Nutrient stoichiometry mediates the patchy coexistence of two subalpine grassland types

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Abstract

Background and Aims

High mountain areas often show high soil heterogeneity that allows for the close coexistence of plant species and communities with contrasting resource requirements. This study investigates the nutritional factors driving the mosaic distribution of *Nardus stricta* L. grasslands and chalk grasslands dominated by forbs in the subalpine southern Pyrenees (Spain).

Methods

The concentrations of C, N, P, S, K, Ca and fiber fractions were analyzed in herbage in relation to soil nutrient availability, soil β–glucosidase, urease, phosphatase and arylsulfatase activity and plant species and functional type composition.

Results

The chalk grassland showed higher N:P ratios in herbage and higher enzyme demand for P relative to N in the soil, which indicate a greater limitation of P versus N compared to the *Nardus* grassland. This limitation was related to the higher soil and plant Ca levels in the chalk grassland, where the calcareous bedrock lies close to the soil surface. In the *Nardus* grasslands, the alleviation of P limitation translated into higher productivity and replacement of forbs with taller graminoids rich in structural carbohydrates, which was accompanied by greater β–D–glucosidase activity. The plant N:K and P:K ratios indicated potential K deficiency in both grasslands, which resulted from a decreased uptake of K due to competition from Ca, as indicated by the correlation between plant K and the soil K^{+}:Ca^{2+} ratio.

Conclusions

Our results highlight the effect of the heterogeneity of soil nutrient constraints, as mediated by their stoichiometry and controlled by local topography, on the biodiversity of high mountain ecosystems.

INTRODUCTION

Soil nutrient availability is, along with water limitation, one of the main determinants of the productivity of terrestrial ecosystems (Schlesinger and Bernhardt 2013; Lambers and Oliveira 2019). It also influences the competition between plant species, thereby affecting biodiversity (Tilman 1982; Koerselman and Meuleman 1996). In high mountain (alpine and subalpine) areas, soils are often nutrient-poor because of low temperatures, which results in slow weathering and organic cycling (Wardle 1985; Gardes and Dahlberg 1996), and due to steep relief and freeze–thaw cycles, which make soils more prone to erosion and leaching (Henry 2007; Nie et al. 2013). Moreover, as they are young soils, their properties vary greatly

depending on the local topography and bedrock (Egli and Poulenard 2016; Körner 2021), which typically gives rise to a mosaic–like landscape of distinct vegetation types with different soil preferences (Vonlanthen et al. 2006; Amagai et al. 2018). The occurrence of microhabitats with contrasting soil conditions within relatively short distances is a characteristic of mountainous landscapes that makes them hotspots for biodiversity and priorities for conservation (Körner 2004; Egli and Poulenard 2016).

The calcareous (as opposed to noncalcareous) nature of the bedrock has been identified as a major factor affecting the distribution of plants in high-mountain habitats (Braun–Blanquet and Jenny 1926; Michalet et al. 2002; Buri et al. 2020). Young soils formed from calcareous materials exhibit characteristic properties such as high calcium (Ca) concentration and pH in soil solution (Foth and Ellis 1997). Plants growing on such calcareous soils must respond and adapt to chemical limitations related to soil nutrient availability (Kishchuk 2000; Lambers and Oliveira 2019). The solubility of phosphorus (P), iron (Fe) and other micronutrients may decrease with increased soil pH and Ca (Kishchuk 2000). Furthermore, high pH favors nitrogen (N) conversion to ammonia and subsequent volatilization, which may lead to low N availability (Cameron et al. 2013). Excess Ca may also result in a potassium (K) limitation due to plant uptake competition for cations (Kishchuk 2000). A number of studies have documented P limitation in high mountain calcareous soils (e.g., Köhler et al. 2001; Sebastià 2004; Arnesen et al. 2007; Kaňa et al. 2011; Bhople et al. 2021), although N limitation (Niklaus et al. 1998; Li et al. 2019) or colimitation of N, P and occasionally K (Niinemets and Kull 2005; Bassin et al. 2012) has also been identified. On the other hand, calcareous reliefs are subject to strong erosion by dissolution, often leading to shallow and skeletal soils in which root growth and water storage are limited (Bamberg and Major 1968; Nicklas et al. 2021). Hence, the lower water availability related to calcareous substrates can be equally or even more important than nutrient availability in explaining the floristic composition (Goldin and Nimlos 1977; Badía–Villas et al. 2002; Michalet et al. 2002; Illa et al. 2017).

Plant communities dominated by mat–grass (*Nardus stricta* L.) (hereafter referred to as *Nardus* grasslands) are among the most widespread natural habitats in Europe, being mostly located in mountain areas (Galvánek and Janák 2008). *Nardus* grasslands (especially high mountain types) host a variety of endemic and threatened plant species; as a result, they are identified as priority habitats for conservation under the Habitat Directive of the European Union (6230*, Council Directive 92/43/CEE). They can be regarded as acidophilous, growing often on base–poor soils on siliceous rocks, but can also be found on soils lying on calcareous rocks where the upper layer is decalcified due to high rainfall and landform stability (Galvánek and Janák 2008; Leuschner and Ellenberg 2017). Armas–Herrera et al. (2020) showed that patches of *Nardus* grassland can coexist within a few meters of patches of grasses and herbs that prefer calcareous soils (usually referred to as chalk grassland), resulting in a repeating vegetation mosaic characteristic of certain high–mountain areas of the Pyrenees. This pattern results from differential soil erosion, which creates soil conditions conducive to either chalk grasslands or *Nardus* grasslands (Badía–Villas et al. 2020). The studies by Armas–Herrera et al. (2020) and Badía–Villas et al. (2020) revealed contrasting soil thickness, stoniness, acid/base status (pH, Ca) and organic matter–related properties among patches of *Nardus* and chalk grasslands. However, the precise soil conditions that influence the occurrence of one or the other grassland type, i.e., limitation by one or more nutrients, are still unknown.

The nutrient status of a habitat is typically assessed by the concentrations of nutrients available to plants in the soil, although the nutrient levels in plant tissues can be equally or more informative, as they provide information about the actual nutrient uptake by plants (Motsara and Roy 2008). The elemental composition of each species varies within narrow ranges that reflect their differential use of elements as an adaptation to their usual habitats (He et al. 2006; Sardans and Peñuelas 2014; Roeling et al. 2018), although individuals can deviate somewhat from the species–typical composition in response to local nutrients or competition with coexisting plants (Sistla et al. 2015; Urbina et al. 2017; Guiz et al. 2018). Considerable attention is given to the stoichiometry of elements, as the use of these elements by plants is coupled, which causes plants to require these elements in fixed proportions; plants experience limitations when one is in short supply (Knecht and Göransson 2004; Ågren 2008). The studies by Koerselman and Meuleman (1996) and Gusewell (2004) established critical values for the N:P ratio that have been widely used for the assessment of relative limitations of N or P, which are the most common limiting nutrients (Vitousek and Howarth 1991), on a community level. Other researchers have derived critical ratios involving other major nutrients, such as sulfur (S) (Sumner 1978; Stevens and Watson 1986; Mahot 2005; Ryant and Skládanka 2009) or K (Dampney 1992; Pegtel et al. 1996; Olde Venterik et al. 2003; Lawniczak et al. 2009), to diagnose possible limitations. The focus has been recently placed on soil enzymes responsible for the cycling of organic C, N, P or S and their activities relative to each other. Soil enzymes are produced by soil microorganisms and to a lesser extent by plants in variable amounts depending on their requirements for the different elements and the availability of nutrients in the environment (Allison and Vitousek 2005; Sinsabaugh et al. 2008). Hence, the enzyme stoichiometry, i.e., the ratios of the activities of enzymes involved in the release of C, N, P and S mirror the relative demand for each enzyme and can be used as an indicator of nutrient limitations (Sinsabaugh et al. 2008; Luo et al. 2017; Cui et al. 2021).

The aim of the present study is to determine whether the patchy coexistence of *Nardus* and chalk grasslands in the Pyrenees reflects the spatially heterogeneous in nutrient limitations and, if this is the case, what nutrients may be limiting for each vegetation type. To this aim, the nutrient status of the two vegetation types was assessed by the chemical composition of the herbage and the potential activities of soil enzymes from the cycles of C, N, P and S and was related to the availability of nutrients in the soil and the plant species composition. Our hypothesis was that different degrees of soil decalcification under the patches of *Nardus* and chalk grasslands may lead to differing limitations of N, P, S and/or K.

MATERIALS AND METHODS

Study area and field work

The study was conducted in the locality of La Estiva de Fanlo, near the National Park of Ordesa and Monte Perdido in the southern Central Pyrenees (province of Huesca, Spain). La Estiva is a summer grazing area located at altitudes from 1,700 to 1,900 m a.s.l. in the subalpine belt of Monte Perdido, the highest calcareous mountain in Europe (3,355 m.a.s.l.). According to the data from the nearest meteorological

station (Online Resource 1), the mean annual temperature is approximately 5°C, and the annual precipitation is approximately 1,700 mm, with snow precipitation from October to May.

The study area has an area of approximately 250 ha and a topography consisting of convex–concave slopes. The bedrock consists of bioclastic limestone with silex from the Ilerdian (early Miocene), which in the middle, and some of the foot slopes are buried beneath a cover of sediments eroded from the rocks higher in the slopes consisting of limestone, marls, marlstones and lutites, also from the Ilerdian. These sediments are affected by uneven erosion, resulting in a mosaic of slightly raised areas with Orthoeutric Cambisols as the prevailing soil type and lower ground areas where Hypereutric Leptosols are predominant. In this mosaic of soil conditions, two different types of vegetation are found (Fig. 1): *Nardus* grassland growing on the Cambisols in soil accumulation areas and chalk grassland on the Leptosols at the bottom of such areas (Badía–Villas et al. 2020).

