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Agriculture, Ecosystems and Environment 387 (2025) 109622

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

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ARTICLE INFO

Dataset link: R code and data for forage mineral quality paper

Keywords:
Forage quality
Extensive grassland
Permanent grassland
Plant diversity

ABSTRACT

Compared to intensive grasslands, semi-natural grasslands host species-rich and functionally diversified plant communities, and thus represent habitats of high conservation interest. Less is known about their ability to provide high-quality forage fitting livestock requirements regarding mineral content in particular, and thus contribute to livestock health. We address this question by measuring forage macro- and micro-mineral content across a range of semi-natural grassland types in nine locations in France and compare it to standards required for cattle health. We also developed a multi-criteria method using forage mineral concentrations and their recommended ranges to determine general mineral concentration quality (MinQ) in forage. We investigated if forage mineral content and MinQ was related to plant community composition, soil characteristics, and land-use intensity of semi-natural grassland forages. Good forage quality in terms of mineral content was found, matching the reference values, in the majority of the extensively managed semi-natural grasslands studied. Plant species evenness was positively correlated with MinQ. The forage mineral quality varied depending on the community weighted mean of the specific leaf area (SLA_{CWM}) and the leaf dry matter content ($LDMC_{CWM}$). It increased with increasing SLA_{CWM} and decreased with increasing $LDMC_{CWM}$. The results demonstrate that a good compatibility between plant evenness and forage mineral quality can be obtained in semi-natural grasslands which suggests that the continued management of these habitats can offer agronomic services as well as conservation values.

1. Introduction

Semi-natural grasslands represent habitats of conservation interest (Habel et al., 2013) and provide an essential ecosystem service by producing feed for livestock (Bengtsson et al., 2019). The value of this provisioning service depends on both the quantity of forage produced and its forage nutritive value (Allen et al., 2011). The mineral content of forage is a particularly important aspect of the forage nutritive value since mineral intake is essential for animal health and growth (Spears, 1994; Suttle, 2010; Yatoo et al., 2013). This is notably reflected by the multiple different mineral requirements published by authorities in various countries (López-Alonso, 2012). Minerals, indeed, partake in the formation of structural components of body organs and tissues, and in the maintenance of physiological functions (Suttle, 2010). They also act as catalysts in enzymes and in endocrine systems, and regulate cell replication and differentiation (Suttle, 2010). Both macro-minerals (Ca, K, Mg, Na, P, Cl, S) and micro-minerals (for example, Cu, Fe, Mn, Zn), two groups that reflect the quantities in which they are found in livestock, are deemed to be essential for animal health (Suttle, 2010).

Mineral deficiencies and excesses may be treated in livestock

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https://doi.org/10.1016/j.agee.2025.109622
Received 28 September 2024; Received in revised form 15 January 2025; Accepted 11 March 2025
Available online 19 March 2025

industrial production systems through controlled feeding and the use of supplements (López-Alonso, 2012). This is not the case in grassland-based feeding livestock farming systems where mineral deficiencies with clinical consequences as well as toxicities due to excesses in mineral uptake can occur (especially during overgrazing periods) as it is harder to control livestock forage uptake composition and quantity (Judson and McFarlane, 1998; López-Alonso, 2012). Investigating the nutritive values of semi-natural grasslands forage, i.e., grasslands with spontaneous and varying plant species composition, as well as their drivers may provide guidelines for the design of livestock feeding systems. If it can be demonstrated that the forage nutritive quality of semi-natural grasslands is deemed satisfactory, then this may constitute a powerful encouragement for maintaining the management of semi-natural habitats that are of interest for biodiversity and nature conservation since it could provide both agronomic and conservation services (Chytrý et al., 2015). Forage mineral content should be considered in nutritive value evaluations; however, other parameters are more often used such as, crude protein content, forage digestibility and net energy content (Lee, 2018). Although these parameters are often considered separately, some studies have performed multivariate analysis to estimated an overall nutritional value (Dindová et al., 2019, Zha et al., 2022). However, these methods are often based on distance matrices where it is assumed that forage nutritive value always increases with increases in parameters used to estimate forage nutritive value. To our knowledge, no method has been used that estimates forage value while taking into account the negative impact that mineral deficiencies and excesses can have on livestock health.

Among the three major types of grasslands that can be distinguished within agricultural production systems, namely natural, semi-natural and improved grasslands (Bullock et al., 2011) only the last two exist in Europe. Semi-natural grasslands have spontaneous and non-sown vegetation but require livestock grazing or hay-cutting for their maintenance, and thus human management, to avoid shrubs and tree development (Queiroz et al., 2014). In semi-natural grasslands, plant communities are constituted by spontaneous and varying plant species composition and their forage may change accordingly (Gardarin et al., 2014, Blaix et al., 2023). Forage quality may be driven by environmental conditions and farming management, directly or through their effect on plant community species composition. The concentration of a mineral that is bioavailable in soil greatly influences its concentration in plant tissues and thus in forage (Spears, 1994). For example, forage mineral profiles differ between calcareous and acidic grasslands, with the availability of P and Fe being lower in calcareous grasslands (Dutoit and Alard, 1997).

Agricultural management may also influence forage mineral content. Mowing or livestock grazing leads to the removal of minerals from the ecosystem and may lead to lower mineral concentration in subsequent forage production (McDowell, 1996; Pavlů et al., 2013). Conversely, increases in forage mineral content have been measured in vegetation regrowth after mowing (Mikhailova, 2000), and grazing (Gardarin et al., 2014; Rossignol et al., 2006), probably due to changes in species composition and renewal of plant tissue. As part of the nutrients assimilated by grazing animals is excreted *in situ*, and thereafter partly recycled by plants (White et al., 2001). This could lead to contrasts in mineral concentration of forage among grazed pastures and mowed grasslands.

Mineral content differs among plant species (Pirhofer-Walzl et al., 2011; Lindström et al., 2012) and also among phenological stages (Lindström et al., 2014). This probably explains a substantial part of the variations in mineral content recorded between plant communities of semi-natural grasslands (Pirhofer-Walzl et al., 2011; Dudek et al., 2020). Apart from phylogenetic differences, nutrient content in species can be driven by their strategies for resource acquisition (Craine et al., 2002). Species efficiency for mineral uptake follows the resource competition principle (Tilman, 1982), with species having a greater capacity at acquiring certain resources at the expense of their capacity to acquire others. Such contrasts may be approached by plant functional types that identify different resource acquisition strategies (i.e., grasses, legumes and non-leguminous forb species). Unlike the other two functional types, non-fixing forbs are polyphyletic and consist of species belonging to numerous botanical families. They are grouped together in studies on grasslands because their functioning and response to management distinguish them from both grasses and legumes (Dumont et al., 2009; Johnson et al., 2020; Zhou et al., 2024). The role of forbs on forage quality may be heterogeneous, but this diversity has not really been explored. Accordingly, the content of numerous minerals, such as Ca, Cu, Fe, K, P, Zn, was found to be greater in non-nitrogen fixing forbs and legumes than in grasses (Pirhofer-Walzl et al., 2011; Lindström et al., 2012; Schlegel et al., 2016). On the other hand, the concentration of manganese (Mn) has been found to be greater in grasses than in forbs and legumes (Spears, 1994; Pirhofer-Walzl et al., 2011). Nevertheless, the relationship between forage mineral content and traits associated with the leaf economics spectrum (Wright et al., 2004) appears to have been little studied up until now.

