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Author: Ifigenia Urbina Jordi Sardans Carl Beierkuhnlein Anke Jentsch Sabrina Backhaus Kerstin Grant Juergen Kreyling Josep Peñuelas

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

Ifigenia Urbina[1,2], Jordi Sardans[1,2*], Carl Beierkuhnlein[3], Anke Jentsch[4], Sabrina Backhaus[4], Kerstin Grant[4], Juergen Kreyling[3], Josep Peñuelas[1,2]

[1]CSIC, Global Ecology Unit CREAF-CEAB-CSIC-UAB, 08913 Cerdanyola del Vallès, Catalonia, Spain.

[2]CREAF, 08913 Cerdanyola del Vallès, Catalonia, Spain.

[3]Biogeography, BayCEER, University of Bayreuth, 95440 Bayreuth, Germany.

[4]Disturbance Ecology, BayCEER, University of Bayreuth, 95440 Bayreuth, Germany.

*Corresponding author. Tel. 34 93 581 2934. Fax: 34 93 581 4151. E-mail address: j.sardans@creaf.uab.es.

Highlights

- -Each species had a particular elemental composition in all circumstances

- -Species had more plastic stoichiometry when growing in more diverse communities

- -The stoichiometric shifts due to drought were more community- than species-dependent

- -Drought in all cases decreased K, N, P, Mg and S, and increased C and Fe concentrations

ABSTRACT

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

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

1. Introduction

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

The main goal of ecological stoichiometry is to understand the relationships of environments and C:N:P ratios with the lifestyles of organisms and the structures and functions of ecosystems (Sterner and Elser, 2002; Elser and Hamilton, 2007). These relationships have been widely observed in planktonic communities, where low N:P ratios in the medium are correlated with high growth rates in organisms and are consequently important to the structure of trophic webs (Elser et al., 1996; Peñuelas et al., 2013). Terrestrial plants, however, are larger than planktonic organisms, and N and P can be allocated to functions other than growth, such as storage, defense or anti-stress systems (Peñuelas and Sardans, 2009). Moreover, the concentrations or ratios of other elements such as K, Mg, Ca and Fe in terrestrial plant communities are much more involved in important physiological functions of organisms (Wilkinson et al., 1990; Leung et al., 1994; Lombini et al., 2003; Murren et al., 2006; Wu et al., 2009; Lourido et al., 2010; Hashimoto and Kudla, 2011; Sardans et al., 2012a, 2012b; Sardans and Peñuelas, 2012; Stael et al., 2012; Yang et al., 2012; Perea-García et al., 2013) and consequently should also be included in ecological stoichiometric studies. The biogeochemical niche hypothesis claims that different sympatric plant species occupy different biogeochemical niches, as manifested by the complete spectra of the contents of macro- and micronutrients in their tissues (Peñuelas et al., 2008, 2010) and not only by N and P concentrations or N:P ratios. The elemental composition of a species should thus be determined, in part, by long-term genetic adaptation to a particular abiotic environment and also by specific ecological strategies such as the optimal successional stage. The success of a plant species, however, requires some degree of flexibility to allow plants to respond to abiotic and biotic shifts. Stoichiometric flexibility enables organisms to adjust their elemental ratios while maintaining a constant function (Sistla and Schimel, 2012). Species with higher stoichiometric flexibility tend to have higher concentrations of N and P and lower N:P ratio (Yu et al., 2011).

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

The production of more sclerophyllous leaves and a slow growth rate are physiological responses to high C:nutrient ratios, drought conditions and dry environments (Feller, 1995; Fernández and Reynolds, 2000; Gonçalves-Alvim et al., 2004 and 2006; Sardans et al., 2011). The various possible responses to water stress depend on the dominant mechanism of water uptake (isohydric vs anisohydric), the functional type, the species, the soil nutrient resource and the nature of episodes of water stress (duration or frequency) (DeLucia and Schlesinger, 1991; Tradieu and Simonneau, 1998; Schultz, 2003).