Nardus grassland belongs to the phytosociological alliance *Nardion strictae* Br.–Bl. 1926. At the time of sampling in early summer, it was characterized by a dominance of *N. stricta* accompanied by *Campanula scheuchzeri* Vill., *Eryngium bourgatii* Gouan, *Potentilla erecta* (L.) Raeusch., *Ranunculus amplexicaulis* L. and *Trifolium alpinum* L. The chalk grassland is made up of a mosaic of species characteristic by three communities (phytosociological alliances): *Bromion erecti* Koch 1926 (dominant), *Festucion gautieri* Br.–Bl. 1948 and *Primulion intricatae* Br.–Bl. *ex* Vigo 1972. Similar to the *Nardus* grassland, the chalk grassland has a coverage close to 100% but with a remarkable diversity of grasses, legumes and other species. The study area has a livestock density of approximately one large animal unit (LAU) per hectare comprising approximately 200 cows grazing from July to October and sheep flocks remaining during the autumn until the first snowfall (Armas–Herrera et al. 2020). In general, the livestock spends more time in the chalk grassland than in the *Nardus* grassland due to the lower fodder quality of the latter (García–González et al. 1990; Badía et al. 2008).

Field work was performed in early July 2018. Six plots were chosen at intervals of 100 m along a 600 m transect placed at mid-slope. In each plot, two sampling points were located at a distance no greater than 3 m in adjacent patches of *Nardus* grassland and chalk grassland. At each sampling point, a quadrat of 1 x 1 m was placed where the plant species were identified, and their coverages were visually assessed on a percent scale. The plant species were categorized by family into three functional groups: legumes (Fabaceae), graminoids (Poaceae and Cyperaceae), and forbs (all other herbaceous species), and the coverage for each group was calculated by summing the coverage values of all species in that group. All the herbage in the quadrat was clipped at ground level using hand shears and collected. Three soil subsamples were collected from the top 10 cm of the soil and mixed to form one composite sample per sampling point. In total, 12 samples of vegetation and soil were collected (6 plots x 2 types of patches). From the soil samples, a subset was selected for biochemical analysis comprising samples from points located at intervals of 200 m along the transect (3 plots x 2 types of patches = 6 samples).

Laboratory procedures

The herbage was weighed before and after drying in a forced–air drier at 60°C for 48 h to determine the dry matter content and the dry biomass per unit area, ground to < 1 mm using a cutting mill (Retsch Mühle, Haan, Germany) and stored at room temperature until analysis. The concentrations of C, N and S were determined using a CNS elemental analyzer (Vario MAX CNS, Hanau, Germany). The concentrations of the other elements were determined after ashing at 550°C and dissolution in aqua regia: P by the molybdate–blue method (Murphy and Riley 1962), Ca by complexometry with EDTA and K by flame spectrophotometry. Based on the concentrations of C, N, P, S, K and Ca, the following mass ratios were calculated: C:N, C:P, C:S, C:K, C:Ca, N:P, N:S, N:K, N:Ca, P:S, P:K, P:K, S:K and S:Ca.

The fiber composition of the herbage was analyzed with an Ankom 200 Fiber Analyzer (Ankom Technol., Fairport NY, USA) according to the method of Van Soest et al. (1991). This procedure sequentially removes the cell contents, hemicellose and cellulose, yielding neutral detergent fiber (NDF), acid detergent fiber (ADF) and an acid–insoluble residue, respectively, which is then ashed at 550°C to remove the acid–detergent lignin (ADL) and obtain the acid–detergent ash (ADA). The contents of ADL, cellulose and hemicellulose were then calculated as the differences between the insoluble residue and ADA, ADF and ADL, and NDF and ADF, respectively. The herbage was also analyzed for the concentrations of lipids (ether extract) by a 12–h Soxhlet extraction with petroleum ether and for the total concentrations of minerals (crude ash) by ashing at 550°C (AOAC 2006). The acid–soluble ash (ASA) fraction was calculated by subtracting the ADA from the crude ash content. The concentration of crude protein was calculated by multiplying the N concentration by 6.25 (AOAC 2006). The concentration of nonstructural carbohydrates (NSC) was assessed by the following calculation: NSC (g kg^{-1}) = 1000 – [crude ash + ether extract + crude protein + hemicellulose + cellulose + ADL] (g kg^{-1}). The water content was determined by drying a subsample in an oven at 103°C to a constant weight. The results of the analyses were corrected for the water and ash contents to express them on an ash–free oven–dry weight basis.

The soil samples were air–dried, sieved to < 2 mm and stored at room temperature until analysis, except for a portion that was sieved while fresh and stored at 4°C for determination of ammonium–N, microbial biomass C and enzyme activities. Soil pH was measured in 1:2.5 soil:water and soil:1 M potassium chloride suspensions, and the electrical conductivity ($EC_{1:5}$) was measured in 1:5 soil:water extracts. Exchangeable cations (Ca, Mg, K, Na) were extracted using ammonium acetate (pH 7.0) and determined by atomic absorption spectrometry (Ca, Mg) and flame photometry (K, Na). The cation exchange capacity was determined by sodium acetate saturation (pH 8.2) and Na determination by flame photometry. The concentrations of C and N were determined using a CNS elemental analyzer (Vario MAX CNS, Hanau, Germany). Ammonium–N was extracted with a 2 M potassium chloride solution and determined with steam distillation through the magnesium oxide–Devarda alloy method (Bremner 1965). Plant–available P (Olsen–P) was extracted using a sodium hydrogen carbonate solution (pH 8.5) and then measured colorimetrically (Murphy and Riley 1962; Watanabe and Olsen 1965). Sulfate–S was extracted using a 1:5 soil:water ratio and determined by turbidimetry as described by AOAC (2006). Soil C:N and K^+:Ca^{2+} ratios were calculated based on the total contents of C and N and the exchangeable contents of K^+ and Ca^{2+}, respectively.

Soil microbial biomass C (MBC) was determined by the fumigation–extraction method (Vance et al. 1987). Four soil enzymes were assayed: β–D–glucosidase (GLU) by the method of Eivazi and Tabatabai (1988), urease (URE) by the method of Tabatabai and Bremner (1972), acid phosphatase (PHO) following Saá et al. (1993) and arylsulfatase (SUL) by the method of Tabatabai and Bremner (1970), each involved in the cycling of C, N, P and S, respectively. These enzymes are widely distributed in nature and extensively used for the assessment of soil nutrient cycling and availability (Karaca et al. 2010; Adetunji et al. 2017). The enzyme activities were expressed as total activities per dry soil unit. From the values of the enzyme activities, the GLU:URE, GLU:PHO, GLU:SUL, URE:PHO, URE:SUL and PHO:SUL ratios were calculated, hereafter termed the C:N, C:P, C:S, N:P, N:S and P:S enzyme ratios.

Statistical analysis

Univariate and bivariate tests were performed using SPSS for Windows version 22 (IBM Corporation, Armonk NY, USA). The composition values of the herbage and the soil in the *Nardus* grassland and chalk grassland were compared using Student's t tests after verifying the assumptions of normality and homogeneity of variance using Kolmogorov–Smirnov and Levene tests, respectively. When the assumptions were not confirmed, appropriate data transformations (cubic, square, square root, natural log, inverse square root, inverse or inverse square) were applied. For ease of interpretation, tables and figures present non–transformed data. The soil biochemical parameters were compared using Mann–Whitney U tests because the normality and homogeneity of variance could not be reliably tested due to the low number of samples. Correlations between selected variables were analyzed by Pearson's test.

Multivariate analyses were performed using CANOCO v. 4.5 (Microcomputer, Ithaca NY, USA) to explore the relationships within and between the various datasets. A principal component analysis (PCA) was performed to examine the variation in the chemical composition of herbage among the *Nardus* and chalk grasslands, and the obtained principal components were then used as explanatory variables in a canonical correspondence analysis (CCA) of the plant species data in relation to the herbage chemical composition. In a similar manner, a PCA was applied to the soil variables to identify the main soil gradients, and then the principal components obtained were used in a redundancy analysis (RDA) of the chemical composition of the herbage in relation to soil factors. RDA was also used to analyze the relationships of the elemental stoichiometry of herbage with the soil enzyme stoichiometry. In the CCA and RDA, the most suitable explanatory variables were selected by a forward stepwise procedure based on Monte Carlo tests using 999 permutations and a threshold *P*–value of 0.10. Additionally, in both analyses, statistical significance was tested for the first canonical axis and for all the canonical axes using Monte Carlo tests with 999 permutations.

RESULTS

Plant chemical composition and its relation to species composition

The herbage of the *Nardus* grassland (Table 1) was richer in fiber (NDF, ADF) and particularly in structural carbohydrates (CEL, HEM) but was lower in ADL and NSC than the herbage of the chalk grassland. Both vegetation types showed similar concentrations of C, N, S and K but differed significantly with respect to P, which was greater in the *Nardus* grassland, and Ca, which was greater in the chalk grassland. With regard to elemental stoichiometry (Table 2), the two vegetation types differed mainly in the ratios of Ca and P among them and in other elements: the *Nardus* grassland had greater ratios of P to C, N, K and Ca than the chalk grassland, which in turn had greater ratios of Ca to C, P, S and K.

