Mosaic coexistence of two subalpine grassland types as a consequence of soil nutrient heterogeneity

Juan Luis Mora [a,*], Cecilia Armas–Herrera [b], Daniel Gómez [c], David Badía–Villas [b]

[a] *Grupo GEOFOREST – Instituto de Investigación en Ciencias Ambientales (IUCA), Departamento de Ciencias Agrarias y del Medio Natural, Facultad de Veterinaria, Universidad de Zaragoza. C/ Miguel Servet 177, 50013 Zaragoza, Spain*
[b] *Grupo GEOFOREST – Instituto de Investigación en Ciencias Ambientales (IUCA), Departamento de Ciencias Agrarias y del Medio Natural, Escuela Politécnica Superior de Huesca, Universidad de Zaragoza. Carretera de Cuarte, s/n, 22071 Huesca, Spain*
[c] *Instituto Pirenaico de Ecología (CSIC), Avda. Ntra. Sra. de la Victoria 16, 22700 Jaca, Spain*

A B S T R A C T

High-mountain areas often exhibit high soil heterogeneity, which allows for the close coexistence of plant species and communities with contrasting resource requirements. This study investigated the nutritional factors driving the mosaic distribution of *Nardus stricta* L. grasslands and chalk grasslands dominated by forbs in the subalpine southern Pyrenees (Spain). The concentrations of C, N, P, S, K, Ca and fiber fractions in herbage were analyzed in relation to soil nutrient availability; soil β-glucosidase, urease, phosphatase and arylsulfatase activities; and plant species and functional type compositions. The chalk grassland showed higher N:P ratios in herbage and higher enzyme demand for P relative to N in the soil, which indicates a greater limitation of P versus N compared to *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 increased productivity and the replacement of forbs with taller graminoids rich in structural carbohydrates, which was mirrored by greater β-D-glucosidase activity. The plant N:K and P:K ratios indicated potential K deficiency in both grasslands, which resulted from decreased uptake of K in competition with Ca, as indicated by the correlation between plant K and the soil $K^+:Ca^{2+}$ ratio. Our results highlight the effect of the heterogeneity of soil nutrient constraints, mediated by stoichiometry and dependent on microtopography, on the biodiversity of high-mountain ecosystems.

1. Introduction

Soils of high-mountain (alpine and subalpine) areas are typically young soils whose properties vary greatly depending on the local topography and bedrock (Egli and Poulenard, 2016; Körner, 2021). As a result, mountain landscapes are often mosaic-like, with distinct vegetation types growing under contrasting soil conditions within relatively short distances (Vonlanthen et al., 2006; Amagai et al., 2018). This contributes to making them hotspots of biodiversity and priorities for conservation (Körner, 2004; Egli and Poulenard, 2016).

The calcareous (as opposed to noncalcareous) nature of 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), which is mainly explained by the occurrence of high calcium (Ca) concentrations and basic pH conditions

in young calcareous soils (Körner, 2021). The plants growing on such soils must respond and adapt to limitations in the availability of phosphorus (P), iron (Fe) and other micronutrients, the solubility of which may decrease with increased soil pH and Ca (Kishchuk, 2000; Lambers and Oliveira, 2019). Furthermore, a 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 potassium (K) limitation due to plant uptake competition for cations (Kishchuk, 2000). Several studies have documented P limitation in high-mountain soils formed on calcareous bedrock (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 by both N and P and occasionally by K (Niinemets and Kull, 2005; Bassin et al., 2012) have also been identified.

Plant communities dominated by matgrass (*Nardus stricta* L.)

(hereafter referred to as *Nardus* grasslands) are among the most widespread natural habitats in Europe and are mostly located in mountain areas (Galvánek and Janák, 2008). *Nardus* grasslands (especially the high-mountain types) host a variety of endemic and threatened plant species, resulting in them being identified as priority habitats for conservation under the Habitats 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 with 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). Studies by Armas-Herrera et al. (2020) and Badía-Villas et al. (2020) revealed contrasting soil thicknesses, stoninesses, acid/base statuses (pH, Ca) and organic matter-related properties among patches of *Nardus* and chalk grasslands. However, the specific soil conditions that influence the occurrence of one or the other grassland type, i.e., limitation by one or more nutrients, have not been determined.

The nutrient status of a habitat is regularly assessed by the concentrations of nutrients available to plants in the soil, although the nutrient levels in plant tissues can also be informative, as they provide information about the actual nutrient uptake by plants (Motsara and Roy, 2008). An important issue is the stoichiometry of nutrients, whose use by plants is coupled to each other's, which causes plants to require them in regular proportions and to undergo limitations when one is in shorter supply (Knecht and Göransson, 2004; Ågren, 2008). The studies by Koerselman and Meuleman (1996) and Güsewell (2004) established critical values for the N:P ratio of plants that have been widely used for the assessment of relative limitation by N or P, which are the most common limiting nutrients on a community level. Other studies have derived critical ratios involving other major nutrients, such as sulfur (S) (Sumner, 1978; Stevens and Watson, 1986; Mathot, 2005; Ryant and Skládanka, 2009) or K (Dampney, 1992; Pegtel et al., 1996; Olde Venterik et al., 2003; Lawniczak et al., 2009), to determine their possible limitations. Another point of interest is the stoichiometry of the soil enzymes responsible for the cycling of organic C, N, P and S. These enzymes are produced by soil microorganisms and plants in variable amounts depending on their requirements and the availability of these nutrients in the environment (Allison and Vitousek, 2005; Sinsabaugh et al., 2008). Hence, the ratios of the activities of enzymes involved in the release of C, N, P and S mirror the relative demand for each nutrient and can be used as indicators of nutrient limitations (Sinsabaugh et al., 2008; Luo et al., 2017; Cui et al., 2021).

Based on the assumption that the different soil decalcifications under the patches of *Nardus* and chalk grasslands may lead to differing limitations by N, P and/or K, we investigated the following: (1) whether the patchy coexistence of *Nardus* and chalk grasslands in the Pyrenees runs parallel to spatially heterogeneous nutrient limitations and (2) if this is the case, what nutrients may be limiting for each vegetation type? To this end, 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.

2. Materials and methods

2.1. Study area and field work

The study was conducted at 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 ranging 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 (**Supplementary material, Figure S1**), the mean annual temperature is approximately 5 °C, and the annual precipitation is approximately 1,700 mm, with snow precipitation occurring from October to May.

The study area covers approximately 250 ha and has a topography 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 is buried beneath a cover of sediments eroded from the rocks higher in the slopes consisting of limestone, marls and lutites, also from the Ilerdian. These sediments are affected by uneven erosion, resulting in a mosaic of slightly raised areas and lower ground areas where two different types of vegetation are found (Fig. 1): *Nardus* grassland growing on Orthoeutric Cambisols on the soil accumulation packages and chalk grassland on Hypereutric Leptosols at the bottom of such packages (Badía-Villas et al., 2020).

The *Nardus* grassland fits to the phytosociological alliance *Nardion strictae* Br.–Bl. 1926 and was characterized by the dominance of *N. stricta* (**Supplementary material, Table S1**). The chalk grassland is made up of a mosaic of species characteristic of three communities (phytosociological alliances): *Bromion erecti* Koch 1926 (dominant), *Festucion gautieri* Br.–Bl. 1948 and *Primulion intricatae* Br.–Bl. *ex* Vigo 1972, and has a remarkable diversity of grasses, legumes and other species (**Supplementary material, Table S1**). Both types of grassland had plant cover close to 100 %. The stocking rate (for cattle and sheep) reaches approximately one large animal unit per hectare (Armas-Herrera et al., 2020).

The field work was performed in early July 2018. Six plots were established at intervals of 100 m along a 600-m transect placed at the mid-slope, which was deemed adequate for representing the spatial variation in both grassland types. In each plot, two sampling points were located in adjacent patches of *Nardus* grassland and chalk grassland at a distance no greater than 3 m. At each sampling point, a quadrat of 1 × 1 m was placed where the plant species were identified, and their covers were visually assessed on a percent scale. The plant species were categorized into three functional groups commonly used in pasture studies: legumes (Fabaceae), graminoids (Poaceae and Cyperaceae), and forbs (all other herbaceous species); the cover for each group was calculated by summing the cover values of all the species in that group. Summaries of the plant species and functional groups of the *Nardus* and chalk grasslands are given in the **Supplementary material, Tables S1 and S2**.

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 × 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 × 2 types of patches = 6 samples).

