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1 **Mycorrhizal symbiosis balances rootstock-mediated growth-defence tradeoffs**

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28 **Abstract**

29 It is well known that AM symbiosis provides several ecosystem services leading to plant adaptation
30 in different environmental conditions and positively affects physiological and production features.
31 Although beneficial effects from grapevine and AM fungi interactions have been reported, the impact
32 on growth-defence tradeoffs features has still to be elucidated. In this study, the potential benefits of
33 an inoculum formed by two AM fungal species, with or without a monosaccharide addition, were
34 evaluated on young grapevine cuttings grafted onto 1103P and SO4 rootstocks. Inoculated and non-
35 inoculated plants were maintained in potted vineyard substrate under greenhouse conditions for three
36 months. Here, agronomic features were combined with biochemical and molecular techniques to
37 assess the influence of the different treatments. Despite the opposite behaviour of the two selected
38 rootstocks, in AM samples the evaluation of gene expression, agronomic traits and metabolites
39 production, revealed an involvement of the whole root microbiome in the growth-defence tradeoffs
40 balancing. Noteworthy, we showed that rootstock genotypes and treatments shaped the root-
41 associated microbes, stimulating plant growth and defence pathways. Progresses in this field would
42 open new perspectives, enabling the application of AMF or their inducers to achieve a more
43 sustainable agriculture also in light of the ongoing climate change.

44

45 **Keywords**

46 AM fungi, trade-off, plant priming, stress tolerance, N, growth-defence balance

47

48 **Declarations**

49 ***Conflict of interest***

50 The authors declare that they have no conflict of interest.

51 ***Availability of data and material***

52 Sequences were deposited in NCBI database under the BioProject PRJNA718015, BioSamples
53 SAMN18520793 to SAMN18520808 and SRR14089924 to SRR14089939.

54 ***Author contributions***

55 WC, RMB and LN designed the experimental system. LN, GQ, GG, LM, NB, LL, RP, MG, MS,
56 FG, RMB and WC conducted the wet lab experiments and performed data elaboration. LN, GQ, RMB
57 and WC performed RT-qPCR analyses. LN, GG and WC performed the microbiome data analysis of
58 root endophytes. LN, RMB and WC wrote the first draft of the manuscript. All the authors carefully
59 revised the final version.

60 **Introduction**

61 Grapevine is one of the most cultivated crop worldwide since its great economic importance resulting
62 from grape and wine production, and commercialization (Chitarra et al. 2017). For this reason, over
63 the years viticulture industry has selected several cultivars showing different traits (*i.e.*, flavour,
64 yields, colour) influenced by geology, soil-scape and climate features, driving some major wine
65 peculiarities (Priori et al. 2019). These components, and their interactions, concur to define the *terroir*
66 of a particular environment (Resolution OIV/VITI 333/2010). Besides scion variety features,
67 rootstocks are able to strongly affect scion performances by means of water transport, biochemical
68 and molecular processes, impacting the whole plant functions and its response to biotic/abiotic stress
69 factors (Chitarra et al. 2017). In the last decade, research on scion/rootstock interactions strongly
70 increased, aiming to develop more sustainable practices against pests and ameliorating plant
71 adaptability to the ongoing climate change (Lovisolo et al. 2016; Warschefsky et al. 2016; Zombardo
72 et al. 2020). Key drivers influencing defence features and adaptive traits are thought to be the
73 microbial communities residing in plant tissues. To date, several studies reported evidence about their
74 influence on physiological performances (*e.g.*, production of flavours, hormones, VOCs) in many
75 plants, including grapevine, where residing microbiota contribute to defining the *microbial terroir*
76 (Gilbert et al. 2014).

77 According to the Intergovernmental Panel on Climate Change (IPCC 2014), an increase in the
78 global surface temperature is expected over the next years, affecting crop production as a
79 consequence of the predicted occurrence of biotic and abiotic stresses (Mittler and Blumwald 2010).
80 To achieve resilience to stress, numerous efforts have been done over the years, such as the adoption
81 of specific breeding programs and genetic engineering approaches (Cushman and Bohnert 2000).
82 Researchers have been focusing just recently their attention on the exploitation of ‘native’ plant
83 defence mechanisms (*e.g.* hormone signalling, plant immunity activation) against biotic and abiotic
84 stressful factors (Feys and Parker 2000; Jones and Dangl 2006; Hirayama and Shinozaki 2007). The
85 triggering of these responses can occur using chemical treatments (Balestrini et al. 2018), root-
86 associated microorganisms and RNA interference technologies (Alagna et al. 2020), leading plants
87 in a state of alertness - ‘Primed state’ or ‘Priming’ – and enabling them to respond more quickly and
88 robustly in case of the exposure to a stress (Beckers and Conrath 2007).

89 Among soil beneficial microorganisms, arbuscular mycorrhizal fungi (AMF) establish
90 symbioses with the majority of land plants showing an important role in providing nutrients,
91 particularly phosphate and N, but also water and other elements to the host plant (Jacott et al. 2017;
92 Balestrini and Lumini 2018). Mycorrhizal symbiosis is able to influence plant growth and
93 productivity and enhance the tolerance to biotic and abiotic stresses as demonstrated in many crops

94 (Balestrini and Lumini 2018; Balestrini et al. 2018; Alagna et al. 2020). In addition, AM fungi are
95 able to increase aggregation of soil surrounding roots, improving soil matrix stability and
96 physicochemical characteristics (Uroz et al. 2019). Grapevine roots are naturally colonized by native
97 AM fungi with a great impact on growth, yield, quality and development performances (Deal et al.
98 1972; Karagiannidis et al. 1995; Linderman and Davis 2001; Trouvelot et al. 2015). Thanks to the
99 application of metagenomics approaches to soil and roots, new insights about the AMF living in
100 symbiosis with grapevine have been discovered (Balestrini et al. 2010; Holland et al. 2014; Balestrini
101 and Lumini 2018).

102 Rootstocks-mediated adaptation to a specific environment is based on the growth-defence
103 trade-offs-mediated mechanisms (Chitarra et al. 2017). Trade-off phenomenon was firstly observed
104 in forestry plants-insect interaction studies and is based on the idea that the limited carbon resources
105 produced by photosynthesis are allocated toward growth or defence processes in order to maximize
106 the adaptation strategies and fitness costs in diverse environments (Huot et al. 2014; Chitarra et al.
107 2017; Züst and Agrawal 2017). Stresses impair plant growth, redirecting energy and carbon sources
108 toward defence, reducing growth and reproduction performances (Bandau et al. 2015; Züst and
109 Agrawal 2017). Recently, it was suggested that through a meta-analysis, that the increased plant
110 resistance promoted by *Epichloë* fungal endophytes does not compromise plant growth, eliminating
111 the trade-off between growth and defence (Bastías et al. 2021). A role in tradeoffs balance has been
112 demonstrated also for AM symbioses, improving nutrient uptake, disease tolerance and abiotic stress
113 resilience (Jacott et al. 2017).

114 In this study, we aimed to evaluate if AM fungi and rootstocks can concomitantly contribute
115 to fine-tuning growth-defence tradeoffs features in grapevine, thus enabling plants to trigger earlier
116 and enhanced defence responses against a potential stressor. The use of specific molecules that can
117 promote the AM fungal colonization have been proposed to improve mycorrhizal inoculum
118 applications under practical field condition (Bedini et al. 2018). In this context, an affordable strategy
119 is the application at low doses of oligosaccharides (*i.e.*, glucose, fructose, and xylose) that have a
120 stimulant effect on AM symbiosis colonization (Lucic and Mercy 2014 - Patent application
121 EP2982241A1). These compounds, initially called as elicitors, in relation to the impact on plant
122 defense, can promote mycorrhizal performances and, for this reason, the term “inducer” was proposed
123 (Bedini et al. 2018). In this work, the impact of an inoculum formed by two AMF species
124 (*Funneliformis mosseae* and *Rhizophagus irregularis*), already reported among the species present in
125 vineyards (Berruti et al. 2018), with or without the addition of a monosaccharide (D-glucose) at low
126 dose (the so called inducer), has been evaluated on young grapevine cuttings *cv.* Glera grafted onto
127 1103 Paulsen and SO4 rootstocks, well known to trigger an opposite growth-defence behaviour in

128 the scion. The effect of the several treatments on the root-associate microbiota has been also
129 evaluated, to verify the response mediated by the AM and its recruited mycorrhizosphere.

130

131 **Materials and methods**

132

133 **Biological materials and experimental set-up**

134 Two hundred one year-old dormant vines of ‘Glera’ cultivar grafted onto 1103 Paulsen (1103P) and
135 SO4 rootstocks certified as ‘virus free’ were purchased from an Italian vine nursery (Vivai
136 Cooperativi Rauscedo, Italy; <http://www.vivairauscedo.com>). Vine roots were washed with tap water
137 and cut to about 4 cm before plantation in 2 L pot containers filled with not sterilized substrate mixture
138 of vineyard soil/*Sphagnum* peat (8:2, v:v) to better simulate the field conditions. The substrate
139 composition was a sandy-loam soil (pH 7.8; available P 10.4 mg kg⁻¹; organic matter 1.80 %; cation
140 exchange capacity 20.11 mew 100 g⁻¹).

141 Grapevine cuttings were inoculated with AMF mixed inoculum (INOQ GmbH, Germany,
142 238,5 Million propagule per kg inoculum) at planting time by placing it in the hole and in contact
143 with the roots following the manufacturer’s instructions. Mycorrhizal inoculum, a powder based
144 mycorrhizal root fragment (Advantage Grade II, 2016 - INOQ GmbH) contained 50% *Rhizoglossum*
145 *irregulare* (syn. *Rhizophagus irregularis*; 450 million propagules per Kg) and 50 % *Funneliformis*
146 *mosseae* (27 million propagules per Kg). The fungal lines were produced *ex vitro*, on *Zea mays* and
147 *Plantago lanceolata* (sand/vermiculite, v/v). Both AMF inoculum and D-glucose at low dose (i.e.,
148 the Inducer) were prepared by Louis Mercy (INOQ GmbH; patent EP2982241A1). The containers
149 were prepared according to treatments as follow: i) 25 plants for each rootstock as uninoculated
150 control plants (C); ii) 25 plants for each rootstock inoculated with 50 mg/L of AMF mixed inoculum
151 (M); iii) 25 plants for each rootstock inoculated with 50 mg/L of AMF mixed inoculum + inducer
152 (M+I); iv) 25 plants for each rootstock amended with 50 mg/L of inducer to stimulate the exploitation
153 of native AMF symbiosis (I). Daily watered grapevine plants were kept under partially climate-
154 controlled greenhouse, under natural light and photoperiod conditions for three months.

155 After three months, at the end of the experiment, engraftment, growth index and chlorophyll
156 content were recorded. Leaf and root samples for molecular and biochemical analysis were collected
157 from at least three randomly selected plants and immediately stored at -80°C. A part of the root
158 apparatus was used to estimate the level of mycorrhiza formation as described (Balestrini et al. 2017).
159 Morphological observations in the colonized fragments of thin roots allowed to identify the presence
160 of the typical structures of the symbiosis, regardless of the thesis. However, the patchy level of
161 colonization, and the quality of the root segments after the staining, made morphological

162 quantification difficult, and therefore the AMF presence has been assessed by molecular analyses
163 (see below).

164

165 **Growth index, engraftment, and chlorophyll content**

166 At the end of the experiment, phenological stages were recorded and classified according to
167 Biologische Bundesanstalt, bundessortenamt und CHEmische industrie (BBCH) scale (from 00 to 12,
168 from dormancy to 9 or more leaves unfolded, respectively). BBCH scales have been developed for
169 many crops, including grapevine, and it is based on a decimal code system that identify the growth
170 stage (Lancashire et al. 1991), engraftment % (i.e. rooting %) were visually determined for each plant
171 and treatment. Chlorophyll content was determined using a portable chlorophyll meter SPAD (Konica
172 Minolta 502 Plus). Readings were collected from the second or third leaf from the top on at least three
173 leaves per plant on five randomly selected vines for each experimental condition (Chitarra et al.
174 2016).

175

176 **Targeted metabolite analyses**

177 Contents of *trans*-resveratrol, viniferin and abscisic acid (ABA) were quantified on at least three
178 biological replicates per condition according to the protocol previously described (Pagliarani et al.
179 2019, 2020; Mannino et al. 2020). Leaves and roots from two randomly selected plants were pooled
180 to form a biological replicate, immediately frozen in liquid nitrogen, freeze-dried and stored at -80°C
181 until use. Briefly, about 100 mg of freeze-dried sample (leaf or root) were transferred with 1 mL of
182 methanol:water (1:1 v/v) acidified with 0.1 % (v/v) of formic acid in an ultrasonic bath for 1 h.
183 Samples were centrifuged for 2 min at 4°C and 23.477 g, and the supernatant was analysed by high-
184 performance liquid chromatography (HPLC). Original standards of resveratrol (purity ≥ 99 %),
185 viniferin (purity ≥ 95 %) and ABA (purity ≥ 98.5%, Sigma-Aldrich) were used for the identification
186 by comparing retention time and UV spectra. The quantification was made by external calibration
187 method. The HPLC apparatus was an Agilent 1220 Infinity LC system (Agilent R, Waldbronn,
188 Germany) model G4290B equipped with gradient pump, auto-sampler, and column oven set at 30°C.
189 A 170 Diode Array Detector (Gilson, Middleton, WI, United States) set at 265 nm (ABA and IAA)
190 and 280 nm (for stilbenes) was used as detector. A Nucleodur C18 analytical column (250x4.6 mm
191 i.d., 5 µm, Macherey Nagel) was used. The mobile phases consisted in water acidified with formic
192 acid 0.1% (A) and acetonitrile (B), at a flow rate of 0.500 mL min⁻¹ in gradient mode, 0-6 min: from
193 10 to 30 % of B, 6-16 min: from 30 % to 100 % B, 16-21 min: 100% B. Twenty µL was injected for
194 each sample.

195

196 **Total N, soluble carbohydrate content in leaf and net nitrate uptake in root**

197 The Kjeldahl method was performed according to method 981.10 of the AOAC International (2016),
198 using VELP Scientifica DKL 20 Automatic Kjeldahl Digestion Unit and UDK 159 Automatic
199 Kjeldahl Distillation and Titration System. Approximately 0.2 g of leaf raw material was hydrolyzed
200 with 15 mL concentrated sulfuric acid (H₂SO₄) containing one catalyst tablets (3.47 g K₂SO₄ + 0.003
201 Se, VELP Scientifica, Italy) in a heat block (DK Heating Digester, VELP Scientifica, Italy) at 300°C
202 for 2 h. After cooling, H₂O was added to the hydrolysates before neutralization with NaOH (30%)
203 and subsequently distilled in a current of steam. The distillate was collected in 25 mL of H₃BO₃ (1%)
204 and titrated with HCl 0.1 M. The amount of total N in the raw materials were calculated.

205 Leaf soluble carbohydrate content was quantified (Chitarra et al. 2018).

206 At the end of the experiment, white non-lignified roots (0.5 – 1 g) were collected from four
207 randomly selected plants for each treatment and rootstock. Root samples were washed in 0.5 mmol
208 L⁻¹ CaSO₄ for 15 min, then transferred to a 20 mL aerated uptake solution containing 0.5 mmol L⁻¹
209 Ca(NO₃)₂ and 0.5 mmol L⁻¹ CaSO₄. Net uptake of NO₃⁻ was measured removing samples of uptake
210 solution (aliquot of 200 µL) for its determination every 2 min for 10 min (Tomasi et al. 2015). The
211 aliquots were carefully mixed with 800 µL of salicylic acid (5% w/v in concentrated H₂SO₄) and
212 incubated for 20 min at room temperature following the addition of 19 ml of 2 mol L⁻¹ NaOH. After
213 cooling, nitrate concentration was measured at the absorbance of 410 nm (Shimadzu UV Visible
214 Spectrophotometer UVmini-1240. Kyoto, Japan) and the net nitrate uptake was expressed as µmol (g
215 FW h⁻¹).

216

217 **RNA isolation and RT-qPCR**

218 Expression changes of target transcripts were profiled on root and leaf samples (three independent
219 biological replicate for each treatment) by quantitative real-time PCR (RT-qPCR) (Chitarra et al.
220 2018). Total RNA was isolated from the same lyophilized samples (leaves and roots) used for HPLC-
221 DAD analysis and cDNA synthesis was performed as previously reported (Chitarra et al. 2016). The
222 absence of genomic DNA contamination was checked before cDNA synthesis by qPCR using *VvUBI*
223 specific primers of grapevine. RT-qPCR reactions were carried out in a final volume of 15 µL
224 containing 7.5 µL of Rotor-GeneTM SYBR[®] Green Master Mix (Qiagen), 1 µL of 3 µM specific
225 primers and 1:10 of diluted cDNA. Reactions were run in the Rotor Gene apparatus (Qiagen) using
226 the following program: 10 min preincubation at 95°C, followed by 40 cycles of 15 s at 95°C, and 30
227 s at 60°C. Each amplification was followed by melting curve analysis (60–94°C) with a heating rate
228 of 0.5°C every 15 s. All reactions were performed with at least two technical replicates. The
229 comparative threshold cycle method was used to calculate relative expression levels using plant

230 (elongation factors, actin and ubiquitin, *VvEF* and *VvUBI* for root and *VvACT* and *VvEF* for leaf
231 tissue) reference genes. While *R. irregularis* and *F. mosseae* elongation factors (*RiEF1*, *FmEF*,
232 respectively) were used to normalized the expression of the AMF phosphate transporter (*PT*) genes.
233 Oligonucleotide sequences are listed in Supplementary Table 1. Gene expression data were calculated
234 as expression ratio (Relative Quantity, RQ) to Control 1103P plants (C 1103P).

