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1 **Contrasting patterns in leaf traits of Mediterranean shrub communities along an elevation**
2 **gradient: measurements matter**

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36 ABSTRACT

37 We assessed the changes in community weighted mean (CWM) and variability of specific leaf area
38 (SLA) and leaf area (LA) of different Mediterranean shrub communities along an elevation gradient
39 in the island of Sardinia (Italy). Furthermore, we explored the relative contribution of species
40 turnover and intraspecific variation to shifts in CWM values along the gradient.

41 Forty sampling units (5x5m) were selected in a probabilistic way along a 1,300 m elevation gradient
42 which crossed four thermotypes (thermometric belts). Leaf traits were measured in each sampling
43 unit. ANOVA and a trend test for monotonic changes in variance were used to assess respectively
44 CWM differences and variability in both the leaf traits across thermotypes. Variance decomposition
45 of CWM values was used to identify the role of inter- and intraspecific variation.

46 SLA and LA responded differently along the studied gradient in terms of abundance weighted mean
47 values and variability: CWM of SLA showed the lowest values in the driest thermotype, while LA
48 in the more humid one; SLA variability showed a significant increasing trend with increased water
49 availability, while LA variability did not show any pattern. The contribution of intraspecific trait
50 variation was significant for both the leaf traits, but higher for SLA, where negative covariation
51 between inter- and intraspecific variation was detected.

52 We highlight the importance of simultaneously considering measurements of both leaf traits to
53 understand the functional response of communities in Mediterranean environments. Moreover,
54 neglecting intraspecific variation in leaf traits, even along steep gradients with relevant species
55 compositional changes, can result in the underestimation of the amount of trait variation in response
56 to environmental changes.

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58 **Keywords:** community weighted mean; interspecific and intraspecific variation; leaf area; plant
59 functional traits; specific leaf area, trait-environment relationship.

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61 **Running head:** Leaf traits variation in Mediterranean shrublands

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72 **INTRODUCTION** A large number of studies have identified relationships between traits and
73 environmental conditions (Garnier et al. 2016 and references therein), such as climate and soil
74 properties at different scales and in different regions and systems (e.g. Ackerly 2004; Wright et al.
75 2004; Ordonez et al. 2009). However, the extent to which such patterns can be generalized beyond
76 the studied scale/region/system is still unknown (Shipley et al. 2016). In particular, despite the
77 Mediterranean biogeographic region being one of the world's most important reservoirs of plant
78 diversity (Medail and Quezel 1999) and one of the most vulnerable areas of the planet to climate
79 change (Thuiller et al. 2005; Cuttelod et al. 2009; Moreno and Oechel 2012), the trait-environment
80 relationship is still poorly investigated (Chelli et al. 2019). Here temperature is expected to increase
81 (Senatore et al. 2011) together with an increase in weather extremes in both the sense of heavy
82 rainfall and summer heat waves, with a high incidence of drought (ISAC-CNR 2009). In this
83 context, shedding light on the trait-environment relationship along temperature and water stress
84 gradients can help to interpret vegetation response under changing climatic conditions (Scherrer et
85 al. 2017).

86 In the Mediterranean biogeographic region, shrublands are one of the most important and
87 widespread vegetation types (Naveh and Whittaker 1979; Cowling et al. 1996; Shoshany and
88 Karnibad 2011). Such vegetation is dominated by evergreen shrubs usually with small and
89 sclerophyllous leaves as part of their drought tolerance strategy (Mooney and Dunn 1970).

90 Of all plant traits, those of leaves are among the most important when it comes to water-use
91 strategies (Wilson et al. 1999; Vendramini et al. 2002). Specific Leaf Area (SLA, the light-
92 capturing surface area per unit of dry biomass, Poorter et al. 2009), is generally recognized as one
93 of the most significant trait to study water and nutrient availability impacts on plant growth (Wright
94 et al. 2001; Reich et al. 2014; Wellstein et al. 2017). Leaf Area (LA) is another trait which has
95 significant implications regarding the regulation of leaf temperature and water-use efficiency during
96 photosynthesis (Ackerly et al. 2002). SLA and LA are two fundamental traits influencing
97 community structure and functioning of Mediterranean ecosystems (Gross et al. 2013).

98 In community level studies based on average trait values for species, LA and SLA usually showed
99 parallel changes, which suggests that these traits are linked in their functional strategy associated
100 with water and/or nutrient availability (Ackerly et al. 2002 and references therein). Accordingly,
101 global studies showed a decrease in LA and SLA along with decreasing water and/or nutrient
102 availability (Poorter et al. 2009; Wright et al. 2017). However, comparative studies accounting for
103 species trait variability suggested that LA and SLA may not be strictly related (e.g. Ackerly et al.
104 2002), thus highlighting the importance of approaches taking into account both leaf traits and their
105 intraspecific variation along environmental gradients.

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107 Decreased plant growth rates are associated with lower SLA values (Wellstein et al. 2017 and
108 references therein), and are often found under conditions of water stress (Chapin 1991), as they can
109 improve water-use efficiency (Wright et al. 2001). LA shows a similar trend, with small leaves
110 reducing resistance in the boundary layers and allowing the plant to maintain lowered temperatures
111 and higher photosynthetic water-use efficiency when solar radiation is high and water availability is
112 low (Wright et al. 2001; Ackerly et al. 2002). In short, SLA and LA are good proxies of broad
113 resource-use strategies and are negatively correlated with many other hard and soft leaf traits (e.g.,
114 leaf toughness, leaf longevity, leaf dry matter content, stomatal conductance; Wright et al. 2002;
115 Reich et al. 2003; Wright et al. 2004; Hoffmann et al. 2005).

