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# Plasticity in plant populations may be constrained by performance costs, complex environments and weakly integrated phenotypes

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1 **Original paper**

2 **Plasticity in plant populations may be constrained by performance costs,**  
3 **complex environments and weakly integrated phenotypes**

4

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6

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9 *Plant population plasticity constrained by costs, environments, integration*

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16 **Abstract**

17 • **Background and Aims** One response of plants to climate warming is plasticity of traits, but  
18 plasticity might come at a cost and might be limited by the integration among traits or by  
19 simultaneous shift of another environmental condition such as shading. Empirical studies  
20 treating simultaneously such costs and limitations of plasticity across populations or maternal  
21 lineages within species, and how they depend on the environmental context remain few.

22 • **Methods** We studied three plant species from the sub-Antarctic, a region currently facing one  
23 of the fastest warming worldwide. For multiple populations or maternal lineages we identified  
24 (i) plasticity by exposing seeds from a given source population to different temperature and light  
25 treatments, (ii) performance (photosynthesis or morphological performance) and (iii)  
26 morphological integration of traits in young plants.

27 • **Key Results** We found that plants from more plastic source populations performed poorly. Plants  
28 from more integrated source populations were more plastic. Exposure to shade rendered plants  
29 less plastic to a warming trend. Moreover, simultaneous shading and warming, rather than *sole*  
30 shading or *sole* warming, reduced plant performance.

31 • **Conclusions** Our results suggest that phenotypic integration of intraspecific lineages  
32 surprisingly might favour rather than limit plasticity. However, our results also suggest that  
33 plasticity in response to climate warming may be limited by parallel increase in shading from  
34 other plants including competitors, and itself does not ensure success due to induced performance  
35 costs.

36

37 **Key words:** *Pringlea antiscorbutica*, *Ranunculus biternatus*, *Ranunculus pseudotrullifolius*,  
38 abiotic treatment, costs, environments, performance, phenotypic integration, phenotypic plasticity,  
39 plant species, plant populations

40

## INTRODUCTION

41 Climate change alters the environments and plants, being sessile organisms, have to cope with  
42 these alterations (Sultan, 2000). Phenotypic plasticity is the capacity of a given genotype to result  
43 in different phenotypes under different environmental conditions, and plants are well known for  
44 having generally high plasticity (Bradshaw, 1965; Schlichting, 1986; Sultan, 2000). Phenotypic  
45 plasticity is one of the major means by which plants cope with environmental heterogeneity  
46 (Valladares *et al.*, 2007; Matesanz *et al.*, 2021) and is now considered as part of a species' adaptive  
47 potential to environmental changes (Ghalambor *et al.*, 2007; Hoffmann and Sgro, 2011; Callahan  
48 *et al.*, 2008). However, the expression of a phenotype under a certain environment is an integrative  
49 result of all local responses of many modular traits and their interactions, so plasticity should be  
50 analysed within a frame of trait variation and co-variation to understand phenotypic response and  
51 evolution (Pigliucci and Preston, 2004; Wang and Zhou, 2021). For instance, plasticity is not  
52 always adaptive, only plasticity producing phenotypes in the same direction as natural selection,  
53 and thus with the ability to increase plant fitness, is considered adaptive (Bell and Galloway, 2008),  
54 a currently developing research question (Murren *et al.*, 2015; Molina-Montenegro *et al.*, 2016;  
55 Diamond and Martin, 2021; Fox *et al.*, 2019). If plasticity is too costly or too limited by other  
56 constraints, then plasticity may reduce rather than increase performance and finally fitness, but our  
57 understanding of the costs or limitations of plasticity is still rather low (Pigliucci, 2005; Snell-Rood  
58 *et al.*, 2018; Wei *et al.*, 2021; Auld *et al.*, 2009).

59 A plant may be only little plastic because of energy-allocation constraints: plasticity is costly  
60 and may reduce performance (Auld *et al.*, 2009; Bongers *et al.*, 2017). Plastic response is dependent  
61 upon physiological and morphological machinery that involves the acquisition of environmental  
62 information, transduction of the signal, and the expression of a phenotypic response (Vinocur and

63 Altman, 2005; DeWitt *et al.*, 1998). Such machinery induces costs in organisms, which may be  
64 classified in two kinds: constitutive and induced (Callahan *et al.*, 2008; Murren *et al.*, 2015).  
65 Constitutive costs include the costs of maintaining physiological machinery and signal acquisition,  
66 and exist independent of whether the plant performs a plastic response or not. Induced costs include  
67 the costs of the realized phenotypic changes and depend on the intensity of the phenotypic response  
68 (Sultan and Spencer, 2002; Auld *et al.*, 2009). Costs of plasticity might reduce plant fitness, and  
69 even challenge plant survival (Pigliucci, 2005; Valladares *et al.*, 2007; Ghalambor *et al.*, 2007;  
70 Bongers *et al.*, 2017). In view of the great role played by phenotypic plasticity in plant response to  
71 global change, trying to evaluate plasticity and performance together remains a key challenge  
72 (Givnish, 2002; Valladares *et al.*, 2007; Auld *et al.*, 2009; Bongers *et al.*, 2017).

73 Another limit to plasticity in plants may lay in the multifactorial aspect of environmental  
74 variation in nature (Valladares *et al.*, 2007). Plastic response to such complex environmental  
75 changes is more difficult to decipher than responses to simple laboratory environments under which  
76 plasticity was often studied, with only one condition varying at a time (Valladares *et al.*, 2007;  
77 Westneat *et al.*, 2019; Callahan *et al.*, 2008). In a complex environment, different conditions, for  
78 instance drought and shade, may lead to opposite plant responses (Sack and Grubb, 2002), hence  
79 hiding the overall response. Also, costs involved in the response to a first condition may limit  
80 responses to other conditions, due to constraints on energy allocation (Auld *et al.*, 2009). How plant  
81 phenotype is changed in response to synchronous changes of multiple environmental conditions  
82 and the consequences on plant performance are topics that still require investigation (Westneat *et*  
83 *al.*, 2019).

84 Finally, plasticity may be limited by the integration among traits. Phenotypic integration is the  
85 pattern and magnitude of character correlations due to genetic, developmental, and/or functional

86 connections among traits and allowing organism consistency (Pigliucci and Preston, 2004).  
87 Integration may be strongly affected by a change in the environment (Pigliucci *et al.*, 1995; Liu *et*  
88 *al.*, 2007; Wood and Brodie, 2015; Benavides *et al.*, 2021). Integration has been suggested to  
89 possibly constrain plasticity. Indeed, linkage with other traits was assumed to limit a trait's range  
90 of variation and so, expression of plasticity for a given trait was assumed to be limited by its overall  
91 integration (Valladares *et al.*, 2007). However, this relationship is far from being solved (Wang  
92 and Zhou, 2021; Matesanz *et al.*, 2021). We here suggest an alternative hypothesis: that in poorly  
93 integrated organisms, extreme plastic modification of one trait may not be consistent with the  
94 modification of other traits, limiting overall plasticity, whereas in highly integrated organisms,  
95 extreme modification of one trait value may be consistent with extreme modification of the value  
96 of other traits. Such consistency in highly integrated organisms might facilitate the plasticity of the  
97 first trait and increase the plasticity of other traits, increasing the overall degree of plasticity of the  
98 organism. Hence phenotypic integration might in theory both, limit and facilitate plasticity. So far  
99 only few studies considered the possible effect of integration of traits on their plasticity (Gianoli  
100 and Palacio-López, 2009; Matesanz *et al.*, 2021). The hypothesis of integration as a constraint to  
101 plasticity was supported by a study showing that plasticity in response to shading or drought was  
102 lowest in traits that were strongly integrated with other traits (Gianoli and Palacio-Lopez, 2009).  
103 The hypothesis of lack of integration as a constraint to plasticity was partly supported by Matesanz  
104 *et al.* (2021) showing that phenotypic plasticity of a given trait to drought was positively associated  
105 with phenotypic integration of this trait with other traits (both in the optimum and the stressful  
106 environments). Inevitably, such studies remain correlative as integration cannot be manipulated  
107 without damaging an organism. One can only compare integration prior to treatment with plasticity  
108 in response to treatment. Importantly, these few existing studies tried to identify whether more  
109 integrated traits are more or less plastic than less integrated traits. However, plasticity varies also

110 among populations or other intraspecific lineages, and to our knowledge no study has tried to  
111 identify whether more integrated lineages respond more or less plastically to the environment than  
112 less integrated lineages.