The frequency and magnitude of extreme climatic events such as drought will increase as the global climate changes (IPCC, 2012). Several specific responses to extreme droughts at the community level, however, remain to be studied. Water shortage should lead to a decline in water potential and to water stress. Plants should be able to alter their functions to increase the efficiencies of their mechanisms of water uptake and use efficiency. These adaptations should depend on the composition of the community, with heathlands being very resistant (Gordon et al., 1999; Peñuelas et al., 2004) and grassland communities being more sensitive to extreme droughts (Grime et al., 2000; Kreyling et al., 2008a). The capacity to adapt to and resist drought should be accompanied by stoichiometric changes that further allow changes in the allocation of elements to different functions and structures. A species growing in communities with different species compositions should have distinct capacities to access different nutrients, thereby changing its capacity to respond to extreme droughts. We hypothesized that changes in the availability of water will be reflected in changes in foliar stoichiometry, which in turn will ultimately help us to understand the processes involved in the response to water stress. This response can also vary due to the biotic relationships of plants. Previous studies have shown that plants of the same species exhibit different elemental compositions depending on the species composition of the plant community in which they live (Urbina et al., 2014).

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

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

2. Materials and Methods

2.1. Experimental area and design

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

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

The experimental setup consisted of five replicates of each factorial combination, with a total of 30 2×2 m plots for the two species of dwarf shrubs (Table 1) (two pre-exposed treatments and three community compositions) and a total of 40 2×2 m plots for the grass species (two pre-exposure treatments and two community compositions). The factors were applied in a split-plot design with the community compositions blocked and randomly assigned within each of the five replicates of the pre-exposure treatments. All communities grew in soil 80 cm in depth, including 20 cm of topsoil, sourced from a nearby sand quarry. The topsoil had a total carbon content of 2% and a pH of 4.5 (measured in 1 M KCl), and the lower soil layer had a total carbon content of 0.2% and a pH of 6.2. The texture of the soil was loamy sand (82% sand, 13% silt and 5% clay). The total concentrations of the various elements in the soil are provided in Table S1.

2.2. Recurrent and final very severe droughts

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

The severe drought was effected by large rain-out shelters (Haygrove Tunnels Ltd., Ledbury, United Kingdom; total area 50 m in length × 31.5 m in width × 3.75 m in height) over the entire surface of the experiment that permitted nearly 90% transmission of photosynthetically active radiation through a transparent polyethylene sheet 0.18 mm in thickness (UV M 42; folitec Agrarfolien-Vertriebs GmbH, Westerburg, Germany). Unwanted greenhouse effects were avoided by leaving gaps between the ground and the shelters 1.05 m along the sides and 2.55 m on the front and back, allowing for near-surface air exchange. Before drought manipulation began, the plants were watered with a total of 46.6 mm of water in two applications (11 and 13 May 2011) to adjust all plants to the same initial conditions. The amount of 46.6 mm was the difference between the long-term average precipitation for April and the amount of natural rainfall for April 2011 (from the start of the growing season to the start of the severe drought). Lateral surface runoff was prevented by plastic sheets around the treated plots buried to a depth of 10 cm. This study focused on the effects of the very severe drought.

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

2.3. Experimental communities – composition and diversity

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

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

2.4. Biomass and elemental analyses

Photosynthetic tissues were dried and pulverized in a heater for 48 h at 70 ºC and conserved in desiccators until analysis (<15 days). For the analyses of C and N, 0.7 mg of dried, pulverized samples was weighed with a Microbalance MX5 Mettler Toledo, and the concentrations of the elements were determined by combustion coupled to gas chromatography (Smith and Tabatabai, 2004) with a CHNS Eurovector 3011 Elemental Analyzer and a Thermo Electron NA 2100 Gas Chromatograph (C.E. instruments-Thermo Electron, Milan, Italy).