2.2. Laboratory procedures

The herbage was weighed before and after drying in a forced–air dryer at 60 °C for 48 h to determine the dry matter content and the dry biomass per unit area. The material was subsequently ground to < 1 mm using a cutting mill (Retsch Mühle, Haan, Germany), after which the samples were 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 dissolving in aqua regia: P by the molybdate–blue method (Murphy and Riley, 1962) and 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

Fig. 1. Top and closer 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.

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 content, hemicellulose and cellulose, yielding respectively the neutral detergent fiber (NDF), acid detergent fiber (ADF) and an acid-insoluble residue, which are 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 (NSCs) 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. The 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 was determined 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 via 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 (Olsen et al., 1954; Murphy and Riley, 1962). Sulfate–S was extracted using a 1:5 soil:water ratio and determined by turbidimetry as described by AOAC (2006). The 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.

The soil microbial biomass C was determined via the fumigation–extraction method (Vance et al., 1987). The following 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 of which are 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. The GLU:URE, GLU:PHO, GLU:SUL, URE:PHO, URE:SUL and PHO:SUL ratios were calculated from the enzyme activity data and are hereafter termed the C:N,C:P, C:S, N:P, N:S and P:S enzyme ratios, respectively.

2.3. 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 tests after verifying the assumptions of normality and homogeneity of variance using Kolmogorv–Smirnov and Levene tests, respectively. When the assumptions were not confirmed, appropriate standard data transformations (power and log) were applied. For ease of interpretation, tables and figures present nontransformed 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 small 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 datasets. Separate principal component analyses (PCAs) were performed on the chemical composition of the herbage and on the soil properties. The principal components obtained from the soil data were subsequently used in a redundancy analysis (RDA) of the herbage chemical composition in relation to the soil factors. The most suitable explanatory variables for the RDA were selected by a forward stepwise procedure based on Monte Carlo tests using 999 permutations. The statistical significance of the RDA was tested with Monte Carlo tests of 999 permutations.

3. Results

3.1. Plant chemical composition

The herbage of the *Nardus* grassland (Table 1) was significantly richer in fiber (NDF, ADF) and particularly in structural carbohydrates (CEL, HEM) but was lower in ADL and NSC than was the herbage of the chalk grassland. The two vegetation types exhibited 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 terms of the ratios of Ca and P and other elements. Specifically, the *Nardus* grassland had greater ratios

Table 1

Descriptive statistics and comparisons (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	ns
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	ns
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	*
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. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. 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 comparisons (t tests) of the mass ratios of major elements (C, N, P, S, K and Ca) in the herbage of the *Nardus* and chalk grasslands studied.

	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	ns	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	ns	
P: S	**0.748**	0.039	0.582	0.858	**0.617**	0.047	0.445	0.745	2.21	ns	
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	
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. * = $P < 0.05$, ** = $P < 0.01$.

of P to C, N, K and Ca than did the chalk grassland, which in turn had greater ratios of Ca to C, P, S and K.

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 %. Fig. 2 shows 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 shown in Fig. 2, Composition Factor 1 was strongly negatively correlated ($P < 0.001$) with the abundance of graminoids 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 abundance of legumes, whereas Composition Factor 4 had high positive loading for ether extract.

3.2. 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 were the soils of the chalk grassland (Table 3). The PCA summarized the variation in soil properties into three 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
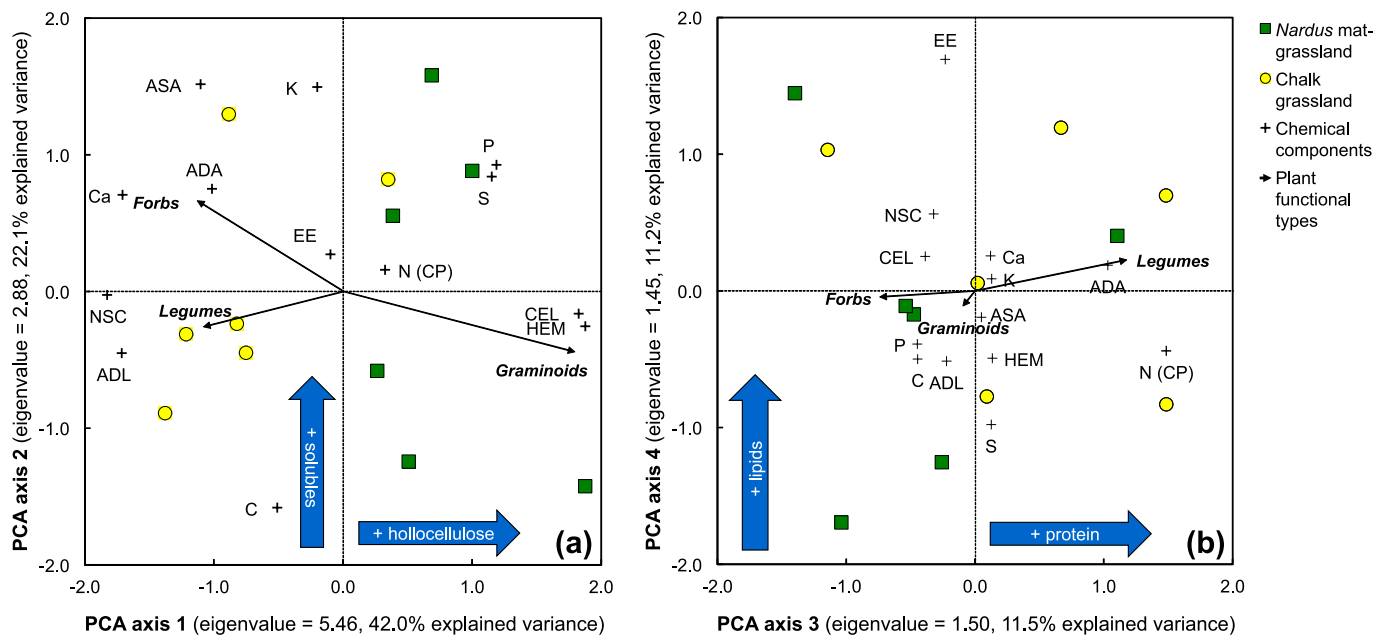
Fig. 2. Main gradients of variation in the chemical composition of the herbage, as obtained by principal component analysis (PCA).ADA = acid detergent ash, ADL = acid detergent lignin, ASA = acid-soluble ash, EE = ether extract, CEL = cellulose, CP = crude protein, HEM = hemicellulose, NSC = nonstructural carbohydrates.

Table 3
Descriptive statistics and comparisons (t tests) of the soil (0–10 cm) 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	0–.31	Ns
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, * = $P < 0.05$, ** = $P < 0.01$. $EC_{1:5}$ = electric conductivity at a 1:5 soil:water ratio, CEC = cation exchange capacity.

interpreted as a leaching gradient that separated the soils of *Nardus* grassland from those of chalk grassland (Fig. 3). Soil Factor 2 was positively related to characteristics related to organic matter (organic C, total N, cation exchange capacity) and exchangeable K. Soil Factor 3 was positively related to the amount of soluble ions (electric conductivity, sulfate–S).

RDA of the herbage chemical composition in relation to the 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. 4, which shows that the herbage becomes leaner in Ca, ADL and NSCs and richer in structural carbohydrates, P and S as the soil undergoes decalcification with the 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 the herbage showed little relation with those of the same nutrients in the soil. Only the herbage Ca concentration was positively correlated with the soil exchangeable Ca^{2+} concentration (r = 0.70, $P < 0.05$), whereas the herbage N, P, S and K levels were unrelated to the plant-available Ca^{2+} concentration 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.