235

236 **Root DNA isolation and sequencing**

237 Root samples were lyophilized prior to DNA extraction. About 30 to 40 mg of freeze-dried and
238 homogenized material were used to extract total DNA following manufacturer instruction of
239 plant/fungi DNA isolation kit (Norgen Biotech Corp., Thorold, ON, Canada) as previously reported
240 (Nerva et al. 2019). Total DNA was quantified using a NanoDrop One spectrophotometer (Thermo
241 Fisher Scientific, Waltham, MA, USA), and DNA integrity was inspected running the extracted
242 samples on a 1% agarose electrophoretic gel. Before sending DNA to sequencing a further
243 quantification was performed using a Qubit 4 Fluorometer (Thermo Fisher Scientific, Waltham, MA,
244 USA).

245 To inhibit plant material amplification, we added a mixture of peptide nucleotide acid (PNA)
246 blockers oligos (Kaneka Eurogentec S.A., Belgium) targeted at plant mitochondrial and chloroplast
247 16S rRNA genes (mitochondrial and plastidial) and plant 5.8S nuclear rRNA. Mitochondrial
248 sequence was derived from (Lundberg et al. 2013) with a 1bp mismatch, mitochondrial sequence was
249 derived from (Cregger et al. 2018). PNA was custom-designed for *V. vinifera* (VvpPNA:
250 GGCTCAACCCTGGACAG; Vv-ITS-PNA: CGAGGGCACGCCTGCCTGG; Vv-mPNA:
251 GGCAAGTGTTCTTCGGA). Thermal cycler conditions were maintained as suggested by the
252 Illumina protocol as previously reported (Nerva et al. 2019).

253 Sequences were deposited in NCBI database under the BioProject PRJNA718015,
254 BioSamples SAMN18520793 to SAMN18520808 and SRR14089924 to SRR14089939.

255

256 **Rhizoplane metapangenomic analyses, taxonomic distributions**

257 A first strict quality control on raw data was performed with PrinSeq v0.20.4 (Schmieder and
258 Edwards 2011) and then processed with Qiime2 (Bolyen et al. 2019). A previously reported and
259 specific pipeline was used for fungal analysis: retained reads were used to identify the start and stop
260 sites for the ITS region using the hidden Markov models (HMMs) (Rivers et al. 2018), created for
261 fungi and 17 other groups of eukaryotes, which enable the selection of ITS-containing sequences.
262 Briefly, the software allows to distinguish true sequences from sequencing errors, filtering out reads
263 with errors or reads without ITS sequences. To distinguish true sequences from those containing

264 errors, sequences have been sorted by abundance and then clustered in a greedy fashion at a threshold
265 percentage of identity (97%). Trimmed sequences were analyzed with DADA2 (Callahan et al. 2016)
266 and sequence variants were taxonomically classified through the UNITE (Abarenkov et al. 2010)
267 database (we selected the reference database built on a dynamic use of clustering thresholds). For
268 graphic representation, only genera with an average relative abundance higher than the settled
269 threshold (1%) were retained.

270 A 16S specific pipeline was used for bacteria: quality filtering was performed with DADA2
271 which is able to perform chimera removal, error-correction and sequence variant calling with reads
272 truncated at 260 bp and displaying a quality score above 20. Feature sequences were summarized and
273 annotated using the RDP classifier (Cole et al. 2014) trained to the full length 16S database retrieved
274 from the curated SILVA database (v132) (Quast et al. 2012).

275

276 **Statistics**

277 Metagenome analyses were performed using R version 3.6.3 (2020-02-29). Fungal and bacterial data
278 were imported and filtered with Phyloseq package (version 1.28.0) (McMurdie and Holmes 2013),
279 keeping only the operational taxonomic units (OTUs) with a relative abundance above 0.01 in at least
280 a single sample. Differential abundance of taxa due to the effects of rootstock-treatment interaction
281 was then tested using DESeq2 (version 1.24.0) (Love et al. 2014) package.

282 For phenotypic, biochemical and RT-qPCR data, when ANOVA indicated that for either
283 Rootstock (R, 1103P and SO4), Inducer (I, NI) and Myc inoculum (M, Myc and NMyc) factors or
284 their interaction was significant, mean separation was performed according to Tukey's HSD test at a
285 probability level of $P \leq 0.05$. ANOVA and Tukey's HSD test were also used to analyze the treatments
286 effects for each rootstock individually. The standard deviation (SD) or error (SE) of all means were
287 calculated.

288

289 **Results**

290

291 **Growth, primary metabolism and N uptake and accumulation**

292 The impact of an AM inoculum, an inducer and a combination of both was evaluated on growth
293 parameters (both rooting % and growth stages coded by BBCH scale) in two grapevine rootstock
294 genotypes (R, 1103P and SO4). Four conditions for each genotype were considered: C, not inoculated
295 plants; I, plants treated with the inducer (I); M, AM-inoculated plants; M+I, AM-inoculated plants +
296 inducer.

297 Results showed a similar impact of the three treatments on the cutting growth parameters (Fig.
298 1, Table S2), independently from the genotype. Particularly, in SO4 genotype both the rooting % and
299 the BBCH values were higher in treated plants with respect to the control (Fig. 1a,b). Chlorophyll
300 Content Index (CCI) has been evaluated at the end of the experiment, showing no strong differences
301 among the genotypes and treatments (Fig. 1c), although it was significantly influenced by root
302 colonization (M), the inducer (I) and the M x I interaction in both rootstock genotypes.

303 Treatments generally led to slightly lower values of carbohydrates content in leaves with the
304 exception of M, and only R and I factors significantly influenced this measurement (Fig. 1d). In detail,
305 for each rootstock I and M+I plants showed significant lower levels of carbohydrates (Fig. 1d).

306 Net Nitrate uptake (NNU) was evaluated (Fig. 2a Table S2), showing that it was significantly
307 affected by M factors and the interaction M x I with lower values in treated samples for both
308 genotypes, particularly in M SO4 plants with respect to C SO4 ones (Fig. 2a).

309 As for the CCI, only slight differences in total N content in leaves were evident among
310 genotypes and treatments, although was significantly affected by the M factor and the M x I
311 interactions (Fig. 2b).

312

313 **ABA Content and the Expression of ABA-related Genes**

314 To complete the physiological characterization of the two genotypes in response to treatments, the
315 concentration of ABA was quantified in roots and leaves (Fig. 3, Table S2). ABA levels showed a
316 complex scenario in roots where all treatments led to higher ABA levels with respect to the control
317 with the greater significant increase recorded in M SO4. Statistical analyses showed that factors
318 influencing its level were R and M, alone or in the interactions with I (R x I, M x I, R x M x I) (Fig.
319 3a). ABA content in leaves was under the detection limit among the treatments (data not shown).

320 To better understand the role of ABA in our system, the expression of ABA-related genes was
321 analyzed in both leaves and roots. Relative expression of: i) a gene encoding for a 9-cis-
322 epoxy-carotenoid dioxygenase potentially involved in ABA biosynthesis (*VvNCED3*,
323 VIT_19s0093g00550 previously reported as *VvNCEDI*); ii) a gene coding for an enzyme involved in
324 conversion of ABA to 8'-hydroxy ABA (*VvABA8OHI*); iii) a β -glucosidase (BG) involved in free
325 ABA biosynthesis *via* hydrolysis of ABA glucose ester to release the ABA active form (*VvBGI*; Jia
326 et al. 2016); iv) a gene encoding an ABA glucosyltransferase (*VvGT*; Sun et al. 2010) were evaluated
327 in leaves and roots. In leaves, *VvNCED3* expression was not affected by rootstock genotype whereas
328 M samples showed significantly higher expression levels with respect to the other samples (Fig. 3b).
329 No significant difference was detected for *VvABA8OHI* expression in leaves although 1103P
330 generally showed higher values with respect to SO4 (Fig. 3c). By contrast, *VvNCED3* expression in

331 roots was influenced by R, M and I factors as well as by R x I interaction, and values for each
332 rootstock genotype were lower in all treatments when compared to C plants (Fig. 3d). Similar to that
333 observed in leaves, M+I treatment led to the significant lowest *VvNCED3* transcripts level in root
334 samples (Fig. 3d). Two pathways promote free ABA accumulation: (1) NCED-mediated *de novo*
335 synthesis (Qin and Zeevaart 1999) and (2) BG-mediated hydroxylation (Lee et al. 2006). Looking at
336 *VvBGI* gene, its expression was significantly influenced by R and I in leaves, while the presence of
337 the AMF was not significantly relevant. In roots all the factors and interactions, significantly affected
338 *VvBGI* expression level, with the highest level in C SO4 samples (Fig. 3e,g). Finally, *VvGT* showed
339 a trend similar to *VvBGI* in leaves where its expression was significantly influenced by R, I and I x
340 M with the exception of SO4 samples where its expression was significantly higher only in M SO4
341 with respect to C SO4 (Fig. 3f). Conversely, in roots *VvGT* transcript levels were significantly lower
342 in all the conditions with respect to the C 1103P plants (Fig. 3h).

343 Although *VvABA8OHI*, coding for an enzyme involved in ABA conversion, was not
344 significantly regulated among genotypes and treatments in leaves, it results to be affected by all the
345 considered factors and interactions in roots (Fig. 3i) where it appeared significantly upregulated in M
346 1103P, M SO4 and M+I SO4 plants with respect to their C (Fig. 3i). It is worth noting the low
347 expression in I root samples, suggesting that the inducer may affect ABA catabolism independently
348 from the genotype and the presence of the AM inoculum.

349

350 **Defense**

351 Stilbenes are the main defense-related metabolites synthesized in grapevine. In this study *trans-*
352 resveratrol and viniferin levels were measured in leaves among the several conditions tested (Fig. 4,
353 Table S2). Particularly, resveratrol was only affected by the MxI interaction, showing in parallel
354 significantly higher levels in I and M plants, independently from genotype, with respect to M+I and
355 C plants (Fig. 4a). Viniferin, which was not detectable in C plants, was affected by the M x I
356 interaction and by the I factor alone. I, M and M+I treated plants presented in fact significantly higher
357 values of viniferin than C plants in both rootstocks (Fig. 4b). To correlate biochemical data with
358 molecular responses, expression levels of genes coding for two stilbene synthases (*VvSTS1* and
359 *VvSTS48*) were assessed. Results showed that in both rootstocks *VvSTS1* was upregulated mainly in
360 M 1103P whereas in SO4 plants was observed an upregulation in both I and M with respect to the
361 other treatments (Fig. 4c). *VvSTS48* expression was influenced by all the factors and their interactions,
362 with the highest expression value in leaves of I-treated SO4 plants (Fig. 4d). Looking independently
363 at each rootstock, in 1103P only I and M induced significant overexpression of *VvSTS48* while in
364 SO4 plants all the treatments showed enhanced gene expression compared to their controls (Fig. 4d).

365 RT-qPCR was also applied to detect the expression levels of several target genes as markers
366 of diverse defense response pathways (Fig. S1, Table S2). Two genes were studied both in leaves and
367 roots (a sugar transporter, *VvSPT13* and a class III chitinases, *VvChitIII*), three genes only in leaves
368 (a callose synthase, *VvCAS2*; a lipoxygenase *VvLOX*, and the Enhanced Disease Susceptibility 1,
369 *VvEDSI*) (Fig. S1a-g). Expression of all the considered genes were influenced by I factor, while
370 influence by M was more variable, suggesting a different impact of the treatments on plant
371 metabolism. Among these genes, *VvSPT13*, encoding a sugar transporter, in leaves of both rootstocks
372 was significantly upregulated in all treatments with respect to their C plants (Fig. S1a) while in root
373 only M-treated plants showed significantly higher expression values (Fig. S1). *VvChitIII* showed a
374 different pattern in leaves and roots. In leaves, *VvChitIII* transcript was significantly induced in M-
375 and M+I-treated plants (Fig. S1c) while in roots an upregulation was observed only in M-treated ones
376 (Fig. S1d). *VvCAS2*, coding for a callose synthase (Santi et al. 2013), showed a downregulation in all
377 the treatments, while *VvLOX* gene, encoding a lipoxygenase involved in the jasmonic acid
378 biosynthesis, was upregulated in all the treatments: among them, the lowest value was observed in M
379 SO4 plants (similar to the C 1103P leaves), suggesting a different response to symbioses in the two
380 genotypes (Fig. S1e-f). *VvEDSI*, selected as marker of Systemic Acquired Responses (SAR)
381 mediated by Salicylic Acid (SA), was influenced by I and M, showing an upregulation trend in I-
382 treated leaves. Conversely, this gene was downregulated in M-treated plants (Fig. S1g).

383

384 **Rhizoplane metaphylogenomic analyses**

385 Bacterial community was analyzed at both order and genus level: the number of retained sequences
386 after chimera removal and taxonomical assignment was always above 35,000 (detailed results of
387 sequencing are reported in Table S3). Shannon index diversity indicated that the only significant
388 difference was observed for the I SO4 samples which show higher index values (Table S4). No
389 significant differences were observed among samples comparing the Shannon index on the fungal
390 community (Table S5). Similar to Shannon index, non-metric multidimensional scaling (NMDS)
391 based on Bray-Curtis dissimilarity matrixes showed that the bacterial community (Fig. 5a) is more
392 affected by treatments than the fungal one (Fig. S2).

393 The bacteria community composition for each sample type at order and genus levels are
394 reported in Table S6. Statistical results of pairwise comparisons among genera are reported in Table
395 S7. To simplify, results are described for the orders and genera that represent at least the 1% of the
396 bacterial community (Fig. 5b). Comparison of the bacterial community between the two rootstocks
397 (1103P vs SO4) revealed that 1103P has a significant higher relative abundance of *Pseudomonas*
398 species whereas SO4 has a significant higher relative abundance of *Bacillus* species. Among the

399 bacterial genera, which display significant differences among the treatments, M 1103P vines
400 stimulated the presence of *Bacillus* species but repressed the interaction with *Pseudomonas* ones. In
401 parallel, when comparing treatments on SO4 rootstock, a positive interaction between the mycorrhizal
402 inoculation and the *Pseudomonas* abundance was observed, whereas the inducer treatment showed a
403 negative impact on *Flavobacterium* abundance.

404 The fungal community composition for each sample type at order and genus levels are
405 reported in Table S6. Statistics of the pairwise comparisons among genera are reported in Table S8.
406 Results for the fungal orders and genera that represent at least the 1% of the fungal community are
407 reported in Fig. S3. Focusing on AMF, results confirm the presence of *Rhizophagus* and
408 *Funneliformis* in inoculated plants. However, AMF were detected also in the I-treated plants (Fig.
409 6a). Despite the presence of AMF associated to these roots, gene expression analysis on fungal PT
410 genes showed the presence of *RiPT* and *FmPT* transcripts only in M-inoculated plants. Surprisingly,
411 absent or low expression levels were detected in I-treated plants (Fig. 6b,c; Table S2). Indeed, fungal
412 *PT* genes were expressed in a different way in the two genotypes, suggesting a different symbiosis
413 efficiency of the two rootstocks. This finding was further confirmed by a plant PT gene (*VvPTI-3*),
414 which expression level was mainly affected by R and M factors, and by 'R x I' interaction. It was up-
415 regulated in 1103P roots, independently by treatment, with respect to C 1103P and strongly up-
416 regulated in M SO4 ones (Fig. 6d, Table S2).

417 Comparing the fungal composition in C, 24 genera with significant differences of relative
418 abundance were observed. Among the analyzed genera, *Clonostachys* displayed a significant negative
419 correlation with all the treatment in both rootstock genotypes. Focusing on significant genera, usually
420 involved in pathogenic interaction, such as *Fusarium*, *Rhizoctonia* and *Ilyonectria* (Fig. S4), the
421 concomitant use of mycorrhizal inocula with the inducer brought to a significant reduction of
422 *Ilyonectria* in both rootstocks. Conversely, *Fusarium* abundance was stimulated in all treatments
423 except for the inoculation with AMF in the 1103P rootstock. Finally, *Rhizoctonia* genus was
424 positively influenced by the inducer, but only in the SO4 rootstock.

425

426 **Discussion**

427 **Treatments and genotypes differently shape the root-associated bacterial and fungal** 428 **communities**

429 The importance of root-associated microbes was extensively demonstrated in several crops including
430 grapevine, with the potential to exploit biocontrol strategies that rely on the beneficial traits of plant
431 growth-promoting microorganisms (PGPBs) naturally associated with plants (Verbon and Liberman
432 2016; Marasco et al. 2018; Yu et al. 2019). Among them, AMF and their impacts on diverse plant

433 species, including economically important crops, have been largely studied highlighting the
434 importance of this relationship that can positively affect both growth and defense traits (Jacott et al.
435 2017). However, despite these advantages, grapevine breeders normally focus their attention more on
436 phenotypic or metabolic peculiarities rather than on the improvement of the interactions with root-
437 associated microbes (Marín et al. 2021).

438 Grapevine roots are commonly colonized by different AMF taxa depending on the considered
439 environment, season and soil management making them relevant in defining the ‘microbial terroir’
440 of a specific grape cultivar (Massa et al. 2020). Svenningsen et al. (2018) reported that AMF
441 ecosystem services might be suppressed by some bacterial groups belonging to Acidobacteria,
442 Actinobacteria, Firmicutes, Chitinophagaceae, and Proteobacteria. Our results showed an inverse
443 correlation between the presence of some of these bacteria (*i.e.*, Acidobacteria, genus *Vicinamibacter*
444 and Actinobacteria genus *Gaiella*) and AMF “functionality”, although ITS sequencing showed a
445 similar level in term AMF abundance. It is also necessary to consider that, ITS was used in the present
446 work as universal fungal marker (Schoch et al. 2012; Lindahl et al. 2013) to better define the overall
447 fungal population despite ribosomal large subunit (LSU) region consistently shows greater utility for
448 taxonomic resolution for AMF (Xue et al. 2019a). Despite the latter approach can give better results,
449 it has rarely been used in environmental studies of AMF because of sequencing and bioinformatics
450 challenges (Delavaux et al. 2021). Similarly, for a better description of the AMF population, it was
451 recently reported that, the use of AMF specific primers, coupled to nested PCR, can greatly help in
452 better define the AMF population (Suzuki et al. 2020).

