

## Plant community composition affects the species biogeochemical niche

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**Abstract.** Nutrients are essential for plant development, and their availability and stoichiometric ratios can influence the composition of plant communities. We investigated the possibility of the reverse influence: whether the conditions of contrasting species coexistence determine foliar element concentrations and plant stoichiometry, that is, species biogeochemical niche (BN). The experiment was conducted at the Ecological-Botanical Garden of the University of Bayreuth, Germany. We analyzed foliar element concentrations of two dwarf shrubs (*Calluna vulgaris* and *Vaccinium myrtillus*) and two grasses (*Holcus lanatus* and *Arrhenatherum elatius*) growing in different community compositions (monocultures and various mixed stands). Foliar nutrient concentrations and stoichiometry (taken as a proxy of species BN) were species specific; each species showed its own BN in all communities. Furthermore, *V. myrtillus* and *H. lanatus* species shifted their BN in response to changes in their community, accomplishing the “biogeochemical niche displacement” hypothesis. We conclude that plants can readjust their foliar element concentration if they grow in communities with contrasting plant composition, suggesting a differential use of element resources when the patterns of species coexistence change. These results also support the complementary niche hypothesis.

**Key words:** biogeochemical niche; community composition; grass; homeostasis; shrub; stoichiometry.

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### INTRODUCTION

In recent decades, plant nutrient stoichiometry, defined as the relative proportion of C and nutrients in plant tissues (leaves, roots, shoots, and wood), has gained significant importance in ecological studies (Elser and Urabe 1999, Sterner and Elser 2002, Moe et al. 2005, Cross et al. 2005, Elser and Hamilton 2007, Sardans et al. 2012a). It has been found that plant nutrient stoichiometry influences several ecological processes that are pivotal to ecosystem functions and dynamics,

such as plant–herbivore interactions (Daufresne and Loreau 2001, Millett et al. 2005, Newingham et al. 2007), the structure and function of arbuscular mycorrhizae (Johnson 2010), and population dynamics (Andersen et al. 2004). Recent studies have also found a strong relationship between changes in foliar C:N:K ratios and changes in foliar metabolites in response to varying environmental conditions (Peñuelas and Sardans 2009, Rivas-Ubach et al. 2012). However, the link between foliar nutrient concentration and community composition has proven to be

challenging and remains poorly explored. This is understandable because the composition of plant communities depends on several ecological processes and functions such as productivity, resource use, nutrient cycling, biotic interactions (Grime 1974, Tilman et al. 1996, Roem 2000), while plant stoichiometry is influenced by several factors such as soil fertility, source and quantity of water supply, phylogenetic affiliation, and climatic conditions (Sterner and Elser 2002). Additionally, soil-layer interactions and competition with microbial communities make it difficult to establish a direct link between soil resources and plant diversity (Hooper and Vitousek 1997), increasing even further the difficulties associated with studying the link between plant stoichiometry and community composition.

In this context, Peñuelas et al. (2008) shed light into the relationship between plant stoichiometry and plant coexistence. The authors proposed the “biogeochemical niche hypothesis” where species have a species-specific stoichiometry in a multivariate space generated by the contents of macro- and micronutrients in plant tissues. This hypothesis is based on coexisting plant species tending to use the main nutrients N, P, and K (and other essential nutrients such as Ca, Mg, and S) in differing proportions (Peñuelas et al. 2008, 2010). Since different plant structures and metabolic processes have distinct and divergent requirements for each of the essential nutrients, the species-specific biogeochemical niches (BNs) should be the result of species specialization to particular abiotic and biotic conditions. Biogeochemical niche should reflect the different species-specific strategies of growth and uptake of resources and the differences in soil space and time occupation reducing direct competition among sympatric species and optimizing the nutrient use by the overall community. Biogeochemical niche also hypothesized that the existence of stoichiometric flexibility (the capacity to change the BN) of a species is an important trait, as it is related to the quality of plants tissues and their capacity to cope with changes in environmental conditions. Building further on this hypothesis, Yu et al. (2010) argued that species exhibit stoichiometric flexibility in response to environmental changes (including ontogenic and seasonal changes) and competitive situations, probably under a tradeoff between the

stoichiometry flexibility and homeostatic regulation (the maintenance of constant internal conditions in the face of externally imposed variation). Recent studies in Spanish and European forests have shown that elemental stoichiometries of different forest species were strongly determined genetically, which is consistent with their long-term adaptation to specific abiotic and biotic environments leading to optimized metabolic and physiological functions and morphological structures determining the specific use of various nutrients (Sardans et al. 2015, 2016). These studies have also shown that current climate is also an important driver of uptake and nutrient use and that BN should reflect trade-offs among several functions such as plant growth, resource storage, and/or anti-stress mechanisms for maximizing plant fitness in each of particular climate. More differences in foliar composition stoichiometry among sympatric species than among allopatric species have been reported (Sardans et al. 2015, 2016). The BN hypothesis thus claims that all species are adapted, to some extent, to different combinations of environmental factors implying a trade-off between different basic functions such as growth rates, reproduction, and stress tolerance. The different BN can also be taken as a final result of an optimum adaptation to environmental gradients and can be compared to the proposed ecological strategies as, for example, the Grime R-C-S model (1977) where different species can be placed in an specific “space” in the gradient among these different ecological strategies.

