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Co-inoculation with arbuscular mycorrhizal fungi differing in carbon sink strength induces a synergistic effect in plant growth

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Abstract

Arbuscular mycorrhizal (AM) fungi play a key role in determining ecosystem functionality. Understanding how diversity in the fungal community affects plant productivity is therefore an important question in ecology. Current research has focused on understanding the role of functional complementarity in the fungal community when the host plant faces multiple stress factors. Fewer studies, however, have investigated how variation in traits affecting nutrient exchange can impact the plant growth dynamics, even in the absence of environmental stressors. Combining experimental data and a mathematical model based on ordinary differential equations, we investigate the role played by carbon sink strength on plant productivity. We simulate and measure plant growth over time when the plant is associated with two fungal isolates with different carbon sink strength, and when the plant is in pairwise association with each of the isolates alone. Overall, our theoretical as well as our experimental results show that co-inoculation with fungi with different carbon sink strength can induce positive non-additive effects (or synergistic effects) in plant productivity. Fungi with high carbon sink strength are able to quickly establish a fungal community and increase the nutrient supply to the plant, with a consequent positive impact on plant growth rate. On the other side, fungi with low carbon sink strength inflict lower carbon costs to the host plant, and support maximal plant productivity once plant biomass is large. As AM fungi are widely used as organic fertilizers worldwide, our findings have important implications for restoration ecology and agricultural management.

Keywords : Arbuscular mycorrhizal fungi (AMF), multiple mutualist effects (MMEs), carbon sink strength, synergy, productivity, agriculture, nutrient exchange, inoculum, mutualism.

1 Introduction

2 Arbuscular mycorrhizal (AM) fungi are microscopic symbionts found inside the roots of
3 the vast majority of terrestrial plants (Smith and Read, 2010). AM fungi exert a strong
4 positive influence on plant growth and fitness, by facilitating plant access to limiting
5 nutrients (e.g., phosphorus) (Jeffries et al., 2003; Smith and Read, 2010), providing
6 pathogen protection (Pozo and Azcón-Aguilar, 2007; Shrivastava et al., 2015), or tol-
7 erance to abiotic stress (Latef et al., 2016; Augé et al., 2015). The plant, in exchange,
8 provides AM fungi with fixed carbon (Smith and Read, 2010). In nature, multiple
9 species of AM fungi coexist in the roots of the same host plant (Verbruggen et al.,
10 2012; Vandenkoornhuysen et al., 2007) and it is proposed that variation of functional
11 traits can be observed among coexisting fungi (Powell and Rillig, 2018; Lee et al., 2013;
12 Mensah et al., 2015; Smith et al., 2000; Feddermann et al., 2010).

13 With functional complementarity among fungi one could expect plant benefit to be
14 higher in the presence of multiple AM fungi than in the presence of any of the species
15 (or isolates) separately (Koide, 2000; Gianinazzi et al., 2010). However, experimental
16 observations on plant growth show that simultaneous colonization by multiple AM fungi
17 does not always lead to a greater benefit for the plant, and in some cases productivity
18 can be larger in the presence of a single, most beneficial fungus (Gustafson and Casper,
19 2006; Jansa et al., 2008; Violi et al., 2007; Thonar et al., 2014; Maherali and Klironomos,
20 2007; Gosling et al., 2016; Jansa et al., 2008; Alkan et al., 2006). As AM fungi play a key
21 role in determining ecosystem productivity and function (Van der Heijden et al., 1998),
22 a better understanding of the relationship between diversity in the fungal community
23 composition and plant productivity is of particular importance.

24 Experimental studies have focused on understanding the link between diversity and
25 productivity in multistressed environments, where the presence of taxonomically distant
26 AM fungal species can act as insurance to support plant growth despite challenging
27 abiotic conditions (Yang et al., 2016; Thonar et al., 2014; Crossay et al., 2019; Gosling
28 et al., 2016; Maherali and Klironomos, 2007). Few studies, however, have looked at
29 how functional complementarity in the fungal community may affect nutrient exchange
30 processes, even in the absence of stress factors (Mensah et al., 2015; Ibrahim, 2018;
31 Jansa et al., 2005). AM fungi can differ in their ability to provide phosphorus to the
32 plant (Drew et al., 2003; Giovannini et al., 2020), or in their access to host carbon (Zhu
33 and Miller, 2003), and these differences should by themselves result into differences in
34 the plant growth dynamics (Afkhami et al., 2014). While some studies have investigated
35 how plant productivity is affected by fungi to plant supply (i.e., phosphorus transfer),
36 (Jansa et al., 2005; Mensah et al., 2015; Ibrahim, 2018; Martignoni et al., 2020a),
37 changes in productivity due to carbon transfer (i.e., plant to fungi supply) have remained
38 unexplored.

39 The need for a framework to study the mechanisms associated with the presence
40 of multiple, functionally different mutualists has been recently highlighted also from
41 the theoretical point of view (Afkhami et al., 2014). Theoretical work, however, has
42 focused on explaining stable coexistence among mutualists (Bachelot and Lee, 2018;
43 Johnson and Bronstein, 2019; Martignoni et al., 2020a), rather than on investigating
44 the consequences of intraguild diversity on productivity. Multiple mutualists effects
45 can potentially increase, reduce or have no repercussion on host performance, but it is
46 unclear how interactions with the environment, direct and indirect interactions among

47 mutualists, or nutrient exchange may contribute to the final outcome (Afkhami et al.,
48 2014; Stanton, 2003).

49 Here, we test whether differences in the benefit provided by the plant to fungal mu-
50 tualists can induce positive non-additive effects (or synergistic effects) in plant biomass,
51 which in our work will be used as a measure of productivity (Xu and Mage, 2001). The
52 factors driving carbon allocation from host to fungus are largely unknown (di Fossalunga
53 and Novero, 2019), but might be important determinants of plant growth. The level of
54 carbon transfer has been hypothesized to be a result of plant discrimination between
55 associated fungi, where fungi providing more nutrients to the plant are rewarded with
56 more carbon in exchange (Kiers et al., 2011; Kafle et al., 2019). However, it remains
57 challenging to explain how a plant can discriminate between symbionts in natural con-
58 ditions, where multiple fungi share a single root system (Walder and van der Heijden,
59 2015). For this reason, in our study we do not assume any association between carbon
60 transfer and the reward received from the fungus. We consider the ability of a fungus
61 to access plant carbon (or its ‘carbon sink strength’) to be intrinsic characteristic of the
62 fungal species (or fungal isolate), determined by the physiological capacity of the fungus
63 to acquire carbon (Řezáčová et al., 2017; Pearson and Jakobsen, 1993), and linked, for
64 example, to the proportion of intercellular hyphae involved in carbon transfer (Smith
65 and Read, 2010; Hodge et al., 2010).

66 We use an ordinary differential equation (ODE) model to investigate the coupled
67 plant-fungi growth dynamics. The model allows us to disentangle the effects on plant
68 growth driven by differences in fungal carbon sink strength, from effects caused by
69 environmental stressors. We then compare our theoretical predictions with in vivo
70 experiments. We use two fungal isolates with different carbon sink strength and measure
71 plant growth over time in monocultures and in mixed cultures.

72 **Models, materials, and methods**

73 **Differences in carbon sink strength and productivity**

74 To investigate plant productivity in the presence of two fungal isolates with different
75 carbon sink strength we compare growth when the plant is in pairwise association with
76 each of the fungi separately (see Fig. 1, left panel), to growth when the plant is in
77 simultaneous association with the two fungi (Fig. 1, right panel). First, we identify the-
78 oretically under which circumstances the synergy occurs, using the mathematical model
79 described below. We then compare our theoretical results with in vivo experiments and
80 statistical analysis of experimental data.

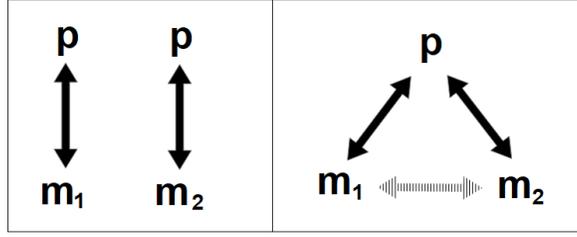


Fig. 1: Schematic representation of the scenarios used to address questions 1 (left panel) and 2 (right panel), through numerical simulation. On the left we consider a plant interacting separately with two different isolates of AM fungi, m_1 and m_2 , taken one at a time. On the right the plant interacts with the two fungal isolates simultaneously. Fungi m_1 and m_2 share the carbon supplied by the plant and therefore interact indirectly with each other.

81 **Theoretical work:**

82 **Model description:** To study the plant-fungi growth dynamics we used the math-
 83 ematical model developed by Martignoni et al. (2020a). This model is based on a
 84 consumer-resource framework for mutualisms (Holland and DeAngelis, 2010), and con-
 85 sideres the coupled dynamics of a mutualist guild (i.e., the fungal mutualists) sharing a
 86 resource supplied by the same host (i.e., plant carbon).