453 Additionally, results obtained from the microbiome analysis confirm that the response of
454 microbial communities to the different treatments are genotype dependent (Marasco et al. 2018). This
455 is particularly clear for the bacterial community, where the addition of the mycorrhizal inoculum
456 promoted the *Pseudomonas* genus in 1103P and the *Bacillus* genus in SO4. It is important to remind
457 that both these genera were largely investigated in grapevine because of their ability to protect vine
458 plants against several fungal pathogens. *Pseudomonas* genus was studied for its ability to impair
459 *Botrytis*, *Neofusicoccum*, *Ilyonectria*, *Aspergillus*, *Phaeoconiella* and *Phaeoacremonium* genera,
460 which are all well-known grape fungal pathogens (Andreolli et al. 2019; Niem et al. 2020). On the
461 other hand, *Bacillus* species were studied for their ability to reduce the impact of black foot disease
462 (mainly due to infection by *Cylindrocarpon* and *Ilyonectria* species) and downy mildew on grapes
463 (Zhang et al. 2017; Russi et al. 2020). These studies well fit with our data where we observed the
464 lower *Ilyonectria* abundance in M+I 1103P and concomitantly the higher abundance of *Bacillus*
465 species. Looking at the fungi, all the treatments promoted the presence of different AMF species,
466 suggesting the recruitment of native AM fungal communities by the I-treated roots, independently

467 from the rootstock genotypes. In detail, it is worth noting a higher diversity in AMF colonization in
468 I 1103P with respect to I SO4 plants, independently from the presence of the AMF inoculum,
469 confirming a diverse recruitment pattern for the two genotypes. Interestingly, *Clonostachys* genus
470 negatively correlated with all the treatments. This genus was extensively studied for its promising
471 exploitation as biological control agents against soil and root pathogens (Nygren et al. 2018; Sun et
472 al. 2020). Considering that in all treatments the *Rhizophagus* genus was more abundant than in C, we
473 can confirm that a mutual exclusion between *Clonostachys* and *Rhizophagus* genera is present.
474 Although a full explanation for this reciprocally inhibitory interaction is still missing, the complex
475 microbial community modulation mediated by the AM fungi could impair the ability of *Clonostachys*
476 to endophytically colonize the host plant (Ravnskov et al. 2006; Akyol et al. 2018; Xue et al. 2019b).
477 These findings, in accordance with the increase in defense-related metabolites and the expression
478 data on defense-related genes, well fit with the concept of mycorrhizal-induced resistance (MIR)
479 (Cameron et al. 2013) as a cumulative effect of direct and indirect (i.e. mediated by mycorrhizosphere
480 associated microorganisms) defense responses. Recently, Emmett et al. (2021) also demonstrated that
481 a conserved community is associated to AMF extraradical hyphae, suggesting an influence on the
482 plant-fungal symbiosis.

483

484 **AM fungi and root-associated microbes balance rootstocks growth traits showing a different** 485 **pattern of functional symbioses**

486 The impact of the different treatments on two different rootstock genotypes was evaluated. The
487 selected rootstocks (i.e. 1103P and SO4) were well characterized at both agronomic and molecular
488 level (Chitarra et al. 2017), showing opposite growth and defense attitudes. Among rootstock
489 features, fine root development and density, imparting vigor to the scion, varied considerably with an
490 impact on water and nutrient uptake as well as on the interaction with soil microorganisms. AM
491 colonization showed that SO4 consistently presented higher levels of root colonization, together with
492 Kober 5BB and Ruggieri 140, with respect to the others (Chitarra et al. 2017). This is in agreement
493 with previous works (Bavaresco and Fogher 1996; Bavaresco et al. 2000), who showed a variation in
494 the range of AM-colonized grape rootstocks among genotypes, which could be considered the main
495 factor driving AM recruitment. However, functional symbiosis was strongly influenced also by scion
496 requirements, soil fertility and soil pH (Bavaresco and Fogher 1996; Bavaresco et al. 2000). Here,
497 both rooting and growth parameters, and partially the CCI, clearly showed a compensation effect in
498 the less vigorous SO4 with respect to 1103P, reaching similar values in all the treatments. A role
499 could be attributed to AMF particularly in SO4. To attest this hypothesis, considering that high-
500 affinity PTs in AM have been characterized and it has clearly been demonstrated that plants possess

501 a symbiotic Pi uptake pathway (Berruti et al. 2016), AM fungal PT genes (*RiPT* and *FmPT*) have
502 been tested showing a highly expression in M SO4 for both, and also in M+I SO4 for *FmPT*.
503 Similarly, the plant gene *VvPT1-3*, homolog of mycorrhiza-inducible inorganic phosphate
504 transporters such as *LePT4* and *OsPT11* (Balestrini et al. 2017), was significantly up-regulated in M
505 SO4. The positive effects exerted by AM symbiosis in growth and physiological features were largely
506 documented in several plants (*e.g.*, Chitarra et al. 2016; Balestrini et al. 2020). Surprisingly, although
507 the ITS sequencing showed a certain abundance of AM genera in both I and M+I, the inducer seemed
508 to lower the expression of plant and fungal genes generally involved in symbiosis functioning. This
509 should be related to presence of bacteria reported to diminish AMF functionality (Svenningsen et al.
510 2018). As well, an impact of the inducer on the number of fine roots, which are those colonized by
511 AMF, cannot be excluded also considering that IAA was not detectable in I samples. Looking at the
512 whole microbial community, in addition to a selection based on the rootstock genotype, it is worth
513 noting that I treatment (particularly I SO4) was able to significantly increase diversity of the
514 microbiota (Table S4). Samples treated with the inducer showed higher bacterial diversity hosting
515 many groups of PGPBs such as *Burkholderiaceae* that might be linked to potassium (K) and
516 phosphorous (P) solubilization and availability (Gu et al. 2020); *Pseudomonas* and *Bacillus* spp. able
517 to produce siderophores, auxin, cytokinins and characterized as phosphate-solubilizing bacteria (Saad
518 et al. 2020; Subrahmanyam et al. 2020) (Table S7). These findings could explain the bacteria-
519 mediated growth effects in I treatments particularly for the SO4 genotype. By contrast, the whole
520 fungal diversity was not significantly affected among the treatments.

521 Nitrogen (N) is an essential element for all grapevine processes and N transporters were found
522 among the genes upregulated by both a single AMF and a mixed bacterial-fungal inoculum through
523 transcriptomics in grapevine roots (Balestrini et al. 2017). However, although AMF may positively
524 influence plant N compound uptake and transport (Balestrini et al. 2020), negative, neutral or positive
525 AMF effects on N nutrition has been reported (Bücking and Kafle 2015). Due to the fact that several
526 nitrate transporters were found to be regulated by an AMF inoculum (Balestrini et al. 2017), the
527 attention was mainly focused on nitrate uptake. Lower values of nitrate uptake with respect to controls
528 were observed among all treatments, independently from the considered genotypes. Furthermore, any
529 relevant effect on N accumulation in leaves was observed, suggesting that a positive correlation
530 between N content and growth is not relevant in our system or likely due to a biomass dilution effect
531 since the higher growth index recorded particularly in SO4-treated plants. AMF have been reported
532 to show NH_4^+ preference to be assimilated in extraradical mycelium and translocated to plant roots
533 after completion of the GS-GOGAT cycle (Balestrini et al. 2020). In this respect, to the plants side

534 the lower NNU observed in M inoculated plants suggest a role of AMF in regulating root N uptake
535 strategies helping plants in acquire N.

536 The plant hormone ABA is a chemical signal involved in the plant response to various abiotic
537 environmental factors, but it can also play a role in interactions with phytopathogens by modulating
538 tissue colonization depending on microorganism type, site and time of infection (Ton et al. 2009). An
539 impact of ABA on AMF colonization has been also reported at diverse colonization stages (Bedini et
540 al. 2018). A role for ABA in the mechanisms by which AM symbiosis influences stomata
541 conductance under drought stress was also suggested (Chitarra et al. 2016). Here, ABA levels were
542 affected by both the genotype and the AMF inoculum. A significant effect of the M treatment was
543 found on the expression of a key gene involved in the ABA synthesis in leaves (*VvNCED3*), showing
544 a positive correlation with the ABA levels in roots. Our result is in accordance with the fact that ABA
545 produced in leaves is then translocated in roots where it might act as a signal to promote root growth
546 (McAdam et al. 2016). AMF presence led to higher ABA content in M SO4 roots, despite the fact
547 that generally SO4 rootstock was reported to have a low endogenous content (Chitarra et al. 2017),
548 suggesting a potential enhanced tolerance to abiotic stresses in M SO4. As already reported by
549 (Ferrero et al. 2018), the relationship between biosynthetic and catabolic processes may be complex
550 and diverse in the different plant organs. Our results showed a different expression pattern of most of
551 the considered genes involved in ABA synthesis and catabolism in leaves and roots. A gene coding
552 for an ABA 8'-hydroxylase (*VvABA8OHI*), belonging to the CYP707A gene family and with a
553 primary role in ABA catabolism, showed an opposite trend in M and I root apparatus, in agreement
554 with the ABA root accumulation. Overall, obtained data are in accordance with that reported by
555 Martín-Rodríguez et al. (2016) showing that both ABA biosynthesis and catabolism are finely tuned
556 in AM-colonized roots. Although with the activation of different mechanisms depending on the
557 treatment, an impact on ABA homeostasis can be suggested particularly in SO4 genotype.

558
559 **AM symbiosis triggers defence-related transcripts and metabolites more in 1103P than in SO4**
560 **rootstock**

561 Plants finely tune the immune system to control both pathogen infection and beneficial
562 microorganism accommodation. Soil bacteria and fungi play a double role in promoting growth and
563 defense response, helping in maintaining the homeostasis in the whole microbial communities
564 associated to the roots through the Induced Systemic Resistance (ISR) pathways (Liu et al. 2020). In
565 grapevine, stilbenes are phytoalexins with proved antifungal activities (Chalal et al. 2014). Here,
566 resveratrol content was higher in I and M leaves with respect to untreated controls, while viniferin,
567 that is highly toxic for grape foliar pathogens such as downy and powdery mildew (Chitarra et al.

2017), has a similar trend in all the treatments while it was not detected in C plants. These patterns clearly highlight a stimulating effect mediated by root-associated microbes (native or inoculated), with differences that might be related to the diverse microbiome composition. Among the genes involved in stilbene synthesis, *VvSTS48*, coding for a stilbene synthase reported as induced by downy mildew infection, showed the highest expression value in I SO4 plants, suggesting a different modulation among treatments and genotypes.

Carbohydrate metabolism is also involved in plant defense responses against foliar pathogens (Sanmartín et al. 2020). In tomato, AM symbiosis was reported to be involved in *Botrytis cinerea* resistance through the mycorrhiza-induced resistance (MIR) mediated by callose accumulation. A tomato callose synthase gene (*PMR4*) was in fact upregulated by mycorrhization mainly upon biotic infection (Sanmartín et al. 2020). In the present study, attention has been focused on the homolog grape gene *VvCAS2*. Conversely to that previously observed, *VvCAS2* showed a downregulation trend in all the treatments with respect to control plants. These findings suggest a primary role in microbe-mediate stimulating of defense responses against biotic factors in grape. Since a correlation between MIR and sugar signaling pathway was reported (Sanmartín et al. 2020), the expression of a grapevine sugar transporter gene (*VvSTP13*), homolog to the *Arabidopsis STP13*, involved in intracellular glucose uptake and in *B. cinerea* resistance, was followed in leaves and roots. Although total soluble carbohydrates were not affected by treatments in leaves, *VvSTP13* expression showed an upregulation trend in all the treatments, particularly in both I sample and M 1103P leaves, suggesting an effect of AMF inoculum in the susceptible genotype. Looking at the roots, *VvSTP13* upregulation trend was observed mainly in mycorrhizal roots, in agreement with the fact that expression of genes from the STP family was revealed in arbuscule-containing cells of *Medicago truncatula* (Hennion et al. 2019). The same trend observed for *VvSTP13* was also found for a gene coding for a class III chitinase (*VvChitIII*). Class III chitinases have been already reported to be markers of functional symbioses (Balestrini et al. 2017), being localized in arbuscule-containing cells (Hogekamp et al. 2011). Finally, the expression of two target genes (*VvLOX* and *VvEDSI*), respectively involved in ISR mediated by jasmonate and SAR mediated by salicylic acid, although differently modulated by the inducer and AM fungi, confirmed the role of the whole microbiome on the plant immunity system in the scion of both rootstock genotypes (Cameron et al. 2013).

597

598 **Conclusion**

599 Overall, our results allowed to provide new insights into growth-defense tradeoffs responses in a
600 model fruit crop (Fig. 7). Although molecular mechanisms at the basis of plant priming are still matter
601 of debate, several hypotheses have been proposed. In this study, a finely tune regulation of growth

602 and defence traits have been highlighted considering three main influencing factors, *i.e.*, the plant
603 genotype, an AM inoculum and an oligosaccharide described as involved in AMF colonization
604 induction. The attention has been focused on two rootstocks characterised by opposite trade-offs.
605 Growth traits have been improved mainly in the low vigour genotype (SO4) by all the treatments
606 probably through the activation of diverse pathways by the root associated microbes. It is worth
607 noting that all the treatments shaped the microbial communities associated to the roots in both the
608 genotypes. Looking at the defence response, a positive impact on immunity system has been revealed
609 both by the AMF inoculum and the oligosaccharide, although with the activation of different
610 pathways. Results suggest that AM symbiosis triggers a mycorrhiza-induced resistance (MIR) also
611 in a model woody plant such as grapevine.

612

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619

620

621 **References**

- 622 Abarenkov K, Henrik Nilsson R, Larsson K, Alexander IJ, Eberhardt U, Erland S, Hoiland K, Kjoller
623 R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedresoo L, Ursing BM, Vralstad T, Liimatainen K,
624 Peintner U, Koljalg U (2010) The UNITE database for molecular identification of fungi—recent
625 updates and future perspectives. *New Phytol* 186:281–285. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-8137.2009.03160.x)
626 [8137.2009.03160.x](https://doi.org/10.1111/j.1469-8137.2009.03160.x)
- 627 Akyol TY, Niwa R, Hirakawa H, Maruyama H, Sato T, Suzuki T, Fukunaga A, Sato T, Yoshida S,
628 Tawaraya K, Saito M, Ezawa T, Sato S (2018) Impact of introduction of arbuscular mycorrhizal fungi
629 on the root microbial community in agricultural fields. *Microbes Environ* 34:23-32.
630 <https://doi.org/10.1264/jsme2.ME18109>
- 631 Alagna F, Balestrini R, Chitarra W, Marsico AD, Nerva L (2020) Getting ready with the priming:
632 Innovative weapons against biotic and abiotic crop enemies in a global changing scenario. In Hossain
633 MA, Liu F, Burritt DJ, Fujita M, Huang B (Eds) *Priming-Mediated Stress and Cross-Stress Tolerance*
634 *in Crop Plants*. Academic Press, Elsevier, London, pp 35–56.
- 635 Andreolli M, Zapparoli G, Angelini E, Lucchetta G, Lampis S, Vallini G (2019) *Pseudomonas*
636 *protegens* MP12: A plant growth-promoting endophytic bacterium with broad-spectrum antifungal
637 activity against grapevine phytopathogens. *Microbiol Res* 219:123–131.
638 <https://doi.org/10.1016/j.micres.2018.11.003>
- 639 Balestrini R, Brunetti C, Chitarra W, Nerva L (2020) Photosynthetic Traits and Nitrogen Uptake in
640 Crops: Which Is the Role of Arbuscular Mycorrhizal Fungi? *Plants* 9:1105.
641 <https://doi.org/10.3390/plants9091105>
- 642 Balestrini R, Chitarra W, Antoniou C, Ruocco M, Fotopoulos V (2018) Improvement of plant
643 performance under water deficit with the employment of biological and chemical priming agents. *The*
644 *Journal of Agricultural Science* 156:680–688. <https://doi.org/10.1017/S0021859618000126>
- 645 Balestrini R, Lumini E (2018) Focus on mycorrhizal symbioses. *Applied soil ecology* 123:299–304
- 646 Balestrini R, Magurno F, Walker C, Lumini E, Bianciotto V (2010) Cohorts of arbuscular mycorrhizal
647 fungi (AMF) in *Vitis vinifera*, a typical Mediterranean fruit crop. *Environmental microbiology reports*
648 2:594-604. <https://doi.org/10.1111/j.1758-2229.2010.00160.x>
- 649 Balestrini R, Salvioli A, Dal Molin A, Novero M, Gabelli G, Paparelli E, Marroni F, Bonfante P
650 (2017) Impact of an arbuscular mycorrhizal fungus versus a mixed microbial inoculum on the
651 transcriptome reprogramming of grapevine roots. *Mycorrhiza* 27:417-430.
652 <https://doi.org/10.1007/s00572-016-0754-8>

653 Bandau F, Decker VHG, Gundale MJ, Albrechtsen BR (2015) Genotypic tannin levels in *Populus*
654 *tremula* impact the way nitrogen enrichment affects growth and allocation responses for some traits
655 and not for others. PLoS One 10:e0140971. <https://doi.org/10.1371/journal.pone.0140971>

656 Bastías DA, Gianoli E, Gundel PE (2021) Fungal endophytes can eliminate the plant growth-defence
657 trade-off. New Phytol 230:2105-2113. <https://doi.org/10.1111/nph.17335>

658 Bavaresco L, Cantù E, Trevisan M (2000) Chlorosis occurrence, natural arbuscular-mycorrhizal
659 infection and stilbene root concentration of ungrafted grapevine rootstocks growing on calcareous
660 soil. J Plant Nutr 23:1685–1697. <https://doi.org/10.1080/01904160009382133>

661 Bavaresco L, Fogher C (1996) Lime-induced chlorosis of grapevine as affected by rootstock and root
662 infection with arbuscular mycorrhiza and *Pseudomonas fluorescens*. Vitis 35:119–123.
663 <https://doi.org/10.5073/vitis.1996.35.119-123>

664 Beckers GJ, Conrath U (2007) Priming for stress resistance: from the lab to the field. Curr Op Plant
665 Biol 10:425–431. <https://doi.org/10.1016/j.pbi.2007.06.002>