Following Peñuelas et al.’s (2008) BN hypothesis, we here investigated the inverse relationship between the changes in species coexistence and the BN of species. We aimed to determine whether the composition of plant communities affects the BN of individual species. We included as many elements as possible in the analysis because of the well-known importance of several elements such as K, Mg, S, and Ca in plant physiology in water stress avoiding strategies, photosynthetic machinery, regulation of ion balance (homeostasis) in chloroplast and vacuoles, transport of sugar into the phloem, and secondary metabolism (Sawhney and Zelitch 1969, Knight et al. 1991, Bourgis et al. 1999, Romeis et al. 2001, Shaul 2002, Franceschi and Nakata 2005, Cakmak 2005, Gill and Tuteja 2011, Sardans and Peñuelas 2015). Our main research question was as follows:

“Are plants able to modify their foliar stoichiometry (species BN) in response to varying species coexistence?” To address this question, we conducted a comparative experiment in which different species were grown in different plant communities in order to test whether the species BN changed in response to their neighboring co-occurring plants. We hypothesized that stoichiometric flexibility could occur in three different ways (see Fig. 1): (1) BN *expansion*, which would represent an increase in the stoichiometry spectrum of a species as a consequence of the competition with neighboring individual plants; (2) BN *contraction*, where species specialize in absorbing nutrients in a more narrow and concrete proportion as a strategy to reduce competition; and (3) BN *displacement*, where certain physiological functions (e.g., more woody biomass production or lower growth rate) are re-adjusted as a result of the shift of competition scenarios.

## METHODS

### Experimental setup

The experiment was conducted at the Ecological-Botanical Garden of the University of Bayreuth, Germany (49°55'19" N, 11°35'55" E; 365 m a.s.l.), as part of the EVENT I experiment (Jentsch et al. 2007, 2011). Mean annual precipitation at the site is 709 mm, with a major peak in June/July and a second peak in December/January. The communities were established in April 2005 by planting pre-grown individuals of all species in 2 × 2 m plots. All species were harvested in May 2011, after 6 yr of pre-grown phase. Four different types of plant communities were studied (monocultures, heath, and grass; Table 1), and a total of seven community compositions were sampled: monocultures (MCs) of two dwarf shrubs (*Calluna vulgaris* and *Vaccinium myrtillus*) and of two grass species (*Holcus lanatus* and *Arrhenatherum*

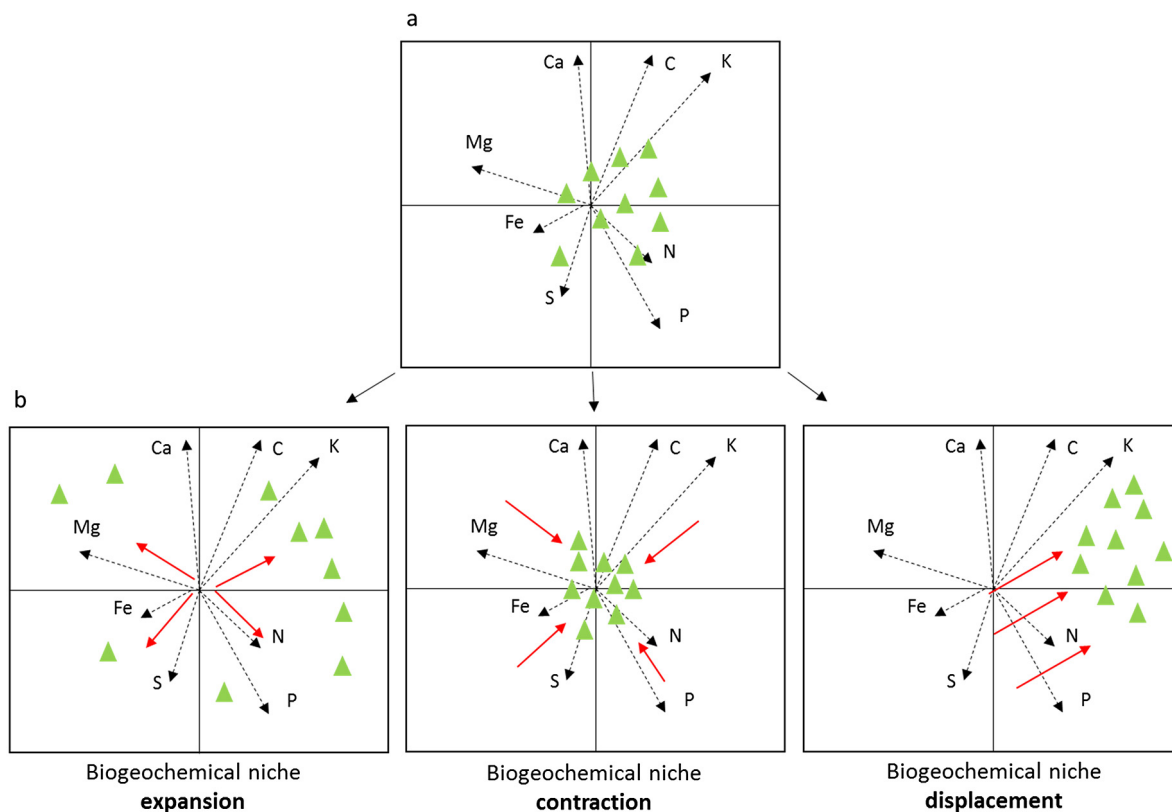


Fig. 1. Hypothetical stoichiometry spectrum (in the principal component analyses) based on the foliar elemental concentrations and the possible hypothetical shifts proposed in this article. (a) Species growing in a monoculture and (b) the possible hypothetical shifts (expansion, contraction, displacement) in the stoichiometry spectrum in response to different community composition. Filled triangles show individuals (replicas) of a same species.

Table 1. Characterization of the plant communities of the EVENT I experiment.

Community	Description	Species
MC	Monocultures of the four target species	<i>Calluna vulgaris</i> , <i>Vaccinium myrtillus</i> , <i>Arrhenatherum elatius</i> , <i>Holcus lanatus</i>
H2	Two species, one functional group (dwarf shrub)	<i>C. vulgaris</i> , <i>V. myrtillus</i>
H4	Four species, two functional groups (dwarf shrub and grass)	<i>C. vulgaris</i> , <i>V. myrtillus</i> , <i>Agrostis stolonifera</i> , <i>Deschampsia flexuosa</i>
G4	Four species, three functional groups (grass, herb, and legume)	<i>A. elatius</i> , <i>H. lanatus</i> , <i>Plantago lanceolata</i> , <i>Lotus corniculatus</i>

Note: The species studied are highlighted in boldface.

