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Mineral concentrations of fresh herbage from mixed grassland as influenced by botanical composition, harvest time and growth stage



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Abbreviations:

DM, dry matter

CP, crude protein

ADF_{OM}, acid detergent fibre

aNDF_{OM}, neutral detergent fibre

Ca, calcium

P, phosphorus

Mg, magnesium

K, potassium

Na, sodium

Cl, chlorine

Cu, copper

Fe, iron

Mn, manganese

Zn, zinc

Co, cobalt

Se, selenium

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ABSTRACT

The mineral concentrations in herbage from mixed grassland are variable which lead to challenging situations for animal's mineral supplementation. The aim of this study consisted in assessing macro and micro mineral concentrations in fresh herbage from mixed grassland, according to a classification method used for nutrients and nutritive values. This classification consists of six classes of botanical composition, two classes of harvest time and seven classes of growth stage. Two hundred and thirty six samples of fresh herbage from mixed grassland were collected from one experimental site during five seasons. The analysed mineral concentrations were modelled using botanical composition and harvest time as fixed effects and growth stage as linear and quadratic effects. Whereas the repeatability of the models for calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sulphur (S) and copper (Cu) were similar to the ones for crude protein and fibre, the repeatability for sodium (Na), chlorine (Cl), iron (Fe) and manganese (Mn) were low. Cobalt (Co, 0.113 ± 0.173 mg/kg DM) and selenium (Se, 0.020 ± 0.022 mg/kg DM) concentrations were hardly detected. Except Fe, mineral concentrations were influenced ($P < 0.05$) by class of botanical composition. Herbage with increasing presence of grasses and thus a reduced presence of legumes and other herbaceous plants, had lower ($P < 0.05$) Ca, P, K, Cu and zinc (Zn) concentrations. Herbage with ryegrass predominance had lower ($P < 0.01$) Mg, S and Mn concentrations. Mineral concentrations, except Fe, were lower ($P < 0.05$) in the first seasonal harvest than in subsequent harvests. With increasing growth stage, P ($P < 0.05$, linear), Mg ($P < 0.001$, linear and quadratic), K ($P < 0.001$, quadratic), S ($P < 0.001$, linear) and Cu, Mn and Zn ($P < 0.001$, linear and $P < 0.05$ quadratic) concentrations progressively decreased. Sodium and Cl concentrations responded quadratically ($P < 0.05$) to the developing growth stages and Ca concentrations remained stable ($P > 0.10$). The developed models may be considered to assess reference values in order to take into account the native mineral concentrations of fresh herbage from mixed grassland when optimizing herbivore diets. This, is a contribution for improving the sustainability of mineral supplementation.

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1. Introduction

Grassland supplies a major part of the feed used by herbivores in fresh or conserved form. In numerous countries, grazing pastures or feeding freshly cut herbage is of common practice during the vegetation period. Herbage contains minerals essential to livestock, but their concentrations are influenced by climatic, soil and agronomic factors leading to challenging situations for animal's mineral supplementation. Mineral supplementation consists in providing the necessary minerals not covered by native contents in herbage and other feeds. An excessive dietary mineral supply may lead to mineral accumulations in soils, when using manure fertilizers (Öborn et al., 2008; Flisch et al., 2009). For certain minerals, such as P, Cu or Zn such increased soil concentrations can affect important microbial processes (Giller et al., 1998, 2009) and increase the risk of environmental water pollution (Römken et al., 2008). An excessive mineral supply also increases feed costs and may lead to metabolic deficiencies of other minerals by antagonistic effects (Suttle, 2010). An insufficient mineral supply may lead to physiological dysfunctions (Suttle, 2010). An efficient and sustainable mineral supplementation therefore requires a good knowledge of native mineral concentrations in herbage and their influencing factors within a given geographic area.

Analyzing fresh herbage for its mineral concentrations is not common in practice. Thus, there is a need for reference values for mineral concentrations, as they exist for other major nutrients such as absorbable protein or net energy. Reference values for nutrient concentrations in herbage from fresh mixed grassland can be organized according to botanical composition, harvest time (first seasonal harvest and subsequent harvests) and growth stage to cover the main influencing agronomic factors. Concentrations of several macro and micro minerals in forage species either grown alone or in mixed grassland were determined according to botanical composition (Hasler and Besson, 1972; Kessler and Jolidon, 1998; Stünzi, 1998; Daccord et al., 2001; Pirhofer-Walzl et al., 2011; Lindström et al., 2012, 2014), harvest time (Daccord et al., 2001; Wyss and Kessler 2002; Govasmark et al., 2005a,b; Pirhofer-Walzl et al., 2011) or growth stage (Kirchgessner et al., 1968; Stünzi, 1973; Casler et al., 1987; Brink et al., 2006; Nordheim-Viken et al., 2009). However, little is known regarding the importance of the combined effects of botanical composition, harvest time and growth stage in fresh mixed herbage, especially regarding micro mineral concentrations.

