

1 **Bottom-up effects of host-plant species diversity and top-down effects of ants interactively**
2 **increase plant performance**

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4 Running head: Bottom-up vs. top-down effects
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28 Word count main text (I + MM + R + D): 4,397

29 Abstract: 154

30 Introduction: 753

31 Material and Methods: 1,598

32 Results: 1,024

33 Discussion: 1,022

34 Acknowledgments: 132

35 References: 1,330
36
37
38
39

40 Number of total pages: 25

41 Number of references: 50

42 Number of Tables: 2

43 Number of Figures: 4
44

45 **ABSTRACT**

46 While plant diversity is well known to increase primary productivity, whether these bottom-up
47 effects are enhanced by reciprocal top-down effects from the third trophic level is unknown. We
48 studied whether pine tree species diversity, aphid-tending ants and their interaction determined
49 plant performance and arthropod community structure. Plant diversity had a positive effect on
50 aphids, but only in presence of mutualistic ants, leading to 3-fold greater number of both groups
51 in the tri-specific cultures than in monocultures. Plant diversity increased ant abundance not
52 only by increasing aphid number, but also by increasing ant recruitment per aphid. The positive
53 effect of diversity on ants in turn cascaded down to increase plant performance; diversity
54 increased plant growth (but not biomass), and this effect was stronger in the presence of ants.
55 Consequently, bottom-up effects of diversity within the same genus and guild of plants and top-
56 down effects from the third trophic level (predatory ants) interactively increased plant
57 performance.

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59 **Keywords:** *arthropod community structure, bottom-up effects, plant diversity, plant growth,*
60 *top-down effects*

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70 1. INTRODUCTION

71 The consequences of plant species diversity on ecosystem function and on the structure of
72 associated communities of consumers have been increasingly recognized [1-7]. There is
73 growing evidence that a greater diversity of plant species may stabilize the multitrophic
74 arthropod community interacting with plants [3, 4, 7], increase net primary production [5, 8],
75 and even provide resistance to biological invasions [9]. In particular, a greater diversity of plant
76 species was found to positively affect plant growth and the abundance and diversity of
77 associated arthropods in grasses, legumes, forbs and other herbaceous plants [3, 4, 7]. In this
78 sense, two non-exclusive hypotheses have been proposed to explain ecological consequences of
79 host-plant species diversity on the multi-trophic communities that plants support. First, the
80 resource specialization hypothesis argues that increasing plant species diversity will provide a
81 greater diversity of resources and, therefore, would attract greater diversity of herbivore species
82 [10, 11]. Alternatively, the more individuals hypothesis postulates that a high diversity of plant
83 species increases the productivity of plant populations and, consequently, would increase the
84 abundance of consumers and the probability of observing higher species diversity at the
85 community level [12].

86 Recent studies have focused more mechanistically on how plant-neighbour interactions
87 in plots of different plant diversity may affect the associated communities [3, 4, 7, 13-16]. A few
88 studies have shown that the bottom-up effects of plant diversity cascade up to higher trophic
89 levels, including the third trophic level (e.g. [3, 16]). Particularly interesting, Haddad *et al.* [3]
90 observed marked increases in the ratio of predator-to-herbivore abundance associated with
91 increasing plant diversity. However, what remains unclear is the generality of such findings, and
92 whether these effects may affect plant fitness, such that the bottom-up effects of plant diversity
93 interact with the top-down effects from the third trophic level.

94 Ants, by acting as predators, mutualists or ecosystem engineers, have large ecological
95 effects and can play an important role in determining the structure and function of entire
96 communities (e.g. [17-19]). An interesting case is the food-for-protection mutualistic interaction
97 established between ants and honeydew-producing hemipteran insects such as aphids. In these
98 interactions, ants “tend” aphids, feeding upon their sugary honeydew exudates in exchange for
99 protection from predators and parasites (reviewed by Stadler & Dixon [20]). Ant-aphid
100 interactions have been proposed to be keystone interactions [18], because aphid-attracted ants
101 can have marked community-wide effects. Specifically, the presence/absence of ants affects the
102 abundance of aphids, but also population dynamics of other arthropods in the community, such
103 as aphid predators and other untended herbivores [18, 21, 22], which may in turn affect plant
104 growth and fitness [23, 24]. In addition to this, because aphid-tending ants may contribute to
105 defend plants against their enemies, plants, in presence of ants, would benefit from reducing the
106 allocation of resources to expensive chemical defences, leaving them available for other vital
107 strategies [25]. As a consequence, the factors that mediate ant-aphid interactions can have broad
108 effects themselves. Although there are several studies showing that ant-aphid interactions vary
109 across plant genotypes [26-29], the effects of plant diversity (both intra- and inter-specific) on
110 this mutualistic interaction remain unstudied.

111 The aim of this study was to test for the effects of host-plant species diversity (within the
112 genus *Pinus*), mutualistic ants and the interaction between these factors on plant
113 performance/productivity/defences and the structure of associated aboveground arthropod
114 communities. To test for these effects, we performed a factorial field experiment where we
115 manipulated host-plant species diversity (three levels: monocultures, dicultures and tricultures)
116 and the presence of mutualistic ants (two levels: exclusion and presence). We measured plant
117 growth, conducted arthropod counts and quantified the defensive and nutritional status of pine
118 seedlings. Pine seedlings are especially vulnerable to herbivore attack, and dynamics at this

119 stage affect survival and competitive dynamics among species regenerating in forest gaps that
120 may have long-term effects on forest structure. We specifically addressed four questions: (i)
121 what are the bottom-up effects of pine species diversity on plant growth and arthropod
122 communities, (ii) what are the top-down effects of ants on plant growth and arthropod
123 communities, (iii) what is the relative strength of these two effects?, and (iv) do they interact?
124 We hypothesized that high plant species diversity should lead to increased plant productivity,
125 which in turn benefits aphids directly and ants indirectly. We further hypothesized that ants
126 could provide a positive effect on plant growth, as their effect facilitating aphid population could
127 be outweighed by reduced non-aphid herbivores in ant tended plants [18]. This study thus
128 provides the first test for multi-trophic interactions between plant diversity and predator effects.