For the other elements (P, S, Ca, Mg and Fe), 0.25 g of dried, pulverized sample was diluted in an acidic mixture of HNO_3 (60%) and H_2O_2 (30% w/v) and digested in a microwave system (MARSXpress, CEM Corporation, Matthews, NC). The digested solutions were dissolved in a 1% HNO_3 solution and then brought to final volume of 50 mL with ultrapure water. Blank solutions (5 mL of HNO_3 and 2 mL H_2O_2 but no sample biomass) were regularly analyzed. After digestion, the concentrations of Ca, K, Mg, S, P and Fe were analyzed by ICP-OES (Inductively Coupled Plasma - Optical Emission Spectrometry) (Priester et al., 2011) with a spectroscopy Optima 4300DV (Perkin-Elmer Inc., Wellesley, MA). We used the standard certified biomass NIST 1573a (tomato leaf) to assess the accuracy of the biomass digestion and analytical procedures.

2.5. Statistical analyses

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

We also conducted multivariate ordination analyses by using principal component analyses (PCAs) including the elemental concentrations and the N:P, N:K and P:K ratios to determine: (i) whether the species growing in different communities have different elemental composition and whether the changes in response to drought were different depending on the community where the target species grew, by analyzing species growing in different communities, and (ii) whether each of the three factors studied (species, community and length of drought) had by themselves significant effects on elemental composition and stoichiometry and whether species-specific identity remained in all community and drought situations by analyzing the data of all species and communities together.

We also conducted an ANOVA with the scores of the first six PC axes as dependent variables and the various plant communities as fixed categorical independent factors to determine the global effects of the community on elemental concentrations and stoichiometries. We tested for normality and homogeneity of variance of the residuals prior to the statistical analyses by examining the residuals versus the expected plots and the normal qq-plots of the linear models. The data were log-transformed if the required conditions were not met. The significance level was set at $P<0.05$. We also correlated biomass survival (ratio of live to dead biomass) during the drought with the elemental and stoichiometric PC scores to determine how mortality affected stoichiometric composition. All statistical analyses were performed using Statistica 6.0 (StatSoft, Inc. 1984-2001, Tulsa, OK, USA).

3. Results

3.1. Effects of the drought on the communities

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

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

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

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

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

The PCA for the four species growing in monoculture indicated comparable responses to those of the H4 community, where the scores in the shrubs *V. myrtillus* and *C. vulgaris* generally differed from those in the two grass species *H. lanatus* and *A. elatius* along the PC1 axis ($P<0.0001$) (Figure 1g,h). *V. myrtillus* and *C. vulgaris* were separated along the PC2 axis ($P<0.0001$). *H. lanatus* and *A. elatius* were also separated along PC2 axis ($P<0.0001$). The death of many individuals of *V. myrtillus* and *A. elatius* did not allow an analysis for the advanced stages of the drought. The variables that mainly loaded PC1 axis were the concentrations of S, K and P, which decreased, and C concentrations that increased towards shrubs, and N and Ca concentrations that decreased in C. vulgaris and *H. lanatus*, during the drought advance (Figure 1 g,h). These changes were also consistent with the observed in univariate analyses (Table S2).

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

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

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

The elemental concentrations and ratios in all species differed significantly among the three sampling times during the drought (Figures 1-4, Table S2), but the differences depended on the community in which the species grew. This interaction between the drought and community composition was species-specific (Table S2). The interaction between the duration of the drought and community composition significantly affected the elemental compositions and stoichiometric ratios in three of the four species that were growing in two or more different communities, showing that the community had a significant influence on the shifts in elemental composition during the drought. Individuals of *C. vulgaris* and *H. lanatus* in the monocultures shifted their elemental concentrations and ratios mainly linearly during the drought, unlike the individuals growing in more diverse communities where elemental concentrations and stoichiometries change more abruptly and irregularly.