Based on the published data, a positive relation between species plant diversity and forage mineral contents of semi-natural grasslands is expected. Furey and Tilman (2021), indeed, showed an increase in shoot mineral content of plant assemblages for N, Mg, P, and Ca with increasing species diversity, from one to sixteen species in an experimental plots design. The mechanisms underlying such a trend have not yet been clarified. Functional diversity can increase niche complementarity and improve the soil nutrient uptake by the plant community. Complementarity effect may occur spatially. For example, roots of different species types may prospect different zones of the soil, avoiding direct resource competition and increasing overall nutrient uptake (Schenk et al., 1999). Grasses and forbs, which generally co-occur in semi-natural grasslands, are known to differ in root traits which may lead to a complementarity in mineral acquisition. A temporal complementarity effect may also be observed in species-rich communities as seasonality of production differs among species, and thus the timing of nutrition uptake as well (Craine et al., 2003). On this basis, it is thus expected that an even distribution among plant functional types may increase forage general mineral uptake (Ravenek et al., 2016). However, a negative impact of increased plant species diversity may also be expected, as it may be associated with a reduction of the frequency of the better performing plants in terms of mineral uptake (dilution effect, Chanteloup and Bonis, 2013)

We investigated the relationships between plant community composition and forage mineral content, considering a large range of temperate semi-natural grasslands, which has never been studied to our knowledge. These grasslands occurred across a variety of environmental conditions in France and therefore correspond to different habitat types *sensu* Eunis (Chytrý et al., 2020). We investigated if plant diversity and plant traits at the community level impacted forage mineral content considering both macro- and micro-mineral nutrients. We also analysed the relationships between forage mineral content and soil parameters (pH, texture, and fertility), as well as soil mineral content and land-use intensity. Furthermore, we evaluated whether the measured forage mineral contents fit within the recommended forage mineral requirement ranges defined for beef cattle (NASEM, 2021). Finally, we developed a multi-criteria method to determine general mineral concentration quality in forage (MinQ) that allowed us to test the relationship between MinQ and land-use intensity, as well as between MinQ and plant community traits and diversity.

2. Materials and methods

2.1. Study sites

A total of 218 plots were sampled in a large range of grassland habitat types, related to six different habitat types *sensu* EUNIS level 2, in nine regions in France, all within or close to Natura 2000 sites, i.e., areas which are part of a network of sites protected by the 1979 Bird Directive and the 1992 Habitat Directive (Table 1. See Fig. S1 for a map displaying the location of the study sites). The 9 sites were: AQU: Atlantic dry grasslands located in the Aquitaine region; CAM: saline meadows and dry grasslands located in the Camargue natural region; COT: marshes located in the Cotentin peninsula; CRA: Mediterranean sub-steppic dry grasslands in the Crau plain; LOR: mesic grasslands and wet grasslands located in the Lorraine historical region; POI: marshes and wet grasslands in the Poitevin natural region; PUY: dry grasslands and heathlands, and mesic grasslands in the Chaîne des Puys mountain range; SOM: dry grasslands in the Somme department; VER: dry grasslands and heathlands, and mesic grasslands in the Vercors mountain range. Out of the 218 plots that were sampled, 188 were located in Natura 2000 sites. Plots were either grazed by cattle (69 plots), sheep (91 plots), or only mowed (58 plots).

Plots were also allocated to climate and soil moisture groups to measure potential environmental effects on soil mineral concentrations. Three climate groups were identified based on average annual temperature and average daily minimum and maximum temperature data, and an aridity index. Group 1 contains continental and Atlantic sites AQU, COT, LOR, POI, and SOM; group 2 included the Mediterranean sites CAM and CRA, and group 3 included the mountain to sub-mountain sites VER and PUY. Two soil moisture groups were distinguished based on grain size of the soil texture, topography, and precipitation data. Group 1 corresponded to wet grasslands and included all the plots of COT, LOR, and POI, as well as 9 plots sampled in CAM, and group 2 was composed of mesophilic to xerophilic grasslands and included all the plots of AQU, CRA, PUY, SOM, and VER, as well as 6 plots sampled in CAM. See Pacé et al. (2024) for more details on the climate and soil moisture classifications.

2.2. Forage sampling and chemical analysis

Forage sampling was carried out during the vegetation growth season and before the beginning of the grazing period. This corresponded to around 500–600 growing degree days (GDD) in most sites, with the exception of CRA (210 and 220 GDD) where early drought impacts the growth in spring and CAM (1100–1200 GDD) and COT (between 1000 and 1140 GDD) where flooding delays the growing period. In each 10 m x 10 m sampling plot (n = 218), five forage samples were harvested in 20 cm× 20 cm randomly distributed quadrats. Samples were collected in 2019, apart from 16 plots in CRA which were sampled in early spring 2020 due to unexpected grazing of the studied plots during winter 2018–2019.

Forage sampling consisted in harvesting plant biomass at 1 cm above ground, then biomass was immediately dried in an oven at 60 °C for at least 48 h until a constant mass was obtained, and then weighted. In some cases, additional forage samples were collected to obtain at least 40 g of total dry biomass per plot, i.e., the quantity required to perform the chemical analyses. Biomass chemical analyses were performed at the agronomic laboratory of Normandie (LANO, Saint-Lô, France) on samples ground at 1 mm. Inductively coupled plasma optical emission spectrometry was used to measure contents in calcium (Ca, g/kg), copper (Cu, mg/kg), iron (Fe, mg/kg), potassium (K, g/kg), magnesium (Mg, g/kg), manganese (Mn, mg/kg), sodium (Na, g/kg), phosphorus (P, g/kg) and zinc (Zn, mg/kg) in fodder samples (NF EN 15510 and NF EN ISO 11885).

Table 1
Description of the study sites. Habitat types correspond to level 2 EUNIS habitat types (Chytrý et al., 2020). Habitats are classified according to the latest review available for each habitat type at the time of writing (EEA, 2023).

Site	EUNIS habitat type	Soil moisture group	Climatic group	Number of plots
AQU	(R1) Dry grassland	Mesophilic to xerophilic	Continental and Atlantic	11
CAM	(R1) Dry grassland, (MA2) Littoral biogenic habitat	Wet, and mesophilic to xerophilic	Mediterranean	15
COT	(R3) Seasonally wet and wet grasslands, (D4) Base-rich fens and calcareous spring mires	Wet	Continental and Atlantic	24
CRA	(R1) Dry grassland	Mesophilic to xerophilic	Mediterranean	30
LOR	(R2) Mesic grasslands, (R3) Seasonally wet and wet grasslands	Wet	Continental and Atlantic	15
POI	(MA2) Littoral biogenic habitat, (R3) Seasonally wet and wet grasslands	Wet	Continental and Atlantic	38
PUY	(R1) Dry grassland, (R2) Mesic grasslands, (S4) Temperate shrub heathland	Mesophilic to xerophilic	Mountain and submountain	37
SOM	(R1) Dry grassland,	Mesophilic to xerophilic	Continental and Atlantic	27
VER	(R1) Dry grassland, (R2) Mesic grasslands, (S4) Temperate shrub heathland	Mesophilic to xerophilic	Mountain and submountain	21

2.3. Soil and management data

Details on soil sampling procedures and methods used for measurements are available in Blaix et al. (2023). The following parameters were measured: soil texture, pH, the cation-exchange capacity (CEC), total nitrogen (N, g/kg) and carbon (C, g/kg), organic C (g/kg), Olsen phosphorus (P, g/kg), and calcium (Ca, g/kg), magnesium (Mg, g/kg), potassium (K, g/kg), and sodium (Na, g/kg) using ammonium acetate extraction.

Information on grassland management was obtained from surveys conducted with land owners or managers for the sampling year. The information includes whether the sites were grazed, mowed, or both, the herbivore species and the annual stocking rate when grazed, the number of cuts per year when mown, and whether or not the sites were fertilised. A land-use intensity index (LUI, Blüthgen et al., 2012) was calculated for each plot as a measure of grazing intensity, by summing the standardised values (values divided by the overall mean) of the number of cuts per year as a measure of mowing intensity and the annual stocking rate (livestock unit per days of grazing ha^{-1} $year^{-1}$). Fertilisation intensity was not used in the equation as none was reported for in any of the studied sites.

2.4. Plant traits and diversity

Methods for the acquisition and calculation of plant community traits and plant diversity are described in detail in Blaix et al. (2023). The following variables were used in the analyses: community-weighted means for leaf dry matter content ($LDMC_{CWM}$) and specific leaf area (SLA_{CWM}), plant functional type (PFT) cover in terms of graminoids (grasses, rushes, and sedges), legumes, and non-N_2 fixing forbs (hereafter forbs), species richness, Shannon diversity, Pielou evenness index, functional richness and functional evenness indices for both LDMC ($LDMC_{FRic}$, $LDMC_{FEv}$) and SLA (SLA_{FRic}, SLA_{FEv}). The functional richness index describes the size of the functional space, and the functional evenness index estimates the distribution of abundance in the functional space (Mason et al., 2005). Additionally, PFT evenness was calculated using the Pielou evenness index.