116 Few papers deal with changes in leaf traits along environmental gradients of shrubs communities in
117 Mediterranean-type climates (e.g., Ackerly et al. 2002; Ackerly 2004; Anacker et al. 2011,
118 conducted in North American chaparral), confirming the above described patterns of SLA and LA.
119 Additional studies used leaf traits to explore assembly rules along aridity gradients in shrublands of
120 the Mediterranean basin (e.g., Gross et al. 2013; Le Bagousse-Pinguet et al. 2015).

121 Looking at a single trait allows the range and distribution of values to inform the relationship
122 between ecosystem function and community structure (Ackerly et al. 2002). In order to summarize
123 the functional composition of plant communities for a certain trait taking into account species
124 abundances, Garnier et al. (2004) proposed the so-called ‘community-weighted mean trait values’
125 (CWMs).

126 Changes in community composition along climate and soil gradients are expected to be strongly
127 represented in leaf traits, due to their reflection of water-use strategies (Le Bagousse-Pinguet et al.
128 2017). Increased variability in these leaf traits is expected when conditions are favorable for plant
129 life, e.g., water and temperature stress are reduced; soils are more fertile (Ackerly et al. 2002;
130 Hoffmann et al. 2005; Cornwell and Ackerly 2009). In contrast, habitat filtering may occur in the
131 face of extreme aridity or a reduction in water availability, reducing the distribution of SLA and LA
132 values within the community, and suggesting that species with high stress tolerance do not
133 generally inhabit broad environmental ranges (Thuiller et al. 2004).

134

135 Most studies focusing on trait-environment relationships along gradients used only mean trait
136 values per species (usually weighted by species proportions), assuming that between-species trait
137 variation is generally considerably greater (e.g. Cornelissen et al. 2003) than within species
138 variation. However, it is clear that some traits are more variable than others (Siefert et al. 2015), and
139 a growing number of studies confirm that intraspecific variation could play a relevant role to shifts
140 in community-level trait measures (i.e., Lepš et al. 2011; Kichenin et al. 2013) and assembly rules
141 (i.e., Jung et al. 2010; Le Bagousse-Pinguet et al. 2015) along environmental gradients. In

142 particular, both SLA (Wellstein et al. 2013, 2017; Liancourt et al. 2015) and LA (Fraser et al. 2009)
143 showed a certain intraspecific variation, with SLA demonstrating a larger one (Rozendaal et al.
144 2006; Siefert et al. 2015).

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146 In this paper, we aimed to assess the patterns of community level leaf traits (namely, SLA and LA)
147 along an elevation gradient (1,300 m) spanning four thermotypes or thermometric belts, defined on
148 the basis of temperature data condensed in two indexes (It = thermometric index; Tp = positive
149 annual temperature), following Rivas-Martínez et al. (2011) method, and mapped by Canu et al.
150 (2015) in Mediterranean shrubland ecosystems in the island of Sardinia (Italy). We measured leaf
151 traits at plot level in order to take into account intraspecific variability.

152 In particular, we propose the following hypotheses:

153 **H1** SLA and LA at community-level should be significantly lower in the most arid climate
154 (thermotype 1) compared to the most cool and moist environment (thermotype 4).

155 **H2** The variability of both leaf traits (in terms of variance) should increase (niche differentiation)
156 toward the cooler and moister end of the gradient (thermotype 4).

157 Furthermore, we disentangled the contribution of interspecific (i.e. species turnover) and
158 intraspecific trait variation to shifts in CWM values along the gradient, hypothesizing (**H3**) a
159 significant influence of intraspecific variation in both leaf traits.

160

161 **MATERIALS AND METHODS**

162 **Study area**

163 Sardinia, the second largest island in the Mediterranean Sea (ca. 24,090 km²), has a unique flora as
164 a result of the unique geological history that played an essential role in shaping plant diversity
165 (Medail and Quezel 1999; Mansion et al. 2008). The Sardinian flora consists of 2,301 native
166 vascular plants (Bartolucci et al. 2018), of which more than 170 are endemic to the island:
167 concentrated mainly in the mountains (Cañadas et al. 2014). This study has been conducted in the
168 NE part of Sardinia (Figure 1), locally known as the Gallura subregion, with constant geology in the
169 altitudinal gradient, being characterized by Hercynian granites, having homogeneous slopes and
170 patterns of degradation. Furthermore, the selected area is also considered homogeneous from a
171 biogeographic standpoint (Fenu et al. 2014). Altitude range in this area is from 0 (coast) to 1354 m
172 (top of Mt. Limbara massif). This altitudinal gradient allows five different thermotypes to occur in
173 the study area (Canu et al. 2015). Thermotypes are described on the basis of thermicity indexes
174 developed by the Worldwide Bioclimatic Classification System (Rivas-Martínez et al. 2011).
175 Annual mean temperature ranged from 16.6°C in the coastal area to 10.5°C at the top of Mt.
176 Limbara; annual rainfall ranged from 560 mm to 1160 mm.

177 Potential natural vegetation of the area (sensu Farris et al., 2010) is represented by different forest
178 types depending on the thermotypes: the coastal thermo-Mediterranean belt is characterized by the
179 presence of Phoenician juniper vegetation (*Oleo-Juniperetum turbinatae* and *Erico-Juniperetum*
180 *turbinatae* on southern and northern slopes, respectively). Wild olive (*Olea europaea*) vegetation is
181 prevalent in the lower meso-Mediterranean belt, holm-oak (*Quercus ilex*) vegetation prevails at
182 both the upper meso-Mediterranean (*Galio-Quercetum ilicis*) and sub-Mediterranean and
183 Temperate belts (*Saniculo-Quercetum ilicis*).