The ANOVA of PC1 scores in *C. vulgaris* showed a separation of samples over the course of the drought. PC1 axis was mainly loaded by N, K, S and P, and N:P ratio, showing that N, K, S and P concentrations decreased and C concentrations and N:P ratios increased over the course of the drought (Figure 2a,b), consistently with the results of univariate analyses (Table S2). N:K and P:K ratios peaked in the last week of the drought. The shifts in elemental composition over the course of the drought, though, differed among the communities containing *C. vulgaris*. The N:K and P:K ratios and in general all elemental concentrations in this shrub increased in response to drought more in the H4 community than in the other two communities (Figure 2a,b, Table S2). The K concentration decreased in the shrub *V. myrtillus* in week 6 but increased toward the end of the drought. P and S concentrations decreased, and the N:P ratios increased, during the drought. This species, however, could not be studied appropriately due the lack of samples from the monoculture for the final week of the drought from high mortality (Figure 2c,d). Large changes in elemental composition were observed between the second and sixth weeks of the drought, whereas the differences between the sixth and twelfth weeks were not significant. The elemental compositions in *V. myrtillus* plants generally varied among the different communities

during the drought as observed in the corresponding PC2 axis (*P*<0.0001). Despite of these differences depending on the communities where these two shrubs grew (Figure 2 a-d, Table S2), the experimental drought decreased P and S concentrations in both species independently of the community where they grew (Table S2).

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

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

K concentrations and N:K and P:K ratios in *V. myrtillus* and *A. elatius*, though, changed in the same direction over the course of the drought independently of the community. *C. vulgaris* and *H. lanatus* showed significant interactions between sampling time and community. K concentrations and N:K and P:K ratios did not change in the monocultured plants but changed in those growing in H2 and H4. Moreover, the distribution of the means over the PC axes reflected a minor change in the foliar elemental concentrations and stoichiometries for the individuals of the four species growing in monocultures. All these results suggest less stoichiometric flexibility during the drought in plants growing in monocultures than in those growing in more diverse communities (Figures 1-3).

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

When we conducted a PCA with the data of all four studied communities altogether we observed that all three studied independent factors (community, species and time of drought) had effects on overall elemental composition and stoichiometry (Figure S1). This further showed that there is a species-specific elemental composition and an effect of drought changing the species elemental composition, but species-specific identity in elemental composition and stoichiometry always remains despite the level of drought.

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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Figure captions:

Figure 1. PCA plots of the mean ± 95% confidence interval of the 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**).

Figure 2. PCA plots of the mean ± 95% confidence interval of the scores of the first two PCs for *Calluna vulgaris* (a) and *Vaccinium myrtillus* (c) growing in different communities (diversity levels) during the course of the severe drought, and the corresponding variable distributions (b) and (d), respectively. 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). Communities (H2 community = **H2**, H4 community = **H4**, Monoculture = **M**).

Figure 3. PCA plots of the mean ± 95% confidence interval of the scores of the first two PCs for *Holcus lanatus* (a) and *Arrhenatherum elatius* (c) growing in different communities (diversity levels) during the course of the severe drought, and the corresponding variable distributions (b) and (d), respectively. 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). Communities (G4 community = **G4**, Monoculture = **M**).

Figure 4. PCA plots of the mean ± 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.

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 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 and Y axes legends indicate the of variance explained by the corresponding PC.

Table 1. Species compositions of the communities.