113 Investigating plant plasticity and integration in response to environmental variation is  
114 particularly relevant in areas with drastic ongoing climate change. The sub-Antarctic region is  
115 characterized by cool and windy climate all year round. However, this region is subject to a rapid  
116 and intense climate change (Le Roux and McGeoch, 2008; Favier *et al.*, 2016; Verfaillie *et al.*,  
117 2021). Such is the case in the Kerguelen Islands located in the southern Indian Ocean (Fig. 1) and  
118 with typical sub-Antarctic climate (mean annual temperature of 4.6°C and mean wind speed of 9.7  
119 m.s<sup>-1</sup> at “Port-aux-Français” from 1951 to 2018; (Verfaillie *et al.*, 2021)). Climate change there  
120 resulted in a significant drying trend associated with a marked shift in the storm track location  
121 (Favier *et al.*, 2016; Verfaillie *et al.*, 2019), whereas a large increase in the mean temperature was  
122 also observed e.g. (Favier *et al.*, 2016; Verfaillie *et al.*, 2021). Direct impacts of climate change are  
123 already visible in the Kerguelen Islands, with more frequent and more prolonged summer droughts,  
124 the rise of the snow line and the drastic reduction of the Cook ice cap (Hennion *et al.*, 2006;  
125 Lebouvier *et al.*, 2011; Verfaillie *et al.*, 2015). Plant species in Iles Kerguelen show signs of stress  
126 such as leaf wilting or increased shoot necrosis suggesting they are particularly sensitive to ongoing  
127 changes (Chapuis *et al.*, 2004; Frenot *et al.*, 2006; Marchand *et al.*, 2021). As the Iles Kerguelen  
128 are geographically very isolated, species can hardly migrate to track changing environments in  
129 space, and so plants have to respond mainly by plasticity or adaptive evolution (Hoffmann and  
130 Sgro, 2011). We studied three indigenous species from Iles Kerguelen: two *Ranunculus* species:  
131 *R. biternatus* and *R. pseudotrullifolius* (Ranunculaceae) and the Brassicaceae *Pringlea*  
132 *antiscorbutica*. *Ranunculus* species are known to show high plastic responses (Garbey *et al.*, 2004),



133 and especially heterophylly that is considered as adaptive plasticity (Wells and Pigliucci, 2000).  
134 *Ranunculus biternatus* and *pseudotrullifolius* are known to show trait plasticity between plants  
135 from the field and such under controlled conditions (Hennion *et al.*, 1994). *Pringlea antiscorbutica*  
136 is known to show decreased root or shoot growth in response to salt or heat (Hummel *et al.*, 2004  
137 a, b) and shows plasticity in shoot growth in response to cultivation conditions (Hennion *et al.*,  
138 2006).

139 We studied lineages (populations, maternal lines) within each of these three subantarctic species  
140 in a phytotron, subjecting plants to variation of different abiotic conditions such as warming  
141 treatment, and (in *P. antiscorbutica*) shading treatment and combination of both warming and  
142 shading treatment. We investigated the variation of morphological traits and the morphological or  
143 physiological performance of individuals. We aimed at testing the predictions of the above  
144 hypotheses that plasticity of intraspecific lineages might be costly, limited by environmental  
145 complexity and by either high or low phenotypic integration: (i) Intraspecific lineages of high  
146 plasticity have low performance. (ii) Intraspecific lineages show a lower degree of plasticity in  
147 response to variations of two environmental conditions than in response to variation of one single  
148 condition; (iii) Intraspecific lineages that show high phenotypic integration prior to environmental  
149 treatment, respond either less plastically or more plastically to this treatment.

150

151

## MATERIALS AND METHODS

152 *Species under study*

153 The Iles Kerguelen (49°20'00" S, 69°20'00" E) are situated in the southern Indian Ocean within  
154 the sub-Antarctic region (Lebouvier and Frenot, 2007) (Fig. 1). We studied *R. biternatus* Smith, *R.*

155 *pseudotrullifolius* Skottsberg, and *Pringlea antiscorbutica*. R.Br. ex Hook.f. All are perennial  
156 plants. *R. biternatus* and *R. pseudotrullifolius* are two species of austral distribution with a  
157 magellanic origin (Lehnebach *et al.*, 2017) while *P. antiscorbutica* is endemic from the southern  
158 Indian Ocean province (Bartish *et al.*, 2012). On the Iles Kerguelen these species occupy different  
159 habitats (Hennion and Walton, 1997a). *Ranunculus biternatus* is widespread on the island  
160 occurring in habitats below 300m above sea level. In contrast, *R. pseudotrullifolius* being  
161 halophilous has more restricted distribution and occurs within a short distance of the coast,  
162 occupying peaty or sandy shores and ponds (Hennion and Walton, 1997a). On the Iles Kerguelen  
163 *P. antiscorbutica* occurs in habitats ranging from coastal meadows to montane fellfields (Hennion  
164 and Bouchereau, 1998).

165

#### 166 *Seed collection*

167 To grow plants under controlled conditions, we used seeds from different natural populations in  
168 the three species. Populations were defined as continuous groups of plants living at a given site.  
169 Seeds from *P. antiscorbutica* were collected in the field in 2013 in six populations spread across  
170 the region covering Ile Australia, Mont Crozier and Ile Mayes (Fig. 1). Ten individuals were  
171 randomly selected within each population. In each of the ten individuals, fifteen siliques were then  
172 randomly collected from the median part of the longest infructescence where seed size is maximum  
173 and least variable (Hennion, Schermann-Legionnet and Atlan, unpubl.). Seeds were also collected  
174 from *P. antiscorbutica* plants growing from four populations in a phytotron in 2013. In *R.*  
175 *biternatus* and *R. pseudotrullifolius*, seeds were collected in 2014 from 20 individuals across three  
176 populations in Isthme Bas, the same in which phenotypic measurements had been performed in

177 2012. All seeds were stored dry with silica gel at 4°C until their use according to an established  
178 protocol (Hennion and Walton, 1997b).

