
298 increased mycorrhizal growth response in alfalfa but decreased it in rice compared to
299 low-P conditions ($P < 0.01$, Supporting Information Fig. S2c, d).

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

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

310 Exogenous myristate induced a variety of differentially expressed genes (DEGs,
311 Myr^+ *vs.* Myr^-) in mycorrhizal alfalfa (LP: 308, HP: 137) and rice (LP: 590, HP: 855)
312 roots (Supporting Information Fig. S3c, d). Gene Ontology (GO) analysis of these
313 DEGs enriched several GO terms related to plant stress or defense, most of which were
314 downregulated by exogenous myristate (Fig. 7a, b). These included "defense response",
315 "response to salicylic acid", "peroxidase activity", etc., in rice, and "glycosyltransferase

316 activity" and "alternative oxidase activity" in alfalfa.

317 We subsequently analyzed the expression profiles of the component genes
318 associated with enriched GO terms that are related to defense or stress, using the RNA-
319 seq dataset (Fig. S4a, b). In alfalfa, exogenous myristate significantly downregulated
320 several key genes involved in reactive oxygen species balance (*alternative oxidase 2*
321 (*AOX2*), *AOX3*) and stress tolerance (*UDP-glycose flavonoid glycosyltransferase*
322 (*UFGT*), *UDP-glycosyltransferase* (*UGT*) *73C3*, *UGT73C6*) under low-P conditions,
323 as well as typical stress-responsive genes (*peroxidase* (*PRX*) *P7*, *cytochromes P450*
324 (*CYP*) *83B1*), and gene families such as *geraniol 8-hydroxylase-like* (*G8HL*),
325 *CYP71D10*, and *CYP736A12* under high-P conditions (Supplementary Fig. S4a). In rice,
326 exogenous myristate downregulated several typical defense-associated transcriptional
327 factors (*TGA2*, *TGA4*, *WRKY45*, *WRKY62*, *WRKY76*, and *bZIP79*) and marker genes
328 (*pathogenesis-related 5-like* (*PR5L*), *protein phosphatase 2C 35* (*OsPP2C35*), *1-*
329 *aminocyclopropane-1-carboxylate synthase 2* (*ACS2*), *DWARF4*, *pyricularia oryzae*
330 *resistance-ta* (*PITA*), *mildew resistance locus O2* (*MLO2*), *NRR repressor homologue*
331 *1* (*RH1*), and *chitinase 1* (*CHT1*)) under low-P conditions (Supplementary Fig. S4b).
332 However, most of these genes were upregulated following myristate application under
333 high-P conditions.

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

336 To verify the impact of exogenous myristate on the AM-activated defense response,
337 qRT-PCR was performed to analyze the expression of defense marker genes in both
338 plant species. In alfalfa roots, exogenous myristate significantly decreased the
339 expression of *alternative oxidase 3 (MsAOX3)* and *UDP-glycosyltransferase 85A*
340 (*MsUGT85A*) ($P < 0.05$), while its effect on *peroxidase P7* (*MsPRXP7*) depended on

341 soil P level (Myr × P: $P < 0.05$): exogenous myristate significantly decreased *MsPRXP7*
342 expression under high-P conditions ($P < 0.05$) but had no effect under low-P conditions
343 (Fig. 7c). In rice roots, the effect of exogenous myristate on *chitinase 1* (*OsCHT1*) was
344 also dependent on soil P level (Myr × P: $P < 0.05$): it marginally decreased *OsCHT1*
345 expression under low-P conditions ($P = 0.053$) but increased it under high-P conditions
346 ($P = 0.050$). Exogenous myristate significantly suppressed the expression of defense-
347 related transcriptional factor *OsTGA2* and *peroxidase 39* (*OsPRX39*) under low-P and
348 high-P conditions, respectively ($P < 0.05$, Fig. 7d). Overall, the qRT-PCR results largely
349 confirmed the inhibitory effects of myristate on AM-activated defense responses in
350 alfalfa and low-P rice.
351
352 **DISCUSSION**
353 AMF are obligate biotrophs that rely on host-derived symbiotic carbohydrates for
354 reproduction (Smith and Read, 2008). However, whether symbiotic AMF can uptake
355 external non-symbiotic organic C remains poorly understood (Rillig et al., 2020; Liu et
356 al., 2023). Under asymbiotic conditions, Sugiura et al. (2020) found that among eight
357 saturated or unsaturated fatty acids (C12 to C18) and two β-monoacylglycerols supplied,
358 only myristate led to an increase in the biomass of *R. irregularis*. In our study, we
359 observed slight yet statistically significant ^{13}C enrichments in the extraradical hyphae
360 of three AMF species from both hyphal and root compartments during the $^{13}C_1$-
361 myristate tracing experiment (Fig. 1c), indicating that the extraradical hyphae of the
362 tested AMF species can absorb external non-symbiotic myristate in the presence of
363 plant-derived symbiotic carbohydrates. Several additional observations further
364 supported this finding. First, exogenous myristate specifically induced the formation of
365 branched absorbing structures in AMF extraradical hyphae (Fig. 1b), which may serve

366 as preferential sites for myristate uptake (Bago et al., 1998; Sugiura et al., 2020).
367 Second, exogenous myristate activated the transcription of marker genes associated
368 with fatty acid uptake and metabolism in extraradical hyphae (Fig. 2). Third, myristate
369 application frequently enhanced both intraradical and extraradical AMF biomass (Figs.
370 2, 3, and 4), even when the symbiotic C supply from the plant was reduced (Figs. 4 and
371 5), accompanied by decreased mycorrhizal P responses (Fig. 6). In a priority study,
372 myristate application also increased colonization intensity, but not the extraradical
373 biomass of AMF in tomato roots (Liu et al., 2023). Overall, our findings indicate that
374 the extraradical hyphae of symbiotic AMF can uptake external non-symbiotic organic
375 C sources like myristate beyond those directly delivered by the host, at least under the
376 conditions studied.

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

391 litter is higher than that in soil (Fig. 4b).

392 During AM establishment, plant roots typically activate low-level defense
393 responses to limit excessive C consumption caused by over-colonization (Blilou et al.,
394 2000; Feng et al., 2019). In our study, myristate increased both intraradical and
395 extraradical biomass of the symbiotic AMF and promoted the formation of normal-
396 sized secondary spores (Figs. 2 and 3), unlike the small spores induced by myristate
397 under asymbiotic conditions (Sugiura et al., 2020). This suggests that plant roots supply
398 additional substances to AMF beyond myristate, the identification of which is essential
399 for the pure culture of AMF. Meanwhile, exogenous myristate transcriptionally
400 downregulated plant genes associated with AM-activated defenses under low-P
401 conditions (Fig. 7), suggesting that myristate may interfere with normal defense
402 mechanisms in host roots to facilitate AMF colonization. This effect contrast with AM
403 signals like strigolactones and quercetin, which enhance plant defenses (Sudheeran et
404 al., 2020; Kusajima et al., 2022). Further research is required to clarify myristate's role
405 in regulating AMF colonization and development.

