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

Lara A. Quaglino,  Florencia A Yannelli, Isabella Gandolfi, Andrea Franzetti, Sarah Caronni,  Chiara Montagnani,  Clinton Carbutt,  Jonathan M. Jeschke,  Sandra Citterio,  Rodolfo Gentili

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

3 Lara A. Quaglini<sup>1,2\*</sup>, Florencia A. Yannelli<sup>3,4,5\*</sup>, Isabella Gandolfi<sup>2</sup>, Andrea Franzetti<sup>2</sup>,  
4 Sarah Caronni<sup>2</sup>, Chiara Montagnani<sup>2</sup>, Clinton Carbutt<sup>6,7</sup>, Jonathan M. Jeschke<sup>3,4</sup>,  
5 Sandra Citterio<sup>2</sup>, Rodolfo Gentili<sup>2</sup>

6 \* These authors contributed equally to this work

7 <sup>1</sup> Natural History Museum of Milan, Corso Venezia 55, 20121, Milan, Italy

8 <sup>2</sup> Department of Earth and Environmental Sciences, Università degli Studi di Milano-  
9 Bicocca, Piazza della Scienza 1, 20126 Milan, Italy

10 <sup>3</sup> Department of Biology, Chemistry, Pharmacy, Institute of Biology, Freie Universität  
11 Berlin, Königin-Luise-Str. 1-3, 14195 Berlin, Germany

12 <sup>4</sup> Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Müggelseedamm  
13 310, 12587 Berlin, Germany

14 <sup>5</sup> Argentine Institute for Dryland Research, CONICET and Universidad Nacional de  
15 Cuyo, Av. Ruiz Leal s/n, 5500 Mendoza, Argentina

16 <sup>6</sup> School of Life Sciences, University of KwaZulu-Natal, Scottsville 3209, South Africa

17 <sup>7</sup> Scientific Services, Ezemvelo KZN Wildlife, Cascades 3202, South Africa

18 **Corresponding author:** Florencia A. Yannelli, [florenciayannelli@gmail.com](mailto:florenciayannelli@gmail.com)

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## 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  $-20^{\circ}\text{C}$  until processing.

241 *DNA extraction and Next Generation sequencing*

242 Genomic DNA was extracted using the FastDNA<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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.

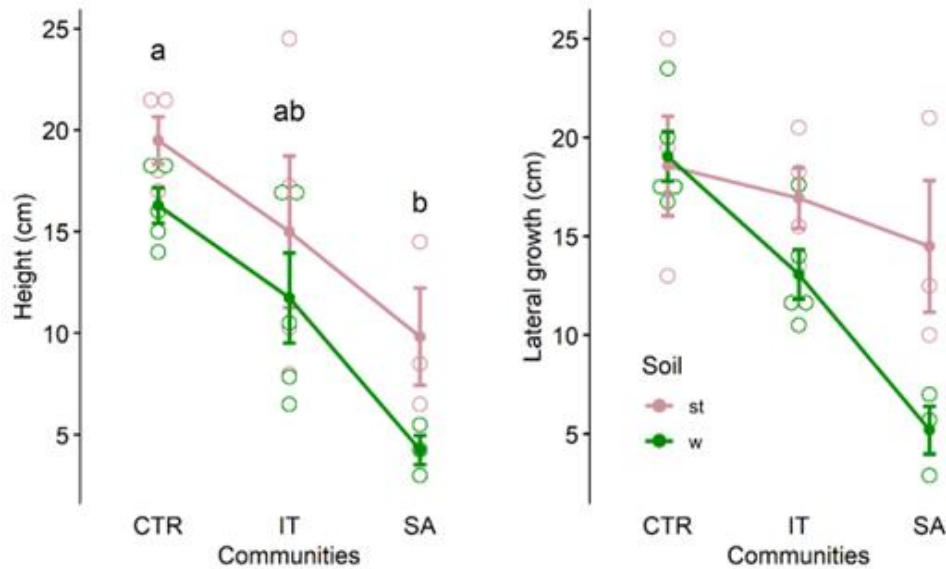
279 We used Non-metric Multidimensional Scaling (NMDS) analysis based on Bray-Curtis  
280 dissimilarity distances (Bray and Curtis 1957) to visualize differences in soil bacterial  
281 community structure according to the treatments using the *metaMDS* function. We  
282 carried out a PERMANOVA test 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*