129

130 **2. MATERIALS AND METHODS**

131 **(a) *Study area and species***

132 We used three focal species belonging to the *Pinus* clade which are broadly planted worldwide,
133 particularly in the study area, the NW of the Iberian Peninsula: Maritime pine (*Pinus pinaster*
134 Ait.), Monterrey pine (*P. radiata* D. Don.) and Scots pine (*P. sylvestris* L.). These pine species
135 coexist in mixed forests found throughout the study area, with overlapping distributions ranging
136 from altitudes of 400 to 800 m. Six month-old seedlings were provided by a local nursery
137 (Norfor Nursery Ltd., Pontevedra, viverofigueirido@norfor.es).

138 The experimental plantation was established at a small agricultural plot located in
139 Pontevedra (Galicia, NW Spain, 42.26° N 8.39° W). The climate in this area is temperate humid
140 Atlantic, with annual precipitation of about 1,620 mm and mean annual temperature of 15.4°C.
141 Previous inspections of the study site confirmed the presence of ant-tended aphids (mainly
142 *Cinara* spp.) and aphid-tending ants (*Lasius grandis*) on the pine trees surrounding the plot.

143

144 **(b) *Experimental design***

145 In early spring 2011 we planted six-month-old pine seedlings manipulating the plant diversity
146 by creating three assemblages of different pine species diversity (see Fig. 1): (i) monocultures of
147 the three pine species, (ii) all possible dicultures with those three species (three different
148 combinations), and (iii) tricultures. The triculture treatment consisted of three different
149 combinations, one with the three species studied in mono- and dicultures, and two additional
150 combinations including a fourth pine species (*P. pinea*, also native from the study area) not
151 included in mono- or dicultures (Figure 1). Each experimental unit (hereafter “combination”)
152 consisted of six plants in two parallel rows of three plants each (Fig. 1). Neighbouring plants
153 were separated by approximately 10 cm, and combinations were spaced at least 1 m apart, with
154 the positioning of plants within the combination being randomized. The experiment followed a
155 randomized split-plot design replicated in four blocks, with ant treatment (two levels: presence
156 or absence) as the whole plot factor and species diversity (mono-, di- and tricultures) as the split
157 factor, with three different combinations of each diversity treatment for a total of nine
158 combinations per block. All blocks were separated by at least 3 m. In total, there were 432 pine
159 seedlings, corresponding to 4 blocks \times 2 ant treatments \times 3 species diversity treatments \times 3
160 combinations for each diversity treatment \times 6 plants in each combination.

161 On April 18, two days after plantation, we measured stem height of all the plants and we
162 carefully placed a piece of tape around the shoot (2 cm wide) of each plant. Ants were excluded
163 from half of the plants by coating the outside surface of the band with a sticky paste
164 (Tanglefoot®, Tanglefoot Company, Michigan, USA). Control plants, with tape but without
165 sticky paste, allowed ant access.

166

167 **(c) *Sampling, plant measurements and chemical analysis***

168 We recorded the number of arthropods on each tree on August 25, when aphid populations peak
169 in this area (X. Moreira, personal observation). Arthropods were identified to species or to the
170 taxonomic level necessary to determine their trophic level by consulting relevant literature and
171 with the help of taxonomist Alberto Gayoso (entomologist from Xunta de Galicia). Arthropods
172 were classified as: ant-tended aphids, ants, untended (non-aphid) herbivores or aphid predators.
173 Some aphid parasitoids were also found, but in very low numbers. Ant-tended aphids consisted
174 either of *Cinara maritima* (95%) or *C. pini* (5%). These species of aphids forms small colonies
175 on terminal shoot and branches of young and mature pine trees (X. Moreira, personal
176 observation). Ants always consisted of aphid-tending *Lasius grandis* (Hymenoptera:
177 Formicidae) all from the same ant nest. Non-aphid herbivores consisted of phloem-feeders
178 [*Pissodes castaneus* (Coleoptera: Curculionidae)] and sap feeders [*Stictocephala bisonia*
179 (Hemiptera: Membracidae), *Leucaspis pini* (Hemiptera: Coccidae) and *Pentatoma rufipes*
180 (Hemiptera: Pentatomidae)]. Aphid predators consisted of wasps [*Dolichovespula media*
181 (Hymenoptera: Vespidae)], ladybirds [*Adalia bipunctata* (Coleoptera: Coccinellidae) and
182 *Coccinella septempunctata* (Coleoptera: Coccinellidae)], one species of assassin bug
183 (Hemiptera: Reduviidae) and spiders [Araneae, various families].

184 On August 26, plant height was measured and all pine seedlings were harvested,
185 transported to the lab in ice coolers and immediately sampled for aboveground biomass
186 determination and further chemical analyses. One fresh 5 cm-long piece of the terminal shoot of
187 each plant was sampled, weighed, immediately frozen and preserved at -80°C for analysis of
188 non-volatile resin and antioxidant activity. Another subsample of terminal shoot was
189 immediately weighed, oven-dried (45°C to constant weight) and manually ground in a mortar
190 with liquid nitrogen for analyses of phenolic compounds, nitrogen and non-structural
191 carbohydrates.