184 The intense past sylvo-pastoral management, that included the use of fire and extensive plantations
185 of alien trees, changed the landscape and determined the current situation where the shrublands
186 represent the most abundant plant communities present in the area.

187

188 **Sampling design**

189 To study a strong climatic gradient, we needed strata that could represent the range of temperatures
190 and water stresses characterizing our study area, stretching from the coastline to the inner areas of
191 Sardinia. To describe this gradient, we adopted a modified version of the thermotypes recognized in
192 the bioclimatic map of Sardinia (Canu et al. 2015) where we unified the upper belts into a unique
193 “temperate” cacuminal belt, to obtain 4 different thermotypes describing the whole transect (Figure
194 1): upper Thermomediterranean (T1); lower Mesomediterranean (T2); upper Mesomediterranean
195 (T3) and lower Supratemperate (T4).

196 The study area was defined creating a grid of 1 km² units including the Limbara massif and
197 extending eastward toward the Olbia coastline (40 km gradient): regarding the other relevant
198 environmental factors, we can consider geology (also soil), erosion patterns, history (including fire
199 regime and cutting) and animal influence as constant along the bioclimatic gradient.

200 From the original 495 quadrats, we excluded i) all the mixed quadrats crossing two thermotypes,
201 and ii) all quadrats showing a land cover characterized by less than 50% of shrubs and garrigues
202 (CLC Regione Sardegna). Among the remaining quadrats, within each of the four strata we selected
203 five sampling quadrats; in each sampling quadrat, we performed a random selection of 2 sampling
204 units 5 x 5 m. As a whole, we sampled 40 sample units of 25 m² divided in 4 thermotypes (2 sample
205 units x 5 quadrats x 4 thermotypes) where we registered the complete species list and visually
206 estimated the species coverage (%) during May 2016 (Appendix 1).

207 In each sampling unit, we selected all the species contributing to reach a relative cumulative
208 coverage of 80%. For each species, five leaves in full sun were sampled from five mature and
209 healthy individuals; such sampling size adequately captures leaf traits intraspecific variability in
210 Mediterranean contexts (Petruzzellis et al. 2017). In total, 900 individuals belonging to 33 species,
211 including shrubs and herbs, were selected for leaf traits measurements (Appendix 1). We measured

212 SLA and LA following standard protocols (Pérez-Harguindeguy et al. 2013): leaf area was
 213 measured within few hours after collection; subsequently leaves were oven-dried for 72 hours at
 214 70°C and leaf dry mass was measured to calculate SLA. Community weighted mean values (CWM,
 215 Garnier et al. 2004) were then calculated according to species plot-level cover values (i.e., we
 216 averaged the species trait values from individual measurements taken in a given plot and used it to
 217 calculate CWM of that plot). According to recent methodological studies (e.g. Peres-Neto et al.
 218 2017; Zelený 2018), measuring traits for each species at each site, and using these plot-level trait
 219 measurements to calculate CWM, avoid the classic problem with inflated Type I error.

220

221 **Data analysis**

222 We analyzed the data to test the differences existing along the gradient, according to the following
 223 variables: **i) Community composition** – to observe differences in terms of species composition and
 224 abundance we performed a NMDS (standardized samples by total, Bray-Curtis resemblance). We
 225 integrated NMDS results with PERMANOVA (Anderson et al 2001; fixed factor: thermotypes,
 226 9999 permutation, unrestricted permutation of raw data, Bray-Curtis resemblance) and a
 227 PERMDISP analyses, a test of the homogeneity of multivariate dispersions within groups, as
 228 PERMANOVA makes the implicit assumption that dispersions are roughly constant across groups;
 229 **ii) Changes of CWM values** - to test if CWM values of SLA and LA significantly changed
 230 according to the four thermotypes (H1), we used ANOVA for multiple samples and Bonferroni
 231 post-hoc test; **iii) Trend in leaf traits variance** - to test for changes in the variance of leaf traits
 232 along the gradient (H2), we used a trend test for monotonic trend in variance suggested by
 233 Neuhauser and Hothorn (2000). The test is based on the finite-intersection approach, the Brown–
 234 Forsythe transformation, and Kendall’s tau coefficient (Noguchi and Gel 2010; Gastwirth et al.
 235 2015). The finite-intersection approach (Mudholkar et al. 1993) combines p values of the
 236 component test statistics, which correspond to a finite number of nested hypotheses. Fisher’s p
 237 value combination method (Fisher 1934) is used in this study. Finally, to distinguish **iv) the relative**
 238 **contribution of inter- and intraspecific variation on community-level trait values** (H3) we
 239 followed the approach proposed by Lepš et al. (2011). This method is based on the decomposition
 240 of the total sum of squares (SS_{specific}) of the plot-level trait variance related to a certain
 241 environmental variable into “interspecific” (or “fixed”) ($SS_{\text{interspecific}}$), “intraspecific” ($SS_{\text{intraspecific}}$) and
 242 “covariation” (SS_{cov}) components, so that $SS_{\text{specific}} = SS_{\text{interspecific}} + SS_{\text{intraspecific}} + SS_{\text{cov}}$ (Kichenin et al.
 243 2013). Firstly, for each plot, we calculated “specific” plot-average trait values using species trait
 244 values as measured on that plot (which includes both inter- and intraspecific components), and
 245 “interspecific” (or “fixed”) plot-average trait values using species trait values averaged over all
 246 plots along the selected environmental gradient (which takes into account only species turnover and

removes the intraspecific variability component). Secondly, we calculated “intraspecific” plot averages as the difference between “specific” and “interspecific” plot-average trait values (thus removing the component of interspecific variability). Thirdly, the “covariation” component (i.e., the effect of covariation between interspecific and intraspecific trait variation) was obtained as follows: $SS_{cov} = SS_{specific} - SS_{interspecific} - SS_{intraspecific}$. As environmental variables we used both categorical (i.e., thermotype) and continuous ones (i.e., temperature seasonality and Potential EvapoTranspiration, PET; Canu et al. 2015).