406 Despite increased arbuscule abundance (Fig. 4)—where symbiotic nutritional
407 exchange occurs (Smith and Read, 2008)—exogenous myristate unexpectedly reduced
408 the mycorrhizal P response and downregulated plant and fungal mycorrhizal P
409 transporter gene expression in both alfalfa and rice roots (Fig. 6), indicating a
410 diminished mycorrhizal P benefit through inactivation of mycorrhizal P pathway
411 transcription. Meanwhile, exogenous myristate also reduced C supply to AMF from
412 both alfalfa and rice, except in low-P rice (Fig. 5). Together, these results suggest that
413 exogenous myristate disrupts the symbiotic C-P exchange, which is functionally
414 coupled in AM symbiosis (Kiers et al., 2011). This disruption could be attributed to
415 several factors. First, it may result from AMF partially shifting from utilizing symbiotic

416 C to absorbing external C (myristate, Fig. 1), thus reducing their competition for
417 symbiotic C at the AM interaction interface (Bitterlich et al., 2014). Correspondingly,
418 this decline in symbiotic C supply to AMF may decrease mycorrhizal P benefit, since
419 AMF can modulate P transport based on the rate at which plants supply C (Kiers et al.,
420 2011; Argüello et al., 2016). Second, exogenous myristate may interfere with AM
421 signaling, as implied by the interference in normal AM-activated defenses (Fig. 7),
422 thereby limiting nutrient exchange in the symbiosis. Third, AMF P transport proteins
423 expressed in arbuscular cells can re-uptake P from the symbiotic interface, creating P
424 competition between the plant and AMF (Xie et al., 2022). In our study, the increased
425 fungal biomass with exogenous myristate suggests a higher P demand by AMF,
426 reducing the feasibility of nutritional trade.

427 In contrast to its effect under high-P treatment, myristate increased, rather than
428 decreased, the allocation of rice-derived C to AMF under low-P conditions,
429 accompanied by enhanced expression of marker genes for fatty acid (but not sugar)
430 allocation from rice to AMF (Fig. 5b, f, g). These results highlight that myristate's
431 impact on AM symbiosis is influenced by soil P status, a significant regulatory factor
432 of the symbiosis by coordinating the direct P pathway and mycorrhizal P pathway in
433 AM plants (Shi et al., 2021; Das et al., 2022). The notable effects of soil P status on
434 myristate's impact on C-P exchange were also observed in alfalfa (Figs. 5 and 6), where
435 the unexpectedly higher AMF intraradical biomass (Fig. 4) and greater mycorrhizal
436 growth response (Fig. S2) in the high-P compared to the low-P treatment are likely due
437 to the inhibitory effects of extremely low-P environments on AMF colonization and
438 functioning (Janos, 2007; Wang et al., 2020). Our findings regarding the disruptive
439 effects of myristate on symbiotic C-P exchange, along with the broad yet varied
440 presence of myristate in different soil components (Fig. 4), imply that myristate may be

441 a critical influencing factor in AM symbiosis. Interestingly, myristate also exhibited
442 inhibitory effects on rhizospheric *Devosia*, which is responsible for promoting growth
443 and suppressing disease in *Arabidopsis* (Liu et al., 2024). Further research is required
444 to clarify the mechanisms underlying the disruptive effect of exogenous myristate on
445 symbiotic C-P exchange and its interaction with soil P.

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

459

460 **CONCLUSIONS**

461 In summary (Fig. 8), our study shows that symbiotic AMF can uptake external
462 non-symbiotic C sources such as myristate, which is commonly present in soil
463 components. Exogenous myristate enhanced both intraradical and extraradical biomass
464 of symbiotic AMF, possibly linked to the suppression of AM-activated defense
465 responses in host roots. Unexpectedly, exogenous myristate disrupted the C-P exchange

466 in AM symbiosis, resulting in reduced mycorrhizal P benefits for plants and decreased
467 allocation of symbiotic C to AMF, although these effects varied depending on soil P
468 conditions. These findings provide new insights into the nutritional interactions in AM
469 symbiosis, which are crucial for understanding and managing this ancient relationship
470 in a changing world.
471

472 **MATERIALS AND METHODS**

473 **Biological materials**

474 *In vitro* cultures of AMF species *Rhizophagus irregularis* (strain: MUCL 41833),
475 *R. intraradices* (strain: MUCL 49410), and *R. diaphanus* (strain: MUCL 49416)
476 associated with carrot (*Daucus carota*) hairy roots were generously supplied by the
477 Glomeromycota *In vitro* Collection (GINCO). Our study consisted of three
478 interconnected and complementary experiments.

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

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

488 When dense AMF extraradical hyphae were observed in the hyphae compartment
489 (HC), 12–14 weeks later, 200 μL of 0.1 mM myristate with 98% $^{13}C_1$ labeling ($^{13}C_1$-
490 Myr) or unlabeled (^{12}C-Myr, control) myristate was added weekly to the HC until

491 harvest, two or three weeks later. At harvest, AMF extraradical hyphae from the HC
492 and root compartment (RC) were collected separately by dissolving the medium in 10
493 mM sodium citrate, filtering through a 37 μm nylon mesh, and thoroughly rinsing five
494 times with sodium citrate followed by ultrapure water. To check for possible
495 contamination of ^{13}C-myristate from the HC to the RC, a small portion of the MSR
496 medium from the RC was also collected randomly from three $^{13}C_1$-Myr and three ^{12}C-
497 Myr (control) treatments, with roots and AMF hyphae removed using tweezers under a
498 stereomicroscope, and then used for determination of ^{13}C enrichment. During the
499 experiment, the MSR medium in the RC was not contaminated by ^{13}C-myristate from
500 the HC, as evidenced by the identical $^{13}C/^{12}C$ ratios of the MSR medium collected in
501 the RC in $^{13}C_1$-Myr (1.0966 ± 0.0012) and ^{12}C-Myr (1.0967 ± 0.0032) treatments.

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

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

507 In Exp. 1, the expression of fungal marker genes related to fatty acid uptake
508 (*RiFAT1* and *RiFAT2*, Brands and Dörmann, 2022) and metabolism—β-oxidation (*fatty*
509 *acid degradation 1* (*RiFAD1*)), palmitvaccenic acid synthesis (*oleate desaturase-like*
510 *enzyme 1* (*RiOLE1*), Brands et al., 2020), and the TCA cycle (*citrate synthase 1*
511 (*RiCIT1*))—in extraradical hyphae of *R. irregularis* from the HC, with or without
512 myristate addition, was assessed, using the elongation factor-1 beta gene (*RiEF-1β*) as
513 the reference gene (Das et al., 2022). Relative gene expression levels were calculated
514 using the comparative $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), with four
515 independent biological replicates and three technical replicates per biological replicate.

516 The collection of extraradical hyphae from the HC and qRT-PCR procedures is detailed
517 in the Supporting Information (Method S3, Table S1).
518 Carrot hairy roots in the RC from the two-compartment AMF-hairy root co-culture
519 system (Exp. 1) were also harvested using sterile tweezers and used to assess AMF
520 colonization intensity (see Supporting Information Methods S4).