192 Concentration of non-volatile resin in the stem was estimated gravimetrically as
193 described in Sampedro *et al.* [30] and Moreira *et al.* [31] (see Appendix 1 in supplementary
194 material), and expressed as mg of non-volatile resin * g⁻¹ stem on a dry weight basis (d.w.).
195 Total phenolics in the stem were estimated by the Folin-Ciocalteu assay as described by
196 Sampedro *et al.* [30] and Moreira *et al.* [31] (see Appendix 1 in supplementary material), and
197 expressed as mg of tannic acid equivalent * g⁻¹ d.w. stem. These variables have been proved
198 useful for identifying differences in resistance in previous studies [32, 33]. The antioxidant
199 capacity in aqueous extracts of stem tissue was measured by a modification of the method
200 described by Noguera *et al.* [34] and Erel [35] (see Appendix 1 in supplementary material), and
201 expressed as mg of Trolox equivalent * g⁻¹ d.w. stem. The concentrations of soluble sugars and
202 starch in the stem were determined colorimetrically by the anthrone method [30, 36] (see
203 Appendix 1 in supplementary material) using glucose and potato starch, respectively, as
204 standards and expressed as mg * g⁻¹ d.w. Total N was determined with a CN-2000 macro
205 elemental analyzer (LECO Corporation, St. Joseph, MI, USA) at the central facilities of
206 Universidade de Vigo, Spain (<http://webs.uvigo.es/cactiweb/>), and expressed in mg * g⁻¹ d.w. of
207 tissue. To reduce the analytical effort to reasonable levels, nutrient concentration and
208 antioxidant activity in the stem were analysed in a subsample of 48 selected pine trees.
209 Specifically, we only analysed one plant per combination in the three monocultures (*P.*
210 *pinaster*, *P. radiata* and *P. sylvestris*) one plant of each species in the triculture including those
211 three pine species.

212

213 **(d) Statistical analyses**

214 Data analysis was performed with mixed linear models for plant growth and defensive and
215 nutritional status traits and generalized linear mixed models for arthropod abundance, using the
216 Mixed and Glimmix procedures, respectively (SAS 9.2 System, SAS, Cary, NC). The main

217 effects of Ants (A), Diversity (D), and the $A \times D$ interaction were treated as fixed factors. The
218 effect of the different Combinations within each diversity treatment (C) and the $A \times C$
219 interaction were also included as fixed factors nested within the diversity treatments, in order to
220 account for the variation between combinations and the effect of the species identity within each
221 combination. The effects of Block (B) and $A \times B$ interaction (i.e. the whole plots) were
222 considered random factors in order to analyze the main effects of the split-plot design with the
223 appropriate error terms [37]. To avoid confounding effects associated with size differences
224 between pine species final height was included as covariate in the analysis of arthropod
225 abundance, defences and carbohydrates. Initial height was included as a covariate for the
226 analysis of plant growth. Pearson correlations were used to evaluate the relationships among all
227 traits separately in control and ant-excluded pine trees. Data are shown as mean \pm standard error.

228 Diverse plots may have greater performance or arthropod abundance because of the
229 increased probability of including species with distinct performance or communities (additive or
230 sampling effects; [38, 39]). Alternatively, plant species diversity may modify plant performance
231 and the structure of arthropod community via positive or negative interactions among
232 neighboring plant species (non-additive effects; [38, 39]). We structured our models not only to
233 test for overall effects of diversity (and ant \times diversity interactions), but also to determine
234 whether such effects occurred through non-additive dynamics. Data were first analyzed as plot
235 means (i.e. the mean of six plants within a combination), including the combinations of each
236 diversity treatment (nested within the diversity treatment) in the statistical model. By accounting
237 for variation among combinations within diversity treatments, a significant diversity effect
238 indicates such effects are independent of the contribution coming from any single species
239 combinations and thus that such effects are non-additive (i.e. synergistic or antagonistic effect
240 among species). In addition, we also analyzed data for each species separately according to the
241 same statistical models (see results in electronic Supplementary Material). In these tests,

242 significant diversity effects were also indicative of non-additive dynamics, as they showed
243 differences based upon the diversity environment within which that single species occurs. Using
244 these two approaches, we tested for diversity effects (and ant-by-diversity interactions) with
245 three focal pine species (*P. pinaster*, *P. radiata* and *P. sylvestris*) in mono- di- and tricultures.
246 Because all combinations of tricultures would by necessity be uniform in species combination,
247 we incorporated variation in triculture species composition by adding two combinations with
248 one additional species, *P. pinea* (see above). Although we lack mono- and diculture treatments
249 of *P. pinea*, excluding the two triculture treatments that contained *P. pinea* trees from our
250 analyses did not alter the direction or significance of any of our results (results not shown).

251 After determining the spatial position (x, y) of each plant, we performed an analysis of
252 the semivariance of the residuals of the mixed models for all the studied variables to check
253 whether spatial heterogeneity in the natural distribution of ants, aphids or soil properties could
254 be affecting our results [40]. We observed no significant deviation from random spatial
255 distribution (see semivariograms in the Figure S1 of the Supplementary Material).

256

257 **3. RESULTS**

258 **(a) Consequences of host-plant species diversity and ants on pine performance**

259 Host-plant species diversity significantly affected pine primary growth (Table 1). Specifically,
260 final height was 12% and 16% greater in pine dicultures and tricultures, respectively, than in
261 pine monocultures (Fig. 2a). In contrast, we found that pine aboveground biomass was not
262 significantly affected by host-plant species diversity after the 4 months of experiment (Table 1,
263 Fig. 2b).

264 The presence of ants had significant effects on pine primary growth (Table 1). After 4
265 months of growth, final height was 10% greater in pines with ants than ant-excluded pines (Fig.
266 2a). Moreover, the effect of ants on pine primary growth depended on species diversity

267 treatment (significant ant \times species diversity interaction, Table 1, Fig. 2a). While plant diversity
268 effect were significant for both control plants ($F_{2,23} = 23.60$, $P < 0.001$) and ant-excluded plants
269 ($F_{2,23} = 7.15$, $P = 0.003$), the magnitude of plant diversity effects was greater for control plants
270 (Fig. 2a). Pine aboveground biomass was not significantly affected by the presence of ants (vs.
271 exclusion), nor by the interaction between ant and species diversity treatments (Table 1, Fig.
272 2b).

