

1 **Shifts in the elemental composition of plants during a very severe drought**

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20 **ABSTRACT**

21 Diverse plant functions (e.g. growth, storage, defense and anti-stress mechanisms) use
22 elements disproportionately. We hypothesized that plants growing under different
23 abiotic and biotic conditions would shift their elemental compositions in response to a
24 very severe drought. We tested this hypothesis by investigating the changes in foliar
25 stoichiometry and species composition from a very severe drought. We also tested the
26 effects of previous droughts (acclimation) on this response. Different species growing
27 in the same community responded more similarly to a very severe drought than did
28 individual species growing in different communities. The stoichiometric shifts were
29 thus more community-dependent than species-dependent. The results also suggested
30 that plants grown in monoculture were less stoichiometrically plastic during the
31 drought than plants grown in a more diverse community. Previous exposure to long-
32 term drought treatments in the same communities did not significantly affect the
33 stoichiometric shifts during the new drought. Differential use of resources may have
34 been responsible for these responses. Monocultured plants, which used the same
35 resources in similar proportions, had more difficulty avoiding direct competition when
36 the resources became scarcer. Moreover, each species tested had a particular
37 elemental composition in all communities and climatic treatments. The differences in
38 foliar elemental compositions were largest between plant functional groups (shrubs
39 and grasses) and smallest among species within the same functional group. Global
40 principal components analyses (PCAs) identified a general tendency for all species,
41 independently of the community in which they grew, toward lower concentrations of
42 K, N, P, Mg and S, and to higher concentrations of C and Fe as the drought advanced.
43 This study has demonstrated the utility of analyses of differences and shifts in plant

44 elemental composition for understanding the processes underlying the responses of
45 plants to changes in biotic and abiotic environmental conditions.

46 Keywords: Biogeochemical niche; Ecological stoichiometry; elemental composition;
47 extreme drought; nitrogen; phosphorus.

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62 **1. Introduction**

63 Climate change is modifying the patterns of global precipitation and the intensity and
64 frequency of droughts (IPCC, 2013), with important consequences to plant physiology,
65 community diversity and ecosystemic productivity in terrestrial biomes (Allen et al.,
66 2010; Trnak et al., 2010; McDowell et al., 2011; Weißhuhn et al., 2011; IPCC., 2013;
67 Peñuelas et al., 2013). The capacity to shift elemental compositions in response to
68 diverse types of drought stress could therefore be expected to vary among plant
69 species.

70 The main goal of ecological stoichiometry is to understand the relationships of
71 environments and C:N:P ratios with the lifestyles of organisms and the structures and
72 functions of ecosystems (Sterner and Elser, 2002; Elser and Hamilton, 2007). These
73 relationships have been widely observed in planktonic communities, where low N:P
74 ratios in the medium are correlated with high growth rates in organisms and are
75 consequently important to the structure of trophic webs (Elser et al., 1996; Peñuelas et
76 al., 2013). Terrestrial plants, however, are larger than planktonic organisms, and N and
77 P can be allocated to functions other than growth, such as storage, defense or anti-
78 stress systems (Peñuelas and Sardans, 2009). Moreover, the concentrations or ratios of
79 other elements such as K, Mg, Ca and Fe in terrestrial plant communities are much
80 more involved in important physiological functions of organisms (Wilkinson et al.,
81 1990; Leung et al., 1994; Lombini et al., 2003; Murren et al., 2006; Wu et al., 2009;
82 Lourido et al., 2010; Hashimoto and Kudla, 2011; Sardans et al., 2012a, 2012b; Sardans
83 and Peñuelas, 2012; Stael et al., 2012; Yang et al., 2012; Perea-García et al., 2013) and
84 consequently should also be included in ecological stoichiometric studies. The

85 biogeochemical niche hypothesis claims that different sympatric plant species occupy
86 different biogeochemical niches, as manifested by the complete spectra of the
87 contents of macro- and micronutrients in their tissues (Peñuelas et al., 2008, 2010) and
88 not only by N and P concentrations or N:P ratios. The elemental composition of a
89 species should thus be determined, in part, by long-term genetic adaptation to a
90 particular abiotic environment and also by specific ecological strategies such as the
91 optimal successional stage. The success of a plant species, however, requires some
92 degree of flexibility to allow plants to respond to abiotic and biotic shifts.
93 Stoichiometric flexibility enables organisms to adjust their elemental ratios while
94 maintaining a constant function (Sistla and Schimel, 2012). Species with higher
95 stoichiometric flexibility tend to have higher concentrations of N and P and lower N:P
96 ratio (Yu et al., 2011).

97 K is strongly correlated with several physiological functions in plants such as
98 water-use efficiency and stomatal control and with above- and belowground biomass
99 and likely has an important role in photosynthesis (Leigh and Wyn Jones, 1984;
100 Andersen et al., 1992; Egilla et al., 2001; Cakmak, 2005, Fernandez et al., 2006, Sardans
101 et al., 2012c). Some studies have observed higher foliar K contents in drier periods,
102 especially in plants typical of dry environments (Rivas-Ubach et al., 2012; Sardans et
103 al., 2012c; 2013). Increases in C:nutrient ratios in leaves due to increases in C-rich
104 compounds linked to the avoidance of water stress are well-known stoichiometric
105 responses to decreases in water availability, at least in typically water-stressed
106 environments (Gonçalves-Alvim et al., 2004 and 2006; Sardans and Peñuelas, 2013).
107 The production of more sclerophyllous leaves and a slow growth rate are physiological
108 responses to high C:nutrient ratios, drought conditions and dry environments (Feller,

109 1995; Fernández and Reynolds, 2000; Gonçalves-Alvim et al., 2004 and 2006; Sardans
110 et al., 2011). The various possible responses to water stress depend on the dominant
111 mechanism of water uptake (isohydric vs anisohydric), the functional type, the species,
112 the soil nutrient resource and the nature of episodes of water stress (duration or
113 frequency) (DeLucia and Schlesinger, 1991; Tradieu and Simonneau, 1998; Schultz,
114 2003).

115 The frequency and magnitude of extreme climatic events such as drought will
116 increase as the global climate changes (IPCC, 2012). Several specific responses to
117 extreme droughts at the community level, however, remain to be studied. Water
118 shortage should lead to a decline in water potential and to water stress. Plants should
119 be able to alter their functions to increase the efficiencies of their mechanisms of
120 water uptake and use efficiency. These adaptations should depend on the composition
121 of the community, with heathlands being very resistant (Gordon et al., 1999; Peñuelas
122 et al., 2004) and grassland communities being more sensitive to extreme droughts
123 (Grime et al., 2000; Kreyling et al., 2008a). The capacity to adapt to and resist drought
124 should be accompanied by stoichiometric changes that further allow changes in the
125 allocation of elements to different functions and structures. A species growing in
126 communities with different species compositions should have distinct capacities to
127 access different nutrients, thereby changing its capacity to respond to extreme
128 droughts. We hypothesized that changes in the availability of water will be reflected in
129 changes in foliar stoichiometry, which in turn will ultimately help us to understand the
130 processes involved in the response to water stress. This response can also vary due to
131 the biotic relationships of plants. Previous studies have shown that plants of the same

132 species exhibit different elemental compositions depending on the species
133 composition of the plant community in which they live (Urbina et al., 2014).