2.5. Recommended mineral requirements for cattle and grassland reference values

The mineral concentration requirements used were for Holstein dry cows of 740 kg between 60 and 21 days prepartum (NASEM, 2021). Although more plots were grazed by sheep than cattle, we chose mineral recommendations for cattle because they were more up-to-date and easier to access than recommendations for sheep. Recommendations of cattle maximum tolerable requirements published by the National Academies of Sciences, Engineering, and Medicine (NASEM) were used (NRC, 2005). Mineral forage concentration for permanent grasslands sampled between 470 GDD and 685 GDD in the Normandy region in France were considered as a reference value for comparisons (INRA, 2018). Distribution of mineral concentrations were also compared between climate groups and soil moisture groups using the Kruskal-Wallis means-comparison test. Dunn's Kruskall-Wallis multiple comparison test with Holm's correction was also used for pairwise comparisons for the climate classification which contains more than two groups.

2.6. Data analysis

A partial redundancy analysis (RDA) was performed to assess the variation of the forage mineral concentrations explained by plant community descriptors (species richness, Shannon diversity, species evenness, $LDMC_{CWM}$, SLA_{CWM}, $LDMC_{FRic}$, $LDMC_{FEv}$, SLA_{FRic}, SLA_{FEv}, PFT evenness, and proportions of graminoids, forbs, and legumes), soil parameters (pH, organic C, C/N, CEC, total N, and proportions of clay and sand), and land-use intensity, while controlling for variation between sampling sites. Mineral concentration variables were standardised for the analysis. The RDA was tested for multicollinearity using the variance inflation factor (VIF). Shannon diversity, a plant community descriptor, was removed from the analysis due to a high VIF (i.e., >10). A permutation test with Holm's correction was conducted to determine the statistical significance of the axes. Statistically significant axes were retained for interpretation of results.

A Multi-Criteria Decision Analysis (MCDA) was performed to score the sampled plots from best to worst in terms of general forage mineral content. This type of analysis is useful as it allows to compare plots using multiple heterogeneous, sometimes conflicting, criteria (Belton and Stewart, 2002; Mendoza and Martins, 2006). In this study, we developed a method to evaluate each grassland plot in terms of the distance of each forage mineral content to the recommended forage mineral concentrations for beef cattle (NASEM, 2021). This method is similar to the Technique of Order Preference Similarity to the Ideal Solution (TOPSIS) which calculates the Euclidean distance between criteria values and an ideal best and an ideal worst value in order to calculate a performance score (Hwang and Yoon, 1981). A detailed procedure of the technique employed is available in Appendix 1. The score from this analysis represents the forage mineral content quality (MinQ). The R code used to perform this novel MCDA technique is available in the supplementary information (SI Code).

A mixed-effect model was used to test the effect of species diversity, functional diversity, community weighted means of functional traits, the proportion of plant functional types, and land-use intensity on MinQ. We compared models containing all the possible combinations of explanatory variables using the Akaike Information Criterion (AIC) and selected the best performing model. The sampling sites variable was included in the model as a random effect. However, we also want to test if there was an interaction effect between the sampling site factor and the model predictors on MinQ. We tested the interaction of each fixed effect term in the best performing model with sampling site in separate models with no random effect with the other terms from the best performing model included as co-factors. These models were then compared to the model with no interactions using AIC to determine if the effect of the predictors varied between sampling sites. We considered that a significant difference between models existed if the delta of AIC was greater than 2. Post-hoc tests on pairwise comparisons were then performed for all combinations of pairs of sampling sites with *p* values adjusted using the Tukey-Kramer method. Similar to the RDA, the initial full model was tested for multicollinearity, and Shannon diversity was omitted from the model due to a high VIF (i.e., >10). All analyses were performed on R v.4.2.2 (R Core Team, 2022) and the following packages were used: lme4 v. 1.1–32 (Bates et al., 2018) for the multi-level models, the vegan v. 2.6–4 package (Oksanen et al., 2022) was used for the RDA and to calculate taxonomic diversity indices, and the FD v. 1.0–12 package (Laliberté et al., 2014) was used to calculate functional diversity indices and community-weighted means. Graphs were rendered using the ggplot2 package v. 3.5.1 (Wickham, 2016).

3. Results

3.1. Mineral concentrations in study sites

Differences in distributions of forage mineral concentrations between climatic or soil moisture groups (as defined in Table 1) were measured for various minerals (Fig. S3-S6). Forage from mountain and sub-mountain sites were significantly different from forage of continental and Atlantic sites in term of K ($Z = 3.23$, $p < 0.01$), Mn ($Z = 2.68$, $p < 0.05$), Na ($Z = -8.94$, $p < 0.001$), P ($Z = 3.14$, $p < 0.01$), and Zn ($Z = 3.02$, $p < 0.01$) concentrations. Differences were also observed between mountain and sub-mountain sites and Mediterranean sites for Fe ($Z = -6.97$, $p < 0.001$), Mn ($Z = 4.53$, $p < 0.001$) and Zn ($Z = 5.19$, $p < 0.001$), and between the continental and Atlantic sites and the Mediterranean sites for Fe ($Z = -6.44$, $p < 0.001$), Mn ($Z = 2.59$, $p < 0.01$), Na ($Z = 8.23$, $p < 0.001$), and Zn ($Z = 3.00$, $p < 0.01$). Significant differences were found between the mesophilic to xerophilic group and the wet grassland group for Ca ($\chi^2 = 25.25$, $p < 0.001$), Fe ($\chi^2 = 16.72$, $p < 0.001$), Mg ($\chi^2 = 8.66$, $p < 0.01$), Na ($\chi^2 = 102.06$, $p < 0.001$), P ($\chi^2 = 4.64$, $p < 0.05$), and Zn ($\chi^2 = 36.18$, $p < 0.001$) forage concentrations. Measured mineral concentration values were within the defined mineral thresholds recommended for cattle health for most minerals, except for Cu, Na, and P which were mostly underneath the lower thresholds. Some forages had concentrations of Ca, Cu, Fe, K, Mg, and P above the upper threshold indicating an excess of these elements compared to the recommendation for cow health.

3.2. Effects on forage mineral concentrations

The result of the RDA performed on forage mineral data constrained by plant community, soil characteristics, and management intensity showed that the constraining variables explained 18.72 % of variation in mineral concentration values, all minerals considered. The first two axes of the RDA were statistically significant ($p < 0.05$) in the permutation test. The conditioning variable (sampling site) explained 36.92 % of the variation, while the first axis explained 14.65 % of variation, the second axis 6.15 %. The axis 1 scores for forage mineral concentration showed that (listed by decreasing absolute scores) K, P, Mg, Ca, and Cu were the minerals that had the greatest correlation with this axis. Mn, Na, Mg, K, and Zn had the greatest correlation with axis 2 (Fig. 1). Mn was the mineral with the most positive correlation with axis 1 and also had the greatest axis 2 values which results in it being isolated on the biplot from the other minerals.