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

523 In Exp. 2, we assessed the effects of exogenous myristate at varying concentrations
524 on spore germination and the development of *R. irregularis* intraradical and extraradical
525 structures associated with carrot hairy roots in a single-compartment co-culture system.
526 Briefly, three 4-cm-long sterile carrot hairy root segments were cultivated in MSR
527 medium with or without *R. irregularis* inoculation (200 sterilized spores per Petri dish).
528 Potassium myristate solution (0, 0.1, 0.5, 1, 5, or 10 mM, 200 μL) was applied to the
529 plates weekly until harvest, with five biological replicates (plates) per treatment. The
530 germination rate of AMF spores, as well as extraradical hyphal branching and length,
531 were monitored under a stereomicroscope (M250FA, Leica, Germany) and calculated
532 using ImageJ at 4 days post-inoculation (Schneider et al., 2012). Spore density was
533 monitored every 2 weeks starting 8 weeks after AMF inoculation.
534 Carrot hairy roots and MSR medium were harvested at 14 weeks post-AMF
535 inoculation. The roots and solid culture medium were carefully separated using sterile
536 tweezers. The medium was weighed and dissolved in 10 mM sodium citrate, and the
537 AMF collection was preliminarily separated into hyphae and spores using a blender,
538 followed by filtration through a 250 μm and 37 μm nylon meshes, respectively, and
539 manual selection with tweezers under a stereomicroscope. Biomasses of the
540 extraradical hyphae and spores were oven-dried at 80°C for 48 hours and quantified

separately. To evaluate the development of AMF intraradical structures in the carrot hairy roots, hyphal, vesicle, and arbuscular colonization intensities were assessed (Supporting Information Methods S4).

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

In Exp. 3, a field survey and a pot experiment were conducted. The field survey aimed to evaluate the environmental distribution of myristate in soil and plants. We analyzed myristate concentrations in bulk soil (0-10 cm) and in plant leaves, roots, leaf litter, and rhizosphere soil from the dominant species *O. sativa*, *Axonopus compressus*, and *Morus alba* in a paddy field, grassland, and woodland, respectively. The procedures regarding sample collection, pretreatment, and analysis of myristate were detailed in Supporting Information Methods S5.

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

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

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

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

574 In Exp. 3, RNA extracted from alfalfa or rice roots was reverse-transcribed into
575 cDNA using the QuantiTect Reverse Transcription Kit (Qiagen, Valencia, CA). qRT-
576 PCR was performed, with three independent biological replicates (randomly selected
577 from ten replicates) for each treatment and three technical replicates per biological
578 replicate. The relative expression levels of plant and fungal genes involved in C delivery
579 from plants to AMF (*MsSTR2*, *OsSTR2*, *RiFAT1*, *RiFAT2*, and *RiMST2*) (Brands and
580 Dörmann, 2022; Das et al., 2022), the mycorrhizal P pathway (*MsPT8*, *OsPT11*, and
581 *RiPT*) (Yang et al., 2012; Fiorilli et al., 2013), and AM-activated plant defense—
582 specifically, reactive oxygen species balance (*MsAOX3*), stress responses (*MsUGT85A*,
583 *MsPRXP7*, and *OsPRX39*), and typical defense marker (*OsCHT1* and *OsTGA2*)
584 (Rehman et al., 2018; Suleman et al., 2021; Zhang et al., 2021)—were analyzed to
585 characterize the impact of exogenous myristate on C-P nutritional exchange in AM
586 symbiosis and the underlying mechanisms at the transcriptional level. The alfalfa *actin*
587 *2* (*MsACT2*), rice *cyclin 2* (*OsCyc2*), or *R. irregularis elongation factor-1 beta* (*RiEF-*
588 *1β*) genes were used as reference genes (Yang et al., 2012; Das et al., 2022), with the
589 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 AM denotes the observed value for the target parameter (biomass or P concentration) in mycorrhizal plants, and NM represents the mean value of nonmycorrhizal plants. Multiple comparisons among different treatments were conducted using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (equal variances) or Games-Howell's post hoc (unequal variances) test. A univariate two-way ANOVA, followed by a post hoc Tukey test, was employed to assess the effects of myristate, P level, and their interactions on AMF colonization intensity and plant P concentration. The significant differences between AM and NM groups were determined using Student's t test (for normally distributed data) or Mann-Whitney U-test (for non-normally distributed data).

Author Contributions

Hanwen Chen: Investigation, Formal analysis, Methodology, Visualization. **Tian Xiong:** Investigation, Formal analysis, Data curation, Methodology. **Baoxing Guan:** Writing – original draft, Investigation, Formal analysis, Data curation, Visualization. **Jiaqi Huang:** Investigation, Methodology, Resources. **Danrui Zhao:** Investigation, Resources. **Yao Chen:** Investigation, Resources. **Haoran Liang:** Investigation, Visualization. **Yingwei Li:** Investigation, Resources. **Jingwen Wu:** Investigation, Resources. **Shaoping Ye:** Resources. **Ting Li:** Resources. **Wensheng Shu:** Supervision. **Jin-tian Li:** Supervision. **Yutao Wang:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing, Funding acquisition, Supervision, Project administration.

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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.