*elatius*); two heath communities, one composed of the two shrub species used in the monocultures (H2); and the other one (H4) with the same two shrub species and two naturally co-occurring grass species (*Agrostis stolonifera* and *Deschampsia flexuosa*); and one grass community (G4) with the two grass species (*H. lanatus* and *A. elatius*) combined with a naturally co-occurring herb (*Plantago lanceolata*) and legume (*Lotus corniculatus*). All community compositions were selected because they represent the most prevailing and widespread species in Europe, and have significant landscape and ecological importance. The original species composition of each community was maintained throughout the experiment by removing invading species at least twice per growing season.

Each plot was divided using a 20 × 20 cm grid, with one individual planted in each grid square (for a total of 100 individuals per plot). Initial species density for plot in the monocultures resulted in one dwarf shrub individual or one grass individual per grid square. In H2 individual were planted alternating species within the grid, resulting in 50 individuals of each species (half density of *species* in the monocultures). Similarly, in the four-species communities (H4, G4), individual species were planted alternatively within the grid, resulting in 25 individuals of each species (one-quarter density of *species* in the monocultures). All the communities (four monocultures and three mixed stands) had five replicates, for a total of 35 plots. In one H4 replicate, one species (*V. myrtillus*) was lost at the time of sampling.

All plots were established on 60 cm of homogenized sandy soil overlaid with 20 cm of topsoil. The topsoil had a total carbon content of 2% and a pH of 4.5 (measured in 1 mol/L KCl), and the lower sandy layer had a total carbon content of 0.2% and a pH of 6.2. A gravel bed and drainage

tubes below 80 cm averted any influence from irregular groundwater. The soil texture was loamy sand (82% sand, 13% silt, 5% clay). Soils were collected from a nearby sand quarry (the topsoil of the quarry) where all study plants are found. Four soil samples were collected in three replicas of each community (MC, H2, H4, and G4) with soil cores of 28 mm in diameter to a depth of 100 mm at the same time of harvest event in May 2011. The four soil samples of each community were bulked into one sample, for three bulked samples for each community and a total of 21 soil samples (3 replicas × 7 communities) that were used in stoichiometric analyses. The stoichiometry of the soil nutrients did not differ significantly among the communities; the total element concentrations are presented in the supporting information section (Appendix S1: Table S1).

#### Sampling, chemical analysis, and biomass calculation

All species were harvested in May 2011 for chemical analyses. An equal number of top and bottom leaves were collected from mature, reproductive individuals. We collected a total of 15 foliar samples from each species of dwarf shrub and a total of 10 foliar samples from each grass species. Sampling was conducted in two differing ways depending on the occurrence or not of regeneration within the plots. When no regeneration occurred (true for the both dwarf shrubs *C. vulgaris* and *V. myrtillus*), 20 × 40 cm sampling frames were centered in two dwarf shrubs for H2 and H4 and a 20 × 20 sampling frame centered in each shrub for the monocultures. When regeneration occurred (G4 and grasses monocultures), the 20 × 40 sampling frames were placed randomly in every community composition because it was not possible to locate originally planted individuals. The biomass of

photosynthetic tissues per area was calculated in  $20 \times 40$  cm ( $0.08 \text{ m}^2$ ) of sampling area. In the dwarf shrubs monoculture, because the sampling was carried out with  $20 \times 20$  frame, the value was corrected by multiplying by two, resulting in the same area.

The collected leaves were oven-dried at  $70^\circ\text{C}$  for 72 h, pulverized, re-dried at  $70^\circ\text{C}$  for 48 h, and stored in desiccators until analyzed (<15 d). C and N concentrations were determined for 0.7–0.9 mg of pulverized dried samples by combustion coupled to gas chromatography with an Elemental Analyzer CHNS Eurovector 3011 Thermo Electron Gas Chromatograph, model NA 2100 (CE Instruments/Thermo Electron, Milan, Italy). The concentrations of the other elements (P, K, Ca, Mg, S, and Fe) were determined for 0.25 g of pulverized dried sample dissolved with an acidic mixture of  $\text{HNO}_3$  (60% w/v) and  $\text{H}_2\text{O}_2$  (30% w/v) and digested in a MARSXpress microwave system (CEM GmbH, Kamp-Lintfort, Germany). The digests were then diluted to a final volume of 50 mL with ultrapure water and 1%  $\text{HNO}_3$ . Blank solutions (5 mL of  $\text{HNO}_3$  and 2 mL of  $\text{H}_2\text{O}_2$  with no sample) were regularly analyzed. The concentrations of Ca, K, Mg, S, P, and Fe after digestion were analyzed by ICP-OES inductively coupled plasma optical emission spectrometry (Optima 4300 DV, Perkin-Elmer, Waltham, USA). The accuracy of the biomass digestions and analytical procedures was assessed with a certified biomass NIST 1573a (tomato leaf, NIST, Gaithersburg, Maryland, USA) standards.

### Statistical analysis

Principal component analyses (PCAs) were conducted based on the foliar stoichiometric data of each species in order to explore the possible different groups formed by the community factor. A complementary PERMANOVA was conducted to test the statistical significance of the groups.

One-way ANOVAs were conducted using element concentrations and C:N, C:P, C:K, N:P, N:K, and P:K ratios as dependent variables and community as a categorical factor for each species in order to evaluate the differences in element concentrations within the different communities. ANOVA was also applied to test differences in species biomass per area in the different communities. We used Tukey's honestly significant difference procedure test and the pairwise test

with Bonferroni corrections to differentiate community groups. Prior to the statistical analyses, we tested for normality and homogeneity of variances of element concentrations using the Shapiro–Wilk normality test. Also, the residuals vs. the expected plots and the normal qq plots of the linear models were examined. All statistical analyses were conducted with R packages in R 3.2.1. FactoMineR package was used for the PCA, Vegan package for the ANOVAs, Permute package for the PERMANOVA, and ggplot2 for graphics.

