Invasive populations of *Senecio pterophorus* are neither more productive nor more plastic than the native populations

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La realització d'aquest treball va començar a finals de Desembre de 2012 amb la introducció de l'alumne al context de l'estudi, la presentació l'equip de treball de la Unitat de Toxicologia (Facultat de Veterinària) i una visita als camps experimentals on es va dur a terme el *common garden* de l'estudi. L'alumne també va realitzar una visita a títol personal per la zona del riu Ripoll on es poden trobar algunes poblacions de *Senecio pterophorus* introduïdes a Europa. A partir del gener de 2013 l'alumne es va dedicar a realitzar el treball de laboratori, processant les mostres de fulles que s'enviarien a l'estranger per a l'anàlisi de la composició isotòpica de carboni i contingut total de carboni i nitrogen. Una vegada realitzades les tasques de laboratori es va continuar amb el tractament de les bases de dades pre-existents, l'anàlisi estadístic d'aquestes, la interpretació dels resultats i la redacció progressiva del manuscrit presentat. El format de l'article elaborat ha estat elaborat seguint les instruccions de publicació de la revista Biological Invasions (Springer)

Contribució de l'alumne a les diferents parts del treball realitzat:

- **Preparació del *common garden*/mostreigs de camp:**
  Realitzat per altres membres de l'equip. La realització del *common garden*, l'obtenció de dades de camp i la presa de mostres es va realitzar al llarg dels anys 2011-2012.

- **Anàlisis de laboratori:**
  Parcialment realitzat per part de l'alumne. Les tasques d'obtenció de les mesures de biomassa i altres variables de l'experiment es van realitzar al llarg dels anys 2011-2012 per altres membres de l'equip. Les tasques de laboratori referents a la preparació de les mostres de fulles per a les anàlisis isotòpiques es van realitzar per part de l'alumne.

- **Recopilació de bases de dades:**
  Parcialment realitzat per part de l'alumne. L'alumne va completar les bases de dades elaborades durant 2011-2012 amb la informació dels resultats de les anàlisis isotòpiques realitzats.

- **Tractaments estadístics:**
  Totalment realitzat per part de l'alumne i supervisat pels directors del treball

- **Elaboració de models:**
  Totalment realitzat per part de l'alumne i supervisat pels directors del treball

- **Redacció del manuscrit, taules i figures:**
  Totalment realitzat per part de l'alumne i supervisat pels directors del treball

1   **Invasive populations of *Senecio pterophorus* are neither more productive nor more**

2   **plastic than the native populations.**

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17   **Abstract**

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19   Successful plant invaders may have specific morphological and physiological

20   traits that promote invasion in a new habitat. The Evolution of Increased Competitive

21   Ability (EICA) hypothesis predicts that plants released from natural enemies in the

22   introduced habitats are more competitive and perform better than plants from the native

23   populations. An increased phenotypic plasticity may also favour invasion because it

24   allows plants to function under a wider range of environments. In this study we used

25   *Senecio pterophorus* (Asteraceae) to test whether introduced plant populations are 1)

26   more competitive and 2) more plastic compared with the native populations. We

27   conducted a common garden experiment using plants from the native range (South

28   Africa, Eastern Cape), an expanded range (South Africa, Western Cape) and two

29   introduced ranges (Australia and Europe) under different conditions of water

30   availability. Contrary to the EICA and the increased plasticity hypotheses, plants from

31   the invasive and expanded populations grew less and responded less to watering than

32   those from their native range. These results may be caused by a depleted competition as

33   well as the presence of genetic bottlenecks in the newly invaded areas.

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36   **Keywords: biological** invasions, alien species, EICA hypothesis, phenotypic plasticity,

37   *Senecio pterophorus*.

Introduction

The introduction of novel plant species can alter the structure, function and dynamics of an ecosystem, endangering the survival of native species (Mack et al. 2000; Pimentel et al. 2001). The number of introductions of exotic plant species has risen dramatically during the last decades (Rejmánek et al. 2005). However, only few of the introduced species get established and spread into the new habitat becoming successful invaders (Williamson 1996; Kolar and Lodge 2001). Determining what traits are related to a higher invasive potential provides useful information to improve management and predict the outcomes of future invasions (Van Wilgen et al. 2001; Sol et al. 2012). The characterization of the invasive species, however, has proved difficult in part because successful strategies may vary among ecosystem types (Sakai et al. 2001; Pyšek and Richardson 2007).

One mechanism increasingly explored during the last decade as an important determinant of invasion success is the rapid adaptation of exotic plants to the new environmental conditions (Thompson 1998, Mooney and Cleland 2001; Sakai et al. 2001; Reznick and Ghalambor 2001). Plant genotypes with morphological and physiological traits that promote invasion, such as a higher RGR, biomass, reproductive capacity, competitive ability and overall fitness, may increase their frequency in the newly established populations as a result of natural selection (Crawley 1987; Lachmuth et al. 2010; Richards et al. 2006). The increase in plant performance in alien populations could be driven by the release of natural herbivores from the habitat of origin (Keane and Crawley 2002). The Evolution of Increased Competitive Ability hypothesis (EICA) (Blossey and Notzold 1995) predicts that, in the absence of herbivores, genotypes allocating more resources to growth and reproduction and less to chemical defences

63 would be favoured over less competitive and more defended plants, increasing their

64 frequency in the population .

65   To date, the EICA hypothesis has only been supported by some experimental

66 studies, as plants with a greater competitive ability have been observed in some invasive

67 areas (Blossey and Notzold 1995; Jakobs et al. 2004; Willis and Blossey 1999), but not

68 by others (Van Kleunen and Schmid 2003; Vilà et al. 2003; Willis et al. 2000). This

69 diversity of results may occur, at least in part, because tests of the EICA hypothesis

70 normally use relatively low sample sizes not covering the whole distributional area of

71 the plant species in their native and invasive ranges. Common garden experiments

72 comparing genetic differences between native and exotic plants across a low number of

73 populations may not use the appropriate controls, particularly when plant introduction

74 pathways are unknown (Bossdorf et al. 2005). An optimal test to the EICA should

75 evaluate differences in plant traits between the introduced populations and their founder

76 populations at the native area or, alternatively, cover a substantial part of the plant

77 biogeographical distribution to incorporate large amounts of genetic variation. To our

78 knowledge, no study has been performed to date testing the EICA hypothesis across the

79 entire known geographical distribution of a species.

80   Invasion success may also be determined by changes in the *plastic response* of

81 traits related to plant performance (Richards et al. 2006). Phenotypic plasticity, which is

82 the ability of an organism to express distinct phenotypes depending on the

83 environmental conditions, expands the ecological niche of a species facilitating

84 colonization of novel habitats (Bradshaw 1965, Richards et al. 2006). Plants with high

85 phenotypic plasticity could better adapt to a variety of environments compared with

86 plants with low plasticity (Berg and Ellers 2010, Richards et al. 2006). Accordingly,

87 since phenotypic plasticity is a genetically-based subject to selection, invasive plants

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88   may evolve an elevated plasticity in comparison to plants from the habitat of origin

89   (Kaufman and Smouse 2001; Bossdorf et al. 2005). The hypothesis of the evolution of

90   increased phenotypic plasticity after invasion, however, has been scarcely explored

91   (Bossdorf et al. 2005; Vanderhoeven et al. 2010; Godoy et al. 2011; Godoy et al. 2012).

