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Don't be naïve: Eco-evolutionary experience better explains invasion success of *Senecio inaequidens* than soil conditions

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1 **Don't be naïve: Eco-evolutionary experience better explains invasion success of**
2 ***Senecio inaequidens* than soil conditions**

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19

20 **Abstract**

21 Introduced species encounter novel biotic and abiotic conditions that influence their
22 success in new environments. Their advantage is often linked to reduced competition
23 from native species that lack eco-evolutionary experience, as well as to their ability to
24 pre-empt resources. Once established, their success can also be shaped by changes in
25 soil conditions, particularly through interactions with soil microbial communities.
26 Understanding how these factors influence invasion success can provide valuable
27 insights into predicting future invasions under global change. In this study, we examined
28 how eco-evolutionary experience and soil bacterial communities influenced the
29 performance of the invasive subshrub *Senecio inaequidens* DC. We conducted a fully
30 factorial experiment in growth chambers consisting of two factors: competing community
31 identity with three levels (plant species from its native range (South Africa), from its
32 invasive range (Italy) and a control with only *S. inaequidens*) and soil biota conditions
33 with two levels (wild soil and autoclaved soil with lower microbial load). Our results
34 showed that plant community identity had the strongest effect on *S. inaequidens* growth
35 (height and lateral spread), with the smallest individuals occurring in competition with
36 South African species. Growing on autoclaved soil had no major impact on plant height,
37 suggesting that reduced competition played a greater role than soil bacterial differences
38 in determining plant performance. Suppression was stronger when the competing native
39 species were more closely related to *S. inaequidens*. Soil bacterial communities were
40 influenced by both plant identity and soil treatment, and *S. inaequidens* performed
41 better in soils with lower bacterial diversity, possibly due to reduced pathogen pressure.
42 These findings suggest that invasive species management could be improved by

43 fostering competition with evolutionarily experienced native species and maintaining or
44 enhancing soil microbial diversity to limit invader success.

45 Keywords: eco-evolutionary experience; phylogenetic similarity, plant traits; relatedness;
46 soil bacteria; South African ragwort

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60 **Introduction**

61 Upon entering new environments, introduced species encounter novel biotic and abiotic
62 conditions that can either facilitate or hinder the invasion process (Heger et al. 2019).
63 To succeed, these species must effectively establish new interactions, make use of and
64 compete for available resources to eventually become invasive (Funk and Vitousek
65 2007). For example, introduced plants may encounter new competitive, pathogenic or
66 herbivore pressures (Funk et al. 2008), lose critical mutualistic interactions (Mitchell et
67 al. 2006) or face novel traits such as allelochemical release, to which they lack eco-
68 evolutionary experience (Callaway and Ridenour 2004). Understanding the mechanisms
69 underlying the success of species introduced in new areas can provide valuable insights
70 into predicting other processes, such as the expansion of species ranges and potential
71 future invasions in the context of global change (Fristoe et al. 2021).

72 The outcome of novel interactions between introduced and native species may be
73 shaped by their evolutionary history, particularly their past experiences interacting with
74 specific species or traits (Saul et al. 2013; Saul and Jeschke 2015). In this context, the
75 invasion success of introduced plants might be hindered if the recipient community
76 includes closely related species, as these species or other community members are
77 more likely to have eco-evolutionary experience with similar competitors, predators, or
78 other antagonistic interactions (Saul et al. 2013). Conversely, functionally dissimilar
79 introduced species with novel physiological and morphological traits, such as the ability
80 of exuding allelopathic compounds or the ability to exploit untapped resources, may
81 gain a competitive advantage in communities where native species lack eco-
82 evolutionary experience with these traits. This experience can influence how native

83 species respond to novel competitors, thereby shaping invasion dynamics (Heger and
84 Treppl 2003; Heger et al. 2019; Novoa et al. 2020).

85 At the community level, niche overlap and resource preemption due to competition for
86 limited resources, are key factors in the success of introduced plant species
87 (MacDougall et al. 2009). Darwin's naturalization hypothesis posits that introduced
88 species would struggle to establish in communities with closely related native species
89 but have a higher invasion success when they are more phylogenetically distant from
90 the resident flora (Darwin 1859; Daehler 2001; Yannelli et al. 2025). The underlying
91 assumption is that niche similarity among species is phylogenetically conserved
92 (Prinzing 2001), with phylogenetic relatedness reflecting shared traits that influence
93 species ability to coexist (Blomberg and Garland 2002). This success is further shaped
94 by the species competitive ability; both in terms of its competitive effect, or its capacity
95 to suppress other individuals by depleting resources, and its competitive response, or its
96 ability to tolerate growth suppression from neighboring plants (Goldberg 1990). While
97 the hypothesis has shed light on initial establishment (Park and Potter 2013; Yannelli et
98 al. 2017), inconsistencies arise from temporal variations and shifts in traits among
99 closely related species (Burns and Winn 2006; Thuiller et al. 2010; Li et al. 2015). To
100 better understand the drivers of invasion success, it is crucial to test this hypothesis with
101 species from both native and invasive ranges, a perspective that has yet to be fully
102 explored (but see e.g. Zheng et al. 2018).

103 Resource availability and competition can be influenced by belowground dynamics
104 linked to plant traits and microbial communities. Soil organisms play a crucial role in
105 mediating interactions between native and invasive plant species, affecting e.g.,

106 competitive, mutualistic, and pathogenic interactions, and ultimately invasion success
107 (Abbott et al. 2015; Fahey and Flory 2022). The competitive strength of invasive plants
108 may be increased by altered microbial communities if they lose coevolved specialist
109 pathogens from their native range (Mitchell and Power 2003; Fahey and Flory 2022) or
110 serve as reservoirs for pathogens that disproportionately affect native species (Eppinga
111 et al. 2006; Mangla and Callaway 2008). New interactions with pathogenic or mutualistic
112 microorganisms can be established with invasive species in the new area based on
113 similarities in traits to those of native species, even without any previous evolutionary
114 experience (Eppinga et al. 2006; Diez et al. 2010). Trait differences in invasive plants
115 compared to the recipient communities, particularly those associated with acquisitive
116 strategies, can also lead to shifts in the microbial community from fungi- to bacteria-
117 dominated (Ehrenfeld 2003; Wardle et al. 2004; Torres et al. 2021). Nevertheless, the
118 role of soil bacteria in mediating competition and plant community assembly, in
119 particular through pathogenic interactions, seems highly limited (van der Putten et al.
120 2007; Dawson and Schrama 2016).

121 We selected the invasive plant *Senecio inaequidens* DC., commonly known as “South
122 African ragwort” or “Canary Weed”, as our study species (hereafter sometimes referred
123 to just with its generic name). This perennial chamaephyte native to South Africa, and
124 invasive in areas of the country outside of its native range, was introduced to Europe in
125 the late 19th century (Ernst 1998) and has become invasive in disturbed areas. *Senecio*
126 *inaequidens* has been found to produce allelopathic defenses in the form of pyrrolizidine
127 alkaloids (Joosten and van Veen 2011), which can protect it against both above- and
128 belowground herbivory (Caño et al. 2009; Thoden et al. 2009), influencing soil microbial

129 communities (Harkes et al. 2017). *Senecio inaequidens* traits were also found to
130 correlate with rhizosphere biota, with bacterial diversity being positively associated with
131 resource allocation to belowground growth and late flowering (Thébault et al. 2010).
132 Furthermore, Van De Walle et al. (2022) found *S. inaequidens* to modify soil abiotic
133 conditions, increasing nutrient concentrations via litter deposition and eliciting increased
134 growth of co-occurring native species in nutrient-poor habitats. However, the impact of
135 these traits and soil community alterations on its competitive success in the invasive
136 versus native range has remained unexplored.

137 In this study, we investigated *S. inaequidens* competitive response to native plant
138 species from both its native and invasive ranges under controlled experimental
139 conditions. The experimental communities included species with which *S. inaequidens*
140 shares a history of eco-evolutionary interactions (native range) and species to which it is
141 evolutionarily naïve (invasive range), lacking such historical associations. We also
142 examined how soil conditions, including the presence or absence (by autoclaving) of
143 soil biota from the invasive range, influenced these competitive interactions.
144 Specifically, we explored whether eco-evolutionary experience, phylogenetic
145 relatedness, and soil biota could explain *Senecio's* performance when competing with
146 native plant communities. We hypothesized that: (i) *S. inaequidens* performance will
147 depend on the identity of the competing plant communities, and it will perform better
148 when competing with naïve species from the invasive range compared to experienced
149 species from the native range; (ii) an increase in phylogenetic relatedness between *S.*
150 *inaequidens* and the competing species in the community will result in lower
151 performance of *S. inaequidens*, following Darwin's naturalization hypothesis; and (iii)

152 soil biota will influence the competitive interactions between *S. inaequidens* and native
153 plant communities, with reduced biotic effects in autoclaved soils leading to better
154 performance of *S. inaequidens* compared to non-autoclaved soils.

155 **Materials and Methods**

156 *Plant and soil material collection*

157 *Senecio inaequidens* is a subshrub that belongs to the Asteraceae family, often
158 reaching 40-100 cm in height. Native to South Africa's highlands, it was introduced to
159 Europe as a wool contaminant (Ernst 1998). It thrives in disturbed areas like roadsides,
160 railways, and quarries, as well as dry grasslands, pastures, and vineyards (Heger and
161 Böhmer 2005; López-García and Maillet 2005). In its native range, the species exists in
162 diploid ($2n = 20$) and tetraploid ($2n = 40$) forms, but only tetraploids are found in Europe
163 (Lafuma et al. 2003).