All statistical analyses were performed in R, version 3.2.2 (R Development Core Team 2015). In particular the following R packages were used: package *vegan* (function *betadisper*) for the analysis of multivariate homogeneity of group dispersion; package *lawstat* (function *neuhausser.hothorn.test*) for the trend test for monotonic trend in variance (Neuhauser and Hothorn 2000); Package *cati* (function *traitflex.anova*) for the analysis on the relative contribution of inter- and intraspecific variation on community weighted mean values (Lepš et al. 2011).

261

262 RESULTS

263 Community composition and species assemblages

We found shrub communities, physiognomically referred to as *Euphorbio dendroidis-Calicotometum villosae* prevalent at the upper Thermomediterranean belt (T1), *Pistacio lentisci-Calicotometum villosae* dominant at the lower Mesomediterranean (T2), *Erico-Arbutetum* widespread at the upper Mesomediterranean (T3) and *Ericetum scopario-arboreae* dominating at sub-Mediterranean and Temperate belts (T4, Farris et al. 2007; Galié et al. 2015). Plant communities differ between thermotypes (PERMANOVA $p < 0.05$ for all pairwise tests; PERMDISP $p > 0.05$). The sub-Mediterranean and Temperate *Ericetum scopario-arboreae* (belt T4) is the community that mostly diverged from the others (NMDS, figure 2), on the contrary *Euphorbio dendroidis-Calicotometum villosae* of the Thermomediterranean belt (T1) is the community that, being statistically different from the others, appeared less distinct from *Pistacio lentisci-Calicotometum villosae* and *Erico-Arbutetum* (T2 and T3)

275

276 Community-level traits variation

Significant differences between community-level SLA values have been found ($p < 0.05$). In detail, T1 (SLA=8.83 mm²*mg⁻¹) and T4 (SLA=13.51 mm²*mg⁻¹) were significantly different according to Bonferroni post-hoc test ($p < 0.05$; Figure 3a). Furthermore, we observed a significant increase in variability along the gradient from T1 to T4 (Test Statistic=3.115; $p < 0.01$). Regarding LA, ANOVA showed significant differences between thermotypes ($p < 0.05$). Differences were found between T2

282 (LA=410.04 mm²), T3 (LA=407,87 mm²) and T4 (LA=102,33 mm²; p<0.05; Figure 3b), with the
283 lowest mean values found at T4. No significant trends in LA variability were detected along the
284 gradient.

285

286 **Contribution of inter- and intraspecific variation**

287 The contributions of inter- and intraspecific variability in explaining the response of community-
288 level SLA and LA variation to the three environmental variables (i.e., thermotype, temperature
289 seasonality, PET) were both significant only for “thermotypes” (p<0.01; Figure 4). On the contrary,
290 along the temperature seasonality gradient the contribution of species turnover was significant only
291 for SLA (p<0.05), while along the PET gradient both the components were not significant for both
292 leaf traits. For SLA, the total variability effects were significant for all the three variables (p<0.05)
293 and was highest for the variable “thermotype” (19.61%). Here species turnover and intraspecific
294 variability showed a similar contribution (turnover = 42.05%; intraspecific = 36.04%), but there
295 was a strong negative covariation (-58.48%) between the effects of inter- and intraspecific
296 variability on CWM values of SLA. For LA the total variability effects were significant only for
297 “thermotype” (26.36%; p<0.01). Here species turnover showed a higher contribution (23.88%) with
298 respect to intraspecific variability (12.25%), with a negative covariation (-9.77%).

299

300 **DISCUSSION**

301 Our study explored the leaf trait-environment relationship along an elevation gradient across four
302 thermotypes in Mediterranean shrubland ecosystems. The large environmental gradient included
303 four types of compositionally different plant communities. SLA and LA showed different patterns
304 in both abundance-weighted values and variability along the gradient. Additionally, the studied leaf
305 traits differed in the relative contribution of species turnover and intraspecific variation to their
306 changes at community level along the gradient, with SLA showing a relevant contribution of
307 intraspecific variation, higher with respect to LA. Overall, our results highlight the importance of
308 approaches considering simultaneously different traits and their intraspecific variability, even in
309 studies encompassing steep environmental gradients (Lepš et al. 2011; Kichenin et al. 2013;
310 Derroire et al. 2018; Garnier et al. 2018).

311

312 **Changes in CWM values and variability**

313 Our results showed significantly different values of community-level SLA between the two
314 extremes of the gradient, with lower values in the driest thermotype. On the contrary, community-
315 level LA values were significantly different only between T2-3 and T4, leading us to confirm H1 of
316 lower leaf traits values in the most arid thermotype only for SLA. Regarding trait variability, our

317 hypothesis (H2) of increasing variability of both leaf traits toward the cooler and moister end of the
318 gradient (T4) is confirmed only for SLA, while LA showed the lowest level of variability in the
319 cooler and moister extreme of the gradient. Variation in the patterns of SLA and LA in the present
320 study across the climatic gradient confirm the expectation that the two leaf traits are not strictly
321 correlated if species variability is properly accounted. The advantage of looking at variation within
322 a trait (instead of using average and fixed trait values) allows us to separate the behaviour of the two
323 traits (as in Ackerly et al. 2002).