273 Results for each pine species when analysed individually were consistent with those
274 found at the plot level. We observed that plant species diversity significantly increased primary
275 growth in all pine species (Table S1, Fig. S2). Primary growth was higher in the presence than
276 absence of ants for all three species, although the effect was only significant for *P. sylvestris*
277 and marginally for *P. radiata* (Table S1, Fig S2). As we observed at the plot level, primary
278 growth was greater in diverse treatments with ants (control treatment), but ant \times diversity
279 interaction was not significant (Table S1, Fig. S2).

280

281 **(b) Consequences of host-plant species diversity and ants on arthropod abundance**

282 Four months after establishing the ant exclusion treatments, we recorded 1,440 arthropods
283 which were classified as 561 ants (39%), 634 ant-tended aphids (44%), 215 aphid predators
284 (15%) and 30 non-aphid herbivores (2%).

285 Plant species diversity significantly affected the abundance of associated arthropods
286 (Table 2). Specifically, the mean number of ant-tended aphids was approximately 2-fold and 3-
287 fold greater in pine dicultures and tricultures, respectively, than in pine monocultures (Fig. 3a).
288 Similarly, the mean number of ants was approximately 2-fold and 3-fold greater in pine
289 dicultures and tricultures, respectively, compared with pine monocultures (Fig. 3b). In addition
290 to influencing ants through changes in aphid abundance, diversity also affected significantly the
291 rate of ant recruitment to aphids (Table 2). Specifically, we observed that the rates of ant

292 recruitment (ant:aphid ratios) increasing with increasing diversity (0.62 ± 0.09 in monocultures,
293 0.95 ± 0.08 in dicultures and 1.05 ± 0.10 in tricultures). The mean number of aphid predators and
294 non-aphid herbivores were not significantly affected by species diversity treatment (Table 2;
295 Fig. 3c, 3d).

296 Ant presence increased aphid abundance approximately 4-fold compared with ant-
297 exclusion treatment (Table 2, Fig. 3a). Interestingly, the effect of ant treatment on aphid
298 abundance depended on species diversity treatment (significant ant \times species diversity
299 interaction, Table 2, Fig. 3a). Analysing ant-excluded and control plants separately we observed
300 that the effect of host-plant species diversity was significant in control plants ($F_{2,23} = 18.57$, $P <$
301 0.001), but not in ant-excluded plants ($F_{2,23} = 1.12$, $P = 0.344$). The presence of ants decreased
302 aphid predator abundance by approximately 1.7-fold compared with ant-exclusion treatment
303 (Table 2, Fig. 3c). This effect was similar in all species diversity treatments as revealed by the
304 non-significant ant \times species diversity interaction (Table 2, Fig. 3c). The mean number of non-
305 aphid herbivores was not significantly affected by ant treatment, nor by the interaction between
306 ant and species diversity treatments (Table 2, Fig. 3d).

307 In most cases the effects of plant species diversity and ants on arthropod abundance for
308 each pine species analysed individually mirrored the effects found at the plot level (Tables S2,
309 S3, Fig. S3). Plant species diversity significantly increased the abundance of ants for all pine
310 species and that of aphids in *P. pinaster* and *P. radiata* (Tables S2, S3, Fig. S3). Ant presence
311 significantly increased the abundance of aphids in the three studied species (Tables S2, Fig. S3).
312 As we observed at the plot level, aphid abundance was higher in high diverse treatments with
313 ants (control treatment), but ant \times diversity interaction was not significant (Table S2, Fig. S3).

314

315 **(c) Consequences of host-plant species diversity and ants on pine defensive status, nitrogen**
316 **and non-structural carbohydrates in the stem**

317 Host-plant species diversity did not significantly affect the concentration of quantitative pine
318 chemical defences (measured as total phenolics and non-volatile resin, Table S4, Fig. 4),
319 antioxidant capacity (Table S6, Fig. S5), nitrogen (Table S6, Fig. S5) and non-structural
320 carbohydrates (measured as soluble sugars and starch, Table S4, Fig. 4) in the stem. Similarly,
321 the presence of ants did not affect the concentration of pine chemical defences, antioxidant
322 activity, nitrogen or non-structural carbohydrates (Tables S4, S6, Figs. 4, S5). However, the
323 interaction between ant and species diversity treatments was significant for the concentration of
324 soluble sugars in the stem (Table S4, Fig. 4c). Comparing ant exclusion and control (with ants)
325 treatments across the three host-plant species diversity treatments, we observed that ants
326 slightly increased stem soluble sugars on pine dicultures, while they decreased it on pine
327 monocultures and tricultures (Fig. 4c).

328 Results for each pine species analysed individually were markedly close to those
329 observed at the plot level (Table S5, Fig. S4).

330

331 **(d) *Correlation between arthropod abundance and pine performance***

332 We observed that the abundance of mutualistic ants was positively correlated with the
333 abundance of ant-tended aphids ($r = 0.85$, $P < 0.001$, $N = 216$; Table S7), and negatively
334 correlated with the abundance of aphid predators ($r = -0.50$, $P < 0.001$, $N = 216$; Table S7) and
335 non-aphid herbivores ($r = -0.23$, $P = 0.001$, $N = 216$, Table S7).

336 We also observed that the relative primary growth of pine trees was positively correlated
337 with the abundance of ants ($r = 0.55$, $P < 0.001$, $N = 216$, Table S7) and with the abundance of
338 ant-tended aphids ($r = 0.55$, $P < 0.001$, $N = 432$, Table S7), but only in the presence of ants
339 (control plants).

340

341 **4. DISCUSSION**

342 This study demonstrates that diversity within the same genus and guild of plant and mutualistic
343 ants interactively determined arthropod community structure and ecosystem functioning. Three
344 results are noteworthy. First, plant species diversity had strong positive effects on the abundance
345 of aphids, but this effect only occurred in the presence of aphid-mutualist ants. Second, this
346 bottom-up effect of diversity on aphids in turn cascaded up to the third trophic level, increasing
347 ant abundance. And third, diversity effects on ants in turn fed-back to influence plant
348 performance. While plant diversity consistently increased pine primary growth, ants increased
349 the magnitude of these diversity effects. Taken together, these results demonstrate the
350 importance of a multi-trophic perspective for a complete understanding of the consequences and
351 mechanisms behind plant diversity effects.