164 Seeds of *S. inaequidens* were collected from a population located in the former quarry
165 of Collepdrino, Northern Italy, which is currently heavily invaded by this species
166 (Bergamo, 45°46'37.4"N 9°31'09.5"). To design the competing native communities, we
167 chose five species known to co-occur with *S. inaequidens* in each range (i.e., native and
168 invasive). To assess this in the native range of the invasive species (i.e., South Africa),
169 we used the National Collections database (<http://posa.sanbi.org/>) to select species
170 documented in the area where tetraploid populations of *S. inaequidens* have been
171 reported (Lafuma et al. 2003). Upon availability in local seed companies, we refined the
172 list of native species by a second check against the results of vegetation surveys (Du
173 Preez and Bredenkamp 1991), to be sure that all natives would have co-occurred in a

174 plot-sized area. As a result, we selected a multi-species suite comprising *Aristida*
175 *congesta* Roem. & Schult. (Poaceae), *Hibiscus trionum* L. (Malvaceae), *Salvia disermas*
176 L. (Lamiaceae), *Wahlenbergia androsacea* A. DC. and *Wahlenbergia undulata* (L.f.) A.
177 DC. (Campunulaceae) for the native range. Seed material for the native range was
178 purchased at the local seed company Silverhill (Cape Town, South Africa). In the
179 invasive range (i.e. Italy), we selected native species according to known co-occurrence
180 in the Collepedrino quarry (Gentili et al. 2020) and collected seeds in the same area.
181 The list included *Bromopsis erecta* (Huds.) Fourr. (Poaceae), *Hypericum perforatum* L.
182 s.l. (Hypericaceae), *Onobrychis viciifolia* Scop. (Fabaceae), *Poterium sanguisorba* L. s.l.
183 (Rosaceae) and *Trifolium repens* L. (Fabaceae). We performed germination tests for all
184 native species to find the best conditions for their germination (Supplementary
185 Information, Section S1, Tab. S1).

186 Soil used for the experiment was collected in the same quarry. It was placed in open dry
187 bags and stored at room temperature until setting the experiment. We prepared the
188 experimental substrate by mixing the quarry soil, which was highly rocky, with common
189 potting substrate (TERCOM potting soil) in a 1:1 ratio to favor plant growth under
190 controlled conditions (growth chamber). Before setting the pot experiment, we
191 autoclaved half of this soil mix at 120°C for 45 minutes.

192 *Experimental design and setting*

193 Our experiment consisted of a fully factorial design with a combination of two factors:
194 competing community and soil biota. The competitive communities' identity had three
195 levels, namely species from the native range considered to be experienced (SA; South

196 Africa), species from the invasive range considered to be naïve (IT; Italy) and the
197 control (CTR) with *S. inaequidens* individuals growing alone. Soil biota conditions had
198 two levels, i.e. autoclaved (st) and non-autoclaved soil (w; henceforth “wild”). Each
199 treatment combination was replicated five times, making up a total of 30 experimental
200 pots. Before the experiment, all seeds were stratified by placing them in paper bags at
201 4°C for about 1 month. Two different methods of germination were used to ensure the
202 survival of the seeds and high germination rates, according to results of germination
203 tests performed before the experiment: (1) Directly in plastic cups with a mix of
204 autoclaved common potting soil and sand with a 1:1 ratio; (2) in Petri dishes with
205 moistened filter paper, which were transplanted to plastic cups filled with autoclaved
206 common potting soil and sand at a 1:1 ratio, a few days after germination (see protocols
207 in Supplementary information, Section S1). When the seedlings were about 20 days old,
208 we placed two individuals of the invasive *S. inaequidens* in the middle of 2L pots filled
209 with a mix of quarry soil and common potting soil. At the same time, in all treatment
210 combinations that required competition with natives, we added 5 individuals for each
211 native species distributed at the edges of the pot. We then completely randomized the
212 pots and placed them in a growth chamber with an average temperature of 29°C,
213 relative humidity of 42%, and a day-night cycle of 14 and 10 hours, respectively. These
214 values were consistent with the growth conditions of *S. inaequidens* when invading
215 ruderal dry habitats (railways, roadsides, etc.). Plants were watered every other day for
216 the first days, and twice a week for the rest of the experiment. The experiment ran for
217 84 days, when some *S. inaequidens* individuals started to die.

218

219 *Measurements and data processing*

220 After 84 days from the start of the experiment, we collected data on plant vegetative
221 fitness and survivorship, as a proxy for success. Specifically, we measured the
222 maximum height as the shortest distance between the upper boundary of the main
223 photosynthetic tissues on a plant and the ground level, and the lateral growth of each
224 individual of *S. inaequidens* as the maximum width of the canopy (Pérez-Harguindeguy
225 et al. 2013). At this point, we also recorded the number and identity of the native
226 species that survived in each pot.

227 To assess the effect of relatedness on *S. inaequidens* performance, we calculated the
228 phylogenetic distances among all species in our experiment from a phylogenetic tree for
229 angiosperms as a backbone (Zanne et al. 2014) that was pruned from all species that
230 were not included in our experiment (Supplementary information, Fig. S1). We then
231 calculated community-weighted phylogenetic distances to the invader (CWMPD) by
232 weighting the native community-invasive distances with the proportion (based on the
233 number of individuals alive) of each species (in terms of number of individuals) in the
234 pot. Further, we also obtained the distance of the most abundant species in each
235 community to the invader (DMANS) to examine the effect of these species on *S.*
236 *inaequidens* growth. In the case of more than one species dominating the community in
237 the same abundance, we used total mean phylogenetic distances to every dominant
238 native. To characterize the soil bacterial communities in each treatment combination,
239 we collected soil samples at the end of the experiment (after 84 days) from three
240 randomly selected pots ($n = 18$). The samples were stored at -20°C until processing.

241 *DNA extraction and Next Generation sequencing*

242 Genomic DNA was extracted using the FastDNA[®] Spin Kit for Soil (MP Biomedicals,
243 Solon, OH, USA) following the manufacturer's instructions. A first PCR amplification
244 was carried out using the 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 519R (5'-
245 GWATTACCGCGGCKGCTG-3') primers (Frank et al. 2008; Hollister et al. 2011) on the
246 original DNA extract and on the 1:10, 1:100, 1:1000 and 1:10000 dilutions, to detect the
247 possible presence of PCR inhibitors. Amplification conditions were: initial denaturation
248 at 95°C for 4 min, 29 cycles at 95°C for 30 s, 55°C for 45 s and 72°C for 45 s, and a
249 final extension at 72°C for 5 min. A second PCR was then performed using 783F and
250 1046R primers on the V5-V6 hypervariable regions of the bacterial 16S rRNA gene, with
251 customized oligonucleotide barcodes (6bp, see sequence in Table S2) added to their 5'
252 end (Gandolfi et al. 2024). We used GoTaq[®] Green Master Mix (Promega Corporation,
253 Madison, WI, USA) and 1 µM of each primer, for a final volume of 2 x 50 µL for each
254 sample. This second amplification was performed under the following conditions: initial
255 denaturation at 94°C for 4 min, 28 cycles at 94°C for 50 s, 47°C for 30 s and 72°C for
256 30 s, and a final extension at 72°C for 5 min. The PCR products were purified using the
257 Wizard[®] SV Gel and PCR Clean-Up System (Promega Corporation, Madison, WI,
258 USA), following the manufacturer's instructions, and the DNA content was quantified
259 with the Qubit[®] 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA). Amplicon
260 libraries were prepared with nine samples each, identifiable due to different barcode
261 pairs. Library preparation with the addition of standard Nextera indices (Illumina, Inc.,
262 San Diego, CA, USA) and sequencing with the MiSeq Illumina platform (Illumina, Inc.,
263 San Diego, CA, USA), using a 2 x 250 bp paired-end protocol, was performed at the

264 Consorzio per il Centro di Biomedicina Molecolare (CBM), located in Trieste, Italy.
265 Amplicon Sequence Variants (ASVs) were inferred through the DADA2 algorithm
266 (Callahan et al. 2016), as described in Gandolfi et al. (2024).

267 *Data analysis*

268 All statistical analyses were performed using R version 4.3.1 (R-Core-Team 2023) and
269 the *vegan* package (Oksanen et al. 2022), unless stated otherwise. We used two-way
270 ANOVA to assess if the average height and lateral growth of *S. inaequidens* were
271 affected by the competing community, soil conditions and their interaction. Since there
272 was an imbalance in the experimental replication due to the mortality of *S. inaequidens*
273 in some replicates, we used the Type III test. We then performed post-hoc pairwise
274 comparisons with Tukey tests. In the same way, we used one-way ANOVA to test the
275 effect of native species identity on the height of *S. inaequidens* to explore the impact of
276 the presence of individual species. To evaluate the effect of CWMPD and DMANS in
277 each community on average *S. inaequidens* height and lateral growth, we used linear
278 regressions.