134 Several studies have observed that plants subjected to recurrent extreme
135 climatic events are better able to cope with subsequent extreme events (Bruce et al.,
136 2007; Lambers et al., 2008; Walter et al., 2011). Such a capacity for ecological
137 “memory”, though, has not been consistently observed (Zavalloni et al., 2008).
138 Modified responses after pre-exposure to extreme climatic events are also dependent
139 on the type of impact, and the opposite effect, worse performance, can also occur,
140 mainly in perennial plants; extreme frosts (in timing or intensity) can hurt perennial
141 plants and substantially reduce their vitality. Lloret et al. (2004) observed that
142 reproduction and survival were lower in *Quercus ilex* trees after a second extreme
143 drought in 1995 than after an earlier drought in 1985. The response of plants to an
144 extreme drought can thus also be negatively influenced by previous exposure.
145 Stoichiometry may be a key to understanding the underlying physiological processes
146 such as resource depletion or hormonal regulation. We thus aimed to determine if
147 plants subjected to previous recurrent periods of drought had different stoichiometric
148 responses to a new extreme drought.

149 We analyzed four typical central European grass and shrub communities to
150 investigate: (i) the species-specific foliar stoichiometric changes in response to an
151 extreme drought, (ii) the role of community composition in plant responses and (iii)
152 the role of previous droughts (acclimation) in these responses.

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155 **2. Materials and Methods**

156 *2.1. Experimental area and design*

157 This research is part of the ongoing EVENT I experiment (Jentsch et al., 2007, 2011) at
158 the Ecological-Botanical Garden of the University of Bayreuth, Germany (49°55'18"N,
159 11° 34'55"E; 365 m a.s.l.) that studies the effects of extreme climatic events. The
160 experiment focuses on important perennial temperate plant communities. Mean
161 annual air temperature at the site is 8.2 °C. Mean annual precipitation is 724 mm and
162 is distributed bi-modally with a major peak in June/July and a secondary peak in
163 December/January (data from the German Weather Service).

164 Our experiment had three fully crossed factors: (1) recurrent drought over five
165 years (drought and control), (2) four community compositions (two species of one
166 functional group, four species of two functional groups, four species of three
167 functional groups and four of these species in monocultures; see Section 2.3) and (3)
168 duration of a very severe drought for all plots, irrespective of their previous exposure
169 to drought. Three levels of duration were tested by sampling at three times during the
170 three-month drought (at two, six and twelve weeks after the onset of the drought).

171 The experimental setup consisted of five replicates of each factorial
172 combination, with a total of 30 2×2 m plots for the two species of dwarf shrubs (Table
173 1) (two pre-exposed treatments and three community compositions) and a total of 40
174 2×2 m plots for the grass species (two pre-exposure treatments and two community
175 compositions). The factors were applied in a split-plot design with the community
176 compositions blocked and randomly assigned within each of the five replicates of the
177 pre-exposure treatments. All communities grew in soil 80 cm in depth, including 20 cm

178 of topsoil, sourced from a nearby sand quarry. The topsoil had a total carbon content
179 of 2% and a pH of 4.5 (measured in 1 M KCl), and the lower soil layer had a total
180 carbon content of 0.2% and a pH of 6.2. The texture of the soil was loamy sand (82%
181 sand, 13% silt and 5% clay). The total concentrations of the various elements in the soil
182 are provided in Table S1.

183 *2.2. Recurrent and final very severe droughts*

184 The climatic manipulations consisted of two phases of drought, recurrent droughts
185 over five years and a very severe drought of three months in the final year of the
186 experiment. The controls were exposed to ambient conditions. The severity of the
187 droughts was determined by statistical extremity in a historical reference period
188 (extreme value theory) independent of its effects on organisms (Jentsch et al. 2007). In
189 particular, the intensity of the treatments was based on the local 100-year extreme
190 droughts in 2005, 2006 and 2007 and on the local 1000-year extreme droughts in
191 2008, 2009 and 2010. Gumbel distributions (Gumbel, 1958) were fitted to the annual
192 extremes, and the 100-year and 1000-year recurrent droughts were calculated.
193 Drought was defined as the number of consecutive days with less than 1 mm of daily
194 precipitation. Accordingly, a recurrent drought of 32 days (2005–2007) and of 42 days
195 (2008, 2009 and 2010) was applied during the peak growing season in June. The very
196 severe drought was applied in all plots (control and drought) from 17 May to 28 August
197 2011.

198 The severe drought was effected by large rain-out shelters (Haygrove Tunnels
199 Ltd., Ledbury, United Kingdom; total area 50 m in length × 31.5 m in width × 3.75 m in
200 height) over the entire surface of the experiment that permitted nearly 90%

201 transmission of photosynthetically active radiation through a transparent polyethylene
202 sheet 0.18 mm in thickness (UV M 42; folitec Agrarfolien-Vertriebs GmbH, Westerburg,
203 Germany). Unwanted greenhouse effects were avoided by leaving gaps between the
204 ground and the shelters 1.05 m along the sides and 2.55 m on the front and back,
205 allowing for near-surface air exchange. Before drought manipulation began, the plants
206 were watered with a total of 46.6 mm of water in two applications (11 and 13 May
207 2011) to adjust all plants to the same initial conditions. The amount of 46.6 mm was
208 the difference between the long-term average precipitation for April and the amount
209 of natural rainfall for April 2011 (from the start of the growing season to the start of
210 the severe drought). Lateral surface runoff was prevented by plastic sheets around the
211 treated plots buried to a depth of 10 cm. This study focused on the effects of the very
212 severe drought.

213 This drought consisted of nearly 3.5 months of no precipitation, which was
214 outside the calculated probabilities based on recorded weather data. Defining
215 “extreme” climates based on historical data, however, has limited value, and the
216 sensitivities of plant species to the same event may differ substantially (Hegerl et al.,
217 2011). The length of the drought in this experiment was not intended to represent a
218 certain future likelihood of duration but was rather considered as an experimental tool
219 to identify plant responses during the ongoing episode of drought (Kreyling et al.,
220 2014).