Table 1
Descriptive statistics and comparison (*t*–tests) of the chemical composition of the herbage of the *Nardus* and chalk grasslands

	Nardus grassland (n = 6)				Chalk grassland (n = 6)				t_{10}	Sig.
	Mean	SE	Min.	Max.	Mean	SE	Min.	Max.		
Biomass (kg dry matter ha^{-1})	**1671**	149	1316	2324	**931**	100	704	1348	4.53	**
NDF (g kg^{-1} dry matter)	**533**	22	472	629	**417**	18	372	486	4.16	**
ADF (g kg^{-1} dry matter)	**274**	3	262	285	**235**	10	204	265	4.32	**
ADL (g kg^{-1} dry matter)	**36.3**	2.1	26.4	40.1	**68.4**	9.5	42.9	105	–4.07	**
CEL (g kg^{-1} dry matter)	**238**	4	225	252	**166**	8	150	204	7.90	***
HEM (g kg^{-1} dry matter)	**259**	21	196	350	**182**	13	150	239	3.07	*
NSC (g kg^{-1} dry matter)	**234**	24	137	301	**314**	22	217	359	–2.46	*
ADA (g kg^{-1} dry matter)	**2.47**	0.48	1.07	4.40	**8.10**	2.27	3.54	18.7	–1.88	#
ASA (g kg^{-1} dry matter)	**73.3**	8.1	42.3	99.6	**90.2**	3.7	80.7	107	–2.31	ns
Crude ash (g kg^{-1} dry matter)	**75.8**	8.3	44.4	102.8	**98.3**	5.9	84.2	126	–2.21	#
Crude protein (g kg^{-1} dry matter)	**133**	8	120	167	**148**	8	116	175	–1.32	ns
Ether extract (g kg^{-1} dry matter)	**24.1**	0.7	22.2	26.3	**23.6**	0.6	21.7	26.1	0.49	ns
C (g kg^{-1} dry matter)	**473**	3	463	481	**476**	5	456	492	–0.46	ns
N (g kg^{-1} dry matter)	**21.3**	1.2	19.1	26.6	**23.6**	1.3	18.6	28.1	–1.32	ns
P (g kg^{-1} dry matter)	**1.64**	0.16	1.17	2.21	**1.26**	0.06	1.07	1.44	2.30	*

	Nardus grassland (n = 6)				Chalk grassland (n = 6)				t_{10}	Sig.
	Mean	SE	Min.	Max.	Mean	SE	Min.	Max.		
S (g kg^{-1} dry matter)	**2.18**	0.13	1.78	2.58	**2.07**	0.11	1.92	2.60	0.67	Ns
K (g kg^{-1} dry matter)	**1.16**	0.12	0.78	1.56	**1.25**	0.09	0.96	1.59	– 0.58	Ns
Ca (g kg^{-1} dry matter)	**10.5**	1.3	4.7	13.7	**16.3**	1.1	13.2	19.9	– 3.35	**

SE = standard error of the mean, Min. = minimum, Max. = maximum, t_{10} = Student's *t* value with 10 degrees of freedom, Sig. = significance, ns = not significant, # = $P < 0.10$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. NDF = neutral detergent fiber, ADF = acid detergent fiber, ADL = acid detergent lignin, CEL = cellulose, HEM = hemicellulose, NSC = non–structural carbohydrates, ADA = acid detergent ash, ASA = acid–soluble ash.

Table 2
Descriptive statistics and comparison (*t*–tests) of the mass ratios of major elements (C, N, P, S, K and Ca) in the herbage of the studied *Nardus* and chalk grasslands

	Nardus grassland (n = 6)				Chalk grassland (n = 6)				t_{10}	Sig.	Critical values
	Mean	SE	Min.	Max.	Mean	SE	Min.	Max.			
C:N	22.5	1.1	18.0	25.1	20.5	1.4	16.5	26.5	1.10	ns	
C:P	302	31	209	411	383	21	324	458	2.23	*	
C:S	220	13	179	268	233	11	179	256	–0.67	ns	
C:K	434	50	303	615	394	32	292	501	0.68	ns	
C:Ca	51.2	10.3	33.7	101.3	29.9	2.1	24.0	35.6	2.66	*	
N:P	13.4	1.2	8.8	16.4	18.9	1.2	17.2	24.3	–2.77	*	10–20[1] 14–16[2] 14.5[3]
N:S	9.8	0.6	7.6	11.4	11.4	0.5	9.7	13.0	–2.10	#	13–17[4] 14[5]
											16[6]
N:K	19.7	3.1	14.1	34.2	19.3	1.3	15.2	23.8	0.13	ns	2.1[3] 1.3[7] 1.2[8] 1.75[9]
N:Ca	2.41	0.66	1.42	5.63	1.49	0.14	1.14	2.12	–1.98	#	
P: S	0.748	0.039	0.582	0.858	0.617	0.047	0.445	0.745	2.21	#	
P:K	1.48	0.18	1.12	2.23	1.04	0.09	0.73	1.38	2.33	*	
P:Ca	0.178	0.040	0.111	0.368	0.079	0.005	0.057	0.094	–4.61	**	
S:K	1.99	0.24	1.46	2.99	1.69	0.11	1.34	2.12	1.12	ns	

	Nardus grassland (n = 6)				Chalk grassland (n = 6)				t_{10}	Sig.	Critical values
	Mean	SE	Min.	Max.	Mean	SE	Min.	Max.			
S:Ca	0.238	0.053	0.150	0.492	0.131	0.014	0.100	0.196	– 3.03	**	
K:Ca	0.117	0.015	0.086	0.165	0.079	0.010	0.054	0.120	– 2.38	*	

[1]Güsewell (2004), [2]Koerselman and Meuleman (1996), [3]Olde Venterik et al. (2003), [4]Mahot et al. (2005), [5]Stevens and Watson (1986), [6]Ryant and Skládanka (2009), [7]Dampney (1992), [8]Pegtel et al. (1996), [9]Lawniczak et al. (2009).

SE = standard error of the mean, Min. = minimum, Max. = maximum, t_{10} = Student's *t* value with 10 degrees of freedom, Sig. = significance, ns = not significant, # = $P < 0.10$, * = $P < 0.05$, ** = $P < 0.01$.

The variation in herbage composition was summarized by PCA into four components (termed Composition Factors 1, 2, 3 and 4) with a total explained variance of 87%. Figure 2 depicts the PCA scores for the chemical components and samples, with the cover of the plant functional types displayed as supplementary variables. Composition Factor 1 had large negative loadings for the concentrations of HEM, CEL, P and S and positive loadings for ADL, NSC and Ca. As seen in Fig. 2, Composition Factor 1 was strongly negatively correlated ($P < 0.001$) with the proportion of graminoids in the vegetation and tended to separate the samples of *Nardus* grassland, located mostly on the negative side, from those of chalk grassland, positioned on the positive side. Composition Factor 2 had large positive loadings for soluble minerals (ASA) and K and negative loadings for C. Composition Factor 3 had its main loading in the concentration of N (or crude protein) and was strongly correlated ($P < 0.001$) with the proportion of legumes in the vegetation, whereas Composition Factor 4 had high positive loading for ether extracts.

The plant species and functional groups of the *Nardus* and chalk grasslands are summarized in Online Resources 2 and 3. The CCA of the plant species data in relation to herbage chemical composition revealed that Composition Factors 1 and Factor 3 were related ($P < 0.001$ and < 0.10, respectively) to species composition, but Composition Factors 2 and 4 added little to the explained variance ($P > 0.10$) and were not added to the ordination model. This ordination was found to be significant for both the first canonical axis (eigenvalue = 0.53, F = 2.81, $P < 0.001$) and all the canonical axes (trace = 0.72, F = 2.17, $P < 0.001$). Figure 3 depicts the CCA scores for species and sites and the vectors for composition Factors 1 and 3. The CCA reconfirmed the relationship between herbage chemical composition and plant functional type at the level of individual species. Graminoid species tend to locate closer to the vector of Composition Factor 1 than forbs or legumes, which indicates that the greater proportion of graminoids is associated with larger concentrations of cellulose, hemicellulose, P and S and lower concentrations of Ca, ADL and NSC. In turn, legume species are associated with greater concentrations of protein–N and are therefore displayed closer to Composition Factor 3 than forbs or graminoids.

Soil properties and their relation to plant chemical composition

The soils of the *Nardus* grassland were significantly more acidic, richer in exchangeable Mg^{2+} and ammonium–N and poorer in exchangeable Ca^{2+} and Na^{+} than the soils of the chalk grassland (Table 3). The PCA summarized the variation in soil properties into three axial components (termed soil Factors 1, 2 and 3), explaining 89% of the variability. Soil Factor 1 had large positive loadings for pH, Ca^{2+} and Na^{+} and negative loadings for Mg^{2+} and ammonium–N and was interpreted as a debasification gradient that separates the soils of the *Nardus* grassland from those of the chalk grassland (Fig. 4). Soil Factor 2 was positively related to characteristics related to organic matter (organic C, total N, CEC) and exchangeable K. Soil Factor 3 was positively related to the amounts of soluble ions (electric conductivity, sulfate–S).