352 Ecological theories, such as the resource specialization hypothesis [10, 11] and the more
353 individuals hypothesis [12] predicts that plant diversity is one of the primary mechanisms
354 explaining the structure of multi-trophic communities and ecosystem processes. In particular,
355 these ecological theories propose that greater plant diversity generate greater productivity and
356 diversity of resources and, therefore, would attract greater diversity and abundance of associated
357 arthropods. However, our findings show that the positive effects of plant diversity on the
358 structure of arthropod communities and plant performance may be strongly mediated by top-
359 down control from the third trophic level. In particular, our results suggest that bottom-up
360 effects of plant diversity (i) interact with top-down effects of higher trophic levels and modify
361 the patterns of species interactions (i.e. plant-herbivore-predator interactions) and (ii) cascade up
362 the food web to promote positive effects on higher trophic levels which in turn positively
363 influence plant growth (positive effects beget positive effects).

364 Our results showed that plant species diversity increased aphid abundance, but only in
365 the presence of ants, and these diversity effects in turn indirectly increased ant abundance. There
366 are different potential mechanisms to explain these effects: (i) the positive direct effect of

367 diversity on plant growth may indirectly increase aphid abundance and, in turn, indirectly
368 increase the abundance of tending ants. Because aphids depend on ants for protection, these
369 effects are only observed in the presence of ants (bottom-up effect of diversity on plant
370 performance plus the direct effect of mutualistic ants on aphids). Some other plant properties
371 that affect aphid performance could be potentially trading off with plant growth in more diverse
372 assemblages (in instance plant defences [41]), however we did not find evidences of altered
373 defensive or oxidative status in plants growing in more diverse species mixtures with ants. (ii)
374 Plant diversity could be positively increasing aphid populations directly due to greater attraction
375 of dispersing aphids to airborne volatiles from more diverse assemblages, as has been reported
376 elsewhere [15, 42]. (iii) Finally, the effect of plant diversity could be mediated by the third
377 trophic level, such that greater aphid abundance could be due to an increase in the protective
378 services of ants provided to aphids in the context of more diverse host plant resources. Aphid
379 honeydew varies by host plant species (e.g. [43, 44]), and a mixture of honeydew types may be
380 thus more attractive to ants than any single honeydew type due to a more complete nutritive
381 value. Our results are consistent with this last hypothesis since the rate of ant recruitment (i.e.
382 ant/aphid ratio) was about 1.5-fold higher in diverse plots than in monocultures. All these direct
383 bottom-up effects could be potentially contributing to greater aphid populations on more diverse
384 assemblages, and in fact interacting with the direct top-down effects of ants on aphid-predators,
385 non-aphid herbivores, aphid-performance and subsequently leading to the observed pattern of
386 increased plant performance .

387 Consistent with past biodiversity-ecosystem function (BEF) studies [7, 45-48], our
388 results showed that plant species diversity increased plant performance (measured as primary
389 growth), presumably through niche partitioning. Competition for limiting resources (carbon,
390 water, light, nutrient, etc.) is lower among- than within species, so plants in diverse species
391 mixtures may occupy more niches and more efficiently uptake the limiting resources [45, 49].

392 As this study was based upon relatively small seedlings, the marked differences observed in
393 pine growth between poly- and monocultures after just 4 months were unlikely associated with
394 light competition, but rather were likely due to belowground interactions (i.e. water acquisition
395 [50]).

396 In addition to the strong direct effects of plant species diversity on pine growth, our
397 results offer clear support that the presence of mutualistic ants enhanced the strength of
398 diversity effects on plant performance. In particular, we observed that plant diversity promoted
399 greater ant abundance, and that these ants in turn increased pine primary growth. Similarly,
400 observations from a long-term BEF experiment suggest that the positive effect of plant species
401 diversity on plant productivity might be due entirely, or in part, to stronger top-down
402 suppression of herbivores in diverse plots [4]. Although we did not detect effects of ants on
403 non-aphid herbivores in our late-August sampling, they were relatively rare at this time and we
404 speculate that ant effects were likely stronger and indirectly promoted pine growth earlier in the
405 season. Contrary to our early predictions, we found that plant species diversity and the presence
406 of mutualistic ants had no detectable effects on aboveground biomass production, probably
407 because it is necessary more time than four months in a growing season to find significant
408 differences on aboveground biomass production [2]. Nevertheless, primary growth may be the
409 most important measure of plant performance in forest seedlings in terms of long-term
410 consequences for individual plant performance and forest structure due to the need to overcome
411 understory vegetation.

412 In summary, this study showed that host-plant species diversity, even within the same
413 genus and plant guild, strongly influenced plant performance and the associated arthropod
414 community at several trophic levels. We found greater plant growth rates and more ants and
415 aphids in the most diverse assemblages, independently of which species compose them.
416 However, in absence of mutualistic ants, plant species diversity did not have marked effects on

417 the community structure of associated arthropods, neither in plant growth. These results together
418 suggest that plant diversity effects cascaded up to higher trophic levels which generated, at least
419 in part, a positive feedback on plant performance.

420

421 **ACKNOWLEDGEMENTS**

422 We thank Joaquín Moreira and Silvia Portela for their technical assistance in the experimental
423 setup, assessments and plant sampling. We also thank César Cendán, Luz Pato and Rocío
424 Campañó for their help with chemical analyses, and Alberto Gayoso, Fina Lombardero, Jorge
425 Tizado and Xavier Espadaler for their guidance with arthropod identification. Special thanks to
426 Dr. José Carlos Noguera and Andrea Tato for their help and guidance with the antioxidant
427 capacity assays. Comments and suggestions by Jessica Pratt, Luis Abdala-Roberts, Will Petry
428 and Tadj Schreck helped to improve the manuscript. This research was supported by funding by
429 the Spanish Association of Terrestrial Ecology (www.aeet.org) and AGL2010-18724
430 COMPROPIN grant from the Spanish Ministry of Education and Science. LS and XM received
431 financial support from DOC-INIA and Postdoctoral Fulbright/Ministry of Education grant
432 programs, respectively.