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222 *2.3. Experimental communities – composition and diversity*

223 Four levels of diversity with different compositions of grasses, shrubs and herbs were
224 tested. The species are widespread in central Europe and are of fundamental
225 importance for agriculture and nature conservation (Table 1) (Jentsch *et al.* 2007,
226 2011). All species are perennials and have life spans of several years. The compositions
227 of the communities were maintained by periodic weeding three times per year. The
228 four community compositions were: (1) H2, composed of two dwarf shrubs of the
229 same functional group, *Calluna vulgaris* and *Vaccinium myrtillus*; (2) G4, composed of
230 two grasses of the same functional group, *Arrhenatherum elatius* and *Holcus lanatus*,
231 and two forbs of the same functional group, *Plantago lanceolata* and *Lotus*
232 *corniculatus*; (3) H4, composed of the two dwarf shrubs in H2, the hairgrass
233 *Deschampsia flexuosa* and the bentgrass *Agrostis stolonifera*, the latter two species
234 belonging to different functional groups, and (4) monocultures of *C. vulgaris*, *V.*
235 *myrtillus*, *A. elatius* and *H. lanatus*.

236 Biomass samples were collected three times during the drought on 30 May in
237 week 2, 27 June in week 6 and 8 Aug in week 12. The biomass data were calculated as
238 vegetative biomass in g per 0.08 m² (20 × 40 cm). The number of samples that could be
239 collected progressively decreased after week 2 due to increasing mortality.

240 2.4. Biomass and elemental analyses

241 Photosynthetic tissues were dried and pulverized in a heater for 48 h at 70 °C and
242 conserved in desiccators until analysis (<15 days). For the analyses of C and N, 0.7 mg
243 of dried, pulverized samples was weighed with a Microbalance MX5 Mettler Toledo,
244 and the concentrations of the elements were determined by combustion coupled to
245 gas chromatography (Smith and Tabatabai, 2004) with a CHNS Eurovector 3011

246 Elemental Analyzer and a Thermo Electron NA 2100 Gas Chromatograph (C.E.
 247 instruments-Thermo Electron, Milan, Italy).

248 Table 1. Species compositions of the communities.

Community	Species
H2	<i>Calluna vulgaris</i> , <i>Vaccinium myrtillus</i>
G4	<i>Arrhenatherum elatius</i> , <i>Holcus lanatus</i> , <i>Plantago lanceolata</i> , <i>Lotus corniculatus</i>
H4	<i>Calluna vulgaris</i> , <i>Vaccinium myrtillus</i> , <i>Deschampsia flexuosa</i> , <i>Agrostis stolonifera</i>
Monocultures	<i>Calluna vulgaris</i>
	<i>Vaccinium myrtillus</i>
	<i>Arrhenatherum elatius</i>
	<i>Holcus lanatus</i>

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250 For the other elements (P, S, Ca, Mg and Fe), 0.25 g of dried, pulverized sample
 251 was diluted in an acidic mixture of HNO₃ (60%) and H₂O₂ (30% w/v) and digested in a
 252 microwave system (MARSPress, CEM Corporation, Matthews, NC). The digested
 253 solutions were dissolved in a 1% HNO₃ solution and then brought to final volume of 50
 254 mL with ultrapure water. Blank solutions (5 mL of HNO₃ and 2 mL H₂O₂ but no sample
 255 biomass) were regularly analyzed. After digestion, the concentrations of Ca, K, Mg, S, P
 256 and Fe were analyzed by ICP-OES (Inductively Coupled Plasma - Optical Emission
 257 Spectrometry) (Priester et al., 2011) with a spectroscopy Optima 4300DV (Perkin-Elmer
 258 Inc., Wellesley, MA). We used the standard certified biomass NIST 1573a (tomato leaf)
 259 to assess the accuracy of the biomass digestion and analytical procedures.

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261 2.5. Statistical analyses

262 We conducted univariate analysis by factorial analysis of variance (ANOVA) with
263 species, community composition and time of sampling as independent variables, and
264 chemical characteristics as dependent variables for the species growing in two or more
265 communities. For the species present only in one community, we conducted ANOVAs
266 with time of sampling as an independent variable and chemical characteristics as
267 dependent variables. We used Fisher's post-hoc tests to compare the different levels
268 of the independent variable.

269 We also conducted multivariate ordination analyses by using principal
270 component analyses (PCAs) including the elemental concentrations and the N:P, N:K
271 and P:K ratios to determine if the same species growing in different communities had
272 different changes in overall elemental concentrations and stoichiometries during the
273 drought.

274 We also conducted an ANOVA with the scores of the first six PC axes as
275 dependent variables and the various plant communities as fixed categorical
276 independent factors to determine the global effects of the community on elemental
277 concentrations and stoichiometries. We tested for normality and homogeneity of
278 variance of the residuals prior to the statistical analyses by examining the residuals
279 versus the expected plots and the normal qq-plots of the linear models. The data were
280 log-transformed if the required conditions were not met. The significance level was set
281 at $P < 0.05$. We also correlated biomass survival (ratio of live to dead biomass) during
282 the drought with the elemental and stoichiometric PC scores to determine how

283 mortality affected stoichiometric composition. All statistical analyses were performed
284 using Statistica 6.0 (StatSoft, Inc. 1984-2001, Tulsa, OK, USA).

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301 3. Results

302 3.1. Effects of the drought on the communities

303 Species responded to drought with a general trend of decreasing P, S and K
304 concentrations and increasing C concentrations in all four species studied in most
305 communities, and with decreasing N in three of the four studied species. Only *V.*
306 *myrtillus* N concentrations did not change in any community in response to drought
307 time increase (Table S2).

308 The PCAs indicated that overall elemental compositions generally varied in the
309 shrubs *C. vulgaris* and *V. myrtillus* in the H2 community with the advance of the
310 drought period (Figure 1a,b). Plants of different communities were separated along
311 the PC1 axis. The overall elemental composition of both species shifted towards lower
312 concentrations of P, S and K and higher C concentrations with increasing drought.

313 These two species had similar responses in foliar elemental composition in the
314 H4 community. This community also included two grasses, *D. flexuosa* and *A.*
315 *stolonifera*, whose overall elemental compositions differed from those of the two
316 shrubs along the PC1 axis ($P < 0.0001$) (Figure 1c,d). The PC2 axis separated *C. vulgaris*
317 from *V. myrtillus* ($P < 0.0001$), within the same functional group, and *D. flexuosa* from *A.*
318 *stolonifera* ($P < 0.0001$), in different functional groups (Figure 1c,d). The responses of
319 the species to the drought were consistent with the univariate analyses (Table S2);
320 there was a trend towards lower S, and P concentrations and higher C concentrations
321 across PC2 axis with increasing drought. All four species tended to occupy a specific
322 space in the plot of the first two PCs, indicating species-specific stoichiometries despite
323 shifts in elemental compositions during the drought.

324 The PCA for the G4 community with the four herbaceous species of two
325 functional groups, *H. lanatus*, *A. elatius*, *P. lanceolata* and *L. corniculatus*, separated all
326 four species in at least one of the first three PCs. In all four species we observed an
327 overall shift in elemental composition along the PC2 axis mainly loaded by decreasing P
328 and K concentrations and increasing C concentrations as the drought progressed
329 (Figure 1e,f), results consistent with univariate analyses that further confirmed these
330 changes with increasing drought (Table S2).