3.3. Soil enzyme activity and its relation to plant chemical composition

The soils of the *Nardus* grassland had greater microbial biomass C and higher potential activities for most of the enzymes examined, except for SUL, which showed no significant differences ($P > 0.05$, Mann–Whitney *U* test) (Table 4). GLU was found to be related to the fiber composition of the herbage, increasing with increasing levels of
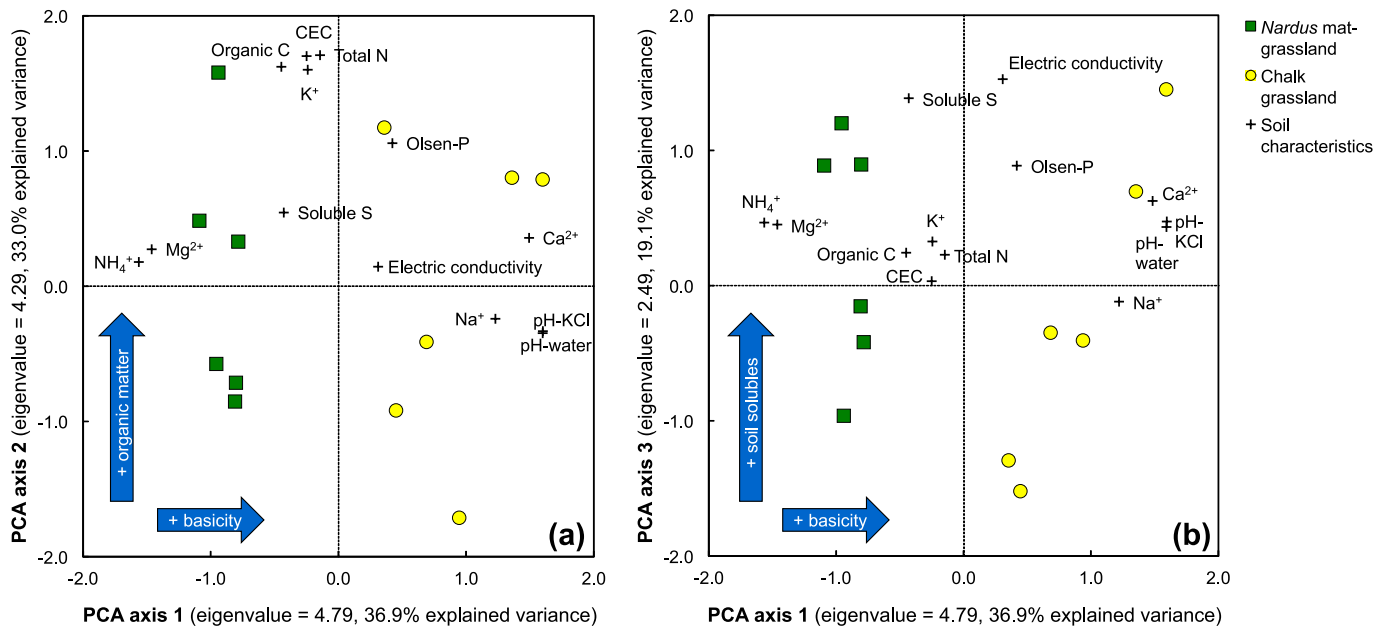
Fig. 3. Main gradients of variation in the soil properties, as obtained by principal component analysis (PCA).
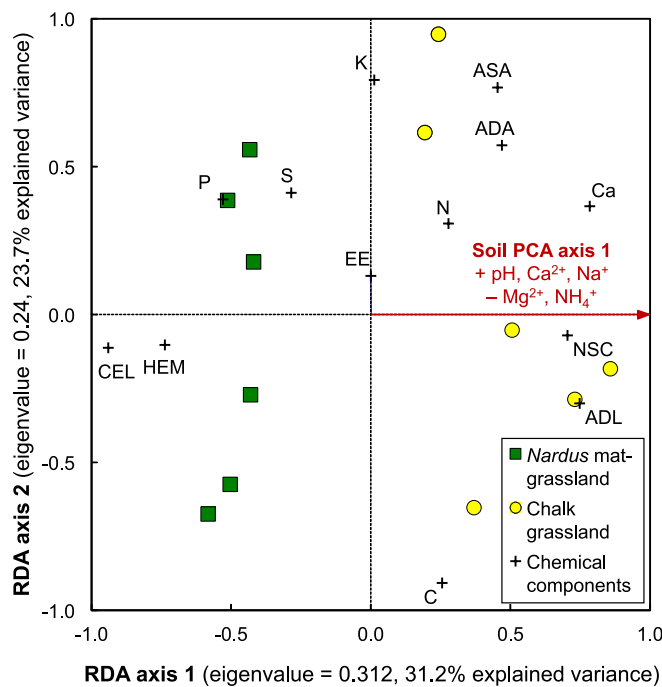

Fig. 4. Relationships between the chemical composition of the herbage and the main gradients of variation in the soil properties, as obtained by redundancy analysis (RDA). ADA = acid detergent ash, ADL = acid detergent lignin, ASA = acid-soluble ash, EE = ether extract, CEL = cellulose, CP = crude protein, HEM = hemicellulose, NSC = nonstructural carbohydrates.

cellulose (r = 0.87, $P < 0.05$; Pearson correlation) and hemicellulose (r = 0.96, $P < 0.01$) and decreasing levels of NSCs (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 analysis of enzyme stoichiometry revealed significant

differences only for the enzyme C:N ratio, which was greater for the chalk grassland than for the *Nardus* grassland, suggesting a greater enzymatic demand for N versus C at the *Nardus* than at the chalk grassland. The enzyme ratios of C, N, P and S showed no correlation with the ratios among the same elements in herbage, with the important exception of the N:P ratios, for which a negative correlation was found between the herbage and the soil enzyme values (r = −0.91, $P < 0.05$) (Fig. 5). According to this correlation, as the N:P ratio increased in herbage (indicating increasing limitation by P versus N), the enzyme N:P ratio decreased (indicating increasing enzymatic demand for P versus N).

4. Discussion

4.1. Nutrient limitations inferred from elemental stoichiometry

Nardus and chalk grasslands are considered oligotrophic environments with low availability of N and P (Smith, 1980; Schelfhout et al., 2017). In the present study, the mean ratios of N to P in the herbage of both grassland types were within the range of 10–20, within which, according to Güsewell (2004), no clear limitation by N or P occurs; rather, there is colimitation by both nutrients. 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 Bedia, 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 colimitation by N. Conversely, Bobbink et al. (1989) reported N:P values of 14–18 and above for *Bromion* chalk grasslands, which denote limitation or colimitation by P. Moreover, the soil enzyme C:P ratio was < 1 in both the *Nardus* and chalk grasslands, indicating more effort to acquire and cycle P relative to processing of 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 cold

Table 4

Descriptive statistics and comparison (Mann–Whitney U tests) of the soil (0–10 cm) biochemical properties and enzyme stoichiometry of the *Nardus* and chalk grasslands.

	Nardus grassland (n = 3)				Chalk grassland (n = 3)				U	Sig.
	Mean	SE	Min.	Max.	**Mean**	SE	Min.	Max.		
Microbial biomass C (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,* = $P \leq 0.05$, GLU = β–D–glucosidase activity, PNP = *p*–nitrophenol, URE = urease activity, PHO = acid phosphatase activity, SUL = arylsulfatase activity.
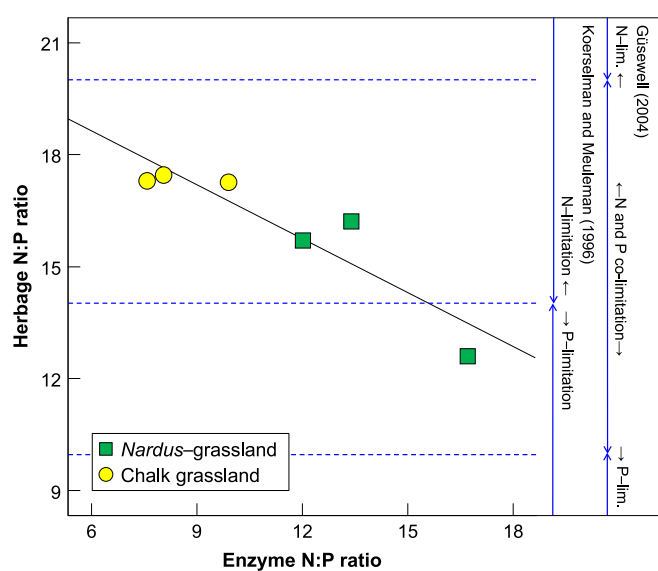
Fig. 5. Relationships between the N:P ratio of the herbage composition and the soil enzyme activities. Critical levels for N versus P limitation according to Koerselman and Meuleman (1996) and Güsewell (2004) are provided as references.

(Hawkesford et al., 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, both types of grasslands have been shown to spread with decreasing P (Klaudisová et al., 2009) or increasing N (Bobbink et al., 1989; Wilson et al., 1995; Leith et al., 1999; Stevens et al., 2011) availability. 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 of Badía-Villas et al. (2020) that ^{15}N fractionation was greater in chalk grasslands than in *Nardus* grasslands, which suggests that nutrients other than N are more strongly limited in chalk grasslands (Xu et al., 2014).

The N:S ratios of the herbage were below the critical values established by Stevens and Watson (1986), Mathot et al. (2005) and Ryant and Skládanka (2009), which indicates the sufficiency of S in both vegetation types. In contrast, the N:K and P:K ratios were well above the various critical values proposed by 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.

4.2. 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+} exhibited significant differences between the two grassland types.