666 Bedini A, Mercy L, Schneider C, Franken P, Lucic-Mercy E (2018) Unraveling the initial plant
667 hormone signaling, metabolic mechanisms and plant defense triggering the endomycorrhizal
668 symbiosis behavior. Front Plant Sci 9:1800. <https://doi.org/10.3389/fpls.2018.01800>

669 Berruti A, Desirò A, Visentin S, Zecca O, Bonfante P (2017) ITS fungal barcoding primers versus
670 18S AMF-specific primers reveal similar AMF-based diversity patterns in roots and soils of three
671 mountain vineyards. Env Microbiol Rep 9:658-667. <https://doi.org/10.1111/1758-2229.12574>

672 Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ,
673 Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT,
674 Callahan BJ, Mauricio Caraballo-Rodríguez A, Chase J, Cope EK, Da Silva R, Dorrestein PC,
675 Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM,
676 Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley
677 GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Bin Kang K, Keefe CR, Keim P, Kelley ST,
678 Knights D, Koester I, Kosciolk T, Kreps J, Langille MGI, Lee J, Ley R, Liu Y, Loftfield E, Lozupone
679 C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC,
680 Morton JT, Turan Naimey A, Navas-Molina JA, Felix Nothias L, Orchanian SB, Pearson T, Peoples
681 SL, Petras D, Lai Preuss M, Pruesse E, Buur Rasmussen L, Rivers A, Robeson MS, Rosenthal P,
682 Segata N, Shaffer M, Shiffer A, Sinha R, Jin Song S, Spear JR, Swafford AD, Thompson LR, Torres
683 PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y,
684 Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD,
685 Willis AD, Zech Xu Z, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG (2019) Reproducible,

686 interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotech* 37:852–
687 857. <https://doi.org/10.1038/s41587-019-0209-9>

688 Bücking H, Kafle A (2015) Role of arbuscular mycorrhizal fungi in the nitrogen uptake of plants:
689 current knowledge and research gaps. *Agronomy* 5:587–612.
690 <https://doi.org/10.3390/agronomy5040587>

691 Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: high-
692 resolution sample inference from Illumina amplicon data. *Nature Methods* 13:581–583.
693 <https://doi.org/10.1038/nmeth.3869>

694 Cameron DD, Neal AL, van Wees SC, Ton J (2013) Mycorrhiza-induced resistance: more than the
695 sum of its parts? *Trends Plant Sci* 18:539–545. <https://doi.org/10.1016/j.tplants.2013.06.004>

696 Chalal M, Klinguer A, Echairi A, Meunier P, Vervandier-Fasseur D, Adrian M (2014) Antimicrobial
697 activity of resveratrol analogues. *Molecules* 19:7679–7688.
698 <https://doi.org/10.3390/molecules19067679>

699 Chitarra W, Cuzzo D, Ferrandino A, Secchi F, Palmano S, Perrone I, Boccacci P, Pagliarani C,
700 Gribaudo I, Mannini F, Gambino G (2018) Dissecting interplays between *Vitis vinifera* L. and
701 grapevine virus B (GVB) under field conditions. *Mol Plant Path* 19:2651–2666.
702 <https://doi.org/10.1111/mpp.12735>

703 Chitarra W, Pagliarani C, Maserti B, Lumini E, Siciliano I, Cascone P, Schubert A, Gambino G,
704 Balestrini R, Guerrieri E (2016) Insights on the impact of arbuscular mycorrhizal symbiosis on tomato
705 tolerance to water stress. *Plant Physiol* 171:1009–1023. <https://doi.org/10.1104/pp.16.00307>

706 Chitarra W, Perrone I, Avanzato CG, Minio A, Boccacci P, Santini D, Gilardi G, Siciliano I, Gullino
707 ML, Delledonne M, Mannini F, Gambino G (2017) Grapevine grafting: Scion transcript profiling and
708 defense-related metabolites induced by rootstocks. *Front Plant Sci* 8:654.
709 <https://doi.org/10.3389/fpls.2017.00654>

710 Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, Brown CT, Porras-Alfaro A, Kuske CR,
711 Tiedje JM (2014) Ribosomal Database Project: data and tools for high throughput rRNA analysis.
712 *Nucleic Ac Res* 42:D633–D642. <https://doi.org/10.1093/nar/gkt1244>

713 Cregger MA, Veach AM, Yang ZK, Crouch MJ, Vilgalys R, Tuskan GA, Schadt CW (2018) The
714 *Populus* holobiont: dissecting the effects of plant niches and genotype on the microbiome.
715 *Microbiome* 6:31. <https://doi.org/10.1186/s40168-018-0413-8>

716 Cushman JC, Bohnert HJ (2000) Genomic approaches to plant stress tolerance. *Curr Op Plant Biol*
717 3:117–124. [https://doi.org/10.1016/S1369-5266\(99\)00052-7](https://doi.org/10.1016/S1369-5266(99)00052-7)

718 Deal D, Boothroyd C, Mai W (1972) Replanting of vineyards and its relationship to vesicular-
719 arbuscular mycorrhiza. *Phytopathology* 62:172:175.

720 Delavaux CS, Sturmer SL, Wagner MR, Schütte U, Morton JB, Beer JD (2021) Utility of large
721 subunit for environmental sequencing of arbuscular mycorrhizal fungi: a new reference database and
722 pipeline. *New Phytol* 229:3048–3052. <https://doi.org/10.1111/nph.17080>

723 Emmett BD, Lévesque-Tremblay V, Harrison MJ (2021) Conserved and reproducible bacterial
724 communities associate with extraradical hyphae of arbuscular mycorrhizal fungi. *ISME J* 15:2276-
725 2288. <https://doi.org/10.1038/s41396-021-00920-2>

726 Ferrero M, Pagliarani C, Novák O, Ferrandino A, Cardinale F, Visentin I, Schubert A (2018)
727 Exogenous strigolactone interacts with abscisic acid-mediated accumulation of anthocyanins in
728 grapevine berries. *J Exp Bot* 69:2391–2401. <https://doi.org/10.1093/jxb/ery033>

729 Feys BJ, Parker JE (2000) Interplay of signaling pathways in plant disease resistance. *Trends in*
730 *Genetics* 16:449–455. [https://doi.org/10.1016/S0168-9525\(00\)02107-7](https://doi.org/10.1016/S0168-9525(00)02107-7)

731 Gilbert JA, van der Lelie D, Zarraonaindia I (2014) Microbial terroir for wine grapes. *Proc Nat Acad*
732 *Sci USA* 111:5–6. <https://doi.org/10.1073/pnas.1320471110>

733 Gu Y, Dong K, Geisen S, et al (2020) The effect of microbial inoculant origin on the rhizosphere
734 bacterial community composition and plant growth-promotion. *Plant Soil* 452:105–117.
735 <https://doi.org/10.1007/s11104-020-04545-w>

736 Hennion N, Durand M, Vriet C, Doidy J, Maurousset L, Lemoine R, Pourtau N (2019) Sugars en
737 route to the roots. Transport, metabolism and storage within plant roots and towards microorganisms
738 of the rhizosphere. *Physiol Plant* 165:44–57. <https://doi.org/10.1111/ppl.12751>

739 Hirayama T, Shinozaki K (2007) Perception and transduction of abscisic acid signals: keys to the
740 function of the versatile plant hormone ABA. *Trends Plant Sci* 12:343–351.
741 <https://doi.org/10.1016/j.tplants.2007.06.013>

742 Hogekamp C, Arndt D, Pereira PA, Becker JD, Hohnjec N, Küster H (2011) Laser microdissection
743 unravels cell-type-specific transcription in arbuscular mycorrhizal roots, including CAAT-box
744 transcription factor gene expression correlating with fungal contact and spread. *Plant Physiol*
745 157:2023–2043. <https://doi.org/10.1104/pp.111.186635>

746 Holland TC, Bowen P, Bogdanoff C, Hart MM (2014) How distinct are arbuscular mycorrhizal fungal
747 communities associating with grapevines? *Biol Fertil Soils* 50:667–674.
748 <https://doi.org/10.1007/s00374-013-0887-2>

749 Huot B, Yao J, Montgomery BL, He SY (2014) Growth–defense tradeoffs in plants: a balancing act
750 to optimize fitness. *Mol Plant* 7:1267–1287. <https://doi.org/10.1093/mp/ssu049>

751 Jacott CN, Murray JD, Ridout CJ (2017) Trade-offs in arbuscular mycorrhizal symbiosis: disease
752 resistance, growth responses and perspectives for crop breeding. *Agronomy* 7:75.
753 <https://doi.org/10.3390/agronomy7040075>

754 Jia H, Wang C, Zhang C, Haider MS, Zhao P, Liu Z, Shangguan L, Pervaiz T, Fang J (2016)
755 Functional analysis of *VvBGL* during fruit development and ripening of grape. *J Plant Growth Reg*
756 35:987–999. <https://doi.org/10.1007/s00344-016-9597-y>

757 Jones JD, Dangl JL (2006) The plant immune system. *nature* 444:323–329.
758 <https://doi.org/10.1038/nature05286>

759 Karagiannidis N, Nikolaou N, Mattheou A (1995) Wirkung dreier VA-Mykorrhizapilze auf Ertrag
760 und Nährstoffaufnahme von drei Unterlagen. *Vitis* 34:85–89

761 Lancashire PD, Bleiholder H, Van Den Boom T, Langelüddeke P, Stauss R, Weber E, Witzemberger
762 A (1991) A uniform decimal code for growth stages of crops and weeds. *Ann App Biol* 119:561–601.
763 <https://doi.org/10.1111/j.1744-7348.1991.tb04895.x>

764 Lee KH, Piao HL, Kim H-Y, Choi SM, Jiang F, Hartung W, Hwang I, Kwak JM, Lee I, Hwang I
765 (2006) Activation of glucosidase via stress-induced polymerization rapidly increases active pools of
766 abscisic acid. *Cell* 126:1109–1120

767 Lindahl BD, Nilsson RH, Tedersoo L, Abarenkov K, Carlsen T, Kjølner R, Kõljalg U, Pennanen T,
768 Rosendahl S, Stenlid J, Kauserud H (2013) Fungal community analysis by high-throughput
769 sequencing of amplified markers—a user’s guide. *New Phytol* 199:288–299.
770 <https://doi.org/10.1111/nph.12243>

771 Linderman RG, Davis EA (2001) Comparative response of selected grapevine rootstocks and
772 cultivars to inoculation with different mycorrhizal fungi. *Am J Enol Vit* 52:8–11.

773 Liu H, Brettell LE, Qiu Z, Singh BK (2020) Microbiome-mediated stress resistance in plants. *Trends*
774 *Plant Sci* 25:733–743. <https://doi.org/10.1016/j.tplants.2020.03.014>

775 Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-
776 seq data with DESeq2. *Genome biology* 15:550. <https://doi.org/10.1186/s13059-014-0550-8>

777 Lovisolò C, Lavoie-Lamoureux A, Tramontini S, Ferrandino A (2016) Grapevine adaptations to
778 water stress: new perspectives about soil/plant interactions. *Theor Exp Plant Physiol* 28:53–66.
779 <https://doi.org/10.1007/s40626-016-0057-7>

780 Lucic E, Mercy L (2014) A method of mycorrhization of plants and use of saccharides in
781 mycorrhization. European Patent Office EP2982241A1

782 Lundberg DS, Yourstone S, Mieczkowski P, Jones CD, Dangl JL (2013) Practical innovations for
783 high-throughput amplicon sequencing. *Nature Methods* 10:999–1002.
784 <https://doi.org/10.1038/nmeth.2634>

785 Mannino G, Nerva L, Gritli T, Novero M, Fiorilli V, Bacem M, Berteà CM, Lumini E, Chitarra W,
786 Balestrini R (2020) Effects of Different Microbial Inocula on Tomato Tolerance to Water Deficit.
787 *Agronomy* 10:170. <https://doi.org/10.3390/agronomy10020170>

788 Marasco R, Rolli E, Fusi M, Michoud G, Daffonchio D (2018) Grapevine rootstocks shape
789 underground bacterial microbiome and networking but not potential functionality. *Microbiome* 6:3.
790 <https://doi.org/10.1186/s40168-017-0391-2>

791 Marín D, Armengol J, Carbonell-Bejerano P, Escalona JM, Gramaje D, Hernández-Montes E,
792 Intrigliolo DS, Martínez-Zapater JM, Medrano H, Mirás-Avalos JM, Palomares-Rius JE, Romero-
793 Azorín P, Savé R, Santesteban LG, de Herralde F (2021) Challenges of viticulture adaptation to global
794 change: tackling the issue from the roots. *Austral J Grape Wine Res* 27:8–25.
795 <https://doi.org/10.1111/ajgw.12463>

796 Martín-Rodríguez JA, Huertas R, Ho-Plágaro T, Ocampo JA, Turečková V, Tarkowská D, Ludwig-
797 Müller J, García-Garrido JM (2016) Gibberellin–abscisic acid balances during arbuscular mycorrhiza
798 formation in tomato. *Frontiers Plant Sci* 7:1273. <https://doi.org/10.3389/fpls.2016.01273>

799 Massa N, Bona E, Novello G, Todeschini V, Boatti L, Mignone F, Gamalero E, Lingua G, Berta G,
800 Cesaro P (2020) AMF communities associated to *Vitis vinifera* in an Italian vineyard subjected to
801 integrated pest management at two different phenological stages. *Sci Rep* 10:9197.
802 <https://doi.org/10.1038/s41598-020-66067-w>

803 McAdam SA, Brodribb TJ, Ross JJ (2016) Shoot-derived abscisic acid promotes root growth. *Plant*
804 *Cell Env* 39:652–659. <https://doi.org/10.1111/pce.12669>

805 McMurdie PJ, Holmes S (2013) phyloseq: an R package for reproducible interactive analysis and
806 graphics of microbiome census data. *PloS one* 8:e61217.
807 <https://doi.org/10.1371/journal.pone.0061217>

808 Mittler R, Blumwald E (2010) Genetic engineering for modern agriculture: challenges and
809 perspectives. *Ann Rev Plant Biol* 61:443–462. <https://doi.org/10.1146/annurev-arplant-042809-112116>

811 Nerva L, Pagliarani C, Pugliese M, Monchiero M, Gonthier S, Gullino ML, Gambino G, Chitarra W
812 (2019) Grapevine phyllosphere community analysis in response to elicitor application against
813 powdery mildew. *Microorganisms* 7:662. <https://doi.org/10.3390/microorganisms7120662>

814 Niem JM, Billones-Baaijens R, Stodart B, Savocchia S (2020) Diversity profiling of grapevine
815 microbial endosphere and antagonistic potential of endophytic pseudomonas against grapevine trunk
816 diseases. *Front Microbiol* 11:477. <https://doi.org/10.3389/fmicb.2020.00477>

817 Nygren K, Dubey M, Zapparata A, Iqbal M, Tzelepis GD, Durling MB, Jensen DF, Karlsson M
818 (2018) The mycoparasitic fungus *Clonostachys rosea* responds with both common and specific gene
819 expression during interspecific interactions with fungal prey. *Evolut Applic* 11:931–949.
820 <https://doi.org/10.1111/eva.12609>

821 Pagliarani C, Boccacci P, Chitarra W, Cosentino E, Sandri M, Perrone I, Mori A, Cuozzo D, Nerva
822 L, Rossato M, Zuccolotto P, Pezzotti M, Delledonne M, Mannini F, Gribaudo I, Gambino G (2019)
823 Distinct metabolic signals underlie clone by environment interplay in “Nebbiolo” grapes over
824 ripening. *Front Plant Sci* 10:1575. <https://doi.org/10.3389/fpls.2019.01575>

825 Pagliarani C, Moine A, Chitarra W, Meloni GR, Abbà S, Nerva L, Pugliese M, Gullino ML, Gambino
826 G (2020) The molecular priming of defense responses is differently regulated in grapevine genotypes
827 following elicitor application against powdery mildew. *Int J Mol Sci* 21:6776.
828 <https://doi.org/10.3390/ijms21186776>

829 Priori S, Pellegrini S, Perria R, Puccioni S, Storchi P, Valboa G, Costantini EAC (2019) Scale effect
830 of terroir under three contrasting vintages in the Chianti Classico area (Tuscany, Italy). *Geoderma*
831 334:99–112. <https://doi.org/10.1016/j.geoderma.2018.07.048>

832 Qin X, Zeevaart JA (1999) The 9-cis-epoxycarotenoid cleavage reaction is the key regulatory step of
833 abscisic acid biosynthesis in water-stressed bean. *Proc Nat Ac Sci USA* 96:15354–15361.
834 <https://doi.org/10.1073/pnas.96.26.15354>

835 Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner O (2012) The
836 SILVA ribosomal RNA gene database project: improved data processing and web-based tools.
837 *Nucleic Ac Res* 41:D590–D596. <https://doi.org/10.1093/nar/gks1219>

838 Ravnskov S, Jensen B, Knudsen IM, Bødker L, Jensen DF, Karliński L, Larsen J (2006) Soil
839 inoculation with the biocontrol agent *Clonostachys rosea* and the mycorrhizal fungus *Glomus*
840 *intraradices* results in mutual inhibition, plant growth promotion and alteration of soil microbial
841 communities. *Soil Biol Bioch* 38:3453–3462. <https://doi.org/10.1016/j.soilbio.2006.06.003>

842 Rivers AR, Weber KC, Gardner TG, Liu S, Armstrong SD (2018) ITSxpress: software to rapidly trim
843 internally transcribed spacer sequences with quality scores for marker gene analysis. *F1000Research*
844 7:1418. <https://doi.org/10.12688/f1000research.15704.1>

845 Russi A, Almança MAK, Grohs DS, Schwambach J (2020) Biocontrol of black foot disease on
846 grapevine rootstocks using *Bacillus subtilis* strain F62. *Trop Plant Pathol* 45:103-111.
847 <https://doi.org/10.1007/s40858-019-00319-7>

848 Saad MM, Eida AA, Hirt H (2020) Tailoring plant-associated microbial inoculants in agriculture: a
849 roadmap for successful application. *J Exp Bot* 71:3878–3901. <https://doi.org/10.1093/jxb/eraa111>