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

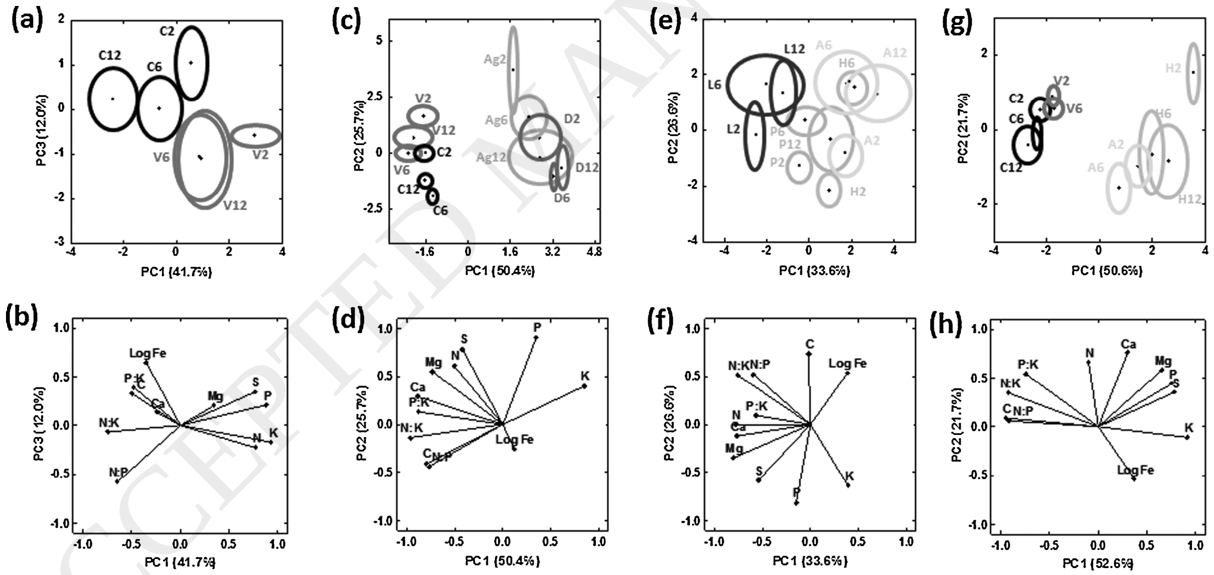
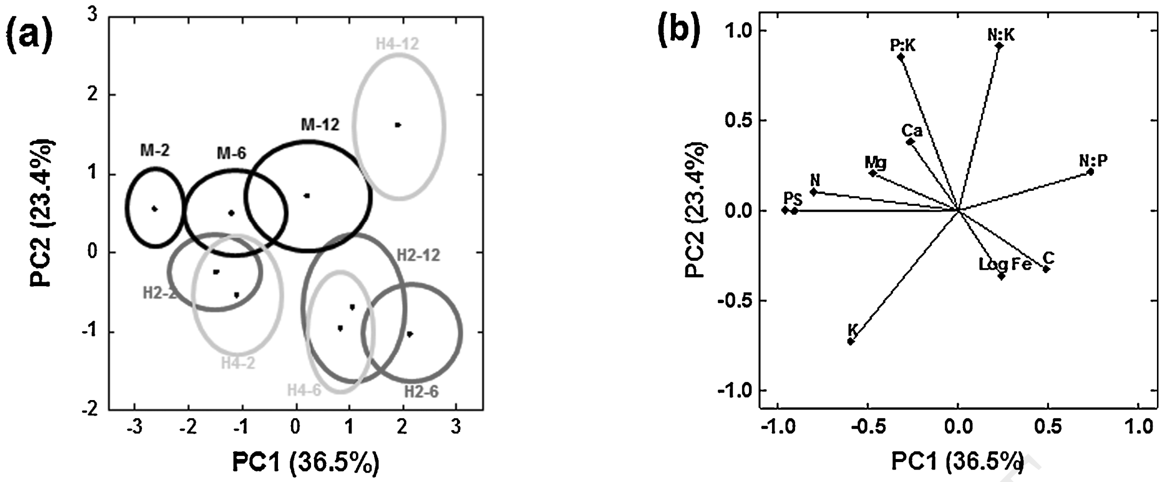