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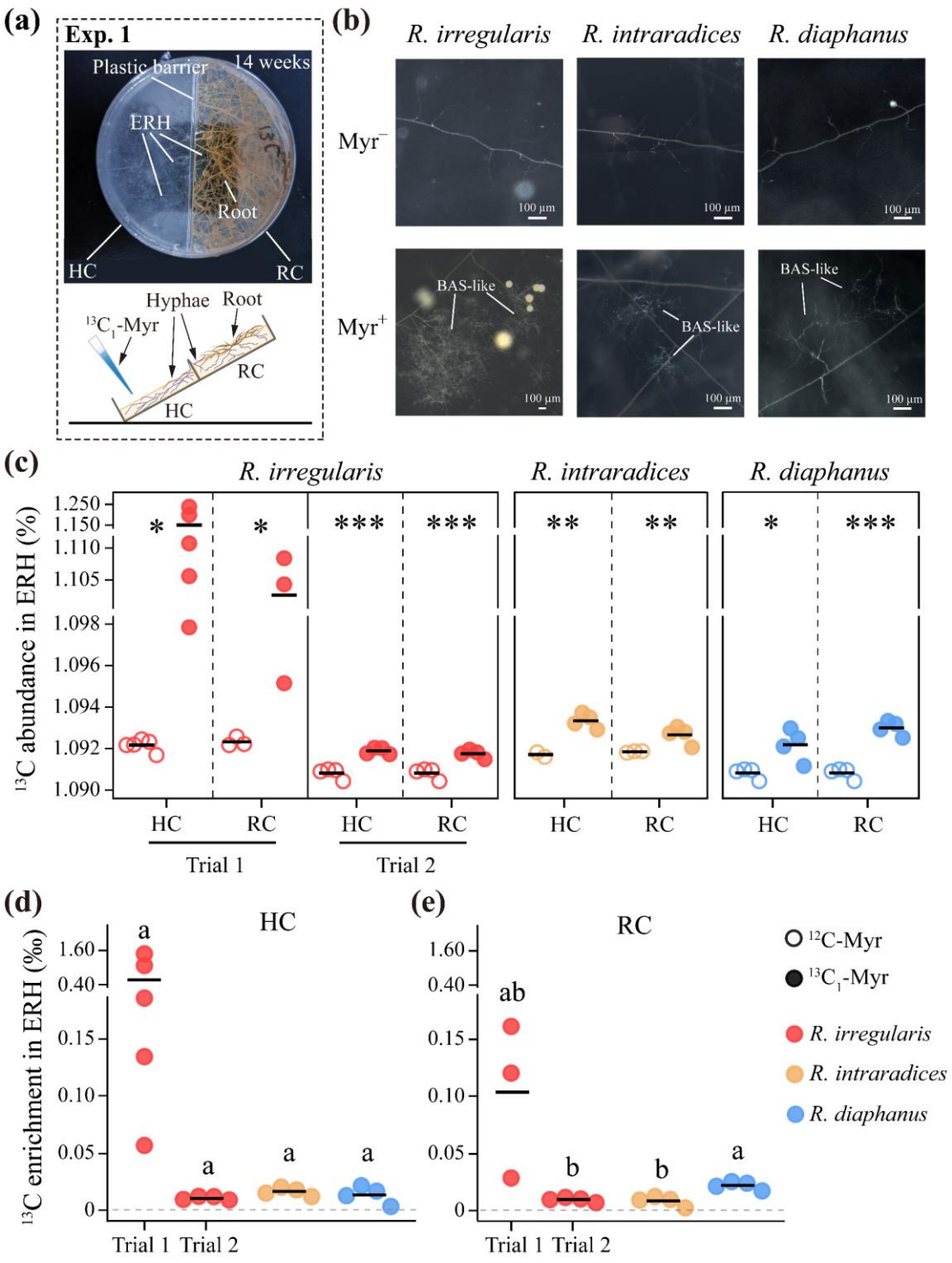

Fig. 1 Uptake of exogenous myristate by extraradical hyphae (ERH) of arbuscular mycorrhizal fungi (AMF) under symbiotic conditions in Exp. 1. **(a)** Schematic illustration of the two-compartment AMF–carrot hairy root co-cultivation system and the addition of myristate to the hyphae compartment (HC).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

843 compartment (RC). Twelve to fourteen weeks later, 0.1 mM of $^{13}C_1$-labeled ($^{13}C_1$-Myr),
844 non-labeled (^{12}C-Myr) myristate, or ddH_2O (Myr^-, control) was added weekly to the
845 HC with well-developed AMF extraradical hyphae (ERH) until harvest. **(b)** Branched
846 absorbing structures (BAS) of *R. irregularis*, *R. intraradices*, and *R. diaphanus* were
847 observed in the HC only when treated with exogenous myristate (Myr^+). **(c)** $^{13}C/^{12}C$
848 ratio of the harvested ERH from HC or RC of *R. irregularis*, *R. intraradices*, and *R.*
849 *diaphanus* when $^{13}C_1$-labeled ($^{13}C_1$-Myr) or non-labeled (^{12}C-Myr, background)
850 myristate was added to HC. Two independent experimental trials were performed for
851 *R. irregularis.* **(d, e)** Calculated ^{13}C enrichment in ERH of *R. irregularis*, *R.*
852 *intraradices*, and *R. diaphanus* in the HC and RC under ^{13}C-Myr treatment. Black
853 horizontal lines represent mean value from 2–5 biological replicates. *, **, and ***
854 indicate statistical significance between $^{13}C_1$-labeled and non-labeled treatments at the
855 0.05, 0.01, and 0.001 probability levels (Mann-Whitney U-test), respectively. Different
856 letters indicate significant difference at the 0.05 probability level (one-way ANOVA
857 followed by Games-Howell's post hoc test). Source data are provided as a Source Data
858 file.

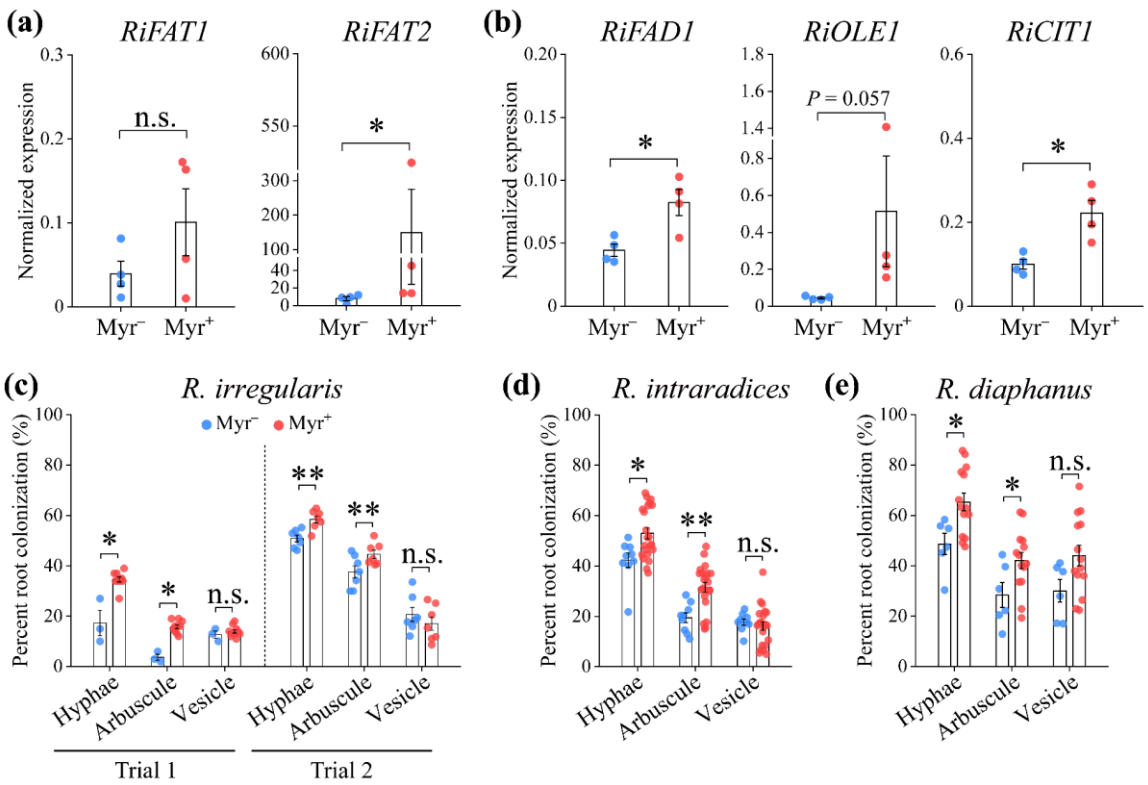

Fig. 2 Effects of exogenous myristate on the expression of fungal fatty acid uptake and metabolism genes in the extraradical hyphae (ERH) and on root colonization by arbuscular mycorrhizal fungi (AMF) in Exp. 1. (**a, b**) Normalized expression (relative to *RiEF-1β*) of AMF genes involved in **(a)** 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.