87 In the model, plant growth depends on the amount of phosphorus that the plant
 88 receives from the fungi, on the carbon that the plant provides to the fungi, on plant
 89 growth in the absence of mutualism, and on the amount of nutrients that need to be
 90 reserved for maintenance of the existing plant biomass. Similarly, the growth of each
 91 fungus depends on the amount of carbon received from the plant, on the amount of
 92 phosphorus that the fungus transfers to the plant, and on the maintenance costs. We
 93 refer to ‘fungal isolates’ instead of ‘fungal species’, as for the experimental testing of
 94 our hypotheses we will select different isolates of the same fungal species (i.e., different
 95 isolates of *Rhizophagus irregularis*, as described in the next section).

The system of equations describing the variation over time of plant biomass p and of the biomass of one associated fungal isolate m_i (where $i = 1, 2$) is given by

$$\underbrace{\frac{dp}{dt}}_{\text{change in plant biomass}} = \underbrace{q_{hp} \left(\sum_i \alpha_i m_i \right) \frac{p}{d+p}}_{\text{phosphorus from AM fungi}} - \underbrace{q_{cp} \left(\sum_i \beta_i m_i \right) p}_{\text{carbon to AM fungi}} + \underbrace{q_{hp} r_p p}_{\text{intrinsic growth}} - \underbrace{\mu_p p^2}_{\text{maintenance cost}}, \quad (1a)$$

$$\underbrace{\frac{dm_i}{dt}}_{\text{change in fungal biomass}} = \underbrace{q_{cm} \beta_i p m_i}_{\text{carbon from plant}} - \underbrace{q_{hm} \alpha_i \frac{p}{d+p} m_i}_{\text{phosphorus to plant}} - \underbrace{\mu_m m_i^2}_{\text{maintenance cost}}. \quad (1b)$$

96 A brief description of all model parameters is given in Table 1. The model assumes
 97 nutrient transfer to be biomass dependent, where the functional responses chosen are
 98 justified in detail in Martignoni et al. (2020a). We assume a linear relationship between
 99 fungal biomass and phosphorus transfer. Indeed the amount of phosphorus transferred
 100 is expected to increase with increasing hyphal length and root colonization (Sawers
 101 et al., 2017; Treseder, 2013). When plant biomass is low, the amount of phosphorus
 102 that can be received by the plant is limited by its small size, and we assume phosphorus

Table 1: Brief description of the variables and parameters of the model of Eq. (1) and default values used for simulations. Default values are based on the statistical analysis of the experimental data (see Appendix C). Values in brackets correspond to case variations shown in Fig. 3.

Symbol	Description	Default value
p	Plant biomass	–
m_i	Fungal biomass	–
p_0	Plant initial biomass	0.08
m_{0i}	Fungal initial biomass of isolate i	0.05 (0.1)
μ_p	Plant maintenance rate	1.44 (1.7)
μ_m	Fungal maintenance rate of isolate i	1.05 (1.3)
q_{hp}	Conversion phosphorus to plant biomass	2.93
q_{cm}	Conversion carbon to fungal biomass of isolate i	2.93
q_{cp}	Conversion carbon to plant biomass	1
q_{hm}	Conversion phosphorus to fungal biomass of isolate i	1
d	Half-saturation constant	1.07
r_p	Plant intrinsic growth rate	0.33 (0.66)
α_i	Phosphorus supply from fungal isolate i	2.2 (2.0)
β_i	Carbon sink strength of fungal isolate i	1.0-4.0

103 transfer to depend on both plant and fungal biomass (see ‘phosphorus from AM fungi’
104 and ‘phosphorus to plant’ terms in Eq. (1)). Carbon transfer from the plant to the
105 fungi depends on carbon fixation and on the extent of root colonization by AM fungi
106 (Thomson et al., 1990; Vierheilig et al., 2002), and in the model we assume a linear
107 relationship between carbon transfer and fungal and plant biomass (see ‘carbon to AM
108 fungi’ and ‘carbon from plant’ terms in Eq. (1)). Fungal isolates therefore do not
109 directly compete for host carbon, but each fungus accesses a different proportion of
110 host carbon depending on its biomass.

111 The nutrient transfer terms show the same biomass dependence for all fungi, how-
112 ever, the exact amount of nutrients transferred varies among isolates, and depends on
113 the ability of a fungus to transfer phosphorus to the plant (parameter α_i) and on its
114 access to plant carbon (parameter β_i). Fungi with higher carbon sink strength can
115 therefore be characterised by a larger β_i parameter. Other fungal parameters are the
116 carbon and phosphorus to biomass conversion rates (q_{cm} and q_{hm}), and the rate at which
117 a fungus deviates resources to maintenance of its existing biomass (μ_m). Parameters
118 characterising plant growth are the phosphorus and carbon to biomass conversion rates
119 (q_{hp} and q_{cp}), the intrinsic growth rate in the absence of mutualism (r_p), and the plant
120 maintenance rate (μ_p). The presence of stress factors can be modeled as an increase in
121 the amount of resources reserved for maintenance, reflected in a higher value of the μ_p
122 and μ_m parameters.

123 **Relating carbon sink strength to plant growth:** To investigate how the plant
124 growth rate and final size are affected by carbon sink strength (parameter β) we first
125 considered a plant associated with a single fungus. For this purpose, we solved Eq. (1)
126 for $i = 1$ and considered a range of β parameters, while other parameters were kept
127 constant at their default value (see Table 1). The final size corresponds to plant biomass

128 at equilibrium. The average plant growth rate was computed as

$$129 \int_0^{T_{95}} \frac{1}{T_{95}} \frac{1}{p} \left(\frac{dp}{dt} \right) dt, \quad (2)$$

130 where T_{95} is the time needed to reach 95% of the plant biomass at equilibrium, and
 131 dp/dt represents plant biomass over time (defined in Eq. (1a)).

132 Additionally, we compared plant growth in the presence of one isolate (with either
 133 high or low carbon sink strength) with plant growth in the presence of both isolates
 134 simultaneously. In the model, fungal maintenance costs are non-additive as they depend
 135 on fungal biomass (see last term in Eq. (1b)). Therefore, in order to compare plant
 136 growth in the presence of one and two fungal isolates, we assumed the plant p to be
 137 always in association with two fungal isolates (i.e., $i = 1, 2$), where the two fungi can be
 138 the same isolate (i.e., characterised by the same parameters), or different isolates (i.e.,
 139 characterised by different β_i parameters).

140 Finally, we compared the effect of variations in the carbon sink strength with vari-
 141 ations in phosphorus supply. We repeated the procedure described in the previous
 142 two paragraphs to investigate how phosphorus supply (parameter α_i) affects the plant
 143 growth rate and final size, and simulated plant growth over time in the presence of one
 144 and two fungal isolates differing in their phosphorus supply to the plant.

145 ***Sensitivity of parameter values:*** We solved Eq. (1) for $i = 1, 2$, where fungus
 146 1 and 2 are characterised by different β_1 and β_2 parameters, while other parameters
 147 were kept constant at their default values (see Table 1). Default values were based on
 148 the statistical analysis of the experimental data described below. We determined the
 149 (β_1, β_2) parameter regions where a synergy in plant growth was observed and for each of
 150 these we computed the intensity and temporal length of the synergistic effect. We define
 151 the synergistic effect as a percentage gain in productivity, quantified as the percentage
 152 increase in plant biomass observed when the plant is in combination with two isolates,
 153 with respect to when the plant is in combination with the isolate that leads to the
 154 highest plant biomass. We found that a synergy in plant growth was observed when
 155 the plant was associated with two isolates with higher or lower carbon sink strength,
 156 characterised by parameters β_H and β_L respectively. Mathematically, we write:

$$157 \quad \% \text{ gain in productivity}(t) = \frac{p_{H+L}(t) - \max(p_H(t), p_L(t))}{\max(p_H(t), p_L(t))} \times 100, \quad (3)$$

158 where $p_H(t)$ and $p_L(t)$ refer to plant biomass at each point in time when the plant is
 159 associated with the isolate with high or low carbon sink strength respectively, while
 160 $p_{H+L}(t)$ refers to plant biomass when the plant is associated with both isolates. When
 161 the maximal gain in productivity is positive, i.e., $p_{H+L}(t) > \max(p_H(t), p_L(t))$, we say
 162 that a synergistic effect is observed. The temporal length of the synergistic effect refers
 163 to the number of days for which the gain in productivity is positive. The intensity of
 164 the synergistic effect refers to the maximal gain in productivity observed during the
 165 time interval considered.

166 We considered variations from the default scenario, by choosing a higher intrinsic
 167 growth rate of the plant (parameter r_p), a higher amount of initial fungal biomass
 168 (higher m_{0_i}), higher environmental stress (reflected in an increase in parameters μ_p

169 and μ_m , the plant and fungal maintenance rates), and a reduced ability of the fungal
170 isolate to transfer phosphorus to the plant (lower α_i parameters). Parameter ranges
171 were chosen based on the significance of the results. Simulations were performed using
172 the Matlab (R2020b) solver ode45.

173 **Experimental work:**

174 ***Selection of AM fungal isolates:*** To identify isolates with different carbon sink
175 strength but similar ability to transfer phosphorus to the plant, we conducted a prelim-
176 inary study using nine isolates of *Rhizophagus irregularis* (Schenck and Smith, 1982).
177 Additional information about this experiment is available in Appendix A. We identi-
178 fied two isolates of *R. irregularis* that matched the needed requirements: 1) Cuba8,
179 which demonstrated a high carbon sink strength under the growing conditions of our
180 study, and 2) CC4 (DAOM 229457), which demonstrated significantly lower carbon
181 sink strength. No statistically significant difference was found in the phosphorus trans-
182 fer ability of Cuba8 and CC4 (Fig. A.1).