331 We also found differences during the course of the drought in *P. lanceolata*
332 and *L. corniculatus* that were growing only in G4 community. In *P. lanceolata*, C and Fe
333 concentrations were higher in week 6 and decreased toward the end of the drought.
334 The K and P concentrations responded oppositely, decreasing in the middle of the
335 drought and increasing toward the end (Figure 1e,f). The leguminous forb *L.*
336 *corniculatus* decreased its N concentration throughout the course of the drought (Data
337 not shown). The Ca concentration decreased in the grass *D. flexuosa*, and the Fe
338 concentration and N:P ratio increased (Data not shown). We could not conduct the
339 analysis for *A. stolonifera* due to the absence of replicates caused by the high mortality
340 in the first sampling of the severe drought.

341 The PCA for the four species growing in monoculture indicated comparable
342 responses to those of the H4 community, where the scores in the shrubs *V. myrtillus*
343 and *C. vulgaris* generally differed from those in the two grass species *H. lanatus* and *A.*
344 *elatius* along the PC1 axis ($P < 0.0001$) (Figure 1g,h). *V. myrtillus* and *C. vulgaris* were
345 separated along the PC2 axis ($P < 0.0001$). *H. lanatus* and *A. elatius* were also separated
346 along PC2 axis ($P < 0.0001$). The death of many individuals of *V. myrtillus* and *A. elatius*

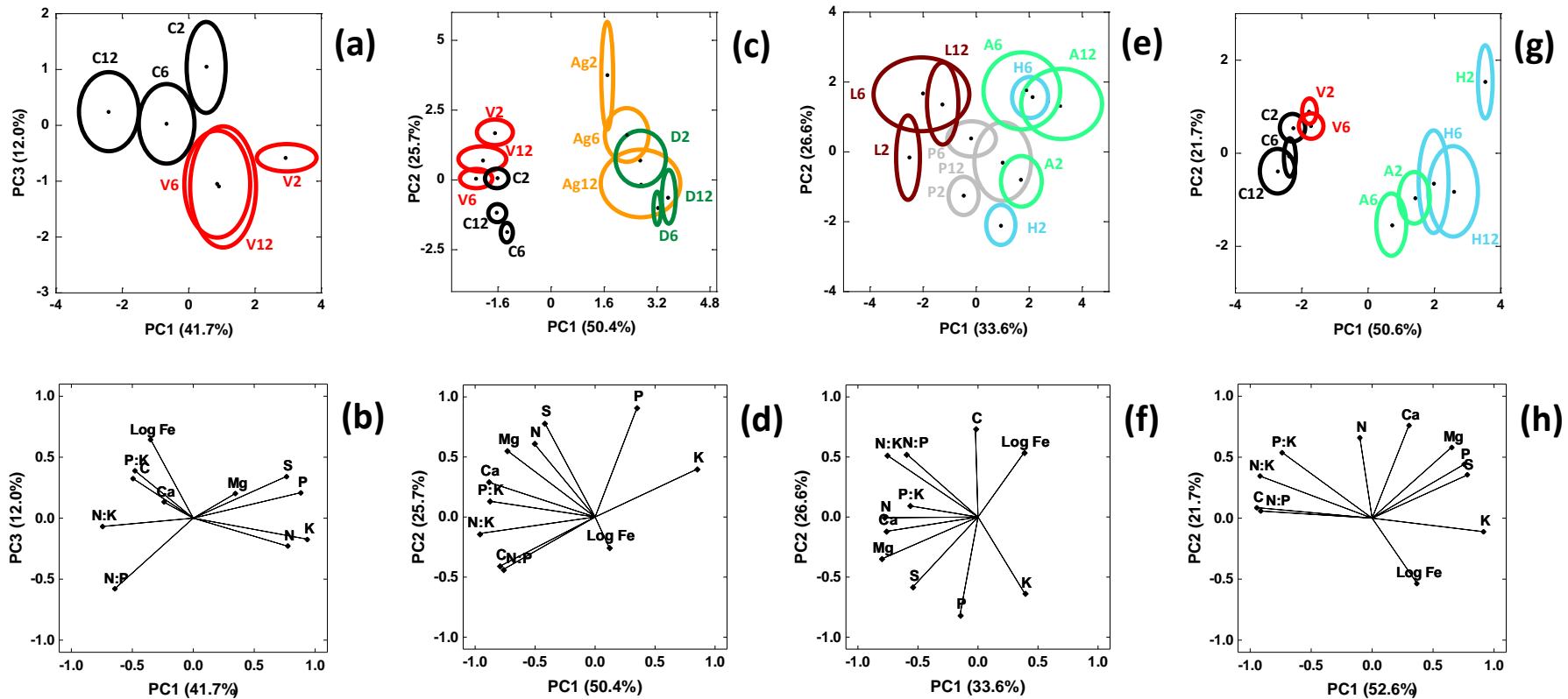
347 did not allow an analysis for the advanced stages of the drought. The variables that
348 mainly loaded PC1 axis were the concentrations of S, K and P, which decreased, and C
349 concentrations that increased towards shrubs, and N and Ca concentrations that
350 decreased in *C. vulgaris* and *H. lanatus*, during the drought advance (Figure 1 g,h).
351 These changes were also consistent with the observed in univariate analyses (Table
352 S2).

353 Fe concentrations increased in all species, although not always significantly, as
354 the drought advanced. These increases may have been a response to the high Fe
355 content of the soil (6 mg/g) and probably to a concentration effect from the lack of
356 water during the drought.

357 The elemental compositions of the species in all communities tended to have
358 comparable shifts as the drought progressed. There were peculiar differences in these
359 shifts depending on the community, but N, P, K, Mg and S concentrations generally
360 tended to decrease and C concentrations to increase during the drought.

361 3.2. Effects of community composition on the responses of the species to the drought

362 The elemental concentrations and ratios in all species differed significantly
363 among the three sampling times during the drought (Figures 1-4, Table S2), but the
364 differences depended on the community in which the species grew. This interaction
365 between the drought and community composition was species-specific (Table S2). The
366 interaction between the duration of the drought and community composition