92   *Senecio pterophorus* (Asteraceae) is a perennial shrub native to the Eastern Cape

93   and KwaZulu-Natal Provinces in South Africa that expanded its distribution to the

94   Western Cape a century ago (Levyns 1950). *S. pterophorus* was introduced to Australia

95   (> 70-100 years ago) and Europe (> 30 years ago), probably by wool commerce

96   (Castells et al. 2013). An analysis of the insect seed predation in *S. pterophorus* across

97   the native, the expanded and the two invasive ranges, showed that plants from the

98   introduced areas were released from herbivores in comparison to the native range, and

99   this release was more intense in Europe, the region with a shorter introduction time

100   (Castells et al. 2013).

101   Here we used *Senecio pterophorus* as a model species to determine whether

102   introduced plants released from natural enemies have evolved after invasion to a higher

103   performance, as predicted by the EICA hypothesis, and to a higher plasticity, as

104   predicted by the increased plasticity hypothesis. We conducted a common garden

105   experiment using 47 populations of *S. pterophorus* from the native, expanded and two

106   introduced ranges spanning the entire known distributional area of this species (Castells

107   et al. 2013). According to the EICA hypothesis, *S. pterophorus* from the introduced

108   populations (Australia, Europe) should have a higher performance in comparison to the

109   native populations, and this increase should be stronger in Europe, where the decrease

110   in herbivory has been more intense. We also expect a higher phenotypic plasticity in the

111   introduced populations in response to environmental stress compared with the native

112   populations.

114 **Materials and methods**

116 Model species and field sampling

118 *Senecio pterophorus* (Asteraceae) is a perennial shrub of 0.4 to 2 m in height.
119 Leaves are lanceolate to linear with serrated margins 5 to 14 cm long and 0.5 to 2.5 cm
120 wide. Each individual plant produces annually up to 1200 capitula (Morante et al.
121 unpublished). The capitula are grouped into terminal corymbose inflorescences with 8-
122 13 ray deep yellow florets of 2-4 mm in its periphery (Hilliard 1977; Pino et al. 2000).

123 *S. pterophorus* is native to the Natal province in Eastern Cape, South Africa
124 (Hilliard 1977) and was introduced into the Western Cape Province circa 1918 (Levyns
125 1950). The first reference of *S. pterophorus* in Australia is since 1908, but it became
126 invasive in 1930 along the southern coast of Australia (Parsons and Cuthbertson 1992).
127 Both South African and Australian *S. pterophorus* populations colonize disturbed
128 environments, such as roads and forest margins and grasslands (Parsons and
129 Cuthbertson 1992). In 1994, *S. pterophorus* was classified as a Declared Noxious Weed
130 subject to eradication by the Department of Environment and Primary Industries,
131 Victoria (Australia).

132 In Europe, *S. pterophorus* was first found in 1913 in the United Kingdom as an
133 adventitious and rare species in field margins (Stace 1997; Preston et al. 2002), but its
134 presence was erratic and infrequent and it is now considered extinct in that country
135 (Castells et al. 2013). In continental Europe *S. pterophorus* was first found near
136 Barcelona, in the north-eastern Iberian Peninsula, in 1982 (Casasayas 1989) and on the
137 ligurian coast in north western Italy in 1990 (Barberis et al. 1998). Due to the ability to

138   colonize river beds of the Besòs and Tordera river basins near Barcelona (Casasayas

139   1989; Pino et al. 2000; Chamorro et al. 2006) *S. pterophorus* has been recently

140   catalogued as an invasive species (Andreu et al. 2012).

141   Seeds of *Senecio pterophorus* were sampled in 2009 and 2010 in the native

142   range (Eastern Cape in South Africa), the expanded range (Western Cape in South

143   Africa) and two invasive ranges (Australia and Europe). This sampling was part of a

144   larger project to test the Enemy Release hypothesis (Castells et al. 2013). Populations

145   were chosen at least 30 km apart in South Africa and Australia, and at least 5 km apart

146   in Europe, where the plant is more localized. More details on the sampling procedure

147   can be found in Castells et al. (2013). Four additional populations not included in

148   Castells et al. (2013) were sampled in January 2010 to increase the number of

149   populations in central parts of the Eastern Cape Province. On the contrary, populations

150   from Liguria (Italy) could not be included in the present study. A total of 47 populations

151   (18 from the native range in South Africa, 5 from the extended range in South Africa,

152   12 from Australia and 12 from Europe) throughout the species' known distributional

153   area were used for this study (Fig. 1, Table S1).

154

155   Common garden experiment

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157   In November 2010, seeds from 6 individuals from each sampled population (47

158   populations x 6 individuals = 282 individuals, hereafter referred as mother plants) were

159   germinated separately in a mixture of *Sphagnum*, perlite and vermiculite (2:1:1)

160   neutralized at pH = 6 under standard conditions of temperature and humidity at the

161   greenhouse facilities of the Faculty of Biology, University of Barcelona (Spain). Soil

162   was watered regularly with a Hoagland nutrient solution. In February 2011, when the

163   seedlings had 4-5 true leaves (100 days old approximately), two seedlings from each

164   mother plant were transplanted to an experimental field at the Autonomous University

165   of Barcelona (41°29'53.3''N, 02°06'9.6''E). The experimental field is located in an old

166   cultivated area surrounded by a *Pinus halepensis* forest. The soil is a typic calcixerept

167   following the classification by the Natural Resources Conservation Service, U.S.

168   Department of Agriculture (Josep M. Alcañiz, personal comment). The mean annual

169   temperature in the area is 14.9 ºC and the mean annual precipitation 562.8 mm (Digital

170   Climatic Atlas of Catalonia, Ninyerola et al. 2003). The weather in 2011, when the

171   experiment was performed, was hotter and wetter than average with a mean annual

172   temperature of 15.6 ºC and 853.1 mm of annual rainfall (535.1 mm during the

173   experiment) (Meteorological Service of Catalonia, observatory code VT). Seedlings

174   from the same mother plant were full or half-sibs, but for the sake of simplicity we

175   referred them as half-sibs along this study.

176   The field was divided in six blocks of 58 m^2. Three blocks were assigned to a

177   drip irrigation treatment (Watered, W) set at 4.5 L/day/plant and three blocks were left

178   without irrigation but receiving rainfall (Not Watered, NW). Drought was selected as

179   the stress treatment to measure plasticity, as growth and survival of *S. pterophorus* is

180   strongly limited by water availability (Caño et al. 2007). Each treatment (W and NW)

181   contained one half-sib per mother plant, with a total of 282 individuals per treatment (47

182   populations x 6 mother plants/population) distributed randomly across blocks within a

183   treatment. Thus, a total of 564 individuals were planted. Individual plants within blocks

184   were separated by 75 cm and blocks were separated by 1.5 m. After transplanting, the

185   field was covered with straw to minimize the impact of cold conditions during winter,

186   prevent weed growth and minimize the effects of extreme drought during the summer

187   months.