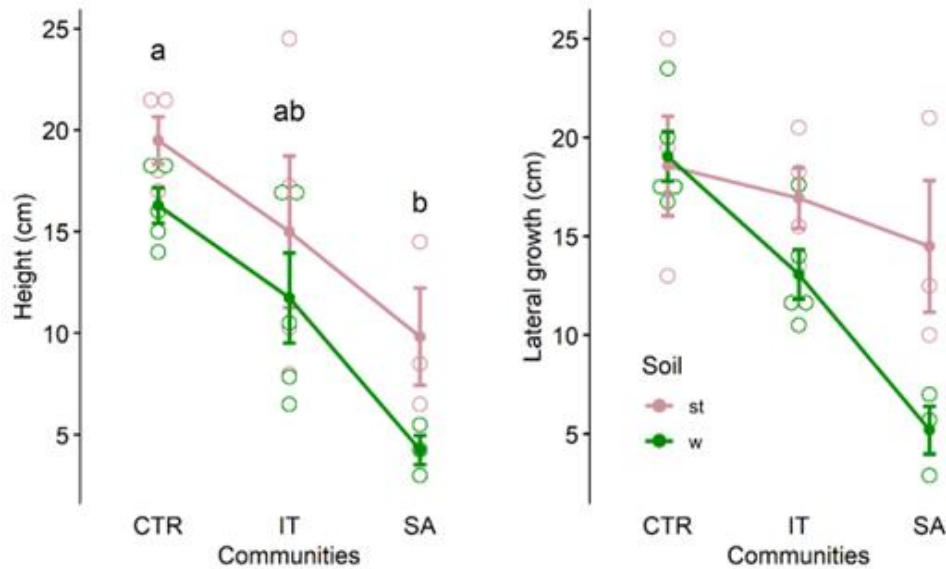
279 We used Non-metric Multidimensional Scaling (NMDS) analysis based on Bray-Curtis
280 dissimilarity distances (Bray and Curtis 1957) to visualize differences in soil bacterial
281 community structure according to the treatments using the *metaMDS* function. We
282 carried out a PERMANOVA test using the *adonis2* function to assess treatment
283 combination effects on soil bacterial communities. Before performing these multivariate
284 analyses, we transformed the bacterial ASV abundance matrix with Hellinger distance
285 to reduce the emphasis on ASV abundances while highlighting their presence or

286 absence and mitigate the double-zero issue when comparing ASV compositions across
287 samples (Bocard et al. 2018). We calculated ASV richness and Shannon index for each
288 treatment combination on the rarefied bacterial data, which were based on the sample
289 with the lowest reading depth (2293). We then evaluated the effects of our treatments
290 on ASV richness using generalized linear models with a quasi-Poisson distribution to
291 correct for overdispersion present in the data (Cameron and Trivedi 1990) and used
292 ANOVA for the Shannon index. Finally, we explored the effect of bacterial alpha-
293 diversity, i.e., ASV richness and Shannon index, on the height and lateral growth of *S.*
294 *inaequidens* using a linear model.

295 **Results**

296 *Effect of competition and soil biota conditions on Senecio performance*

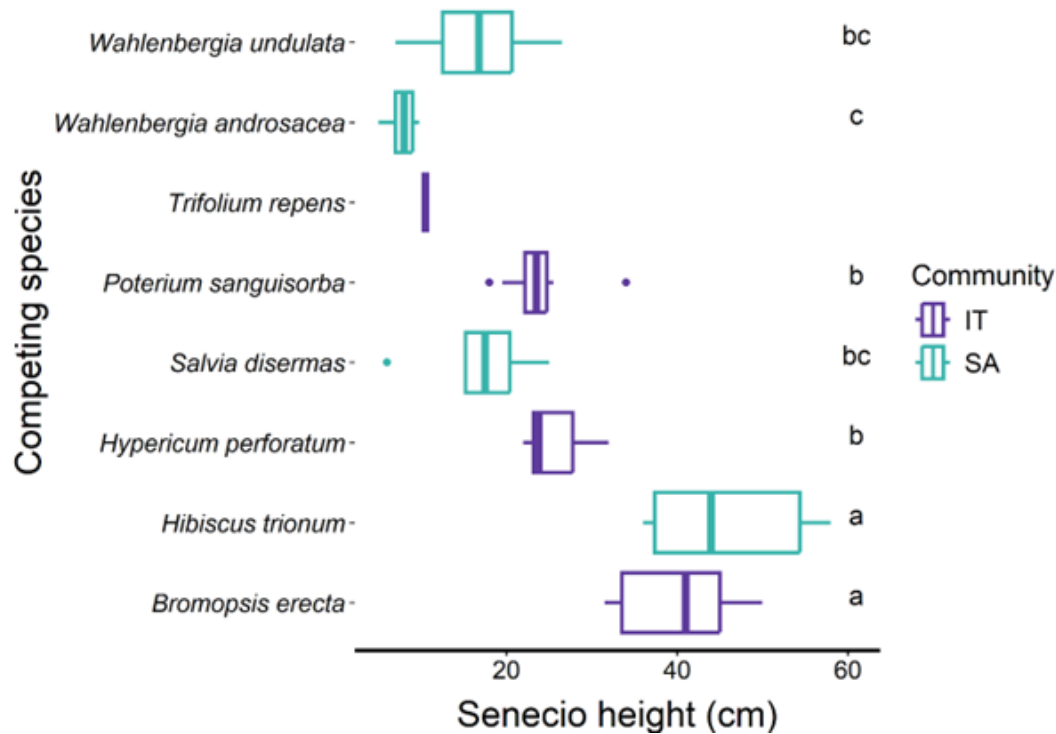
297 *Senecio inaequidens* performance was affected by the community it was growing along
298 with more than soil conditions, compared to the control treatment in pots where it grew
299 without competition. Specifically, in terms of *S. inaequidens* maximum height at day 84,
300 only community identity had a significant effect (ANOVA: Community: $F = 4.31$, $p <$
301 0.03 ; Fig.1; Supplementary information, Table S3). We did not find an effect of
302 community and soil conditions on lateral growth (ANOVA $p > 0.05$; Fig. 1).



303

304 **Figure 1.** Differences among community and soil treatments in height (left panel) and
 305 lateral growth (right panel) of *Senecio inaequidens* 84 days after the experiment started.
 306 CTR represents the control treatment with no native species growing with *Senecio*, IT is
 307 the naïve community from the invasive range in Italy, and SA is the experienced
 308 community from the native range in South Africa (ANOVA: Community $F = 4.31$, $p <$
 309 0.03). Autoclaved soil is represented in light pink (st) and wild one (not autoclaved; “w”)
 310 in green. Different letters indicate significant differences among treatments ($p < 0.05$).

311 We found a significant effect of the identity of the native species competing with *S.*
 312 *inaequidens* on its performance (ANOVA: Species $F = 27.11$, $p < 0.001$; Fig. 2;
 313 Supplementary information, Table S4). *Trifolium repens* was not considered in the
 314 analysis because the species only survived in one pot. *Senecio inaequidens* had the
 315 smallest individuals when competing with *Wahlenbergia androsacea* (SA community)
 316 and the largest when competing with *Hibiscus trionum* and *Bromopsis erecta* (SA and IT
 317 community, respectively).



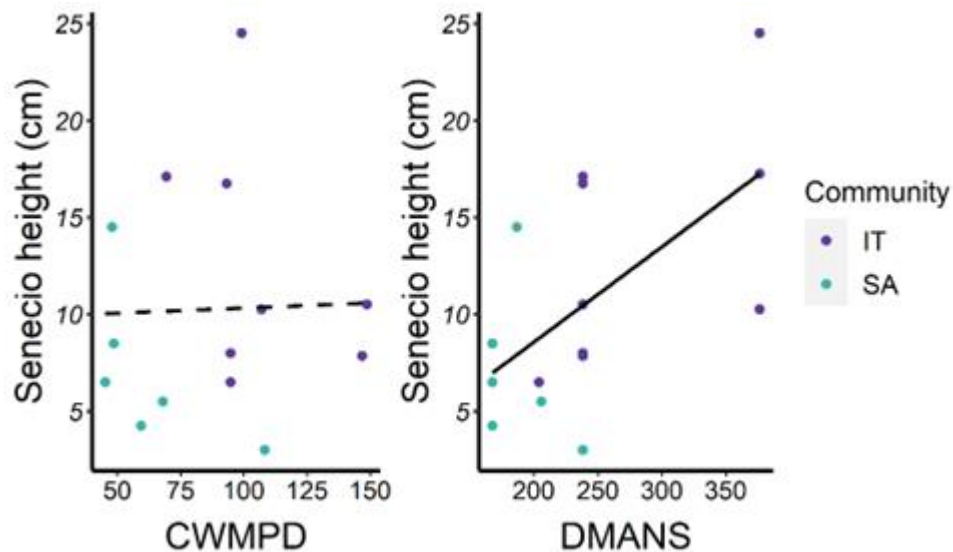
318

319 **Figure 2.** Variation in *Senecio inaequidens* height based on the identity of the
 320 competing species in the community (ANOVA: Species $F = 27.11$, $p < 0.001$). IT
 321 represents the naïve community from the invasive range (in purple) and SA is the
 322 experienced community from the native range (in turquoise). Different letters indicate
 323 significant differences among treatments ($p < 0.05$).

324 *Senecio performance in relation to phylogenetic distance from the native community*

325 When exploring the effect of phylogenetic distance between the native species and *S.*
 326 *inaequidens*, we applied two measures of phylogenetic distance. In the first one, we
 327 weighted the abundances of native species. We first eliminated an outlier here since
 328 distances were above 1.5 times the interquartile range. This number resulted from the
 329 dominance of one native species and the mortality of all other natives in one
 330 community. After this procedure, we did not find a significant effect of weighted
 331 phylogenetic native-invasive distances (CWMPD) on either maximum height or lateral
 332 growth (LM: $R\text{-squared}_{\text{height}} = -0.08$, $p = 0.91$, $R\text{-squared}_{\text{lat. growth}} = -0.08$, $p = 0.88$; Fig. 3;

333 Supplementary information, Table S5). For the second measure of phylogenetic
 334 distance, the distance of the most abundant native species in each community to the
 335 invasive *S. inaequidens* (DMANS), we found a significant relationship with the height of
 336 *S. inaequidens* (R -squared = 0.32, p = 0.02; Fig. 3.; Supplementary information, Table
 337 S5), though for lateral growth the effect was not significant (R -squared = 0.19, p = 0.06;
 338 Supplementary information, Table S4). *Wahlenbergia undulata* and *Wahlenbergia*
 339 *androsacea* (SA community) were the most phylogenetically similar species to *S.*
 340 *inaequidens* (Supplementary information, Fig. S1).