Table 3
Descriptive statistics and comparison (*t*–tests) of the soil characteristics of the *Nardus* and chalk grasslands

	***Nardus* grassland (n = 6)**				**Chalk grassland (n = 6)**				t_{10}	**Sig.**
	Mean	**SE**	**Min.**	**Max.**	**Mean**	**SE**	**Min.**	**Max.**		
pH–water	**5.18**	0.10	4.74	5.36	**6.28**	0.23	5.53	7.01	–4.41	**
pH–KCl	**4.31**	0.13	3.78	4.68	**5.73**	0.30	4.78	6.66	–4.29	**
$EC_{1:5}$ (µS cm^{-1})	**135**	10	111	169	**129**	17	75	193	0.29	Ns
Na^+ ($cmol_c$ kg^{-1})	**0.03**	0.00	0.02	0.04	**0.04**	0.00	0.03	0.04	–2.73	*
Mg^{2+} ($cmol_c$ kg^{-1})	**1.05**	0.13	0.75	1.58	**0.40**	0.08	0.26	0.80	4.32	**
K^+ ($cmol_c$ kg^{-1})	**0.26**	0.03	0.19	0.36	**0.24**	0.03	0.15	0.32	0.66	Ns
Ca^{2+} ($cmol_c$ kg^{-1})	**4.78**	0.53	2.68	6.37	**9.92**	1.43	6.13	14.75	–3.36	*
K^+:Ca^{2+} ratio	**0.063**	0.015	0.031	0.135	**0.025**	0.002	0.020	0.034	4.67	**
CEC ($cmol_c$ kg^{-1})	**22.3**	1.6	18.9	29.0	**20.5**	2.2	13.6	27.1	0.65	Ns
Base saturation (%)	**28.7**	3.9	13.2	39.4	**51.6**	4.1	37.2	63.3	–4.05	**
Organic C (g kg^{-1})	**66.3**	6.5	49.3	88.7	**52.2**	10.0	20.5	78.2	1.19	Ns
Total N (g kg^{-1})	**5.65**	0.59	4.23	8.00	**5.06**	0.86	2.10	6.96	0.56	Ns
C:N ratio	**11.8**	0.2	11.1	12.7	**10.1**	0.3	9.2	11.3	4.34	**
Ammonium–N (mg kg^{-1})	**0.24**	0.01	0.22	0.26	**0.12**	0.01	0.11	0.14	7.82	**
Olsen–P (mg kg^{-1})	**2.79**	0.35	1.31	3.54	**2.99**	0.54	1.32	4.76	–.31	Ns

	Nardus grassland (n = 6)				Chalk grassland (n = 6)				t_{10}	Sig.
	Mean	**SE**	**Min.**	**Max.**	**Mean**	**SE**	**Min.**	**Max.**		
Sulfate–S (mg kg^{-1})	**77.5**	6.1	55.4	99.5	**55.3**	11.1	30.3	97.6	1.75	Ns

SE = standard error of the mean, Min. = minimum, Max. = maximum, t_{10} = Student's *t* value with 10 degrees of freedom, Sig. = significance, ns = not significant, # = $P < 0.10$, * = $P < 0.05$, ** = $P < 0.01$. $EC_{1:5}$ = electric conductivity at a 1:5 soil:water ratio, CEC = cation exchange capacity.

The RDA of the herbage chemical composition in relation to soil factors revealed a very significant effect ($P < 0.001$) of soil Factor 1, whereas the addition of soil Factors 2 and 3 spatial variables did not provide additional explanatory power ($P > 0.10$). The ordination based only on soil Factor 1 was statistically significant (trace = 0.31, F = 4.54, $P < 0.01$), explaining 31% of the variance in the chemical composition of the herbage. A biplot of the results of this RDA is presented in Fig. 5, which shows the herbage becoming leaner in Ca, ADL and NSC and richer in structural carbohydrates, P and S as the soil undergoes debasification with a shift from chalk to *Nardus* grassland.

Despite the overall relationship between the herbage chemical composition and soil properties, the levels of the individual nutrients in herbage showed little relation to those of the same nutrients in the soil. Only the herbage Ca was positively correlated with soil exchangeable Ca^{2+} (r = 0.70, $P < 0.05$), whereas the herbage levels of N, P, S and K were unrelated to their plant-available concentrations in the soil (r = − 0.47 to 0.01, $P > 0.10$). The herbage concentration of K was found to correlate positively (r = 0.58, $P < 0.05$) with the soil K^+/Ca^{2+} ratio but not with the soil K^+ or Ca^{2+} concentrations.

Soil biological activity and its relation to plant chemical composition

The soils of the *Nardus* grassland had larger microbial biomass C and higher potential activities for most of the enzymes examined, except for SUL, which showed no significant difference ($P > 0.05$, Mann–Whitney U test) (Table 4). Analysis of enzyme stoichiometry demonstrated significant differences only for the enzyme C:N ratio, which was higher for the chalk grassland than for the *Nardus* grassland, suggesting a larger enzymatic demand for N versus C in the *Nardus* grassland relative to the chalk grassland.

Table 4
Descriptive statistics and comparison (Mann–Whitney U tests) of the soil biochemical properties and enzyme stoichiometry of the studied *Nardus* and chalk grasslands

	Nardus grassland (n = 3)				Chalk grassland (n = 3)				U	Sig.
	Mean	SE	Min.	Max.	Mean	SE	Min.	Max.		
MBC (g kg^{-1})	1.31	0.02	1.28	1.35	1.00	0.16	0.68	1.21	0	*
GLU (μmol PNP g^{-1} soil h^{-1})	6.08	0.51	5.48	7.10	3.88	0.24	3.43	4.24	0	*
URE (μmol NH_3 g^{-1} soil h^{-1})	0.37	0.02	0.33	0.41	0.17	0.02	0.15	0.21	0	*
PHO (μmol PNP g^{-1} soil h^{-1})	14.0	1.4	12.0	16.7	8.5	0.7	7.6	9.9	0	*
SUL (μmol PNP g^{-1} soil h^{-1})	8.52	0.47	7.87	9.43	6.17	1.11	4.51	8.27	2	ns
Enzyme C:N ratio	16.6	0.8	15.1	17.4	23.3	1.9	20.2	26.8	0	*
Enzyme C:P ratio	0.43	0.01	0.42	0.46	0.46	0.04	0.40	0.53	4	ns
Enzyme C:S ratio	0.72	0.09	0.60	0.90	0.67	0.14	0.48	0.94	3	ns
Enzyme N:P ratio	0.026	0.002	0.024	0.030	0.020	0.003	0.015	0.026	2	ns
Enzyme N:S ratio	0.044	0.005	0.035	0.052	0.030	0.009	0.018	0.047	2	ns
Enzyme P:S ratio	1.67	0.23	1.42	2.12	1.43	0.18	1.20	1.78	2	ns

SE = standard error of the mean, Min. = minimum, Max. = maximum, U = Mann–Whitney U value, Sig. = significance, ns = not significant, * = $P \leq 0.05$, MBC = microbial biomass C, GLU = β–D–glucosidase activity, PNP = *p*–nitrophenol, URE = urease activity, PHO = acid phosphatase activity, SUL = arylsulfatase activity.

GLU was found to be related to the fiber composition of the herbage, increasing with increasing levels of cellulose (r = 0.87, $P < 0.05$, Pearson's correlation) and hemicellulose (r = 0.96, $P < 0.01$) and decreasing levels of NSC (r = − 0.94, $P < 0.01$) and ADL (r = − 0.83, $P < 0.05$). GLU was also correlated with the quality of soil organic matter as measured by the soil C:N ratio (r = 0.87, $P < 0.05$). In turn, SUL and URE exhibited highly significant positive correlations with the soil concentrations of their products sulfate–S and ammonium–N (r = 0.98 and r = 0.93, respectively, $P < 0.01$), but PHO showed no correlation with Olsen–P (r = 0.24, $P > 0.05$). The enzyme ratios of C, N, P and S showed no correlation with the ratios among the same elements in herbage, with an important exception being the N:P ratios, for which a negative correlation was found between the herbage and the soil enzyme values (r = − 0.91, $P < 0.05$). According to this correlation, as the N:P ratio increased in herbage (indicating increasing limitation by P rather than N), the enzyme N:P ratio decreased (indicating increasing enzymatic demand for P relative to N) (Fig. 6).

Figure 7 shows the ordination diagram of the RDA of the enzyme ratios of C, N, P and S with respect to the stoichiometry of these elements in the herbage. The N:P ratio was the major and only significant ($P < 0.01$) elemental ratio of herbage influencing the soil enzyme stoichiometry. Using the herbage N:P as a single explanatory variable, a significant ordination (trace = 0.70, F = 9.47, $P < 0.01$) was obtained that explained 70.3% of the variance in enzyme stoichiometry. The first canonical axis of the RDA separated the samples of the *Nardus* grassland, which formed a tight cluster on its negative side, from those of the chalk grassland, which were scattered toward the positive side. The enzyme N:P ratio increased along this canonical axis with decreasing herbage N:P ratio, mirroring the increasing demand for P relative to N from chalk to *Nardus* grassland. Additionally, along this axis, the enzyme ratios of C to N, P and S decreased, suggesting a greater demand for these nutrients relative to C, and the enzyme ratios of C, N and P to S decreased, suggesting a substantially greater demand for S in the *Nardus* grassland relative to the chalk grassland.

DISCUSSION

Nutrient limitations inferred from elemental stoichiometry

Nardus and chalk grasslands are both considered oligotrophic environments with low levels of available N and P (Smith 1980; Schelfhout et al. 2017). In the present study, the mean ratios of N to P in both grassland types were within the range of 10–20, within which, according to Güsewell (2004), no clear limitation by N or P occurs but a co–limitation by both nutrients is observed. However, according to the criteria of Koerselman and Meuleman (1996), the N:P ratio of the herbage of the chalk grassland was above the critical value of 16, which indicates a relative limitation by P, whereas the average N:P ratio for the *Nardus* grassland was slightly below the critical value of 14, indicating a limitation by N. Other studies conducted in other European mountain areas (Kirkham 2001; Klaudisová et al. 2009; Busqué and Bedía 2013) yielded mean N:P ratios of 12–14 for *N. stricta* and *Nardus* grasslands that are similar to those of the *Nardus* grassland in the present study and indicate limitation or co–limitation by N. Conversely, Bobbink et al. (1989) reported N:P values of 14–18 and above for *Bromion* chalk grasslands, which indicate limitation or co–limitation by P.