## RESULTS

### Monoculture communities

The PERMANOVA separated the species and the functional groups (Appendix S2: Table S1). The PCA of the four monocultures showed the separation of the dwarf shrub and grass functional groups on the first axis ( $P < 0.0001$ ), which explained 56.4% of the variability (Fig. 2a, b). Furthermore, the grass species were separated along the PC1 axis ( $P < 0.0001$ ), while the shrubs were not separated.

### Heath communities (H2 vs. H4 vs. MCs)

The PERMANOVA for *Vaccinium myrtillus* discriminated among the different communities ( $P < 0.001$ ; Appendix S2: Table S1). The BNs of *V. myrtillus* individuals in the different communities were separated in the first two PCA axes, PC1 ( $P < 0.01$ ) and PC2 ( $P < 0.0001$ ; Fig. 3a, b). H2 was separated from H4 and MC on the PC1 axis and MC from H4 and H2 on the PC2 axis. These two principal component (PC) axes explained 37.9% and 25.6% of the variability, respectively. *Calluna vulgaris* individuals were separated marginally (Appendix S2: Table S1). H4 and the MC individuals were separated ( $P = 0.05$ ) along PC1, which explained 41.4% of the variability (Fig. 3c, d).

K and Fe concentrations of *V. myrtillus*, as well as the C:K, N:K, P:K ratios, varied when grown in different communities (Fig. 4, Appendix S2: Table S2). K concentration was significantly higher in H2, followed by H4 and lowest in MC. Fe concentration was lowest in H2, and C:K, N:K, and P:K ratios were higher in the monoculture. Mg concentration was marginally significantly higher in H4. For *C. vulgaris*, we found only marginal differences. P concentration was lower in H4 relative to

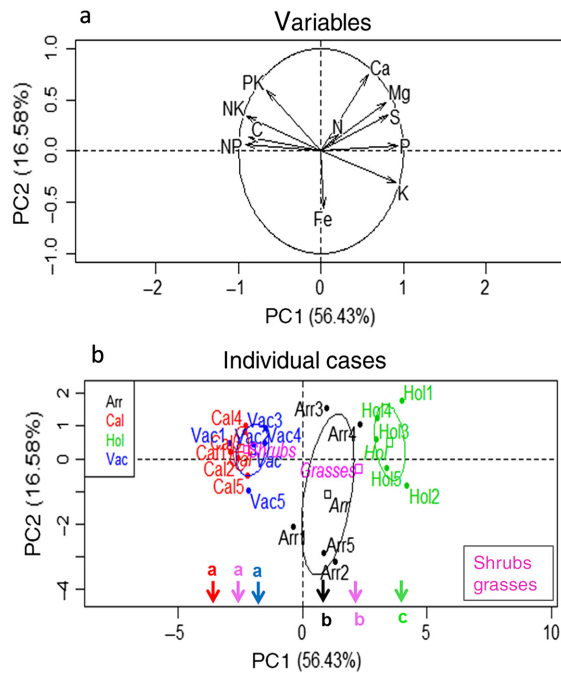


Fig. 2. Principal component analyses of foliar C, N, P, K, Ca, S, Mg, and Fe concentrations and N:P, N:K, and P:K ratios. (a) PC1 vs. PC2 loadings for the included variables. (b) Estimated site scores for the monocultures of *Calluna vulgaris* (Cal), *Vaccinium myrtillus* (Vac), *Holcus lanatus* (Hol), and *Arrhenatherum elatius* (Arr) in monoculture community. Numbers displayed right to the species acronym indicate the replica of each species. Shrubs and grass functional groups were separated significantly in PC1. Between the four species, *H. lanatus* was separated significantly from *Arrhenatherum*. *C. vulgaris* and *V. myrtillus* were not separated. Differently colored arrows and letters indicate significant differences.

H2 and the monoculture, and the C:P ratio was higher in H4 relative to the monoculture (Fig. 5; Appendix S2: Table S2).

The PERMANOVA for both species together discriminated groups by species ( $P < 0.0001$ ), by community ( $P < 0.0001$ ), and the interaction between them ( $P < 0.0001$ ; Appendix S2: Table S1). The BN of *C. vulgaris* and *V. myrtillus* occupied different PC spaces on the first two PCA axes (PC1 and PC2,  $P < 0.0001$ ), which explained 36% and 21.6% of the variability, respectively (Fig. 6a, b). Also, the *V. myrtillus* in MC was separated on the PC2 ( $P < 0.001$ ) axis

from the H2 and H4 communities. The ANOVA reflected differences in element concentrations by species and differences in P and C:P, N:P, and N:K ratios by community (Tables 2, 3).

#### Grassland communities (G4 vs. monocultures)

For *Holcus lanatus*, the PERMANOVA discriminated between the different communities ( $P < 0.01$ ; Appendix S2: Table S1). The BNs of *H. lanatus* in G4 were separated ( $P < 0.001$ ) from the monoculture on the PC1, which explained 43.6% of the total variance (Fig. 7a, b). In contrast, the PERMANOVA for *Arrhenatherum elatius* found no differences between groups, and the PCA did not separate the species when growing in monoculture or in G4.

The P, S, and Fe concentrations of *H. lanatus* were higher, and the C:P and N:P ratios were lower in the monoculture than in G4 (Fig. 8; Appendix S2: Table S2). Concentrations of N and Mg, and the C:N ratio, showed only marginal differences. For *A. elatius*, only the N:K ratio was higher in the monoculture compared to the G4 community (Fig. 9; Appendix S2: Table S2).

The PERMANOVA for *H. lanatus* and *A. elatius* together discriminated by species ( $P < 0.001$ ) and by community ( $P < 0.01$ ), but the interaction between them was not significant (Appendix S2: Table S1). The PCA separated the BNs of the two species on PC1, which explained 36.2% of the variability, and *H. lanatus* individuals from G4 were separated from monoculture individuals on PC1 (Fig. 6c, d). The two grass species showed differences in their element concentrations due to the species and community factor (Table 2).