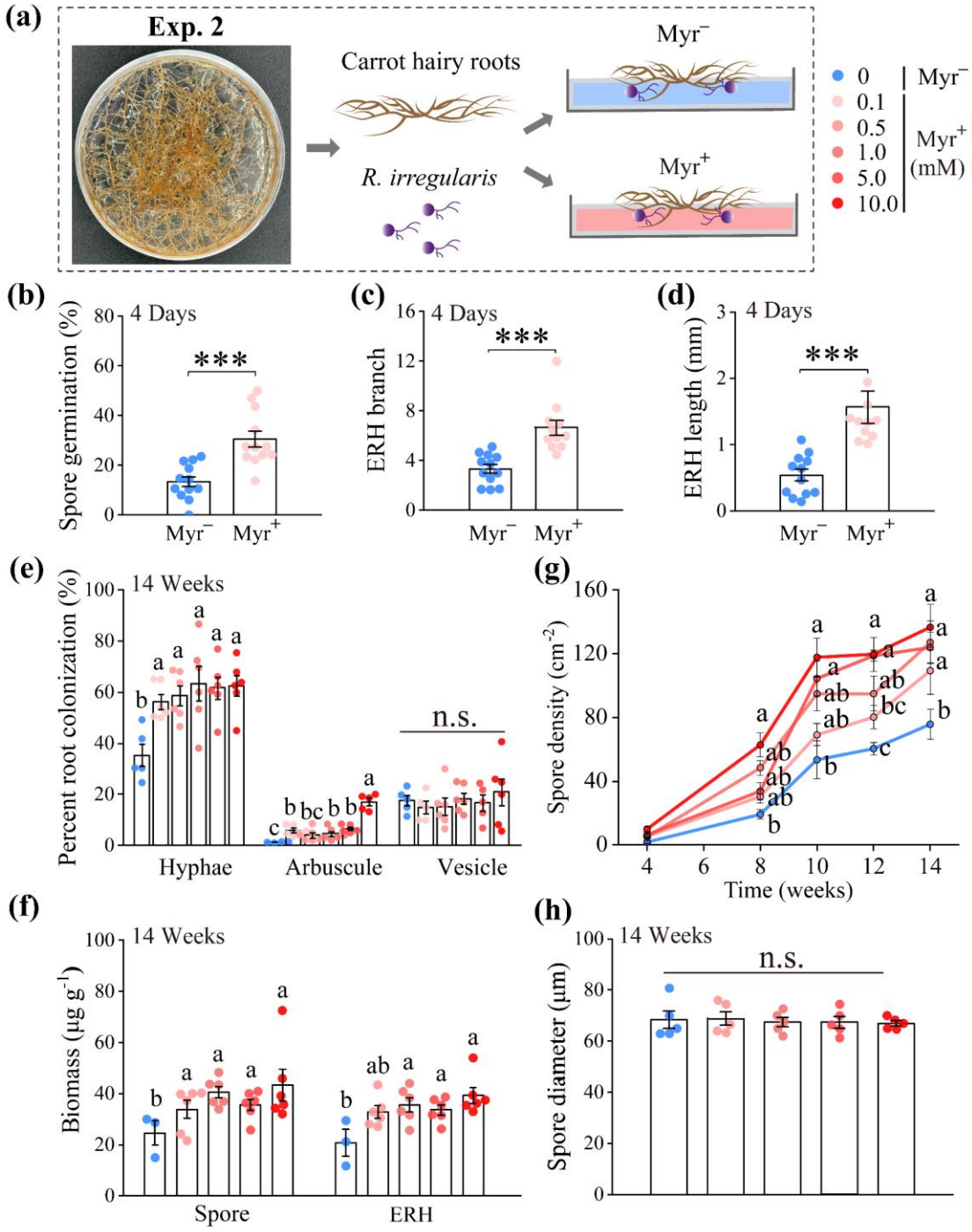

Fig. 3 Effects of exogenous myristate on the development of intraradical and extraradical structures of AMF (*Rhizophagus irregularis*) in the single-compartment AMF-carrot hairy root co-cultivation system (Exp. 2). **(a)** Schematic illustration of the single-compartment *R. irregularis*-hairy root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

880 cultivation system with (Myr^+) or without (Myr^-) myristate treatment at 4 days post-
881 inoculation (n = 11−12). **(e)** AMF percent root colonization in carrot hairy roots and **(f)**
882 biomass of the AMF extraradical spore and hypha (per gram of medium) at 14 weeks
883 post-inoculation (n = 3−6). **(g)** Dynamics of AMF spore density over time (per square
884 centimeter, n = 3−5); and **(h)** AMF spore diameter at harvest with 0, 0.1, 0.5, 1, 5, or
885 10 mM myristate treatment under symbiotic conditions (n = 5, calculation from > 900
886 spores in each replicate). Means ± SE; different letters indicate significant differences
887 at $P < 0.05$ level (One-way ANOVA followed by Tukey's post hoc test); *** indicates
888 statistical significance at the 0.001 probability level (Student's *t*-test). Source data are
889 provided as a Source Data file.