179

### 180 *Controlled conditions*

181 We grew plants in a phytotron at Rennes. Seeds were sterilized and germinated in Petri dishes  
182 at 25°C for *P. antiscorbutica* and 21°C for *Ranunculus* species, under low light (1kLux) following  
183 an established protocol (Hennion and Walton, 1997b). To render the overall design more  
184 homogeneous we kept a constant number of seedlings from each population. In each species, three  
185 hundred seedlings were planted in vermiculite substrate and fertilized with ½ Hoagland solution  
186 (Hoagland and Arnon, 1938). For the *R. pseudotrullifolius*, NaCl was added to Hoagland solution  
187 to reach a concentration of 1g.L<sup>-1</sup>, being consistent with the salinity in the field (Labarrere, 2017).  
188 Plants were grown hydroponically in a phytotron under conditions (light exposure, photoperiod,  
189 temperature and air humidity) mimicking austral summer in Iles Kerguelen at best, according to a  
190 previous protocol (Hennion and Bouchereau, 1998; Hennion *et al.*, 2006). Specifically, plants were  
191 grown in a photoperiod of 14h with an irradiance of 15klux from a combination of Philips TLD 79  
192 fluorescent and Philips Aquarelle TLD 89 tubes. Temperature was of 9°C during the day and 4°C  
193 during the night with a relative humidity of 75/85% day/night.

194

### 195 *Experimental treatments*

196 The experimental design is summarized in Fig. 2. Plants were grown under standard, “control”  
197 conditions for 5 months at 9°C; 15klux. We then subjected subsets of plants to different treatments  
198 during 3 months. In *P. antiscorbutica*, we subjected plants to four conditions: (i) warming (13°C /  
199 +4°C), (ii) shading (5klux), (iii) both warming and shading (13°C; 5klux) and (iv) control (9°C;

200 15klux). In *R. biternatus* and *R. pseudotrullifolius*, we subjected plants to warming (13°C). Due to  
201 a low number of available seedlings, *R. biternatus* and *R. pseudotrullifolius* were not subject to  
202 shading treatment or combined warming-shading treatment. In *P. antiscorbutica*, a total 320 plants  
203 (32 plants from each of 10 source populations) were used: per each of four treatments 8 plants from  
204 10 source populations. In *R. biternatus*, a total 152 plants (8 plants from each of 19 mothers) were  
205 grown: per each of two treatments (warming treatment and control) 4 plants from each of 19  
206 mothers for a total of 76 plants. In *R. pseudotrullifolius*, a total 112 plants (8 plants from each of  
207 14 mothers) were grown: per each of two treatments (warming treatment and control) 4 plants from  
208 14 mothers for a total of 56 plants. Placement of plants within a treatment was random with respect  
209 to levels of integration or of performance, thereby avoiding any confounding effect of possible  
210 variation within-treatment variation of microenvironmental conditions and how different lineages  
211 of plants responded to a given treatment. Plants were all 8-month old at the end of experiments.

212

### 213 *Trait measurements*

214 We conducted two sets of plant measurements. First, we measured plants after 5 months growth  
215 while all plants were subjected to control conditions to determine baseline differences between  
216 plants (T0). We measured plants again after 3 months growth in the different abiotic conditions  
217 (T1). Traits considered were height, diameter and number of leaves of the rosette, and of the 2  
218 longest leaves: length and width of lamina and length of petiole. These traits were shown to respond  
219 to increased temperature in previous work (Hennion *et al.*, 2006; Hermant *et al.*, 2013; Labarrere,  
220 2017). In *R. biternatus* and *R. pseudotrullifolius*, diameter changed during plant handling and  
221 therefore was not measured. We also estimated physiological and morphological plant  
222 performances. To determine physiological performance, we used the maximum efficiency of

223 photosystem II (Maxwell and Johnson, 2000; Zhu *et al.*, 2008), a useful performance parameter  
224 known to decrease with stress (Maxwell and Johnson, 2000; Kalaji *et al.*, 2011). Maximum PSII  
225 efficiency (Fv/Fm) was measured using a PAM chlorophyll fluorometer system (Heinz Walz,  
226 Effeltrich, Germany) in saturating pulse mode. After 30 minutes of dark adaptation, the maximum  
227 fluorescence of leaves was measured under dark conditions (Fm) and a subsequent saturating pulse  
228 of light was applied. Minimum fluorescence (F0) was determined under weak light before a pulse,  
229 and variable fluorescence (Fv=Fm-F0) was calculated. To determine morphological performance,  
230 we used leaf spread (Zamora *et al.*, 1998). Leaves that are not fully spread, but curled, indicated  
231 that the plant is not fully performant. Part of leaf that is curled is unexposed to light and therefore  
232 does not contribute to photosynthesis. We measured leaf spread as the ratio between length of leaf  
233 that is not curled and maximum flattened leaf length.

234

### 235 *Data analyses*

#### 236 *Trait means and plasticity*

237 We investigated plant plasticity comparing plant trait values between the different experimental  
238 conditions, *i.e.* a trait change, with in whatever direction, with a change in environment. Prior to  
239 calculating plasticity of traits, we aimed to remove the intrinsic trait value differences among plants  
240 to correct for differences between plants already present prior to exposing them to controls or  
241 treatments during months 5 – 8. Thus, we calculated trait value as standardized trait value  $T_s =$   
242  $(T1-T0)/T0$ . With T0 being the measurement of the trait done during the first set of measurements  
243 - when all plants were subjected to the same conditions – and T1 being the measurement of the trait  
244 done during the second set of measurements, when plants were subjected to different experimental  
245 conditions. In *P. antiscorbutica*, we calculated trait means of these standardized trait values across

246 plants from the same population subjected to same condition. In *R. biternatus* and *R.*  
247 *pseudotrullifolius*, we calculated trait means across plants from the same mother subjected to same  
248 condition. So, to summarize, we had one data point per maternal line (or population) and treatment.  
249 In each species, we calculated - for each population (or mother) and treatment - trait plasticity as  
250 the absolute value of the difference between trait mean under treatment and trait mean under  
251 control. In *P. antiscorbutica* we also calculated the mean degree of plasticity across the three  
252 treatments. Samples sizes did not permit the same calculus for *Ranunculus* species. These means  
253 across individuals per population or per mother could then be compared to phenotypic integration  
254 calculated across individuals per population or per mother. For consistency we used source  
255 populations or mothers as our level of analyses in all further comparisons.

256

### 257 *Phenotypic integration*

258 We calculated phenotypic integration across *P. antiscorbutica* individuals from the same source  
259 populations. We did so prior to treatments, thereby avoiding any effect of environment on  
260 integration (Wang and Zhou, 2021). Samples sizes did not permit the same calculus for *Ranunculus*  
261 species. We focused on the dominant axis of phenotypic integration, quantified as the percentage  
262 of variance explained by the first axis of a Principal Component Analysis (PCA) performed on  
263 traits (Tucic *et al.*, 2013; Haber and Dworkin, 2017; Michaud *et al.*, 2020). PCA was performed  
264 using correlation matrix. Traits were height and diameter of the rosette, and of the 2 longest leaves:  
265 length and width of lamina and length of petiole, leaf 1 older than leaf 2. Trait values were scaled  
266 and PCA conducted using the FactoMineR package of R 4.0 (Lê *et al.*, 2008; R, 2020). Note that  
267 all PCAs are calculated across the same number of traits hence the same total number of axes,  
268 rendering % explained variance directly comparable among PCAs. We investigated whether degree  
269 of phenotypic integration differed before and after the exposure to treatment, but found no

270 differences [ **Supplementary Information** Table S2a ]. Moreover, we found that degree of  
271 phenotypic integration did not differ among treatments [ **Supplementary Information** Table S2b  
272 ].