#### Biomass per unit area

The ANOVA showed no differences in biomass per unit area ( $\text{g}/\text{m}^2$ ) for any species in all the different communities. In other words, the biomass per unit area ( $\text{g}/\text{m}^2$ ) of each species was similar in every community.

## DISCUSSION

We hypothesized that BNs would be species specific and could change in three ways: (1) BN expansion, increasing the variability among individuals without changing the mean foliar stoichiometric spectrum; (2) BN contraction, decreasing the stoichiometric spectrum among individuals; or

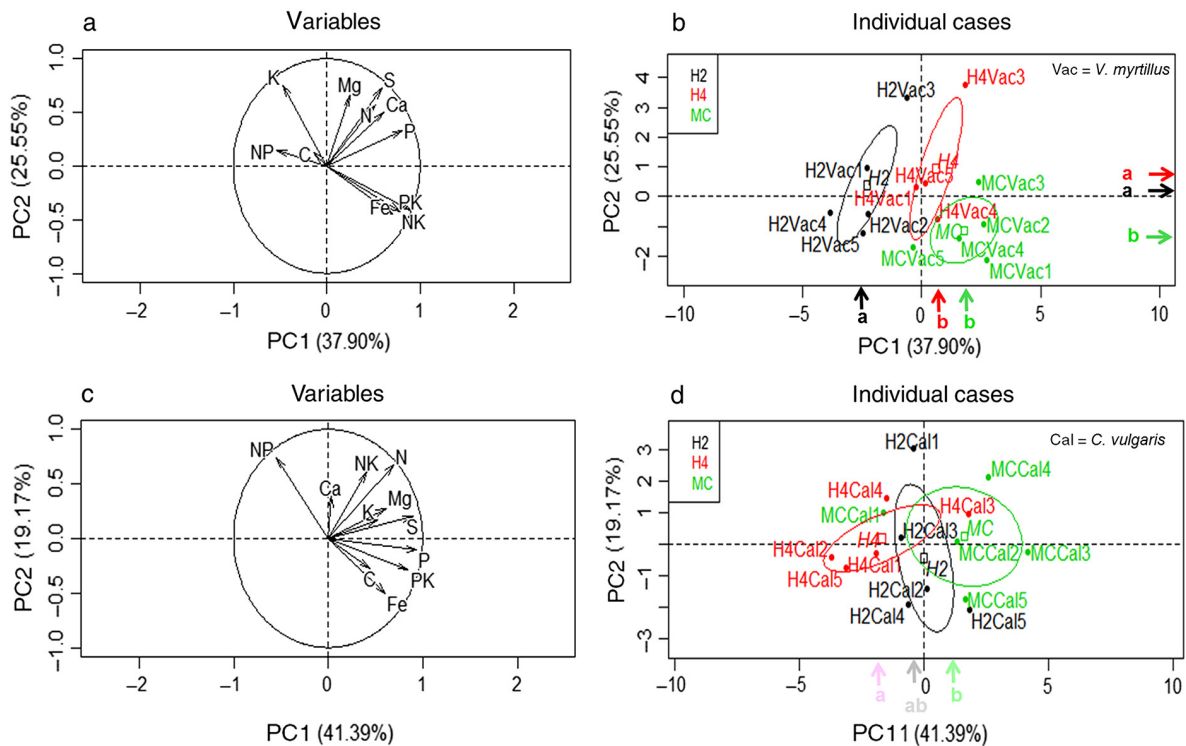


Fig. 3. Principal component analyses of foliar C, N, P, K, Ca, S, Mg, and Fe concentrations and N:P, N:K, and P:K ratios for the shrubs species. (a, c) PC1 vs. PC2 loadings for the included variables. (b) Estimated site scores for the *Vaccinium myrtillus* (Vac) and (d) *Calluna vulgaris* (Cal) in different communities. Community richness is indicated with MC for the monocultures, H2 for the two-species community, and H4 for the four-species community. Numbers displayed right to the species acronym indicate the replica of each species. Differently colored arrows and letters indicate significant differences. Lightly colored arrows and letters indicate marginally significant differences.

(3) BN displacement, shifting the mean foliar stoichiometric spectrum of all individuals. The four monocultures occupied different BNs in the PCA space and all the BN changes observed occurred through *biogeochemical niche displacement*, supporting the third possibility that we postulated.

The anatomical differences and life cycles of grass and shrub species may lead to important differences in the nutrient contents of leaves (Reich and Oleksyn 2004). In our study, the BN spectra of the two functional groups were clearly discriminated (Fig. 1a, b). However, BN did not differ between the two shrub species even though they have different life strategies (evergreen and deciduous). In contrast, the foliar BN of the two grass species *Holcus lanatus* and *Arrhenatherum elatius* was different, probably because of differences in the life spans of their leaves. Both species grew

under the same climatic and soil conditions (soil nutrients did not differ significantly between the communities), so these results can be interpreted as different resource exploitation strategies. The different soil exploitation strategies is a logical explanation for our results and is in line with the complementary niche hypothesis, which claims that different species occupy different ecological niches based on resource acquisition (Loreau and Hector 2001). However, the experimental design with all individuals regularly placed (one in each 20 × 20 cm grid square) could affect life forms differently because of the different root systems of dwarf shrubs and grasses.