324 The lowest community level SLA values were found in the driest thermotype, which reflects the
325 increase in water use efficiency in a more arid environment (Wright et al. 2001; Wellstein et al.
326 2017). Low SLA values and decreased plant growth rates usually reflect water stress (Chapin 1991).
327 Moreover, T1 is characterized by the lowest SLA variability, most likely indicating a strong habitat
328 filtering generating a convergence in leaf strategy to cope with drought in our system (see also
329 Wright et al. 2002). Under drought conditions, leaves tend to have a denser mesophyll layer, and
330 cells with thicker cell walls, this helps avoid wilt (Shield 1950; Poorter et al. 2009; Wellstein et al.
331 2017). The increase in SLA variability along the gradient can be explained by the occurrence of
332 more benign conditions, thus allowing the coexistence of species/individuals with different
333 functional strategies related to water-use based on different mechanisms of coexistence (see
334 Wellstein et al. 2014).

335 Low LA generally leads to high water stress tolerances, whereas species equipped with large leaves
336 are better suited to mesic environments (Westoby et al. 2002). Accordingly, we would expect larger
337 and more variable LA due to more benign conditions in terms of temperature and water availability
338 in T4. However, our results indicated that different habitat filtering mechanisms are acting on this
339 trait leading to both low values of abundance-weighted mean and variability in the two extremes of
340 the gradient. Our findings are consistent with those of Gross et al. (2013), who found a quadratic
341 relationship between functional diversity and mean LA values along a rainfall gradient in
342 Mediterranean shrublands. We propose different explanations for the unexpected strong
343 convergence to low mean LA values in T4. Firstly, LA is a key trait that is strongly influenced by
344 air temperature (Wright et al. 2017). Nocturnal low temperatures in particular seem to have the most
345 significant impact on LA (Wright et al. 2017 and references therein). The T4 is in fact the only
346 thermotype having average lower temperatures in the coldest month approaching 0°C (0.4°C in the
347 locality Vallicciola at 1040 m a.s.l. on Limbara massif), and an average snow cover of 3-4 weeks
348 every year (Farris et al. 2007). Secondly, functional trade-offs between different plant traits could
349 be significant in combined trait responses to increase the variety of ways that plants can respond to
350 environmental stress (Givnish 1984; Marks and Lechowicz 2006; West et al. 2012). Thirdly, LA is
351 more strongly anchored to species identity than SLA, and thus more responsive to species turnover.

352 These results correspond to the ones of Kichenin et al. (2013) who found a major contribution of
353 interspecific variability in explaining the response of LA to elevation. Since T4 is characterized by
354 plant communities that mostly diverged from the others in terms of species composition, here the
355 compositional changes were reflected by dramatic changes in functional strategy in terms of LA.
356 Accordingly, plant communities of T4 were dominated by *Erica arborea* and *Erica scoparia*, both
357 characterized by small persistent linear leaves. On the contrary, for SLA the species compositional
358 changes between T2-3 and T4 were not reflected by a functional shift. In other words, the different
359 plastic behaviour of these two leaf traits could be behind the unexpected pattern of LA in T4
360 outlining the relevance of intraspecific variation. In contrast to the majority of many other leaf
361 traits, SLA is related to the cell volume ratio of leaf parenchyma to epidermis cells, making it more
362 plastic than traits dependent on subcellular processes (Kichenin et al. 2013). Mitchell and Bakker
363 (2014) found SLA variation to be mostly based on plasticity with low relevance of ontogeny or
364 local adaptation. For size dependency of SLA and further discussion see Milla et al. (2008).

365

366 **The relative contribution of intraspecific variation**

367 Our finding of a similar relative contribution of inter- and intraspecific community-level SLA
368 variation (55% and 45%, respectively) along the gradient of four thermotypes, confirms the
369 plasticity of this trait (Liancourt et al. 2015; Siefert et al. 2015; Wellstein et al. 2017). Kichenin et
370 al. (2013) found similar patterns along a 900 m elevation gradient spanning subalpine and alpine
371 plant communities in New Zealand. However, the effect of species turnover can be more marked
372 along other environmental gradients (here, e.g. with temperature seasonality), as also showed by
373 Lepš et al. (2011). Regarding LA, the relative contribution of species turnover is twice as big as
374 intraspecific variation (66.1% and 33.9%, respectively) along the gradient of four thermotypes.
375 Despite the plastic behaviour that this trait has shown (Fraser et al. 2009), we demonstrated that its
376 changes at the community level were mostly dependent on species turnover (Rozendaal et al. 2006;
377 Siefert et al. 2015). These results lead us to confirm H3 on a significant influence of intraspecific
378 variation in both leaf traits, but with a larger role of this component for SLA. It is most likely that
379 SLA varies with high temperature and low water availability at the intraspecific level (Ackerly
380 2004; Poorter et al. 2009; Wellstein et al. 2017) which are known to strongly decrease with
381 increasing elevation in Mediterranean contexts.

382 Moreover, our results provide relevant implications for future studies focusing on leaf trait-
383 environment relationship in Mediterranean contexts. Indeed, the measurements of individual traits
384 within species show that trait-based community responses to environmental changes are not
385 adequately predicted using leaf traits mean values (Lepš et al. 2011). This is in part due to the
386 influence of intraspecific trait variation (Garnier et al. 2018; Kickenin et al. 2013). In particular, the

387 significantly negative covariation between inter- and intraspecific SLA variation suggests that there
388 is a negative compensation between them. For example, in species with high SLA, individuals with
389 lower SLA values may be promoted, and in species of lower SLA, individuals with higher values
390 are promoted. This topic is worthy of further research, as significant uncertainty remains about the
391 complex responses of plant communities to the environment (Lepš et al. 2011).