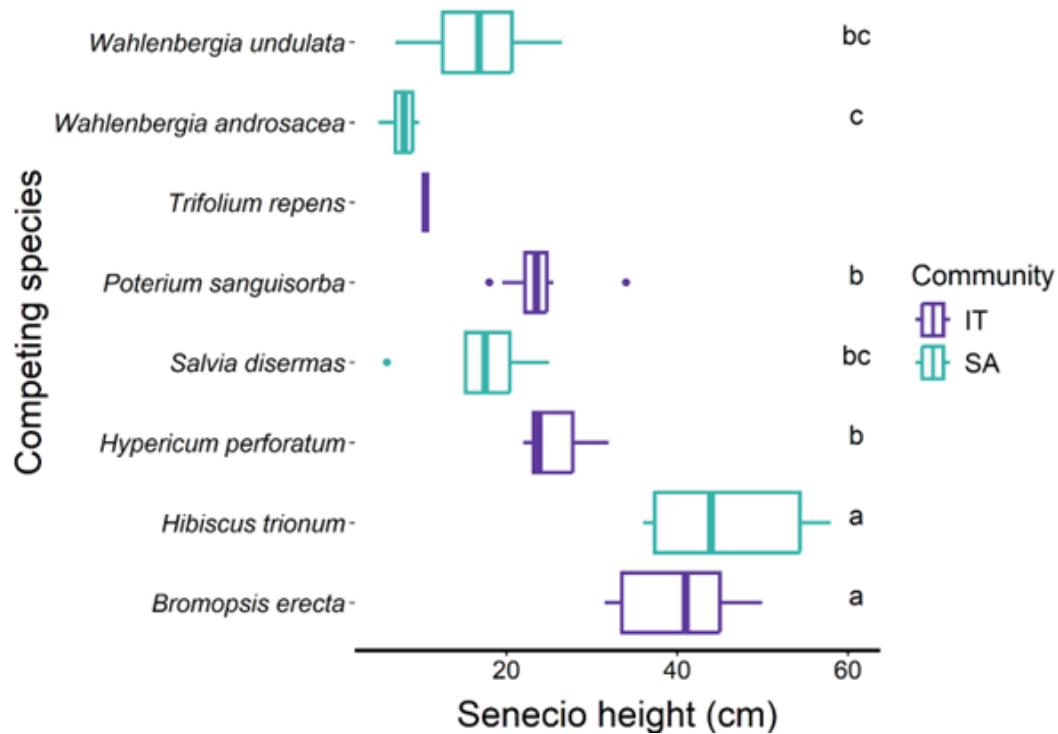
297 *Senecio inaequidens* performance was affected by the community it was growing along  
298 with more than soil conditions, compared to the control treatment in pots where it grew  
299 without competition. Specifically, in terms of *S. inaequidens* maximum height at day 84,  
300 only community identity 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

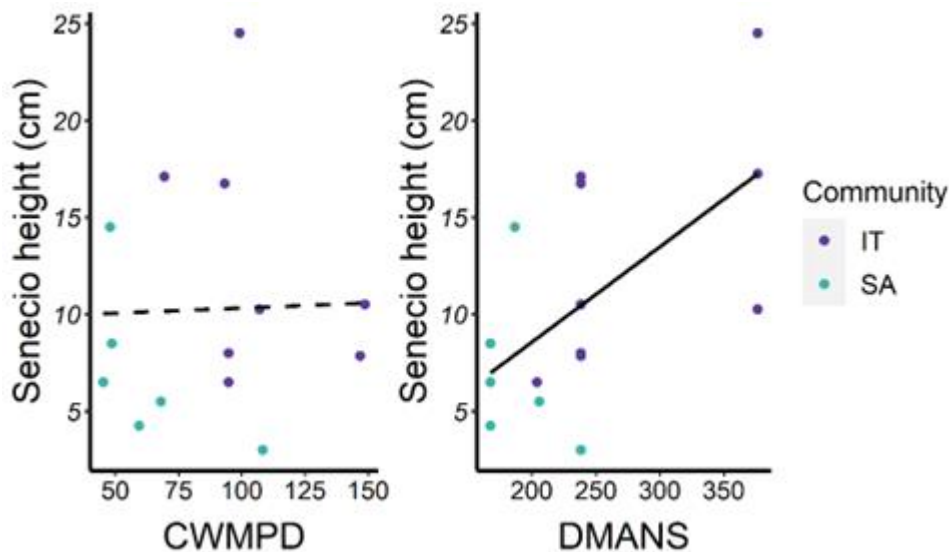
329 dominance of one native species and the mortality of all other natives in one

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).