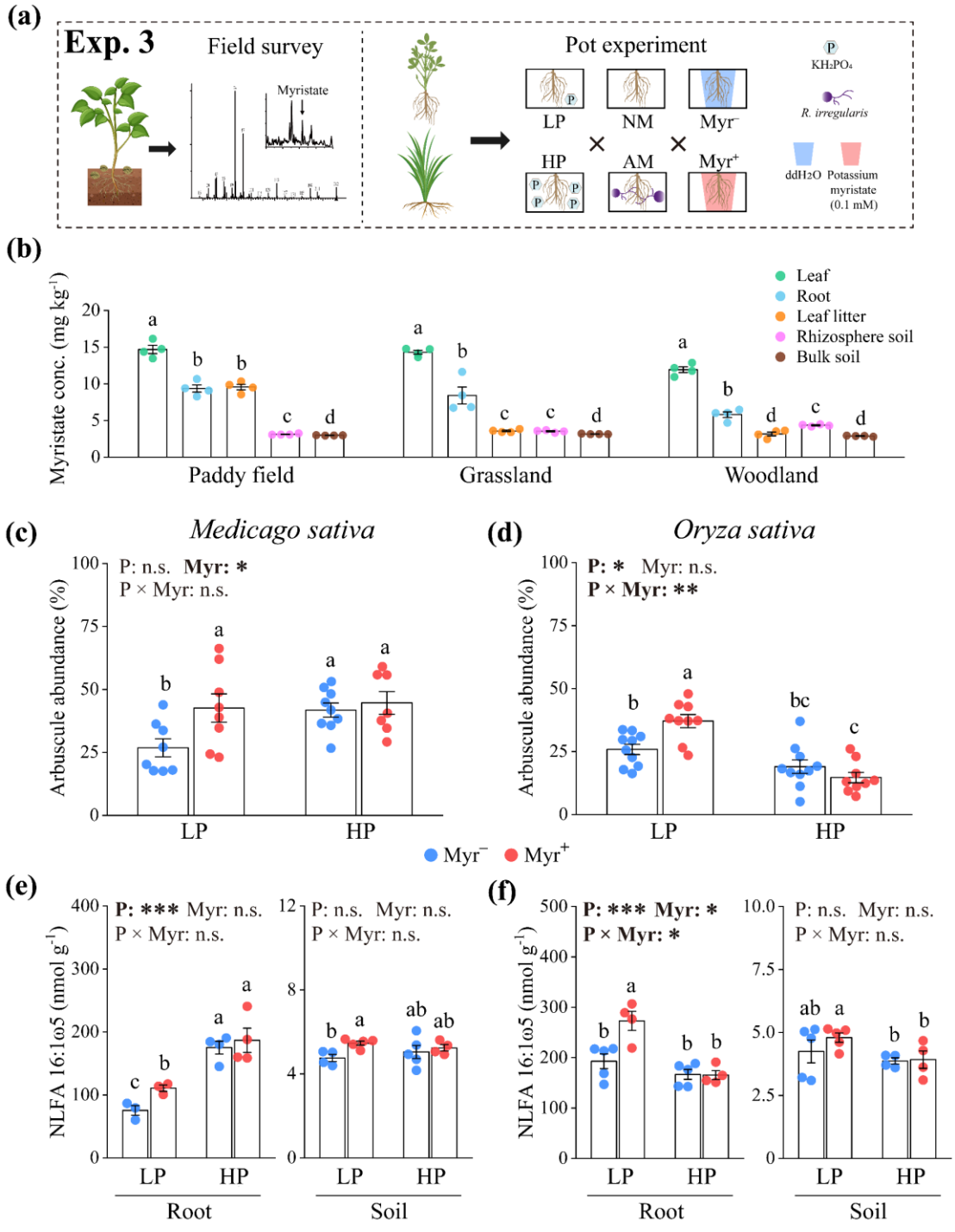

Fig. 4 Environmental myristate levels and the effects of exogenous myristate on arbuscule abundance, as well as the intraradical and extraradical biomass of arbuscular mycorrhizal fungi (AMF, *Rhizophagus irregularis*) associated with *Medicago sativa* or *Oryza sativa* under low (LP) or high (HP) phosphorus conditions (Exp. 3). **(a)** Schematic illustration of the field survey of environmental myristate levels and

896 experimental design in the pot experiment (Exp. 3). **(b)** Myristate concentration in bulk
897 soil, as well as in plant leaves, roots, leaf litter, and rhizosphere soil from the dominant
898 species *O. sativa*, *Axonopus compressus*, and *Morus alba* in the paddy field, grassland,
899 and woodland, respectively (n = 4). **(c, d)** Arbuscule abundance in *M. sativa* and *O.*
900 *sativa* roots treated with 0.1 mM myristate (Myr^+) or ddH_2O ($Myr^–$) (n = 10; vesicle
901 and hyphal abundance are shown in Supporting Information Fig. S1); **(e, f)** AMF
902 biomass, indicated by the AMF signature fatty acid (NLFA 16:1ω5), in AMF-colonized
903 roots and rhizosphere soil of *M. sativa* and *O. sativa* treated with myristate (Myr^+) or
904 ddH_2O ($Myr^–$) (n = 5). Means ± SE; different letters indicate significant differences at
905 $P < 0.05$ level (**b**: Student's *t*-test, **c**–**f**: one-way ANOVA followed by Tukey's post hoc
906 test). Source data are provided as a Source Data file.