Ammonium–N was greater 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 concentrations might result from greater N inputs from livestock excreta, but this is likely not the case in the *Nardus* grassland since livestock generally spend less time in the *Nardus* grassland than in the chalk grassland due to the greater fodder quality of the latter (Badía et al., 2008; García–González et al., 1990). Instead, the higher ammonium concentrations in the *Nardus* grassland soils could be related to more active N cycling (Zhou et al., 2022), as supported by the correlation between ammonium–N and URE and the more acidic conditions, which mitigate volatilization losses (Woodmansee et al., 1981; Cameron et al., 2013).

Calcium was the only element among the nutrients studied that exhibited 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 and can rather lead to stress from other nutrients (Arnesen et al., 2007; Körner, 2021). Plant Ca and soil Ca showed a strong negative relationship with the herbage P levels, suggesting an adverse effect of Ca on P intake, leading to greater limitation by P than by N and higher enzyme demand for P versus N in the chalk grassland than in the *Nardus* grassland. A plausible mechanism for this effect is the precipitation of Ca-phosphates, which is typical of high-pH soils but can also occur at pH values less than 7 in the presence of high exchangeable Ca^{2+} (Penn and Camberato, 2019).

Phosphorus exists in soils in several inorganic (P$_i$) and organic (P$_o$) forms (most of which were not measured in the present study) and only a small portion is labile, i.e. easily accessible to organisms (Yang and Post, 2011; Li et al., 2022). Calcareous-derived soils can contain substantial amounts of P$_i$ in Ca-primary minerals that are low-soluble and, therefore, poorly bioavailable (Martin-Sanz et al., 2021; Li et al., 2022). The slow turnover of organic matter in high-mountain soils favors the buildup of P$_o$ and its stabilization via interaction with soil minerals (Li et al., 2022); however, P$_o$ requires its biochemical conversion to become

bioavailable, and usually only a small fraction is susceptible to release available P after phosphatase reaction (Margalef et al., 2017). Microbial biomass-P constitutes an important pool within P$_o$ that can act as a P source through mineralization, but in high-mountain soils, it often acts as a sink by immobilizing large amounts of P, even larger than the P reservoir in vegetation (Wang et al., 2020). Although the total amount of P in the soils of high-mountain grasslands may be high, plant-available P is usually scarce and often growth-limiting (Zhou et al., 2020). Hence plants in these habitats have evolved strategies to enhance P-acquisition by stablishing mycorrhizal symbioses or secreting compounds that stimulate microbial activity and facilitate P release (Zhou et al., 2021; Andrade-Linares et al., 2023). Phosphorus-acquisition traits differ by plant functional type: graminoids prioritize improving P capture via association with arbuscular mycorrhizal fungi, whereas forbs adopt more diverse strategies including secretion of phosphatase and organic acids to mobilize P from the soil (Zhou et al., 2021; Guan et al., 2024). The present study addressed only one of these mechanisms, namely phosphatase secretion. According to our results, the soils of the chalk grassland (where forbs are predominant) exhibited a higher PHO vs. URE activity ratio, suggesting the secretion of excess phosphatase enzyme as a response to stronger P vs. N limitation. Neither the herbage P nor its ratios to other elements were related to soil Olsen P. Olsen-P only measures a portion of labile P including water-soluble P and some P$_i$ found in Ca-phosphates (Olsen et al., 1954), therefore it may not be an adequate measure of P availability when P inputs are mediated by arbuscular mycorrhizal symbiosis, P$_o$ mineralization or P mobilization by root exudates (Schneider, 2014; Klaus et al., 2016).

The composition of the herbage denoted a severe K deficiency that cannot be solely explained by the 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 during their uptake by plants, so high Ca concentrations in the presence of low–moderate K can decrease K intake enough to induce K deficiency (Cahoon and Crummett, 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).

4.3. Effects of plant traits on plant composition

The organic and elemental compositions of plants are genetically and physiologically controlled and vary between species and, to a more limited extent, among individuals (Kay et al., 2005; Elser et al., 2010). The results of the present study revealed 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* versus 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 CEL and HEM and poorer in ADL, as is characteristic of graminoids compared to nongraminoid herbs (Gordon, 1989; Marinas and García-González, 2006; Poca et al., 2014). The abundance of structural carbohydrates stimulates GLU activity (Sinsabaugh et al., 2008; Wang et al., 2020)), which is positively correlated with the concentrations of CEL and HEM, which act as substrates. A greater amount of structural carbohydrates reflects a greater investment in grass stems (ADL was a minor constituent in both grasslands), indicating greater density and stronger competition for light in *Nardus* grassland than in chalk grassland, which is consistent with less severe nutrient limitation (Irving, 2015; Postma et al., 2021; Rehling et al., 2021). Indeed, *Nardus* grassland tends to be denser and taller than chalk grassland, which could effectively indicate more competition for light.

The greater proportion of graminoids in the *Nardus* grassland was

also related to higher P and lower Ca concentrations in herbage, which agrees with the findings of Marinas and García-González (2006) that higher P and lower Ca levels occur in graminoids than in nongraminoids in subalpine Pyrenean grasslands. A greater investment in stem structures can affect the 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 resulted in lower N:P ratios in the *Nardus* grassland than in the chalk grassland, which contradicts the usual finding that a higher proportion of graminoids is associated with higher N:P ratios and, consequently, with increasing limitation of P versus N (Güsewell, 2004; He et al., 2006). In turn, forbs and legumes are generally reported to contain more Ca than graminoids (Meerts, 1997; Mladkova et al., 2018; Kajzrová et al., 2022), which is related 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; Mladkova et al., 2018).

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

4.4. Topographic controls on elemental stoichiometry

Heterogeneous element 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 diverse niche spaces for plants with different requirements (Antonelli et al., 2018). Microtopography contributes greatly to mountain soil heterogeneity (Hiller and Müterthies, 2005; Holtmeier and Broll, 2018) and has been shown to have a considerable effect on nutrient distribution in calcareous ranges (Sebastiá 2004; Michalet et al., 2002; Giaccone et al., 2019), being even more influential than the calcareous vs. siliceous nature of bedrock (Michalet et al., 2002). Distinguishing topographic effects 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 related to limitations due to excess Ca that were dependent on local topography. Erosion in mountain areas is usually uneven due to the complex topography, with local areas experiencing both soil thinning and thickening (Amundson et al., 2015). 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 decalcification (Amundson et al., 2015). In the study area, uneven erosion has led to the small-scale co-occurrence of soils with contrasting degrees of evolution (e.g., Hypereutric Leptosols and Orthoeutric Cambisols) on a few meters scale within the same slope (Badía-Villas et al., 2020). On the Hypereutric Leptosols, higher Ca results in increased P limitation, causing them to be colonized by the chalk

grassland, which can be regarded as a typical calcicole adapted to (but likely not demanding) extreme Ca contents. In contrast, the *Nardus* grassland, which is not typically calcifuge but still intolerant to excess Ca, grows on Orthoeutric Cambisols where P limitation has been alleviated by decalcification. The lower biomass of the chalk grasslands on Leptosols allows for further soil erosion (Liu et al., 2018) as opposed to the greater stability of the Cambisols of the *Nardus* grassland, which contributes to stabilizing the mosaic of soils and plant communities by positive feedback mechanisms (Armas-Herrera et al., 2020). To our knowledge, reports on Ca-induced P limitation driven by local topography are scarce for upland areas, but this phenomenon is well known 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.

5. 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 the soil and Ca uptake by plants. Among the nutrients studied, Ca was the only one whose concentrations in herbage and soil were correlated, both being greater in the chalk grassland soils than in the *Nardus* grassland soils. High Ca in soil adversely affects P intake (as shown by decreasing plant P with increasing plant Ca and soil Ca), leading to a greater prevalence of P limitation than N limitation (as measured by the plant N:P ratio), which was associated with greater enzyme demand for P relative to N (as assessed from the soil enzyme N:P ratios). In *Nardus* grassland, the alleviation of P limitation results in increased productivity and the development of tall, dense graminoid vegetation, which opposes the general idea that graminoids have a greater N:P ratio than do forbs. The greater investment in stem-like structures in the *Nardus* grassland was mirrored by greater concentrations of C and structural carbohydrates in the herbage and higher potential carbohydrate-degrading activity in the soil. The S status is sufficient in both grasslands, as inferred from its high levels in soil and low plant N:S ratio. In turn, a high Ca:K ratio results in strong K deficiency in both grassland types as a consequence of the antagonistic effect of Ca on K uptake.

CRediT authorship contribution statement

Juan Luis Mora: . **Cecilia Armas–Herrera:** Writing – review & editing, Investigation, Conceptualization. **Daniel Gómez:** Writing – review & editing, Investigation, Conceptualization. **David Badía–Villas:** Writing – review & editing, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

Appendix A. Supplementary material

Supplementary material to this article can be found online at https ://doi.org/10.1016/j.catena.2024.108192.