The aim of this study was to assess the concentrations of essential macro and micro minerals in fresh herbage from mixed grassland as influenced by botanical composition, harvest time and growth stage. This classification methodology for mixed grassland herbage described by Agroscope (2015a) was developed in the 1970's and is similar to the ones recommended in Germany (Jentsch et al., 2003) and France (Beaumont et al., 2011).

2. Material and methods

2.1. Experimental site, climate and soil characteristics

Fresh herbage samples were collected on the experimental farm of Agroscope in Posieux, Switzerland (46°46'N, 07°06'E, 650 m a. s. l.). The experimental farm is located on a cambisol soil type, known to be agriculturally highly productive and covering 12% of the European soils (EC, 2005). According to ten soil analyses from 2014, the sandy-loam soil contained 158 ± 12 g/kg clay, 293 ± 22 g/kg silt and 525 ± 35 g/kg sand and had a pH of 6.8 ± 0.3 . Mean ammonium acetate EDTA extractable P, Mg and K in soils were 66 ± 21 , 159 ± 68 and 139 ± 47 mg/kg, respectively. The herbage plots were fertilized over the vegetation period with up to 180 kg N, 44 kg P and 195 kg K per hectare and year according to their yield, mainly in the form of mixed liquid bovine and porcine manure and of ammonium nitrate. The swards originated from multi-species mixtures including seeds of *Lolium perenne*, *Poa pratensis*, *Dactylis glomerata*, *Phleum pratense*, *Festuca rubra*, *Trifolium repens* and *Trifolium pratense* and herbage growth was measured regularly (Agroscope, 2015b). Within the vegetation period (beginning of April–end–October), the mean temperature at 2 m above ground was 9.7, 13.4, 16.6, 18.0, 17.8, 14.1 and 11.9 °C during the respective months and the mean monthly rainfall was 89 ± 49 mm.

2.2. Sampling procedures and characterisation of herbage

Two hundred thirty six fresh herbage samples were collected in 2008–2011 and in 2014 on the experimental farm. A sample contained approx. 1 kg freshmatter and consisted in pooled subsamples systematically cut at a stubble height of 60–80 mm using a battery grass shearer (Gardena, Husqvarna Schweiz AG, Mägenwil, Switzerland). Subsamples were either collected at intervals of 20 m over the two diagonals of a selected plot (3.0 ha, two thirds of the samples) or collected within a fenced surface (10 × 5 m) inside a selected plot to follow the growth stages within the same harvest (one third of the samples). The class of botanical composition and growth stage were visually evaluated during the sampling procedure according to Agroscope (2015a). A second sample was taken in 72% of the sampling procedures to determine the proportional fresh weight of each botanical group (forage grass with distinction of ryegrass, legume, and other herbaceous plants). The classes of botanical composition depend on the presence of the botanical groups: grass rich (**G**, >70% grass; **G_R**, >70% grass with more than half as ryegrass), equilibrated (**E**, 50–70% grass; **E_R**, 50–70% grass with more than half as ryegrass) and rich in other herbaceous plants (**H**, >50% other herbaceous plants with thin leaves). The harvest time is distinguished between 1st seasonal harvest (**1st**) and subsequent harvests (**+2nd**). The classes of growth stage are the following: **1** (tillering), **2**

(stem elongation), **3** (begin heading), **4** (full heading), **5** (end heading), **6** (flowering) and **7** (fructification) with reference to *Dactylis glomerata* or *Lolium perenne*.