188   Plants from all treatments were watered during the first 7 weeks to minimize

189   mortality and dead plants were replaced as necessary. The experiment was run from

190   April to October 2011. Plants from the W treatment received approximately 2212 L/m^2

191   from drip irrigation throughout the experiment. Precipitation during the course of the

192   experiment was 535 L/m^2 and thus plants from the W treatment received 413% more

193   water than plants from the NW treatment.

194

195   Measurements

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197   Plants were monitored for mortality once a week. To estimate relative growth

198   rate (RGR) plant height was measured at the beginning of the experiment (week 0) and

199   after 10, 16 and 23 weeks. RGR was calculated as the difference in plant height between

200   two consecutive periods (first period: 0-10 weeks, second period: 10-16 weeks, and

201   third period: 16-23 weeks). Shoot biomass was determined at the end of the experiment

202   (September/October 2011) for all surviving individuals. Individuals were cut at ground

203   level and leaves were separated from the stems. Both fractions were oven-dried at 65ºC

204   for 2-3 days and weighed.

205   In September-October 2011, three leaves per plant were collected to estimate

206   Specific leaf area (SLA) and leaf sphericity. We collected one leaf per individual from

207   the primary branch (at approximately half of plant height) and one leaves from two

208   secondary branches. Leaves were healthy, green and without necroses whenever

209   possible. Leaves were immediately scanned to avoid desiccation and images were

210   analyzed using the software ImageJ ® to determine foliar area and sphericity. Leaves

211   were oven-dried for 72 h at 65 °C and weighed.

212   The SLA was calculated by dividing leaf area by dry weight. A high SLA is

213   normally associated with an increased productivity and invasiveness (Reich et al. 1998;

214   Evans and Poorter 2001; Lake and Leishman 2004; Hamilton et al. 2005; Pyšek and

215   Richardson 2007), but also with shorter life-spans and vulnerability to herbivores

216   (Coley et al. 1985; Grime et al. 1996). Leaf sphericity is a measure of leaf shape,

217   estimated as $4 \cdot p \cdot$ (leaf area/leaf perimetre^2), sphericity = 1 for a perfect circle. Leaf

218   margins are associated with strong gradients, particularly where the surface area/volume

219   ratio is high (e.g., serrated leaf margins) (Royer and Wilf 2006), maximizing

220   evaporation and assimilation rates (Schuepp 1993; Baker-Brosh and Peet 1997). Total

221   leaf area was calculated multiplying dried leaf weight and specific leaf area (SLA).

222   A subset of individual plants was used to determine C and N content and C

223   isotopic composition in their leaves. The isotopic composition of carbon ($\delta^{13}C$) was

224   used as a proxy of water-use efficiency (Farquhar et al. 1989), whereas N concentration

225   in leaves was used as a surrogate for maximum photosynthetic capacity and, hence,

226   potential growth (Reich et al. 1998). We selected 6 populations per region in Eastern

227   South Africa, Australia and Europe that were widely spread into the territory, covering

228   the plant distribution limits (Table S1). All 5 populations available from the Western

229   Cape in South Africa were used. Between 4 and 6 individuals per population were

230   randomly selected, with a total of 116 individuals per treatment. Leaf samples were

231   homogenized on a bead-beater and weighed in a microscale (Metter Toledo MX5) at

232   Servei d'Anàlisis Químiques (SAQ), Autonomous University of Barcelona. Analyses of

233     $\delta^{13}C$, and total amount of C and N were carried out at UC Davis Stable Isotope Facility

234   using IRMS (PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa

235   20-20 isotope ratio mass spectrometer). The relationship between carbon stable isotopes

236   was expressed in relation to a Pee-Dee Belemnite (PDB) standard. The accuracy of the

237   measurements was 0.015‰.

238

239   Statistical analyses

240

241   Data analysis was performed using linear mixed effects models. Such models

242   can incorporate the imbalance between groups of observations and random effects

243   associated to the experimental design (hierarchical, nested effects). For response

244   variables that were measured only once (biomass, SLA, leaf sphericity, total leaf area,

245   carbon isotopic composition, N amount, C/N and survival) the structure of the model

246   included Region, Treatment and their interaction as fixed effects; and Block, Population

247   and Individual as random effects (on the intercept of the model). For response variables

248   that were measured repeatedly (RGR) the model included time as a fixed factor. General

249   linear mixed effects models were used in all cases, except in the case of survival

250   because the response variable was binary, in which case a generalized binomial mixed

251   model (logit transformation) was used. Specific leaf area, leaf sphericity, total leaf area,

252   nitrogen amount (as percentage) and carbon/nitrogen ratio variables were normalized by

253   using a logarithmic transformation.

254

255   The residuals of the mixed-effects models described above showed no pattern.

256   The fixed effects β (model coefficients) are given together with their standard errors

257   (SE) and significance. Variance components analysis was used to split the variance at

258   the Region, Bloc, Population and Individual levels. Significance for all statistical

259   analyses was accepted at $P < 0.05$. All models were fitted using the R software (v3.0.0,

260   The R Foundation for Statistical Computing).

261

Results

263

264   Shoot biomass, leaf biomass and stem biomass was significantly lower in plants

265 from the expanded and invasive ranges compared with plants from the native range

266 (Figure 2, Table 2). Watered plants resulted in a higher biomass for all regions in

267 comparison to non-watered plants, and this effect was stronger in the native

268 populations¡. However, only plants from Australia, which had the lowest biomass

269 response to water treatment, were significantly different compared with plants from the

270 native range (Table 2). Survival was significantly decreased in plants from Australia

271 compared with plants from the native range. The effect of watering on survival was

272 similar and non-significant for all regions except Europe. For plants from the European

273 range watering caused a significant reduction in survival, compared with the non-

274 significant positive effect watering had on native populations (Table 2).

275   RGR (relative growth rate) was highest for the first measured period to decline

276 in the following periods (Figure 3, Table 3). No significant differences where found

277 between regions in the first period of growth. A marginally significant decrease in RGR

278 was observed for the second period in Australian plants and Western Cape plants

279 compared with native plants, whereas individuals from European populations grew

280 significantly more (Table 3). On the third period, only Australian plants showed a

281 significant decrease in RGR compared with native populations ($P < 0.05$). The water

282 had a clear significant effect on the 3rd measurement period, and showed no interaction

283 with the region of origin of the plants. The overall results for RGR were thus consistent

284 with the patterns observed for biomass increment.

285   Significant differences among regions were found in SLA, leaf area and leaf

286   sphericity, whereas no differences were observed for $\delta^{13}C$, N concentration and the C/N

287   ratio (Table 4, Fig. 4). SLA was higher in plants from the European range compared

288   with the native individuals. Leaf area was lower in all non-native regions, whereas leaf

289   sphericity was lower in European plants and higher in Australian and expanded South

290   African individuals. The watering treatment increased SLA and leaf area, and reduced

291      $\delta^{13}C$ and the C/N ratio (the latter effect being only marginally significant). Water

292   treatment effects were similar among all regions, except in the case of N concentration

293   and the C/N ratio. For these two variables Australian plants responded by reducing their

294   N concentration (and thus increasing their C/N ratio), and this effect was significantly

295   different to the effect of watering on native plants.