The permutation test showed that $LDMC_{CWM}$ ($F = 19.30$, $p < 0.05$), forb percentage cover ($F = 7.74$, $p < 0.05$), and legume cover ($F = 4.15$, $p < 0.05$) were the three vegetation traits that had a significant effect on the forage mineral concentrations. $LDMC_{CWM}$ was positively correlated with axis 1 and had the greatest absolute axis 1 score amongst the constraining variables. Forb cover had the second greatest absolute axis 1 score and was negatively correlated with the axis and thus showed a positive relationship with K, P, Mg, Ca, and Cu. Legume cover had nearly the same absolute score for axis 1 than axis 2 and had negative values for both axes. Species richness was the only plant community diversity metric with a significant association to forage mineral content as a whole ($F = 6.79$, $p < 0.05$). It had a low, positive score for axis 1 and an axis 2 score close to zero. Soil pH was the only edaphic factor for which a significant association on forage mineral content was detected ($F = 4.82$,

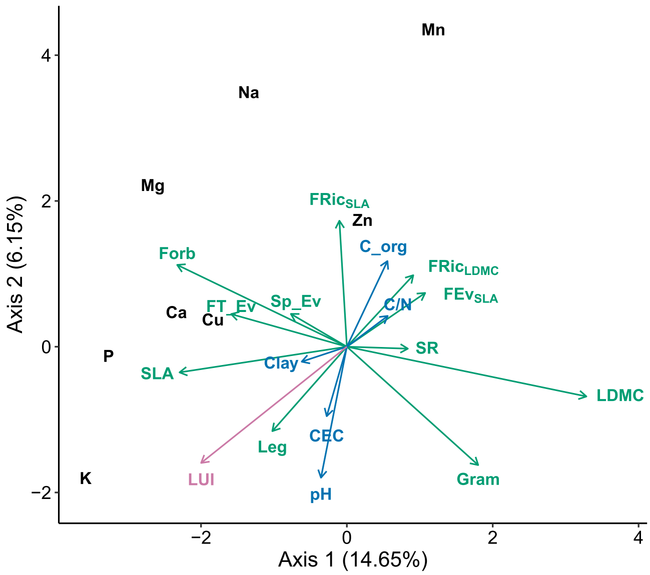

Fig. 1. Biplot of the first two axes from the RDA performed on forage mineral data constrained by plant community metrics, soil characteristics, and management intensity. The forage minerals are displayed in black, the variables associated with vegetation diversity and traits are in green, the soil variables are in blue, and land-use intensity (LUI) is in pink. Gram: graminoids; Leg: legumes; LDMC: community-weighted mean of leaf dry matter content, SLA: community-weighted mean of specific leaf area; FEv_{SLA}: functional evenness in SLA; $FRic_{SLA}$: functional richness in SLA; $FRic_{LDMC}$: functional richness in LDMC; FT_Ev: functional type evenness; Sp_Ev: species evenness; SR: species richness. Fe concentration, proportion of sand, and functional evenness in LDMC, which were located near the origin of the biplot, were omitted from the figure to improve clarity.

$p < 0.05$). It had the greatest axis 2 absolute score, with a negative value for that axis which suggests a positive correlation with forage K content and negative correlations with Mn, Na, Mg, and Zn. Lastly, LUI had a significant effect on forage mineral content ($F = 5.53$, $p < 0.05$), with negative scores for both axes. This suggests a positive relationship between LUI and forage K concentration, and a negative relationship with Mn concentration.

3.3. Effects on forage mineral content quality

Model results demonstrate that plant community traits, vegetation species evenness, and $FRic_{LDMC}$ were significantly associated with variations in MinQ values (Fig. 2; Supp. Info. Table 1). Forage MinQ values increased with increasing SLA_{CWM} ($T = 2.53$, $p < 0.05$), $FRic_{LDMC}$ ($T = 2.45$, $p < 0.05$), and species evenness ($T = 3.06$, $p < 0.01$) in the plant communities. However, the effect of SLA_{CWM} varied between sampling sites with a statistically significant difference found between the slopes for CRA and POI (Fig. S7; $T = 3.43$, $p < 0.05$). On the other hand, MinQ decreased with increasing percentage cover of graminoids ($T = -3.48$, $p < 0.001$) and forbs ($T = -2.65$, $p < 0.01$), and increasing $LDMC_{CWM}$ ($T = -3.25$, $p < 0.01$).

4. Discussion

4.1. Effect of plant community structure on forage mineral concentrations

In semi-natural habitats, species complementarity effects on forage mineral quality are expected as plant communities are functionally and taxonomically diversified. Such effects are expected to enhance forage mineral content, and potentially mediate conflicts between production and nature conservation objectives (Young et al., 2005). Modelling and empirical studies have already suggested that livestock have a better mineral balance, i.e., a higher proportion of mineral concentrations within recommended ranges, when they graze in species-rich rather than in species-poor pasture plant communities (Yoshihara et al., 2013a; Yoshihara et al., 2013b). Although our study did not find species richness to be an important predictor of MinQ, species evenness and $FRic_{LDMC}$ were found to be positively correlated with MinQ across a wide range of semi-natural grassland habitats. It is possible that a high species evenness leads to a dilution effect (Chanteloup and Bonis, 2013), with a reduction of the frequency of the better performing plants in terms of mineral uptake or the least efficient species at mineral uptake, which contributes to mineral concentrations being within recommended thresholds. The dominant species in most plots were graminoids which in turn were found to have a negative correlation with MinQ. This relationship is probably due to lower mineral concentrations found in graminoids (Pirhofer-Walzl et al., 2011; Schlegel et al., 2016; Lindström et al., 2012). Although LDMC was found to be correlated with MinQ, the effect of $FRic_{LDMC}$ on MinQ suggests that it is not only the species with the lowest LDMC value that contribute to MinQ and that having a long LDMC gradient in a plant community can benefit forage mineral quality.

Plant traits are known to be a good predictor of nutrient concentrations in plants (Fernández-Martínez, 2022). We expected legumes and other forbs to be positively correlated with most mineral contents (Kaspari et al., 2021; Zhou et al., 2024) and this was indeed the case. Such a result can be explained by how nutrient acquisition and transport strategies differ between grasses and forbs, especially for macro-nutrients, with micro-nutrient concentrations more impacted by

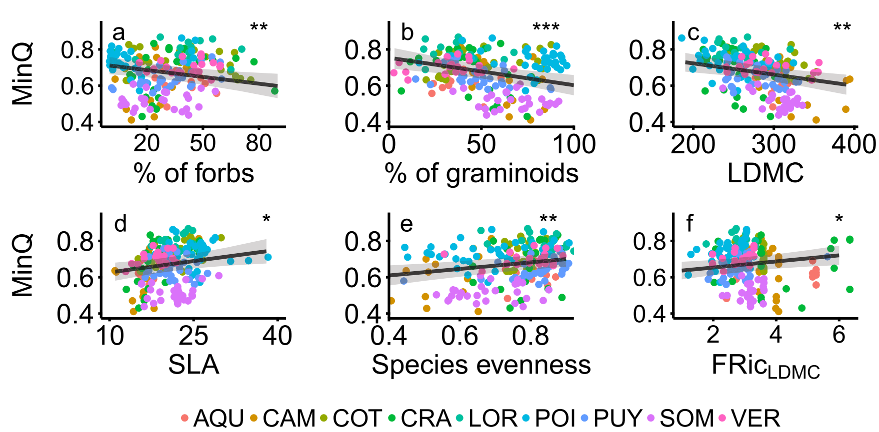

Fig. 2. Predicted response of MinQ to values of community-weighted mean leaf dry matter content (LDMC, a), community-weighted mean specific leaf area (SLA, b), species evenness (c), percentage of graminoid cover (d), and percentage of forb cover (e). Colours indicate sampling sites. See Table 1 for description of sampling sites. The light grey bands represent 95 % confidence intervals. * : $p < 0.05$; * *: $p < 0.01$; * ** : $p < 0.001$.

environmental conditions which would explain the weaker correlations observed for Mn and Zn with plant functional groups (Zhou et al., 2024). Plant functional type (PFT) evenness was not retained in the final model focussing on MinQ, perhaps due to the greater importance of the percentage cover of individual plant functional types. Forb percentage cover was negatively correlated with MinQ despite being seemingly positively correlated with many minerals in the RDA and in the results from other studies (Pirhofer-Walzl et al., 2011; Schlegel et al., 2016; Lindström et al., 2012). This may suggest that their negative effect is due to excesses in the concentrations of certain minerals. In most grasslands, this may not be an issue since graminoids tend to be more dominant. However, in semi-natural grasslands, particularly when they are exploited extensively, the proportion of forbs can reach significant values (Gaujour et al., 2012). In our study, they were the most dominant of the three functional groups in more than a quarter of the plots studied and therefore cannot be considered as a subordinate group when studying their potential role in forage minerals. However, forbs form a polyphyletic group and species may provide different minerals depending on their botanical family or their own specificities. Although considering them as a whole group may weaken the analysis of their effect on nutritional quality, there is a lack of knowledge to justify dividing forbs into different sub-groups. Focusing on plant traits, the $LDMC_{CWM}$ and SLA_{CWM} are associated with variations in forage mineral contents. MinQ decreased with increasing $LDMC_{CWM}$ as did the mineral forage concentrations for most minerals. By contrast, positive correlations were observed between SLA_{CWM} and for most forage mineral contents, as expected since SLA is positively associated with plant relative growth rate (Garnier et al., 1997; Wright et al., 2004) which in turn was reported as being positively associated with plant mineral uptake (Poorter and Bergkotte, 1992). Our results highlight that having a greater concentration in forage mineral content does not equate to having better forage mineral quality. We observe that both forb percentage cover and SLA_{CWM} are positively correlated with most mineral concentrations, however, forb cover is negatively correlated with MinQ while SLA_{CWM} is positively correlated with MinQ.