2.3. Chemical analysis

Following collection, the samples were subsequently dried (60 °C, 15 h) and ground using a mill (Brabender, Duisburg, Germany) equipped with titanium knives and a 1.0 mm sieve to avoid mineral contamination (Dahlin et al., 2012). Dry matter (DM) content was quantified thermo-gravimetrically by heating at 105 °C for 3 h (Leco TGA 601, Mönchengladbach, Germany) and ash content was subsequently determined after incineration at 550 °C until constant weight was attained. Crude protein (CP) was calculated as $6.25 \times$ nitrogen, where nitrogen content was determined using a Kjeldahl method (AOAC, 1995; method number 988.05) where samples were digested (Tecator™ Digestor 20 with Auto lift and Scrubber, Foss Analytical, Hilleroed, Denmark) and titrated (Kjeltec™ 2400 Auto Analyzer Unit, Foss Analytical, Hilleroed, Denmark). Cell wall constituents were analysed using an Ankom 200/220 Fibre Analyzer (Ankom Technology Corporation, Fairport, USA). Acid detergent fibre (ADF_{OM}; AOAC, 1995; method number 973.18) was determined with correction for residual ash obtained after incineration at 500 °C for 1 h, and neutral detergent fibre (aNDF_{OM}) was assayed with the addition of heat-stable amylase and sodium sulfite and with correction for residual ash (Mertens, 2002). Dry ashed samples were solubilized in nitric acid 65% (quality grade for analysis) and their content in Ca, P, Mg, K, Na, Cu, Fe, Mn and Zn were analysed (European Standard EN 155510:2008) using an inductively coupled plasma optical emission spectrometer (ICP-OES, Optima 7300 DV, Perkin-Elmer, Schwerzenbach, Switzerland). Sulfur content was directly determined by infrared absorption after combustion at 1360 °C (TruMac CNS, Leco Instrumente GmbH, Mönchengladbach, Germany). Chloride content was determined by argentometric titration (Mettler Toledo T70, Mettler, Schwerzenbach, Switzerland) after samples were solubilized in an acidified (nitric acid 65%, quality grade for analysis) and deionized water (resistance of 18.2 MΩ). Cobalt and selenium contents were determined by atomic absorption in a graphite oven (GF-AAS Analyst 600 Perkin-Elmer, Schwerzenbach, Switzerland), after samples were solubilized using nitric acid 65% (quality grade for analysis) in a microwave oven (Micro Wave Ultra Clave, MWS GmbH, Heerbrugg, Switzerland). All analyses were performed in the accredited Agroscope laboratories in duplicate, except DM and ash as single analysis. The samples collected during 2014 (n = 55) were not analysed for Cl, Co and Se. The defined mineral limit of quantification was, per kg, 0.12 g for Ca, P, K and Mg, 0.05 g for Na, 0.1 g for S and Cl, 2.5 mg for Cu, Fe, Mn and Zn, 0.1 mg for Co and 0.025 mg for Se. Those were defined according to signal-to-noise approach of a reference sample. If the obtained value was below the limit of quantification, but the signal was positive the mean value of the duplicated signal values was considered and if, at least, one of the obtained signal value was negative, the concentration was defined as zero.

2.4. Statistical analysis

A graphical examination of ash and of all mineral concentrations was performed in relation to origin (plot and year) of the samples before statistical analysis. Through this examination, the Na values (n = 20) from one specific plot were discarded as those presented exceptional high values (1.33 ± 0.63 g/kg DM) and a linear relation of Co and Fe to ash was observed when ash was high, indicative of soil contamination. Descriptive statistics (mean and range of values) were generated for each variable. A piecewise non-linear regression model procedure of SYSTAT 13 (Systat Software GmbH, Erkrath, Germany) was carried out to determine the breakpoint where mineral concentrations increase with increasing ash. The model was $y = (\text{Ash} < X) X_1^*b_0 + (\text{Ash} \geq X) (X^*b_0) + (b_1 * (\text{Ash} - X))$ If $X_i \geq X_{1_i}$, $Y_j = (b_0 * X_{1_i}) + (b_1 * X_i - X_{1_i}) + \varepsilon_i$; If $X_i < X_{1_i}$, $Y_i = b_0 * X_{1_i} + \varepsilon_i$. The expected outcome for the dependent variable Y observed at level i of the independent variable X was Y_i , the independent variable was X_i , the breakpoint was X_{1_i} , the slope of Y on X below and above X_{1_i} were respectively b_0 and b_1 and the residual error was ε_i . Finally, a GLM procedure of SYSTAT 13 (Systat Software GmbH, Erkrath, Germany) was carried out. The model included the class of botanical composition (G_R , G, E_R , E, L and H) and harvest time (1st, +2nd) as fixed effects and the growth stage (1–7) as linear and quadratic effects. Samples with ash concentrations above X_1 were discarded in the GLM model. With significant effects of the class of botanical composition, Tuckey test was used to compare the least squares means. The coefficient of determination (R^2) and the root mean square error (r.m.s.e.) of each generated equation were calculated between the predicted and observed values. Differences were considered significant when $P < 0.05$ and trends were noted at $P < 0.10$.