183 ***AM fungal inoculum:*** Isolates Cuba8 and CC4 were grown with Ri T-DNA trans-
184 formed carrot roots on Petri plates (60-mm diameter and 15-mm height) (VWR) with
185 M medium as described by Bécard and Fortin (1988), solidified with 1% gellan gum
186 (Alfa Aesar). The Petri plates were incubated inverted at 26°C (CONVIRON ADAP-
187 TIS CMP6010) in the dark, until numerous spores were produced. Mature spores were
188 extracted after solubilizing the medium with sterile 10 mM, pH=6 sodium citrate buffer
189 solution (Doner and Bécard, 1991). Cuba8 has a germination rate of 20% while CC4
190 has a significantly higher germination rate of 60% (Kokkoris et al., 2019). To ensure
191 standard inoculum density throughout the experiment we accounted for the variation
192 in the germination rates to ensure the presence of at least six viable spores per pot.
193 For this reason we used 30 Cuba8 spores or 10 CC4 spores per pot when the plant
194 was inoculated with a single isolate, and 30 spores of Cuba8 and 10 spores CC4 when
195 the plant was inoculated with a mix of both isolates. To avoid runoff of spores post
196 inoculation and to attain successful colonization we applied a novel inoculation system
197 that involved the creation of ‘inoculation disks’ (see Appendix B, Fig. B.1).

198 ***Growing conditions:*** For the main experiment, AM fungal isolates and host plants
199 of *Linum usitatissimum* (Flax) were grown in 2L pots containing (v/v=1:1) sterilized
200 medium-fine sand (SAKRETE Play Sand) and vermiculate (in identical conditions as
201 in the preliminary study, see Appendix A). Flax seeds were surface sterilized with 70%
202 ethanol for 1 min, followed by 5 min in 9.6% bleach. Seeds were then washed six times
203 for 1 min each, in sterilized distilled water. The surface sterile seeds were germinated
204 in Petri dishes on wet sterile 11 cm filter papers (VWR) and kept in an incubator
205 (CONVIRON ADAPTIS CMP6010) at 26°C (no light) until a radicle was observed.
206 Inoculated pots were placed in the greenhouse where the temperature ranged from 26
207 to 20°C (day/night) with a photoperiod of 18 h per day, of 200 $\mu\text{mol s}^{-1} \text{m}^{-2}$ per μA
208 as measured with a LI-250A light meter (Biosciences). Two weeks post inoculation
209 and weekly thereafter, plants were fertilized with Low P fertilizer (Miracle-gro 24-8-16)
210 (50mL per plant of 5 mL fertilizer dissolved in 4L water) until harvest. Additional

211 water was applied with emitters directly above the straws, supplying 2 l hr⁻¹ to each
212 pot, ~35 ml every two days.

213 After the first month, eight plants per week and per treatment were harvested from
214 each treatment for the duration of eight weeks, for a total growing period of 12 weeks.
215 At each harvest, roots were washed, dried of excess water and the fresh root weight was
216 recorded. Shoots were weighed for fresh weight.

217 **Statistical analysis:**

218 ***Analysis of experimental data:*** Our first goal was to analyse the experimental
219 data to understand whether a synergy in productivity has occurred, and if so to quantify
220 its duration and intensity. We compared the total biomass of randomly chosen replicates
221 from each experiment with only one fungal isolate (either Cuba8 or CC4), with the total
222 plant biomass of a random replicate from the experiments with a combination of both
223 isolates (Cuba8 and CC4). We computed the number of weeks for which plant biomass
224 from the experiments with both AM fungal isolates was greater than the maximal
225 plant biomass measured when only one AM fungal isolate was present. In addition, we
226 computed the percentage gain in productivity per week (or weekly percentage increase
227 in plant biomass) measured when a synergy occurs (Figs. 5a and 5b). For each weekly
228 measurement of plant biomass in the presence of both isolates ($p_{H+L}(t)$) the gain in
229 productivity was computed as:

$$230 \quad \% \text{ gain in productivity}(t) = \frac{p_{H+L}(t) - \max(M(p_H)(t), M(p_L)(t))}{\max(M(p_H)(t), M(p_L)(t))} \times 100, \quad (4)$$

231 where $M(p_H)(t)$ and $M(p_L)(t)$ refer to the median of the plant biomass weekly mea-
232 surements collected in the presence of a single isolate with high or low carbon sink
233 strength. A synergy is observed if the % gain in productivity is positive.

234 ***Parametrization from experimental data:*** Our second goal was to recover re-
235 source exchange parameters from the experimental data (such as the carbon sink
236 strength β_i and phosphorus transfer ability α_i of the fungal isolates used), to test
237 whether our theoretical predictions could explain experimental findings (Fig. 5c). To
238 do so, we used a statistical model, which describes the experimental measurements,
239 and a mechanistic-statistical model based on the mathematical model of Eq. (1), which
240 describes plant-fungal growth. The model projects the biomass of the plant $p(t)$ at any
241 time t , in association with AM fungi.

242 During our experiments, we measured the biomass of the plant Y_i every week $t_i \in$
243 $\{1, \dots, I\}$ during $I = 8$ weeks. Depending on the experiments, the plant was associated
244 with only one fungal isolate (either Cuba8 or CC4) or with the two different isolates
245 simultaneously (Cuba8 and CC4). The measurement protocol was identical for all
246 the experiments and the measures are independent from each other for any of the
247 experiments. Thus, conditionally on the plant biomass p and the harvesting time $\mathbf{t} =$
248 $\{t_i | 1 \leq i \leq I\}$, the plant biomass measurements Y_i for $i \in \{1, \dots, I\}$ are assumed to be
249 independent random variables following a Gamma distribution with a mean $p(t_i)$ and
250 a variance proportional to the actual biomass of the plant $p(t_i)$. We obtain:

$$251 \quad Y_i | p, \mathbf{t} \sim \text{Gamma} \left(\frac{p(t_i)}{\sigma}, \sigma \right), \quad (5)$$

252 where $\sigma > 0$ is the proportionality constant that determines the variance of the biomass
 253 measurements and $p(t)$ depends on the parameters of the model given in Table 1.

254 We denote by θ the parameters of our mechanistic-statistical model: $\theta = (\beta_1, \beta_2, \alpha_1,$
 255 $\alpha_2, r_p, p_0, m_{1,0}, m_{2,0}, d, \mu_p, \mu_m, q_{hp}, q_{cm}, \sigma)$. Using the definition of the random variables
 256 Y_i , we can state the likelihood function associated to our mechanistic-statistical model
 257 with unknown parameters θ :

$$258 \quad \mathcal{L}(\theta) = \mathbb{P}(Y|\theta, \mathbf{t}) = \prod_{i=1}^8 \frac{1}{Y_i} \left(\frac{Y_i}{\sigma}\right)^{p(t_i)/\sigma} \Gamma\left(\frac{p(t_i)}{\sigma}\right)^{-1} e^{-Y_i/\sigma}. \quad (6)$$

259 For each of the three experiments (plant with Cuba8, plant with CC4, and plant
 260 with Cuba8 and CC4), we have 8 replicates, which therefore provide us with 24 data
 261 sets. For each data set, we obtain an estimator $\hat{\theta}$ of the parameters θ by minimizing the
 262 function $-\log(\mathcal{L}(\theta))$. The minimization was performed using the Matlab[®] constrained
 263 gradient-based minimization algorithm, `fmincon`, with the constraint:

$$264 \quad \hat{\theta} \in [0, 10]^2 \times [0, 20]^2 \times [0, 0.5] \times [0, 0.3] \times [0, 0.5]^2 \times [0, 2] \times [0.01, 10]^2 \times [1, 6] \times [0.5, 5] \times [0, 0.65].$$

265 In the computation of $-\log(\mathcal{L}(\theta))$, the numerical evaluation of the quantities $(p(t_i))_{i=1,\dots,8}$
 266 was based on the numerical computation of the ODE model using the Matlab (R2020b)
 267 `ode45` solver.

268 Results

269 Differences in carbon sink strength and plant productivity:

270 Overall, our theoretical as well as our experimental results show that a temporal synergy
 271 in productivity can be observed when associated fungi present differences in their carbon
 272 sink strength. Below we discuss model predictions and their comparison to experimental
 273 data.