341
 342 **Figure 3.** Relationship between the height of *Senecio inaequidens* and two measures of
 343 phylogenetic distance between *S. inaequidens* and the native species: (left panel)
 344 phylogenetic distance weighted by species abundance (CWMPD; R -squared = -0.07, p =
 345 0.78); (right panel) phylogenetic distance of the most abundant native species in each
 346 community (DMANS); R -squared = 0.32, p = 0.02). IT represents the naïve community
 347 from the invasive range (in purple) and SA is the experienced community from the
 348 native range (in turquoise).

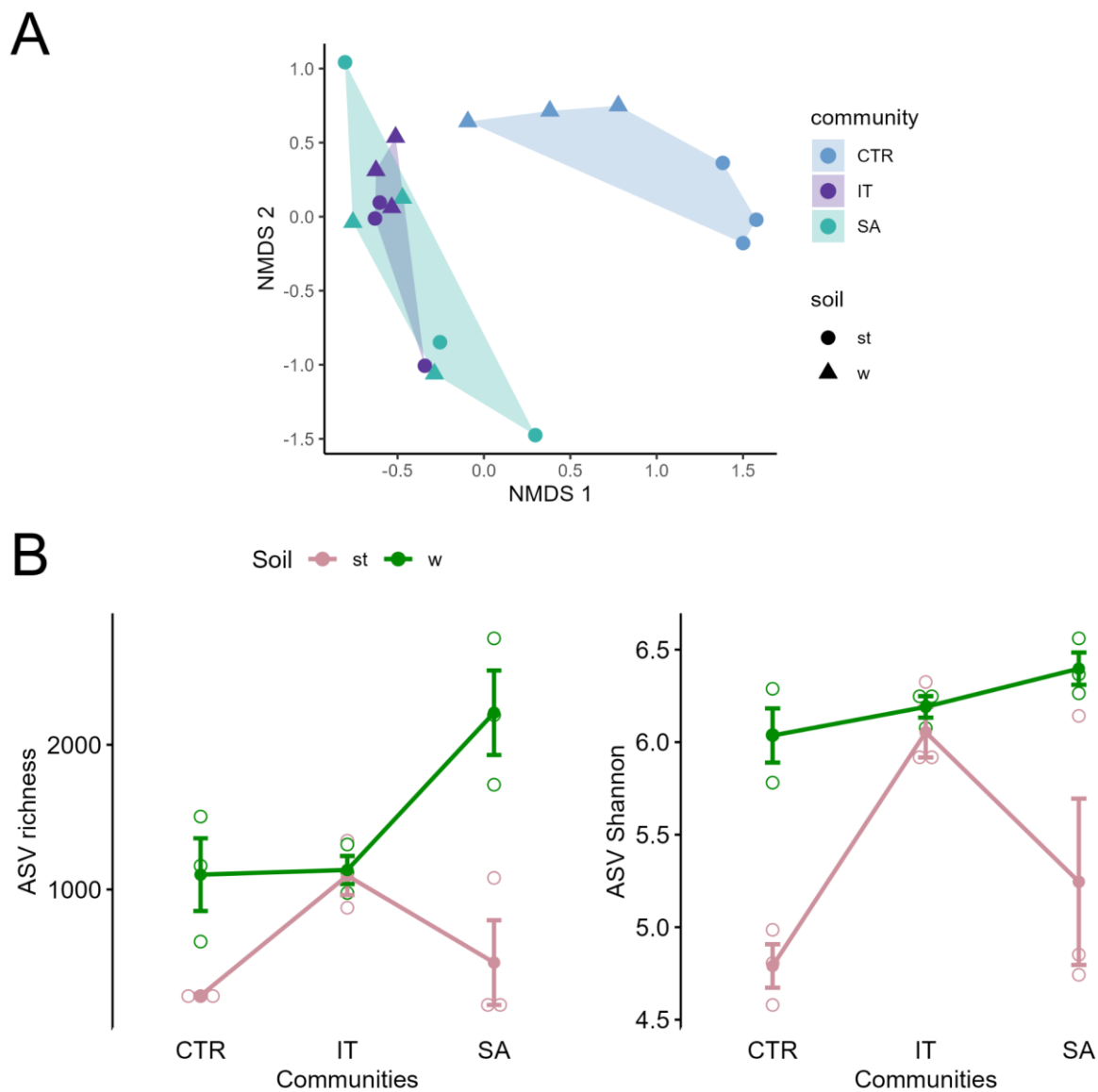
349

350

351 *Soil bacterial community and its influence on Senecio performance*

352 The analysis of soil bacterial communities yielded a total of 687,210 valid sequences,
 353 ranging between 2293 and 199,736 per sample, from which 8471 ASVs were inferred.
 354 At phylum level, $46.8 \pm 6.1\%$ of sequences were classified as *Pseudomonadota*, $20.7 \pm$
 355 7.1% as *Actinomycetota*, and $10.6 \pm 3.1\%$ as *Bacteroidota* (Supplementary information,
 356 Table S6). At genus level, $60.0 \pm 9.6\%$ of sequences could not be classified.
 357 Unclassified Bacteria were particularly abundant ($9.5 \pm 2.7\%$), as well as unclassified
 358 members of classes, *Beta-* and *Gammaproteobacteria* ($5.4 \pm 2.8\%$ and $4.8 \pm 2.0\%$,
 359 respectively). The most abundant classified genus was *Streptomyces*, with $3.9 \pm 3.4\%$
 360 of average abundance (Supplementary information, Table S7). The NMDS analysis had
 361 a stress coefficient under 0.2 at two dimensions (0.128), thus indicating that this number
 362 of dimensions in a plot was a good representation of our data (Clarke 1993). The NMDS
 363 plot showed a clear separation of the samples of autoclaved soil in pots where *S.*
 364 *inaequidens* was growing alone (CTR; Fig. 4. Panel A). Samples from the experienced
 365 community (SA) tended to spread more, while samples from the naïve community (IT) in
 366 any soil condition clustered more closely (Fig. 4. Panel A). Our PERMANOVA test
 367 accounted for 47.27% of the overall variation and indicated an effect of both competing
 368 communities and soil, but not their interaction, on ASV community structure
 369 (PERMANOVA, Community $F = 0.23378$, $p = 0.001$, Soil $F = 0.11063$, $p = 0.003$;
 370 Supplementary information, Table S8).

371



372

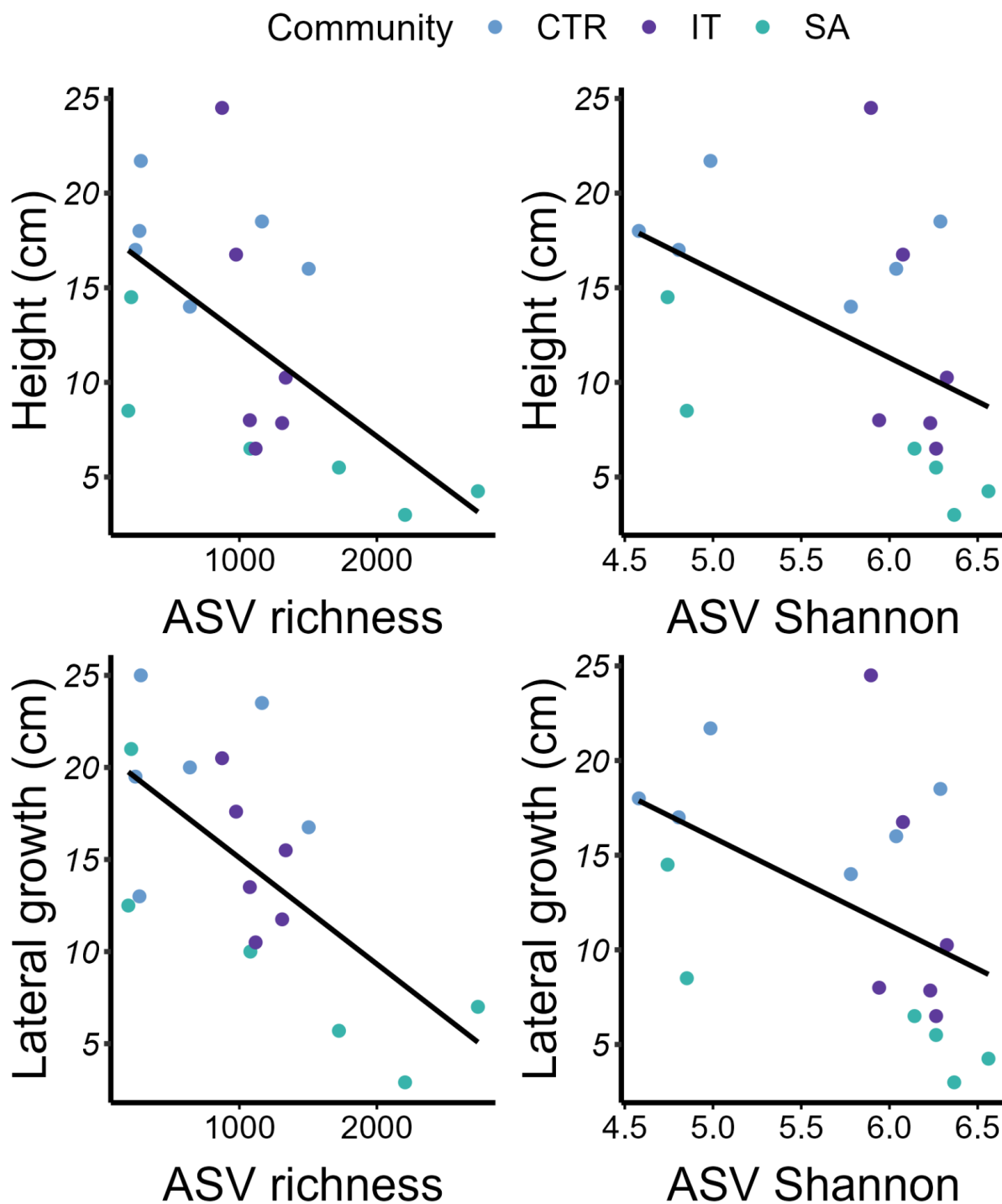
373 **Figure 4.** Panel A: Two-dimensional plot of our non-metric multidimensional scaling
 374 analysis (NMDS) for bacterial ASVs. The control treatment CTR is shown in light blue,
 375 the naïve community from the invasive range IT in purple, and the experienced
 376 community in the native range SA in turquoise. Autoclaved soil treatment is represented
 377 with filled circles (st) and wild soil (not autoclaved) with filled triangles (w). Panel B:
 378 Differences among community and soil treatments in ASV richness and Shannon index
 379 of soil bacterial communities. Autoclaved soil is represented in light pink and wild soil
 380 (not autoclaved) in green. ASV richness: Interaction, $p < 0.05$; Shannon index (ANOVA,
 381 Community $F = 5.699$, $p < 0.05$, Soil $F = 24.082$, $p < 0.001$, Interaction $F = 4.254$, $p =$
 382 0.04).