273

### 274 *Hypothesis Testing*

275 The relationships between trait means, plasticity or integration were tested using simple linear  
276 regression. We determined differences of trait values among treatments using ANOVA. We used  
277 MANOVA to determine whether plant phenotype (accounting for all traits) differed among  
278 treatments. The same traits were tested in MANOVA and ANOVA analyses. Analyses were  
279 conducted in R 4.0 (R, 2020). For multiple testing on the same data set, p-values were corrected  
280 using sequential Bonferroni's correction (Cabin and Mitchell, 2000). In all regression analyses we  
281 verified the assumptions of the analyses using residual plots.

282

283

## RESULTS

### 284 *Effects of treatments on plant traits*

285 In *P. antiscorbutica*, plant phenotype of maternal lineages was significantly larger in warming  
286 treatment than in control ( $p=0.005$ ;  $F=5.9$ ;  $df_{\text{error}}=16$ ; MANOVA). In contrast, plant phenotype  
287 was significantly smaller in response to shading or combined warming-shading treatments than in  
288 control (respectively:  $p=0.0043$ ;  $F=6.8$ ;  $df_{\text{error}}=16$  and  $p=0.012$ ;  $F=5.1$ ;  $df_{\text{error}}=16$ ; MANOVA).  
289 We did not find any difference in plant phenotype between shading and combined warming-  
290 shading treatments ( $p=0.55$ ;  $F=0.93$ ;  $df_{\text{error}}=16$ ; MANOVA). Differences of individual traits  
291 between treatments and the control are indicated Table 1.

292 In *R. biternatus*, number of leaves as well as petiole length and lamina width of leaf 2 of  
293 populations were significantly larger in warming treatment compared to control (Table 2). In *R.*  
294 *pseudotrullifolius*, lamina length of leaf 1 was significantly larger in warming treatment than in  
295 control (Table 2). Overall, phenotype was larger (marginally significant) in warming in *R.*  
296 *biternatus* but not in *R. pseudotrullifolius* (respectively:  $p=0.058$ ;  $F=2.2$ ;  $df_{error}=34$  and  $p=0.57$ ;  
297  $F=0.8$ ;  $df_{error}=24$ ; MANOVA).

298

### 299 *Effects of treatments on plant performance*

300 In *P. antiscorbutica*, we found that morphological performance (percentage of leaf spread) of  
301 maternal lineages was lower in combined warming-shading treatment than in the other treatments  
302 or in the control (Table 3). In *R. biternatus* we found that plant performances of populations did  
303 not differ significantly between control and warming treatment (Table 4). In *R. pseudotrullifolius*,  
304 we found that physiological performance of populations was higher in warming treatment. Yet,  
305 this result is only marginally significant after sequential Bonferroni's correction (Table 4).

306

### 307 *Effect of treatments on the degree of plasticity*

308 In *P. antiscorbutica*, we found that the degree of plasticity of maternal lineages was higher in  
309 response to warming treatment than in response to shading treatment or combined warming-  
310 shading treatment (respectively:  $p=0.05$ ;  $F=2.19$ ;  $df_{error}=16$  and  $p=0.036$ ;  $F=2.21$ ;  $df_{error}=16$ ;  
311 MANOVA). In contrast, the degree of plasticity did not differ between shading treatment and  
312 combined warming-shading treatment ( $p=0.45$ ;  $F=0.46$ ;  $df_{error}=16$ ; MANOVA). Specifically,  
313 degrees of plasticity of plant height and lamina width of the tallest leaf were higher in response to



314 warming treatment than to shading treatment (Table 5). Degrees of plasticity of lamina width of  
315 leaf 1 and leaf 2 were higher in response to warming treatment than in response to combined  
316 warming-shading treatments (Table 5).

317

### 318 *Relationship between degree of plasticity and performance*

319 In *P. antiscorbutica*, we found significant negative relationship between the degree of trait  
320 plasticity of maternal lineages and plant performance (percentage of leaf spread and marginally  
321 photosynthetic efficiency) in response to warming treatment (Fig. 3). Also, we found a significant  
322 negative relationship between trait plasticity and performance (percentage of leaf spread) in  
323 response to combined warming-shading treatment (Fig. 4). We did not find any significant  
324 relationships between the degree of trait plasticity and plant performances in response to shading  
325 treatment ( $p\text{-value} > 0.05$ ; all  $\text{adj.}r^2 < 0.1$ ; simple linear regressions). To explore constituent costs of  
326 being plastic we compared across-treatment means of plasticity per source population to  
327 performances in control condition, where no plasticity is expressed. We found no relationships ( $p\text{-}$   
328  $\text{value} > 0.05$ ; all  $\text{adj.}r^2 < 0.1$ ; simple linear regression).

329 In *R. biternatus* and *R. pseudotrullifolius*, we did not find any significant relationship between  
330 the degree of trait plasticity of populations and plant performance.

331

### 332 *Relationship between phenotypic integration prior to treatment and the degree of plasticity in* 333 *response to treatment*

334 In *P. antiscorbutica*, we found a significant positive relationship between phenotypic integration  
335 of maternal lineages and the degree of plasticity of leaf 1 length in shading treatment (Fig. 5). Also,

336 we found marginally significant positive relationships between phenotypic integration and the  
337 degree of plasticity of leaf 2 length in shading treatment (Fig. 5). We found no significant  
338 relationship between integration and the degree of trait plasticity in other treatments.

339

340

## DISCUSSION

341 As sessile organisms, plants have to face changes of their environmental conditions. Phenotypic  
342 plasticity is considered as a major mean for plants to face environmental change (Ghalambor *et al.*,  
343 2007; Fox *et al.*, 2019). Yet, plasticity has costs for plant performance and limits that have been  
344 recognized in theory but remain to be evidenced in practice (Pigliucci, 2005; Valladares *et al.*,  
345 2007; Molina-Montenegro *et al.*, 2016; Wei *et al.*, 2021; Callahan *et al.*, 2008; Murren *et al.*, 2015).  
346 Also, plasticity was mainly studied analysing the independent response of traits to the variation of  
347 a single environmental condition (Valladares *et al.*, 2007; Matesanz *et al.*, 2021). Yet, in nature  
348 plants are subject to the variation of multiple conditions. Moreover, traits are correlated to each  
349 other (*i.e.* phenotypic integration) and cannot vary independently. We investigated the relationships  
350 between plasticity and performance in experimental treatments of plants. Moreover, we  
351 investigated the effects of combined abiotic conditions on plant plasticity, and examined the  
352 relationship between plant phenotypic plasticity and integration. We showed that plasticity may  
353 decrease performance suggesting strong induced costs of plasticity. Also, we showed that plasticity  
354 of plants may be lower when plants respond to two conditions combined than when plants respond  
355 to a single condition. Finally, some positive correlation between phenotypic plasticity and  
356 integration was evidenced.

357

358 *Trait values under different treatments*

359 Iles Kerguelen face rapid and intense climate change, characterized by an increase of  
360 temperature and a decrease of rainfall (Lebouvier *et al.*, 2011; Favier *et al.*, 2016; Verfaillie *et al.*,  
361 2021). Plants in Iles Kerguelen already exhibit signs of stress such as leaf wilting or increased shoot  
362 necrosis during dry summer periods (Chapuis *et al.*, 2004; Frenot *et al.*, 2006; Marchand *et al.*,  
363 2021). Previous work evidenced plastic responses in the studied species (Hummel *et al.*, 2004b;  
364 Hennion *et al.*, 1994; Hennion and Walton, 1997b). In this study, we showed that all species exhibit  
365 plastic morphological responses to abiotic changes. In *P. antiscorbutica* we found that traits (4 out  
366 of 9 traits) increased in response to warming. This is consistent with the response of plants at the  
367 15-day seedling stage where shoot growth increased in response to a warm temperature treatment  
368 (22°C night/ 25°C day) compared to colder temperature regime mimicking Kerguelen summer  
369 conditions (5°C night/ 10°C day) (Hummel *et al.*, 2004b). In *R. biternatus*, various traits increased  
370 in response to warming (3 out of 8 traits). In contrast, in *R. pseudotrullifolius*, only one out of 8  
371 traits was plastic, *i.e.* increased in response to warming.