274 **Theoretical work:** Our theoretical study predicts that plant final size decreases with
 275 increasing carbon sink strength, while plant average growth rate responds nonmono-
 276 tonically to changes in carbon sink strength (β), increasing when carbon sink strength
 277 is low and decreasing when carbon sink strength is high (Fig. 2a). Thus, if the plant is
 278 in simultaneous association with two isolates of AM fungi differing only in their carbon
 279 sink strength, when the plant growth rate is enhanced by the isolate with low carbon
 280 sink strength, and the plant final size is enhanced by the isolate with high carbon sink
 281 strength, the plant will do better when both isolates are present than in association
 282 with either of the isolates alone (Fig. 2c). A fungus with low carbon sink strength (e.g.,
 283 $\beta \sim 1$) will benefit productivity when plant biomass is large, by increasing the plant
 284 final size. A fungus with higher carbon sink strength (e.g., $\beta \sim 1.5$) will maximize the
 285 average plant growth rate when plant biomass is small. If the plant is in simultaneous
 286 association with two isolates of AM fungi differing only in their carbon sink strength,
 287 the plant final size is enhanced by the isolate with low carbon sink strength, and the
 288 plant final size is enhanced by the isolate with high carbon sink strength. Thus, adding
 289 an isolate with high carbon sink strength to an isolate with low carbon sink strength

290 will lead to an increase in the plant growth rate, and a decrease in the plant final size.
291 Adding an isolate with low carbon sink strength to an isolate with high carbon sink
292 strength will decrease the plant growth rate, but increase the plant final size. Addition-
293 ally, for a certain period of time, co-inoculation of two isolates with different carbon
294 sink strength can lead to higher productivity than when the plant is in association with
295 either isolate separately, and drive a temporal synergistic effect in plant growth, as
296 shown in Fig. 2c.

297 Our theoretical predictions suggest that differences in phosphorus supply do not
298 lead to a synergistic effect on plant growth. The plant growth rate and final size both
299 increase with increasing fungi to plant supply (Fig. 2b) and productivity at any point
300 in time is always higher when the plant is associated with the isolate with the higher
301 phosphorus transfer ability (Fig. 2d).

302 From Fig. 3 we see that the synergistic effect is stronger and can be observed for
303 a longer period of time when the difference in carbon sink strength of the two fungal
304 isolates is large. High initial fungal biomass and high intrinsic growth rate of the plant
305 can reduce the intensity and duration of the synergy. Stress factors, quantified in an
306 increase of the maintenance rates μ_p and μ_m , and the phosphorus transfer ability of the
307 fungi (parameter α) do not affect the synergy duration or intensity.

308 From numerical simulations we expect a synergy to be observed also in the presence
309 of multiple isolates differing in their carbon sink strength (Fig. E.1). Our analysis shows
310 that the gain in productivity is affected by the average as well as by the variability
311 in carbon sink strength observed among isolates (see Appendix E). A comprehensive
312 analysis of the synergistic effect in the presence of multiple fungi is complex, and will
313 be left for future work.

314 **Experimental work & Statistical analysis:** Our theoretical model with the in-
315 ferred parameters from our statistical analysis predicts plant biomass over time to follow
316 a logistic growth curve, where a synergy due to differences in the carbon sink strength of
317 the isolates is expected to occur around weeks 4 or 5 (Fig. 2c). Experimental data also
318 show a tendency toward logistic growth, and a synergy at weeks 4, 5 and 6 was indeed
319 detected in most of the experiments (Fig. 4). Although the statistical significance is
320 low, other statistics performed considering the mean of the experimental observations,
321 instead of the median, show a statistically significant synergy at week 4 (see Appendix
322 D). Our analysis suggests therefore that the synergy predicted by our simulations is
323 also present in the experimental data.

324 A synergy at weeks 1 and 2 was also observed. This early synergy was not expected
325 to occur theoretically, as our model is linear at low density, and non-additive effects
326 (such as a synergy) are not expected to occur when the fungal biomass is small. We
327 can therefore not draw any conclusion from the gain in productivity observed in weeks
328 1 and 2. No synergy was detected at week 3, which suggests that the synergy at weeks
329 1 and 2 and the synergy at weeks 4, 5 and 6 might be due to different mechanisms.

330 In Fig. 5a we can see that a temporary synergy in plant growth in Cuba8-CC4
331 mixed inocula was observed in 75% of the experiments conducted. In half of the cases
332 the synergy was observed for at least 3 out of 8 weeks of measurements. When the
333 synergy occurred, the increase in productivity (or % gain in plant biomass) was higher
334 than 33% in half of the cases (Fig. 5b). The medians of the phosphorus and carbon
335 transfer parameters recovered from the statistical analysis are $\alpha_1 = 2.06$ and $\beta_1 = 1.94$

336 for isolate 1 (Cuba8) and $\alpha_2 = 2.39$ and $\beta_2 = 1.64$ for isolate 2 (CC4) (Fig. 5c).
 337 The two fungal isolates Cuba8 and CC4 were selected because of their different carbon
 338 sink strength and potentially similar phosphorus transfer ability (see Appendix A).
 339 The medians of the resource exchange parameters obtained by fitting our model to the
 340 experiments reflect this difference in carbon sink strength, with Cuba8 having a larger
 341 carbon sink strength than CC4 (β_1 (Cuba8) $>$ β_2 (CC4)). However, the median of the
 342 recovered phosphorus transfer ability of Cuba8 is lower than CC4 (α_1 (Cuba8) $<$ α_2
 343 (CC4)), although the statistical test conducted in the preliminary analysis suggest that
 344 the phosphorus transfer ability of the two isolates might be similar on average (Fig. A.1).

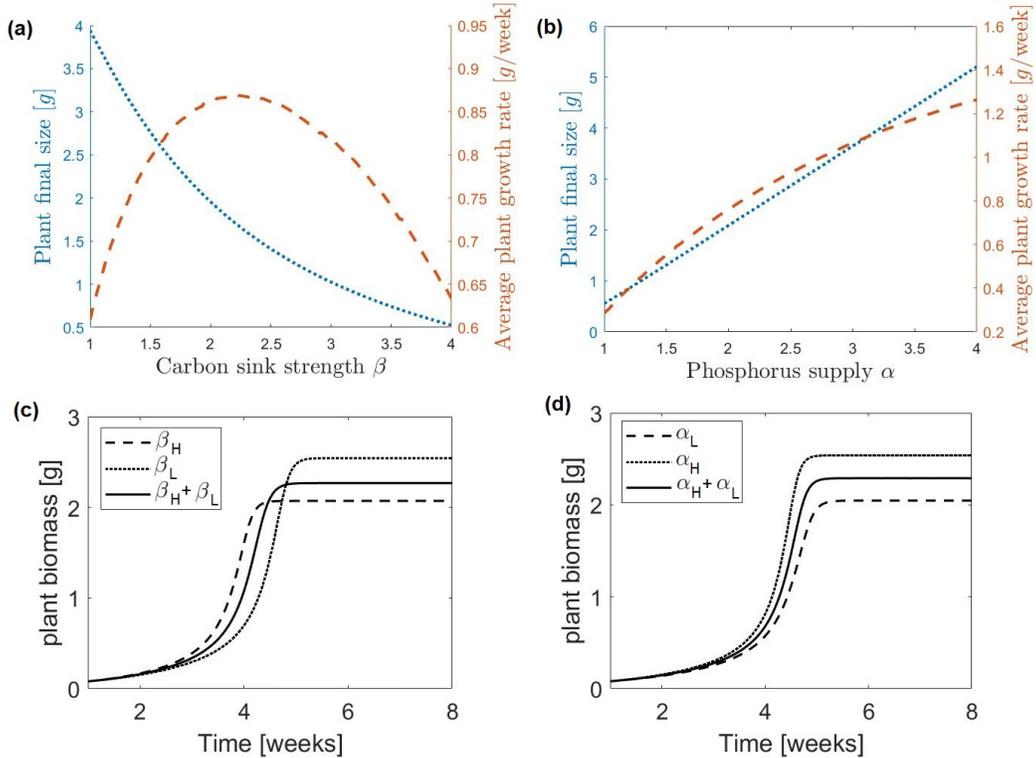


Fig. 2: Projections of plant final size (blue dotted line) and average growth rate (red dashed line) (see Eq. (2)), as a function of (a) carbon sink strength β and (b) phosphorus supply α , when the plant is considered in pairwise association with a single AM fungal isolate. (c) Numerical simulations showing plant biomass over time when the plant is associated with a fungal isolate with high carbon sink strength (β_H , dashed curve), with an isolate with low carbon sink strength (β_L , dotted curve), or with a combination of both isolates (β_{L+H} , solid curve). We assume that the isolates have the same phosphorus exchange ability ($\alpha = (2.06 + 2.39)/2$) and differ only in their carbon sink strength ($\beta_H = 1.94$, $\beta_L = 1.64$). (d) Numerical simulations of plant biomass over time when the plant is associated with a fungal isolate with low phosphorus supply (α_L , dashed curve), with an isolate with high phosphorus supply (α_H , dotted curve), or with a combination of both isolates (α_{H+L}). We assume that the isolates have the same carbon sink strength ($\beta = (1.94 + 1.64)/2$) and differ only in their phosphorus supply ($\alpha_H = 2.39$, $\alpha_L = 2.06$). The chosen parameter values correspond to the parameters recovered from the statistical analysis of the experimental data (Fig. boxplots(c) and Table 1).