850 Sanmartín N, Pastor V, Pastor-Fernández J, Flors V, Pozo MJ, Sánchez-Bel P (2020) Role and
851 mechanisms of callose priming in mycorrhiza-induced resistance. *J Exp Bot* 71:2769–2781.
852 <https://doi.org/10.1093/jxb/eraa030>

853 Santi S, De Marco F, Polizzotto R, Grisan S, Musetti R (2013) Recovery from stolbur disease in
854 grapevine involves changes in sugar transport and metabolism. *Front Plant Sci* 4:171.
855 <https://doi.org/10.3389/fpls.2013.00171>

856 Schmieder R, Edwards R (2011) Quality control and preprocessing of metagenomic datasets.
857 *Bioinformatics* 27:863–864. <https://doi.org/10.1093/bioinformatics/btr026>

858 Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding
859 Consortium (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA
860 barcode marker for Fungi. *Proc Natl Acad Sci USA* 109:6241–6246.
861 <https://doi.org/10.1073/pnas.1117018109>

862 Subrahmanyam G, Kumar A, Sandilya SP, Chutia M, Yadav AN (2020) Diversity, plant growth
863 promoting attributes, and agricultural applications of rhizospheric microbes In Yadav A., Singh J.,
864 Rastegari A., Yadav N. (Eds) *Plant Microbiomes for Sustainable Agriculture. Sustainable*
865 *Development and Biodiversity*, vol 25. Springer, Cham, pp 1-52. [https://doi.org/10.1007/978-3-030-](https://doi.org/10.1007/978-3-030-38453-1_1)
866 [38453-1_1](https://doi.org/10.1007/978-3-030-38453-1_1)

867 Sun L, Zhang M, Ren J, Qi J, Zhang G, Leng P (2010) Reciprocity between abscisic acid and ethylene
868 at the onset of berry ripening and after harvest. *BMC Plant Biol* 10:257. [https://doi.org/10.1186/1471-](https://doi.org/10.1186/1471-2229-10-257)
869 [2229-10-257](https://doi.org/10.1186/1471-2229-10-257)

870 Sun Z-B, Li S-D, Ren Q, Xu JL, Lu X, Sun MH (2020) Biology and applications of *Clonostachys*
871 *rosea*. *J App Microbiol* 129:486–495. <https://doi.org/10.1111/jam.14625>

872 Suzuki K, Takahashi K, Harada N (2020) Evaluation of primer pairs for studying arbuscular
873 mycorrhizal fungal community compositions using a MiSeq platform. *Biol Fertil Soils* 56:853–858.
874 <https://doi.org/10.1007/s00374-020-01431-6>

875 Svenningsen NB, Watts-Williams SJ, Joner EJ, Battini F, Efthymiou A, Cruz-Paredes C, Nybroe O,
876 Jakobsen I (2018) Suppression of the activity of arbuscular mycorrhizal fungi by the soil microbiota.
877 *ISME J* 12:1296–1307. <https://doi.org/10.1038/s41396-018-0059-3>

878 Tomasi N, Monte R, Varanini Z, Cesco S, Pinton R (2015) Induction of nitrate uptake in Sauvignon
879 Blanc and Chardonnay grapevines depends on the scion and is affected by the rootstock. *Austral J*
880 *Grape Wine Res* 21:331–338. <https://doi.org/10.1111/ajgw.12137>

881 Ton J, Flors V, Mauch-Mani B (2009) The multifaceted role of ABA in disease resistance. *Trends*
882 *Plant Sci* 14:310–317. <https://doi.org/10.1016/j.tplants.2009.03.006>

883 Trouvelot S, Bonneau L, Redecker D, van Tuinen D, Adrian M, Wipf D (2015) Arbuscular
884 mycorrhiza symbiosis in viticulture: a review. *Agronomy Sust Dev* 35:1449–1467.
885 <https://doi.org/10.1007/s13593-015-0329-7>

886 Uroz S, Courty PE, Oger P (2019) Plant symbionts are engineers of the plant-associated microbiome.
887 Trends Plant Sci 24:905–916. <https://doi.org/10.1016/j.tplants.2019.06.008>

888 Verbon EH, Liberman LM (2016) Beneficial microbes affect endogenous mechanisms controlling
889 root development. Trends Plant Sci 21:218–229. <https://doi.org/10.1016/j.tplants.2016.01.013>

890 Warschefsky EJ, Klein LL, Frank MH, Chitwood DH, Londo JP, von Wettberg EJB, Miller AJ (2016)
891 Rootstocks: diversity, domestication, and impacts on shoot phenotypes. Trends Plant Sci 21:418–
892 437. <https://doi.org/10.1016/j.tplants.2015.11.008>

893 Xue C, Hao Y, Pu X, Penton CR, Wang Q, Zhao M, Zhang B, Ran W, Huang Q, Shen Q, Tiedje JM
894 (2019a) Effect of LSU and ITS genetic markers and reference databases on analyses of fungal
895 communities. Biol Fertil Soils 55:79–88. <https://doi.org/10.1007/s00374-018-1331-4>

896 Xue L, Almario J, Fabiańska I, Saridis G, Bucher M (2019b) Dysfunction in the arbuscular
897 mycorrhizal symbiosis has consistent but small effects on the establishment of the fungal microbiota
898 in *Lotus japonicus*. New Phytol 224:409–420. <https://doi.org/10.1111/nph.15958>

899 Yu K, Pieterse CM, Bakker PA, Berendsen RL (2019) Beneficial microbes going underground of
900 root immunity. Plant Cell Environ 42:2860–2870. <https://doi.org/10.1111/pce.13632>

901 Zhang X, Zhou Y, Li Y, Fu X, Wang Q (2017) Screening and characterization of endophytic *Bacillus*
902 for biocontrol of grapevine downy mildew. Crop Protec 96:173–179.
903 <https://doi.org/10.1016/j.cropro.2017.02.018>

904 Zombardo A, Mica E, Puccioni S, Perria R, Valentini P, Mattii GB, Cattivelli L, Storchi P (2020)
905 Berry quality of grapevine under water stress as affected by rootstock–Scion interactions through
906 gene expression regulation. Agronomy 10:680. <https://doi.org/10.3390/agronomy10050680>

907 Züst T, Agrawal AA (2017) Trade-offs between plant growth and defense against insect herbivory:
908 an emerging mechanistic synthesis. Ann Rev Plant Biol 68:513–534.
909 <https://doi.org/10.1146/annurev-arplant-042916-040856>

910

911 **Figure legends**

912 **Fig. 1 Growth-related traits and metabolites.** **a** Growth index according to BBCH scale recorded
913 for each treatment at the end of the experiment ($n = 25$). Upper picture showed an overview of the
914 cuttings' development in response to the treatments at the end of the experiment. **b** Rooting % of
915 cuttings at the end of the experiment ($n = 25$). **c** Chlorophyll Content Index (CCI) measured at the
916 end of the experiment ($n = 25$). **d** Quantification of soluble carbohydrates contents in leaves at the
917 end of the experiment ($n = 4$). All data are expressed as mean \pm SD. ns, *, **, and ***: non-significant
918 or significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively. Different lowercase letters above the
919 bars indicate significant differences according to Tukey HSD test ($P \leq 0.05$), considering R x I x M
920 interaction. Analysis of variance on the single variables is reported in Table S2. Different uppercase
921 letters above the bars indicate significant differences according to Tukey HSD test ($P \leq 0.05$)
922 considering the two rootstocks independently. C: Control plants; I: Inducer-treated plants; M: AMF
923 mixed inoculum-treated plants; M+I AMF mixed inoculum + Inducer-treated plants for 1103P and
924 SO4 selected rootstocks.

925

926 **Fig. 2 Net nitrate uptake in roots and total N in leaves.** **a** *in vivo* Net nitrate uptake. **b** Total N in
927 leaves (g kg^{-1} DW). All data are expressed as mean \pm SD ($n = 3$). ns, *, **, and ***: non-significant
928 or significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively. Different lowercase letters above the
929 bars indicate significant differences according to Tukey HSD test ($P \leq 0.05$), considering R x I x M
930 interaction. Analysis of variance on the single variables is reported in Table S2. Different uppercase
931 letters above the bars indicate significant differences according to Tukey HSD test ($P \leq 0.05$)
932 considering the two rootstocks independently. C: Control plants; I: Inducer-treated plants; M: AMF
933 mixed inoculum-treated plants; M+I AMF mixed inoculum + Inducer-treated plants for 1103P and
934 SO4 selected rootstocks.

935

936 **Fig. 3 Expression changes of ABA-related genes and metabolite quantification in both root and**
937 **leaf tissues.** **a** ABA content in roots. **b** *VvNCED3* in leaf. **c** *VvABA8OH1* in leaf. **d** *VvNCED3* in root.
938 **e** *VvBGI* in root. **f** *VvGT* in leaf. **g** *VvBGI* in leaf. **h** *VvGT* in root. **i** *VvABA8OH1* in root. All data are
939 expressed as mean \pm SD ($n = 3$). ns, *, **, and ***: non-significant or significant at $P \leq 0.05$, $P \leq$
940 0.01 , and $P \leq 0.001$, respectively. Different lowercase letters above the bars indicate significant
941 differences according to Tukey HSD test ($P \leq 0.05$), considering R x I x M interaction. Analysis of
942 variance on the single variables is reported in Table S2. Different uppercase letters above the bars
943 indicate significant differences according to Tukey HSD test ($P \leq 0.05$) considering the two
944 rootstocks independently. C: Control plants; I: Inducer-treated plants; M: AMF mixed inoculum-

945 treated plants; M+I AMF mixed inoculum + Inducer-treated plants for 1103P and SO4 selected
946 rootstocks.

947

948 **Fig. 4 Expression changes of stilbenes-related genes and metabolites quantification in leaf**
949 **tissues. a** *trans*-resveratrol quantification. **b** Viniferin quantification. **c** *VvSTS1* gene expression
950 changes. **d** *VvSTS48* gene expression changes. All data are expressed as mean \pm SD ($n = 3$). ns, *, **,
951 and ***: non-significant or significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively. Different
952 lowercase letters above the bars indicate significant differences according to Tukey HSD test ($P \leq$
953 0.05), considering R x I x M interaction. Analysis of variance on the single variables is reported in
954 Table S2. Different uppercase letters above the bars indicate significant differences according to
955 Tukey HSD test ($P \leq 0.05$) considering the two rootstocks independently. C: Control plants; I:
956 Inducer-treated plants; M: AMF mixed inoculum-treated plants; M+I AMF mixed inoculum +
957 Inducer-treated plants for 1103P and SO4 selected rootstocks.

958

959 **Fig. 5 Distinct root associated-bacteria community composition among treatments.** NMDS
960 algorithm based on Bray-Curtis distances matrixes was used to reduce into a bi-dimensional scaling
961 data obtained for bacteria community (**a**). Relative abundance of bacterial genera (**b**) among
962 treatments. Only genera representing at least the 1% over the total number of classified amplicons
963 were retained ($n = 3$). C: Control plants; I: Inducer-treated plants; M: AMF mixed inoculum-treated
964 plants; M+I AMF mixed inoculum + Inducer-treated plants for 1103P and SO4 selected rootstocks.

965

966 **Fig. 6 Mycorrhiza genera and expression changes of plant and fungus Phosphate Transporter**
967 **(PT) genes as markers of functional symbioses. a** Relative abundances of mycorrhiza genera ($n =$
968 3). **b** *RiPT*. **c** *FmPT*. **d** *VvPT1-3*. Gene expression data are expressed as mean \pm SD ($n = 3$). ns, *, **,
969 and ***: non-significant or significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively. Different
970 lowercase letters above the bars indicate significant differences according to Tukey HSD test ($P \leq$
971 0.05), considering R x I x M interaction. Analysis of variance on the single variables is reported in
972 Table S2. Different uppercase letters above the bars indicate significant differences according to
973 Tukey HSD test ($P \leq 0.05$) considering the two rootstocks independently. C: Control plants; I:
974 Inducer-treated plants; M: AMF mixed inoculum-treated plants; M+I AMF mixed inoculum +
975 Inducer-treated plants for 1103P and SO4 selected rootstocks. **Insets:** Microscope images of typical
976 AM symbioses structures in 1103P and SO4 M-colonized roots.

977

978 **Fig. 7 Overview of phenotypic, biochemical, and molecular changes induced by the treatments.**
979 Green arrows indicate responses in 1103 Paulsen (1103P) rootstock whereas orange ones are referred
980 to SO4 genotype. Upward arrows indicate an increase whereas downward arrows represent a decrease
981 in content of metabolites or gene relative expression or relative abundance of microbial taxa with
982 respect to Control (C) plants. NNU: Net Nitrate Uptake; ABA: Abscisic Acid.

983

984 **Supporting information**

985 **Fig. S1 Gene expression changes of defense-related target genes in both leaf and root. a** *VvSTP13*
986 in leaf. **b** *VvSTP13* in root. **c** *VvChitIII* in leaf. **d** *VvChitIII* in root. **e** *VvCAS2* in leaf. **f** *VvLOX* in leaf.
987 **g** *VvEDSI* in leaf. **h** *VvHNT1* in leaf. All data are expressed as mean \pm SD ($n = 3$). ns, *, **, and ***:
988 non-significant or significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively. Different lowercase
989 letters above the bars indicate significant differences according to Tukey HSD test ($P \leq 0.05$),
990 considering R x I x M interaction. Analysis of variance on the single variables is reported in Table
991 S2. Different uppercase letters above the bars indicate significant differences according to Tukey
992 HSD test ($P \leq 0.05$) considering the two rootstocks independently. C: Control plants; I: Inducer-
993 treated plants; M: AMF mixed inoculum-treated plants; M+I AMF mixed inoculum + Inducer-treated
994 plants for 1103P and SO4 selected rootstocks.

995

996 **Fig. S2 NMDS of root-associated fungal communities.** NMDS algorithm based on Bray-Curtis
997 distances matrixes was used to reduce into a bi-dimensional scaling data obtained for and fungi
998 community ($n=3$).

999

1000 **Fig. S3 Distinct root associated-fungal community structure among treatments.** Relative
1001 abundances of bacterial class (a) and genera (b) among treatments. Only genera representing at least
1002 the 1% over the total number of classified amplicons were retained ($n = 3$). C: Control plants; I:
1003 Inducer-treated plants; M: AMF mixed inoculum-treated plants; M+I AMF mixed inoculum +
1004 Inducer-treated plants for 1103P and SO4 selected rootstocks.

1005

1006 **Fig. S4 Relative abundances of fungal pathogens genera.** C: Control plants; I: Inducer-treated
1007 plants; M: AMF mixed inoculum-treated plants; M+I AMF mixed inoculum + Inducer-treated plants
1008 for 1103P and SO4 selected rootstocks ($n = 3$).

1009

1010 **Table S1** Oligonucleotides used in this study.

1011

1012 **Table S2** Analysis of variance (ANOVA) outcomes of target genes, metabolites and nitrogen content
1013 in leaf and root tissues. Different letters within each column indicate significant differences according
1014 to Tukey HSD test ($P \leq 0.05$). Rootstock (R), Inducer (I) and Myc (M) main effects were compared
1015 using the Student's *t*-test ($P \leq 0.05$).

1016

1017 **Table S3** General feature from sequencing results of MiSeq Illumina using specific 16S or ITS
1018 primers together with PNA.

1019

1020 **Table S4** Shannon index for bacterial (16S) communities sampled among the different treatments.

1021

1022 **Table S5** Shannon index for fungal (ITS) communities sampled among the different treatments.

1023

1024 **Table S6** Summary of bacterial and fungal communities composition among treatments.

1025

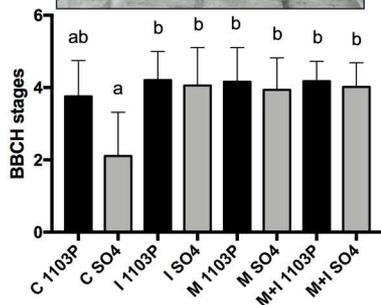
1026 **Table S7** Statistical analysis of the bacterial community among the different treatments.

1027

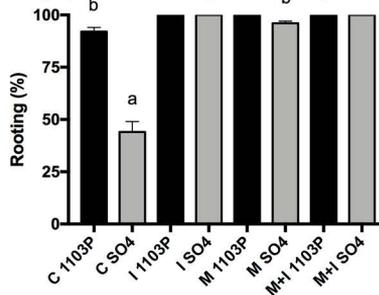
1028 **Table S8** Statistical analysis of the fungal community among the different treatments.