296   The variance components analysis indicates that most of the variability in the

297   measured variables was observed across individuals within populations (48-86 %).

298   Variability among populations and regions ranged between <0.01-29 % and 0.01-21%

299   for the region and population levels (Table S2). The variance associated to the bloc

300   factor was low (< 12%) in most cases, except for SLA, total leaf area and $\delta^{13}C$ (Table

301   S2).

302

303   **Discussion**

304

305   The Evolution of Increased Competitive Ability (EICA) hypothesis predicts that

306   plants released from herbivores in a novel environment perform better than plants from

307   native range (Blossey and Notzold 1995). Additionally, the increased plasticity

308   hypothesis predicts that plants from invasive populations are more plastic than plants

309   from the native populations. Contrary to these hypotheses, *S.pterophorus* from the

310   invasive (Australia and Europe) and expanded ranges (South Africa, Western Cape)

311   grew less and responded less to watering than those from their native range. Individuals

312   from the invasive populations were released from herbivory compared to plants from

313   the native range (Castells et al. 2013), which is the first premise of the EICA hypothesis.

314   However, this decrease in herbivory pressure was not translated into a higher plant

315   performance as predicted by EICA.

316   Our study shows strong genetically-based differences in plant performance and

317   plasticity between the native and invasive populations of *S.pterophorus*. These

318   genotypic differences could result from adaptation to the novel environmental

319   conditions or from neutral events, such as demographic bottlenecks or genetic drift.

320   However, the contribution of neutral events to invasion success is frequently low in

321   comparison to natural selection (Lee 2002). In our case, climatic conditions are similar

322   across the compared regions (Table 1) (Castells et al. 2013) and it is thus unlikely that

323   climate has played a major role in shaping the different responses observed across

324   regions. In addition, we have found similar responses for all non-native regions which

325   suggest that the same directional processes have occurred at least in two occasions.

326   Preliminary results on neutral molecular markers (Vilatersana et al., unpublished) show

327   that European *S. pterophorus* are more closely related to the plants from South Africa

328   than to those from Australia, suggesting the occurrence of two independent invasive

329   processes.

330   A possible explanation of the lower growth rates of *S.pterophorus* in the

331   invasive range could be a weaker pressure from other plant competitors during the first

332   stages of colonization. It is known that resource competition has a cost. Under a low

333   competitive pressure in the invasive range there might be a reduction in biomass, as

334 suggested by the Evolutionary Reduced Competitive Ability (ERCA) hypothesis

335 (Bossdorf et al. 2004).

336   The reduction of plasticity and overall fitness in South African expanded range

337 populations (Western Cape) may be is caused by the initial low number of populations

338 during colonization, which suffered a demographic bottleneck and, in consequence, the

339 reduced genetic variation might result in inbreeding, fixation of deleterious mutations

340 and, finally, in a reduced fitness (Sakai et al. 2001).

341   It is unclear to what extent the contradictory results obtained by studies testing

342 the EICA hypothesis (cf. Introduction) could be a result of including only a small part of

343 the overall genetic variation of the target species (Caño et al. 2008; Caño et al. 2009).

344 Since we covered most of the known distributional range of the study species, our

345 results are not expected to be driven by sampling size or representativeness. However,

346 our study is not free of limitations. Perhaps the most important one is that we only

347 measured aboveground biomass, so we have no way to assess changes in allocation

348 between stems and roots nor can we discard that root growth might have been higher in

349 invasive than in native populations, potentially compensating for the lower aboveground

350 biomass increment in the former. The same could be said for reproductive biomass.

351 Finally, another important aspect that could affect our results is the effect of

352 competition. In our common garden experiment plants from different populations were

353 competing with each other for resources and our design does not allow disentangling

354 competition effects from differences in growth potential across populations.

355   In conclusion, our results show that populations of *S. pterophorus* of invasive

356 and expanded ranges did not have better performance or plasticity than native

357 populations of South Africa. Therefore, the success of alien species is not explained by

358 post-invasive evolution after being released from herbivory, as suggested by the EICA

359   hypothesis. Our results are consistent with other reports showing that growth is not

360   higher in invasive compared to native populations of a given species (Thébaud and

361   Simberloff 2001; Leger and Rice 2003; Vilà et al. 2003; Bossdorf et al. 2004).

362

363   **Acknowledgements**

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526

Table 1. Geographical and climatic characteristics of the distribution areas of *Senecio pterophorus* in the native, expanded and introduced ranges. Mean ± SE

(n = 5-18 populations per region)

Region	Plant Status	Code	Populations and individuals	Altitude (m)	Mean annual temperature (°C)[1]	Min. Annual temperature (°C)[1]	Max. Annual temperature (°C)[1]	Annual Rainfall (mm)[1]
South Africa	Native	Saf-Nat	18 pop, 107 ind	792.72 ± 96.30	16.64 ± 0.24	10.40 ± 1.15	23.50 ± 0.70	746.17 ± 31.82
South Africa	Expanded	Saf-Exp	5 pop, 29 ind	133.00 ± 56.13	16.14 ± 0.38	9.08 ± 0.79	19.46 ± 1.12	856.40 ± 86.50
Australia	Invasive	Aus	12 pop, 70 ind	140.77 ± 46.91	15.14 ± 0.45	7.84 ± 0.70	18.28 ± 1.30	754.38 ± 62.97
Europe	Invasive	Eur	12 pop, 72 ind	244.58 ± 46.94	15.27 ± 0.24	11.39 ± 1.54	18.58 ± 1.71	667.00 ± 19.87

Table 2. Summary of the statistical models testing the effects of region (South Africa-native, Saf-Nat; South Africa Expanded Saf-Exp; Australia, Aus; Europe, Eur) and water treatment (non-watered NW, and watered W) on shoot, leaf and stem biomass and plant survival of *Senecio pterophorus* at the end of the common garden experiment. The fixed effects (β model coefficients) are given together with their standard errors (SE). Intercept corresponds to region Saf-Nat and NW treatment. Asterisks denote significant effects: $^+ P < 0.1$, $^* P < 0.05$, $^{**} P < 0.01$ and $^{***} P < 0.001$.