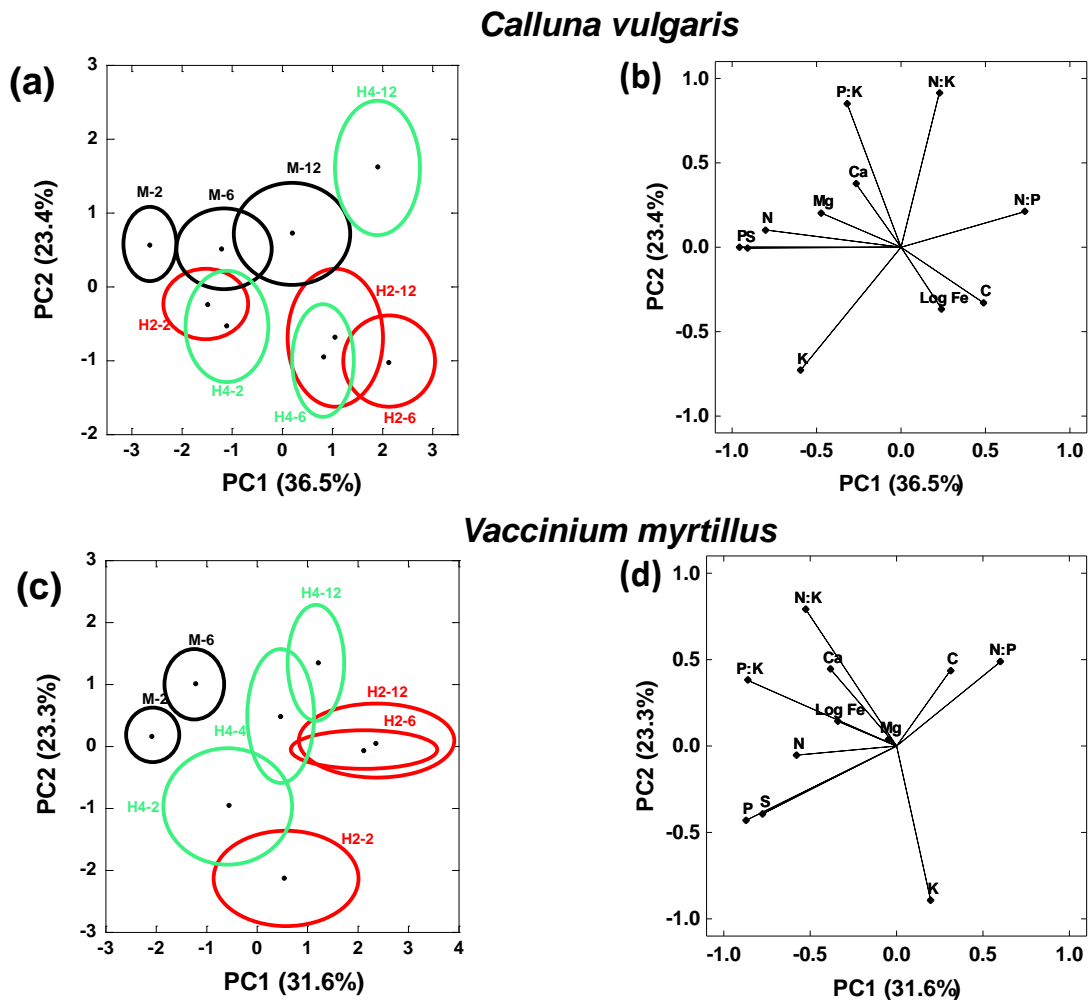
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Figure 1. PCA plots of the mean \pm S.E. scores of first two PCs for the species during the course of the severe drought in the H2 (a), H4 (c) and G4 (e) communities and the monocultures (g), and the corresponding variable distributions in H2 (b), H4 (d), G4 (f) and the monocultures (h). The ellipses represent the 95% confidence regions around the point representing the scores mean of PC1 and PC2 axes for each species. Percentages within brackets in the X and Y axes legends indicate the percentage of variance explained by the corresponding PC. Codes: number of weeks since the beginning of drought (two weeks = 2, six weeks = 6, twelve weeks = 12). Species (*C. vulgaris* = C, *V. myrtilus* = V, *A. stolonifera* = Ag, *D. flexuosa* = D, *H. lanatus* = H, *A. elatius* = A, *L. corniculatus* = L, *P. lanceolata* = P).

374 significantly affected the elemental compositions and stoichiometric ratios in three of
375 the four species that were growing in two or more different communities, showing
376 that the community had a significant influence on the shifts in elemental composition
377 during the drought. Individuals of *C. vulgaris* and *H. lanatus* in the monocultures
378 shifted their elemental concentrations and ratios mainly linearly during the drought,
379 unlike the individuals growing in more diverse communities where elemental
380 concentrations and stoichiometries change more abruptly and irregularly.

381 The ANOVA of PC1 scores in *C. vulgaris* showed a separation of samples over
382 the course of the drought. PC1 axis was mainly loaded by N, K, S and P, and N:P ratio,
383 showing that N, K, S and P concentrations decreased and C concentrations and N:P
384 ratios increased over the course of the drought (Figure 3a,b), consistently with the
385 results of univariate analyses (Table S2). N:K and P:K ratios peaked in the last week of
386 the drought. The shifts in elemental composition over the course of the drought,
387 though, differed among the communities containing *C. vulgaris*. The N:K and P:K ratios
388 and in general all elemental concentrations in this shrub increased in response to
389 drought more in the H4 community than in the other two communities (Figure 3a,b,
390 Table S2). The K concentration decreased in the shrub *V. myrtillus* in week 6 but
391 increased toward the end of the drought. P and S concentrations decreased, and the
392 N:P ratios increased, during the drought. This species, however, could not be studied
393 appropriately due the lack of samples from the monoculture for the final week of the
394 drought from high mortality (Figure 3c,d). Large changes in elemental composition
395 were observed between the second and sixth weeks of the drought, whereas the
396 differences between the sixth and twelfth weeks were not significant. The elemental

397 compositions in *V. myrtillus* plants generally varied among the different communities



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400 Figure 2. PCA plots of the mean \pm S.E. scores of the first two PCs for *Calluna vulgaris* (a) and
 401 *Vaccinium myrtillus* (c) growing in different communities (diversity levels) during the course of
 402 the severe drought, and the corresponding variable distributions (b) and (d), respectively. The
 403 ellipses represent the 95% confidence regions around the point representing the
 404 scores mean of PC1 and PC2 axes for each species. Percentages within brackets in the X
 405 and Y axes legends indicate the percentage of variance explained by the corresponding PC.
 406 Codes: number of weeks since the beginning of drought (two weeks = 2, six weeks = 6, twelve
 407 weeks = 12). Communities (H2 community = **H2**, H4 community = **H4**, Monoculture = **M**).