The only measured edaphic parameter which influenced forage mineral content was pH. It was negatively correlated with forage Mn and Na contents only. Trace elements tend to be more influenced by soil pH than macro elements, and Mn availability has been found to decrease with increasing soil pH (Berbecea et al., 2011; Barker and Culman, 2018). Other soil variables considered did not have an important influence on forage mineral content.

4.2. Evaluation of forage mineral quality

The majority of the semi-natural grasslands studied, ranging from wet grasslands to xerophilic grasslands, had a good forage nutritive value in terms of mineral content for Ca, Fe, K, Mg, Mn, and Zn, which meet the standard requirements for cattle. Unexpectedly, we found that their forage qualities match the reference values for most minerals. This is an interesting result as the reference used is typical of productive permanent grasslands, while the semi-natural grasslands sampled were extensively managed, with no agronomically valuable species sown and little or no fertiliser applied. Furthermore, 17 plots (out of the 218 plots studied) had a higher MinQ than the reference grassland. The fact that extensively managed semi-natural grasslands often reach recommended mineral forage concentrations for cattle supports the view that their management is generally compatible with agronomically sustainable beef breeding production, with good mineral provision in forage. This is reassuring for cattle breeders and demonstrates that semi-natural extensive species-diverse grasslands may together provide forage of sufficiently good mineral quality for beef cattle, and be of ecological and biodiversity value. This thus shows that trade-offs between such services are not systematically observed.

Focusing on individual minerals, concentration values lower than the reference values were recorded for K and P. This may be a consequence of NPK fertilisation in productive grasslands (Ineichen et al., 2020) rather than an intrinsic low content in semi-natural unfertilised grasslands. Although NPK fertiliser is used primarily to boost forage productivity, it may have an undesired effect on forage mineral content, especially considering our findings regarding the positive correlations between mineral content in soil and forage. In the case of K, the majority of forage sampled was within the beef cattle K requirement thresholds (NASEM, 2021) and the reference value was above the maximum recommended threshold. Forage in some plots had slightly lower K concentration than the minimum recommended value which does not suggest a severe K deficiency in cattle diet, and thus the impact of the deficiency would probably only be on milk yield in lactating cows (NASEM, 2021).

We recorded extremely high values for Na in the CAM sampling site, probably due to its proximity to the sea, with some of the sampling plots being located in salt marshes. In CRA, the soil is characterised by high Fe content which probably explains the very high forage concentration values for Fe. This is of concern as a diet too rich in iron increases the intestinal permeability and to reduce feed efficiency and weight gain in calves (Hansen et al., 2010). In CRA, plots are grazed by sheep and not cattle, and farmers did not report detrimental effects in relation to the high Fe content in the forage, which supports Grace and Lee's (1990) findings. Pedo-climatic conditions had an effect on mineral concentrations as expected (Perotti et al., 2021) and also on the response of MinQ to SLA_{CWM} with differences in slope between the dry grasslands of CRA and the marshes and wet grasslands of POI. Grassland management type can also have an effect on forage quality (Oates et al., 2011). We were not able to determine if this was the case for MinQ in our study due to certain practices being site-specific. Management intensity, however, did not have a significant impact on MinQ even though LUI had a positive effect on K concentration and a negative effect on Mn concentration. The management intensity in this study was quite low in general and it would be worthwhile looking at this effect with the inclusion of more intensive grassland systems.

4.3. The use of mineral requirement recommendations to evaluate forage mineral quality

By analysing the mineral concentrations in forages with reference to recommended concentration values for bovine health, while taking into account environmental conditions, this study provides a new perspective for assessing forage quality for a large set of diversified semi-natural grasslands in France.

The metric that we propose to characterise mineral forage quality considers both forage mineral content and recommended mineral concentration thresholds. It, therefore, should allow a better interpretation of forage quality by revealing potential and specific risks for animal health. By carefully taking into account maximum as well as minimum mineral content recommendation, it acknowledges that increase in mineral concentrations must not be considered as a systematic increase in forage quality. A striking result of this study is that the concentration of at least one mineral was above the recommended maximum threshold for 46 % of the plots sampled. Practically, this means that mineral supplementation in livestock ration may not always be justified, and may also be detrimental.

There are limitations associated with using this forage mineral quality metric, notably the fact that it does not take into account the importance of the ratio between certain minerals (Mayland and Shewmaker, 2001). For example, bone formation in cattle is dependent on both Ca and P and optimal performance in that regard can be estimated using the ratio between both minerals (NASEM, 2021), while the K/(Ca + Mg) ratio can help determine the risk of grass tetany (Aydin and Uzun, 2008). There is also,a lack of consensus regarding livestock mineral requirements (López-Alonso, 2012) which presents a challenge for evaluating forage quality by considering the distance from the recommended mineral values as we propose. Here we used thresholds recommended by the collective national academies of the United States of America (NASEM, 2021), as they are the most detailed that we could find. Finally, we make the assumption that the metric accurately reflects the health and well-being of cattle. The value of this metric could be tested by comparing with field measurements on livestock. Recommended mineral requirements could also be further improved by including differences in requirements for multiple breeds of cattle. The concentration value thresholds used in this study are designed for dry Holstein cattle, which is not the more widespread breed in semi-natural grasslands. However, detailed mineral requirements for other breeds were only found for Jersey cattle (NASEM, 2021). It is however known that mineral requirements may differ between breeds (McDowell, 1996), and some recommended thresholds are accurate for only specific breeds and growth status (Costa e Silva et al., 2015).

4.4. Conclusion and perspectives

The results of this study show that, in a large range of environmental conditions and semi-natural grasslands types in France, there is a good compatibility between species diversity and forage mineral quality. Trade-offs between agronomic services and conservation values in semi-natural grasslands are not to be expected for all agronomic facets. Indeed, an increase in species evenness of a grassland community can lead to forage mineral contents approaching their optimum levels. It thus emphasises the importance of semi-natural grasslands for producing high nutritional quality forage for healthy livestock. This study also demonstrated the link between forage mineral content and plant functional traits as well with soil mineral content. Estimation of forage quality could further be improved by considering secondary metabolites, which are known to impact livestock health (Poutaraud et al., 2017). Further studies are needed to investigate the relationships between functional traits and forage mineral content in specific grasslands habitat types and environmental conditions to complement this research which was conducted across a wide range of environmental conditions. Further and more accurate information on animal requirements may help farmers to decide if mineral supplements are required for livestock or to choose a breed that is adapted to the forage nutrient profile of their semi-natural grassland.

CRediT authorship contribution statement

Bonis Anne: Writing – review & editing, Supervision, Conceptualization. **Loucougaray Grégory:** Writing – review & editing, Conceptualization. **Michelot-Antalik Alice:** Writing – review & editing, Conceptualization. **Blaix Cian:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Alard Didier:** Writing – review & editing, Conceptualization. **Chabrerie Olivier:** Writing – review & editing, Conceptualization. **Fontès Hugo:** Writing – review & editing, Conceptualization. **Lemauviel-Lavenant Servane:** Writing – review & editing, Conceptualization. **Diquélou Sylvain:** Writing – review & editing, Conceptualization. **Dutoit Thierry:** Writing – review & editing, 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.