Moreover, the soil enzyme C:P ratio was < 1 in both the *Nardus* and chalk grasslands, indicating that more effort was directed at acquiring and cycling P relative to processing C, which suggests a P deficiency in both grassland types (Liu et al. 2020; Lasota et al. 2022). Phosphorus limitation can occur in high-mountain vegetation even with N:P ratios < 10 (Wang et al. 2017) because of the high demand for P, which is used by plants to protect tissues and membranes against the cold (Marschner 2012). Both the *Nardus* and chalk grasslands are regarded as adapted to P-limited or colimited habitats because of the efficient P uptake and use strategies of their dominant species (Köhler et al. 2001; Van der Krift and Berendse 2002; Hejcman et al. 2014). Consequently, they have been shown to spread with decreasing P (Klaudisová et al. 2009) or increasing N availability (Bobbink et al. 1989; Wilson et al. 1995; Leith et al. 1999; Stevens et al. 2011). The elemental ratios in the present study indicate stronger P versus N limitation in the chalk grassland than in the *Nardus* grassland. This is also supported by the finding in the previous study by

Badía–Villas et al. (2020) of greater ^{15}N fractionation in chalk grasslands compared to *Nardus* grasslands, which suggests a relative excess of N over other more limiting nutrients in the chalk grassland (Xu et al. 2014).

The N:S ratios of the herbage were below the critical values established by Stevens and Watson (1986), Mahot (2005) and Ryant and Skládanka (2009), which indicates a sufficiency of S in both vegetation types. In contrast, the N:K and P:K ratios were well above the various critical values proposed in other studies (Dampney 1992; Pegtel et al. 1996; Olde Venterik et al. 2003; Lawniczak et al. 2009), which suggests a strong limitation by K in both grassland types.

Nutrient uptake as affected by nutrient availability in soil

According to customary ranges for soil nutrients (Jones 2001; Horneck et al. 2011), Olsen–P and ammonium–N were low, sulfate–S was high and exchangeable K^+ was low to moderate in the soils of both grassland types, whereas exchangeable Ca^{2+} varied from low–moderate in the *Nardus* grassland to moderate–high in the chalk grassland. Only ammonium–N and exchangeable Ca^{2+} showed significant differences between the two grassland types.

Ammonium–N was higher in the *Nardus* grassland, with values similar to those reported by Badía et al. (2008) for other *Nardus* grasslands in the same region. Higher ammonium can result from more active N cycling (Zhou et al. 2022), as suggested by the correlation between ammonium–N and URE, and from the more acidic conditions in the *Nardus* grassland, which mitigate volatilization losses (Woodmanse et al. 1981; Cameron et al. 2013).

Calcium was the only element among the nutrients studied that showed a significant correlation between its available concentration in the soil and its levels in the herbage. The concentrations of exchangeable Ca^{2+} in the soils of both grasslands were equal to or above the range of 1–3 $cmol_c\ kg^{-1}$ typical for ‘calcicole’ plant species and well above the value of 0.5 $cmol_c\ kg^{-1}$ below which ‘calcifuge’ species usually occur (Cross and Lambers 2021). Calcareous soils usually exhibit Ca levels that exceed the demand for this element by any plant species but can lead to stress due to a deficiency of other nutrients (Arnesen et al. 2007; Körner, 2021). According to our findings, the high availability of Ca adversely affected P intake, as shown by the decrease in plant P with increasing plant Ca and soil Ca. In the chalk grassland, high Ca resulted in greater limitation by P than by N, as can be inferred from the values of the N:P ratio of the herbage, and led to higher enzyme demand for P relative to N compared to the *Nardus* grassland (Fig. 6). Conversely, in the *Nardus* grassland, decalcification somewhat alleviated P limitation, which, according to the N:P ratios, shifted to N and P colimitation or even N limitation at some sites and increased the enzyme demand for N relative to P (Fig. 6). Contrary to other studies on *Nardus* grasslands (Kirkham, 2001; Klaudisová et al. 2009), the herbage P concentrations were not related to the soil available P, which may occur in calcareous P-limited grasslands because P acquisition depends more on P mobilization by root exudates than on uptake of soluble inorganic P (Klaus et al. 2016).

The results of the RDA (Fig. 7) also suggest that the enzyme demand for S relative to C, N and P may increase as the enzyme demand for P versus N decreases. Enhanced productivity resulting from sufficient P availability might explain a stronger demand for S, which is consistent with the observations that the levels of plant S increase with increasing plant P and decreasing Ca (Fig. 2). The plant dynamics from chalk to *Nardus* grasslands were also related to higher enzyme ratios of C to N, P and S. This is likely to be the consequence of the higher concentration of C-rich structural carbohydrates in the *Nardus* grassland, as suggested by the positive correlation between GLU activity and the concentrations of CEL and HEM, which act as substrates (Wang et al. 2020).

The composition of the herbage indicated a severe K deficiency that cannot be solely explained by soil K availability, which was within the optimal range according to Jones (2001), but appeared instead related to the K^+:Ca^{2+} ratios. Calcium and K are mutually antagonistic in their uptake by plants, so high Ca in the presence of low–moderate K can decrease K intake enough to induce K deficiency (Cahoon and Grummet 1954). The K^+:Ca^{2+} ratio has long been reported to be decisive for the calcicole–calcifuge behavior of plants (De Bilde, 1978; Korcak, 1987) and a major factor influencing the composition of chalk grassland communities (Austin, 1968).

Effect of plant traits on plant composition

The chemical and elemental composition of plants is genetically and physiologically controlled and varies between species and, to a more limited extent, among individuals (Kay et al. 2005; Elser et al. 2010). The results of the present study reveal that the shift between *Nardus* grassland and chalk grassland involved a shift in plant functional groups with contrasting compositional traits. The two grasslands differed mainly in the relative proportions of graminoids, which were more abundant in the *Nardus* grassland mainly due to the dominance of *N. stricta*, relative to forbs and legumes, which were predominant in the chalk grassland. The greater proportion of graminoids affected the organic composition of herbage, which became richer in cellulose and hemicellulose and poorer in lignin, as is characteristic of graminoids compared to nongraminoid herbs (Gordon, 1989; Marinas and García–González 2006; Poca et al. 2014). A larger amount of structural carbohydrates reflects a greater investment in grass stems (lignin was a minor constituent in both grasslands), indicating higher density and stronger competition for light in the *Nardus* grassland than in the chalk grassland, which would be consistent with the less severe nutrient limitation (Irving, 2015; Postman et al. 2020; Rehling et al. 2021). In fact, *Nardus* grasslands tend to be denser, and the plants tend to be taller than those in chalk grasslands, which could effectively mean more competition for light.

The greater proportion of graminoids in the *Nardus* grassland was also related to higher P and lower Ca concentrations, which agrees with the finding by Marinas and García–González (2006) of higher P and lower Ca levels in graminoids compared to non–graminoids in subalpine Pyrenean grasslands. A higher investment in stem structures can affect the plant elemental composition since it means less investment in leaf mesophyll and epidermis, which contain most of the N, P and K in plants (Meerts, 1997). However, in our study, the greater proportion of graminoids and higher concentration of structural carbohydrates were related to higher P and unrelated to N or K. Furthermore, the higher levels of P result in lower N:P

ratios in the *Nardus* grassland compared to chalk grassland, which contradicts the usual finding that a higher proportion of graminoids is associated with higher N:P ratios and, consequently, with an increasing limitation of P relative to N (Güsewell, 2004; He et al. 2006). On the other hand, forbs and legumes are generally reported to show higher Ca concentrations than graminoids (Meerts, 1997; Mládková et al. 2018; Kajzrová et al. 2022), which relates to the large amount of Ca in the form of Ca–pectates in the cell walls of dicotyledons (including legumes and many forbs) (White and Broadley 2003; Mládková et al. 2018).

The plant functional type also influenced the N levels, which increased with increasing amounts of legumes. Legumes are important N fixers, which results in the luxury consumption of N (Freschet et al. 2017) and thus in higher N concentrations and higher ratios of N relative to other elements compared to nonlegumes (He et al. 2007; Di Palo and Fornara 2017). However, the N concentrations were similar in the two grasslands (and the N:P ratios were higher in the *Nardus* grassland) presumably because legumes were minor populations in both plant communities (albeit more abundant in the chalk grassland). The soil C:N ratio was higher in the *Nardus* grassland than in the chalk grassland, but this can likely be explained by the fact that a lower concentration of structural components leads to faster decomposition of organic matter, resulting in lower C:N (Badea et al. 2020). Faster decomposition can also result in lighter $\delta^{13}C$ values in the chalk grassland (Badea et al. 2020), a finding observed in a previous study by Badía–Villas et al. (2020).

Topographical controls on elemental stoichiometry

The small-scale coexistence of *Nardus* grasslands and chalk grasslands was related to contrasting elemental composition and demand, particularly of Ca and P. Heterogeneous elemental composition is a key factor in the coexistence of plant species (He et al. 2008; Hong et al. 2015) and communities (Yan et al. 2019; Lin et al. 2022) in high mountain areas. Differences in elemental composition mirror different nutrient requirements, resulting in niche separation (Sardans and Peñuelas 2014), and the high spatial variability in mountain soils provides a diversity of niche spaces for plants with different requirements (Antonelli et al. 2018). The heterogeneity of mountain soils is largely contributed by microtopography (Hiller and Müterthies 2005; Holtmeier and Broll 2018), which has been shown to have a considerable effect on the nutrient distribution in calcareous ranges (Sebastiá 2004; Michalet et al. 2002; Giaccone et al. 2019) and is even more influential than the calcareous versus siliceous nature of bedrock (Michalet et al. 2002). Distinguishing topographic from geochemical effects is critical for understanding how bedrock geology influences the biodiversity of high mountain ecosystems (Rahbek et al. 2019).