3. Results

The dataset included 37, 93, 28, 58 and 20 samples of the botanical composition G, G_R , E, E_R and H; 57, 63, 40, 31, 22, 9 and 14 samples of the growth stage 1–7; and 81 and 155 samples of 1st harvest and subsequent harvests (+2nd), respectively. The classes of botanical composition and growth stages were equally distributed within 1st harvest and subsequent harvests. Within the 1st harvest, samples were collected between calendar weeks 14 and 25 (beginning of April to mid-June) and the stage of development increased quadratically with the week of sampling ($R^2 = 0.94$, $P < 0.001$). The visually evaluated classes of botanical composition corresponded with the measured proportional weight of each botanical group (Fig. 1). Grasses were predominantly *Lolium perenne* and *Dactylis glomerata*, legumes were mainly *Trifolium* species and the main other herbaceous plants were broadleaved herbs, especially *Taraxacum officinale*. The descriptive nutrient concentrations are presented in Table 1 and coefficients of variation of CP, ADF_{OM} and aNDF_{OM} concentrations were 0.26, 0.16, and 0.16,

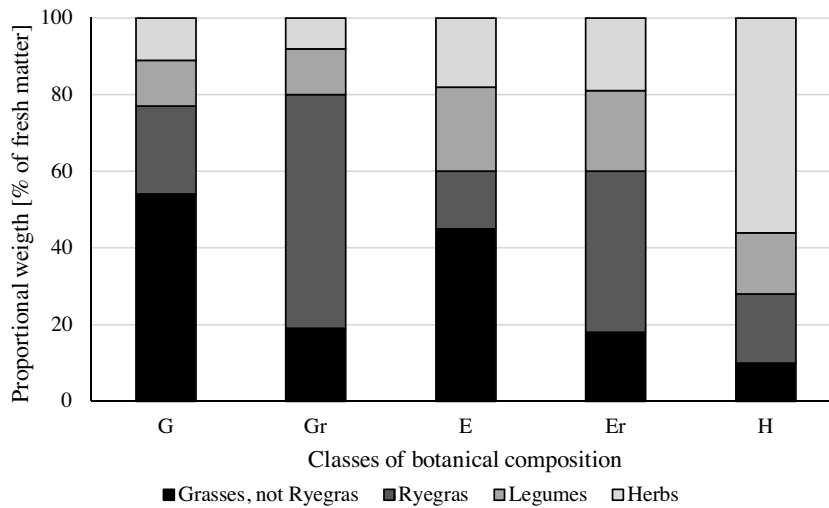


Fig. 1. Proportions of botanical groups and ryegrass in each class of botanical composition.

Table 1

Descriptive statistics of nutrient and mineral concentration in fresh herbage from mixed grassland.¹

		n	Min	Max	Median	Mean	SD
DM	g/kg	236	111	403	181	190	45
CP	g/kg DM	236	62	277	165	164	42
ADF _{OM}	g/kg DM	236	170	371	256	260	41
aNDF _{OM}	g/kg DM	236	274	632	419	419	65
Ash	g/kg DM	236	43	156	94	93	18
Ca	g/kg DM	236	3.66	15.29	6.85	7.17	2.03
P	g/kg DM	236	1.56	6.67	4.18	4.08	0.87
Mg	g/kg DM	236	0.99	4.65	2.07	2.08	0.53
K	g/kg DM	236	9.65	47.87	32.14	31.39	6.70
Na	g/kg DM	216	0.00	0.68	0.23	0.25	0.13
Cl	g/kg DM	181	1.12	15.25	5.88	6.21	3.12
S	g/kg DM	236	0.80	3.92	1.81	1.90	0.59
Cu	mg/kg DM	236	1.9	16.1	8.1	8.1	2.6
Fe	mg/kg DM	236	39.4	1719.1	108.6	207.1	280.1
Mn	mg/kg DM	236	17.2	236.3	49.8	65.4	41.7
Zn	mg/kg DM	236	15.9	53.4	27.2	27.6	5.8
Co	mg/kg DM	181	0.000	1.186	0.061	0.113	0.173
Se	mg/kg DM	181	0.000	0.161	0.015	0.020	0.022