The community composition produced a BN displacement in three of the four species. The BNs of *Vaccinium myrtillus* and *H. lanatus* shifted when growing in different communities, the BN of

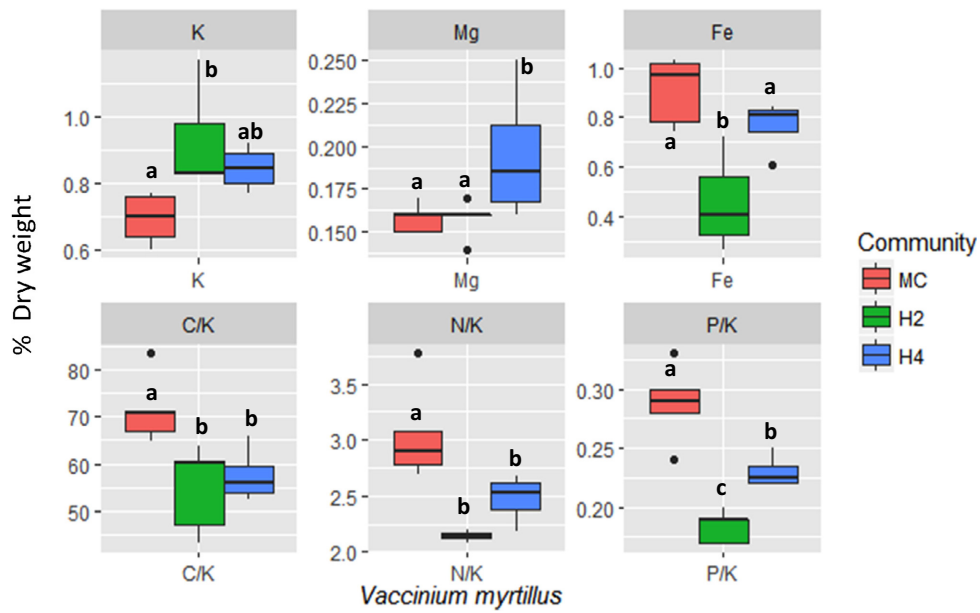


Fig. 4. Foliar elemental concentrations and ratios (mean % dry weight  $\pm$  SE) of *Vaccinium myrtillus* for the different communities. Black line inside the box shows the data median, and the bars indicate the smallest (down) and largest (top) non-outlier value. Different letters indicate significant differences ( $P < 0.05$ ) between communities.

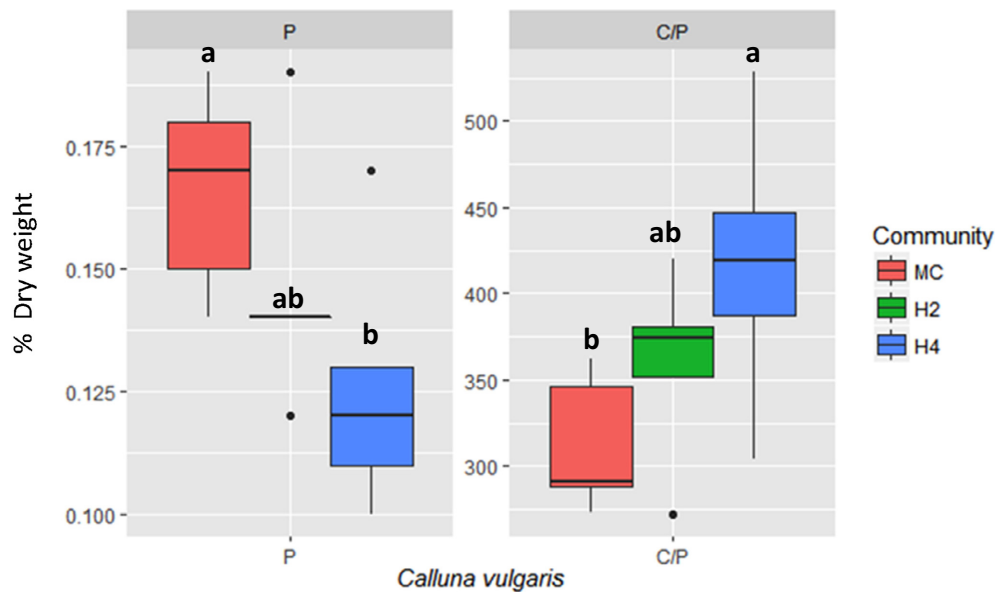


Fig. 5. Foliar elemental concentrations and ratios (mean % dry weight  $\pm$  SE) of *Calluna vulgaris* for the different communities. Black line inside the box shows the data median, and the bars indicate the smallest (down) and largest (top) non-outlier value. Different letters indicate significant differences ( $P < 0.05$ ) between communities.