References

Adetunji, A.T., Lewu, F.B., Mulidzi, R., Ncube, B., 2017. The biological activities of β–glucosidase, phosphatase and urease as soil quality indicators: a review. J. Soil Sci. Plant Nutr. 17 (3), 794–807. https://doi.org/10.4067/S0718-95162017000300018.

Ågren, G.I., 2008. Stoichiometry and nutrition of plant growth in natural communities. Annu. Rev. Ecol. Evol. Syst. 39, 153–170. https://doi.org/10.1146/annurev.ecolsys.39.110707.173515.

Allison, S.D., Vitousek, P.M., 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biol. Biochem. 37 (5), 937–944. https://doi.org/10.1016/j.soilbio.2004.09.014.

Amagai, Y., Kudo, G., Sato, K., 2018. Changes in alpine plant communities under climate change: Dynamics of snow-meadow vegetation in northern Japan over the last 40 years. Appl. Veg. Sci. 21 (4), 561–571. https://doi.org/10.1111/avsc.12387.

Amundson, R., Heimsath, A., Owen, J., Yoo, K., Dietrich, W.E., 2015. Hillslope soils and vegetation. Geomorphology 234, 122–132. https://doi.org/10.1016/j.geomorph.2014.12.031.

Andrade-Linares, D.R., Schwerdtner, U., Schulz, S., Dannenmann, M., Spohn, M., Baum, C., Gasche, R., Wiesmeier, M., García-Franco, N., Schloter, M., 2023. Climate change and management intensity alter spatial distribution and abundance of P mineralizing bacteria and arbuscular mycorrhizal fungi in mountainous grassland soils. Soil Biol. Biochem. 186, 109175 https://doi.org/10.1016/j.soilbio.2023.109175.

Antonelli, A., Kissling, W.D., Flantua, S.G., Bermúdez, M.A., Mulch, A., Muellner-Riehl, A.N., Kreft, H., Linder, H.P., Badgley, C., Fjeldså, J., Fritz, S.A., Rahbek, C., Herman, F., Hooghiemstra, H., Hoorn, C., 2018. Geological and climatic influences on mountain biodiversity. Nat. Geosci. 11, 718–a725. https://doi.org/10.1038/s41561-018-0236-z.

AOAC, 2006. Official Methods of Analysis. Association of Official Analytical Chemists, Gaithersburg MD.

Armas-Herrera, C.M., Badía-Villas, D., Mora, J.L., Gómez, D., 2020. Plant–topsoil relationships underlying subalpine grassland patchiness. Sci. Total Environ. 712, 134483 https://doi.org/10.1016/j.scitotenv.2019.134483.

Arnesen, G., Beck, P.S., Engelskjøn, T., 2007. Soil acidity, content of carbonates, and available phosphorus are the soil factors best correlated with alpine vegetation: evidence from troms. North Norway. Arct. Antarct. Alp. Res. 39 (2), 189–199. https://doi.org/10.1657/1523-0430(2007)39[189:SACOCA]2.0.CO;2.

Austin, M.P., 1968. An ordination study of a chalk grassland community. J. Ecol. 56 (3), 739–757. https://doi.org/10.2307/2258104.

Badea, S.L., Ionete, R.E., Costinel, D., Nechita, C., Mihai, B., Botoran, O.R., 2020. Changes of carbon–isotope ratios in soil organic matter relative to parent vegetation and site specificity. Not Bot Horti Agrobot Cluj Napoca 48 (4), 2085–2094. https://doi.org/10.15835/nbha48412138.

Badía, D., Martí, C., Sánchez, J.R., Fillat, F., Aguirre, J., Gómez, D., 2008. Influence of livestock soil eutrophication on floral composition in the Pyrenees mountains. J. Mt. Sci. 5, 63–72. https://doi.org/10.1007/s11629-008-0063-6.

Badía-Villas, D., Buendía-García, L., Longares-Aladrén, L.A., Martí-Dalmau, C., Peña-Monné, J.L., González-Pérez, J.A., Gómez-García, D., 2020. Soil–geomorphology relationships determine the distribution of the main subalpine grasslands in the Central Pyrenees (NE–Spain). Sci. Total Environ. 734, 139121 https://doi.org/10.1016/j.scitotenv.2020.139121.

Bassin, S., Schalajda, J., Vogel, A., Suter, M., 2012. Different types of sub-alpine grassland respond similarly to elevated nitrogen deposition in terms of productivity and sedge abundance. J. Veg. Sci. 23 (6), 1024–1034. https://doi.org/10.1111/j.1654-1103.2012.01422.x.

Bhople, P., Keiblinger, K., Djukic, I., Liu, D., Zehetner, F., Zechmeister-Boltenstern, S., Joergensen, R.G., Murugan, R., 2021. Microbial necromass formation, enzyme activities and community structure in two alpine elevation gradients with different bedrock types. Geoderma 386, 114922. https://doi.org/10.1016/j.geoderma.2020.114922.

Bobbink, R.D., den Dubbelden, K., Willems, J.H., 1989. Seasonal dynamics of phytomass and nutrients in chalk grassland. Oikos 55 (2), 216–224. https://doi.org/10.2307/3565425.

Boeye, D., Verhagen, B., Van Haesebroeck, V., Verheyen, R.F., 1997. Nutrient limitation in species-rich lowland fens. J. Veg. Sci. 8 (3), 415–424. https://doi.org/10.2307/3237333.

Boyer, M.L.H., Wheeler, B.D., 1989. Vegetation patterns in spring-fed calcareous fens: Calcite precipitation and constraints on fertility. J. Ecol. 77 (2), 597–609. https://doi.org/10.2307/2260772.

Braun-Blanquet, J., Jenny, H., 1926. Vegetations–entwicklung und Bodenbildung in der alpinen Stufe der Zentralalpen. Denkschriften Der Schweizerischen Naturforschenden Gesellschaft 63, 183–349.

Bremner, J.M., 1965. Inorganic forms of nitrogen. In: Black, C.A. (Ed.), Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties. American Society of Agronomy, Madison WI, pp. 1179–1237. https://doi.org/10.2134/agronmonogr9.2.c33.

Buri, A., Grand, S., Yashiro, E., Adatte, T., Spangenberg, J.E., Pinto-Figueroa, E., Verrecchia, E., Guisan, A., 2020. What are the most crucial soil variables for predicting the distribution of mountain plant species? a comprehensive study in the Swiss Alps. J. Biogeogr. 47 (5), 1143–1153. https://doi.org/10.1111/jbi.13803.

Busqué, J., Bedia, J., 2013. Productivity, grazing utilization, forage quality and primary production controls of species-rich alpine grasslands with *Nardus stricta* in northern

Spain. Grass Forage Sci. 68 (2), 297–312. https://doi.org/10.1111/j.1365-2494.2012.00903.x.

Cahoon, G., Crummett, D., 1954. Mineral nutrition of stocks: Need for potassium can be anticipated by soil analysis to determine calcium–potassium ratio. Calif Agric (Berkeley) 8, 10–15.

Cameron, K.C., Di, H.J., Moir, J.L., 2013. Nitrogen losses from the soil/plant system: a review. Ann. Appl. Biol. 162 (2), 145–173. https://doi.org/10.1111/aab.12014.

Cross, A.T., Lambers, H., 2021. Calcicole–calcifuge plant strategies limit restoration potential in a regional semi-arid flora. Ecol. Evol. 11 (11), 6941–6961. https://doi.org/10.1002/ece3.7544.

Cui, Y., Bing, H., Fang, L., Jiang, M., Shen, G., Yu, J., Wang, X., Zhu, H., Wu, Y., Zhang, X., 2021. Extracellular enzyme stoichiometry reveals the carbon and phosphorus limitations of microbial metabolisms in the rhizosphere and bulk soils in alpine ecosystems. Plant and Soil 458, 7–20. https://doi.org/10.1007/s11104-019-04159-x.

Dampney, P.M.R., 1992. The effect of timing and rate of potash application on the yield and herbage composition of grass grown for silage. Grass Forage Sci. 47 (3), 280–289. https://doi.org/10.1111/j.1365-2494.1992.tb02272.x.

De Bilde, J., 1978. Nutrient adaptation in native and experimental calcicolous and siliceous populations of *Silene nutans*. Oikos 31 (3), 383–391. https://doi.org/10.2307/3543666.

Di Palo, F., Fornara, D.A., 2017. Plant and soil nutrient stoichiometry along primary ecological successions: Is there any link? PLoS One 12 (8), e0182569.