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409 during the drought as observed in the corresponding PC2 axis ($P < 0.0001$). Despite of

410 these differences depending on the communities where these two shrubs grew (Figure

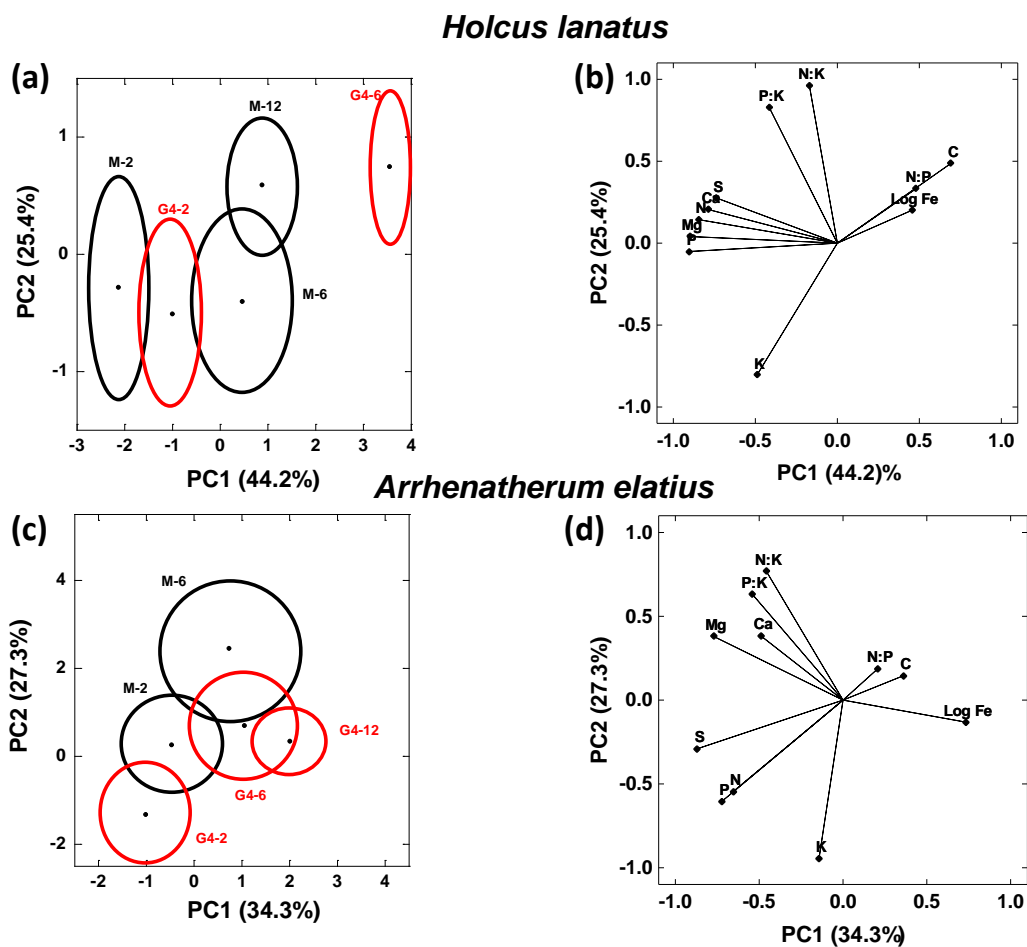
411 3 a-d, Table S2), the experimental drought decreased P and S concentrations in both
412 species independently of the community where they grew (Table S2).

413 The elemental compositions in the two grass species *H. lanatus* and *A. elatius*
414 also generally differed between the two communities in which they grew. The plants
415 growing in monocultures had different scores in the PCA plot of the first two PCs than
416 those growing in the G4 community with the two herb species and were separated
417 significantly along the PC2 axis ($P < 0.001$ and $P < 0.0001$, respectively) (Figure 3). *H.*
418 *lanatus* plants in the monoculture had lower P:K, N:K and N:P ratios and lower K
419 concentration than did those in the more diverse G4 community and consistently with
420 these results we observed in the univariate analyses lower N:K and N:P ratios in
421 monoculture plants (Table S2). Lower K concentrations were observed in *A. elatius*
422 growing in monoculture (Table S2), and consistently this variable had higher loading
423 values on PC2 axis (Figure 3c,d). Both species increased their C and Fe concentrations
424 and N:P ratios and decreased their S, P, N and K concentrations over the course of the
425 drought (Table S2).

426 The PCA for the H4 community showed a general tendency for all species to
427 shift along the PC2 axis, as a result of the overall shift towards lower concentrations of
428 N, P, Mg and S, and to higher concentrations of Fe as the drought advanced (Figure 4).

429 K concentrations and N:K and P:K ratios in *V. myrtillus* and *A. elatius*, though,
430 changed in the same direction over the course of the drought independently of the
431 community. *C. vulgaris* and *H. lanatus* showed significant interactions between
432 sampling time and community. K concentrations and N:K and P:K ratios did not change
433 in the monocultured plants but changed in those growing in H2 and H4. Moreover, the

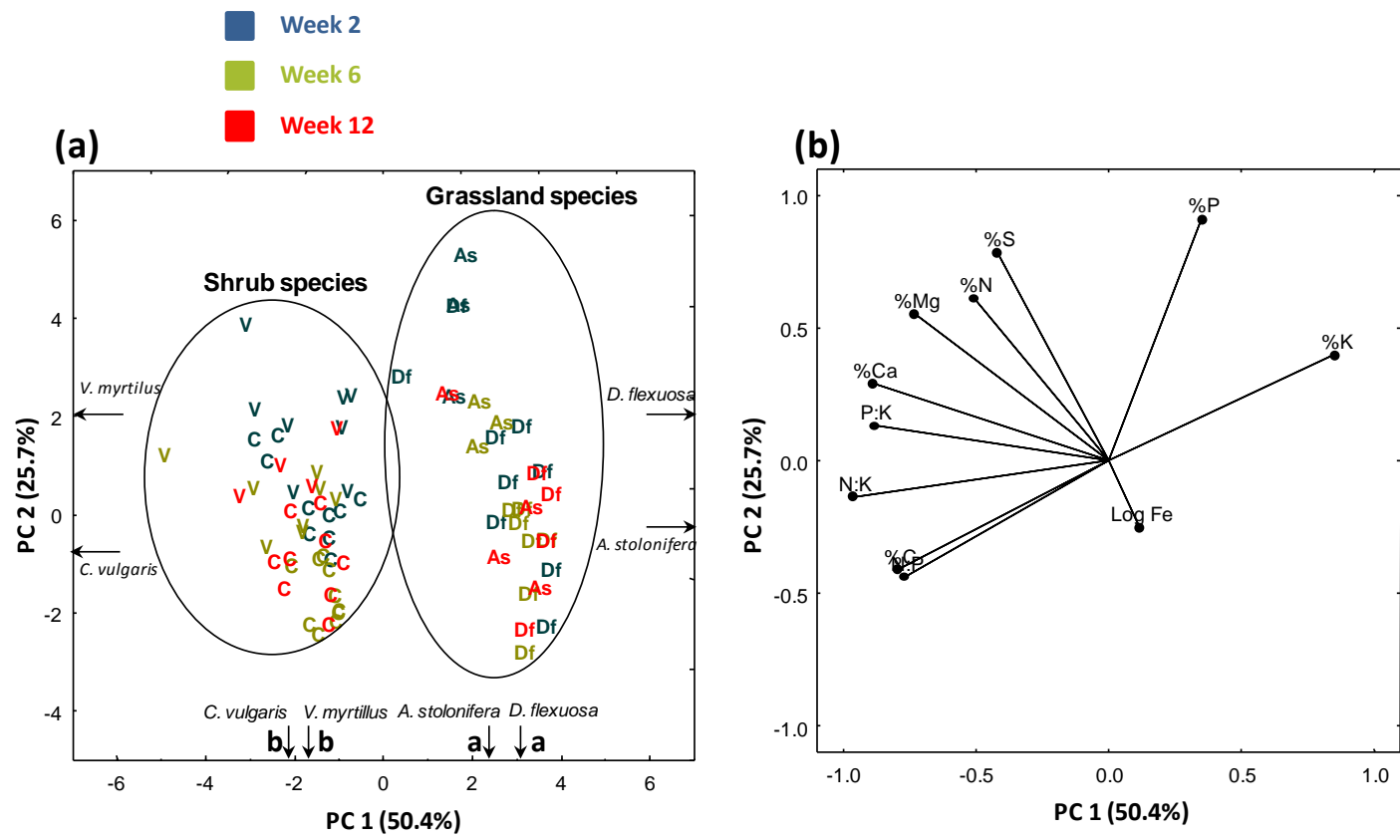
434 distribution of the means over the PC axes reflected a minor change in the foliar
 435 elemental concentrations and stoichiometries for the individuals of the four species
 436 growing in monocultures. All these results suggest less stoichiometric flexibility during
 437 the drought in plants growing in monocultures than in those growing in more diverse
 438 communities (Figures 1-3).