392

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395

396 **Electronic supplementary material**

397 **Appendix 1.** Species x plot matrix.

398

399 **Conflict of interest**

400 The authors declare that they have no conflict of interest.

401

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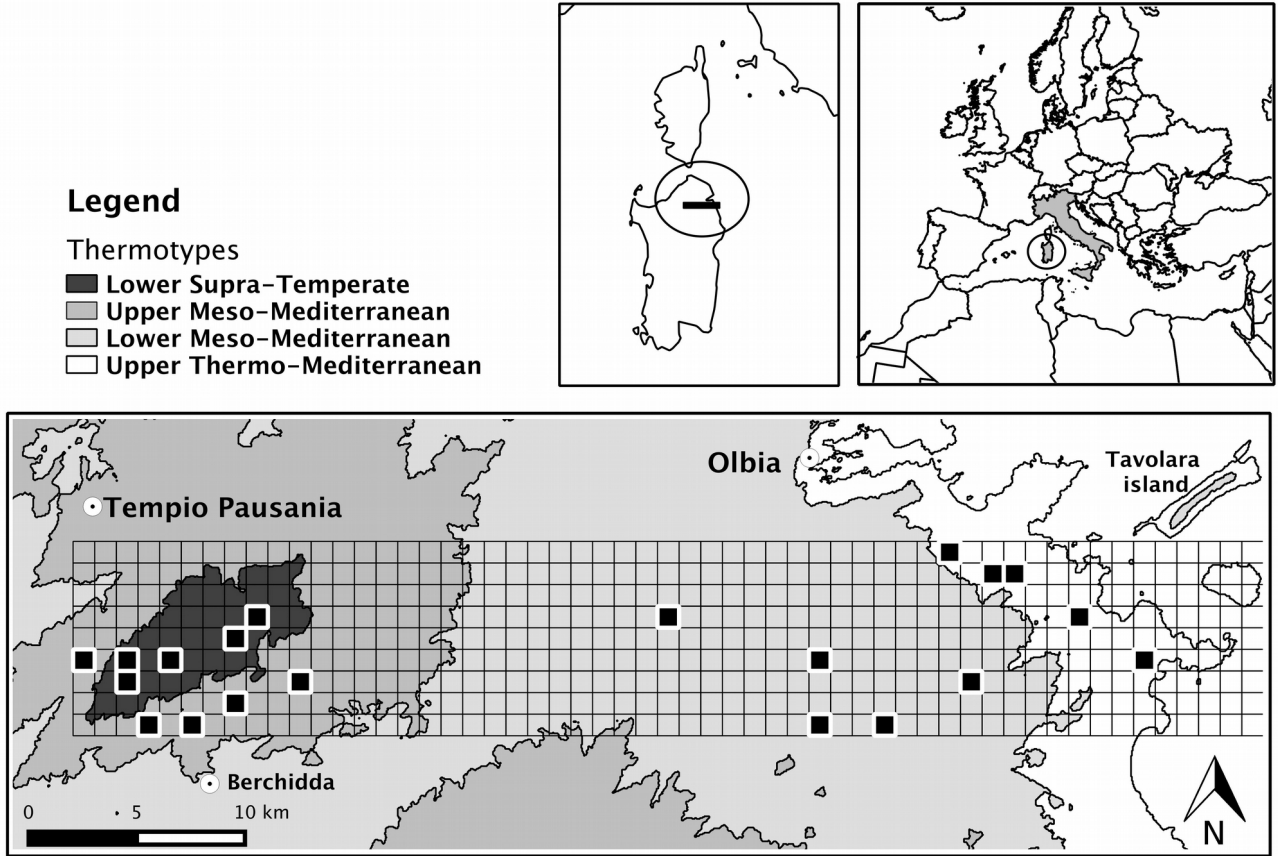
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582 **FIGURES**

583 **Figure 1.** Study area and scheme of the sampling design. T1-T4 gradient is represented by white to
584 dark grey areas. The selected sampling quadrats are highlighted in black; each quadrat includes two
585 sampling units 5 x 5 m.