383

384 We found that the highest richness and diversity (in terms of Shannon index) of ASVs
385 occurred in wild soils from the South African communities and the lowest in the
386 autoclaved soil with no competing species (control). There was a significant interaction
387 between competing community types and soil conditions for bacterial ASVs (GLM, $p <$
388 0.05 , ANOVA, $p < 0.05$; Supplementary information, Table S9). Specifically, the effect
389 that community had on ASV richness was modified by soil conditions, with less ASV
390 richness and diversity in controls and South African communities growing in autoclaved
391 soil, compared to wild conditions. Furthermore, ASV richness and diversity in Italian
392 communities did not differ between soil conditions (Fig. 4. Panel B).

393 There was a statistically significant relationship between both ASV richness and
394 Shannon index and the height and lateral growth of *S. inaequidens* (LM height: Adj-
395 $R^2(\text{ASV richness}) = 0.32$, $p < 0.01$, Adj- $R^2(\text{ASV Shannon}) = 0.18$, $p = 0.04$, LM lateral
396 growth: Adj- $R^2(\text{ASV richness}) = 0.39$, $p < 0.01$, Adj- $R^2(\text{ASV Shannon}) = 0.14$, $p =$
397 0.067 ; Fig. 5, Supplementary information, Table S10). Specifically, *S. inaequidens*
398 individuals were taller and wider when growing in pots with lower soil bacterial diversity.

399



400
 401 **Figure 5.** Linear model results for the relationship between soil bacterial diversity,
 402 represented as ASV richness and Shannon index, and height and lateral growth of
 403 *Senecio inaequidens* (LM height: Adj-R²(ASV richness) = 0.32, $p < 0.01$, Adj-R²(ASV
 404 Shannon) = 0.18, $p = 0.04$, LM lateral growth: Adj-R²(ASV richness) = 0.39, $p < 0.01$,
 405 Adj-R²(ASV Shannon) = 0.14, $p = 0.067$). For reference, the identity of the competing
 406 communities is indicated in different colors.

407 Discussion

408 With our experiment, we investigated how the eco-evolutionary experience of species in
409 the resident community and the soil biotic conditions influence the performance of
410 *Senecio inaequidens*. As hypothesized, the identity of competing plant communities
411 significantly affected *S. inaequidens* growth, supporting the hypothesis that competition
412 with naïve species in the invasive range is less intense than with experienced species
413 from the native range. We only found partial support for our other hypotheses.
414 Specifically, the effect of phylogenetic relatedness in explaining *S. inaequidens*
415 performance was mixed, with no effect of community-wide distances but a significant
416 influence of the most abundant species relatedness to *S. inaequidens*. Although the
417 competitive responses of *S. inaequidens* to the plant communities were not significantly
418 affected by autoclaving the soil in which they grew, soil bacterial diversity still seems to
419 play a role in its performance.

420 *Eco-evolutionary experience and species identity modulates competition*

421 Our results align with previous studies suggesting that naïve species in the invasive
422 range may lack evolved resistance or competitive strategies against introduced species
423 with which they have had no similar interactions in their evolutionary history (Callaway et
424 al. 2011; Saul et al. 2013; Zhang et al. 2018). For instance, in a removal experiment,
425 Callaway et al. (2011) found *Centaurea stoebe* L. populations in their native range
426 (Europe) to exhibit a significantly higher response (6.5- to 7.5-fold) to the removal of
427 neighboring plants compared to populations in their invasive range (North America). The
428 reduced competitive effects associated with the lack of eco-evolutionary experience of

429 the Italian communities could be attributed to several mechanisms, including differences
430 in resource acquisition with naïve native competitors. On the other hand, *S. inaequidens*
431 may be exerting a stronger competitive response, possibly through allelopathic effects
432 that naïve species have not yet adapted to counter. Additionally, *Senecio inaequidens* is
433 known to contain secondary metabolites in its tissues (i.e. pyrrolizidine alkaloids) that
434 are poisonous to some animals (Dimande et al. 2007). Invasive populations may benefit
435 from this chemical defense, as naïve herbivores in the newly colonized environment are
436 unlikely to feed on it, further enhancing its invasion success (Scherber et al. 2003;
437 Misuri et al. 2020). Alternatively, its success could be linked to a subtle temporal
438 advantage, allowing it to grow slightly faster and establish dominance earlier in the
439 competition. Indeed, Delory et al. (2019) found *S. inaequidens* to exhibit strong
440 competitive effects on native plants when it has a temporal advantage due to, for
441 example, the slower growth of competing native species (Delory et al. 2019).

442 We also found that the identity of the species in the community affected *S. inaequidens*
443 performance. In particular, the South African *Wahlenbergia androsacea* had a
444 consistent negative effect on *S. inaequidens* height when present in the community.
445 This pattern was not consistent across species from the native range, indicating that
446 origin or co-occurrence *per se* is not a strong indicator of competitive effects of the
447 native species. Instead, the traits of the competing species may play a more significant
448 role. For example, a study modeling experimentally derived competitive impact and
449 responses of *Acroptilon repens*, a species native to Uzbekistan and invasive in North
450 America, found them to be rather dependent on the traits of the species it was
451 competing with (Xiao et al. 2013). These results together support the idea that invasion

452 success and impact are shaped by both the introduced species traits and the
453 adaptability and competitiveness of the recipient community.

454 Our phylogenetic analyses support the idea that the presence of a dominant, closely
455 related native species (e.g., *Wahlenbergia* sp.) may increase the competitive resistance
456 against *S. inaequidens*, supporting Darwin's naturalization hypothesis. This finding is in
457 line with the assumption that phylogenetic relatedness can be a good proxy for
458 functional trait similarity and resource use overlap, leading to more intense competition
459 (Divišek et al. 2018). Our findings also align with previous research showing that biotic
460 resistance in native plant communities against other invasive Asteraceae species in
461 Europe, such as *Ambrosia artemisiifolia* L. and *Solidago gigantea* Aiton, is strongly
462 influenced by phylogenetic proximity to dominant native species (Yannelli et al. 2017).
463 Therefore, while community phylogenetic similarity may not strongly predict invasion
464 success (Dostál 2011), interactions with key species within the community, particularly
465 the most abundant ones, may play a critical role. Interestingly, a recent observational
466 study carried out in Northern Italy described a negative relationship between *S.*
467 *inaequidens* performance and phylogenetic similarity to resident species in the field
468 (Quaglini et al. 2025), lending support to what is known as the *pre-adaptation*
469 *hypothesis*. The study found that *S. inaequidens* performed better when growing
470 alongside more similar species, particularly in more productive habitats. Such
471 apparently contradictory results could be reconciled by recent reviews suggesting that
472 Darwin's naturalization and pre-adaptation hypotheses are not mutually exclusive, but
473 may operate at different spatial scales (Thuillier et al. 2010; Ma et al. 2016). Namely,
474 successful alien species would be more closely related to natives at broader spatial

475 scales, due to environmental filtering, but more distantly related at finer spatial scales,
476 where competition for limiting resources becomes more important (Ma et al. 2016). In
477 other words, at large scales, environmental filtering selects for invaders that are adapted
478 to the conditions of the new area, while at small spatial scales, the role of competition
479 for limiting resources becomes more important. This highlights the context dependency
480 of biotic resistance, where competition dynamics can shift depending on environmental
481 conditions and resource levels.