439
 440 Figure 3. PCA plots of the mean \pm S.E. scores of the first two PCs for *Holcus lanatus* (a) and
 441 *Arrhenatherum elatius* (c) growing in different communities (diversity levels) during the course
 442 of the severe drought, and the corresponding variable distributions (b) and (d), respectively.
 443 The ellipses represent the 95% confidence regions around the point representing the
 444 scores mean of PC1 and PC2 axes for each species. Percentages within brackets in the X
 445 and Y axes legends indicate the percentage of variance explained by the corresponding PC.
 446 Codes: number of weeks since the beginning of drought (two weeks = 2, six weeks = 6, twelve
 447 weeks = 12). Communities (G4 community = **G4**, Monoculture = **M**).

448

449 Species were grouped by functional group in the PCA analysis (Figure 4),
450 reflecting a strong hierarchical tendency in the foliar elemental contents and
451 stoichiometries determined by a phylogenetic component. Biomass survival (the ratio
452 of live to dead biomass) during the drought was correlated with elemental and
453 stoichiometric PC scores (Figure 5), indicating a relationship, in some cases, between
454 biomass mortality and elemental composition and stoichiometry.



455

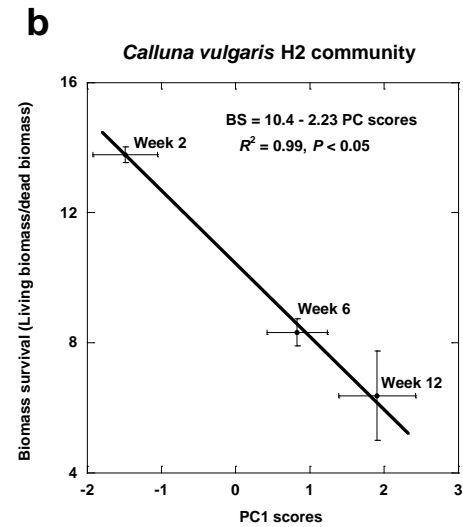
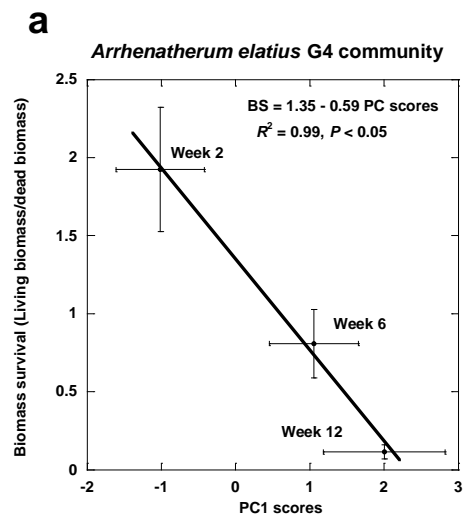
456 Figure 4. PCA plots of the mean \pm S.E. scores of the first two PCs for the two shrub and two grass species (a) growing in the H4 community during the course of the severe drought, and the corresponding variable distributions (b). Arrows on the X axis of the mean PC scores indicate the averages of each species group (shrubs and grasses) when the differences between them were significant ($P < 0.05$). When there are more than two species, different letters indicate statistical significances ($P < 0.05$) of the differences among them. Percentages within brackets in the X and Y axes legends indicate the percentage of variance explained by the corresponding PC.

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463 Figure 5. Relationships between the PC1 scores in Figure 2 and biomass survival (BS; ratio of live to dead biomass) during the course of the severe drought
 464 for *Calluna vulgaris* growing in the H2 community (a) and for *Arrhenatherum elatius* growing in the G4 community (b). Percentages within brackets in the X
 465 and Y axes legends indicate the of variance explained by the corresponding PC.

3.3. Pre-treatment: recurrent droughts

The effects of recurrent droughts on elemental composition were small and not detectable in some species. We found no significant differences in the elemental concentrations and ratios in the two species of dwarf shrubs (*C. vulgaris* and *V. myrtillus*) between the re-current drought and the control plots in the second week of the drought (first sampling) (Table S3). The grass *H. lanatus* had higher K concentrations and lower N:K ratios in response to the recurrent-drought treatment, whereas *A. elatius* had lower Mg concentrations and N:K and P:K ratios (Table S4). These species-specific effects, however, disappeared during the severe drought. The foliar elemental concentrations did not differ between the control and drought plots in the two other species in the grassland community (G4), the herb *P. lanceolata* and the leguminous *L. corniculatus*, and in the grass *D. flexuosa* in the H4 community.

4. Discussion

4.1. Pre-exposure to drought effects in the responses of grassland and shrubland to a very severe experimental drought

Increasing climatic variability within and between years and reduced reliability of seasonal climatic conditions are accepted as key characteristics of the ongoing climate change (IPCC, 2013). Communities of perennial plants such as grasslands and heathlands cover a large part of Europe and are of economic and ecological importance. Such ecosystems are increasingly exposed to novel conditions, leading to uncertainty about their future stability and functioning. Previous treatments in the experimental site and communities of this study have shown surprisingly high resilience in many ecosystemic traits such as biomass, plant cover, community species composition, soil enzyme activity and variability of flowering phenology (Kreyling et al., 2008a; Jentsch et al., 2011). Nevertheless, significant impacts were found on the allocation of carbon in the soil (Mirzae et al., 2008) and on the functioning of plants within their life cycles (Jentsch et al., 2011). Pre-exposure to drought enhanced physiological processes and even led to memory effects (Walter et al., 2011, 2013). In the present study, however, the previous recurrent droughts (pre-treatment) had no significant effect on the shifts in elemental composition during the severe drought.