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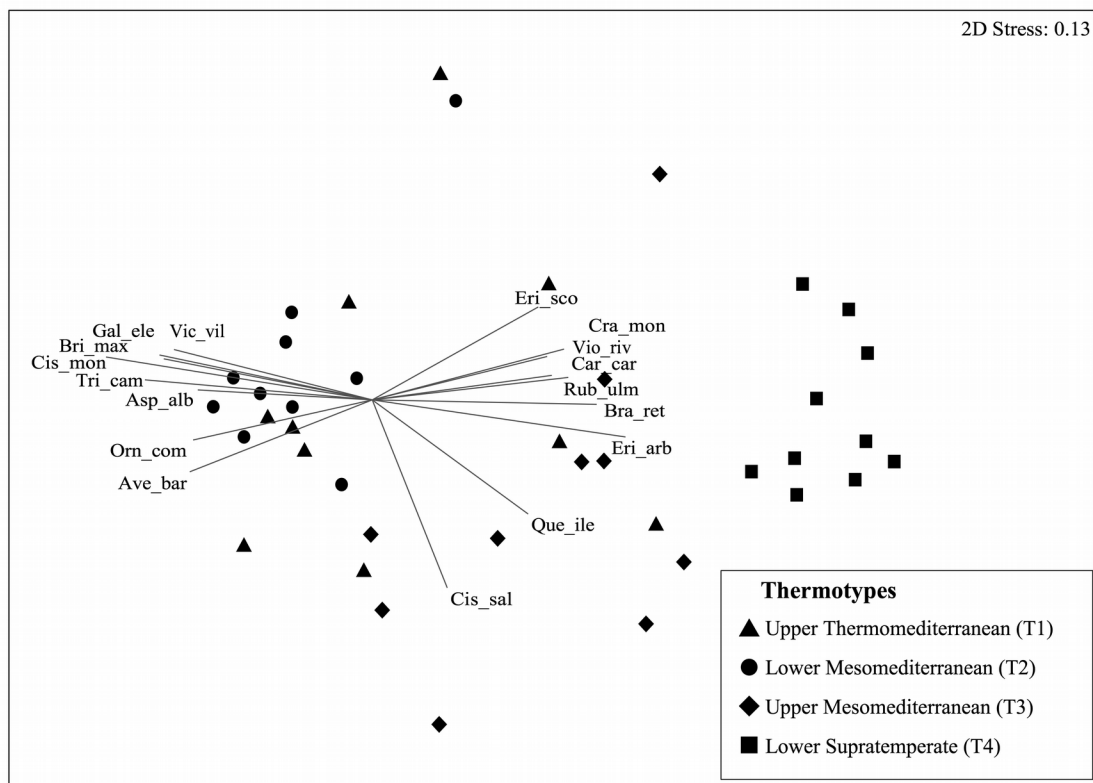
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600 **Figure 2.** Non-metric multidimensional scaling (NMDS) plot of a 2-dimensional solution (Bray-
 601 Curtis similarities, standardized samples by total, abundance data - stress 0.13). We represented the
 602 species showing a higher correlation to the ordination axes (Spearman rho>0,6). Species are
 603 labelled as follows: Ave_bar: *Avena barbata*; Orn_com: *Ornithopus compressus*; Asp_alb:
 604 *Asparagus albus*; Tri_cam: *Trifolium campestre*; Cis_mon: *Cistus monspeliensis*; Bri_max: *Briza*
 605 *maxima*; Gal_ele: *Galactites elegans*; Vic_vil: *Vicia villosa*; Cis_sal: *Cistus salviifolius*; Que_ile:
 606 *Quercus ilex*; Eri_arb: *Erica arborea*; Bra_ret: *Brachypodium retusum*; Rub_ulm: *Rubus ulmifolius*;
 607 Car_car: *Carex caryophyllea*; Vio_riv: *Viola riviniana*; Cra_mon: *Crataegus monogyna*; Eri_sco:
 608 *Erica scoparia*.



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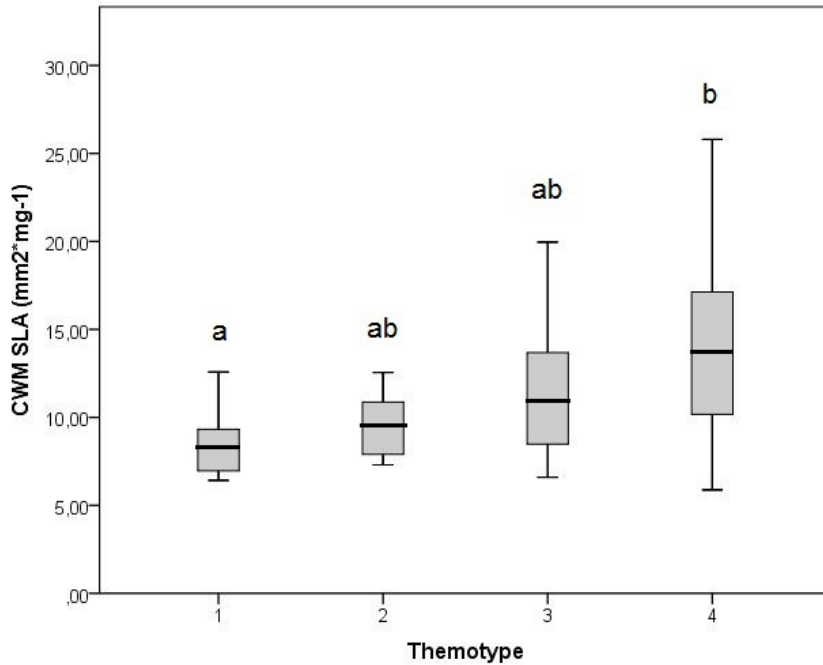
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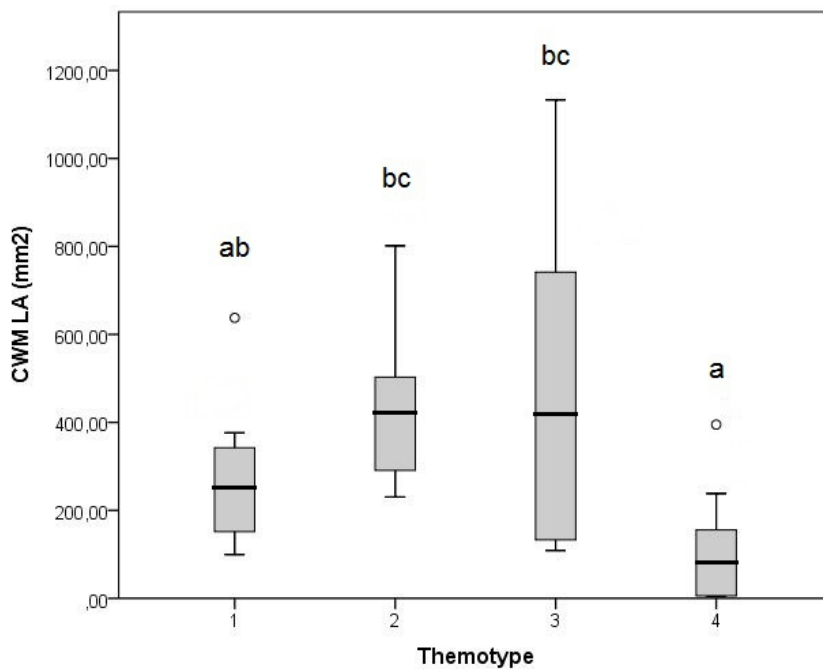
622 **Figure 3.** Boxplots of community-level SLA (a) and LA (b) for each thermotype (increasing
623 elevation and decreasing heat and water stress from T1 to T4). Significant differences between
624 mean values are indicated by different letters according to ANOVA ($p < 0.05$).