Fixed effects $\beta \pm$ SE	Shoot biomass (g DM) (n = 526)	Leaf biomass (g DM) (n = 519)	Stem biomass (g DM) (n = 520)	Survival (n = 558)
Intercept	537.25 ± 54.86***	146.38 ± 15.16***	397.33 ± 37.66***	3.34 ± 0.57***
Region Saf-Exp	-207.10 ± 74.68**	-51.17 ± 19.50**	-159.24 ± 57.18**	0.50 ± 1.30
Region Aus	-184.80 ± 57.09**	-57.58 ± 14.81***	-132.08 ± 43.73**	-1.79 ± 0.64**
Region Eur	-206.79 ± 54.92***	-45.72 ± 14.27**	-157.95 ± 42.36***	1.48 ± 1.27
Treatment W	255.43 ± 77.35*	71.59 ± 21.35*	183.48 ± 53.05*	1.18 ± 1.01
Region Saf-Exp : Treatment W	-107.64 ± 105.66	-31.96 ± 27.41	-80.07 ± 80.92	-1.88 ± 1.68
Region Aus : Treatment W	-185.51 ± 80.82*	-53.79 ± 20.90*	-132.81 ± 61.91*	-1.28 ± 1.03
Region Eur : Treatment W	-113.71 ± 78.17	-32.19 ± 20.23	-84.45 ± 60.30	-3.46 ± 1.53*

Table 3. Summary of the effects of region (South Africa-native, Saf-Nat; South Africa Expanded Saf-Exp; Australia, Aus; Europe, Eur) and water treatment (non-watered NW, and watered W) on Relative Growth Rate for *Senecio pterophorus* plants calculated for three time periods during the course of the common garden experiment (1^{rst} period, 2nd period and 3rd period). See materials and methods for additional details. The fixed effects (β model coefficients) are given together with their standard errors (SE). Intercept corresponds to South Africa native range (Saf-Nat), NW treatment and 1rst Period. Asterisks denote significant effects: $^{+}P < 0.1$, $* P < 0.05$, $** P < 0.01$ and $*** P < 0.001$.

	Relative Growth Rate (cm/day)
Fixed effects $\beta \pm$ SE	
Intercept	0.02290 ± 0.00049***
Region Saf-Exp	0.00101 ± 0.00062
Region Aus	0.00055 ± 0.00048
Region Eur	-0.00034 ± 0.00045
Treatment W	0.00012 ± 0.00065
2nd Period	-0.01892 ± 0.00041***
3rd Period	-0.02052 ± 0.00041***
Region Saf-Exp : Treatment W	-0.00089 ± 0.00062
Region Aus : Treatment W	0.00007 ± 0.00048
Region Eur : Treatment W	0.00064 ± 0.00045
Treatment W : 2nd period	0.00068 ± 0.00044
Treatment W : 3rd period	0.00160 ± 0.00044***
Region Saf-Exp : 2nd period	-0.00145 ± 0.00075^{+}
Region Aus : 2nd period	-0.00109 ± 0.00058^{+}
Region Eur : 2nd period	0.00204 ± 0.00055***
Region Saf-Exp : 3rd period	0.00022 ± 0.00076
Region Aus : 3rd period	-0.00134 ± 0.00059*
Region Eur: 3rd period	-0.00057 ± 0.00055

Table 4. Summary of the effects of region (South Africa-native, Saf-Nat; South Africa Expanded Saf-Exp; Australia, Aus; Europe, Eur) and water treatment (non-watered NW, and watered W) on Specific Leaf Area, total leaf area, leaf sphericity, carbon isotopic composition, nitrogen concentration and C/N ratio on *Senecio pterophorus* growing at the common garden experiment. The fixed effects (β model coefficients) are given together with their standard errors (SE). Intercept region South Africa (native range) (Saf-Nat) and NW treatment. Asterisks denote significant effects: $^{+}P < 0.1$, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

	Log[Specific Leaf Area (cm²/g)] (n = 482)	Log[Total Leaf Area (cm²)] (n = 475)	Log[Leaf Sphericity] (n = 482)	δ13C (n = 232)	Log[N] (%) (n = 232)	Log[C/N ratio] (n = 232)
Fixed effects $\beta \pm$ SE						
Intercept	4.736 ± 0.023***	9.606 ± 0.107***	-2.959 ± 0.038***	-29.791 ± 0.166***	0.774 ± 0.042***	2.985 ± 0.038***
Region Saf-Exp	0.065 ± 0.045	-0.351 ± 0.141*	0.284 ± 0.074***	-0.159 ± 0.241	0.037 ± 0.043	-0.041 ± 0.041
Region Aus	-0.025 ± 0.036	-0.553 ± 0.111***	0.205 ± 0.057***	-0.011 ± 0.229	-0.028 ± 0.041	0.029 ± 0.039
Region Eur	-0.057 ± 0.034⁺	-0.328 ± 0.107**	-0.170 ± 0.055**	-0.258 ± 0.226	-0.031 ± 0.041	0.028 ± 0.039
Treatment W	0.207 ± 0.032***	0.623 ± 0.152*	-0.016 ± 0.054	-1.276 ± 0.235**	0.072 ± 0.060	-0.120 ± 0.053⁺
Region Saf-Exp : Treatment W	0.004 ± 0.065	-0.011 ± 0.202	-0.020 ± 0.106	0.135 ± 0.341	0.009 ± 0.061	0.019 ± 0.058
Region Aus : Treatment W	0.006 ± 0.051	-0.029 ± 0.159	-0.056 ± 0.082	0.169 ± 0.323	-0.129 ± 0.058*	0.117 ± 0.055*
Region Eur : Treatment W	0.044 ± 0.048	-0.143 ± 0.151	0.012 ± 0.079	0.071 ± 0.321	-0.021 ± 0.058	0.020 ± 0.055

Figure captions

Fig. 1

Populations of *S. pterophorus* surveyed at the native range (South Africa, populations 6 to 19), the expanded range (South Africa, populations 1 to 5), and the two invasive ranges (Australia and Europe). Abbreviations for regions or states: in South Africa ECP = Eastern Cape, WCP = Western Cape, KZN = KwaZulu-Natal; in Australia NSW = New South Whales, SA = South Australia, VIC = Victoria; in Europe CAT = Catalonia (Spain). The shaded area indicates *S. pterophorus* distribution based on herbaria databases and literature. Population numbers correspond to the code from Table S1. Adapted from Castells et al. 2013. Populations S20 to S23 were not originally included in the study by Castells et al. 2013.

Fig. 2

Biomass in terms of: a) total weight, b) leaf weight, c) stem weight for plants from the South Africa-native (Saf-Nat), South Africa Expanded (Saf-Exp) and introduced regions Australia (Aus) Europe (Eur) for both treatments (W, watered; NW, non watered) grown under common garden conditions in Europe. The horizontal line inside the boxes indicates the median, the box limits indicate 25th and 75th percentiles, whiskers denote 1.5 x interquartile range (IQR), and additional data points indicate outliers. Percentage survival at the end of the experiment by Region and Treatment is shown in panel d).

Fig. 3

Height growth of plants from the South Africa-native region (Saf-Nat) compared to: a) South Africa Expanded region (Saf-Exp), b) introduced region Australia (Aus) and c) introduced region Europe (Eur). Each data point (linked by segments showing the overall trajectory) indicates the mean and SE for the corresponding treatment (W, watered; NW, not watered) for the four studied periods.