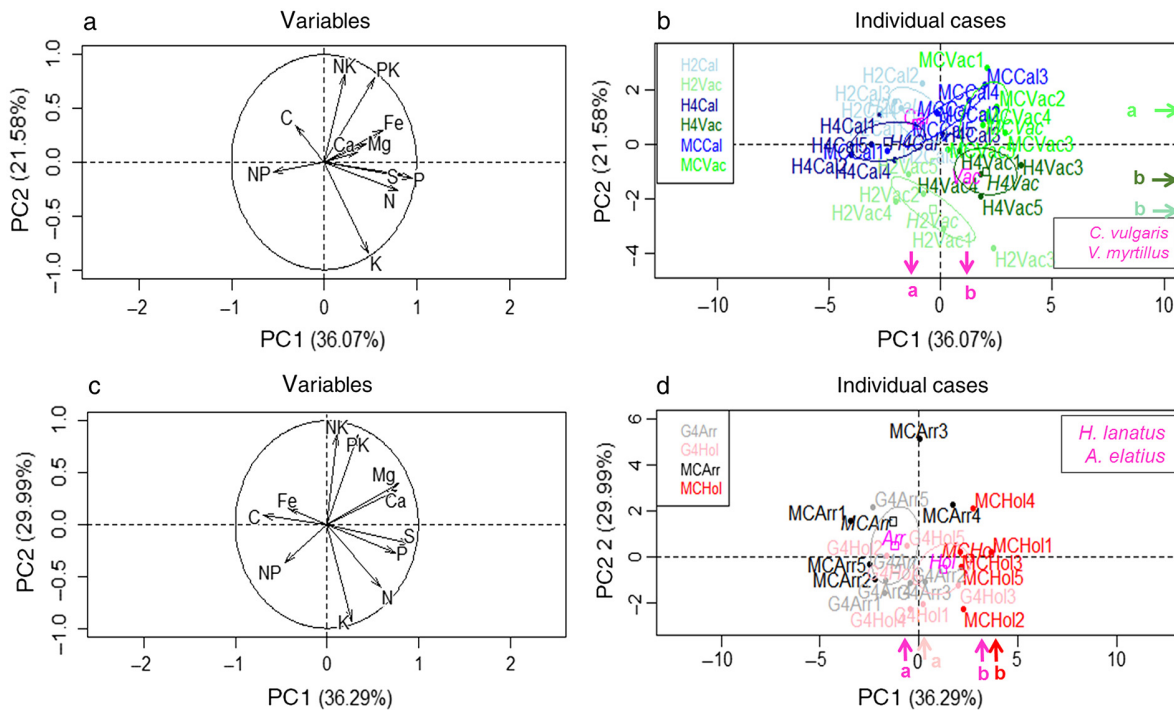


Fig. 6. Principal component analyses of foliar C, N, P, K, Ca, S, Mg, and Fe concentrations and N:P, N:K, and P:K ratios for the shrubs and grass species. (a, c) PC1 vs. PC2 loadings for the included variables. Estimated site scores for *Calluna vulgaris* (Cal) and *Vaccinium myrtillus* (Vac) (b) and *Holcus lanatus* (Hol) and *Arrhenatherum elatius* (Arr) (d) in different communities. Community richness is indicated with MC for the monocultures, H2 for the two-species community, and H4 for the four-species community. Numbers displayed right to the species acronym indicate the replica of each species. Community and species were significantly separated in both analyses. The different species are shown in magenta. Blue color intensity shows different communities for *C. vulgaris*, and green color intensity shows different communities for *V. myrtillus*. Red color intensity shows different communities for *H. lanatus*, and gray color intensity shows different communities for *A. elatius*. Different arrows with distinct letters means significant differences ( $P < 0.05$ ) among the mean scores values on the corresponding axis of the sets of individuals of the same species growing in different communities.

Table 2. ANOVA results for the elemental concentration (C, N, P, K, Ca, Mg, S, and Fe) for the different functional groups studied and the effect of the species and community factor.

Factor	C	N	P	K	Ca	Mg	S	Fe
<b>Shrubs</b>								
Species (Sp.)	<0.01	<0.0001	<0.0001	<0.0001			0.05	<0.001
Community			<0.01	0.05	0.05			<0.01
Sp. × community				<0.001		<0.01		<0.001
<b>Grasses</b>								
Species (Sp.)	<0.001	0.05	0.05		<0.01		<0.0001	<0.01
Community			<0.01				<0.01	<0.01
Sp. × community		0.05	<0.001				<0.001	

Table 3. ANOVA results for the element ratios (C:N, C:P, C:K, N:P, N:K, and P:K) for the different functional groups studied and the effect of the species and community factor.

Factor	C:N	C:P	C:K	N:P	N:K	P:K
<b>Shrubs</b>						
Species (Sp.)	<0.0001	<0.0001	<0.0001			
Community	0.05	<0.01			<0.001	<0.0001
Sp. × community		0.05	<0.001		<0.01	<0.001
<b>Grasses</b>						
Species (Sp.)	0.05	0.05	0.05			<0.01
Community				<0.01	<0.01	<0.001
Sp. × community		<0.01		<0.01		

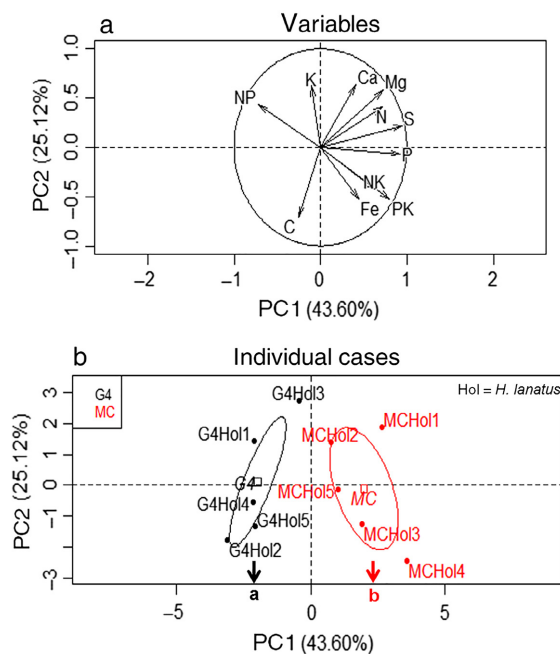


Fig. 7. Principal component analyses of foliar C, N, P, K, Ca, S, Mg, and Fe concentrations and N:P, N:K, and P:K ratios (a) PC1 vs. PC2 loadings for the included variables. (b) Estimated site scores for *Holcus lanatus* (Hol) in different communities. Community richness is indicated with MC for the monocultures, H2 for the two-species community, and H4 for the four-species community. Numbers displayed right to the species acronym indicate the replica, and arrows indicate significant differences.

*Calluna vulgaris* also shifted although only marginally significantly, whereas the BN of *A. elatius* did not shift. The displacement of the BNs indicates differences in the exploitation of soil resources depending on each community

composition. This is in accordance with what Sistla and Schimel (2012) expose as stoichiometric flexibility ecosystem mechanism, where an organism can alter its allometric patterns or tissue chemistry to adjust its overall stoichiometry, which can differ among and within organisms and across trophic groups. Another reasonable explanation could be that stoichiometry changes reflect changes in plant function (e.g., growth, root length, flowering, leaf area index [LAI], and above-ground net primary production) in response to the different abiotic resources available in different communities (Kreyling et al. 2008a, b, Mirzaei et al. 2008, Jentsch et al. 2009). Consistent with the changes in foliar stoichiometry of species depending on community species-specific composition observed in this experiment, some previous studies have observed that species richness and functional group richness affect plant community C:N (Guiz et al. 2016) and C:P and N:P ratios (Abbas et al. 2013). Although these two latter studies were at community and not species level, such as the present study, these results show the link between different species composition and different overall function and the proportional use of different elements.