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627 b)

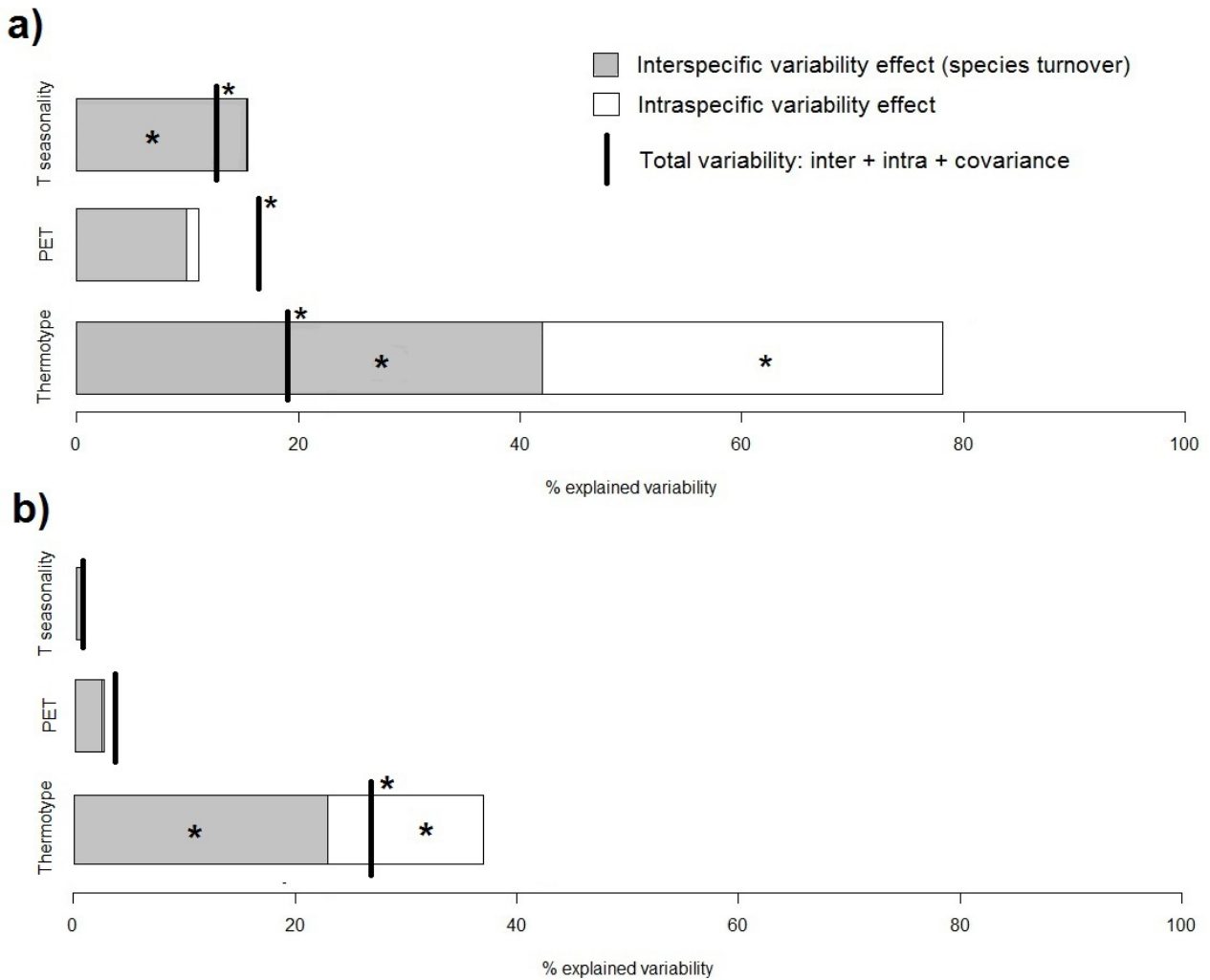


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630 **Figure 4.** Decomposition of total variability in community weighted mean SLA (a) and LA (b)
 631 values into interspecific (species turnover), intraspecific and covariation effect explained by
 632 temperature seasonality, PET and thermotype. Covariation strength is represented by the interval
 633 between the “total variability” and the sum of inter- and intraspecific variability effects. A value of
 634 total variability that is lower than the sum of inter- and intraspecific variability effects indicates
 635 negative covariations, and a value of total variability that is higher than the sum of inter- and
 636 intraspecific variability effects indicates positive covariations. Statistical significance (*) of inter-,
 637 intraspecific and total variability effects are indicated on the graph, when significant.

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