Fig. 4

Leaf attributes of plants from the South Africa-native (Saf-Nat), South Africa Expanded (Saf-Exp) and introduced regions Australia (Aus) Europe (Eur) for both treatments (W, watered; NW, non watered) grown under common garden conditions in Europe: a) Specific Leaf Area, b) Total leaf area, c) Leaf sphericity, d) Carbon isotopic composition, e) Percentage of nitrogen and f) Carbon/nitrogen ratio. The horizontal line inside the boxes indicates the median, the box limits indicate 25th and 75th percentiles, whiskers denote 1.5 x interquartile range (IQR) and additional data points indicate outliers.
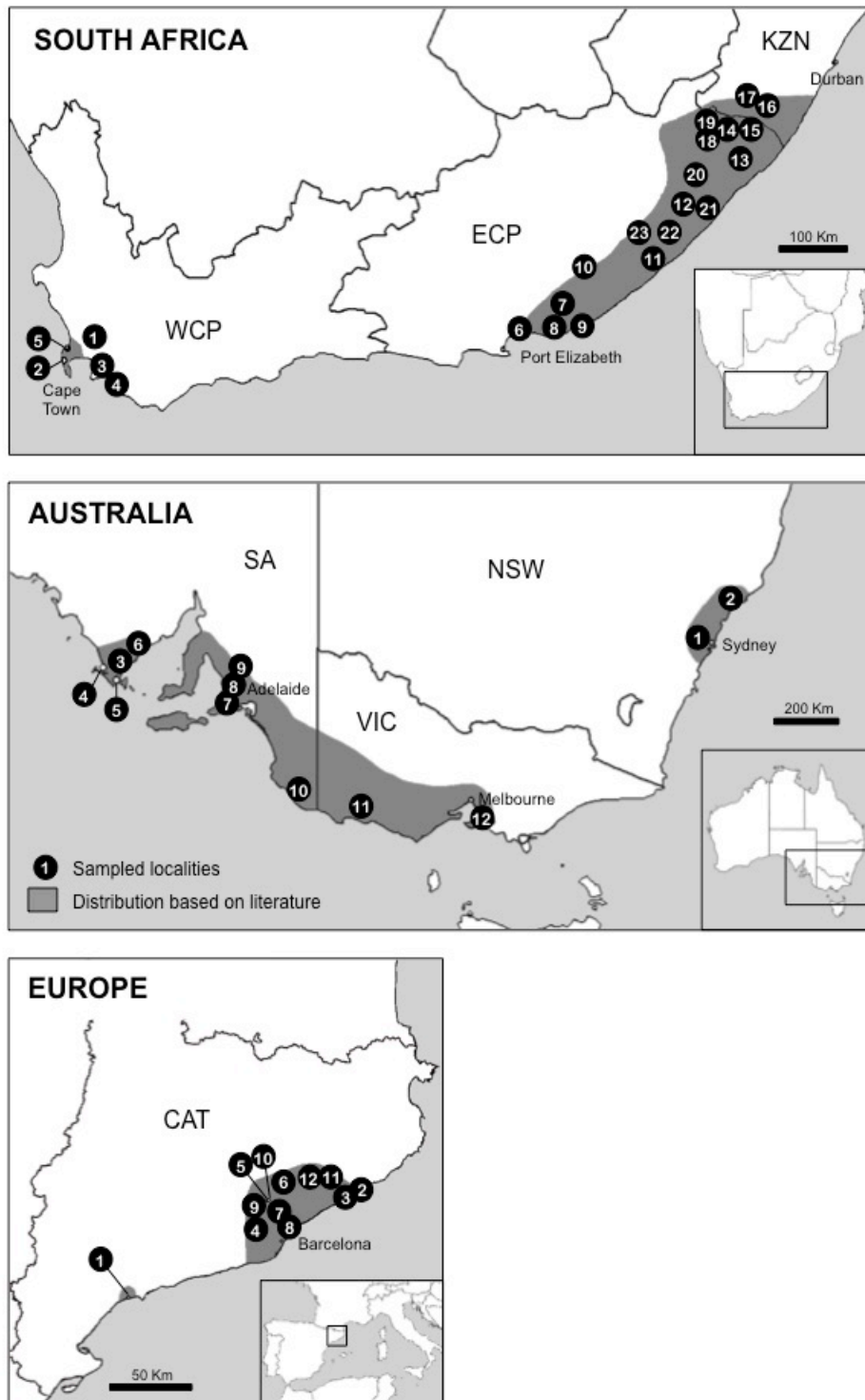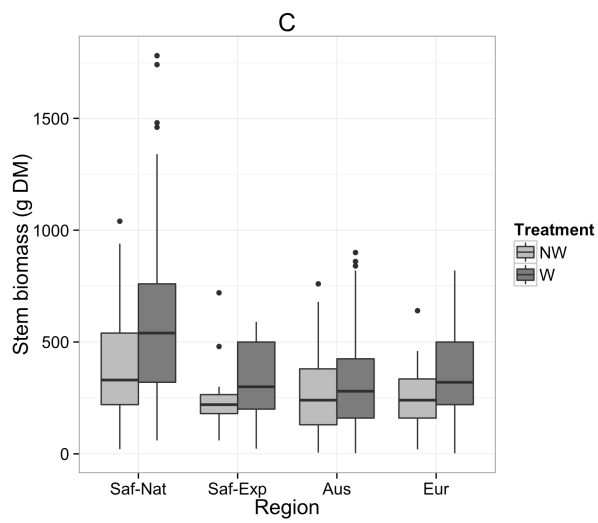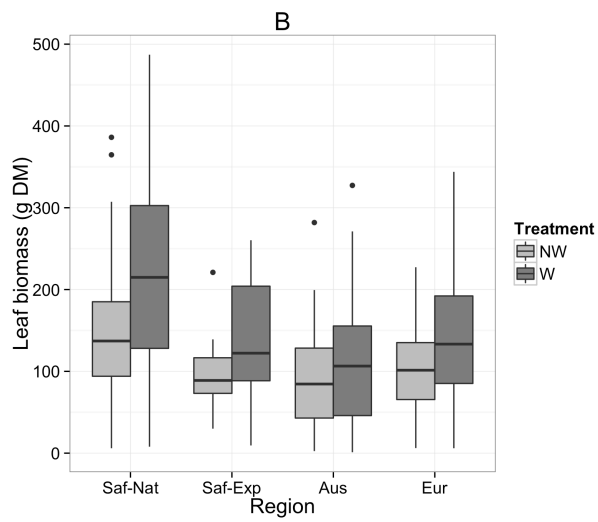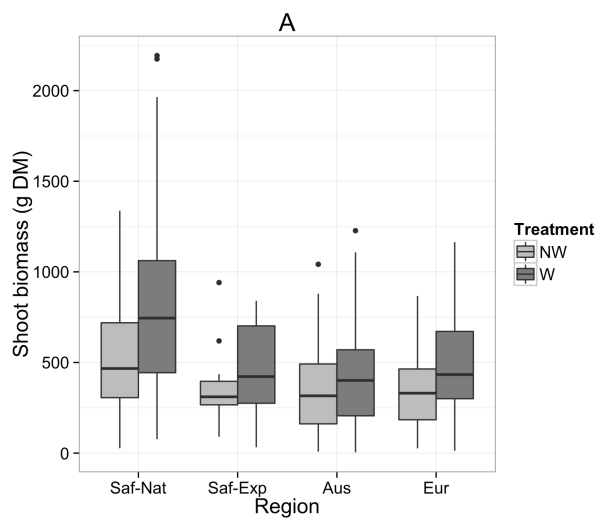
Figure 1