*Vaccinium myrtillus* foliar K concentration was highest in the community where it was competing with the other dwarf shrub (H2). High foliar K concentration is related to drought resistance strategies, such as high insolation rate and stomatal control (Sawhney and Zelitch 1969, Cakmak 2005, Sardans et al. 2012a, Sardans and Peñuelas 2015). Thus, high foliar K concentration in *V. myrtillus* growing in the H2 community suggests that the coexistence with *C. vulgaris* increased the water stress on this species more than when it grew with grasses, or on its own in

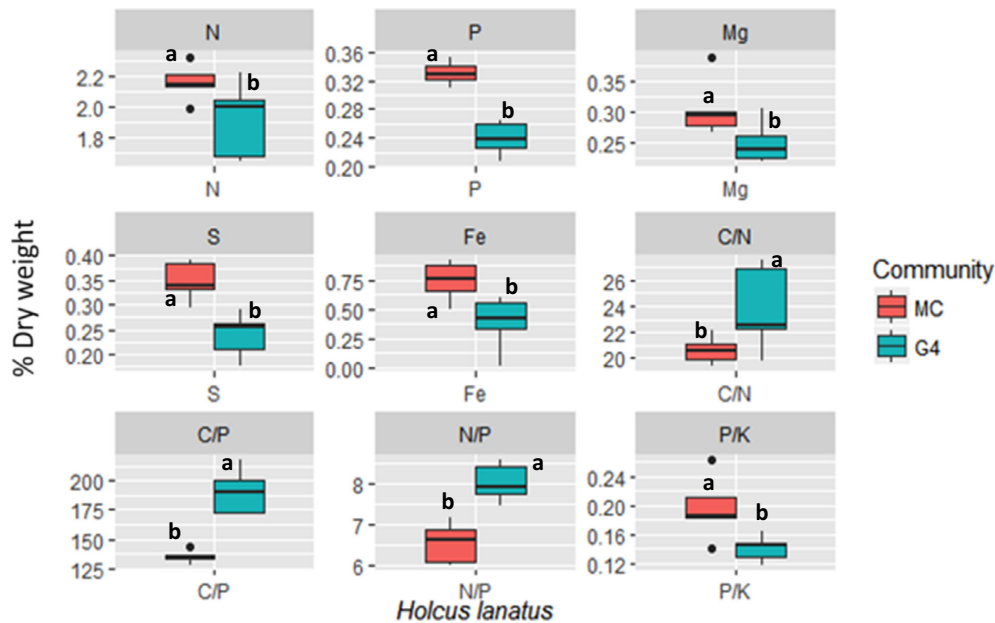


Fig. 8. Foliar elemental concentrations and ratios (mean % dry weight  $\pm$  SE) of *Holcus lanatus* for the different communities. Black line inside the box shows the data median, and the bars indicate the smallest (down) and largest (top) non-outlier value. Different letters indicate significant differences ( $P < 0.05$ ) between communities.

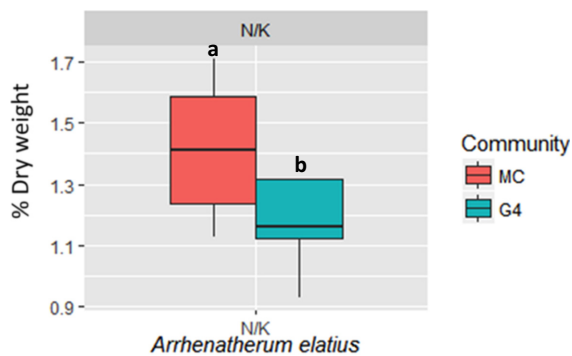


Fig. 9. Foliar elemental concentrations and ratios (mean % dry weight  $\pm$  SE) of *Arrhenatherum elatius* for the different communities. Black line inside the box shows the data median, and the bars indicate the smallest (down) and largest (top) non-outlier value. Different letters indicate significant differences ( $P < 0.05$ ) between communities.

the monoculture. This could have happened because *C. vulgaris* (a shrub) has higher individual biomass than the grass species and therefore demands more water.

*Holcus lanatus* nutrient concentrations were higher in the monoculture than in the mixed stands, suggesting that interspecific competition was detrimental to its nutrient uptake. Foliar element concentrations for *A. elatius* differed very little among the communities. The lack of stoichiometric flexibility in species that did not change their BN indicates a more homeostatic stoichiometry, that is, the ability to maintain a specific elemental composition despite changes in the availability of elements in the surrounding environment (Elser et al. 2010, Yu et al. 2010, Sardans et al. 2012b). This type of stoichiometry could have important implications on the stability and prevalence of some species in changing environments (Sardans et al. 2012b).

Finally, our results provide good examples of the possible stoichiometric responses of several species in different community conditions (displacement of their BN). The compromise between stoichiometric flexibility and homeostasis determined the differential responses of the species. However, this study lacked physiological data that could explain the physiological mechanisms of these shifts. New and more thorough studies

on foliar stoichiometry and community complexity, with a larger number of coexisting species and physiological measurements, are warranted for gaining a better knowledge of the stoichiometry of terrestrial ecosystems.

## CONCLUSION

The study provides evidence that plant stoichiometry responds to changes in plant community composition. The BNs of the species changed in response to the community composition in which they grew, and these changes occurred by displacement of their stoichiometry spectrum. Nevertheless, foliar composition was more homeostatic in *Calluna vulgaris* and especially in *Arrhenatherum elatius*. Regardless of the underlying mechanisms ruling foliar stoichiometry changes, our results suggest that changes in plant composition affect the species foliar stoichiometry. Our results are also a good example of a possible complementary BN hypothesis and highlight the importance of plant stoichiometric flexibility in the ecosystem. We encourage further and deeper studies that contribute to better understanding the relationship between community composition and plant stoichiometry.

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