In the present study, the differences in elemental composition between *Nardus* and chalk grasslands were derived from the calcareous substrate and limitations resulting from excess Ca but were controlled by the local topography. Erosion in mountain areas is usually uneven due to the complexity of the topography, with local areas of both soil thinning and thickening occurring (Amundson et al. 2015). In the study area, the two grassland types settle on different sediment levels separated by a centimeter–scale unevenness produced by gully erosion, which was likely triggered by the removal of the original forest for pasture in the past (Badía-Villas et al. 2021). Soil thinning brings the bedrock closer to the soil surface, which in calcareous ranges leads to increased levels of lime and pH (Tovar et al., 2012; Liu et al. 2018), whereas

thickened soils are more prone to leaching and debasification (Amundson et al. 2005). In the study area, higher Ca results in increased P limitation in the eroded soils, causing them to be colonized by the chalk grasslands, which can be regarded as a typical calcicole adapted to (but likely not demanding) extreme Ca contents. In contrast, *Nardus* grassland, which is not typically calcifuge but is still intolerant to excess Ca, grows in noneroded soils where P limitation is gradually alleviated by debasification. The lower biomass of the chalk grasslands allows for further soil erosion (Liu et al. 2018) as opposed to the greater stability of the soils of the *Nardus* grassland, which contributes to stabilizing the mosaic of communities by positive feedback mechanisms (Armas–Herrera et al. 2020). To our knowledge, reports on Ca–induced P limitation being driven by local topography are limited for upland areas, but it is a well-known phenomenon for calcareous fens (e.g., Boyer and Wheeler 1989; Boeye et al. 1997), where P limitation and plant communities are patchily distributed due to fluctuations in the base-rich water table due to microtopography.

CONCLUSIONS

The small-scale coexistence of *Nardus* grasslands and chalk grasslands in the southern central Pyrenees was related to differing levels of available Ca in soil and uptake by plants. Among the nutrients studied, Ca was the only nutrient that had concentrations in herbage and soil that were correlated, both being higher in the chalk grassland soils (Hypereutric Leptosols) than in the *Nardus* grassland soils (Orthoeutric Cambisols). High Ca adversely affects P intake (as shown by the observation that plant–P decreased with increasing plant Ca and soil Ca), leading to a greater prevalence of P–limitation rather than N–limitation (as measured by the plant N:P ratio), which was associated with a greater enzyme demand for P relative to N (as assessed from the soil enzyme N:P ratios). In the *Nardus* grassland, the alleviation of P limitation results in higher productivity and the development of tall, dense graminoid vegetation, which opposes the general idea that graminoids have a larger N:P ratio than forbs. The greater investment in stem-like structures in the *Nardus* grassland was mirrored by greater concentrations of C and structural carbohydrates in herbage and higher carbohydrate-degrading enzyme activity in the soil. The S levels are sufficient in both grasslands, as inferred from its high levels in the soil and the low plant N:S ratios. On the other hand, excess Ca results in strong K deficiency in both grassland types as a consequence of the antagonistic effect of Ca on K uptake.

Declarations

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Figures

Figure 1

Top and close-up views of the mosaic-patterned landscape with tall graminoid vegetation (*Nardus* grasslands) located on slightly elevated ground and shorter forb vegetation (chalk grasslands) located in relatively lower areas.

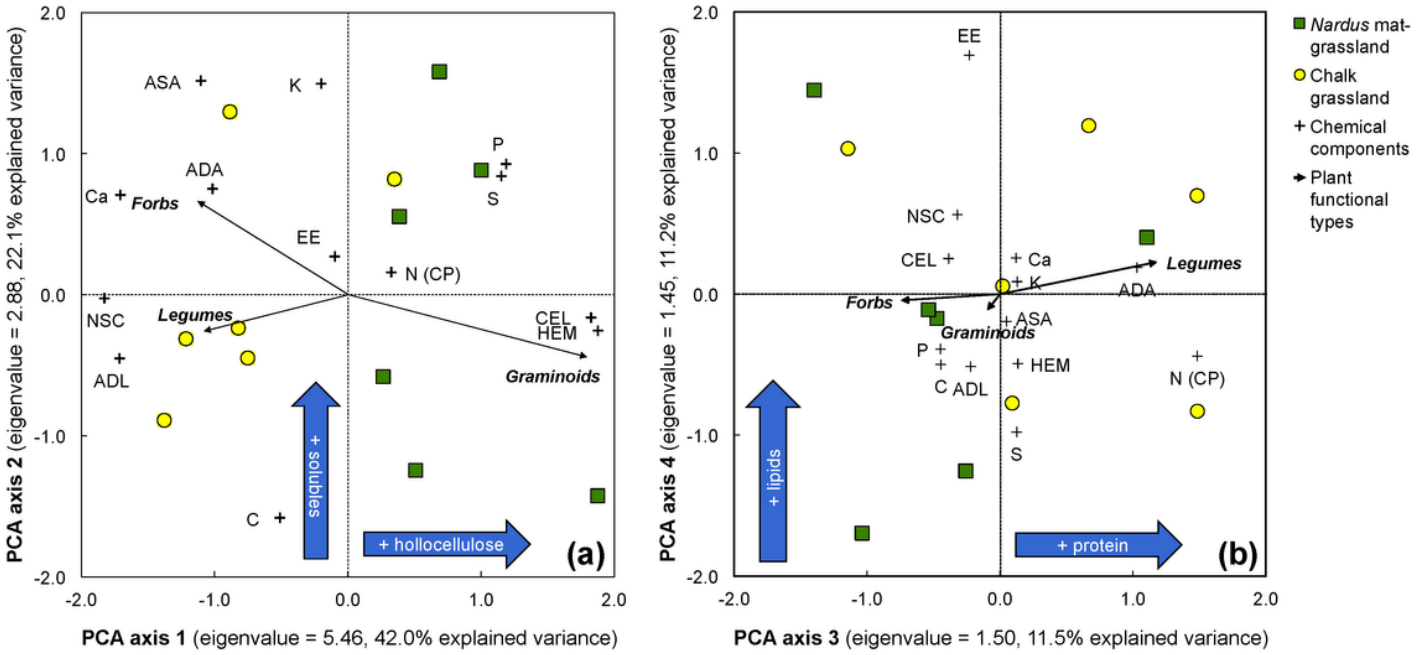

Figure 2

Main gradients of variation in the chemical composition of the herbage, as obtained by principal component analysis(PCA).

ADA = acid–detergent ash, ADL = acid–detergent lignin, ASA = acid–soluble ash, EE = ether extract, CEL = cellulose, CP = crude protein, HEM = hemicellulose, NSC = non–structural carbohydrates

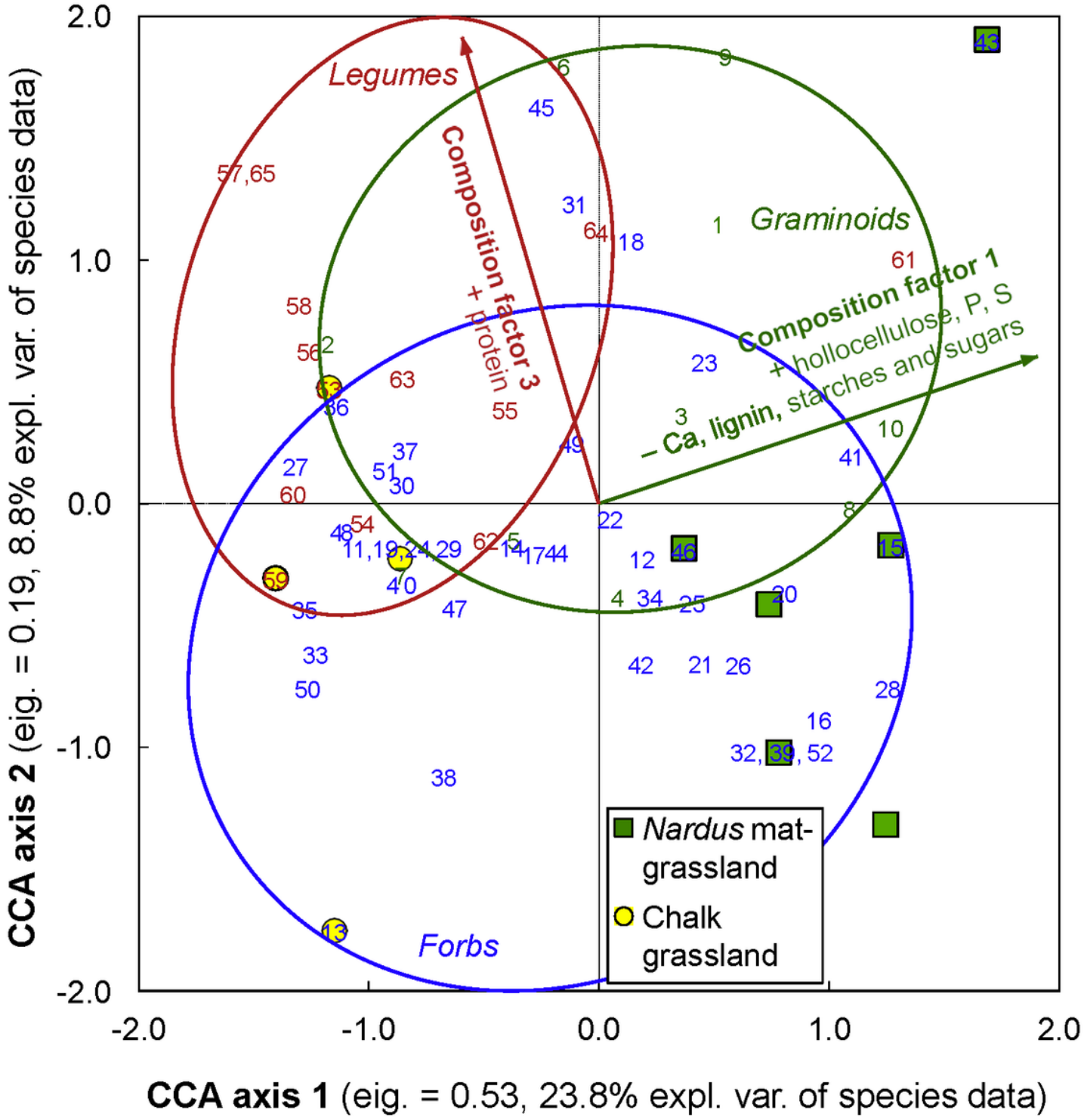

Figure 3

Relationship between the plant species composition and the main gradients of variation in the chemical composition of the herbage, as obtained by canonical correspondence analysis(CCA).