Figure 2
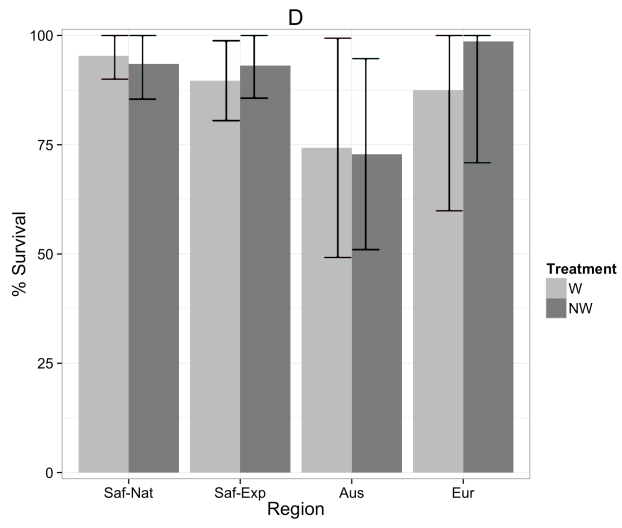
Figure 3

Figure 4

Supplementary material

Table S1. Populations of *Senecio pterophorus* used in a common garden experiment from the native range (South Africa), the expanded range (Western Cape, South Africa) and two introduced ranges (Australia and Europe).
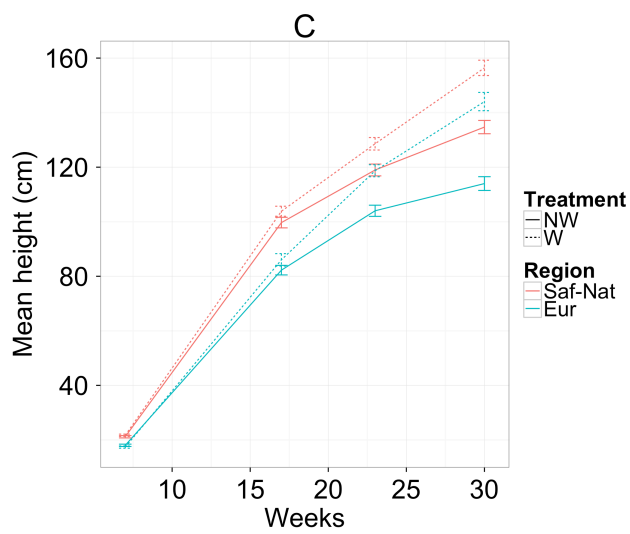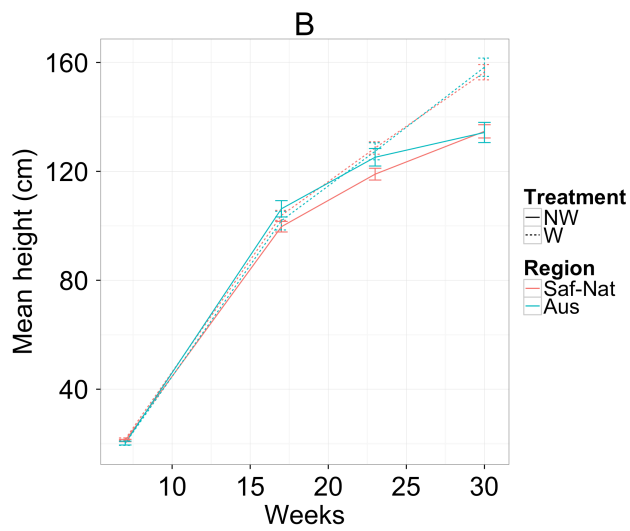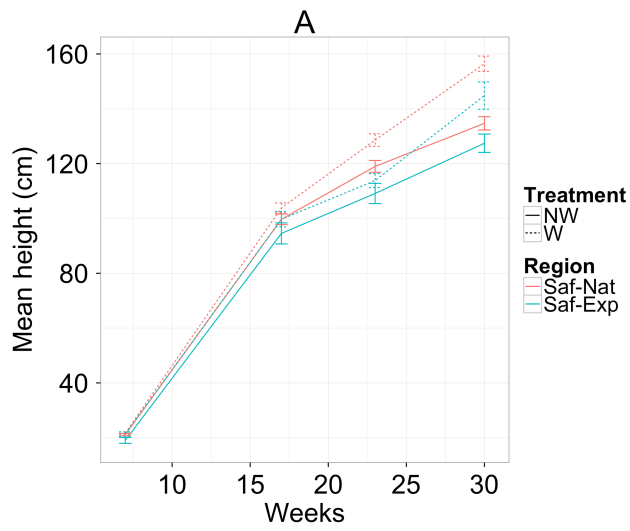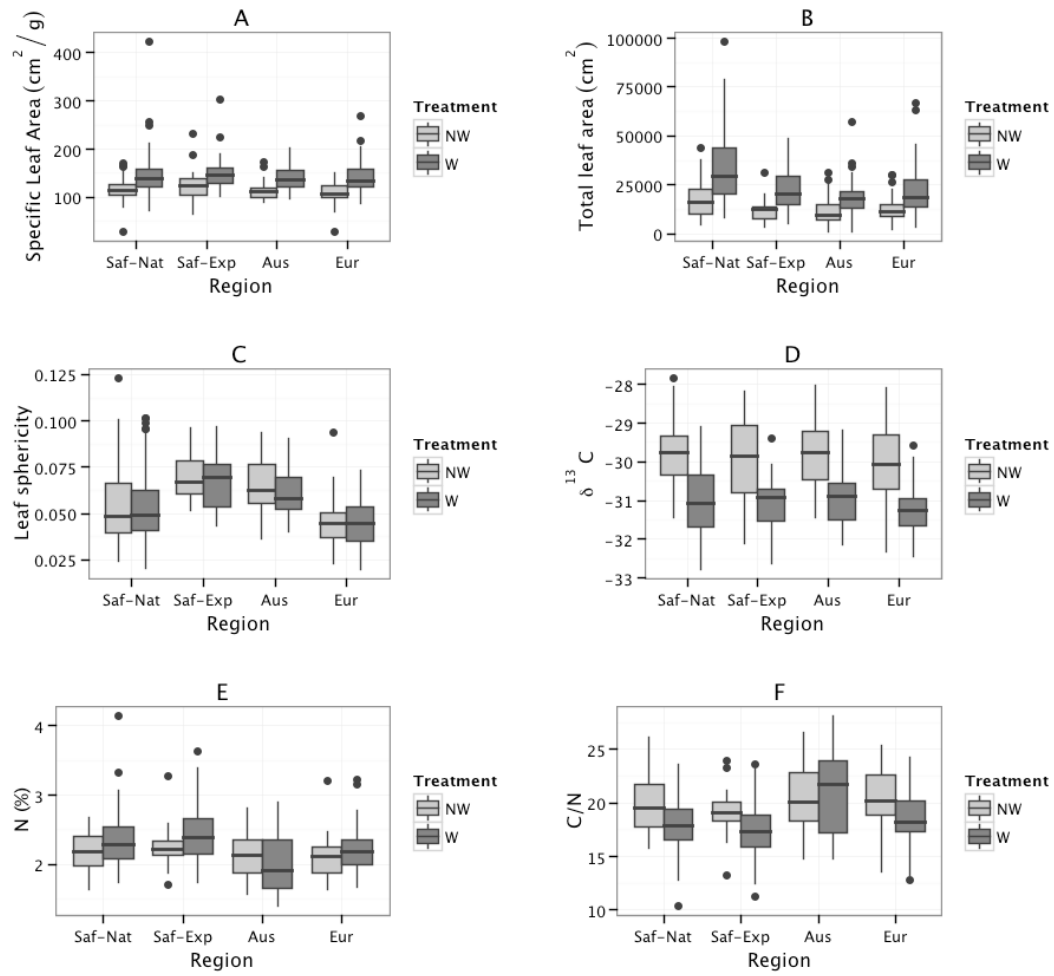
Location[1]	Population	Code[2,3]	Origin[4]	Coordinates	Alt. (m)[5]	Temp. (°C)[5]	Rainfall (mm)[5]
SOUTH AFRICA							
ECP	Colchester	S06*	Nat	33°41'S 25°49'E	6	16.4	440
ECP	Grahamstown	S07	Nat	33°19'S 26°32'E	668	18.5	741
ECP	Alexandria	S08	Nat	33°36'S 26°24'E	124	16.1	548
ECP	Port Alfred	S09*	Nat	33°36'S 26°52'E	61	18.2	595
ECP	Fort Hare	S10*	Nat	32°47'S 26°52'E	535	18.2	593
ECP	Courtlands	S11*	Nat	32°40'S 28°00'E	471	17.9	802
ECP	Umtentu	S12*	Nat	31°51'S 28°30'E	716	17.4	713
ECP	Flargstaff	S13	Nat	31°11'S 29°26'E	884	16.0	912
ECP	Mt. Ayliff	S14*	Nat	30°50'S 29°15'E	1116	15.9	827
ECP	Bizana	S15	Nat	30°50'S 29°35'E	1076	15.6	907
ECP	MountFrere	S18	Nat	30°57'S 28°57'E	1242	15.6	856
ECP	Antioch	S19	Nat	30°42'S 28°51'E	1261	16.0	787
ECP	Sidwadwenii	S20	Nat	31°23'S 28°50'E	1002	16.0	801
ECP	Mqanduli	S21	Nat	31°49'S 28°45'E	785	16.8	748
ECP	Gwadana	S22	Nat	32°12'S 28°17'E	709	16.9	750
ECP	Nobokwe	S23	Nat	31°54'S 27°50'E	963	16.9	650
KZN	Stafford's Post	S16	Nat	30°31'S 29°46'E	1141	15.6	927
KZN	Franklin	S17	Nat	30°23'S 29°38'E	1200	15.5	834