482 *Reduced soil bacterial diversity benefits Senecio performance under competition*

483 We observed distinct proportions of the most abundant bacterial phyla across
484 treatments, mainly *Pseudomonadota* and *Actinomycetota*, with South African soils
485 exhibiting slightly higher levels of *Actinomycetota*, while Italian soils had more
486 *Pseudomonadota*. *Actinomycetota*, a highly diverse and globally widespread bacterial
487 phylum (van Bergeijk et al. 2020), along with *Pseudomonadota*, is commonly found
488 across various habitats in Europe (Labouyrie et al. 2023). Autoclaved soil showed a
489 significantly lowered bacterial diversity compared to wild soil, at least in the control and
490 South African communities. In those conditions, bacterial communities could not recover
491 their original diversity after the sterilizing treatment which eliminated to some degree the
492 existing soil microbial community. The composition of the plant community competing
493 with *S. inaequidens* also affected soil microbial diversity, with the South African
494 communities supporting higher bacterial diversity in wild soil compared to other
495 treatments. Control pots with *S. inaequidens* individuals growing alone maintained the
496 most unique bacterial communities, especially in autoclaved soils, whereas soils with
497 competing native plants showed greater similarity in community structure. For instance,

498 control pots with autoclaved soil were characterized by a generally higher abundance of
499 *Nocardioides* (10.0% on average) compared to the other treatments. Such results
500 suggest that plant community identity influences microbial assemblages, even after a
501 sterilization treatment. One possible explanation for the observed patterns is that
502 introduced plants like *S. inaequidens* may bring along their associated bacteria (e.g. in
503 the seeds), which can aid their invasion by enhancing establishment, nutrient
504 acquisition, growth, or resistance to local biotic pressures (van der Putten et al. 2007; Le
505 Roux et al. 2017; Zhang et al. 2023).

506 Soil autoclaving did not have a significant direct effect on the overall competitive
507 response of *S. inaequidens* to competition, rather bacterial diversity was found to
508 influence its performance. Autoclaved soils generally supported reduced bacterial
509 diversity, and lower bacterial diversity was associated with increased *S. inaequidens*
510 height. This finding is somewhat unexpected, given that higher microbial diversity is
511 typically associated with ecosystem stability and resilience (Ehrenfeld 2003; Wardle et
512 al. 2004). One possible explanation, consistent with our soil autoclaving results, is that
513 reduced microbial diversity may lower the presence or activity of pathogens and
514 competitors, thereby enabling *S. inaequidens* to allocate more resources toward growth.
515 This aligns with the enemy release hypothesis, which posits that invasive species may
516 escape their natural enemies in new environments, reducing their biotic resistance and
517 enhancing their performance (Keane and Crawley 2002; Heger et al. 2024). The
518 enormous diversity of soil microbial communities can harbor generalist pathogens that
519 affect invasive plants but also disadvantage native species through pathogen spillover,
520 especially if exotics are more tolerant (van der Putten et al. 2007; Dawson and Schrama

521 2016). Therefore, another possible explanation for the increased height of *S.*
522 *inaequidens* in soils with low bacterial diversity is that native plants may be less vigorous
523 or competitive under these conditions, possibly due to a shortage of beneficial microbes
524 or disruptions of commensalistic and symbiotic relationships between the soil microbial
525 community and the plants. With less competition from native plants, *S. inaequidens*
526 could allocate more resources to growth, leading to taller individuals. This is supported
527 by other research showing correlations between *S. inaequidens* traits, particularly those
528 related to competitive ability and resource allocation, and bacterial diversity (e.g.
529 Thébault et al. 2010). These findings suggest that shifts in soil microbial diversity could
530 influence *S. inaequidens* ability to outcompete native species, potentially by altering
531 nutrient availability, pathogen pressure, or the presence of beneficial microbial partners.

532 It is important to note that methods like autoclaving can alter soil chemistry, nutrient
533 availability, and physical structure, potentially confounding experimental results by
534 affecting both microbial communities and abiotic factors (Perkins et al. 2013). We note
535 that sterilization does not fully eliminate bacterial DNA, however, its influence is likely
536 minimal, as samples were collected when community shifts dominate and residual DNA
537 from cells killed ~90 days earlier is probably negligible. Finally, the 84-day duration of
538 the experiment provided valuable insights, though longer-term studies could offer a
539 more comprehensive understanding of plant-soil feedbacks and competitive dynamics
540 (Liu et al. 2024).

541

542

543 **Conclusions and implications for management**

544 Our study highlights the interplay between eco-evolutionary experience, plant
545 phylogenetic relationships and soil biotic conditions. By analysing the interactions
546 between this invasive species and plant communities from both its native and invasive
547 ranges, we provide insights into the possible mechanisms driving its invasion success,
548 which seems to be favoured by the inexperience of the community of the invasive range
549 with respect to the invader (*i.e.* naivety). Based on our findings, we argue that selecting
550 few phylogenetically related species at high abundances to outcompete *S. inaequidens*
551 could be a promising practice for management in areas under restoration. In particular,
552 the observation that *S. inaequidens* performs better in the presence of naïve species
553 and lower microbial diversity indicates that restoration efforts might benefit from
554 enhancing the competitive ability of native species and promoting microbial diversity.
555 This could involve the selection of native species that are closely related to the invader
556 or have strong competitive abilities and testing soil amendments to increase microbial
557 diversity and resilience. Furthermore, our findings suggest that management strategies
558 should also consider the composition and functional roles of native communities by
559 selecting multi-species suites of closely related competitors displaying similar trait
560 profiles, as well as the structure of soil microbial communities.

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565 References

- 566 Abbott KC, Karst J, Biederman LA, Borrett SR, Hastings A, Walsh V, Bever JD (2015)
567 Spatial Heterogeneity in Soil Microbes Alters Outcomes of Plant Competition. *Plos One*
568 10: e0125788. <https://doi.org/10.1371/journal.pone.0125788>
- 569 Blomberg SP, Garland T (2002) Tempo and mode in evolution: phylogenetic inertia,
570 adaptation and comparative methods. *Journal of Evolutionary Biology* 15: 899-910.
571 <https://doi.org/10.1046/j.1420-9101.2002.00472.x>
- 572 Borcard D, Gillet F, Legendre P (2018) Canonical ordination. In: *Numerical ecology with*
573 *R*. Springer (New York): 203-297.
- 574 Bray JR, Curtis JT (1957) An Ordination of the Upland Forest Communities of Southern
575 Wisconsin. *Ecological Monographs* 27: 325-349. <https://doi.org/10.2307/1942268>
- 576 Burns J, Winn A (2006) A comparison of plastic responses to competition by invasive
577 and non-invasive congeners in the Commelinaceae. *Biological Invasions* 8: 797-807.
578 <https://doi.org/10.1007/s10530-005-3838-5>
- 579 Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016)
580 DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*
581 13: 581-583. <https://doi.org/10.1038/nmeth.3869>
- 582 Callaway RM, Ridenour WM (2004) Novel weapons: invasive success and the evolution
583 of increased competitive ability. *Frontiers in Ecology and the Environment* 2: 436-443.
584 <https://doi.org/10.2307/3868432>
- 585 Callaway RM, Waller LP, Diaconu A, Pal R, Collins AR, Mueller-Schaerer H, Maron JL
586 (2011) Escape from competition: Neighbors reduce performance at home but not away.
587 *Ecology* 92: 2208-2213. <https://doi.org/10.1890/11-0518.1>
- 588 Cameron AC, Trivedi PK (1990) Regression-based tests for overdispersion in the
589 Poisson model. *Journal of Econometrics* 46: 347-364. [https://doi.org/10.1016/0304-4076\(90\)90014-k](https://doi.org/10.1016/0304-4076(90)90014-k)
- 591 Caño L, Escarré J, Vrieling K, Sans FX (2009) Palatability to a generalist herbivore,
592 defence and growth of invasive and native *Senecio* species: Testing the evolution of
593 increased competitive ability hypothesis. *Oecologia* 159: 95-106.
594 <https://doi.org/10.1007/s00442-008-1182-z>
- 595 Clarke KR (1993) Non-parametric multivariate analyses of changes in community
596 structure. *Australian Journal of Ecology* 18: 117-143. <https://doi.org/10.1111/j.1442-9993.1993.tb00438.x>
- 598 Daehler CC (2001) Darwin's Naturalization Hypothesis Revisited. *The American*
599 *Naturalist* 158: 324-330. <https://doi.org/10.1086/321316>

- 600 Darwin C (1859) The origin of species. J. Murray, London: 1-502.
- 601 Dawson W, Schrama M (2016) Identifying the role of soil microbes in plant invasions.
602 Journal of Ecology 104: 1211-1218. <https://doi.org/10.1111/1365-2745.12619>
- 603 Delory BM, Weidlich EWA, Kunz M, Neitzel J, Temperton VM (2019) The exotic species
604 *Senecio inaequidens* pays the price for arriving late in temperate European grassland
605 communities. Oecologia 191: 657-671. <https://doi.org/10.1007/s00442-019-04521-x>
- 606 Diez JM, Dickie I, Edwards G, Hulme PE, Sullivan JJ, Duncan RP (2010) Negative soil
607 feedbacks accumulate over time for non-native plant species. Ecology Letters 13: 803-
608 809. <https://doi.org/10.1111/j.1461-0248.2010.01474.x>
- 609 Dimande AFP, Botha CJ, Prozesky L, Bekker L, Rösemann GM, Labuschagne L, Retief
610 E (2007) The toxicity of *Senecio inaequidens* DC. Journal of the South African
611 Veterinary Association 78: 142-148. <https://doi.org/10.4102/jsava.v78i3.302>
- 612 Divíšek J, Chytrý M, Beckage B, Gotelli NJ, Lososová Z, Pyšek P, Richardson DM,
613 Molofsky J (2018) Similarity of introduced plant species to native ones facilitates
614 naturalization, but differences enhance invasion success. Nature Communications 9:
615 4631. <https://doi.org/10.1038/s41467-018-06995-4>
- 616 Dostál P (2011) Plant competitive interactions and invasiveness: searching for the
617 effects of phylogenetic relatedness and origin on competition intensity. The American
618 Naturalist 177: 655-667. <https://doi.org/10.1086/659060>
- 619 Du Preez P, Bredenkamp GJ (1991) Vegetation classes of the southern and eastern
620 Orange Free State (Republic of South Africa) and the highlands of Lesotho. Navorsinge
621 van die Nasionale Museum 7: 501-526.
- 622 Ehrenfeld JG (2003) Effects of Exotic Plant Invasions on Soil Nutrient Cycling
623 Processes. Ecosystems 6: 503-523. <https://doi.org/10.1007/s10021-002-0151-3>
- 624 Eppinga MB, Rietkerk M, Dekker SC, De Ruiter PC, Van der Putten WH (2006)
625 Accumulation of local pathogens: a new hypothesis to explain exotic plant invasions.
626 Oikos 114: 168-176. <https://doi.org/10.1111/j.2006.0030-1299.14625.x>
- 627 Ernst W (1998) Invasion, dispersal and ecology of the South African neophyte *Senecio*
628 *inaequidens* in The Netherlands: from wool alien to railway and road alien. Acta
629 Botanica Neerlandica 47: 131-151.
- 630 Fahey C, Flory SL (2022) Soil microbes alter competition between native and invasive
631 plants. Journal of Ecology 110: 404-414. <https://doi.org/10.1111/1365-2745.13807>
- 632 Frank JA, Reich CI, Sharma S, Weisbaum JS, Wilson BA, Olsen GJ (2008) Critical
633 evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes.
634 Applied and Environmental Microbiology 74: 2461-2470.
635 <https://doi.org/10.1128/aem.02272-07>