372

373 *Degrees of plasticity are low under simultaneous change of multiple environmental conditions*

374 In *P. antiscorbutica*, we investigated differences of degree of plasticity among treatments.  
375 Particularly, we investigated to what extent simultaneous variation of multiple environmental  
376 conditions affect the degree of plant plasticity - compared to the variation of a single condition.  
377 Firstly, we found that the degree of plasticity is higher in response to warming than in response to  
378 shading. We yet recognize that the two conditions do not have the same units and difference of  
379 plasticity might result from a difference of the intensity of changes, hardly comparable, between

380 the two conditions. Secondly, we found that the degree of plasticity is lower in response to  
381 warming-shading than in response to warming. Warming and shading have opposite effects on  
382 plant growth. A first explanation for the decreased degree of plasticity in response to multiple  
383 conditions is hence that opposite effects of warming and shading may annihilate each other -  
384 resulting in a lower degree of plasticity than that resulting from one single factor. Further studies  
385 may investigate whether conditions having the same effect on plant growth have cumulative effects  
386 on the degree of plasticity. Another explanation for our result is that constraints of resource  
387 allocation might prevent traits to respond to multiple environmental conditions (Auld *et al.*, 2009).  
388 We showed that the degree of plasticity is higher in response to warming than in response to  
389 shading. We might therefore expect the plant phenotype in warming-shading to be closer to the  
390 plant phenotype in warming than to the plant phenotype in shading. Yet, we found that plant  
391 phenotypes in warming-shading are closer to plant phenotypes in shading than to plant phenotypes  
392 in warming. We hence suggest that, in addition to the opposite effects of shading and warming  
393 treatments, shading imposes a stronger constraint than warming. Resource constraints imposed by  
394 shading might prevent plants from responding to temperature and might possibly explain lower  
395 performance. This result is consistent with the current view that multidimensional phenotypic  
396 plasticity (*i.e.* plasticity of a trait with respect to variation in multiple environmental factors) cannot  
397 be explained solely by scaling up ideas from models of unidimensional plasticity (Westneat *et al.*,  
398 2019). Climate change alters simultaneously multiple environmental factors and plasticity might  
399 hence not suffice to compensate such changes.

400

401 *Plasticity in face of climate-change-induced warming and shading by competitors*

402 Species that show plasticity in response to abiotic conditions are believed better armed face to  
403 climate change (Valladares *et al.*, 2007). A large increase in the mean temperature is observed in  
404 Kerguelen Islands (e.g. Favier *et al.*, 2016; Verfaillie *et al.*, 2021). Plasticity shown in *P.*  
405 *antiscorbutica* and *R. biternatus* in response to warming might hence favour their capacity to face  
406 climate change, whereas *R. pseudotrullifolius* seems less plastic in relation to temperature. Notably,  
407 in the three species this plasticity did not decrease performance. Possibly, this maintained  
408 performance is because our treatment lasted for three months only and we acknowledge that a  
409 persistent increase of temperature might affect plant performance in the long term as suggested by  
410 former experiments. Indeed, under well-watered cultivation in a common garden under a temperate  
411 climate, warmer than the sub-Antarctic, at Brest (France), young plants of *P. antiscorbutica*, *R.*  
412 *biternatus* and *R. pseudotrullifolius* collected from Iles Kerguelen first showed increased growth  
413 but finally collapsed after several months (Hennion, pers. com.). In addition, in the field, plants are  
414 also exposed to the wind and different levels of soil water saturation or soil nutrients. Combined  
415 with these conditions, increased temperature might affect plant performance. Increased  
416 temperatures in Iles Kerguelen are suggested to favour expansion of invasive plant species (Frenot  
417 *et al.*, 2006), which may compete with native species, for instance for light. In *P. antiscorbutica*,  
418 we found that the phenotypes were similar in response to shading or combined warming and  
419 shading, and that individual traits decreased. Yet, we showed that neither shading nor warming did  
420 affect plant performance but combined warming and shading reduced plant performance. *P.*  
421 *antiscorbutica* might thus be sensitive to the combination of climate change and shading due to  
422 colonization by invasive species in Kerguelen at low altitude. We suggest that competition for light  
423 (with native and introduced species) might impact the capacity of *P. antiscorbutica* plants to cope  
424 with warming. Importantly, by triggering plant growth, warming may itself be the cause of  
425 increased light competition.

426

427 *Phenotypic plasticity is high in maternal lineages showing high phenotypic integration prior to*  
428 *treatment*

429 The relationship between the degree of plasticity and phenotypic integration still requires  
430 investigation (e.g. Matesanz *et al.*, 2021; Wang and Zhou, 2021). Before determining the influence  
431 of phenotypic integration on the degree of plasticity in response to a given abiotic condition, we  
432 must verify whether phenotypic integration responds or not to that abiotic condition. Here, we did  
433 not find any response of degree of integration to abiotic conditions. Given that integration does not  
434 respond to treatments, integration might potentially influence trait plasticity to those treatments.  
435 Some authors suggest integration may decrease plasticity (Gianoli and Palacio-López, 2009).  
436 Alternatively, however, we hypothesized that integration might also favour plastic response of a  
437 trait as in highly integrated organisms, extreme variation of one trait value matches extreme  
438 variation of other trait values, allowing extreme trait modification to maintain plant consistency.  
439 Here, we found no relationships between integration of maternal lineages prior to treatment and  
440 plasticity in response to warming or warming-shading treatment. But we did find that maternal  
441 lineages of high integration prior to the treatment responded more plastically to shading treatment.  
442 This latter result may explain positive relationships between plasticity and integration found post-  
443 treatment (density, Wang and Zhou, 2021) by an effect of integration on plasticity. We note,  
444 however, that we cannot rule out more complex causalities where both integration and plasticity  
445 are driven by a third, unknown property of plants. To our knowledge our results are the first  
446 evidence that intraspecific lineages of higher integration react more plastically to certain  
447 environmental changes, suggesting that plastic change of a trait is easier when integrated with other  
448 traits.

449

450 *Costs of plasticity: high degree of plasticity relates to decreased performance*

451 Plasticity helps plants facing environmental changes, yet, plasticity also has costs which may  
452 compromise plant fitness (Valladares *et al.*, 2007; Bell and Galloway, 2008; Auld *et al.*, 2009;  
453 Murren *et al.*, 2015). We investigated whether the degree of plasticity of populations/maternal  
454 lineages statistically increased or decreased performance in *P. antiscorbutica*. We showed that the  
455 degree of plasticity statistically decreased plant performance in high temperature treatment. Yet,  
456 we found no significant relationship between plasticity and performance in the other treatments.  
457 Overall, we found evidence for costs of plasticity, and these costs appeared to depend on the  
458 environmental context. Note that our study is essentially correlative as we did not directly  
459 manipulate neither integration nor plasticity as imposing integration or plasticity risks to damage  
460 the organism and may not reflect levels of integration or plasticity actually achieved in nature.