Egli, M., Poulenard, J., 2016. Soils of mountainous landscapes. In: Richardson, D., Castree, N., Goodchild, M.F., Kobayashi, A., Liu, W., Marston, R.A. (Eds.), International Encyclopedia of Geography: People, the Earth, Environment and Technology. Wiley & Sons, Oxford UK, pp. 1e–e. https://doi.org/10.1002/9781118786352.wbieg0197.

Eivazi, F., Tabatabai, M.A., 1988. Glucosidases and galactosidases in soils. Soil Biol. Biochem. 20 (5), 601–606. https://doi.org/10.1016/0038-0717(88)90141-1.

Elser, J.J., Fagan, W.F., Kerkhoff, A.J., Swenson, N.G., Enquist, B.J., 2010. Biological stoichiometry of plant production: Metabolism, scaling and ecological response to global change. New Phytol. 186 (3), 593–608. https://doi.org/10.1111/j.1469-8137.2010.03214.x.

Freschet, G.T., Valverde-Barrantes, O.J., Tucker, C.M., Craine, J.M., McCormack, M.L., Violle, C., Fort, F., Blackwood, C.B., Urban-Mead, K.R., Iversen, C.M., Bonis, A., Comas, L.H., Cornelissen, J.H.C., Dong, M., Guo, D., Hobbie, S.E., Holdaway, R.J., Kembel, S.K., Makita, N., Onipchenko, V.G., Picon-Cochard, C., Reich, P.B., de la Riva, E.G., Smith, S.W., Soudzilovskaia, N.A., Tjoelker, M.G., Wardle, D.A., Roumet, C., 2017. Climate, soil and plant functional types as drivers of global fine–root trait variation. J. Ecol. 105 (5), 1182–1196. https://doi.org/10.1111/1365-2745.12769.

Galvánek, D., Janák, M., 2008. Management of Natura 2000 habitats. 6230 *Species–rich Nardusgrasslands. European Commission.

García–González, R., Hidalgo, R., Montserrrat, C., 1990. Patterns of livestock use in time and space in the summer ranges of the western Pyrenees: a case study in the Aragon Valley. Mt. Res. Dev. 10 (3), 241–255. https://doi.org/10.2307/3673604.

Giaccone, E., Luoto, M., Vittoz, P., Guisan, A., Mariéthoz, G., Lambiel, C., 2019. Influence of microclimate and geomorphological factors on alpine vegetation in the Western Swiss Alps. Earth Surf. Proc. Land. 44 (15), 3093–3107. https://doi.org/10.1002/esp.4715.

Gordon, I., 1989. Vegetation community selection by ungulates on the Isle of Rhum. I. Food Supply. J Appl Ecol 26 (31), 35–51. https://doi.org/10.2307/2403649.

Guan, Z.H., Cao, Z., Li, X.G., Scholten, T., Kühn, P., Wang, L., Yu, R.P., He, J.S., 2024. Soil phosphorus availability mediates the effects of nitrogen addition on community- and species-level phosphorus-acquisition strategies in alpine grasslands. Sci. Total Environ. 906, 167630 https://doi.org/10.1016/j.scitotenv.2023.167630.

Güsewell, S., 2004. N: P ratios in terrestrial plants: Variation and functional significance. New Phytol. 164 (2), 243–266. https://doi.org/10.1111/j.1469-8137.2004.01192.x.

Hawkesford, M., Horst, W., Kichey, T., Lambers, H., Schjoerring, J., Skrumsager Møller, I., White, P., 2012. Chapter 6 - Functions of Macronutrients. In: Marschner, P. (Ed.), Marschner's Mineral Nutrition of Higher Plants (Third Edition). Academic Press, London, pp. 135–189. doi: 10.1016/B978-0-12-384905-2.00006-6.

He, J.S., Fang, J., Wang, Z., Guo, D., Flynn, D.F., Geng, Z., 2006. Stoichiometry and large–scale patterns of leaf carbon and nitrogen in the grassland biomes of China. Oecologia 149, 115–122. https://doi.org/10.1007/s00442-006-0425-0.

He, J.S., Wang, L., Flynn, D.F., Wang, X., Ma, W., Fang, J., 2008. Leaf nitrogen: phosphorus stoichiometry across Chinese grassland biomes. Oecologia 155, 301–310. https://doi.org/10.1007/s00442-007-0912-y.

Hejcman, M., Jouany, C., Cruz, P., Morel, C., Stroia, C., Theau, J.P., 2014. Subsoil P status could explain the absence of resilience in plant species composition of subalpine grassland 63 years after the last fertilizer application. Sci. Agric. Bohem. 45 (2), 75–84. https://doi.org/10.7160/sab.2014.450201.

Hiller, B., Müterthies, A., 2005. Humus forms and reforestation of an abandoned pasture at the alpine timberline (Upper Engadine, Central Alps, Switzerland). In: Broll, G., Keplin, B. (Eds.), Mountain Ecosystems. Springer, Berlin-Heidelberg, pp. 203–218. https://doi.org/10.1007/3-540-27365-4_8.

Holtmeier, F.K., Broll, G., 2018. Soils at the altitudinal and northern treeline: European Alps, northern Europe, Rocky Mountains—a review. Insights for Res 2 (1), 67–83. https://doi.org/10.36959/948/463.

Hong, J., Wang, X., Wu, J., 2015. Effects of soil fertility on the N: P stoichiometry of herbaceous plants on a nutrient-limited alpine steppe on the northern Tibetan Plateau. Plant and Soil 391, 179–194. https://doi.org/10.1007/s11104-015-2416-6.

Horneck, D.A., Sullivan, D.M., Owen, J.S., Hart, J.M., 2011. Soil interpretation guide. Oregon State University Extension Service, Corvallis OR. Accessed November 3, 2022. https://extension.oregonstate.edu/pub/ec-1478.

Irving, L.J., 2015. Carbon assimilation, biomass partitioning and productivity in grasses. Agriculture 5 (4), 1116–1134. https://doi.org/10.3390/agriculture5041116.

Jones Jr, J.B., 2001. Laboratory guide for conducting soil tests and plant analysis. CRC Press, Boca Raton FL. https://doi.org/10.1201/9781420025293.

Kajzrová, K., Kassahun, T., Pavlů, L., Pavlů, V.V., Fraser, M.D., 2022. The effect of 19 years of restoration managements on forage quality and herbage-soil relationships within improved upland grassland. Grass Forage Sci. 77 (3), 167–174. https://doi.org/10.1111/gfs.12576.

Kaňa, J., Kopáček, J., Camarero, L., Garcia-Pausas, J., 2011. Phosphate sorption characteristics of European alpine soils. Soil Sci. Soc. Am. J. 75 (3), 862–870. https://doi.org/10.2136/sssaj2010.0259.

Karaca, A., Cetin, S.C., Turgay, O.C., Kizilkaya, R., 2010. Soil enzymes as indication of soil quality. In: Shukla, G., Varma, A. (Eds.), Soil Enzymology. Springer, Berlin-Heidelberg, pp. 119–148. https://doi.org/10.1007/978-3-642-14225-3_7.

Kay, A.D., Ashton, I.W., Gorokhova, E., Kerkhoff, A.J., Liess, A., Litchman, E., 2005. Toward a stoichiometric framework for evolutionary biology. Oikos 109 (1), 6–17. https://doi.org/10.1111/j.0030-1299.2005.14048.x.

Kirkham, F.W., 2001. Nitrogen uptake and nutrient limitation in six hill moorland species in relation to atmospheric nitrogen deposition in England and Wales. J. Ecol. 89 (6), 1041–1053. https://doi.org/10.1111/j.1365-2745.2001.00626.x.

Kishchuk, B.E., 2000. Calcareous soils, their properties and potential limitations to conifer growth in southeastern British Columbia and western Alberta: A literature review. Canadian Forest Service, Edmonton AL.

Klaudisová, H., Hejcman, M., Pavlů, V., 2009. Long-term residual effect of short-term fertilizer application on Ca, N and P concentrations in grasses *Nardus stricta* L. and *Avenella flexuosa* L. Nutr. Cycl. Agroecosyst. 85, 187–193. https://doi.org/10.1007/s10705-009-9257-8.

Klaus, V.H., Boch, S., Boeddinghaus, R.S., Hölzel, N., Kandeler, E., Marhan, S., Oelmann, Y., Prati, D., Regan, K.M., Schmitt, B., Sorkau, E., Kleinebecker, T., 2016. Temporal and small-scale spatial variation in grassland productivity, biomass quality, and nutrient limitation. Plant Ecol. 217, 843–856. https://doi.org/10.1007/s11258-016-0607-8.