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587 **TABLE CAPTIONS**

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589 **Table 1.** Summary of the linear mixed model for the effects of plant specific diversity (mono-,
590 di- and tri-cultures) and presence of mutualistic ants (two levels: presence or absence) on plant
591 performance. The effect of the particular combination of pine species (three mono-, three di- and
592 three tricultures) nested in each diversity treatment, was included in the model. Ant treatments
593 spent four months. Initial height was used as covariate. Significant *P* values ($P<0.05$) are typed
594 in bold.

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596 **Table 2.** Summary of the generalized mixed models for the effects of plant specific diversity
597 (mono-, di- and tri-cultures) and presence of mutualistic ants (two levels: presence or absence)
598 on the abundance of the associated arthropod community at several trophic levels. The effect of
599 the particular combination of pine species (three mono-, three di- and three tricultures) nested in
600 each diversity treatment, was included in the model. Ant treatments spent four months. Final
601 height was used as covariate. Significant *P* values ($P<0.05$) are typed in bold.

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611 **Table 1.** Summary of the linear mixed model for the effects of plant specific diversity (mono-,
612 di- and tri-cultures) and presence of mutualistic ants (two levels: presence or absence) on plant
613 performance. The effect of the particular combination of pine species (three mono-, three di- and
614 three tricultures) nested in each diversity treatment, was included in the model. Ant treatments
615 spent four months. Initial height was used as covariate. Significant *P* values ($P < 0.05$) are typed
616 in bold.

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	DF _{num}	DF _{den}	Final height		Aboveground Biomass	
			F	<i>P</i>	F	<i>P</i>
Ant	1	3	27.47	0.014	0.67	0.474
Diversity	2	47	25.59	<0.001	2.04	0.141
Ant × Diversity	2	47	4.23	0.021	1.09	0.344
Combination	6	47	12.40	<0.001	6.52	<0.001
Ant × Combination	6	47	0.61	0.722	0.82	0.559
Initial height	1	47	44.81	<0.001	2.36	0.131

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627 **Table 2.** Summary of the generalized mixed models for the effects of plant specific diversity
628 (mono-, di- and tri-cultures) and presence of mutualistic ants (two levels: presence or absence)
629 on the abundance of the associated arthropod community at several trophic levels. The effect of
630 the particular combination of pine species (three mono-, three di- and three tricultures) nested in
631 each diversity treatment, was included in the model. Ant treatments spent four months. Final
632 height was used as covariate. Significant *P* values ($P < 0.05$) are typed in bold.

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	DF _{num}	DF _{den}	Ant-tended aphids		Aphid predators		Non-aphid herbivores	
			F	<i>P</i>	F	<i>P</i>	F	<i>P</i>
Ant	1	3	172.32	0.001	19.80	0.021	1.58	0.298
Diversity	2	47	25.96	<0.001	2.17	0.126	0.48	0.623
Ant × Diversity	2	47	33.29	<0.001	0.81	0.453	0.88	0.423
Combination	6	47	1.73	0.135	1.44	0.219	1.41	0.230
Ant × Combination	6	47	1.49	0.201	0.90	0.504	0.57	0.749
Final height	1	47	1.93	0.171	2.79	0.101	0.01	0.919
			Ants		Ant:aphid ratio			
	DF _{num}	DF _{den}	F	<i>P</i>	F	<i>P</i>		
Diversity	2	23	19.13	<0.001	5.46	0.011		
Combination	6	23	2.49	0.053	1.35	0.278		
Final height	1	23	1.27	0.272	0.01	0.933		

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640 **FIGURE LEGENDS**

641 **Figure 1.** Schematic representation of one block in the experimental design, showing the ant
642 treatments as whole plots, the three levels of specific diversity as split-plots, and the three
643 different mono-, di- and tri-specific combinations of the three focal pine species. Constructing
644 three different tri-specific combinations was possible by including a fourth native pine species
645 (*P. pinea*, circles with bars). Including “combination” as a factor in the model allowed us to
646 remove the possible effects of including particular species (i.e. sampling effects), thus testing for
647 non-additive diversity effect. In the field, diversity combinations were randomized within the
648 whole plots (not showed here for clarity).

649 **Figure 2.** Effect of host plant species diversity (mono-, di- and tri-specific cultures) and
650 presence of mutualistic ants (two levels: presence or absence) on final (a) height and (b)
651 aboveground biomass. Ant treatments were in place for four months. Initial height was used as
652 covariate in the statistical model. Least-square means \pm SE (N = 72).

653 **Figure 3.** Effect of host plant species diversity (mono-, di- and tri-specific cultures) and
654 presence of mutualistic ants (two levels: presence or absence) on the abundance (mean number
655 per plant) of associated arthropods grouped as (a) ant-tended aphids, (b) ants, (c) aphid predators
656 and (d) non-aphid herbivores. Ant treatments were in place for four months. Final height was
657 used as covariate in the statistical model. Least-square means \pm SE (N = 72).

658 **Figure 4.** Effect of host plant species diversity (mono-, di- and tri-specific cultures) and
659 presence of mutualistic ants (two levels: presence or absence) on the concentration of (a) total
660 phenolics, (b) non-volatile resin, (c) soluble sugars and (d) starch in the stem of the pine trees.
661 Ant treatments were in place for four months. Initial height was used as covariate in the
662 statistical analyses. Least-square means \pm SE (N = 72). Results of the mixed model are shown in
663 the figure where asterisks indicate significant differences ($P < 0.05$).

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679 **Figure 1.** Moreira et al.