461 Costs of plasticity may be dissociated in two kinds of costs, constitutive and induced.  
462 Constitutive costs result from maintaining the basic physiological machinery of plasticity while  
463 induced costs depend on the amount of phenotypic changes (Sultan and Spencer, 2002; Callahan  
464 *et al.*, 2008). Constitutive costs have to be paid even without phenotypic change in a constant  
465 environment (Auld *et al.*, 2009). However, we found that performance under stable conditions did  
466 not relate to the capacity to plastically respond to changing conditions. Plasticity might hence have  
467 only little constitutive costs in our study system. Induced costs, in contrast, reflect the costs of  
468 morphological modification in response to environmental change. High induced costs might  
469 impose resource allocation constraints (Auld *et al.*, 2009), which does not allow plants to maintain  
470 the same degree of performance. Here we found that the degree of plasticity is indeed negatively  
471 related to plant performance post-treatment, notably in warming treatment. Above, we discussed

472 that warming induced a higher degree of plasticity than other treatments. We suggest that a high  
473 degree of plasticity generates important induced costs that impact plant performance. We also  
474 observed a negative plasticity-performance relationship in response to a combined warming-  
475 shading treatment. We suggest that the most stressful environments, such as combined warming  
476 and shading, reduce energy available to plants. In our systems, the complete absence of positive  
477 relationship between the degree of plasticity and performance tends to suggest that more plastic  
478 organisms may not inevitably better sustain environmental change. On the opposite, plants that  
479 show high degree of plasticity may perform poorly. Performance is poor once the environmental  
480 change occurs, not before, *i.e.* reflecting induced rather than constitutive costs.

481

482

## CONCLUSION

483 Although the concept of plastic response to environmental change has long been studied, our  
484 understanding has to be improved about costs and limits of such plastic response in individuals  
485 (Pigliucci, 2005; Matesanz *et al.*, 2021; Murren *et al.*, 2015). Here we showed that simultaneous  
486 shift of two environmental factors may limit plasticity of individuals from different lineages. This  
487 may reduce the capacity of populations to respond to climate warming under increased light  
488 competition. Also, we found some evidence for a positive relationship between phenotypic  
489 plasticity in response to treatment and phenotypic integration prior to treatment suggesting that a  
490 trait can respond to treatment more plastically if changes are well integrated into a body plan.  
491 Moreover, we found that highly plastic individuals performed poorly. Due to induced costs, plant  
492 plastic response to environmental change does not guarantee plant success in the new environment.  
493 Hence, little plastic individuals may be as, or even more, performant in the new environment than



494 highly plastic individuals. Future studies might aim to verify whether the relationships we found  
495 also hold for traits other than vegetative morphology.

496

497

#### 498 SUPPLEMENTARY DATA

499 Supplementary data are available online in the Supporting Information section and consist of the  
500 following. Table S1: MANOVA analyses indicating trait differences among source mothers (*R.*  
501 *biternatus* and *R. pseudotrullifolius*) or source populations (*P. antiscorbutica*) in the control  
502 condition. Table S2: ANOVA analyses indicating in *P. antiscorbutica* whether a) degree of  
503 phenotypic integration differs before and after application of treatments, b) phenotypic integration  
504 differs among treatments.

505 The datasets generated during the current study will be available online on Osuris geonetwork:  
506 [https://www.osuris.fr/geonetwork /srv/fre/catalog.search#/.](https://www.osuris.fr/geonetwork/srv/fre/catalog.search#/)

507

508

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517

518

519

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527

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Table 1. ANOVA analyses indicating trait differences between different abiotic treatments in *P. antiscorbutica*. P-values (P) after sequential Bonferroni's correction and F values (F) are indicated. "</>": trait is lower/larger in the first treatment indicated. dferror =18 source populations.

	Plant									Leaf 1									Leaf 2										
	Plant height			Diameter			Number of leaves			Petiol length			Lamina length			Lamina width			Petiol length			Lamina length			Lamina width				
	P	F	</>	P	F	</>	P	F	</>	P	F	</>	P	F	</>	P	F	</>	P	F	</>	P	F	</>	P	F	</>	P	F
Control vs shading	0.30	2.1		<b>0.001</b>	<b>27.8</b>	>	<b>0.001</b>	<b>24.8</b>	>	0.50	0.47		0.10	6.09	>	<b>0.007</b>	<b>15.7</b>	>	0.88	0.02		0.10	6.84	>	0.29	1.2			
Control vs warming-shading	0.17	1.14		<b>0.06</b>	<b>8.4</b>	>	<b>0.028</b>	<b>21.8</b>	>	0.25	4.54		0.83	0.046		<b>0.008</b>	<b>11</b>	>	0.11	2.85		0.69	0.16		0.21	1.7			
Shading vs warming-shading	0.59	0.3		0.25	1.5		0.34	0.4		0.42	3.78	>	0.15	2.23		0.59	0.29		0.32	5.05	>	0.30	4.37	>	0.88	0.02			
Control vs warming	0.75	0.10		0.18	2		<b>0.001</b>	<b>16.8</b>	<	0.14	2.33		<b>0.02</b>	<b>10.72</b>	<	<b>0.001</b>	<b>28.8</b>	<	0.30	1.14		<b>0.05</b>	<b>8.1</b>	<	<b>0.001</b>	<b>28</b>	<		

Table 2. ANOVA analyses indicating trait differences between warming and control in *R. biternatus* and *R. pseudotrullifolius*. P-values (P) after sequential Bonferoni's correction and F values (F) are indicated. "</>": trait is lower/larger in the control. In *R. biternatus*, dferror = 36 mothers; in *R. pseudotrullifolius*, dferror =26 mothers.

	Plant			Leaf number 1									Leaf number 2														
	Plant height			Number of leaves			Petiol length			Lamina length			Lamina width			Petiol length			Lamina length			Lamina width					
	P	F	</>	P	F	</>	P	F	</>	P	F	</>	P	F	</>	P	F	</>	P	F	</>	P	F	</>	P	F	</>
Control vs warming	0.14	3.62		<b>0.0091</b>	<b>10.94</b>	<	0.085	4.12		0.12	2.44		0.11	4.67		<b>0.03</b>	<b>7.19</b>	<	0.066	1.07		<b>0.0003</b>	<b>5.8</b>	<			
<i>R. biternatus</i>	0.54	0.66		1	1.36		0.43	0.18		<b>0.033</b>	<b>3.1</b>	<	0.077	2.6		0.85	1		0.70	0.39		0.90	0.6				
<i>R. pseudotrullifolius</i>																											

Table 3. ANOVA analyses indicating differences of performance (photosynthetic efficiency and leaf spread) between treatments in *P. antiscorbutica*. P-values (P) after sequential Bonferroni's correction and F values (F) are indicated. "</>": performance is lower/higher in the first treatment indicated. dferror =18 source populations.

	Photosynthetic efficiency			Leaf spread		
	P	F	</>	P	F	</>
Control vs warming	0.11	2.8		0.90	0.014	
Control vs shading	0.19	1.81		0.09	3.32	
Control vs warming-shading	0.24	1.51		<b>0.0002</b>	<b>31.74</b>	>
Shading vs warming-shading	0.58	0.32		<b>0.02</b>	<b>8.02</b>	>
Warming vs warming-shading	0.42	0.69		<b>0.0002</b>	<b>27.92</b>	>

Table 4. ANOVA analyses indicating differences of performance (percentage of survival, photosynthetic efficiency and leaf spread) between warming and control in *R. biternatus* and *R. pseudotrullifolius*. P-values (P) after sequential Bonferroni's correction and F values (F) are indicated. "</>": performance is lower/higher in the control. In *R. biternatus*, dferror = 36 mothers; in *R. pseudotrullifolius*, dferror =26 mothers.