Acknowledgments

The authors would like to thank Sylvain Plantureux, François Mesléard, Marine Pacé, Antoine Tardif and Maxime Burst for contributing to discussions for this study or in data acquisition. We also thank the following people for their help in field work/acquiring data: Nicole Yavercovski, Benjamin Allard, Pauline Boubert, Méghan Boulembert, Lucie Chauvel, Clémence Helleu, Nathan Daumergue, Françoise Dubois, Frédéric Dubois, Adeline François, Claude Gallois, Marion Girardier, Loan Madej, Chloé Malik, Daniel Pavon, Lisa Poisse, Hervé Ramone, Emmanuelle Revardel, Adelaïde Theraroz, Romain Ulmer, and Christel Vidaller. We would like to thank the laboratory "Laboratoire Agronomique Normand" (LANO) for the chemical analysis management. We are grateful to the farmers who provided access to their fields and to the following organisations which provided basic information and facilities for working in the Natura2000 sites: Parc naturel régional (PNR) des Marais du Cotentin et du Bessin, PNR Volcans d'Auvergne, PNR du Vercors, Conservatoire des Espaces Naturels (CEN) Aquitaine, CEN Lorraine, Pôle Lorrain du Futur Conservatoire Botanique National Nord-Est, CEN de Provence-Alpes Côte-d'Azur, Réserve Naturelle Nationale des Coussouls de Crau, the "Coussouls sentinelles" programme of the Conseil Départemental des Bouches-du-Rhône, Chambre d'Agriculture des Bouches-du-Rhône, CEN des Hauts-de-France, Société Nationale de Protection de la Nature, Réserve Naturelle Nationale de Camargue, and Réserve Naturelle Régionale de la Tour du Valat. This work is a result of the SUR-PAS project ("SURveillance de l'état de conservation des habitats agro-PAStoraux pelousaires et prairiaux") funded by Le Ministère de la Transition Écologique et Solidaire (MTES) and driven by UMS PatriNat for the Office Français de la Biodiversité (OFB), within the framework of the "Habitats, Fauna, Flora" directive, Natura 2000 Project. We would also like to thank the two anonymous reviewers for their corrections and advice.

Appendix A

Calculation of the mineral content quality score (MinQ)

Given a set of plots $P = \{p_i | i = 1, 2, \ldots, k\}$, a set of forage minerals $M = \{m_j | j = 1, 2, \ldots, n\}$, let $X = \{x_{ij} | i = 1, 2, \ldots, k; j = 1, 2, \ldots, n\}$ denote the matrix mineral content values for each plot and each mineral where xij is the performance measure of plot pi with respect to the mineral mj, let $T^+ = \{t_j^+ | j = = 1, 2, \ldots, n\}$ denote the positive threshold matrix where t_j^+ is the upper threshold value for mineral mj, let $T^- = \{t^-j | j = 1, 2, \ldots, n\}$ denote the negative threshold matrix where t⁻j is the negative threshold value for mineral mj. Threshold values correspond to recommended forage mineral concentrations for beef cattle.

Step 1: The recommended range medians $v_j$were calculated as

$$v_j = t_j^- + \frac{t_j^+ - t_j^-}{2}, j = 1, |2,| \ldots, n$$

Step 2: The next step involved the following procedures:

(i) Transforming values within recommended thresholds to the median of the lower and upper threshold values.

(ii) Transforming values beneath the lower threshold so that the distance of the values to the median is the same as the distance of the original values to the lower threshold.

(iii) If the distance between the maximum value of mineral content and the upper threshold is less or equal to the value of the lower threshold, the values were transformed so that they were on the same scale as values beneath the lower threshold and inverted. Procedure (ii) was then applied to the new values.

(iv) If the distance between the maximum value of mineral content and the upper threshold is greater than the value of the lower threshold, values above the upper threshold were scaled to the range of 0 and the lower threshold, and the values subtracted from the lower threshold in order to have them on the same scale as the values below the lower threshold. This was done to reduce the influence of high values as these are not bounded, unlike low values which cannot be lower than 0. Procedure (ii) was then applied to the new values.

The following formula describes how the new values y_{ij} were calculated:

$$y_{ij} = \left(t_j^- \le x_{ij} \le t_j^+ \rightarrow v_j\right) \& \left(x_{ij} < t_j^- < x_{ij} + v_j - t_j^-\right) \&$$
$$\left(x_{ij} > t_j^+ \& \left(max(x_j) - t_j^+\right) \le t_j^- \rightarrow v_j + t_j^+ - x_{ij}\right) \&$$
$$\left(x_{ij} > t_j^+ \& \left(max(x_j) - t_j^+\right) > t_j^- \rightarrow \left(\frac{t_j^- - x_{ij} - t_j^+}{max(x_j) - t_j^+}\right) + v_j - t_j^-\right),$$
$$i = 1, 2, \ldots, k; j = 1, 2, \ldots, n$$

Step 3: The new mineral values y_{ij} were then rescaled from 0 to 1 using the following procedure

$$z_{ij} = \frac{y_{ij} - \min(y_i)}{\max(y_i) - \min\left(y_j\right)}, i = 1, 2, \ldots, k; j = 1, 2, \ldots, n$$

Step 4: The sum of the rescaled mineral values divided by the number of minerals was calculated for each plot to obtain the performance score S_i^R

$$s_j^R = \frac{\sum_{j=1}^{n} z_{ij}}{n}, i = 1, 2, \ldots, k; j = 1, 2, \ldots, n$$

The procedure was repeated with the recommended mineral range reduced by half, a common-sense, albeit arbitrary value hereafter referred to as the preferred mineral range S_i^P. This was done in order to obtain a buffer zone as we considered that it was better to have mineral content values around the median of the recommended values than

near the thresholds. The addition of S_i^R and S_i^P divided by two were equal to the final scores (see Fig. S2 for a simulation of the effect of the procedure on the values for one mineral).

$$S_i^F = \frac{S_i^R + S_i^P}{2}$$

No maximum recommended concentration was found for Na in our case study and we therefore considered all the values above the minimum recommended concentration to be of equal value.

The final score (MinQ) was used to assess multi-dimensional forage quality on the basis of the concentrations measured for all minerals.

Appendix B. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.109622.

Data availability

The data used for this study is available in an online repository. R code and data for forage mineral quality paper (Zenodo)

References

Allen, V.G., Batello, C., Berretta, E.J., Hodgson, J., Kothmann, M., Li, X., McIvor, J., Sanderson, M., 2011. An international terminology for grazing lands and grazing animals. Grass Forage Sci. 66 (1), 2. https://doi.org/10.1111/j.1365-2494.2010.00780.x.

Aydin, I., Uzun, F., 2008. Potential decrease of grass tetany risk in rangelands combining N and K fertilization with MgO treatments. Eur. J. Agron. 29 (1), 33–37. https://doi.org/10.1016/j.eja.2008.02.003.

Barker, D.J., Culman, S.W., 2018. Fertilization and nutrient management, 7th Edition, Chapter 14. In: Collins, M., Nelson, C.J., Moore, K.J., Barnes, R.F. (Eds.), Forages. A Blackwell Publishing Company, pp. 268–286.

Bates, D., Mächler, M., Bolker, B.M., Walker, S.C., 2018. Fitting Linear Mixed-Effects Models Using lme4. J. Stat. Softw. 67 (1), 1–48. https://doi.org/10.18637/jss.v067.i01.

Belton, V., & Stewart, T. (2002). Multiple criteria decision analysis: an integrated approach. Springer Science & Business Media.

Bengtsson, J., Bullock, J.M., Egoh, B., Everson, C., Everson, T., O'Connor, T., Lindborg, R., 2019. Grasslands - more important for ecosystem services than you might think. Ecosphere 10 (2), e02582. https://doi.org/10.1002/ecs2.2582.

Berbecea, A., Radulov, I., Sala, F., Crista, F., Lato, A., 2011. Interrelation between metal availability, soil pH and mineral fertilization. Res. J. Agric. Sci. 43, 19–22.

Blaix, C., Chabrerie, O., Alard, D., Catterou, M., Diquelou, S., Dutoit, T., Bonis, A., 2023. Forage nutritive value shows synergies with plant diversity in a wide range of semi-natural grassland habitats. Agric., Ecosyst. Environ. 347, 108369. https://doi.org/10.1016/j.agee.2023.108369.

Blüthgen, N., Dormann, C.F., Prati, D., Klaus, V.H., Kleinebecker, T., Hölzel, N., Weisser, W.W., 2012. A quantitative index of land-use intensity in grasslands: Integrating mowing, grazing and fertilization. Basic Appl. Ecol. 13, 207–220. https://doi.org/10.1016/j.baae.2012.04.001.