¹ Min minimum; Max maximum; SD standard deviation.

respectively. The coefficients of variation (P of Ca, P, Mg, K and Zn were comparable (<0.30), whereas the ones of Na, Cl, S and Cu were between 0.30 and 0.60 and the ones of Fe, Co and Se were >1.00. The obtained R² from the model was between 0.49 and 0.62 for all minerals, except for Na, Cl, Fe Mn and Zn where R² were lower (Tables 2 and 3). The breakpoint X1 for Fe was 106 g ash/kg DM. Models for Co and Se were not calculated as, respectively, only 25 and 27% of the samples obtained values above the defined limit of quantification.

3.1. Effect of botanical composition

The botanical composition influenced all macro mineral (Table 2, P<0.05) and micro mineral concentrations (Table 3, P<0.001), except Fe. Grass rich (G and G_R) herbage was lower (P<0.001) in Ca and P concentrations than that of other botanical composition and was lower (P<0.001) in Cu and Zn than herbage rich in other herbaceous plants (H). Grass rich herbage, with ryegrass predominance (G_R) was lower (P<0.01) in Mg and S than without ryegrass predominance (G) and was lower in K (P<0.05) and higher (P<0.001) in Na than in equilibrated (E, E_R) and other herbaceous plant rich (H) herbage. Equilibrated (E and E_R) herbage was lower (P<0.001) in Ca, Mg and Zn than other herbaceous plants rich (H) herbage. Equilibrated herbage with ryegrass predominance (E_R) was higher (P<0.01) in Cl than without ryegrass predominance (E) and than other herbaceous plants rich (H) herbage. Ryegrass rich herbage (G_R and E_R) had comparable, but numerically lower mineral concentrations (except K, Na and Cl) and lower (P<0.001) Mn concentrations than corresponding classes of botanical composition with less ryegrass (G and E).

Table 2

Adjusted macro mineral response (g/kg DM) to botanical composition, harvest time and growth stage of fresh herbage from mixed grassland.

Model	Ca	P	Mg	K	Na	Cl	S
Intercept	7.89	4.81	2.49	34.40	0.18	4.75	2.62
Botanical composition ¹							
G	-1.012 ^c	-0.165 ^{bc}	-0.083 ^b	-1.245 ^{ab}	0.010 ^{ab}	0.035 ^{ab}	0.107 ^a
G _R	-1.481 ^c	-0.283 ^c	-0.318 ^c	-1.219 ^b	0.074 ^a	0.250 ^{ab}	-0.140 ^b
E	0.457 ^b	0.242 ^a	0.003 ^b	1.305 ^a	-0.026 ^b	-0.901 ^b	-0.014 ^{ab}
E _R	-0.257 ^b	-0.018 ^{ab}	-0.116 ^b	-0.473 ^{ab}	-0.038 ^b	1.564 ^a	-0.087 ^{ab}
H	1.779 ^a	0.223 ^a	0.514 ^a	1.631 ^a	-0.020 ^b	-0.947 ^b	0.134 ^{ab}
Harvest time							
1st	-0.726	-0.313	-0.268	-1.349	-0.029	-1.603	-0.197
+2nd	0.726	0.313	0.268	1.349	0.029	1.603	0.197
Growth stage ²							
Linear	-0.467	-0.199	-0.266	0.830	0.034	0.814	-0.306
Quadratic	0.080	-0.013	0.035	-0.464	-0.005	-0.144	0.014
P values ³							
Bot. composition	***	***	***	*	***	**	**
Harvest time	***	***	***	***	**	***	***
G. Stage linear	n.s.	*	***	n.s.	+	+	***
G. Stage quadratic	+	n.s.	***	***	*	*	+
R ²	0.49	0.62	0.56	0.61	0.18	0.41	0.55
r.m.s.e.	1.47	0.54	0.36	4.24	0.12	2.44	0.40

¹ Class of botanical composition: G (>70% grass), G_R (>70% grass with more than half as ryegrass), E (50–70% grass), E_R (50–70% grass with more than half as ryegrass), and H (>50% other herbaceous plants).

² Class of growth stage: 1 (tillering), 2 (stem elongation), 3 (begin heading), 4 (full heading), 5 (end heading), 6 (flowering) and 7 (fructification) with reference to *Dactylis glomerata* or *Lolium perenne*.