		Percentage of survival			Photosynthetic efficiency			Leaf spread		
		P	F	</>	P	F	</>	P	F	</>
<i>R. biternatus</i>	Control vs warming	0.14	1.01		0.90	0.014		0.51	0.2	
<i>R. pseudotrullifolius</i>	Control vs warming	0.14	0.93		0,08	3.24	<	0.11	1.65	

Table 5. ANOVA analyses indicating differences of the degree of plasticity between treatments in *P. antiscorbutica*. "</>": trait is less/more plastic in the first treatment indicated. P-values (P) and F values (F) are indicated. dferror =16 source populations.

	Plant			Leaf number 1									Leaf number 2											
	Plant height			Number of leaves			Petiole length			Lamina length			Lamina width			Petiole length			Lamina length			Lamina width		
	P	F	</>	P	F	</>	P	F	</>	P	F	</>	P	F	</>	P	F	</>	P	F	</>	P	F	</>
Warming vs shading	<b>0.054</b>	<b>4.31</b>	>	0.13	2.53		0.28	1.23		0.19	1.87		<b>0.036</b>	<b>5.19</b>	>	0.35	0.94		0.38	0.83		0.30	1.18	
Warming vs warming-shading	0.13	2.55		0.54	0.38		0.87	0.03		0.35	0.91		<b>0.012</b>	<b>7.97</b>	>	0.72	0.13		0.06	3.97		<b>0.017</b>	<b>6.99</b>	>
Shading vs warming- shading	0.64	0.22		0.43	0.64		0.23	1.54		0.71	0.15		0.41	0.73		0.25	1.45		0.34	0.95		0.31	1.09	

**Figure 1.** a. Location of Iles Kerguelen in the southern Indian Ocean; b. sampled sites in Iles Kerguelen (map modified from Google maps).

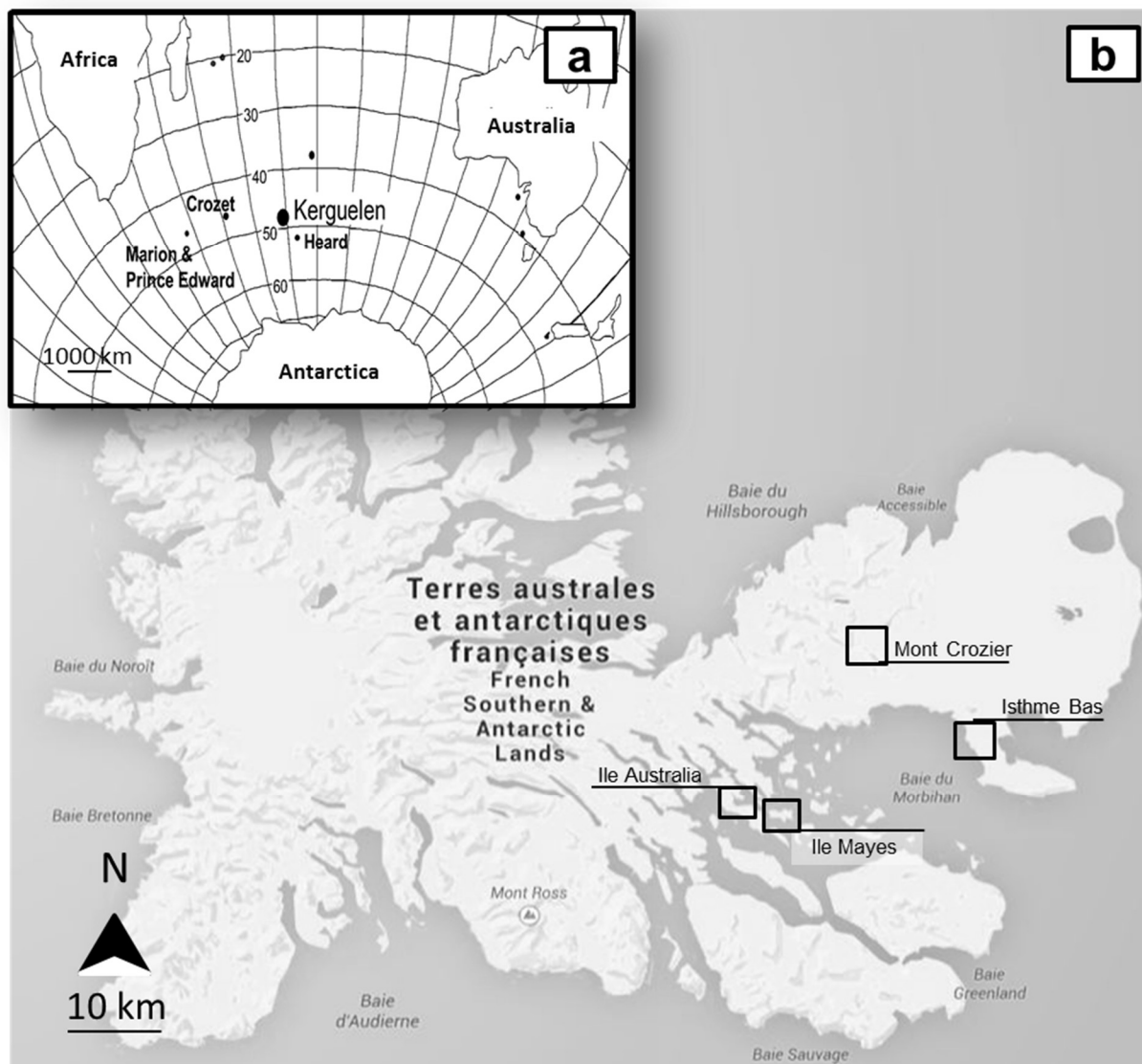
**Figure 2.** Illustration of experimental design. The numbers of plants used per treatment, per species are shown.

**Figure 3.** Negative relationship between the degree of plasticity and performance in the warming treatment across maternal lineages of *P. antiscorbutica*. P-values and adj. $r^2$  are indicated, N=9.