Bullock, J.M., Jefferson, R.G., Blackstock, T.H., Pakeman, R.J., Emmett, B.A., Pywell, R. J., Grime, J.P., Silvertown, J., 2011. Semi-natural grasslands. In: Technical Report: The UK National Ecosystem Assessment. UNEP-WCMC, Cambridge, UK, pp. 162–195. ⟨http://nora.nerc.ac.uk/15322⟩.

Chanteloup, P., Bonis, A., 2013. Functional diversity in root and above-ground traits in a fertile grassland shows a detrimental effect on productivity. Basic Appl. Ecol. 14 (3), 208–216. https://doi.org/10.1016/j.baae.2013.01.002.

Chytrý, M., Dražil, T., Hájek, M., Kalníková, V., Šibík, J., Ujházy, K., Mládek, J., 2015. The most species-rich plant communities in the Czech Republic and Slovakia (with new world records). Preslia 87 (3), 217–278.

Chytrý, M., Tichý, L., Hennekens, S.M., Knollová, I., Janssen, J.A., Rodwell, Schaminée, J.H., 2020. EUNIS Habitat Classification: Expert system, characteristic species combinations and distribution maps of European habitats. Appl. Veg. Sci. 23 (4), 648–675. https://doi.org/10.1111/avsc.12519.

Costa e Silva, L.F., Valadares Filho, S., de, C., Engle, T.E., Rotta, P.P., Marcondes, M.I., Silva, F.A.S., Martins, E.C., Tokunaga, A.T., 2015. Macrominerals and trace element requirements for beef cattle. PLoS One 10 (12), e0144464. https://doi.org/10.1371/journal.pone.0144464.

Craine, J.M., Tilman, D., Wedin, D., Reich, P., Tjoelker, M., Knops, J., 2002. Functional traits, productivity and effects on nitrogen cycling of 33 grassland species. Funct. Ecol. 16 (5), 563–574. https://doi.org/10.1046/j.1365-2435.2002.00660.x.

Craine, J.M., Reich, P.B., Tilman, D.G., Ellsworth, D., Fargione, J., Knops, J., Naeem, S., 2003. The role of plant species in biomass production and response to elevated CO2 and N. Ecol. Lett. 6 (7), 623–625. https://doi.org/10.1046/j.1461-0248.2003.00467.x.

Dindová, A., Hakl, J., Hrevušová, Z., Nerušil, P., 2019. Relationships between long-term fertilization management and forage nutritive value in grasslands. Agric., Ecosyst. Environ. 279, 139–148. https://doi.org/10.1016/j.agee.2019.01.011.

Dudek, T., Wolański, P., Rogut, K., 2020. The content of macro-and micro minerals in the sward of different types of semi-natural meadows of temperate climate in SE Poland. Agronomy 10 (2), 273. https://doi.org/10.3390/agronomy10020273.

Dumont, B., Farruggia, A., Garel, J.P., Bachelard, P., Boitier, E., Frain, M., 2009. How does grazing intensity influence the diversity of plants and insects in a species-rich upland grassland on basalt soils? Grass Forage Sci. 64 (1), 92–105. https://doi.org/10.1111/j.1365-2494.2008.00674.x.

Dutoit, T., Alard, D., 1997. Mineral contents of chalk grasslands in relation with sheep grazing involved in conservation management systems. Rev. D. 'Ecol., Terre Et. Vie 52 (1), 9–20.

EEA (2023). European Environmental Agency. ⟨https://www.eea.europa.eu⟩. Retrieved 25/09/2023.

Fernández-Martínez, M., 2022. From atoms to ecosystems: elementome diversity meets ecosystem functioning. N. Phytol. 234 (1), 35–42. https://doi.org/10.1111/nph.17864.

Furey, G.N., Tilman, D., 2021. Plant biodiversity and the regeneration of soil fertility. Proc. Natl. Acad. Sci. 118 (49), e2111321118. https://doi.org/10.1073/pnas.2111321118.

Gardarin, A., Garnier, É ., Carrère, P., Cruz, P., Andueza, D., Bonis, A., Kazakou, E., 2014. Plant trait–digestibility relationships across management and climate gradients in permanent grasslands. J. Appl. Ecol. 51 (5), 1207–1217. https://doi.org/10.1111/1365-2664.12293.

Garnier, E., Cordonnier, P., Guillerm, J.L., Sonié, L., 1997. Specific leaf area and leaf nitrogen concentration in annual and perennial grass species growing in Mediterranean old-fields. Oecologia 111, 490–498. https://doi.org/10.1007/s004420050262.

Gaujour, E., Amiaud, B., Mignolet, C., Plantureux, S., 2012. Factors and processes affecting plant biodiversity in permanent grasslands. A review. Agron. Sustain. Dev. 32 (1), 133–160. https://doi.org/10.1007/s13593-011-0015-3.

Grace, N.D., Lee, J., 1990. Effect of increasing Fe intake on the Fe and Cu content of tissues in grazing sheep. Proc. N. Z. Soc. Anim. Prod. 50, 265–268.

Habel, J.C., Dengler, J., Janišová, M., Török, P., Wellstein, C., Wiezik, M., 2013. European grassland ecosystems: threatened hotspots of biodiversity. Biodivers. Conserv. 22, 2131–2138. https://doi.org/10.1007/s10531-013-0537-x.

Hansen, S.L., Ashwell, M.S., Moeser, A.J., Fry, R.S., Knutson, M.D., Spears, J.W., 2010. High dietary iron reduces transporters involved in iron and manganese metabolism and increases intestinal permeability in calves. J. Dairy Sci. 93 (2), 656–665. https://doi.org/10.3168/jds.2009-2341.

Hwang, C.L., Yoon, K. (1981). Multiple Attribute Decision Making: Methods and Applications. New York: Springer-Verlag.

Ineichen, S., Marquardt, S., Kreuzer, M., Reidy, B., 2020. Forage quality of species-rich mountain grasslands subjected to zero, PK and NPK mineral fertilization for decades. Grass Forage Sci. 75 (4), 385–397. https://doi.org/10.1111/gfs.12488.

INRA, 2018. Alimentation des ruminants, Éditions Quæ, Versailles, France, 728 p.

Johnson, D.P., Driscoll, D.A., Catford, J.A., Gibbons, P., 2020. Fine-scale variables associated with the presence of native forbs in natural temperate grassland. Austral Ecol. 45 (3), 366–375. https://doi.org/10.1111/aec.12866.

Judson, G.J., McFarlane, J.D., 1998. Mineral disorders in grazing livestock and the usefulness of soil and plant analysis in the assessment of these disorders. Aust. J. Exp. Agric. 38 (7), 707–723. https://doi.org/10.1071/EA97145.

Kaspari, M., De Beurs, K.M., Welti, E.A., 2021. How and why plant ionomes vary across North American grasslands and its implications for herbivore abundance. Ecology 102 (10), e03459. https://doi.org/10.1002/ecy.3459.

Laliberté, E., Legendre, P., & Shipley, B. (2014). FD: measuring functional diversity from multiple traits, and other tools for functional ecology. R package version 1.0-12.3.

Lee, M.A., 2018. A global comparison of the nutritive values of forage plants grown in contrasting environments. J. Plant Res. 131, 641–654. https://doi.org/10.1007/s10265-018-1024-y.

Lindström, B.E.M., Frankow-Lindberg, B.E., Dahlin, A.S., Wivstad, M., Watson, C.A., 2012. Micronutrient concentrations in common and novel forage species and varieties grown on two contrasting soils. Grass Forage Sci. 68 (3), 427–436. https://doi.org/10.1111/gfs.12006.

Lindström, B.E.M., Frankow-Lindberg, B.E., Dahlin, A.S., Wivstad, M., Watson, C.A., 2014.). Micronutrient concentrations in relation to phenological development of red clover (*Trifolium pratense* L.), perennial ryegrass (*Lolium perenne* L.) and timothy (*Phleum pratense* L.). Grass Forage Sci. 69 (2), 276–284. https://doi.org/10.1111/gfs.12068.

López-Alonso, M., 2012. Trace minerals and livestock: not too much not too little. Int. Sch. Res. Not. https://doi.org/10.5402/2012/704825.