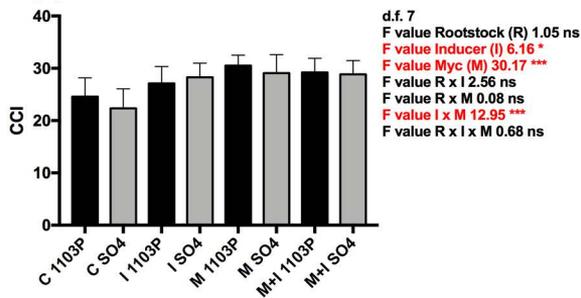
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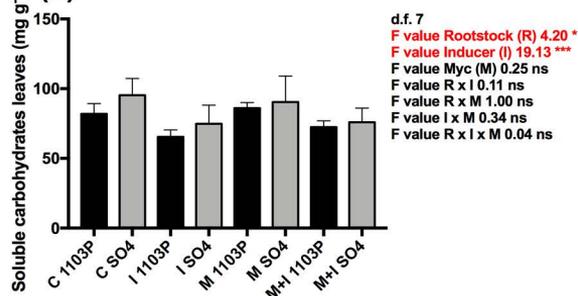
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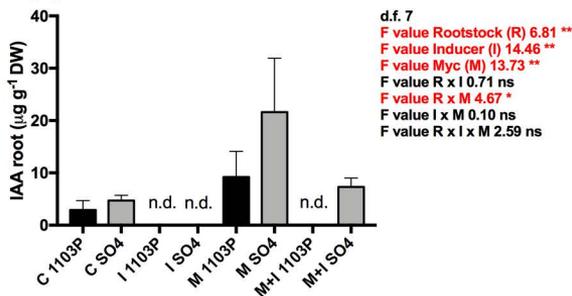
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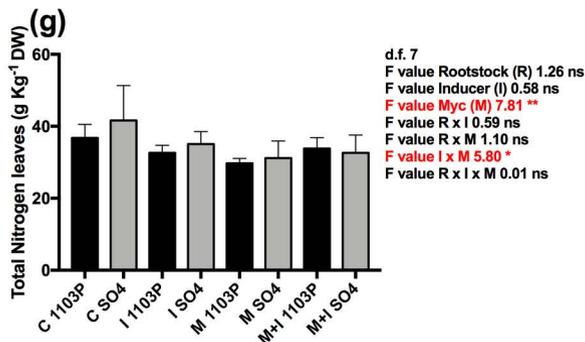
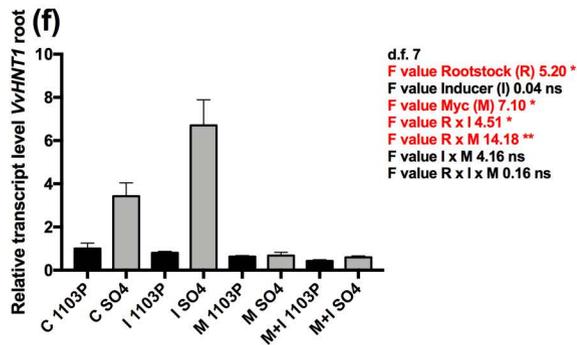
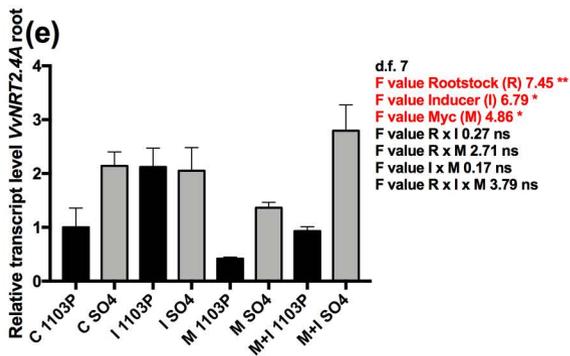
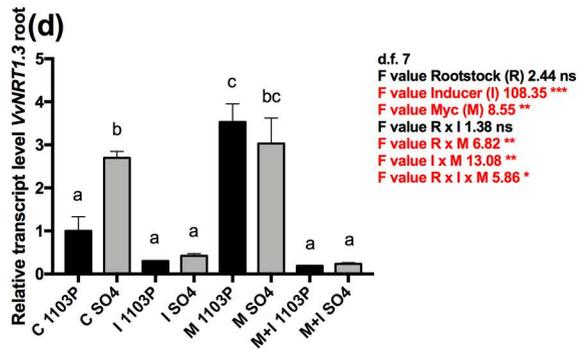
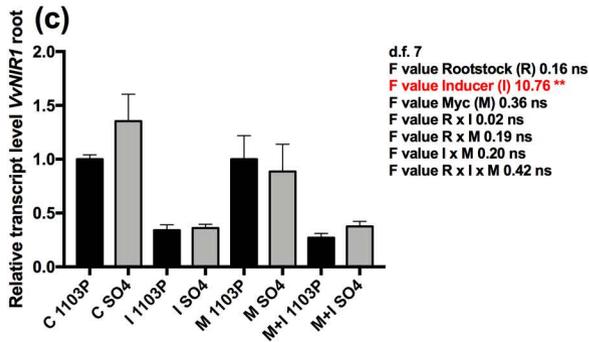
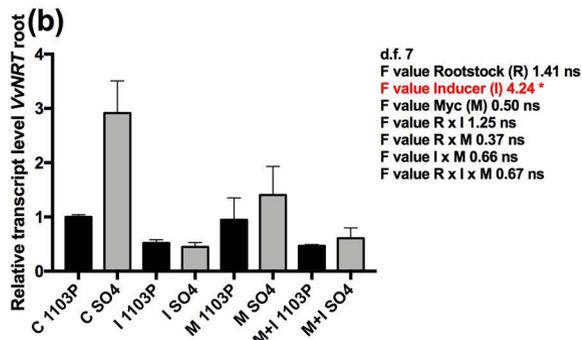
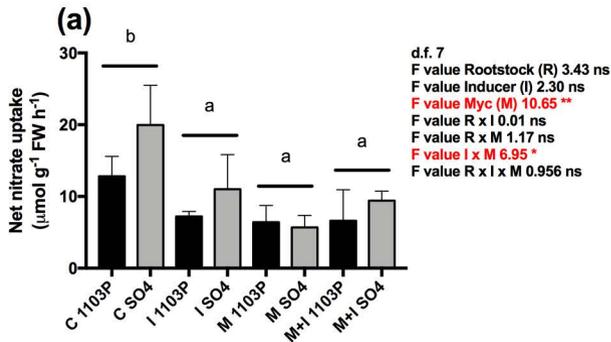


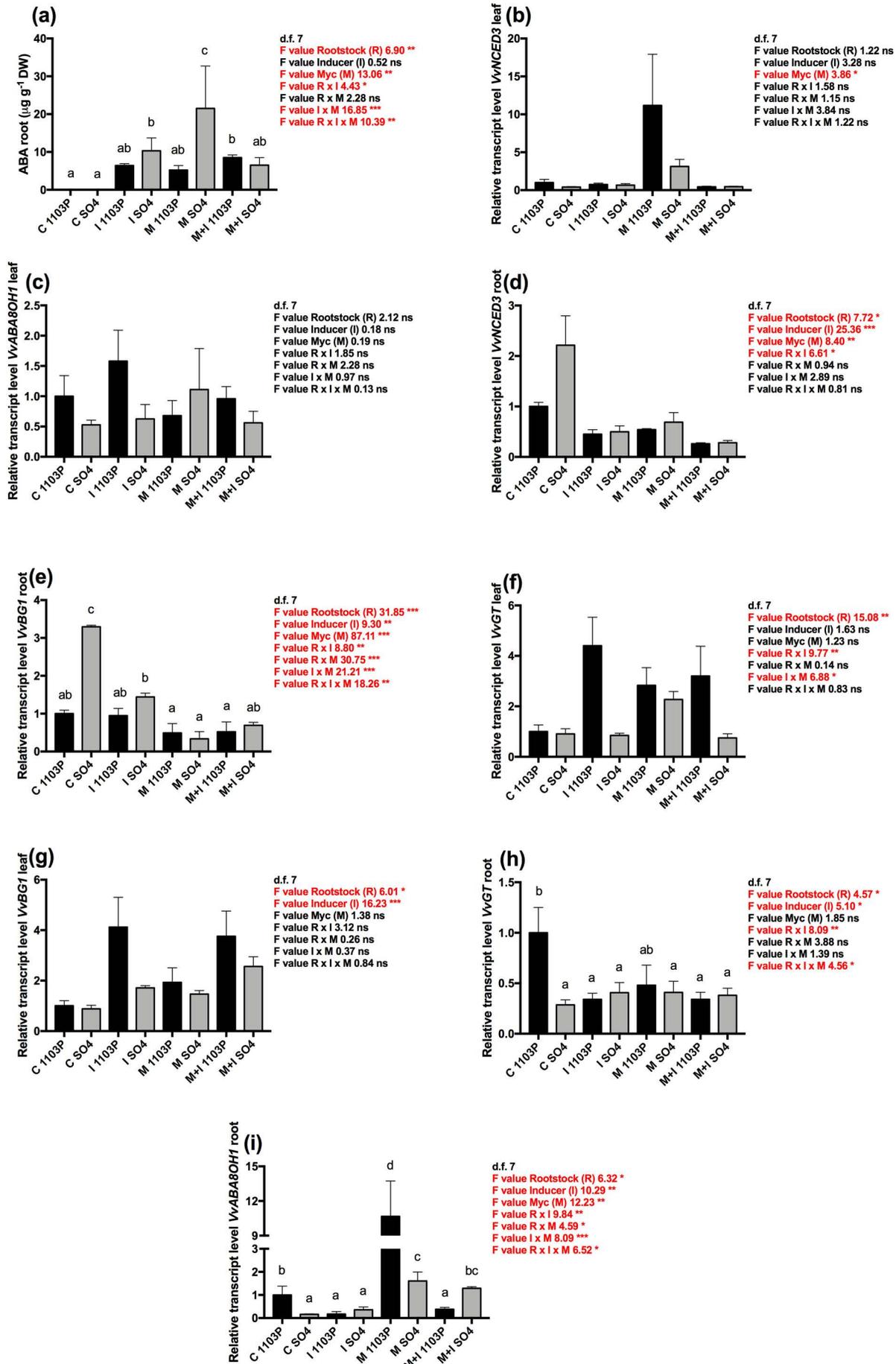
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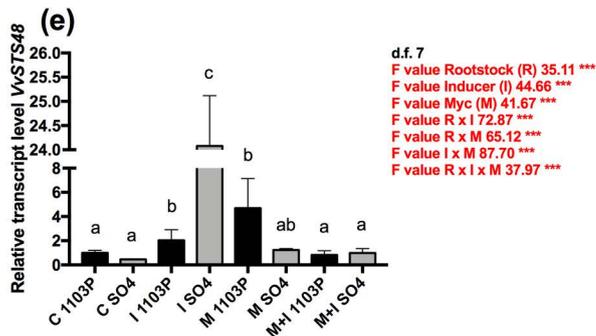
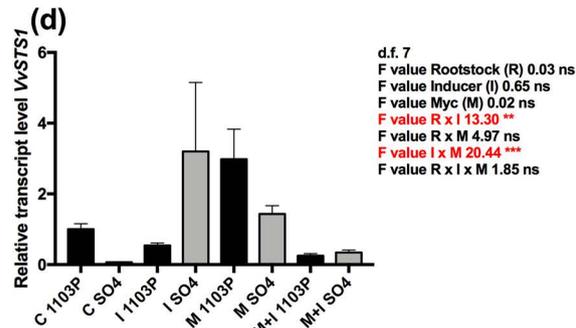
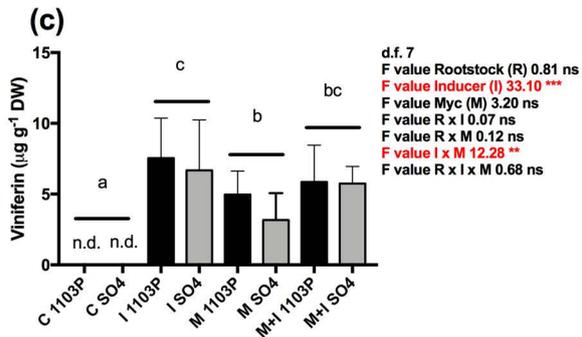
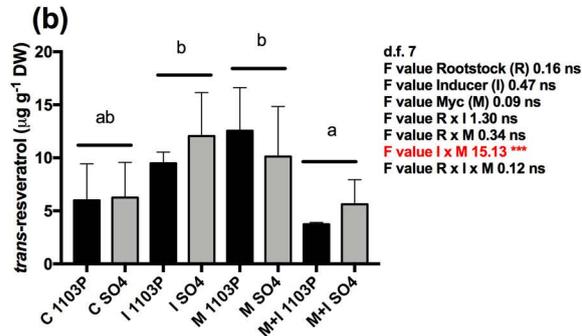
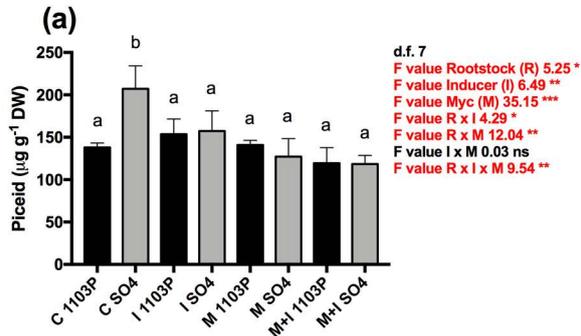


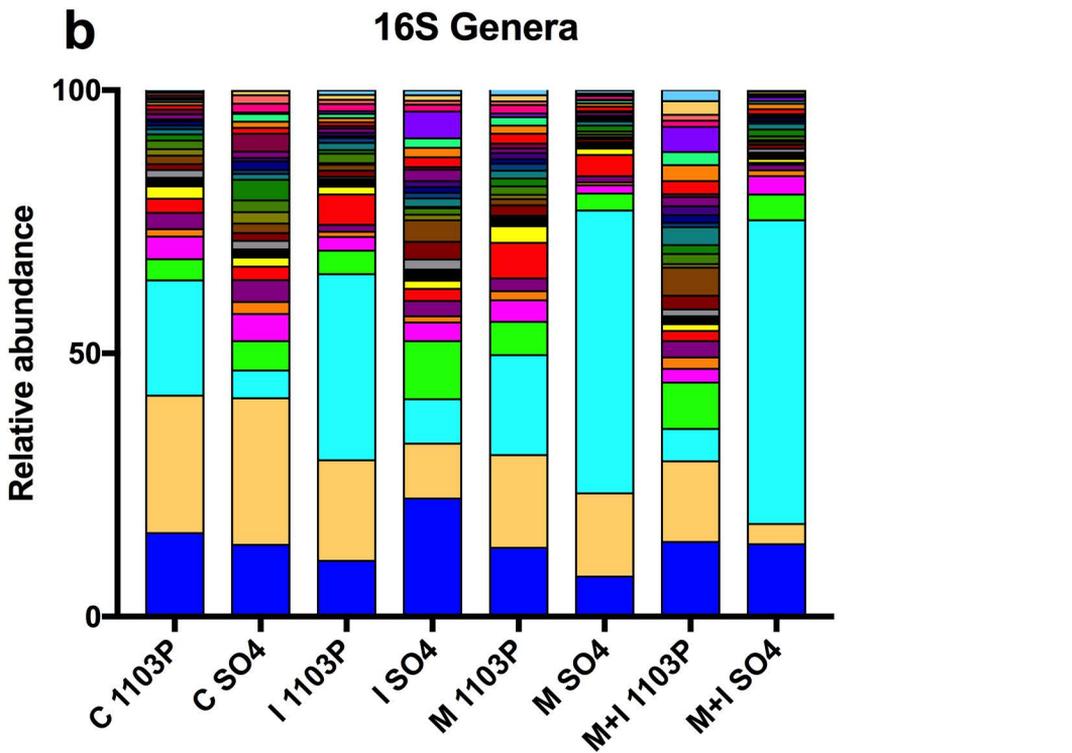
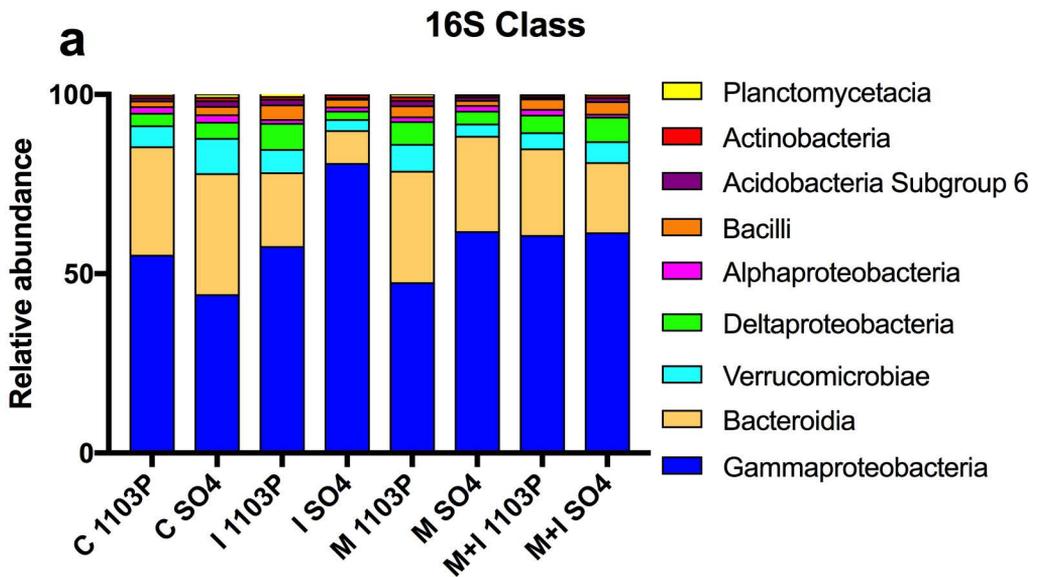
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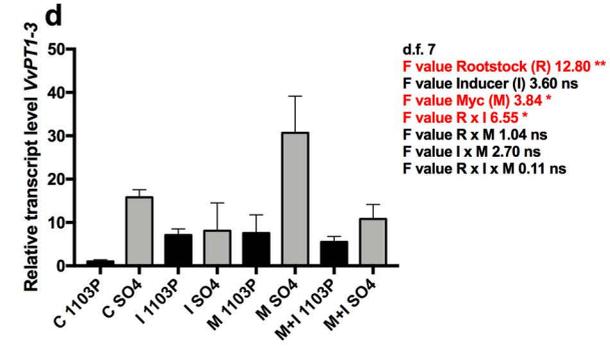
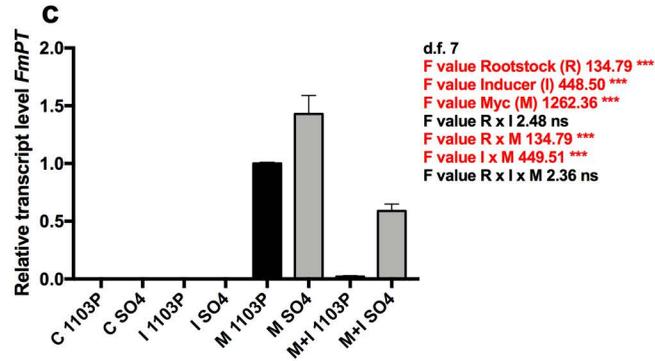
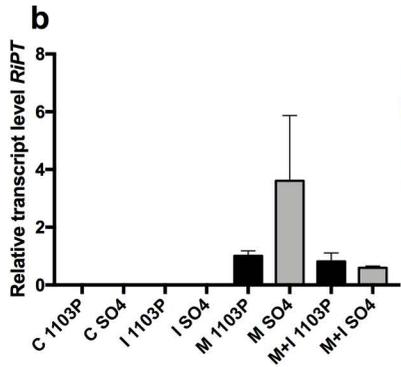
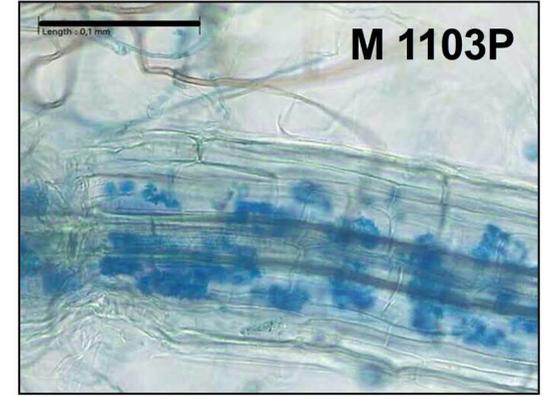
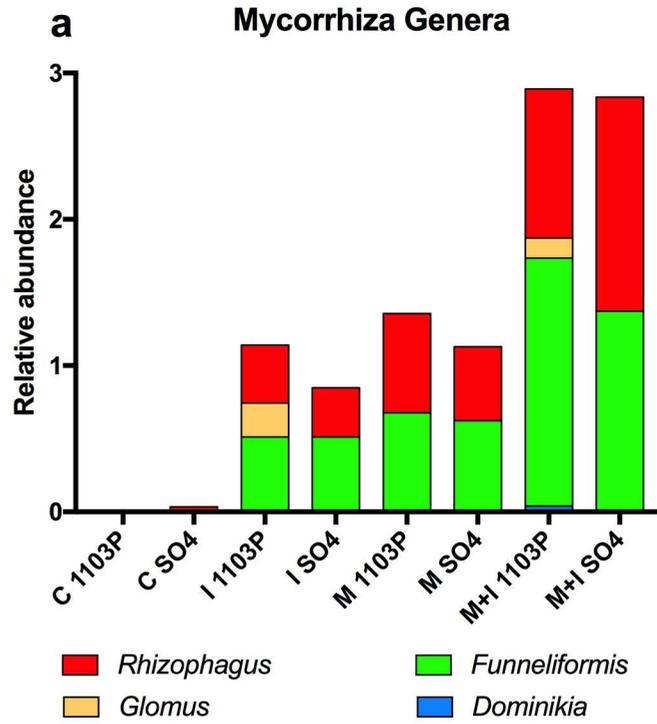
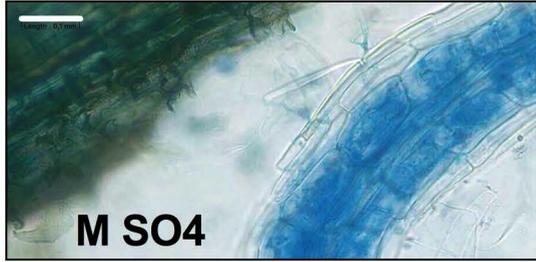