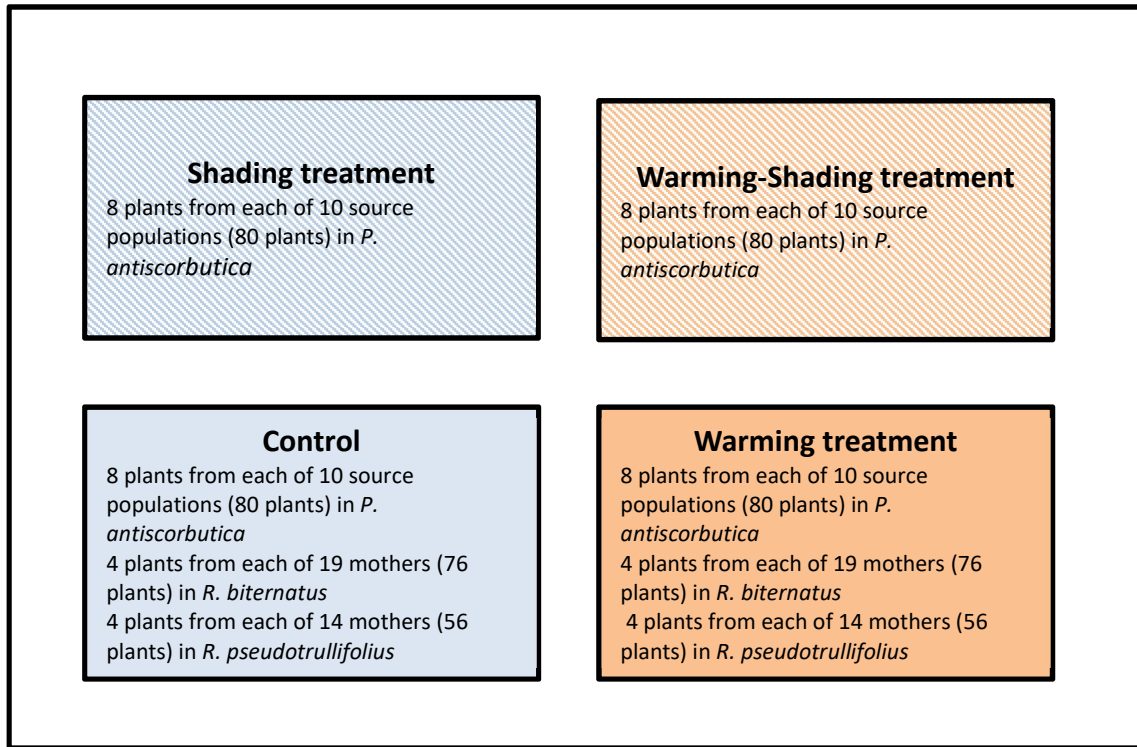
**Figure 4.** Negative relationship between the degree of plasticity and performance in the warming-shading treatment across maternal lineages of *P. antiscorbutica*. P-values and adj. $r^2$  are indicated, N=9.

**Figure 5.** Positive relationship between phenotypic integration and the degree of plasticity of leaf 1 width or leaf 2 length in the shading treatment across maternal lineages of *P. antiscorbutica*. N=9.

Fig. 1.



**Fig. 2.**



**Fig. 3.**

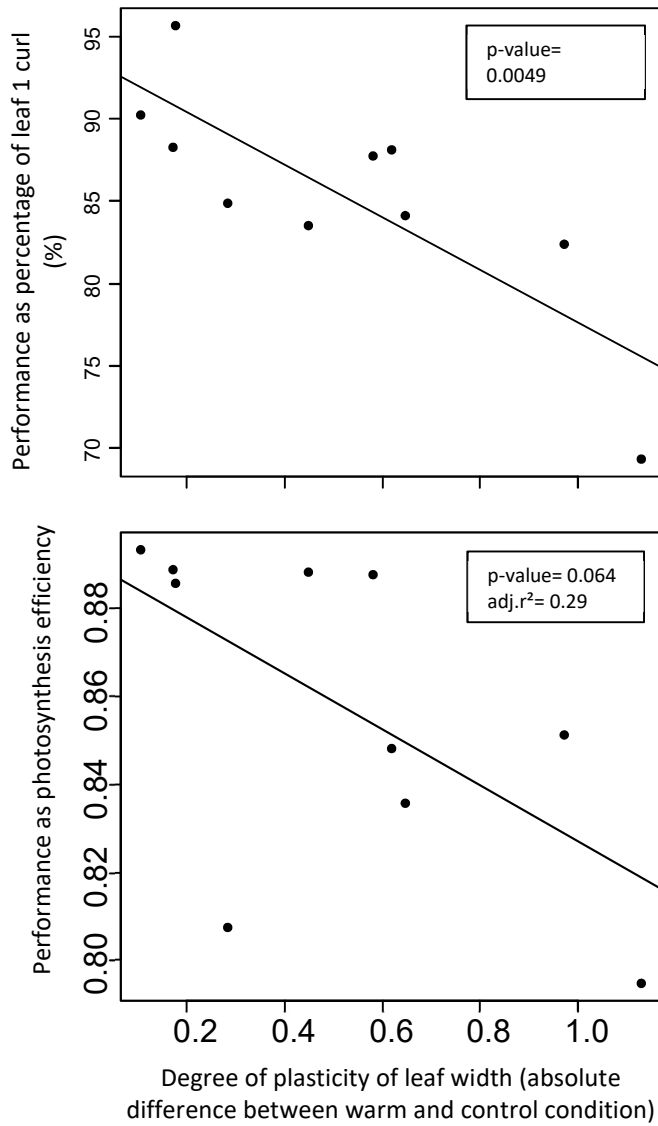




Fig. 4.

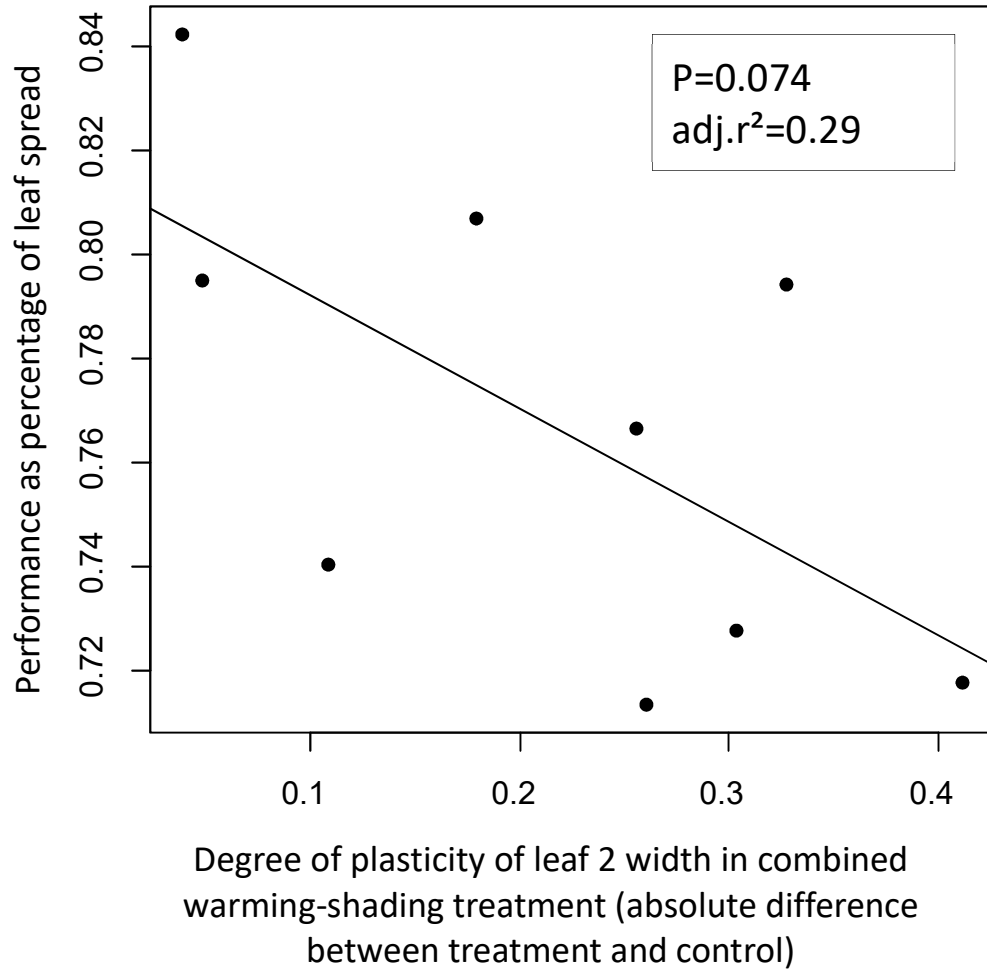


Fig. 5.