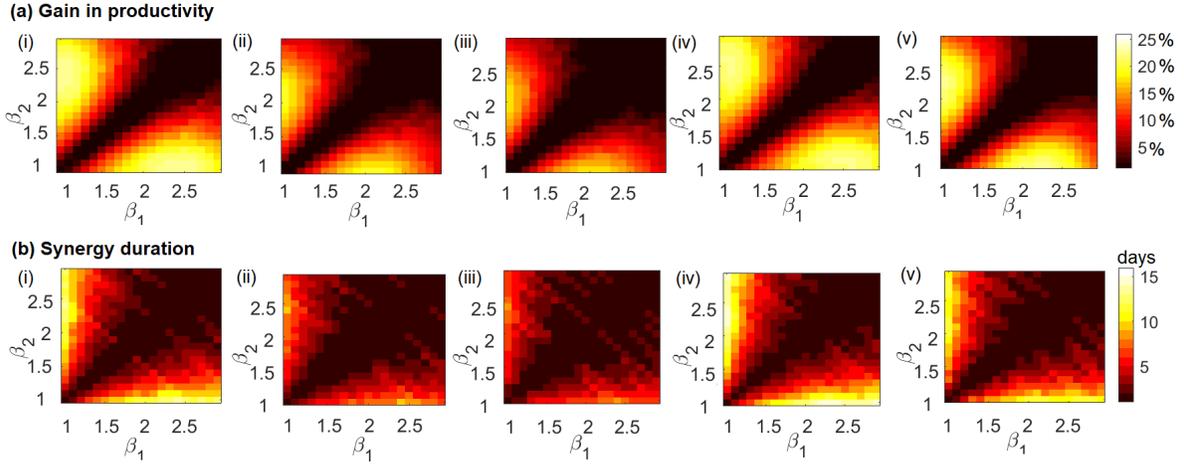


Fig. 3: (a) Maximal gain in productivity (see Eq. (3)) and (b) duration for which a synergy in plant growth is observed (in days) as functions of the carbon sink strength of the two associated fungi (parameters β_1 and β_2 , incremented in steps of size 0.1). (i) Parameters at their default values (see Table 1), (ii) higher plant intrinsic growth rate r_p , (iii) higher initial fungal biomass m_{0i} , (iv) higher plant and fungal maintenance rates μ_p and μ_m , (v) lower phosphorus transfer ability α_i . The darker colors indicate a smaller gain in productivity and a shorter duration of the synergistic effect. Note that figures are symmetric about the $\beta_1 = \beta_2$ diagonal.

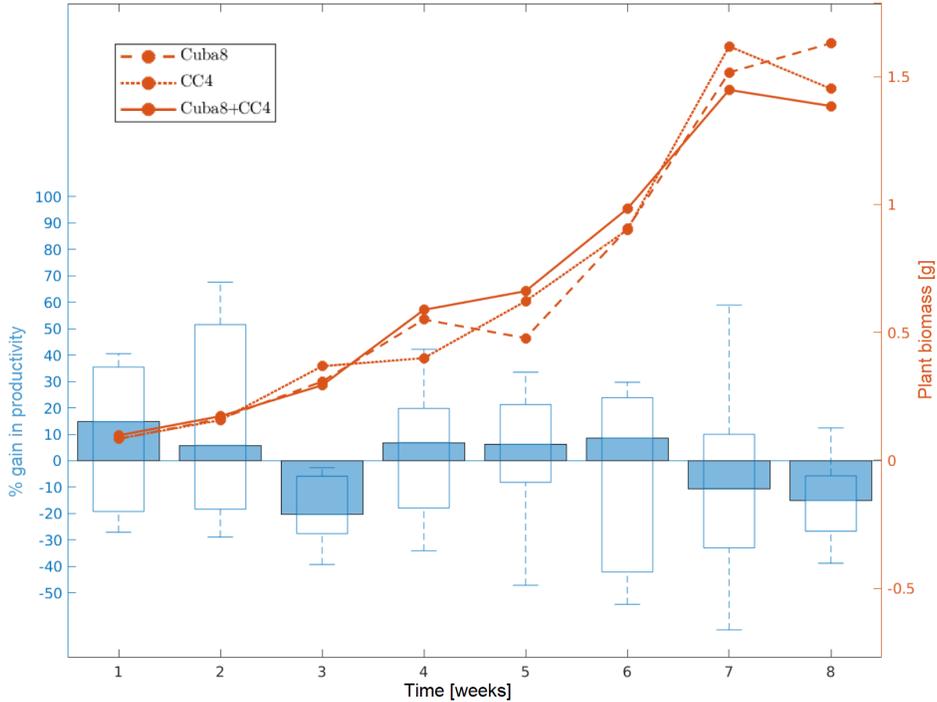


Fig. 4: The red curves represent the median of experimental measurements of plant biomass over 8 weeks when the plant is grown in association with an isolate with high carbon sink strength (Cuba8), with an isolate with low carbon sink strength (CC4), or with a mix of both isolates. The boxplots show the first and third quartile (box edges), the median (bar plots), and the range of the data of the gain in productivity at each sampling date, computed as described in Eq. (4).

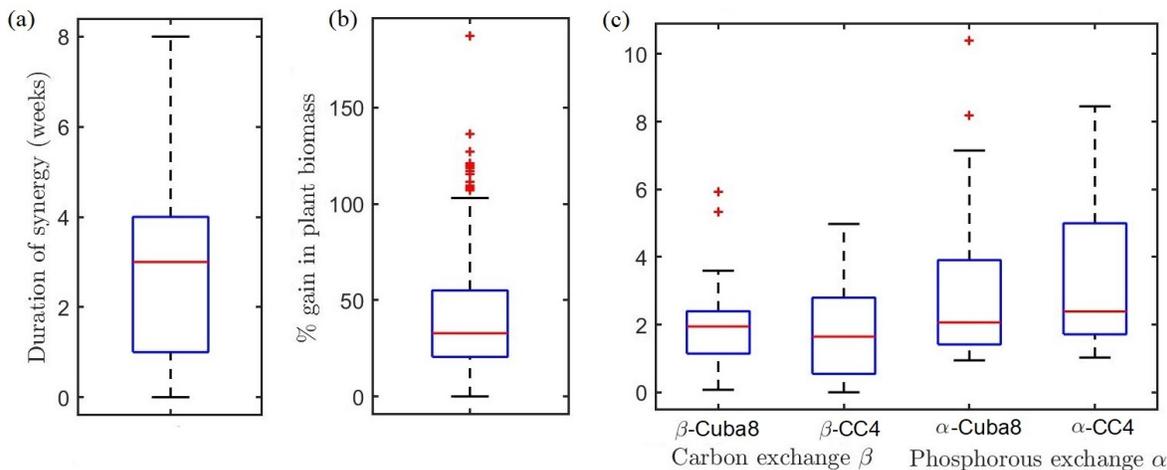


Fig. 5: (a) Average duration of the synergy in plant biomass observed when the plant is associated with two different fungal isolates. (b) Increase in productivity (expressed as % gain in plant biomass) observed when a synergy occurs. (c) Estimation of the α_i parameters (quantifying phosphorus exchange) and the β_i parameters (quantifying carbon exchange) of fungal isolates Cuba8 and CC4, recovered by fitting our model to the experimental data. Boxplots show the first and third quartile (box edges), the median (middle red line), the range of the data (whiskers), and data outliers (red crosses). Other parameters estimated from the statistical analysis are provided in Fig. (C.1).

Discussion

345

346 It has been proposed that complementarity in the reward provided by fungi to their
 347 host plant (i.e., phosphorus transfer) positively correlates with increased plant biomass
 348 (Mensah et al., 2015; Jansa et al., 2005). This hypothesis is supported by studies on
 349 tripartite interactions of legumes with AM fungi and rhizobia, showing that minimal
 350 functional overlap in the rewards provided might not only benefit plant growth, but
 351 also microbe growth, as a fitter host would be able to provide a higher reward in return
 352 (Afkhami et al., 2014; Afkhami and Stinchcombe, 2016) (but see Franklin et al. (2020);
 353 Martignoni et al. (2020a)). Less intuitive, however, is how functional differences in
 354 the reward received from the host (e.g., carbon supply) might ultimately affect host
 355 benefit. It is likely that species providing very distinct rewards to different mutualists
 356 (such as a plant providing pollen to bees and nectar to butterflies) should experience
 357 enhanced benefit (such as pollination from both bees and butterflies) (Afkhami et al.,
 358 2014). However, empirical evidence assessing those potential productivity benefits is
 359 lacking.

360

361 In our work, the amount of plant carbon supplied by the host to each fungal mu-
 362 tualist is determined by an intrinsic characteristic of the fungus (i.e., its carbon sink
 363 strength), and it is independent of the amount of phosphorus that each fungus provides
 364 in return. We show that differences in the rates at which plant carbon is supplied to the
 365 AM fungi can effectively lead to plant productivity benefits, regardless of phosphorus
 366 supply. Fungal isolates with high carbon sink strength are most beneficial when the
 367 total fungal biomass is low. In this case, a higher amount of carbon per biomass intake
 368 by the fungi helps the quick establishment of an abundant fungal community, with a
 369 consequent increase in fungi to plant nutrients supply and thus in the plant growth
 rate. Fungal isolates characterised by low carbon sink strength are not able to support

370 plant growth when fungal biomass is low, but by reducing the carbon cost to the plant
371 they help the plant to reach a higher final size. We expect the synergistic effect to be
372 stronger when fungal biomass is initially low, and when the plant is highly dependent
373 on the AM symbiosis for nutrient provision, as in this case quick establishment of a
374 fungal community is particularly important.