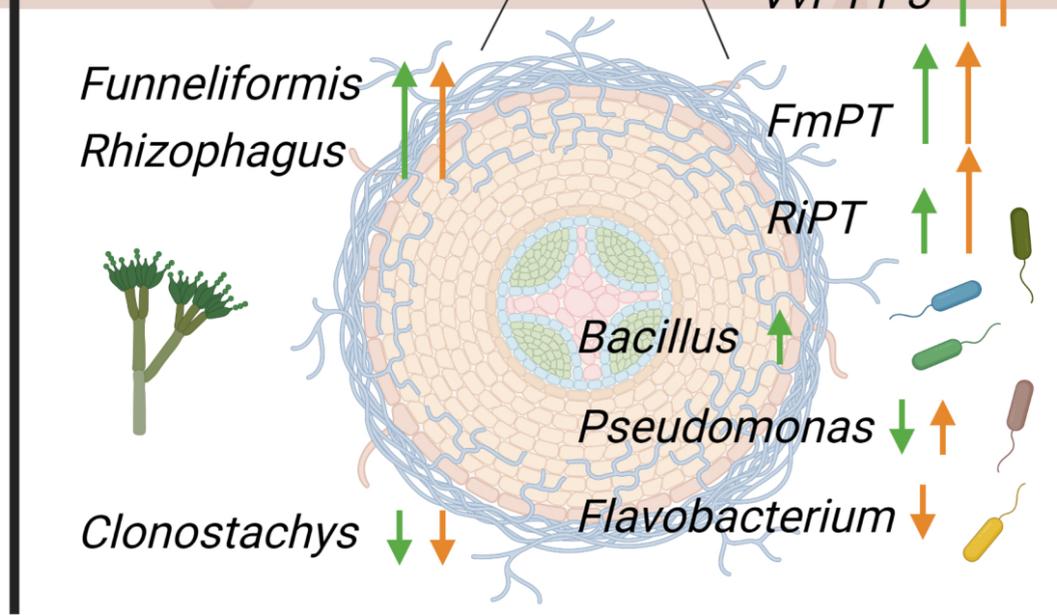
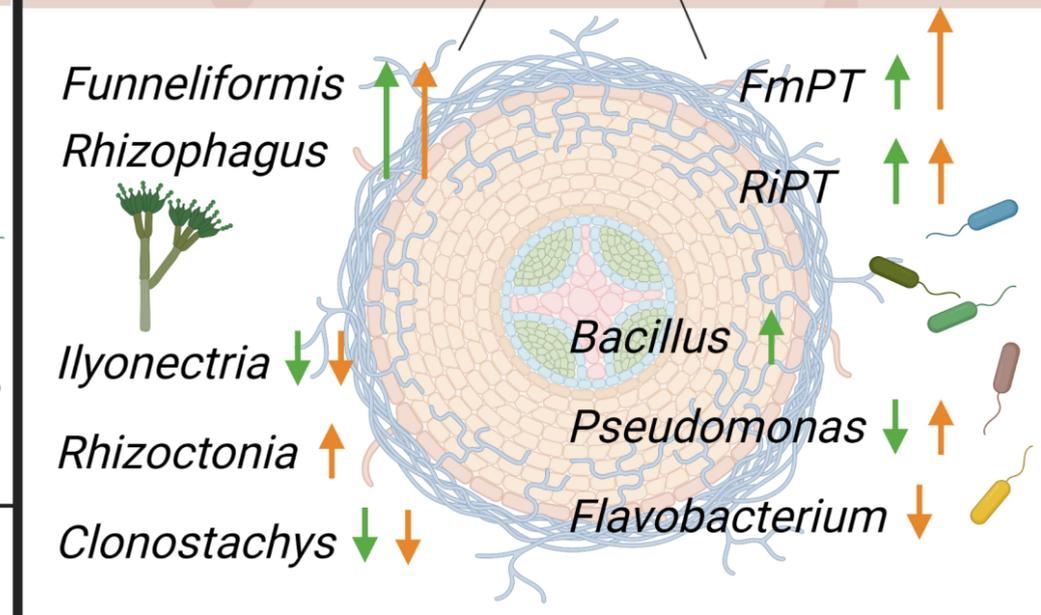
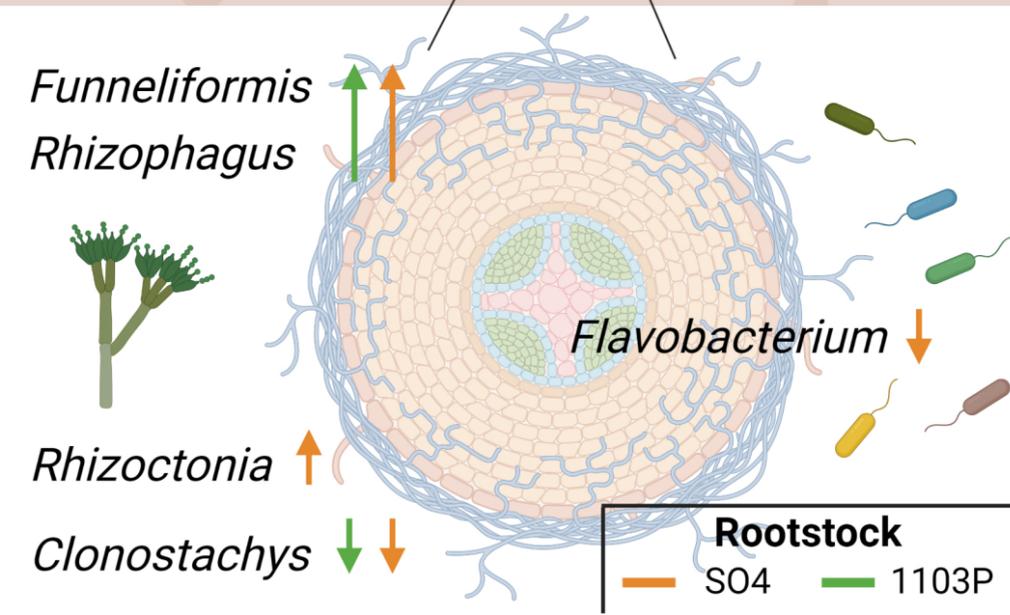
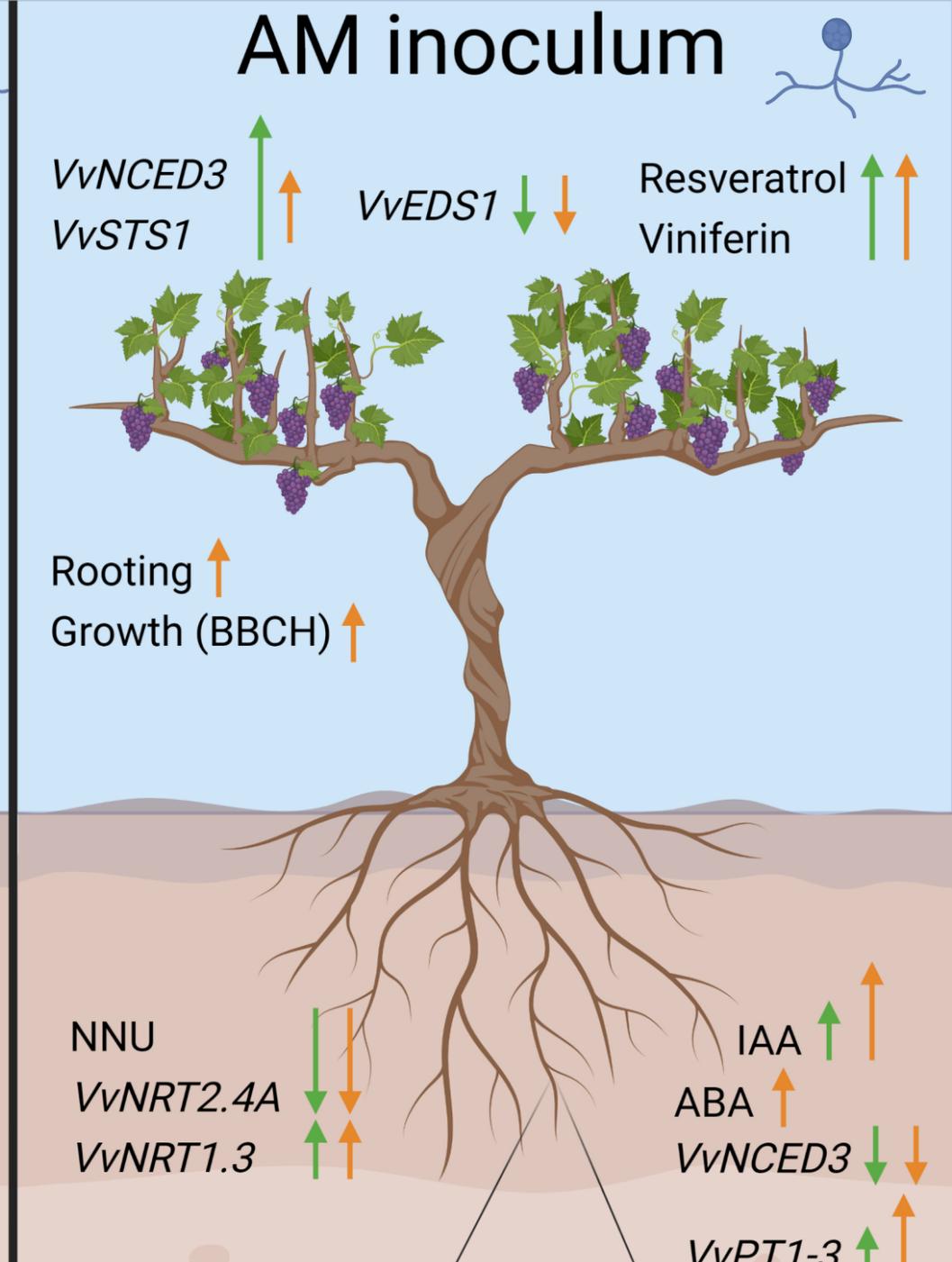
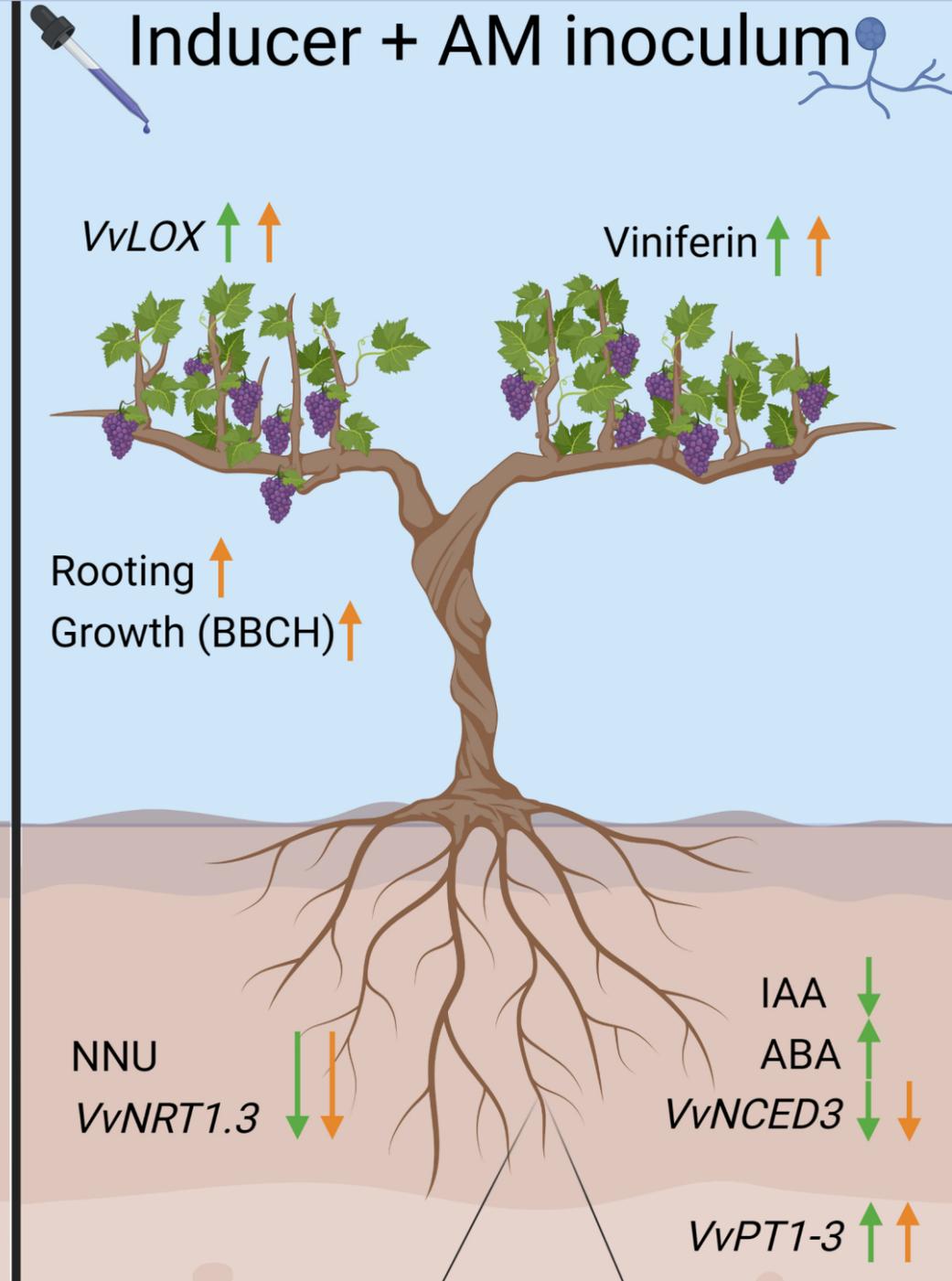
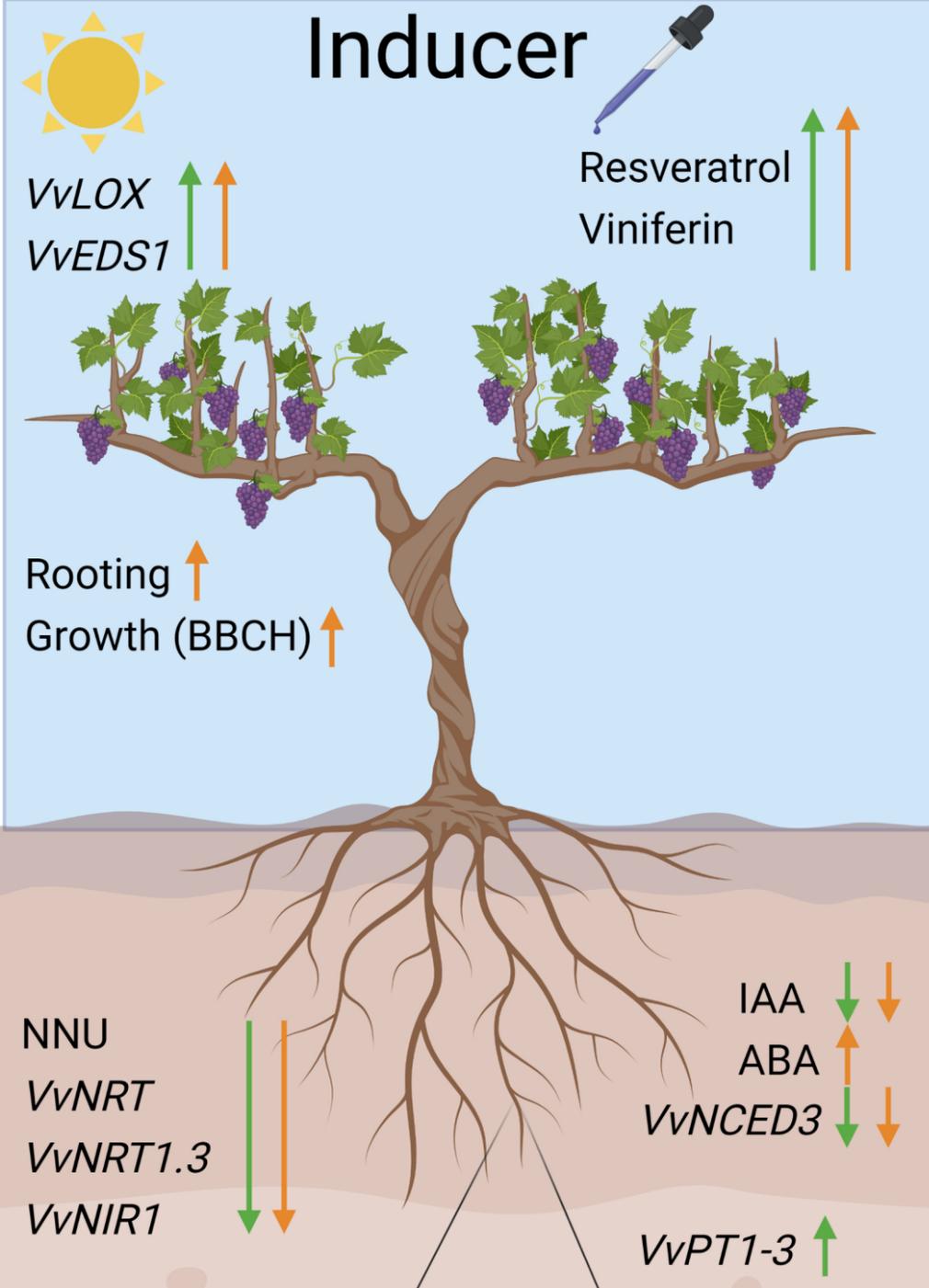