- 636 Fristoe TS, Chytrý M, Dawson W, Essl F, Heleno R, Kreft H, Maurel N, Pergl J, Pyšek
637 P, Seebens H, Weigelt P, Vargas P, Yang Q, Attorre F, Bergmeier E, Bernhardt-
638 Römermann M, Biurrun I, Boch S, Bonari G, Botta-Dukát Z, Bruun HH, Byun C, Čarni A,
639 Carranza ML, Catford JA, Cerabolini BEL, Chacón-Madrigal E, Ciccarelli D,
640 Čušterevska R, de Ronde I, Dengler J, Golub V, Haveman R, Hough-Snee N, Jandt U,
641 Jansen F, Kuzemko A, Kůzmič F, Lenoir J, Macanović A, Marcenò C, Martin AR,
642 Michaletz ST, Mori AS, Niinemets Ü, Peterka T, Pielech R, Rašomavičius V, Rūsiņa S,
643 Dias AS, Šibíková M, Šilc U, Stanisci A, Jansen S, Svenning JC, Swacha G, van der
644 Plas F, Vassilev K, van Kleunen M (2021) Dimensions of invasiveness: Links between
645 local abundance, geographic range size, and habitat breadth in Europe's alien and
646 native floras. *Proceedings of the National Academy of Sciences* 118: e2021173118.
647 <https://doi.org/10.1073/pnas.2021173118>
- 648 Funk JL, Vitousek PM (2007) Resource-use efficiency and plant invasion in low-
649 resource systems. *Nature* 446: 1079-1081. <https://doi.org/10.1038/nature05719>
- 650 Funk JL, Cleland EE, Suding KN, Zavaleta ES (2008) Restoration through reassembly:
651 plant traits and invasion resistance. *Trends in Ecology & Evolution* 23: 695-703.
652 <https://doi.org/10.1016/j.tree.2008.07.013>
- 653 Gandolfi I, Canedoli C, Rosatelli A, Covino S, Cappelletti D, Sebastiani B, Tatangelo V,
654 Corengia D, Pittino F, Padoa-Schioppa E, Báez-Matus X, Hernández L, Seeger M,
655 Saati-Santamaría Z, García-Fraile P, López-Mondéjar R, Ambrosini R, Papacchini M,
656 Franzetti A (2024) Microbiomes of urban trees: unveiling contributions to atmospheric
657 pollution mitigation. *Frontiers in Microbiology* 15: 1470376.
658 <https://doi.org/10.3389/fmicb.2024.1470376>
- 659 Gentili R, Casati E, Ferrario A, Monti A, Montagnani C, Caronni S, Citterio S (2020)
660 Vegetation cover and biodiversity levels are driven by backfilling material in quarry
661 restoration. *Catena* 195: 104839. <https://doi.org/10.1016/j.catena.2020.104839>
- 662 Goldberg DE (1990) Components of resource competition in plant communities. In:
663 Grace JB, Tilman D (Eds) *Perspectives of Plant Competition*. Academic Press
664 (Cambridge): 27-49.
- 665 Harkes P, Verhoeven A, Sterken MG, Snoek LB, van den Elsen S, Mooijman PJW,
666 Quist CW, Vervoort MTW, Bakker J, Mulder C, Helder J (2017) The differential impact
667 of a native and a non-native ragwort species (*Senecioneae*) on the first and second
668 trophic level of the rhizosphere food web. *Oikos* 126: 1790-1803.
669 <https://doi.org/10.1111/oik.04530>
- 670 Heger T, Treppl L (2003) Predicting Biological Invasions. *Biological Invasions* 5: 313-321.
671 <https://doi.org/10.1023/b:binv.0000005568.44154.12>
- 672 Heger T, Böhmer HJ (2005) The Invasion of Central Europe by *Senecio Inaequidens*
673 DC. — A Complex Biogeographical Problem. *Erdkunde* 59: 34-49.
674 <https://doi.org/10.3112/erdkunde.2005.01.03>

- 675 Heger T, Jeschke JM, Bernard-Verdier M, Musseau CL, Mietchen D (2024) Hypothesis
676 Description: Enemy Release Hypothesis. *Research Ideas and Outcomes* 10: e107393.
677 <https://doi.org/10.3897/rio.10.e107393>
- 678 Heger T, Bernard-Verdier M, Gessler A, Greenwood AD, Grossart HP, Hilker M, Keinath
679 S, Kowarik I, Kueffer C, Marquard E (2019) Towards an integrative, eco-evolutionary
680 understanding of ecological novelty: Studying and communicating interlinked effects of
681 global change. *BioScience* 69: 888-899. <https://doi.org/10.1093/biosci/biz095>
- 682 Joosten L, van Veen JA (2011) Defensive properties of pyrrolizidine alkaloids against
683 microorganisms. *Phytochemistry Reviews* 10: 127-136. [https://doi.org/10.1007/s11101-](https://doi.org/10.1007/s11101-010-9204-y)
684 [010-9204-y](https://doi.org/10.1007/s11101-010-9204-y)
- 685 Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release
686 hypothesis. *Trends in Ecology & Evolution* 17: 164-170. [https://doi.org/10.1016/s0169-](https://doi.org/10.1016/s0169-5347(02)02499-0)
687 [5347\(02\)02499-0](https://doi.org/10.1016/s0169-5347(02)02499-0)
- 688 Labouyrie M, Ballabio C, Romero F, Panagos P, Jones A, Schmid MW, Mikryukov V,
689 Dulya O, Tedersoo L, Bahram M, Delgado-Baquerizo M (2023) Patterns in soil microbial
690 diversity across Europe. *Nature Communications* 14: 3311.
691 <https://doi.org/10.1038/s41467-023-37937-4>
- 692 Lafuma L, Balkwill K, Imbert E, Verlaque R, Maurice S (2003) Ploidy level and origin of
693 the European invasive weed *Senecio inaequidens* (Asteraceae). *Plant Systematics and*
694 *Evolution* 243: 59-72. <https://doi.org/10.1007/s00606-003-0075-0>
- 695 Le Roux JJ, Hui C, Keet JH, Ellis AG (2017) Co-introduction vs ecological fitting as
696 pathways to the establishment of effective mutualisms during biological invasions. *New*
697 *Phytologist* 215: 1354-1360. <https://doi.org/10.1111/nph.14593>
- 698 Li SP, Guo T, Cadotte MW, Chen YJ, Kuang JL, Hua ZS, Zeng Y, Song Y, Liu Z, Shu
699 WS, Li JT (2015) Contrasting effects of phylogenetic relatedness on plant invader
700 success in experimental grassland communities. *Journal of Applied Ecology* 52: 89-99.
701 <https://doi.org/10.1111/1365-2664.12365>
- 702 Liu Z, Liang Y, Wei C, Zhu B, Chen W, Lu X (2024) Phylogenetic relatedness, not
703 geographic origin, shapes traits across ontogenetic stages for common native and
704 exotic plants in subtropical China. *Journal of Ecology* 112: 2917-2928.
705 <https://doi.org/10.1111/1365-2745.14432>
- 706 López-García MC, Maillet J (2005) Biological characteristics of an invasive south
707 African species. *Biological Invasions* 7: 181-194. [https://doi.org/10.1007/s10530-004-](https://doi.org/10.1007/s10530-004-8978-5)
708 [8978-5](https://doi.org/10.1007/s10530-004-8978-5)
- 709 Ma C, Li SP, Pu Z, Tan J, Liu M, Zhou J, Li H, Jiang L (2016) Different effects of
710 invader–native phylogenetic relatedness on invasion success and impact: a meta-
711 analysis of Darwin's naturalization hypothesis. *Proceedings of the Royal Society B* 283:
712 20160663. <https://doi.org/10.1098/rspb.2016.0663>