GRAMINOIDS (in green letters): 1 = *Agrostis capillaris* L., 2 = *Briza media* L., 3 = *Carex caryophyllea* Latourr., 4: *C. rupestris* All., 5 = *Festuca nigrescens* Lam., 6 = *Koeleria pyramidata* (Lam.) Beauv., 7 = *K. vallesiana* (Honckeny) Gaudin, 8 = *Nardus stricta* L., 9 = *Poa pratensis* L., 10 = *Tragopogon lamottei* Rouy.

FORBS (in blue letters): 11 = *Alchemilla alpigena* Buser ex Hegi, 12 = *A.* gr. *glaucescens* Wallr., 13 = *Antennaria dioica* (L.) Gaertn., 14 = *Achillea millefolium* L., 15 = *Campanula scheuchzeri* Vill., 16 = *Cerastium fontanum* Baumg., 17 = *Cirsium acaule* (L.) Scop., 18 = *C. eriophorum* (L.) Scop., 19 = *Erigeron alpinus* L., 20 = *Eryngium bourgatii* Gouan, 21 = *Euphorbia cyparissias* L., 22 = *Galium pumilum* Murray, 23 = *G. verum* L., 24 = *Gentiana verna* L., 25 = *Geranium cinereum* Cav., 26 = *Iris latifolia* (Mill.) Voss, 27 = *Leontodon hispidus* L., 28 = *L. pyrenaicus* Gouan, 29 = *Leucanthemum vulgare* Lam., 30 = *Linum catharticum* L., 31 = *Merendera montana* (L.) Lange, 32 = *Phyteuma orbiculare* L., 33 = *Pilosella lactucella* (Wallr.) P.D. Sell & C. West, 34 = *P. officinarum* F.W. Schultz & Schultz Bip., 35 = *Plantago alpina* L., 36 = *P. lanceolata* L., 37 = *P. media* L., 38 = *Polygala vulgaris* L., 39 = *Polygonum viviparum* L., 40 = *Potentilla neumanniana* Rchb., 41 = *P. erecta* (L.) Räuschel, 42 = *Prunella vulgaris* L., 43 = *Ranunculus amplexicaulis* L., 44 = *R. carinthiacus* Hoppe, 45 = *R. gouanii* Willd., 46 = *Rhinanthus pumilus* (Sterneck) Pau, 47 = *Sanguisorba minor* Scop., 48 = *Seseli montanum* L., 49 = *Taraxacum officinale* Weber, 50 = *Thymelaea tinctoria* (Pourr.) Endl., 51 = *Thymus praecox* Opiz, 52 = *Viola pyrenaica* Ramond. LEGUMES (in red letters): 53 = *Anthyllis montana* L., 54 = *A. vulneraria* L., 55 = *Lotus corniculatus* L., 56 = *Medicago suffruticosa* Ramond ex DC., 57 = *Onobrychis pyrenaica* (Sennen) Sirj., 58 = *Ononis cristata* Mill., 59 = *O. striata* Gouan, 60 = *Oxytropis neglecta* Ten., 61 = *Trifolium alpinum* L., 62 = *T. montanum* L., 63 = *T. pratense* L., 64 = *T. repens* L., 65 = *T. thalii* Vill.

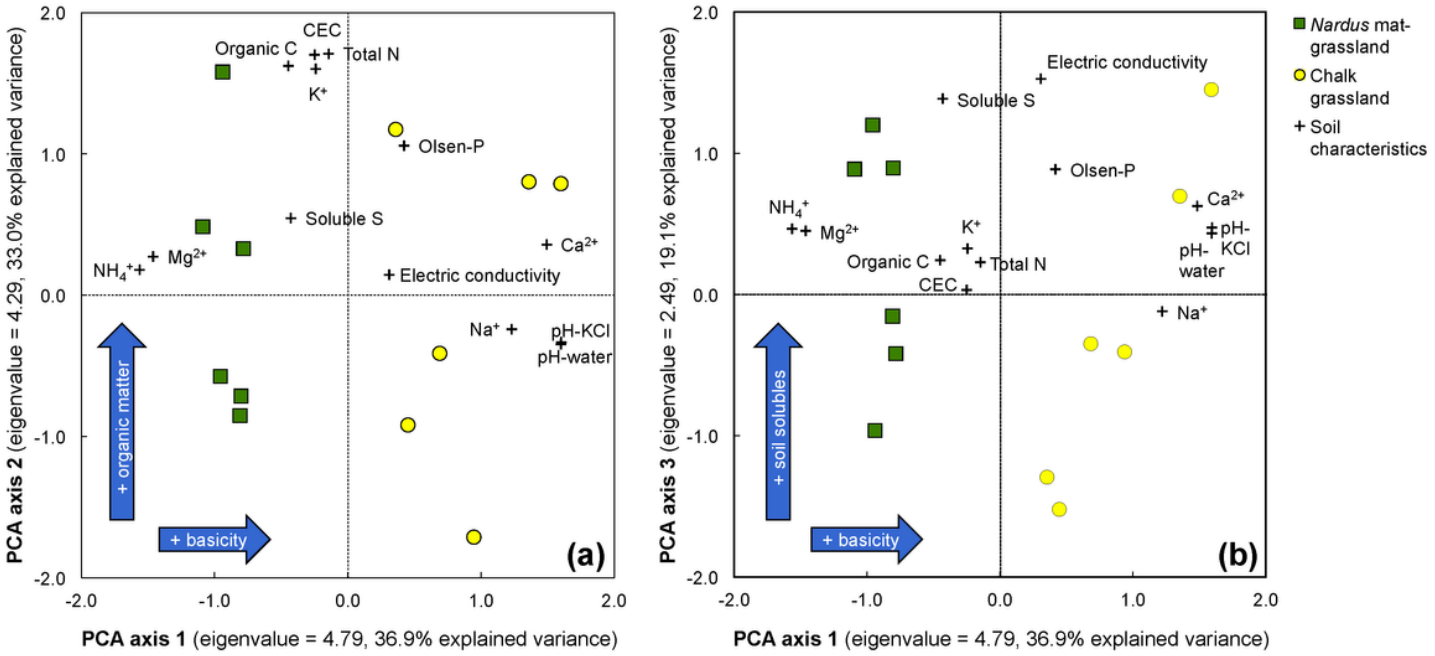

Figure 4

Main gradients of variation in the soil properties, as obtained by principal component analysis (PCA).

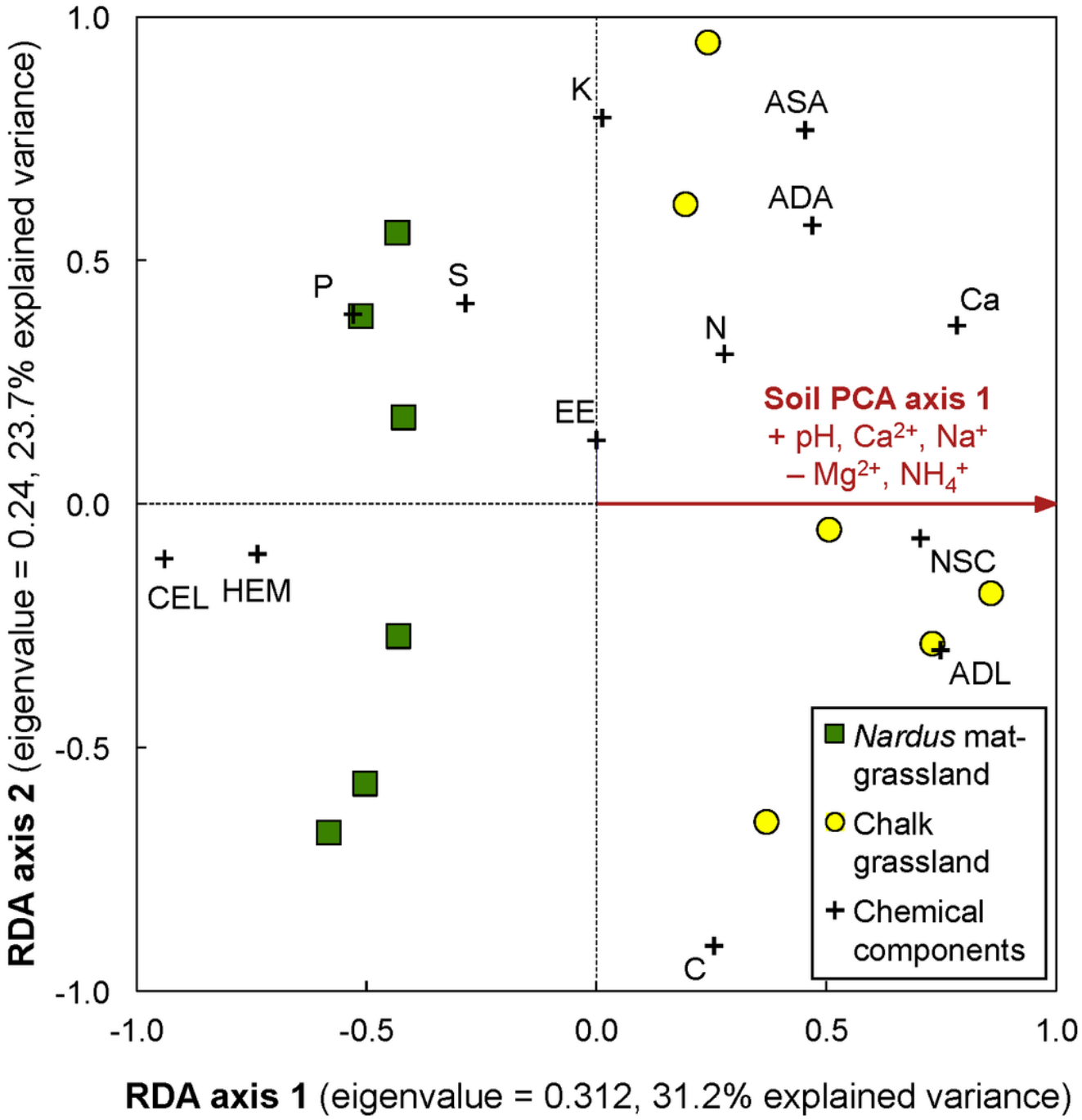

Figure 5

Relationship between the chemical composition of the herbage and main gradients of variation in the soil properties, as obtained by redundancy analysis (RDA).