Mason, N.W., Mouillot, D., Lee, W.G., Wilson, J.B., 2005. Functional richness, functional evenness and functional divergence: the primary components of functional diversity. Oikos 111 (1), 112–118. https://doi.org/10.1111/j.0030-1299.2005.13886.x.

Mayland, H.F., Shewmaker, G.E., 2001. Animal health problems caused by silicon and other mineral imbalances. J. Range Manag. 54, 441–446. https://doi.org/10.2307/4003115.

McDowell, L.R., 1996. Feeding minerals to cattle on pasture. Anim. Feed Sci. Technol. 60 (3-4), 247–271. https://doi.org/10.1016/0377-8401(96)00983-2.

Mendoza, G.A., Martins, H., 2006. Multi-criteria decision analysis in natural resource management: A critical review of methods and new modelling paradigms. For. Ecol. Manag. 230 (1-3), 1–22. https://doi.org/10.1016/j.foreco.2006.03.023.

Mikhailova, E.A., Bryant, R.B., Cherney, D.J.R., Post, C.J., Vassenev, I.I., 2000. Botanical composition, soil and forage quality under different management regimes in Russian grasslands. Agric., Ecosyst. Environ. 80 (3), 213–226. https://doi.org/10.1016/S0167-8809(00)00148-1.

National Academies of Sciences, Engineering, and Medicine (NASEM) (2021). Nutrient Requirements of Beef Cattle: Eighth Revised Edition. Washington, DC: The National Academies Press. https://doi.org/10.17226/19014.

National Research Council (NRC) (2005). Mineral Tolerance of Animals. Washington, DC: The National Academies Press.

Oates, L.G., Undersander, D.J., Gratton, C., Bell, M.M., Jackson, R.D., 2011. Management-intensive rotational grazing enhances forage production and quality of subhumid cool-season pastures. Crop Sci. 51 (2), 892–901. https://doi.org/10.2135/cropsci2010.04.0216.

Oksanen J., Simpson G., Blanchet F., Kindt R., Legendre P., Minchin P., … & Weedon J. (2022). _vegan: Community Ecology Package_. R package version 2.6-4, <⟨https://CRAN.R-project.org/package=vegan⟩> .

Pacé, M., Bonis, A., Alard, D., Blaix, C., Burst, M., Catterou, M., Chabrerie, O., 2024. Identifying generic proxies for assessing ecosystem properties and conservation status of semi-natural grasslands. Ecol. Indic. 158, 111586. https://doi.org/10.1016/j.ecolind.2024.111586.

Pavlů, L., Pavlů, V., Gaisler, J., Hejcman, M., 2013. Relationship between soil and biomass chemical properties, herbage yield and sward height in cut and unmanaged mountain hay meadow (Polygono–Trisetion). Flora-Morphol., Distrib., Funct. Ecol. Plants 208 (10-12), 599–608. https://doi.org/10.1016/j.flora.2013.09.003.

Perotti, E., Huguenin-Elie, O., Meisser, M., Dubois, S., Probo, M., Mariotte, P., 2021. Climatic, soil, and vegetation drivers of forage yield and quality differ across the first three growth cycles of intensively managed permanent grasslands. Eur. J. Agron. 122, 126194. https://doi.org/10.1016/j.eja.2020.126194.

Pirhofer-Walzl, K., Søegaard, K., Høgh-Jensen, H., Eriksen, J., Sanderson, M.A., Rasmussen, J., Rasmussen, J., 2011. Forage herbs improve mineral composition of grassland herbage: Forage herbs improve mineral composition of grassland herbage. Grass Forage Sci. 66, 415–423. https://doi.org/10.1111/j.1365-2494.2011.00799.x.

Poorter, H., Bergkotte, M., 1992. Chemical composition of 24 wild species differing in relative growth rate. Plant, Cell Environ. 15 (2), 221–229. https://doi.org/10.1111/j.1365-3040.1992.tb01476.x.

Poutaraud, A., Michelot-Antalik, A., Plantureux, S., 2017. Grasslands: a source of secondary metabolites for livestock health. J. Agric. Food Chem. 65 (31), 6535–6553. https://doi.org/10.1021/acs.jafc.7b00425.

Queiroz, C., Beilin, R., Folke, C., Lindborg, R., 2014. Farmland abandonment: threat or opportunity for biodiversity conservation? A global review. Front. Ecol. Environ. 12 (5), 288–296. https://doi.org/10.1890/120348.

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL ⟨https://www.R-project.org/⟩.

Ravenek, J.M., Mommer, L., Visser, E.J., van Ruijven, J., van der Paauw, J.W., Smit-Tiekstra, A., de Kroon, H., 2016. Linking root traits and competitive success in grassland species. Plant Soil 407 (1), 39–53. https://doi.org/10.1007/s11104-016-2843-z.

Rossignol, N., Bonis, A., Bouzillé, J.B., 2006. Consequence of grazing pattern and vegetation structure on the spatial variations of net N mineralisation in a wet grassland. Appl. Soil Ecol. 31 (1-2), 62–72. https://doi.org/10.1016/j.apsoil.2005.04.004.

Schenk, H.J., Callaway, R.M., Mahall, B.E., 1999. Spatial root segregation: are plants territorial?. In: In *Advances in ecological research*, 28 Academic Press, pp. 145–180.

Schlegel, P., Wyss, U., Arrigo, Y., Hess, H.D., 2016. Mineral concentrations of fresh herbage from mixed grassland as influenced by botanical composition, harvest time and growth stage. Anim. Feed Sci. Technol. 219, 226–233. https://doi.org/10.1016/j.anifeedsci.2016.06.022.

Spears, J.W., 1994. Minerals in forages. Forage Qual., Eval., Util. 281–317.

Suttle, N.F. (2010). The mineral nutrition of livestock. 4th ed. CAB International: Oxfordshire, UK.

Tilman, D. (1982). Resource Competition and Community Structure. Princeton University Press.

White, S.L., Sheffield, R.E., Washburn, S.P., King, L.D., Green Jr, J.T., 2001. Spatial and time distribution of dairy cattle excreta in an intensive pasture system. J. Environ. Qual. 30 (6), 2180–2187. https://doi.org/10.2134/jeq2001.2180.

Wickham, H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Villar, R., 2004. The worldwide leaf economics spectrum. Nature 428 (6985), 821–827. https://doi.org/10.1038/nature02403.

Yatoo, M.I., Saxena, A., Deepa, P.M., Habeab, B.P., Devi, S., Jatav, R.S., Dimri, U., 2013. Role of trace elements in animals: a review. Vet. World 6 (12), 963. https://doi.org/10.14202/vetworld.2013.963-967.

Yoshihara, Y., Mizuno, H., Yasue, H., Purevdorj, N.O., Ito, T.Y., 2013. Nomadic grazing improves the mineral balance of livestock through the intake of diverse plant species. Anim. Feed Sci. Technol. 184 (1-4), 80–85. https://doi.org/10.1016/j.anifeedsci.2013.06.007.

Yoshihara, Y., Mizuno, H., Ogura, S., Sasaki, T., Sato, S., 2013. Increasing the number of plant species in a pasture improves the mineral balance of grazing beef cattle. Anim. Feed Sci. Technol. 179 (1-4), 138–143. https://doi.org/10.1016/j.anifeedsci.2012.11.009.

Young, J., Watt, A., Nowicki, P., Alard, D., Clitherow, J., Henle, K., Richards, C., 2005. Towards sustainable land use: identifying and managing the conflicts between human activities and biodiversity conservation in Europe. Biodivers. Conserv. 14 (7), 1641–1661. https://doi.org/10.1007/s10531-004-0536-z.

Zha, X., Tian, Y., Ouzhu, Fu, G., 2022. Response of forage nutrient storages to grazing in alpine grasslands. Front. Plant Sci. 13, 991287. https://doi.org/10.3389/fpls.2022.991287.

Zhou, N., Li, H., Wang, B., Rengel, Z., Li, H., 2024. Differential root nutrient-acquisition strategies underlie biogeochemical niche separation between grasses and forbs across grassland biomes. Funct. Ecol. 38, 2286–2299. https://doi.org/10.1111/1365-2435.14629.