- 713 MacDougall AS, Gilbert B, Levine JM (2009) Plant invasions and the niche. *Journal of*
714 *Ecology* 97: 609-615. <https://doi.org/10.1111/j.1365-2745.2009.01514.x>
- 715 Mangla S, Callaway RM (2008) Exotic invasive plant accumulates native soil pathogens
716 which inhibit native plants. *Journal of Ecology* 96: 58-67. <https://doi.org/10.1111/j.1365-2745.2007.01312.x>
717
- 718 Misuri A, Ferretti G, Lazzaro L, Mugnai M, Viciani D (2020) Investigations on ecology
719 and distribution of *senecio inaequidens* DC. (Asteraceae) in Tuscany (Italy). *Atti Della*
720 *Societa Toscana Di Scienze Naturali, Memorie Serie B* 127: 137-150.
721 <https://doi.org/10.2424/ASTSN.M.2020.10>
- 722 Mitchell CE, Power AG (2003) Release of invasive plants from fungal and viral
723 pathogens. *Nature* 421: 625-627. <https://doi.org/10.1038/nature01317>
- 724 Mitchell CE, Agrawal AA, Bever JD, Gilbert GS, Hufbauer RA, Klironomos JN, Maron
725 JL, Morris WF, Parker IM, Power AG, Seabloom EW, Torchin ME, Vázquez DP (2006)
726 Biotic interactions and plant invasions. *Ecology Letters* 9: 726-740.
727 <https://doi.org/10.1111/j.1461-0248.2006.00908.x>
- 728 Novoa A, Richardson DM, Pyšek P, Meyerson LA, Bacher S, Canavan S, Catford JA,
729 Čuda J, Essl F, Foxcroft LC, Genovesi P, Hirsch H, Hui C, Jackson MC, Kueffer C, Le
730 Roux JJ, Measey J, Mohanty NP, Moodley D, Müller-Schärer H, Packer JG, Pergl J,
731 Robinson TB, Saul WC, Shackleton RT, Visser V, Weyl OLF, Yannelli FA, Wilson JR
732 (2020) Invasion syndromes: a systematic approach for predicting biological invasions
733 and facilitating effective management. *Biological Invasions* 22: 1801-1820.
734 <https://doi.org/10.1007/s10530-020-02220-w>
- 735 Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R,
736 Solymos P, Stevens M, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker D,
737 Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R,
738 Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlenn D, Ouellette M, Ribeiro
739 Cunha E, Smith T, Stier A, Ter Braak C, Weedon J (2022) *vegan: Community Ecology*
740 *Package*. R package version 2.6-4. <https://CRAN.R-project.org/package=vegan>
- 741 Park DS, Potter D (2013) A test of Darwin's naturalization hypothesis in the thistle tribe
742 shows that close relatives make bad neighbors. *Proceedings of the National Academy*
743 *of Sciences* 110: 17915-17920. <https://doi.org/10.1073/pnas.1309948110>
- 744 Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-
745 Harte MS, Cornwell WK, Craine JM, Gurvich DE, Urcelay C, Veneklaas EJ, Reich PB,
746 Poorter L, Wright IJ, Ray P, Enrico L, Pausas JG, de Vos AC, Buchmann N, Funes G,
747 Quétier F, Hodgson JG, Thompson K, Morgan HD, ter Steege H, van der Heijden MGA,
748 Sack L, Blonder B, Poschlod P, Vaieretti MV, Conti G, Staver AC, Aquino S,
749 Cornelissen JHC (2013) New handbook for standardised measurement of plant
750 functional traits worldwide. *Australian Journal of Botany* 61: 167-234.
751 <https://doi.org/10.1071/bt12225>

- 752 Perkins LB, Blank RR, Ferguson SD, Johnson DW, Lindemann WC, Rau BM (2013)
753 Quick start guide to soil methods for ecologists. *Perspectives in Plant Ecology,*
754 *Evolution and Systematics* 15: 237-244. <https://doi.org/10.1016/j.ppees.2013.05.004>
- 755 Prinzing A (2001) The niche of higher plants: evidence for phylogenetic conservatism.
756 *Proceedings of the Royal Society of London B: Biological Sciences* 268: 2383-2389.
757 <https://doi.org/10.1098/rspb.2001.1801>
- 758 Quaglini LA, Yannelli FA, Fasano F, Montagnani C, Caronni S, Citterio S, Gentili R
759 (2025) Abiotic and biotic factors shape the invasion success of the alien plant species
760 *Senecio inaequidens* (Asteraceae) in Northern Italy. *Journal of Plant Ecology: rtaf035.*
761 <https://doi.org/10.1093/jpe/rtaf035>
- 762 R Core Team (2023) R: A language and environment for statistical computing. R
763 Foundation for Statistical Computing, Vienna, Austria. <http://www.r-project.org/>
- 764 Saul WC, Jeschke J (2015) Eco-evolutionary experience in novel species interactions.
765 *Ecology Letters* 18: 236-245. <https://doi.org/10.1111/ele.12408>
- 766 Saul WC, Jeschke J, Heger T (2013) The role of eco-evolutionary experience in
767 invasion success. *NeoBiota* 17: 57-74. <https://doi.org/10.3897/neobiota.17.5208>
- 768 Scherber C, Crawley MJ, Porembski S (2003) The effects of herbivory and competition
769 on the invasive alien plant *Senecio inaequidens* (Asteraceae). *Diversity and*
770 *Distributions* 9: 415-426. <https://doi.org/10.1046/j.1472-4642.2003.00049.x>
- 771 Thébault A, Frey B, Mitchell EAD, Buttler A (2010) Species-specific effects of
772 polyploidisation and plant traits of *Centaurea maculosa* and *Senecio inaequidens* on
773 rhizosphere microorganisms. *Oecologia* 163: 1011-1020.
774 <https://doi.org/10.1007/s00442-010-1598-0>
- 775 Thoden TC, Boppré M, Hallmann J (2009) Effects of pyrrolizidine alkaloids on the
776 performance of plant-parasitic and free-living nematodes. *Pest Management Science*
777 65: 823-830. <https://doi.org/10.1002/ps.1764>
- 778 Thuiller W, Gallien L, Boulangéat I, De Bello F, Münkemüller T, Roquet C, Lavergne S
779 (2010) Resolving Darwin's naturalization conundrum: a quest for evidence. *Diversity*
780 *and Distributions* 16: 461-475. <https://doi.org/10.1111/j.1472-4642.2010.00645.x>
- 781 Torres N, Herrera I, Fajardo L, Bustamante RO (2021) Meta-analysis of the impact of
782 plant invasions on soil microbial communities. *BMC Ecology and Evolution* 21: 172.
783 <https://doi.org/10.1186/s12862-021-01899-2>
- 784 van Bergeijk DA, Terlouw BR, Medema MH, van Wezel GP (2020) Ecology and
785 genomics of Actinobacteria: new concepts for natural product discovery. *Nature*
786 *Reviews Microbiology* 18: 546-558. <https://doi.org/10.1038/s41579-020-0379-y>

- 787 Van De Walle R, Massol F, Vandegehuchte ML, Bonte D (2022) The distribution and
788 impact of an invasive plant species (*Senecio inaequidens*) on a dune building engineer
789 (*Calamagrostis arenaria*). *NeoBiota* 72: 1-23. <https://doi.org/10.3897/neobiota.72.78511>
- 790 van der Putten WH, Klironomos JN, Wardle DA (2007) Microbial ecology of biological
791 invasions. *The ISME Journal* 1: 28-37. <https://doi.org/10.1038/ismej.2007.9>
- 792 Wardle DA, Bardgett RD, Klironomos JN, Setälä H, van der Putten WH, Wall DH (2004)
793 Ecological Linkages Between Aboveground and Belowground Biota. *Science* 304:
794 1629-1633. <https://doi.org/10.1126/science.1094875>
- 795 Xiao S, Ni G, Callaway RM (2013) Models of Experimentally Derived Competitive
796 Effects Predict Biogeographical Differences in the Abundance of Invasive and Native
797 Plant Species. *Plos One* 8: e78625. <https://doi.org/10.1371/journal.pone.0078625>
- 798 Yannelli FA, Koch C, Jeschke JM, Kollmann J (2017) Limiting similarity and Darwin's
799 naturalization hypothesis: Understanding the drivers of biotic resistance against invasive
800 plant species. *Oecologia* 183: 775-784. <https://doi.org/10.1007/s00442-016-3798-8>
- 801 Yannelli FA, Dawson W, van Kleunen M, Jeschke JM, Heger T (2025) Hypothesis
802 Description: Darwin's Naturalisation Hypothesis. *Research Ideas and Outcomes* 11:
803 e140548. <https://doi.org/10.3897/rio.11.e140548>
- 804 Zanne AE, Tank DC, Cornwell WK, Eastman JM, Smith SA, FitzJohn RG, McGlinn DJ,
805 O'Meara BC, Moles AT, Reich PB, Royer DL, Soltis DE, Stevens PF, Westoby M,
806 Wright IJ, Aarssen L, Bertin RI, Calaminus A, Govaerts R, Hemmings F, Leishman MR,
807 Oleksyn J, Soltis PS, Swenson NG, Warman L, Beaulieu JM (2014) Three keys to the
808 radiation of angiosperms into freezing environments. *Nature* 506: 89-92.
809 <https://doi.org/10.1038/nature12872>
- 810 Zhang H, Li Q, Sun W, Guo J, Liu W, Zhao M (2023) Microbial communities in the
811 rhizosphere soil of *Ambrosia artemisiifolia* facilitate its growth. *Plant and Soil* 492: 353-
812 365. <https://doi.org/10.1007/s11104-023-06181-6>
- 813 Zheng YL, Burns JH, Liao ZY, Li YP, Yang J, Chen YJ, Zhang JL, Zheng YG (2018)
814 Species composition, functional and phylogenetic distances correlate with success of
815 invasive *Chromolaena odorata* in an experimental test. *Ecology Letters* 21: 1211-1220.
816 <https://doi.org/10.1111/ele.13090>
- 817