375 As AM fungal inoculants are being used as organic fertilizers worldwide ([Gianinazzi](#)
376 [and Vosátka, 2004](#)), our findings constitute an important contribution for agricultural
377 management ([Ryan and Graham, 2018](#)). We suggest that to maximize plant productiv-
378 ity in depleted soils (i.e., where fungal biomass is low), plants should ideally associate
379 with multiple fungal species or isolates with high and low carbon sink strengths, where
380 fungi with high carbon sink strength contribute to an initial increase in the plant growth
381 rate, and fungi with low carbon sink strength support maximal productivity once plant
382 biomass is large.

383 Synergistic effects in productivity due to the presence of a diverse AM fungal commu-
384 nity have been found in plants subjected to multiple stress factors and in the presence
385 of taxonomically distant AM fungal species ([Yang et al., 2016](#); [Thonar et al., 2014](#);
386 [Crossay et al., 2019](#); [Gosling et al., 2016](#); [Maherali and Klironomos, 2007](#)). While the
387 context of these papers is different from the one we studied, they provide evidence of
388 synergistic effects arising from the presence of multiple fungi. Here we show that syn-
389 ergies can be explained by an interaction mechanism that does not require any stress
390 factors. We propose that, even in the absence of stress, differences in fungal carbon
391 sink strength can drive a synergistic effect in productivity. Indeed, our theoretical work
392 shows that variations in environmental stress did not affect the overall dynamics.

393 Competition between fungi is also known to affect productivity benefits ([Berruti](#)
394 [et al., 2016](#); [Gosling et al., 2016](#); [Koch et al., 2017](#)). In particular, our previous studies
395 have shown that strong competition between fungi can negatively affect productivity
396 benefits in mixed cultures ([Martignoni et al., 2020b](#)). If the observed dynamics were due
397 to competitive interactions alone, we would have expected to see a reduction in plant
398 productivity in the presence of two isolates over the whole time window considered,
399 which was not the case in our experimental results.

400 The similarities found between the experimental results and our simulations suggest
401 that, despite its simplicity, our model can be an efficient tool in predicting, and explain-
402 ing the mechanisms behind, the response of plant productivity to a fungal community.
403 Our results lend support to the benefits of having redundancy in biological systems,
404 even if symbionts are ecologically similar, as both the average plant growth rate as well
405 as final plant biomass can be important determinants of productivity. Whether our
406 findings are valid for all conditions is not clear. For example, it would be worthwhile to
407 consider plant growth when resources are limiting, or in the presence of multiple plants.
408 The model could be expanded to consider the consequences of temporal variation in
409 carbon and phosphorus allocation strategies over time ([Lekberg et al., 2013](#)), the influ-
410 ence of enemies on plant performance ([Morris et al., 2007](#)), potential anastomosis and
411 cytoplasmic exchange between the isolates ([Novais et al., 2017](#)), or competition between
412 fungi ([Martignoni et al., 2020b](#); [Franklin et al., 2014](#); [Hammarlund et al., 2020](#)). Inves-
413 tigation of these factors is an important avenue of future work. We hope that the work
414 presented here will call for further studies to understand how productivity responds to
415 co-inoculation with multiple symbionts differing in their resource exchange capacity.

416 Authors contribution

417 MMM developed the theory, developed and analysed the model, helped to design the
418 experiment, interpreted the results, wrote the manuscript.

419 JG performed the statistical analysis, performed the analysis of the experimental data,
420 interpreted the results, contributed to discussions, contributed to writing of the final
421 manuscript.

422 XZ executed the experiment, performed measurements, collected experimental data,
423 and wrote the experimental part of the manuscript.

424 DR helped with measurement and analysis of the experimental study.

425 VK designed preliminary and main experiment, executed and analysed the preliminary
426 study, helped to analyze the main study, and wrote Appendix A.

427 RCT funded the modelling portion of the project, helped with initial model develop-
428 ment, contributed to discussions, contributed to the writing of the final manuscript.

429 MMH funded the experimental portion of the project, supervised the experimental
430 aspect of the project, designed the experiment, contributed to discussions, helped to
431 analyze the main study, contributed to the writing of the final manuscript.

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631 Appendix A: Preliminary analysis for the selection 632 of AM fungal isolates

633 **Experimental design:** In order to test the carbon and phosphorus sink strength
634 of multiple *Rhizophagus irregularis* isolates we performed a greenhouse experiment in
635 a completely randomized block design (n = 10, total 100 experimental units) where
636 we used a single spore-single plant inoculation approach (as described by [Kokkoris
637 et al. \(2019\)](#)) for nine *R. irregularis* isolates and pre-germinated plantlets of *Linum usi-
638 tatissimum* (Flax, var. Bethune). This experiment was conducted at UBC Okanagan
639 greenhouse from May 2018 to September 2018 and was part of a larger study (of 400
640 experimental units) designed to assess life history variation among *R. irregularis* iso-
641 lates. Variation in plant biomass reflects differences between the carbon sink strength
642 of the isolates while % shoot P reflects differences between the P sink strength of each
643 isolate.

644 **Growing conditions:** AM fungal isolates and host plants were grown in 2L pots
645 containing (v/v=1:1) sterilized medium-fine sand (SAKRETE Play Sand) and vermic-
646 ulate. Plants were grown for approximately three months (to flowering stage). Water
647 and fertilizer were as described for the main experiment. The temperature ranged from
648 26 to 20°C (day/night) with a photoperiod of 18 h per day, of 200 $\mu\text{mol s}^{-1} \text{m}^{-2}$ per
649 μA as measured with a LI-250A light meter (Biosciences).

650 **Harvest and Plant responses:** Upon harvest, roots were separated from shoots
651 and wet weight of each part was obtained. Roots were fragmented and stained for AM
652 fungal colonization status assessment (positive vs negative) and shoots were dried at
653 60°C until weight stability was achieved (approximately after 72h). After recording the
654 dry shoot weight, the tissue was pulverized, homogenized, and was used to calculate
655 the % P in the flax shoot using the color development method described in the main
656 manuscript ([Murphy and Riley, 1962](#)).

657 **Statistical analysis:** To evaluate the carbon and phosphorus sink strength of each
658 isolate we first calculated the mycorrhizal response (MR) for the total plant biomass and
659 % shoot P. MR describes any benefit or detriment for a specific plant trait (e.g. biomass
660 and phosphorus in our case) as a result of AM fungal inoculation ([Baon et al., 1993](#);
661 [Sawers et al., 2008](#)). The MR was calculated as: $\text{MR} = \ln (X/Y)$ where X= response
662 of mycorrhizal plants and Y= mean response of non-mycorrhizal (control) plants.

663 We used a mixed effect linear model to examine the variation between the isolates
664 with AM fungal isolate identity as a fixed factor and block as a random factor followed
665 by pairwise comparison (emmeans package – [Lenth et al. \(2020\)](#)) (Fig. A.1). The
666 package uses a p-value adjustment method by default, to ensure that the confidence
667 interval remains large enough when performing multiple comparisons. The analysis
668 was performed in R studio (Version 1.0.136–2009–2016 RStudio, Inc.).