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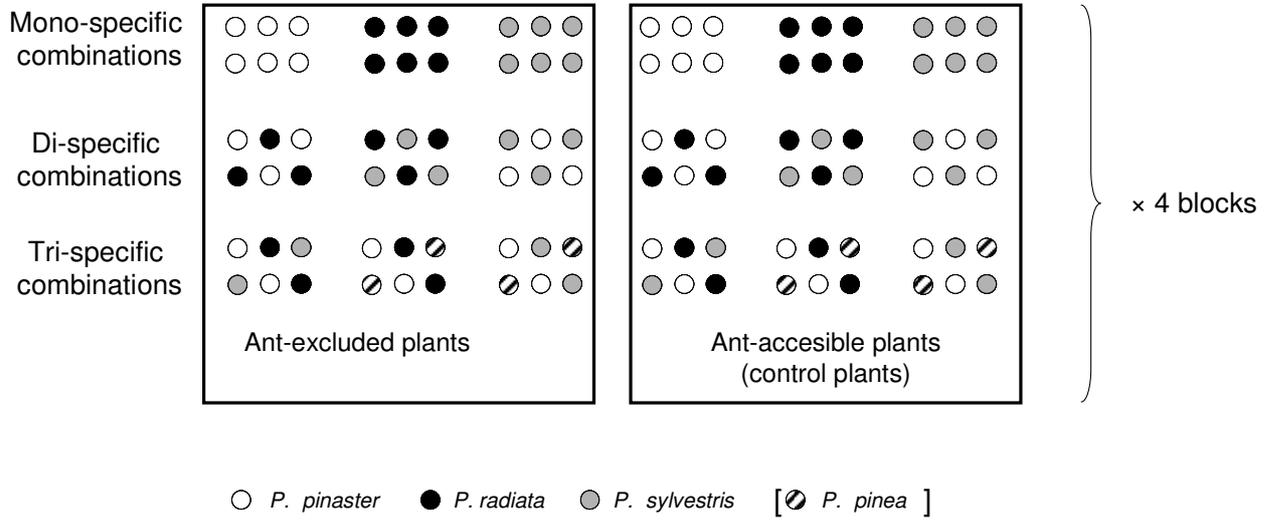
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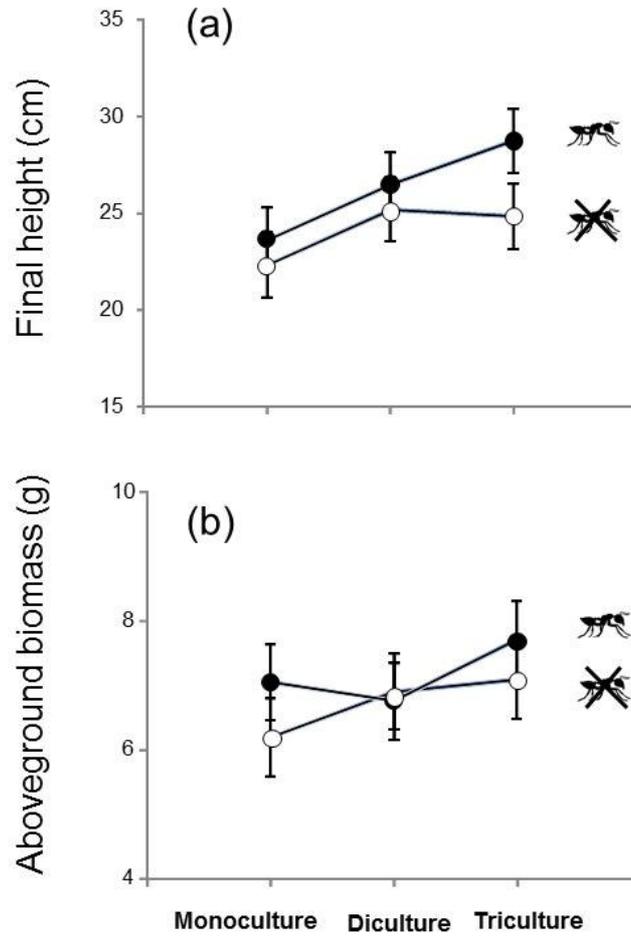
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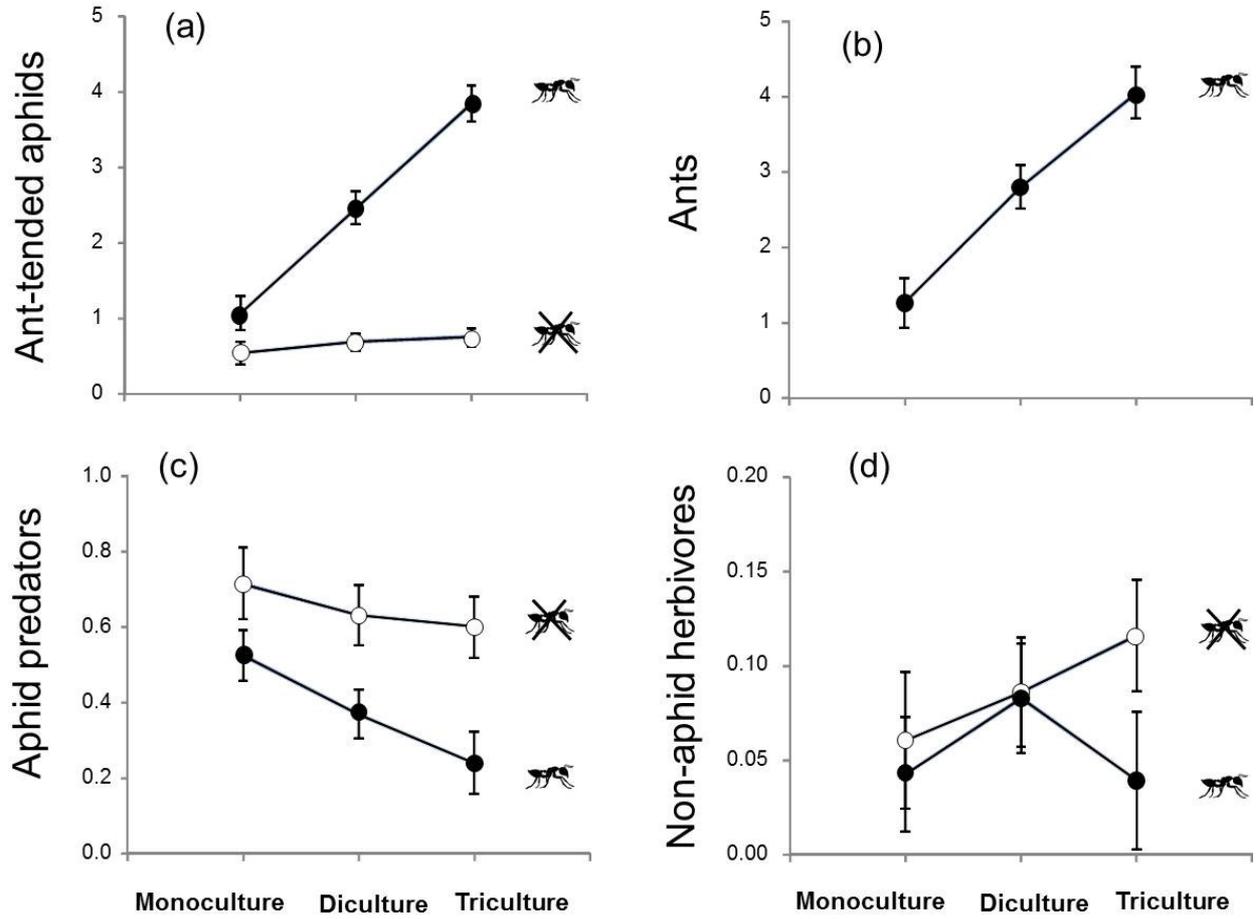
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Figure 2. Moreira et al.