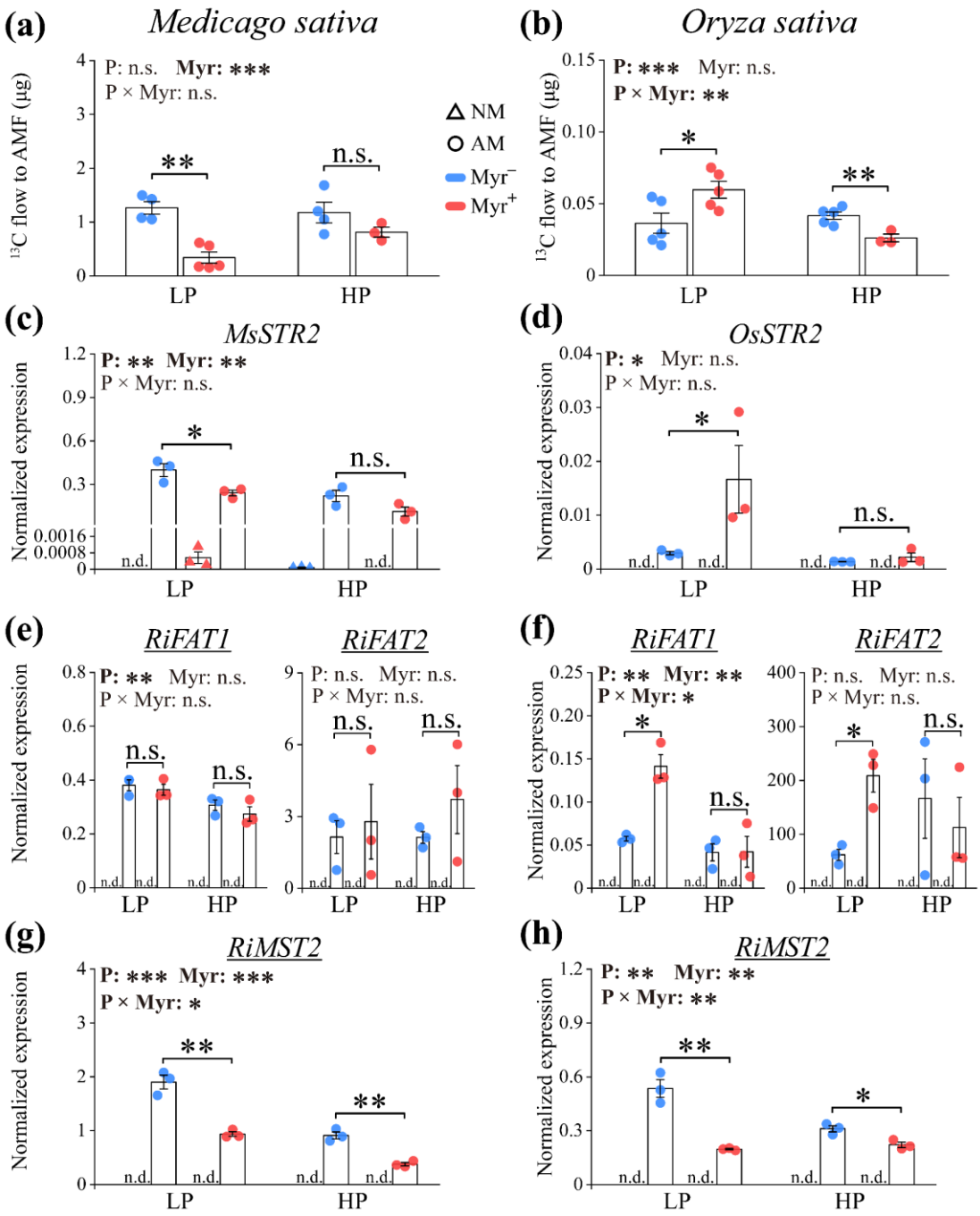

Fig. 5 Effects of exogenous myristate on symbiotic carbon (C) allocation from *Medicago sativa* and *Oryza sativa* to arbuscular mycorrhizal fungi (AMF, *Rhizophagus irregularis*) and on the expression profiles of mycorrhizal C transportation marker genes. **(a, b)** Plant-derived ^{13}C flow to the 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). **(c–h)**

914 Normalized expression of plant and fungal (underlined) marker genes for mycorrhizal
915 C transport in AMF-colonized (AM) and non-colonized (NM) roots treated with 0.1
916 mM myristate (Myr^+) or ddH_2O (Myr^-) (n = 3). The mycorrhizal C transportation genes
917 include the plant symbiotic fatty acid transporter *STR2* **(c, d**), the fungal fatty acid
918 transporters (*RiFAT1, RiFAT2*; **e, f**), and the monosaccharide transporter (*RiMST2*; **g,**
919 **h**). Normalized expression of *M. sativa*, *O. sativa*, and AMF genes was calculated
920 relative to *MsACT2*, *OsCyc2*, and *RiEF-1β*, respectively; n.d., not detected. Two-way
921 ANOVA in each histogram applies only to AM roots. Means ± SE; * and ** indicate
922 statistical significance at the 0.05 and 0.01 probability levels, respectively. Source data
923 are provided as a Source Data file.

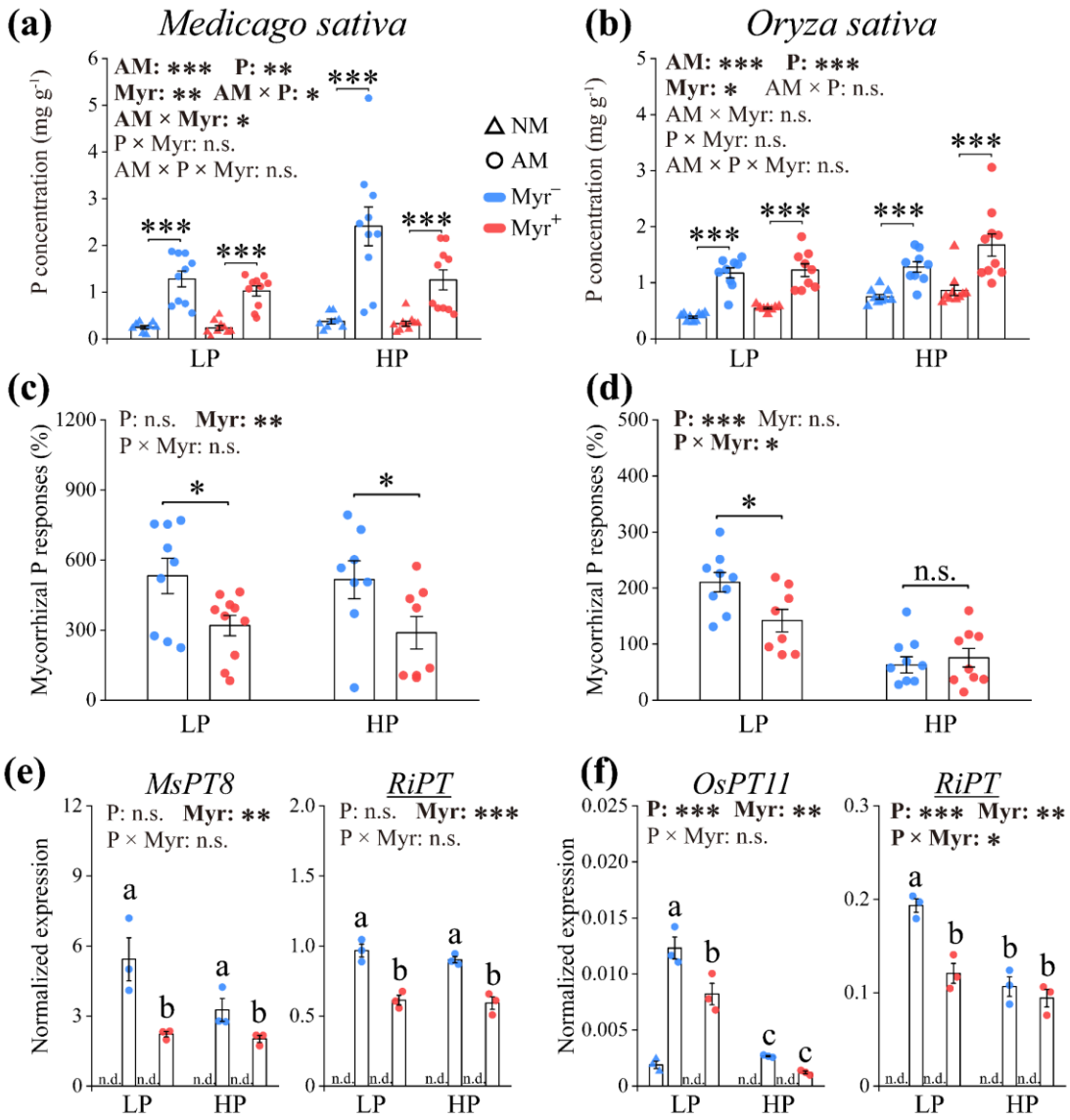

Fig. 6 Effects of exogenous myristate on plant phosphorus (P) concentration, mycorrhizal P response, and expression of plant and fungal (underlined) marker genes for the mycorrhizal P pathway in *Medicago sativa* and *Oryza sativa*. **(a, b)** Plant P concentration (n = 8−10); **(c, d)** mycorrhizal 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. Two-

way ANOVA in each histogram (c−f) applies only to AM roots. Means ± SE; different letters indicate significant differences at $P < 0.05$ level (one-way ANOVA followed by Tukey's post hoc test). * 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.