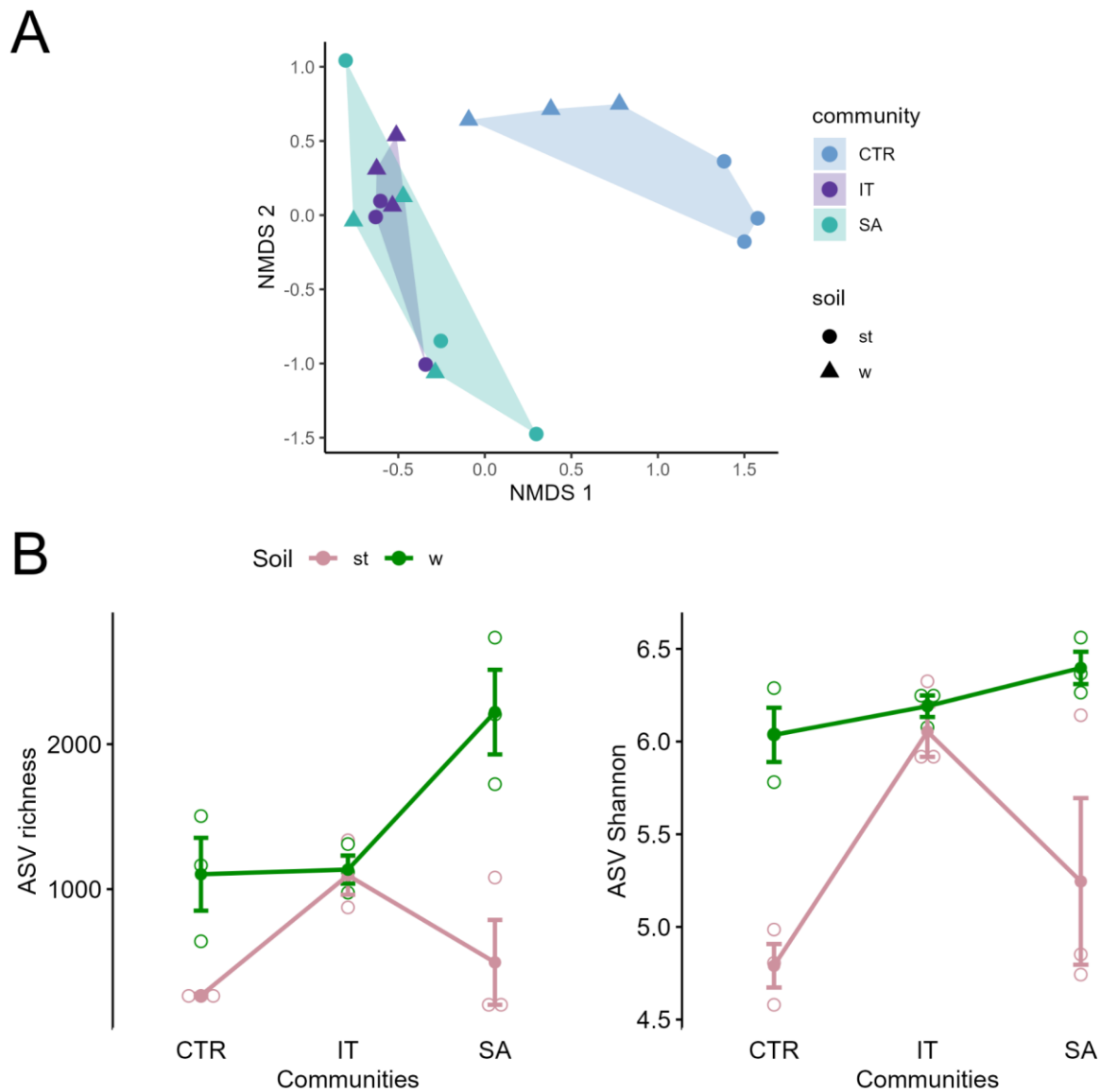
349

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

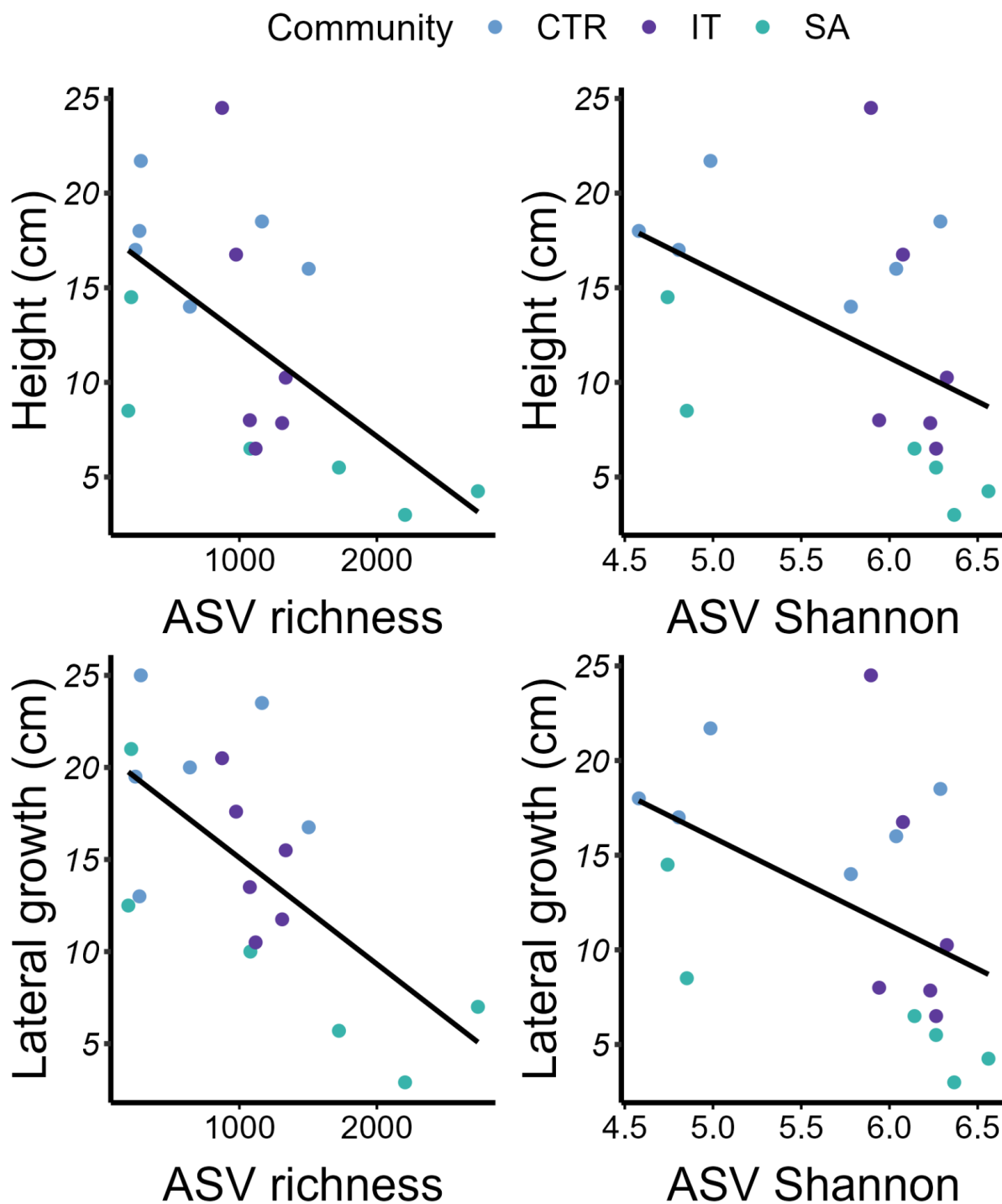
373 **Figure 4.** Panel A: Two-dimensional plot of our non-metric multidimensional scaling  
 374 analysis (NMDS) for bacterial ASVs. The control treatment CTR is shown in light blue,  
 375 the naïve community from the invasive range IT in purple, and the experienced  
 376 community in the native range 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:  $\text{Adj-R}^2(\text{ASV richness}) = 0.32, p < 0.01, \text{Adj-R}^2(\text{ASV}$   
 404  $\text{Shannon}) = 0.18, p = 0.04$ , LM lateral growth:  $\text{Adj-R}^2(\text{ASV richness}) = 0.39, p < 0.01,$   
 405  $\text{Adj-R}^2(\text{ASV Shannon}) = 0.14, p = 0.067$ ). 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 (Divíšek 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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