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709 **Figure 3.** Moreira et al.

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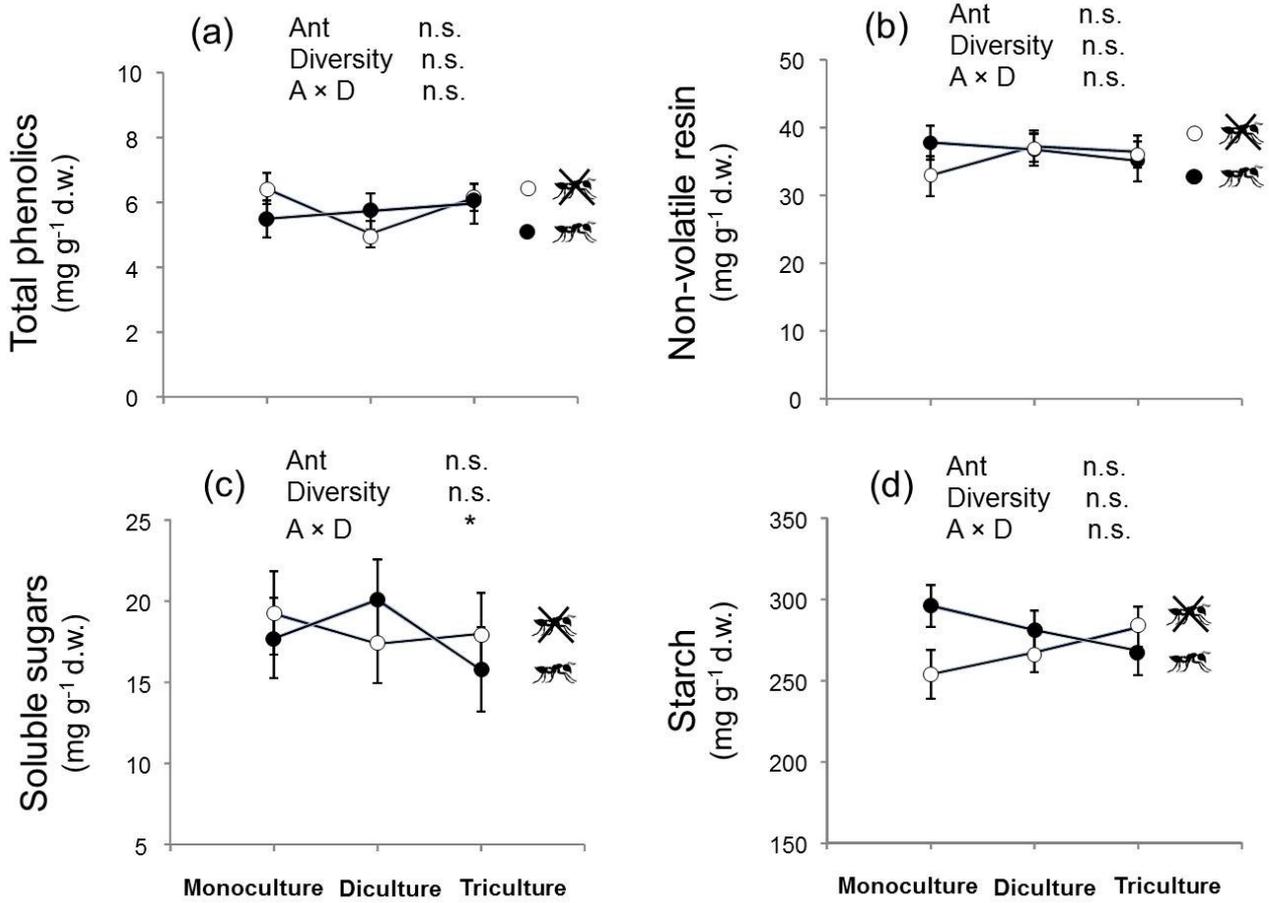
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723 **Figure 4.** Moreira et al.

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