³ *** P < 0.001; ** P < 0.01; * P < 0.05; + P < 0.10; n.s. not significant; r.m.s.e. root mean square error; for the factor botanical composition, values in the same column not followed by the same letter differ significantly.

Table 3

Adjusted micro mineral response (mg/kg DM) to botanical composition, harvest time and growth stage in fresh herbage from mixed grassland.

Model	Cu	Fe ¹	Mn	Zn
Intercept	11.4	251.9	101.4	34.4
Botanical composition ²				
G	-0.292 ^{bc}	26.749	18.971 ^a	-0.669 ^b
G _R	-0.917 ^c	7.944	-8.257 ^b	-1.830 ^b
E	0.365 ^{ab}	-8.249	9.343 ^a	0.083 ^b
E _R	-0.431 ^{bc}	-14.865	-16.176 ^b	-1.755 ^b
H	0.045 ^a	-11.579	-3.882 ^{ab}	4.171 ^a
Harvest time				
1st	-1.079	-3.710	-8.310	-1.836
+2nd	1.079	3.710	8.310	1.836
Growth stage ³				
Linear	-1.593	-55.307	-23.546	-3.578
Quadratic	0.114	4.289	2.913	0.342
P values ⁴				
Bot. composition	***	n.s.	***	***
Harvest time	***	n.s.	**	***
G. Stage linear	***	**	***	***
G. Stage quadratic	**	+	***	***
R ²	0.59	0.15	0.18	0.38
r.m.s.e.	1.7	260.7	38.3	4.6

¹ Values above breakpoint of 106 g ash/kg DM were discarded.

² Class of botanical composition: G (>70% grass), G_R (>70% grass with more than half as ryegrass), E (50–70% grass), E_R (50–70% grass with more than half as ryegrass), and H (>50% other herbaceous plants).

³ Class of growth stage: 1 (tillering), 2 (stem elongation), 3 (begin heading), 4 (full heading), 5 (end heading), 6 (flowering) and 7 (fructification) with reference to *Dactylis glomerata* or *Lolium perenne*.

⁴ *** P < 0.001; ** P < 0.01; + P < 0.10; n.s. not significant; r.m.s.e. root mean square error; for the factor botanical composition, values in the same column not followed by the same letter differ significantly.

3.2. Effect of harvest time

All mineral concentrations, except Fe, were higher (P < 0.01) in subsequent harvests than in the 1st seasonal harvest (Table 2 and 3). In case of an equilibrated (E) herbage at begin of heading, the macro mineral concentrations of Ca, P, Mg, K,

Na, Cl and S were 21, 16, 31, 8, 31, 95 and 24% higher, respectively, than in the 1st harvest. The micro mineral concentrations of Cu, Mn and Zn for that herbage were, 31, 29 and 15% higher, respectively, than in the 1st harvest.

3.3. Effect of growth stage

With the exception of Na, Cl and Ca, the mineral concentrations of herbage were progressively reduced with increasing maturity (Table 2 and 3). With increasing growth stage, P ($P < 0.05$, linear), Mg ($P < 0.001$, linear and quadratic), K ($P < 0.001$, quadratic), S ($P < 0.001$, linear), Fe ($P < 0.01$, linear; $P < 0.10$, quadratic) and Cu, Mn and Zn ($P < 0.001$, linear; $P < 0.05$ quadratic) concentrations progressively decreased. Sodium and Cl concentrations increased up to growth stage 3 and declined afterwards ($P < 0.05$, quadratic). Calcium concentrations remained stable ($P > 0.10$) with increasing growth stage.

4. Discussion

The distribution of the main factors (class of botanical composition, harvest time and growth stage) and the variability of CP, ADF_{OM} and aNDF_{OM} concentrations in the collected samples were sufficient to run the model and permitted to reach R² values of 0.73, 0.55 and 0.48, respectively (data not shown). Calcium, P, Mg, K, S and Cu models reached similar coefficients of determination than CP, ADF_{OM} and aNDF_{OM}. Sodium, Fe and Mn models obtained unsatisfactory R² values (below 0.20) and suggest that other factors than the ones included in the model influence their concentrations. With an ash concentration above 106 g/kg DM, Fe concentrations increased by 30 mg/kg DM per g of ash ($R^2 = 0.79$, $SE = 1.3$). Values below this threshold were still variable (133 ± 109 mg/kg DM), with highest values observed in herbage of early growth stages. Although some unavoidable soil contamination may become part of the plant material when harvested or ingested by the animal, it probably contributed to the low R² value of the Fe model. Sodium concentrations were mostly above the limit of determination, but relatively close to it, probably explaining the weakness of the coefficient of determination.