ADA = acid–detergent ash, ADL = acid–detergent lignin, ASA = acid–soluble ash, EE = ether extract, CEL = cellulose, CP = crude protein, HEM = hemicellulose, NSC = non–structural carbohydrates

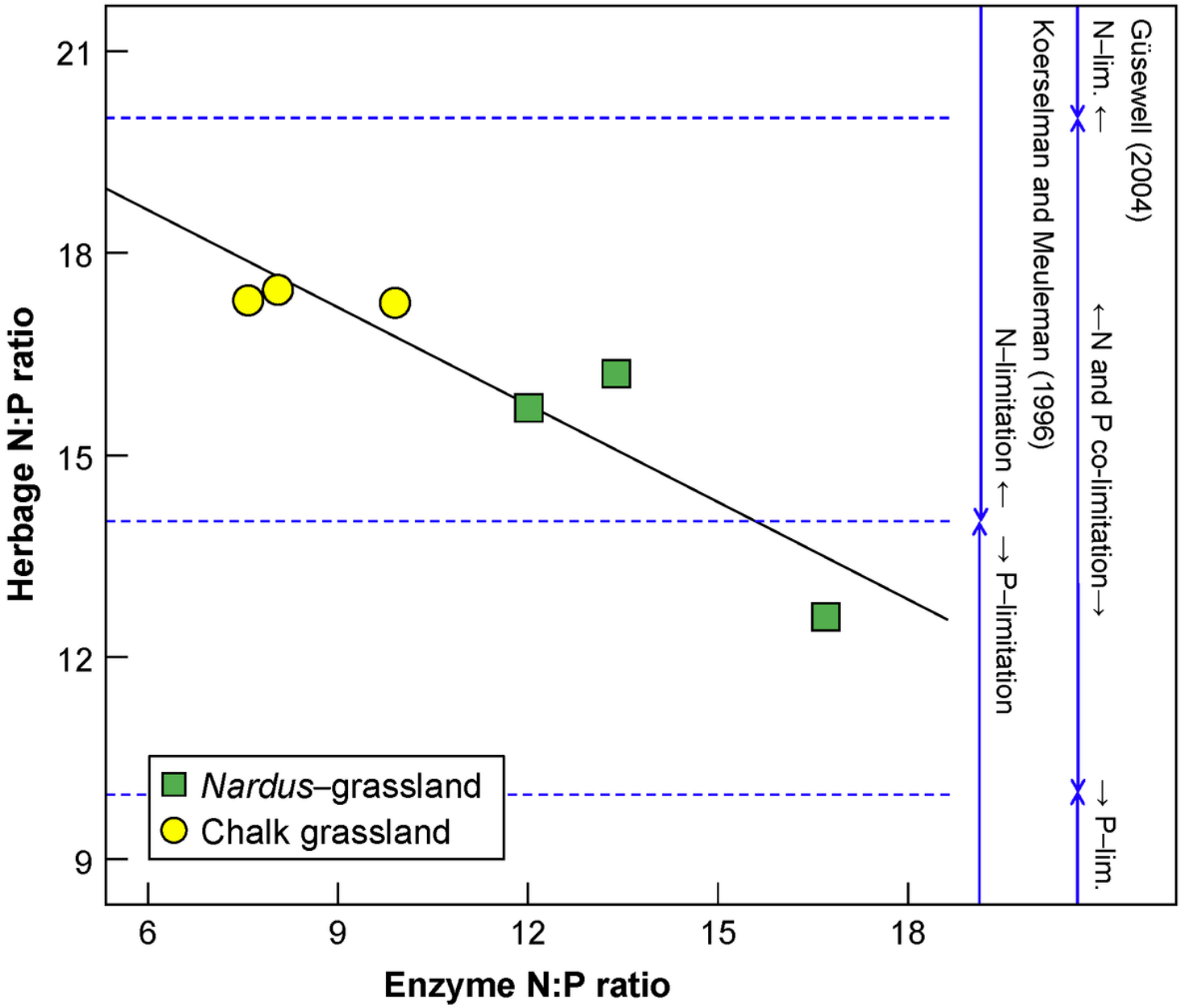

Figure 6

Relationship between the N:P ratio of the herbage composition and of the soil enzyme activities. The critical levels for N versus P limitation according to Koerselman and Meuleman (1996) and Güsewell (2004) are provided as references.

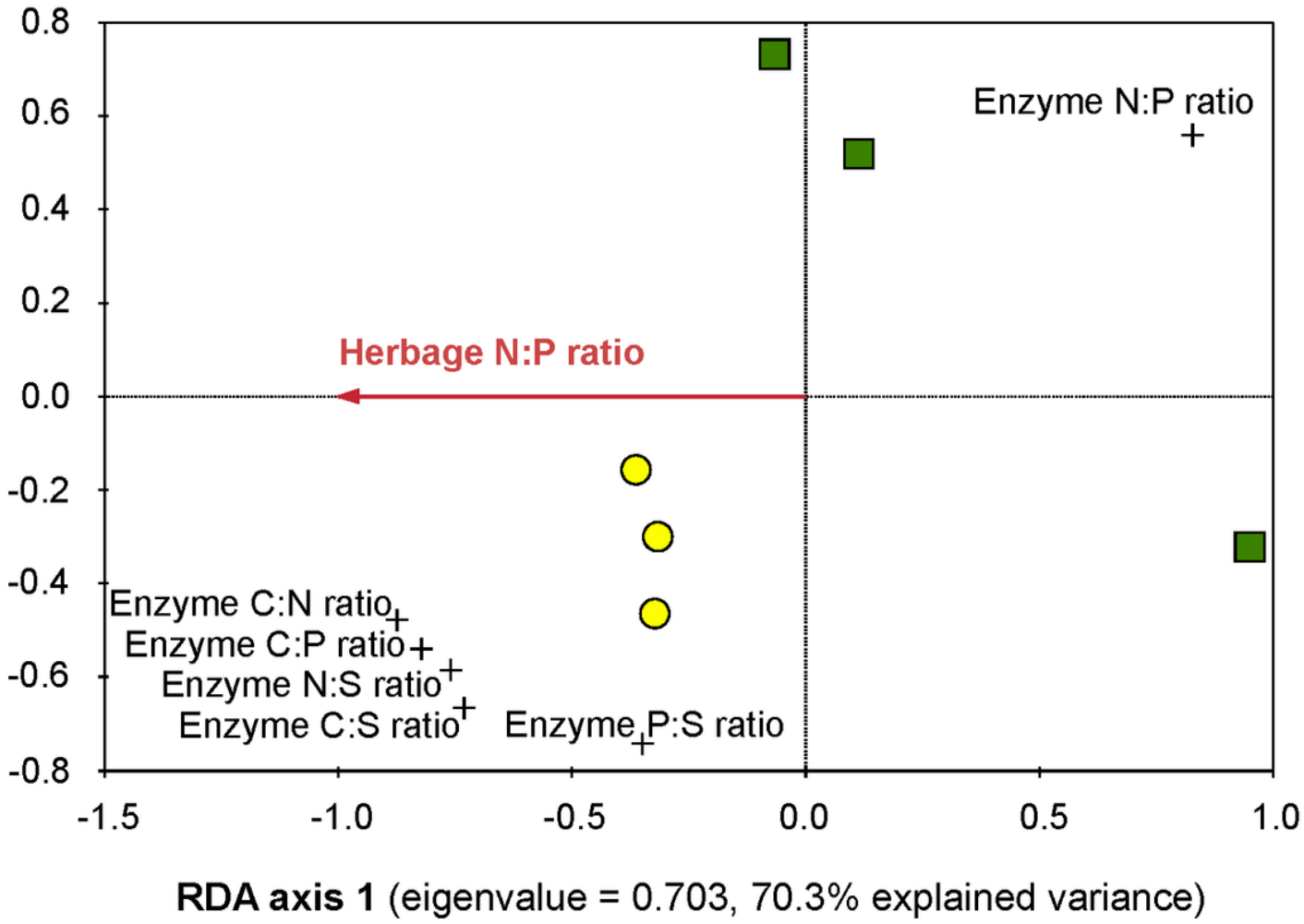

Figure 7

Relationship between the stoichiometry of the soil enzyme activities and the stoichiometry of the herbage elemental composition, as obtained by redundancy analysis(RDA).

Supplementary Files

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