Knecht, M.F., Göransson, A., 2004. Terrestrial plants require nutrients in similar proportions. Tree Physiol. 24 (4), 447–460. https://doi.org/10.1093/treephys/24.4.447.

Koerselman, W., Meuleman, A.F., 1996. The vegetation N: P ratio: a new tool to detect the nature of nutrient limitation. J. Appl. Ecol. 33 (6), 1441–1450. https://doi.org/10.2307/2404783.

Köhler, B., Ryser, P., Güsewell, S., Gigon, A., 2001. Nutrient availability and limitation in traditionally mown and in abandoned limestone grasslands: a bioassay experiment. Plant and Soil 230, 323–332. https://doi.org/10.1023/A:1010335825818.

Korcak, R.F., 1987. Satisfying and altering edaphic requirements for acidophilic plants. J. Plant Nutr. 10 (9–16), 1071–1078. https://doi.org/10.1080/01904168709363635.

Körner, C., 2004. Mountain biodiversity, its causes and function. Ambio 33 (sp13), 11–17. https://doi.org/10.1007/0044-7447-33.sp13.11.

Körner, C., 2021. Alpine plant life: Functional plant ecology of high mountain ecosystems, Third edition. Springer–Verlag, Berlin–Heidelberg. https://doi.org/10.1007/978-3-030-59538-8.

Lambers, H., Oliveira, R.S., 2019. Mineral nutrition. In: Lambers, H., Oliveira, R.S. (Eds.), Plant Physiological Ecology. Springer, Cham, pp. 301–384. https://doi.org/10.1007/978-3-030-29639-1_9.

Lasota, J., Babiak, T., Błońska, E., 2022. C: N: P stoichiometry associated with biochar in forest soils at historical charcoal production sites in Poland. Geoderma Reg. 28, e00482.

Lawniczak, A.E., Güsewell, S., Verhoeven, J.T.A., 2009. Effect of N: K supply ratios on the performance of three grass species from herbaceous wetlands. Basic Appl. Ecol. 10 (8), 715–725. https://doi.org/10.1016/j.baae.2009.05.004.

Leith, I.D., Hicks, W.K., Fowler, D., Woodin, S.J., 1999. Differential responses of UK upland plants to nitrogen deposition. New Phytol. 141 (2), 277–289. https://doi.org/10.1046/j.1469-8137.1999.00333.x.

Leuschner, C, Ellenberg, H., 2017. In: Leuschner, C., Ellenberg, H. (Eds.), Ecology of central European non–forest vegetation: Coastal to alpine, natural to man–made habitats. Vegetation ecology of Central Europe, Volume II. Springer, Cham, pp. 435–494. https://doi.org/10.1007/978-3-319-43048-5_6.

Li, C., Luo, S., Ge, S., Zhao, L., Dong, Q., Li, Q., Zhao, X., 2019. Convergent and divergent responses of topsoil nitrogen and phosphorus content to nutrient addition in natural and restored alpine grasslands around the Qinghai Lake Basin. Agr Ecosyst Environ. 282, 1–12. https://doi.org/10.1016/j.agee.2019.05.010.

Li, Z., Zhang, D., Peng, Y., Qin, S., Wang, L., Hou, E., Yang, Y., 2022. Divergent drivers of various topsoil phosphorus fractions across Tibetan alpine grasslands. J. Geophys. Res.-Biogeo 127 (4), e2022JG006795. https://doi.org/10.1029/2022JG006795.

Lin, L., Cao, G., Xu, X., Li, C., Fan, B., Li, B., Lan, Y., Si, M., Dai, L., 2022. Changes and relationships between components in the plant-soil system and the dominant plant functional groups in alpine *Kobresia* meadows due to overgrazing. Diversity 14 (3), 183. https://doi.org/10.3390/d14030183.

Liu, J., Chen, J., Chen, G., Guo, J., Li, Y., 2020. Enzyme stoichiometry indicates the variation of microbial nutrient requirements at different soil depths in subtropical forests. PLoS One 15 (2), e0220599. https://doi.org/10.1371/journal.pone.0220599.

Liu, S., Zamanian, K., Schleuss, P.M., Zarebanadkouki, M., Kuzyakov, Y., 2018. Degradation of Tibetan grasslands: Consequences for carbon and nutrient cycles. Agr. Ecosyst. Environ. 252, 93–104. https://doi.org/10.1016/j.agee.2017.10.011.

Luo, L., Meng, H., Gu, J.D., 2017. Microbial extracellular enzymes in biogeochemical cycling of ecosystems. J. Environ. Manage. 197, 539–549. https://doi.org/10.1016/j.jenvman.2017.04.023.

Margalef, O., Sardans, J., Fernández-Martínez, M., Molowny-Horas, R., Janssens, I.A., Ciais, P., Goll, D., Richter, A., Obersteiner, M., Asensio, D., Peñuelas, J., 2017. Global patterns of phosphatase activity in natural soils. Sci. Rep. 7 (1), 1337. https://doi.org/10.1038/s41598-017-01418-8.

Marinas, A., García-González, R., 2006. Preliminary data on nutritional value of abundant species in supraforestal Pyrenean pastures. Pirineos 161, 85–110. https://doi.org/10.3989/pirineos.2006.v161.4.

Martin-Sanz, R.C., Pando, V., Bueis, T., Turrion, M.B., 2021. Influence of soil properties on P pools and its effect on forest productivity in mediterranean calcareous soils. Forests 12 (10), 1398. https://doi.org/10.3390/f12101398.

Mathot, M., Lambert, R., Toussaint, B., Peeters, A., 2005. Total sulphur content and N: S ratio as indicators for S deficiency in grasses. In: Jarvis, S.C., Murray, P.J., Roker, J.A. (Eds.), Optimisation of Nutrient Cycling and Soil Quality for Sustainable Grasslands. Wageningen Academic Press, p. 90. https://doi.org/10.3920/978-90-8686-556-7.

Meerts, P., 1997. Foliar macronutrient concentrations of forest understorey species in relation to Ellenberg's indices and potential relative growth rate. Plant and Soil 189, 257–265. https://doi.org/10.1023/A:1004215322004.

Michalet, R., Gandoy, C., Joud, D., Pagès, J.P., Choler, P., 2002. Plant community composition and biomass on calcareous and siliceous substrates in the northern French Alps: comparative effects of soil chemistry and water status. Arct. Antarct. Alp. Res. 34 (1), 102–113. https://doi.org/10.1080/15230430.2002.12003474.

Mladek, J., Mladek, J., Mladek, S., Hejcman, M., Pakeman, R.J., 2018. Calcium plus magnesium indicates digestibility: The significance of the second major axis of plant chemical variation for ecological processes. Ecol. Lett. 21 (6), 885–895. https://doi.org/10.1111/ele.12956.

Motsara, M.R., Roy, R.N., 2008. Guide to laboratory establishment for plant nutrient analysis. Food and Agriculture Organization of the United Nations, Rome.

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5.

Niinemets, Ü., Kull, K., 2005. Co–limitation of plant primary productivity by nitrogen and phosphorus in a species–rich wooded meadow on calcareous soils. Acta Oecol. 28 (3), 345–356. https://doi.org/10.1016/j.actao.2005.06.003.

Niklaus, P.A., Leadley, P.W., Stöcklin, J., Körner, C., 1998. Nutrient relations in calcareous grassland under elevated CO_2. Oecologia 116, 67–75. https://doi.org/10.1007/s004420050564.

Olde Venterik, H., Wassen, M.J., Verkroost, A.W.M., De Ruiter, P.C., 2003. Species richness–productivity patterns differ between N–, P–, and K–limited wetlands. Ecology 84 (8), 2191–2199. https://doi.org/10.1890/01-0639.

Olsen, R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate. In: USDA Circ, 939. United States Division of Agronomy, Washington, DC.

Pegtel, D.M., Bakker, J.P., Verweij, G.L., Fresco, L.E.M., 1996. N, K and P deficiency in chronosequential cut summer–dry grasslands on gley podzol after cessation of fertilizer application. Plant and Soil 178, 121–131. https://doi.org/10.1007/BF00011170.

Penn, C.J., Camberato, J.J., 2019. A critical review on soil chemical processes that control how soil pH affects phosphorus availability to plants. Agriculture 9 (6), 120. https://doi.org/10.3390/agriculture9060120.

Poca, M., Pérez-Harguindeguy, N., Vaieretti, M.V., Cingolani, A.M., 2014. Descomposición y calidad físico–química foliar de 24 especies dominantes de los pastizales de altura de las sierras de Córdoba. Argentina Ecol Austral 24 (2), 249–257. https://doi.org/10.25260/EA.14.24.2.0.28.