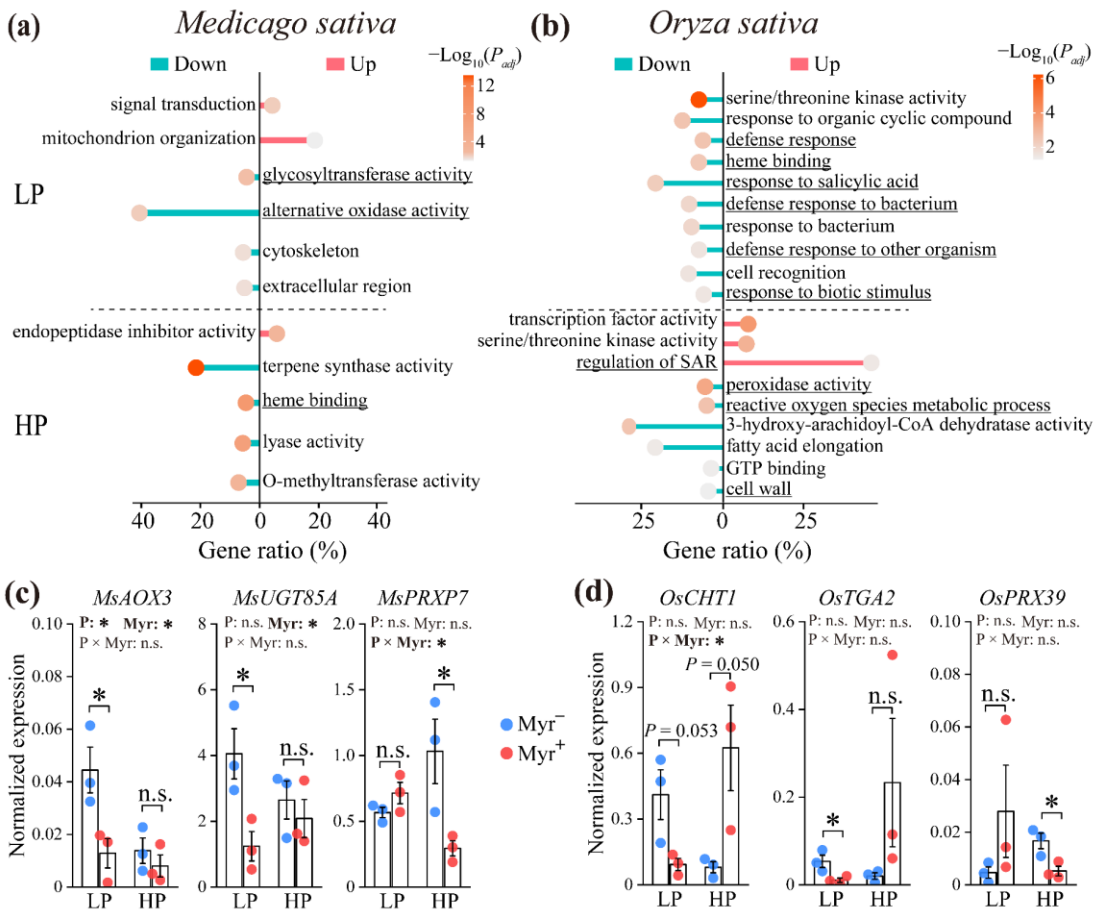

Fig. 7 Effects of exogenous myristate on the global transcriptome and putative AM-activated defense genes in *Medicago sativa* and *Oryza sativa* roots inoculated with arbuscular mycorrhizal fungi (AMF, *Rhizophagus irregularis*) under low (LP) and high (HP) phosphorus conditions. **(a, b)** Significantly 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). Means ± SE; * indicates statistical significance between Myr^+

952 and Myr^{-} treatments at the 0.05 probability level (Student's *t*-test). Source data are

953 provided as a Source Data file.

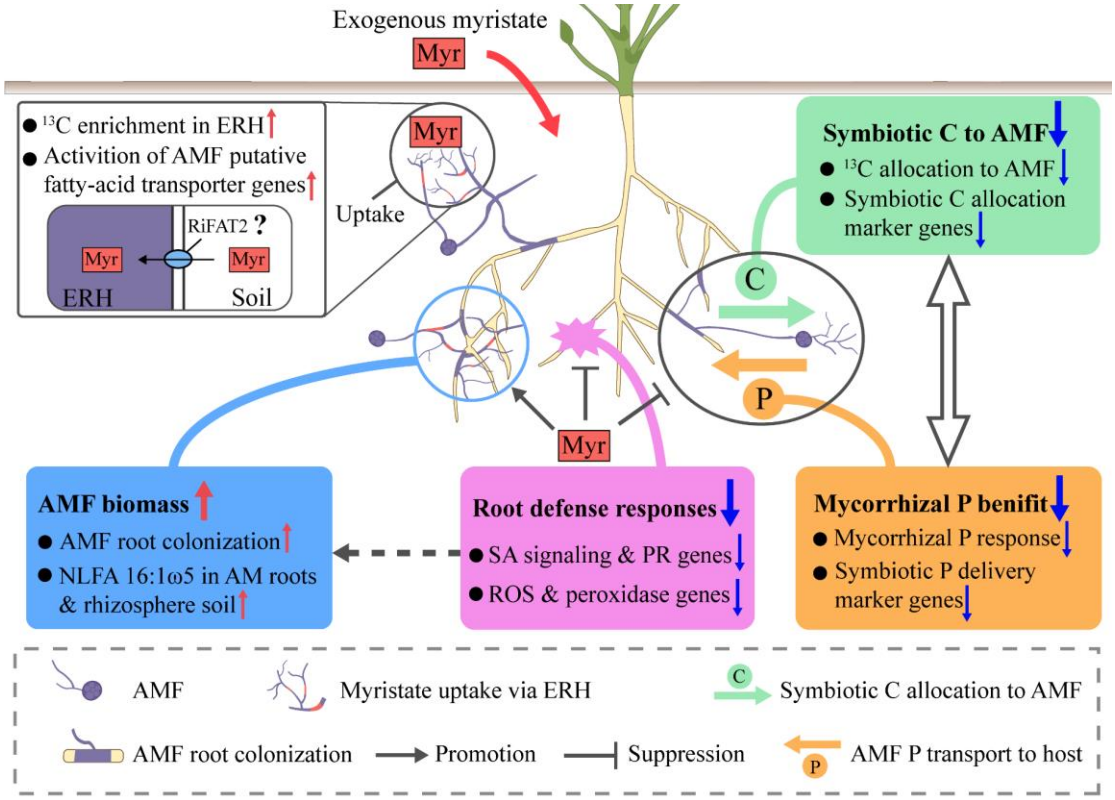

Fig. 8 A proposed model for direct uptake of exogenous myristate by symbiotic arbuscular mycorrhizal fungi (AMF) and its regulatory effects on the arbuscular mycorrhizal symbiosis. Symbiotic AMF can uptake exogenous myristate, possibly through the fungal fatty acid transporters RiFAT2 in the extraradical hyphae (ERH), as indicated by ^{13}C enrichment and transcriptional activation of fatty acid transport and metabolism genes in AMF extraradical hyphae. 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. Question mark or dashed arrow indicates putative or unclear mechanisms. SA, salicylic acid; ROS, reactive oxygen species; PR, pathogenesis-related; NLFA 16:1ω5, neutral lipid fatty acid 16:1ω5 (AMF marker).