Fig. 1

Calluna vulgaris



Vaccinium myrtillus

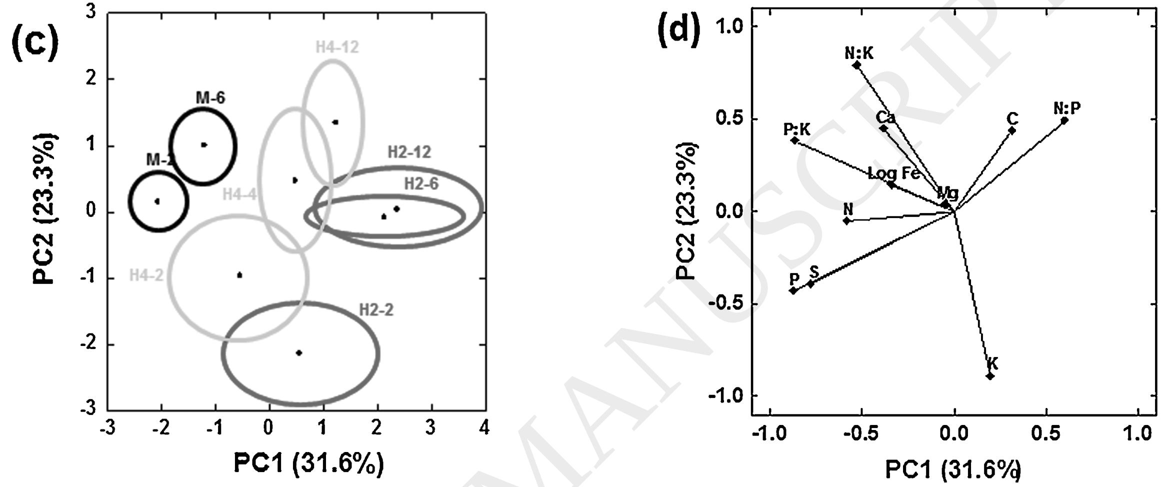


Fig. 2

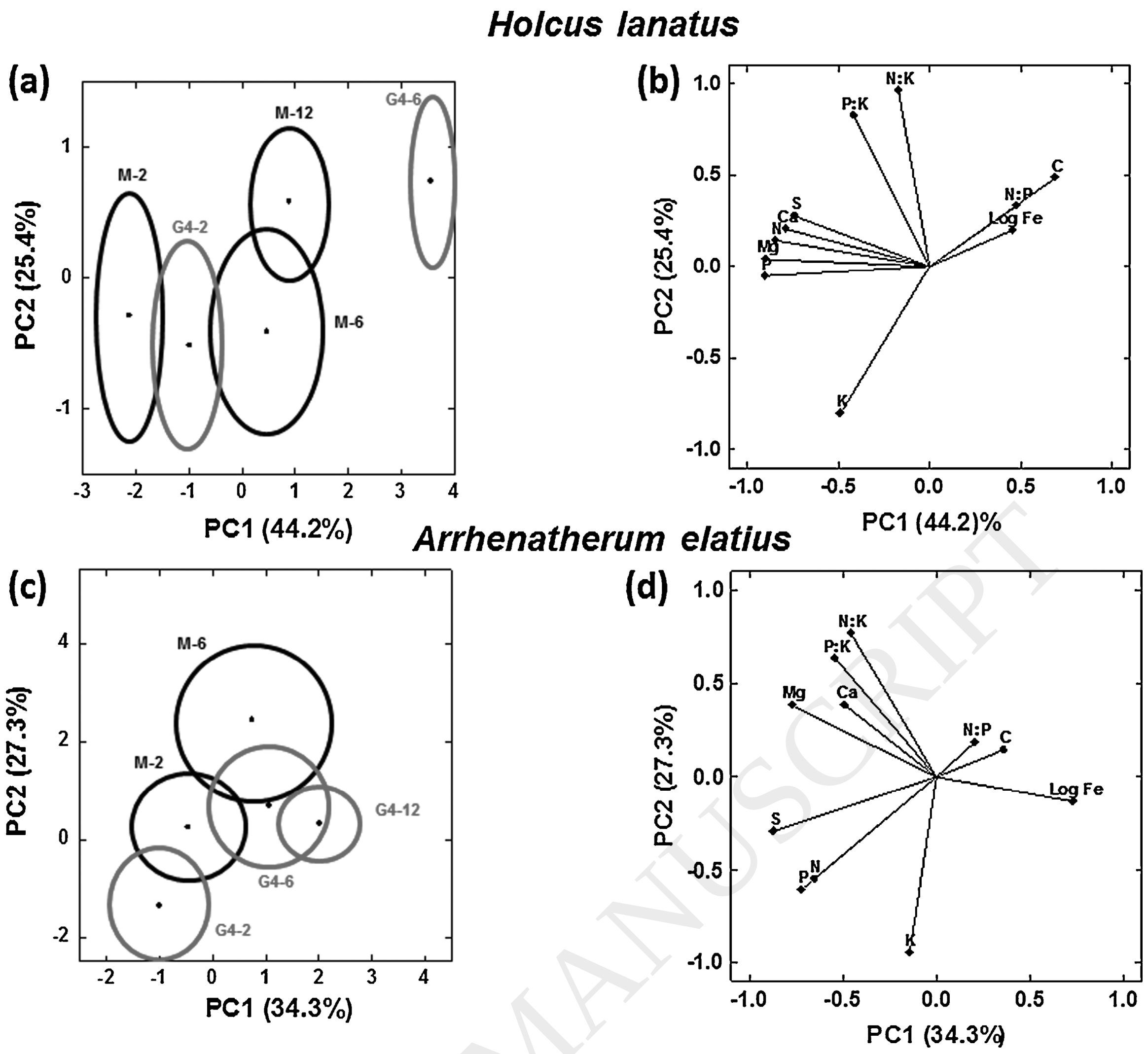

Holcus lanatus
(a)
M-2
G4-2
M-12
M-6
G4-6
PC2 (25.4%)
PC1 (44.2%)
-3
-2
-1
0
1
2
3
4
1
0
-1
(b)
N:K
P:K
C
N:P
Log Fe
S
Ca
N
Mg
P
K
PC2 (25.4%)
PC1 (44.2)%
1.0
0.5
0.0
-0.5
-1.0
Arrhenatherum elatius
(c)
M-6
M-2
G4-12
G4-6
G4-2
PC2 (27.3%)
PC1 (34.3%)
4
2
0
-2
-1
1
3
(d)
N:K
P:K
Mg
Ca
N:P
C
Log Fe
S
P
N
K
PC2 (27.3%)
PC1 (34.3%)