4.2. Species-specific differences

Despite the dependence of the responses to the drought on community composition, the shifts in elemental compositions during the extended severe drought tended to

have common trends among the species. The drought had general effects on the foliar compositions of the species: a decrease in the N, P, K concentrations and an increase in C concentration and in C:nutrient and N:P ratios. As expected, the nutritional status of photosynthetic tissues became increasingly impoverished with the advancement of the drought, coinciding with an increase in mortality and a deterioration of the health status of plants. All species had a better nutritional status at the time of the first sampling two weeks after the onset of the drought than at the final sampling 12 weeks after the onset. This finding can be appreciated in the PCA plots, where all individuals of the first sampling, regardless of community, tended to occupy a PCA space that reflected higher concentrations of macronutrients, irrespective of the dissimilarities among the different communities. The shifts in the foliar stoichiometries of the species, however, became less predictable in the more diverse communities as the drought advanced.

Some degree of species-specific response was evident in this situation of extreme stress. *C. vulgaris* was more drought tolerant than *V. myrtillus*. Its elemental concentrations suggested a large sclerophyllous development in all communities to confront water stress. In contrast, all *V. myrtillus* plants died in the monoculture before the final week of drought, suggesting that cohabitation with other species favored survival, reinforcing the significant role of diversity. The large variation in the foliar stoichiometry of *H. lanatus* during the drought indicated a high capacity to modify elemental concentrations (high stoichiometric flexibility). The high K concentrations in the final week were consistent with the importance of this element for the conservation of water. The other grass species, *A. elatius*, had the highest C and N concentrations by the end of the drought, indicating that this species responded

differently to the water stress and was better adapted to drought conditions. K accordingly plays a fundamental role in terrestrial environments under conditions of water stress. Water availability is the main limiting factor in terrestrial ecosystems, so analyses of K should be included in ecological stoichiometric studies (Rivas-Ubach et al., 2012; Sardans et al., 2012c). Our results are consistent with the postulate by Yu et al. (2011) that plants with lower N:P ratios, the grass species in our study (7-8.5 in *A. elatius*, 7.5-9.2 in *D. flexuosa* and 7-7.5 in *H. lanatus*), should have more flexible stoichiometries than plants with higher N:P ratios, the shrub species (11.4-12.7 in *C. vulgaris* and 10.7-12.6 in *V. myrtillus*).

4.3. Effects of community composition

The changes in elemental compositions during the drought among the various species growing in the same communities were generally quite similar, but the grasses were usually more plastic than the shrubs and herbs. The responses to the drought, however, varied among the different communities. For example, P concentrations in all communities and species decreased in response to the drought, but the changes in K concentration depended on the community. K concentrations did not change in H4 but decreased in H2 over the course of the drought. *C. vulgaris* and *V. myrtillus*, which were present in these two communities, thus had different elemental changes depending on the community composition in which they grew. These results provided strong evidence for higher similarities in the response to the drought in species growing in the same community compared to the same species growing in different

communities, showing that stoichiometric shifts in response to drought are more-community dependent than species-dependent.

The interaction between the time during the drought and community composition was significant for several elemental concentrations in *C. vulgaris* and *H. lanatus* and for one ratio in *A. elatius*. These interactions supported our hypothesis that community composition influences the changes in plant stoichiometry under extreme water stress. The species growing in monocultures tended to have a less plastic response and a more consistent pattern of change in elemental concentrations and stoichiometries during the drought than did the same species growing in communities. The elemental compositions in species growing in a richer community, however, tended to change more abruptly and erratically, probably by adjusting their stoichiometries to the variable demands and requirements under a more variable competitive environment. Some studies have observed that plants in highly diverse communities are able to take up more nutrients than are plants in less diverse communities (Massey et al., 2013). Wisheu et al. (2000) reported that nutrient-poor Mediterranean ecosystems have a high diversity of plants due to the large number of specialists able to take up nutrients in different ways and at different soil depths. Under a scenario of global change with more sudden intense and severe droughts and an increasing probability of nutrient imbalances (Peñuelas et al., 2012; 2013), communities with more species but with fewer individuals of each species could thus be expected to be more successful, at least over short time scales, because these communities would be better able to respond quickly by using the resources more efficiently. A recent review has reported that plant species diversity is consistently

higher in infertile soils, an association linked to functional diversity for nutritional strategies (Lambers et al., 2011).

Community composition and diversity of functional groups had significant influences on tissue dieback in the communities in previous treatments (Kreyling et al., 2008b; Backhaus et al., 2014). We have also observed that the dieback of biomass in some communities and species was closely associated with the changes in elemental stoichiometry over the course of the drought, whereas this relationship was not as clear in the same species growing in other communities. Furthermore, soil microbial communities and their contributions to nutrient cycling and uptake are modified by the presence or absence of particular plant species rather than by species richness (Bremer et al., 2009).

The better status and contents of nutrients in plants growing in communities with more species suggests that interspecific competition can have some advantages over intraspecific competition in times of drought; the more variable demands of soil resources can lead to different abiotic requirements, such as water, nutrients and space that can be freely used by the diverse species that compose the community. A higher stoichiometric flexibility is more likely in a more diverse community, which is advantageous for responding to environmental changes. Species intolerant to stress could take advantage of this higher stoichiometric plasticity to avoid competition with stress-tolerant neighbors that respond to stress by increasing sclerophylly and thus lowering their demands for resources, thereby increasing the availability of resources to the intolerant species. Each species exhibited specific elemental stoichiometries, with elemental compositions more similar between herbs and grasses than between

these species and the shrubs. The differences among species were independent of the composition of the community in which the plants grew and were consistent with those in a previous study conducted before the severe drought (Urbina et al., 2014). Although, the study did not include data of roots, leaves (or photosynthetic tissues) constitute the crucial organ when plant responds to changes in its functions. In this context, we can not define the plant global elemental composition and stoichiometry responses but by knowing the values and changes of foliar biomass stoichiometry we can define the “species biogeochemical niche”. It represents the adequate foliar elemental composition and stoichiometry for the optimal foliar function for a determined species in its environmental circumstances.

5. Final remarks and conclusions

The responses to a very severe drought were more similar in different species growing in the same community than in the same species growing in different communities, indicating that stoichiometric shifts in response to drought were more community-dependent than species-dependent. The degree (range) of the change, however, varied among species, with grasses being more plastic than shrubs or herbs. Previous recurrent droughts (pre-treatments) had no significant effects on the shifts in elemental composition during the severe drought.

This study provides evidence of the suitability of studying the differences and shifts in plant elemental composition to improve our understanding of the processes underlying the responses of plants to different biotic and abiotic environmental circumstances. Species have particular foliar elemental compositions and

stoichiometries, depending in part on their ecological lifestyle, but they also have some degree of flexibility to adapt to changes in abiotic conditions (e.g. drought) and biotic conditions (e.g. community composition). Our results are consistent with the biogeochemical niche hypothesis (Peñuelas et al., 2008; 2010). Complex communities have a higher capacity to respond to drought relative to stands dominated by only one species. Species-specific abilities to exploit the available resources may explain this phenomenon.

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