WCP	Groenfonteinkop	S01*	Exp	33°47'S 18°52'E	176	16.9	786
WCP	CapeTown	S02*	Exp	34°07'S 18°23'E	26	16.4	825
WCP	Elgin	S03*	Exp	34°09'S 19°01'E	326	14.7	996
WCP	Hermanus	S04*	Exp	34°24'S 19°11'E	23	16.2	589
WCP	CapeTown	S05*	Exp	33°56'S 18°26'E	114	16.5	1086
AUSTRALIA							
NSW	Doonside	A01	Int	33°45'S 150°52'E	48	17.2	873
NSW	Newcastle	A02*	Int	32°52'S 151°41'E	13	17.9	1092
SA	Port Lincoln	A03	Int	34°33'S 135°49'E	13	16.0	509
SA	Wangary	A04*	Int	34°30'S 135°25'E	15	16.2	531
SA	Lincoln NP	A05*	Int	34°48'S 135°46'E	6	16.2	516
SA	Hincks NP	A06	Int	33°55'S 136°14'E	84	16.4	399
SA	Mt. Compass	A07	Int	35°20'S 138°36'E	262	14.6	801
SA	Cleland NP	A08*	Int	34°57'S 138°42'E	602	13.0	1101
SA	Warren CP	A09*	Int	34°40'S 138°51'E	257	14.9	616
SA	Mt. Burr	A10*	Int	37°35'S 140°28'E	171	13.3	867
VIC	Mt. Napier NP	A11	Int	37°55'S 142°02'E	223	12.9	757
VIC	Hastings	A12	Int	38°17'S 145°11'E	15	14.5	785
EUROPE							
CAT	Cambrils	C01*	Int	41°04'N 1°04'E	20	16.3	549
CAT	Palafolls	C02*	Int	41°39'N 2°42'E	66	15.6	722
CAT	Calella	C03	Int	41°37'N 2°39'E	144	15.6	717
CAT	Castellbisbal	C04*	Int	41°27'N 1°59'E	51	16.3	589
CAT	Castellar V.	C05*	Int	41°36'N 2°04'E	288	15.3	631
CAT	Bigues i Riells	C06	Int	41°41'N 2°12'E	295	15.1	667
CAT	Sabadell	C07	Int	41°31'N 2°07'E	124	16.1	594
CAT	Ripollet	C08*	Int	41°29'N 2°10'E	61	16.3	599

CAT	Matadepera	C09	Int	41°35'N 2°01'E	413	14.5	662
CAT	Sant Llorenç NP	C10	Int	41°36'N 2°05'E	332	14.9	654
CAT	Campins	C11*	Int	41°43'N 2°28'E	519	14.2	798
CAT	Montseny NP	C12	Int	41°43'N 2°24'E	622	13.0	822

[1] Abbreviation for regions and States. In South Africa: ECP = Eastern Cape, WCP = Western Cape, KZN = KwaZulu-Natal; in Australia: NSW = New South Whales, SA = South Australia, VIC = Victoria; in Europe: CAT = Catalonia (Spain),

[2] Code assigned to each population. Population number corresponds to Fig. 1

[3] Asterisks denote populations used for isotope analyses

[4] Origin: Nat = native, Exp = expanded, Int = introduced

[5] Altitude, mean annual temperature and mean annual rainfall. WorldClim Database (Hijmans et al. 2005)

Table S2. Variance components analysis showing the percentage of variance explained at different hierarchical levels (Block, Region, Population, Individual,

Within-individual or treatment) for all the variables measured in the study.

	Block	Region	Population	Individuals	Within
Shoot Biomass	5.49%	15.10%	18.25%	53.71%	7.45%
Leaf Biomass	6.18%	18.05%	16.76%	51.86%	7.15%
Stem Biomass	4.02%	14.22%	19.58%	54.53%	7.65%
Specific leaf area	23.88%	1.86%	< 0.01%	74.20%	0.06%
Total leaf area	20.40%	12.15%	13.07%	48.19%	6.19%
Leaf sphericity	0.01%	21.30%	29.00%	49.27%	0.43%
$\delta^{13}C$	34.35%	0.01%	< 0.01%	65.65%	< 0.01%
N	7.38%	6.97%	< 0.01%	85.52%	0.13%
C/N	11.59%	4.98%	< 0.01%	83.29%	0.14%