Postma, J.A., Hecht, V.L., Hikosaka, K., Nord, E.A., Pons, T.L., Poorter, H., 2021. Dividing the pie: a quantitative review on plant density responses. Plant Cell Environ. 44 (4), 1072–1094. https://doi.org/10.1111/pce.13968.

Rahbek, C., Borregaard, M.K., Antonelli, A., Colwell, R.K., Holt, B.G., Nogues-Bravo, D., Rasmussen, C.M.Ø., Richardson, K., Rosing, M.T., Whittaker, R.J., Fjeldså, J., 2019. Building mountain biodiversity: Geological and evolutionary processes. Science 365 (6458), 1114–1119. https://doi.org/10.1126/science.aax0151.

Rehling, F., Sandner, T.M., Matthies, D., 2021. Biomass partitioning in response to intraspecific competition depends on nutrients and species characteristics: a study of 43 plant species. J. Ecol. 109 (5), 2219–2233. https://doi.org/10.1111/1365-2745.13635.

Ryant, P., Skládanka, J., 2009. The effect of applications of various forms of sulfur on the yields and quality of grass forage. Acta Agric. Scand. B. Soil Plant Sci. 59 (3), 208–216. https://doi.org/10.1080/09064710802011526.

Saá, A., Trasar-Cepeda, M.C., Gil-Sotres, F., Carballas, T., 1993. Change in soil phosphorus and acid phosphatase activity immediately following forest fires. Soil Biol. Biochem. 25 (9), 1223–1230. https://doi.org/10.1016/0038-0717(93)90218-Z.

Sardans, J., Peñuelas, J., 2014. Climate and taxonomy underlie different elemental concentrations and stoichiometries of forest species: the optimum "biogeochemical niche". Plant Ecol. 215, 441–455. https://doi.org/10.1007/s11258-014-0314-2.

Schelfhout, S., Mertens, J., Perring, M.P., Raman, M., Baeten, L., Demey, A., Reubens, B., Oosterlynck, P., Gibson-Roy, P., Verheyen, K., Schrijver, A.D., 2017. P-removal for restoration of *Nardus* grasslands on former agricultural land: cutting traditions. Restor. Ecol. 25 (S2), S178–S187. https://doi.org/10.1111/rec.12531.

Schneider, K.D., 2014. Understanding Biological Contributions to Phosphorus Availability in Organic Dairy Farm Soils. Ph. D. thesis. University of Guelph, Guelph ON.

Sebastià, M.T., 2004. Role of topography and soils in grassland structuring at the landscape and community scales. Basic Appl. Ecol. 5 (4), 331–346. https://doi.org/10.1016/j.baae.2003.10.001.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecol. Lett. 11 (11), 1252–1264. https://doi.org/10.1111/j.1461-0248.2008.01245.x.

Smith, C.J., 1980. Ecology of the English chalk. Academic Press, London.

Stevens, C.J., Duprè, C., Gaudnik, C., Dorland, E., Dise, N.B., Gowing, D., Bleeker, A., Alard, D., Bobbink, R., Fowler, D., Vandvik, V., Corcket, E., Mountford, J.O., Aarrestad, P.A., Muller, S., Diekmann, M., 2011. Changes in species composition of European acid grasslands observed along a gradient of nitrogen deposition. J. Veg. Sci. 22 (2), 207–215. https://doi.org/10.1111/j.1654-1103.2010.01254.x.

Stevens, R.J., Watson, C.J., 1986. The response of grass for silage to sulphur application at 20 sites in Northern Ireland. J. Agric. Sci. 107 (3), 565–571. https://doi.org/10.1017/S0021859600069732.

Sumner, M.E., 1978. Interpretation of nutrient ratios in plant tissue. Commun. Soil Sci. Plant Anal. 9 (4), 335–345. https://doi.org/10.1080/00103627809366812.

Tabatabai, M.A., Bremner, J.M., 1970. Arylsulfatase activity of soils. Soil Sci. Soc. Am. J. 34 (2), 225–229. https://doi.org/10.2136/sssaj1970.03615995003400020016x.

Tabatabai, M.A., Bremner, J.M., 1972. Assay of urease activity in soils. Soil Biol. Biochem. 4 (4), 479–487. https://doi.org/10.1016/0038-0717(72)90064-8.

Tovar, C., Duivenvoorden, J.F., Sánchez-Vega, I., Seijmonsbergen, A.C., 2012. Recent changes in patch characteristics and plant communities in the jalca grasslands of the Peruvian Andes. Biotropica 44 (3), 321–330. https://doi.org/10.1111/j.1744-7429.2011.00820.x.

Van der Krift, T.A.J., Berendse, F., 2002. Root life spans of four grass species from habitats differing in nutrient availability. Funct. Ecol. 16 (2), 198–203. https://doi.org/10.1046/j.1365-2435.2002.00611.x.

Van Soest, P.V., Robertson, J.B., Lewis, B., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74 (10), 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology Biochem 19 (6), 703–707. https://doi.org/10.1016/0038-0717(87)90052-6.

Vonlanthen, C.M., Bühler, A., Veit, H., Kammer, P.M., Eugster, W., 2006. Alpine plant communities: A statistical assessment of their relation to microclimatological, pedological, geomorphological, and other factors. Phys. Geogr. 27 (2), 137–154. https://doi.org/10.2747/0272-3646.27.2.137.

Wang, R., Cao, Y., Wang, H., Dijkstra, F.A., Jiang, J., Zhao, R., Ma, W., Li, T., Dorodnikov, M., Wang, Z., Sardans, J., Peñuelas, J., 2020. Exogenous P compounds differentially interacted with N availability to regulate enzymatic activities in a meadow steppe. Eur. J. Soil Sci. 71 (4), 667–680. https://doi.org/10.1111/ejss.12906.

Wang, R., Ma, X., Yan, Y., 2017. Effects of soil C:N: P stoichiometry on biomass allocation in the alpine and arid steppe systems. Ecol. Evol. 7 (5), 1354–1362. https://doi.org/10.1002/ece3.2710.

White, P.J., Broadley, M.R., 2003. Calcium in plants. Ann. Bot. 92 (4), 487–511. https://doi.org/10.1093/aob/mcg164.

Wilson, E.J., Wells, T.C.E., Sparks, T.H., 1995. Are calcareous grasslands in the UK under threat from nitrogen deposition? – an experimental determination of a critical load. J. Ecol. 83 (5), 823–832. https://doi.org/10.2307/2261419.

Woodmansee, R.G., Vallis, I., Mott, J.J., 1981. Grassland nitrogen. Ecol Bull. Georgian Academy Sci. 33, 443–462.

Xu, X., Wanek, W., Zhou, C., Richter, A., Song, M., Cao, G., Ouyang, H., Kuzyakov, Y., 2014. Nutrient limitation of alpine plants: Implications from leaf N: P stoichiometry and leaf δ^{15}N. J. Soil Sci. Plant Nutr. 177 (3), 378–387. https://doi.org/10.1002/jpln.201200061.

Yan, Y., Tian, L., Du, Z., Chang, S.X., Cai, Y., 2019. Carbon, nitrogen and phosphorus stocks differ among vegetation patch types in a degraded alpine steppe. J. Soil. Sediment. 19, 1809–1819. https://doi.org/10.1007/s11368-018-2191-0.

Yang, X., Post, W.M., 2011. Phosphorus transformations as a function of pedogenesis: a synthesis of soil phosphorus data using Hedley fractionation method. Biogeosciences 8 (10), 2907–2916. https://doi.org/10.5194/bg-8-2907-2011.

Zhou, H., Ma, A., Zhou, X., Chen, X., Zhang, J., Zhang, Q., Qi, X., Liu, G., Zhuang, G., 2022. Phosphorus shapes soil microbial community composition and network properties during grassland expansion into shrubs in Tibetan dry valleys. Front. Plant Sci. 13, 848691. https://doi.org/10.3389/fpls.2022.848691.

Zhou, J., Zúñiga-Feest, A., Lambers, H., 2020. In the beginning, there was only bare regolith—then some plants arrived and changed the regolith. J. Plant Ecol. 13 (5), 511–516. https://doi.org/10.1093/jpe/rtaa030.

Zhou, J., Li, X.L., Peng, F., Li, C., Lai, C., You, Q., Xue, X., Wu, Y., Sun, H., Chen, Y., Zhong, H., Lambers, H., 2021. Mobilization of soil phosphate after 8 years of warming is linked to plant phosphorus-acquisition strategies in an alpine meadow on the Qinghai-Tibetan Plateau. Glob. Chang. Biol. 27 (24), 6578–6591. https://doi.org/10.1111/gcb.15914.