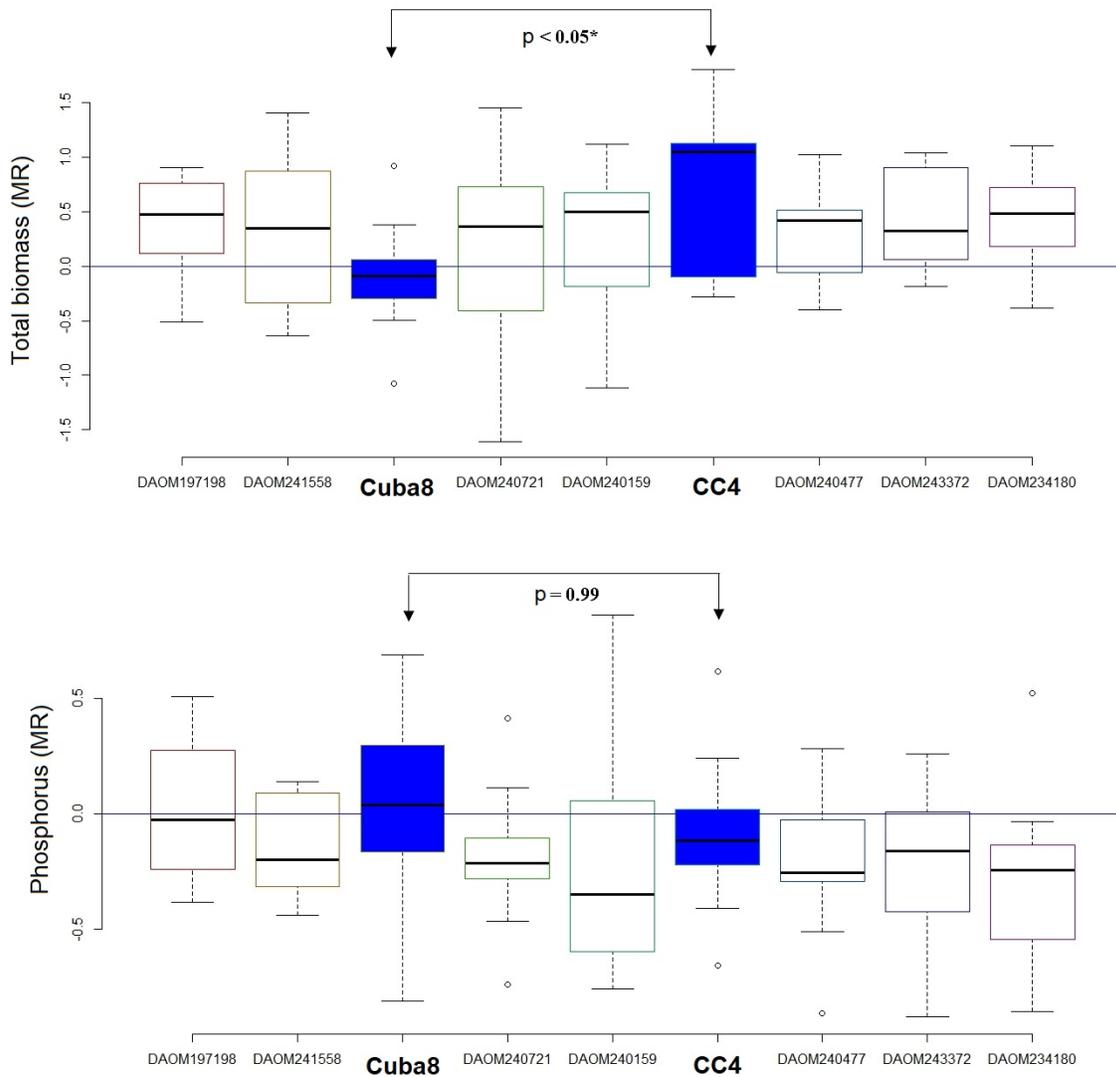


Fig. A.1: *Linum usitatissimum* (Flax) mycorrhizal response of (A) total biomass and (B) % shoot P, for different fungal isolates (x-axis). Cuba8 has a higher carbon sink strength compared to CC4 since the plant biomass was significantly lower ($p < 0.05$) but the two isolates are not different in their P sink strength ($p = 0.99$). Blue line indicates the mean value of the non-mycorrhizal plants. Boxplots show the third and first quartile (box edges), the median (middle black line), the range of the data (whiskers) and data outliers (black open circles).

Appendix B: Inoculation method



Fig. B.1: (1a) AM fungal inoculation method. ‘Inoculation Discs’ were created by mixing sterile quartz sand with 1% gellan gum - H_2O solution. We then inoculated each disc with 30 Cuba8 spores or/and 10 CC4 spores to ensure a minimum of 6 viable spores per pot. (1b) After filling each pot halfway with the growing media (sand-vermiculite) an inoculation disc containing the spores) was embedded in the middle. (1c) To ensure the physical interaction of plant-AM fungi, we placed a straw (5mm diameter and 5cm length) filled with wet sand on the top of the disc and completed the volume of the pot with our substrate. (1d) The germinated seed was placed into the straw, positioning it directly above the AM fungal inoculum disc.

Appendix C: Inferred parameters

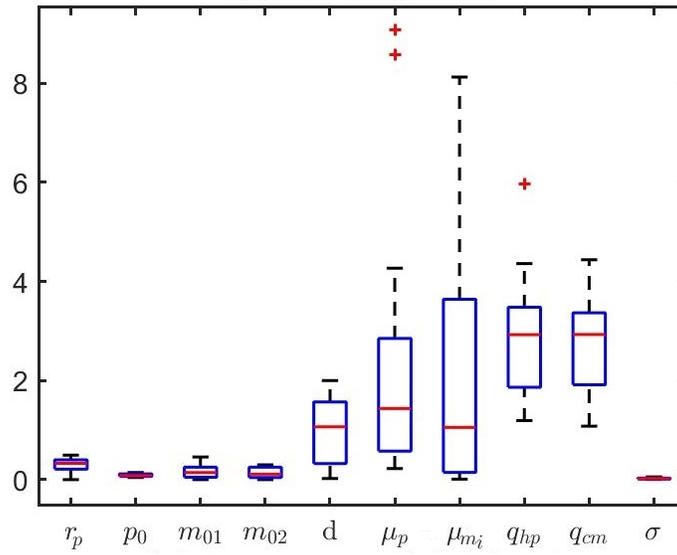


Fig. C.1: Parameters recovered from fitting our model to the experimental data. Median values: $r_0 = 0.33$, $p_0 = 0.08$, $m_{01} = 0.14$, $m_{02} = 0.11$, $d = 1.07$, $\mu_p = 1.44$, $\mu_m = 1.05$, $q_{hp} = 2.93$, $q_{cm} = 2.93$, $\sigma = 0.02$. Parameters q_{cp} and q_{hm} have been set equal to 1.

Appendix D: Statistical analysis based on the mean average of the experimental observations

In this appendix we present the analysis of the experimental data resulting when considering the mean, instead of the median, of the experimental observations. The mean of the synergistic effect is computed as

$$\% \text{ gain in productivity}(w) = \frac{p_{H+L}(t) - \max(\text{mean}(p_H)(t), \text{mean}(p_L)(t))}{\max(\text{mean}(p_H)(t), \text{mean}(p_L)(t))} \times 100, \quad (7)$$

where p_{H+L} represent the weekly measurements of plant biomass in the presence of both isolates, $\text{mean}(p_H)(t)$ and $\text{mean}(p_L)(t)$ refer to the mean of the plant biomass weekly measurements collected in the presence of a single isolate with high or low carbon sink strength. A synergy is observed if the % gain in productivity is positive. Note that Eq. (7) corresponds to Eq. (4) where the median of the experimental measurements has been substituted with the mean.

The confident intervals of the synergistic effect in Fig. 4 are obtained by computing

$$\% \text{ gain in productivity}'(w) = \frac{p_{H+L} - \max(p_H(w), p_L(w))}{\max(p_H(w), p_L(w))} \times 100. \quad (8)$$

We make this choice for two reasons: First, the confident intervals obtained when considering Eq. (7) are large because of the small amount of data (8 data points per weeks), while in Eq. (8) we do have more data available ($8 \times 64 = 512$ data points per weeks). Secondly, the mean of the gain in productivity obtained by using Eqs. (7) and (8) are similar, as the variance of $\max(p_H, p_L)$ is small. We can write:

$$\text{mean}(\% \text{ gain in productivity}'(w)) = \left(\text{mean} \left[\frac{p_{H+L}(w)}{\max(p_H(w), p_L(w))} \right] - 1 \right) \times 100, \quad (9)$$

and we can show that, at each week,

$$\frac{\text{mean}(p_{H+L})}{\text{mean}(\max(p_H, p_L))} \leq \frac{\text{mean}(p_{H+L})}{\max(\text{mean}(p_L), \text{mean}(p_H))}. \quad (10)$$

From the Jensen's inequality we know that, at each week,

$$\frac{\text{mean}(p_{H+L})}{\text{mean}(\max(p_H, p_L))} \leq \text{mean} \left(\frac{p_{H+L}}{\max(p_H, p_L)} \right). \quad (11)$$

Given that the variance of $\max(p_H, p_L)$ is small, we can assume that $\max(p_H, p_L)$ follows a normal distribution. We obtain:

$$\text{mean} \left(\frac{p_{H+L}}{\max(p_H, p_L)} \right) \sim \frac{\text{mean}(p_{H+L})}{\text{mean}(\max(p_H, p_L))} \pm \frac{\text{Var}(\max(p_H, p_L))}{\text{mean}(\max(p_H, p_L))^2} \times \text{mean}(p_{H+L}). \quad (12)$$

As $\text{Var}(\max(p_H, p_L)) \ll 1$, we obtain that at each week:

$$\begin{aligned} \text{mean}(\% \text{ gain in productivity}') &= \\ & \frac{\text{mean}(p_{H+L})}{\text{mean}(\max(p_H, p_L))} - 1 + \text{Var} \left(\frac{\max(p_H, p_L)}{\text{mean}(\max(p_H, p_L))^2} \right) \times \text{mean}(p_{H+L}) \\ & \leq \text{mean}(\% \text{ gain in productivity}). \end{aligned} \quad (13)$$

699 So if $mean(\% \text{ gain in productivity}')$ is significantly larger than zero, so does $mean(\% \text{ gain}$
 700 $\text{ in productivity})$ and the confidence intervals of $(\% \text{ gain in productivity}')$ can be used
 701 to describe the confidence intervals of $(\% \text{ gain in productivity})$.

702 In Fig. D.1 we see that the mean is significantly above zero in weeks 1, 2, and 4,
 703 and significantly below zero in weeks 6, 7 and 8. The gain in productivity at week 3
 704 is not significantly different than zero, while when considering the median (Fig. 4) we
 705 obtained a significant loss in productivity at week 3.