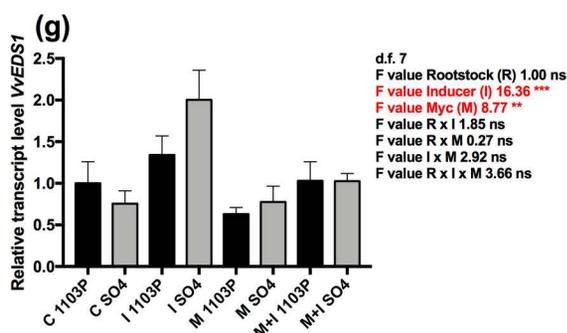
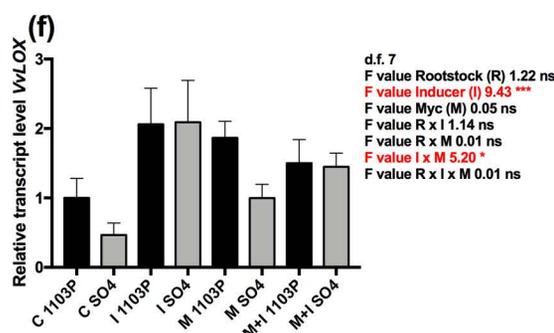
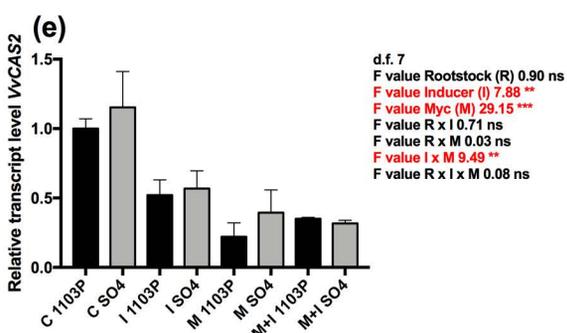
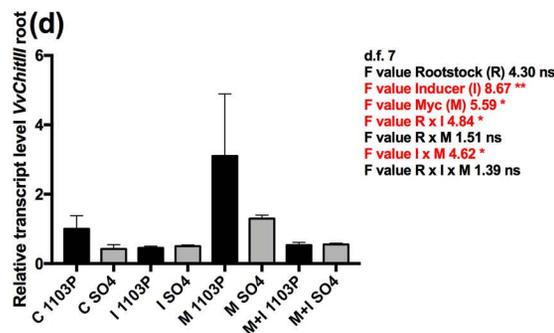
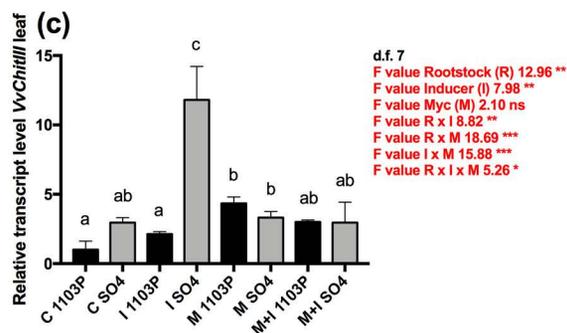
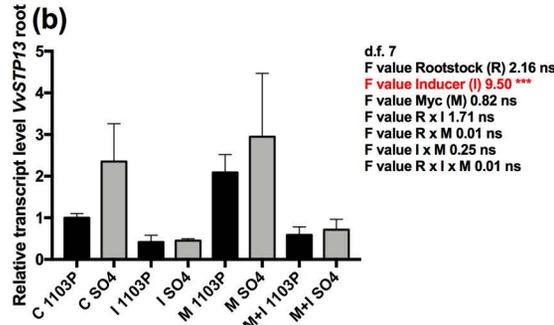
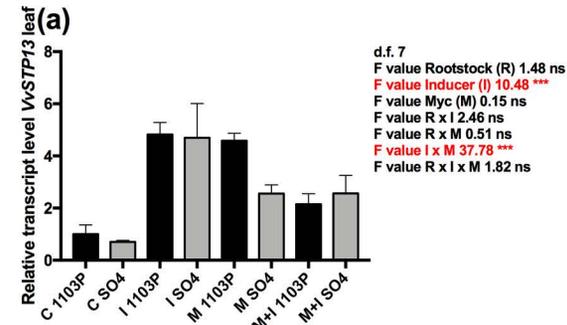






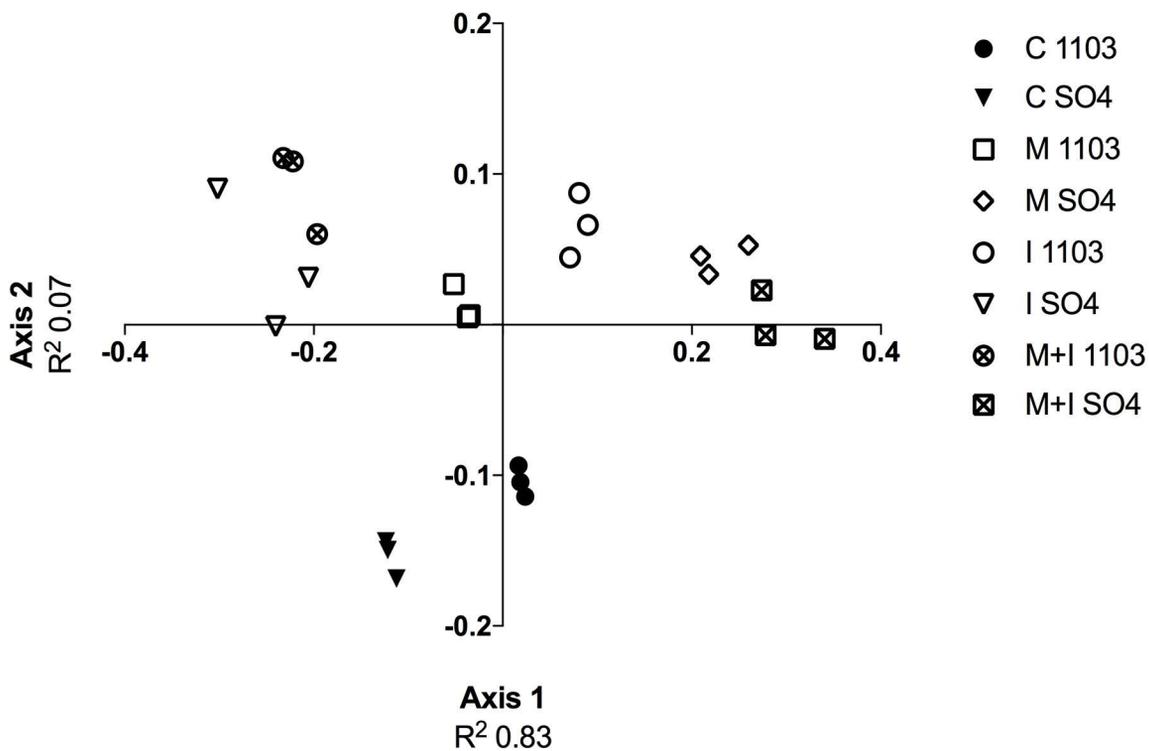






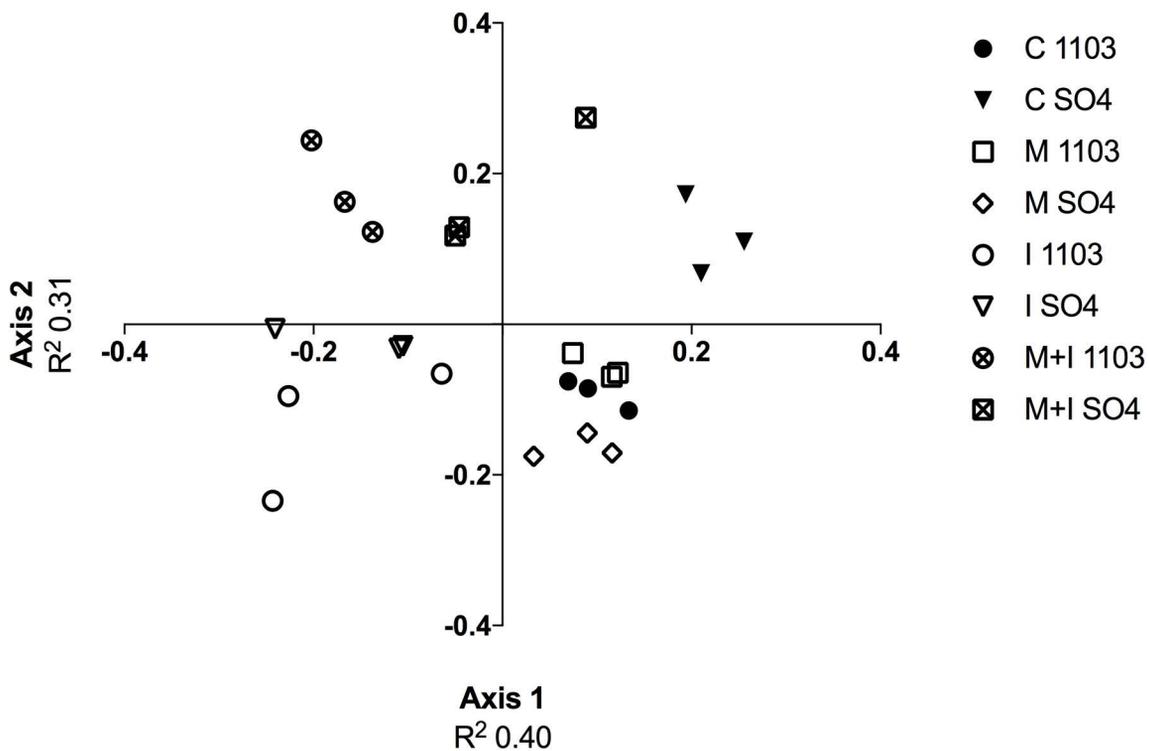
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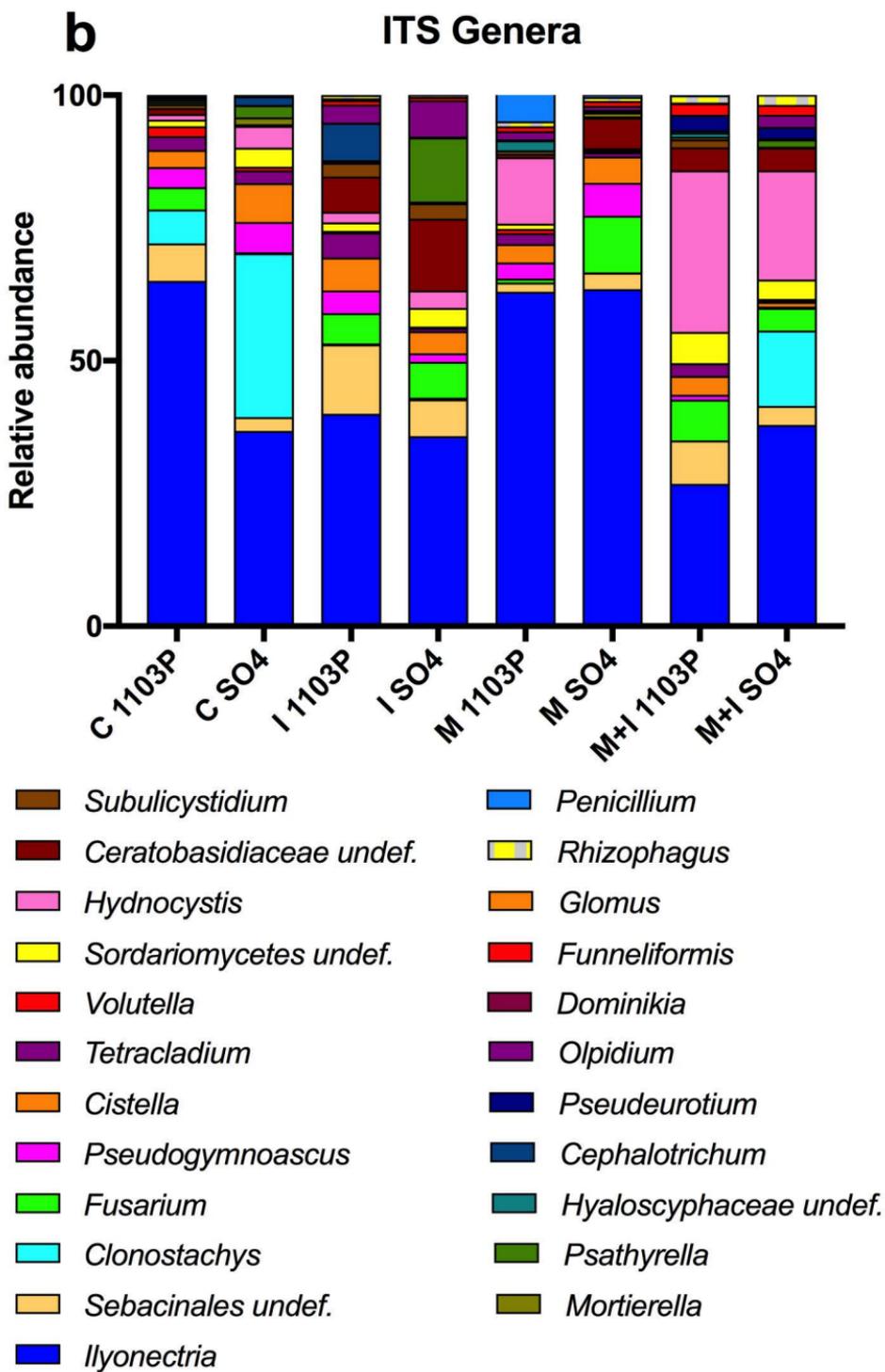
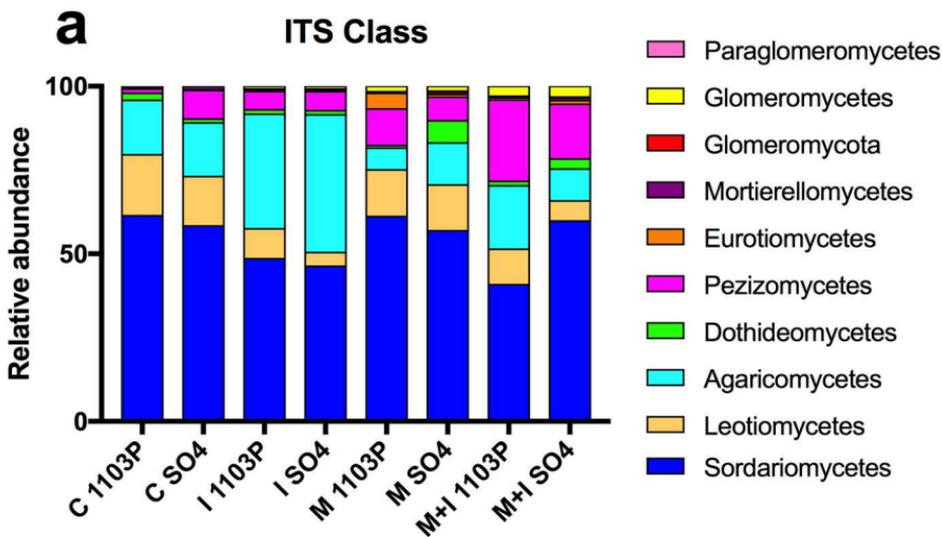
NMDS Bacteria



(b)

NMDS Fungi





Pathogen Genera