Fig. 3

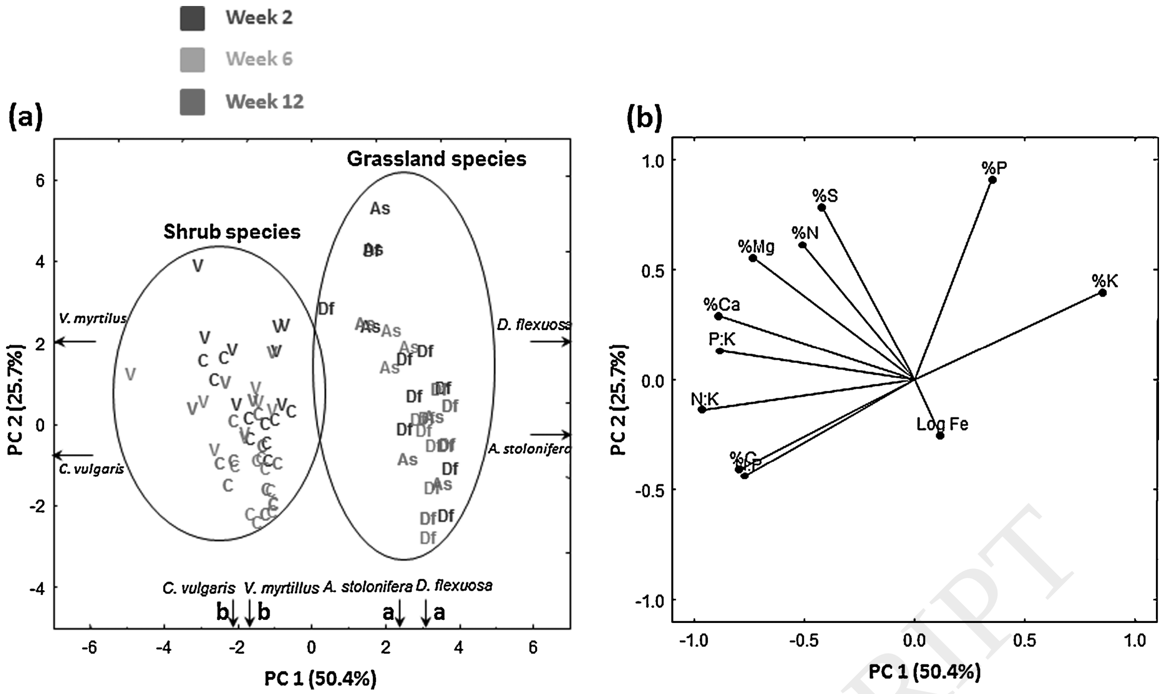

Week 2
Week 6
Week 12
(a)
Grassland species
Shrub species
V. myrtillus
C. vulgaris
D. flexuosa
A. stolonifera
C. vulgaris V. myrtillus A. stolonifera D. flexuosa
b b a a
PC 2 (25.7%)
PC 1 (50.4%)
(b)
%P
%S
%N
%Mg
%K
%Ca
P:K
N:K
Log Fe
%C
C:P
PC 2 (25.7%)
PC 1 (50.4%)

Fig. 4

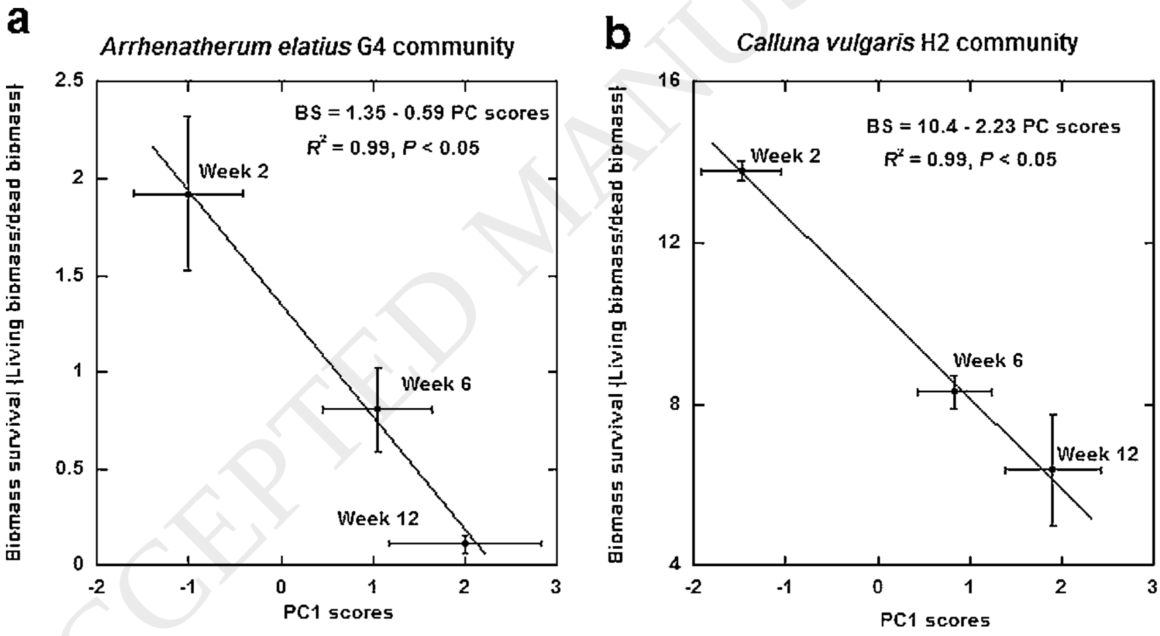

a
Arrhenatherum elatius G4 community
BS = 1.35 - 0.59 PC scores
R² = 0.99, P < 0.05
Week 2
Week 6
Week 12
Biomass survival (Living biomass/dead biomass)
PC1 scores
b
Calluna vulgaris H2 community
BS = 10.4 - 2.23 PC scores
R² = 0.99, P < 0.05
Week 2
Week 6
Week 12
Biomass survival (Living biomass/dead biomass)
PC1 scores

Fig. 5