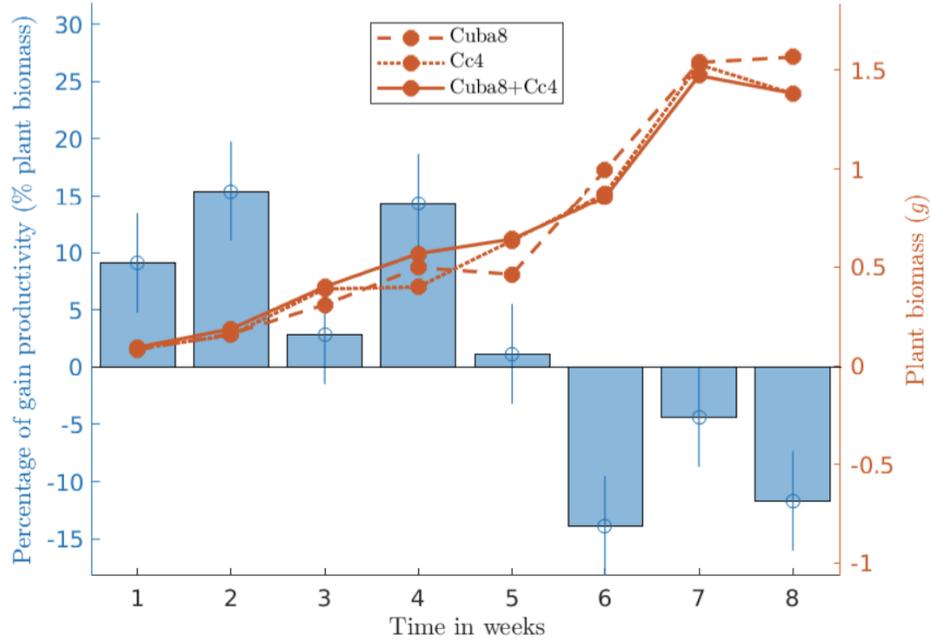


Fig. D.1: Equivalent of Fig. 4 when considering the mean instead of the median of the experimental observations. The red curves represent the mean of experimental measurements of plant biomass over 8 weeks when the plant is grown in association with an isolate with high carbon sink strength (Cuba8), with an isolate with low carbon sink strength (CC4), or with a mix of both isolates. The boxplots show the first and third quartile (box edges), the median (bar plots), and the range of the data of the gain in productivity at each sampling date, computed as described in Eqs. (7) and (8).

Appendix E: Multiple fungal partners

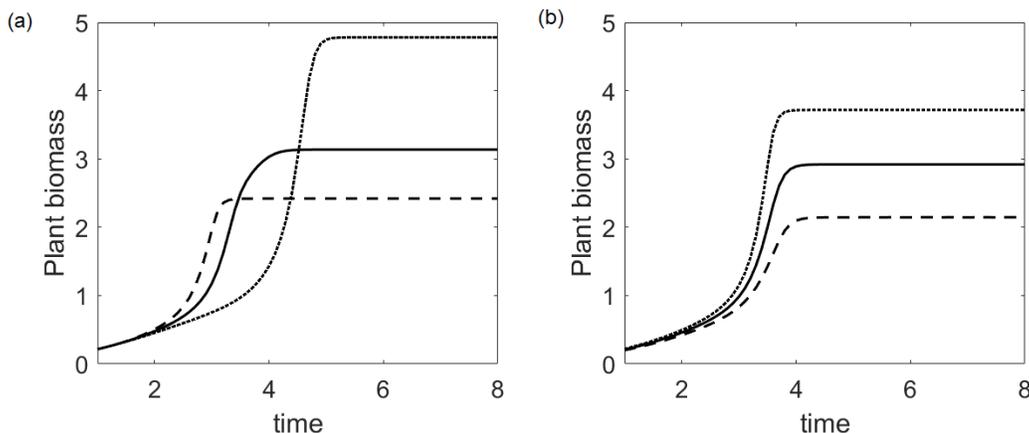


Fig. E.1: Plant growth over time when the plant is associated with five AM fungal partners (i.e., $i = 1, \dots, 5$) with (a) different carbon sink strength and identical phosphorus supply (with $\alpha_i = 2.2$ for all i) or (b) different phosphorus supply and identical carbon sink strength (with $\beta_i = 1.4$ for all i). (a) The solid line represents plant growth when the fungal partners have mixed carbon sink strength ($\beta_1 = 1.0, \beta_2 = 1.2, \beta_3 = 1.4, \beta_4 = 1.6, \beta_5 = 1.8$). The dotted curve represents plant growth when fungal partners have low carbon sink strength ($\beta_i = 1.0$ for all i), and the dashed curve represents plant growth when fungal partners have high carbon sink strength ($\beta_i = 1.8$ for all i). Similarly, in (b) the solid line represents plant growth when the fungal partners provide mixed phosphorus supply ($\alpha_1 = 1.6, \alpha_2 = 1.8, \alpha_3 = 2.0, \alpha_4 = 2.2, \alpha_5 = 2.4$). The dotted curve represents plant growth when fungal partners provide high phosphorus supply ($\alpha_i = 2.4$ for all i), and the dashed curve represents plant growth when fungal partners provide low phosphorus supply ($\alpha_i = 1.6$ for all i).

707 **Analysis of plant final size:** We assume that all fungi provide the same amount of
 708 phosphorus to the plant (i.e., $\alpha_i = \alpha$) but differ in their access to carbon (i.e., β_i differ,
 709 where $\beta = (\beta_1, \dots, \beta_N)$). We want to understand how the plant final size p^* depends
 710 on the carbon sink strength of all mutualists. From Martignoni et al. (2020a) we know
 711 that

$$p^*(\beta) = \frac{q_{hm}\alpha}{q_{cm}\beta} P^*(\beta) - d$$

$$\text{with } P^*(\beta) = \frac{(1+Q)\sum_{i=1}^N \frac{1}{\eta_{M_i}} + \sqrt{(1+Q)^2(\sum_{i=1}^N \frac{1}{\eta_{M_i}})^2 - 4Q\sum_{i=1}^N \frac{\bar{\beta}}{\beta_i\eta_{M_i}}(\sum_{i=1}^N \frac{\beta_i}{\beta\eta_{M_i}} + \eta_{P_i})}}{2\left(\sum_{i=1}^N \frac{\beta_i}{\beta\eta_{M_i}} + \eta_{P_i}\right)}, \quad (14)$$

712

713 where the non-dimensionalized variables P^* and M_i are defined as $P^* = \frac{q_{cm}\beta}{q_{hm}\alpha} p^*$ and

714 $M_i = \frac{q_{cp}\beta_i}{q_{hm}\alpha} m_i$ (Martignoni et al., 2020a). Non-dimensionalized parameters Q , η_{M_i} and

715 η_P are defined as $Q = \frac{q_{cm}q_{hp}}{q_{cp}q_{hm}}$, $\eta_{P_i} = \frac{\mu_p}{q_{cm}\beta_i}$, and $\eta_{M_i} = \frac{\mu_m}{q_{cp}\beta_i}$ (Martignoni et al., 2020a).

716

To go further, we need to come back to the original variable for η_{M_i} and η_{P_i} . We

717 define $\bar{\beta} = \text{mean}(\boldsymbol{\beta})$ and we obtain

$$\begin{aligned}
P^*(\boldsymbol{\beta}) &= \frac{(1+Q)\frac{Nq_{cm}\bar{\beta}}{\mu_m} + \sqrt{(1+Q)^2\left(\frac{Nq_{cm}\bar{\beta}}{\mu_m}\right)^2 - 4Q\frac{Nq_{cm}\bar{\beta}}{\mu_m}\left(\sum_{i=1}^N \frac{q_{cm}\beta_i^2}{\bar{\beta}\mu_m} + \frac{\mu_p}{q_{cm}\bar{\beta}}\right)}}{2\left(\sum_{i=1}^N \frac{q_{cm}\beta_i^2}{\bar{\beta}\mu_m} + \frac{\mu_p}{q_{cm}\bar{\beta}}\right)} \\
&= \frac{(1+Q)\bar{\beta} + \bar{\beta}\sqrt{(1+Q)^2 - 4Q\left(\frac{1}{N}\sum_{i=1}^N \frac{\beta_i^2}{\bar{\beta}^2} + \frac{\mu_p\mu_m}{q_{cm}^2\bar{\beta}^2 N}\right)}}{2\left(\frac{1}{N}\sum_{i=1}^N \frac{\beta_i^2}{\bar{\beta}^2} + \frac{\mu_p\mu_m}{q_{cm}^2\bar{\beta}^2 N}\right)}.
\end{aligned}
\tag{15}$$

718

719 We can see that

$$\frac{1}{N}\sum_{i=1}^N \frac{\beta_i^2}{\bar{\beta}^2} = 1 + \frac{\text{Var}(\boldsymbol{\beta})}{\bar{\beta}^2}.
\tag{16}$$

720

721 Thus we obtain

$$P^*(\boldsymbol{\beta}) = \bar{\beta} \frac{(1+Q) + \sqrt{(1+Q)^2 - 4Q\left(1 + \frac{\mu_p\mu_m}{q_{cm}^2\bar{\beta}^2 N} + \frac{\text{Var}(\boldsymbol{\beta})}{\bar{\beta}^2}\right)}}{2\left(1 + \frac{\mu_p\mu_m}{q_{cm}^2\bar{\beta}^2 N} + \frac{\text{Var}(\boldsymbol{\beta})}{\bar{\beta}^2}\right)},
\tag{17}$$

722

723 and

$$p^*(\boldsymbol{\beta}) = \frac{q_{hm}\alpha}{q_{cm}\bar{\beta}}P^*(\boldsymbol{\beta}) - d.
\tag{18}$$

724

725 Eq. (18) shows that $p^*(\boldsymbol{\beta})$ is decreasing with respect to the mean $\bar{\beta}$ of $\boldsymbol{\beta}$ and decreases
726 with respect to the variance $\text{Var}(\boldsymbol{\beta})$.