The reason for a reduced mineral concentration (except Na, Cl, S and Mn) with increasing proportion of grasses, especially of ryegrass is not completely explained. Haynes (1980) suggested that monocotyledonous species have less cation exchange sites necessary for mineral uptake by the plant than dicotyledonous species. However, the plant specific mineral uptake and the transport mechanisms from the roots to the shoots (Xing et al., 2008) as well as the homeostatic mineral regulations within plants and between species are not yet fully understood (Krämer et al., 2007). Nevertheless, differences in macro mineral (Daccord et al., 2001) and micro mineral (Lindström et al., 2012) concentrations were observed in individually grown species and in botanical groups of mixed grasslands (Hasler and Besson, 1972; Stünzi, 1998; Pirhofer-Walzl et al., 2011; Lindström et al., 2014). The observed lower Cu, Mn and, in a certain way, S concentrations in ryegrass rich herbage (E_R and G_R) illustrate findings that *Lolium perenne* and *Lolium multiflorum* were lower in Cu and Mn than *Dactylis glomerata* (Stünzi, 1998; Lindström et al., 2012) and that *Lolium multiflorum* was lower in S than *Dactylis glomerata* (Stünzi, 1998). The botanical composition affected the Na concentrations of the herbage too. But in respect to the low repeatability of the model, the low Na concentrations in herbage and the lack of similar observations in earlier studies (Kessler and Jolidon, 1998; Pirhofer-Walzl et al., 2011) its relevance is considered as limited.

All mineral concentrations were higher in subsequent harvests than in the 1st seasonal harvest. This is in agreement with Daccord et al. (2001) for Ca and Mg, but not for P, K and Na in single plants. It also agrees with Pirhofer-Walzl et al. (2011) for macro minerals, Cu, Mn and Zn in grasses and other herbaceous plants and for Cu in legumes. In mixed grassland, it is in agreement with Wyss and Kessler (2002) for Ca, P, Mg and in agreement with Govasmark et al. (2005a,b) for Cu, Mn and Zn. The relatively important difference in mineral concentration between the 1st harvest and subsequent harvests may be related to the change from a generative (first harvest) to a vegetative (subsequent harvests) growth as suggested by the increased mineral concentrations in single plants. A second reason is probably the increasing proportion of legumes in mixed grassland during the subsequent harvests. Legumes contain more minerals than grasses and according to the herbage samples in which the botanical groups were not only visually determined but also weighted, the proportional weight of legumes increased in the subsequent harvests within all classes of botanical composition (12.2 and 20.6%, respectively in the 1st and the subsequent harvests, $P < 0.01$). This phenomenon also occurred in the studies of Wyss and Kessler (2002) and Govasmark et al. (2005a,b).

The mineral concentration of herbage (except Ca, Na and Cl) progressively decreased with increasing maturity. This phenomenon is in agreement with other findings for ryegrass (Brink et al., 2006), timothy (Nordheim-Viken et al., 2009), smooth bromegrass (Casler et al., 1987), legumes (Kirchgessner et al., 1968) and with Zn in mixed grassland (Stünzi, 1973). Calcium concentrations remained stable over aging. Calcium is mainly present in cell walls of grasses and legumes (Whitehead et al., 1985). Calcium is considered as immobile and is expected to deviate from mobile elements, such as P, Mg, K and S in its behaviour during plant growth (White, 2012). This is probably the reason for the stable Ca concentrations over aging. As the botanical composition, the growth stage also affected the Na concentrations of the herbage. But in respect to the low repeatability of the model, the low Na concentrations in herbage and the lack of similar observations in earlier studies (Wyss and Kessler, 2002) its relevance is considered as limited.

Finally, the present data show that fresh herbage from mixed grassland represents a major mineral source to herbivores. Indeed, a typical grazing herbage (equilibrated botanical composition, growth stage between 2 and 3, 1st/subsequent harvest ratio of 30/70) covered between 75 and 100% of Ca, P, K, Cl, S, Fe and Mn and between 50 and 75% of Mg, Co, Cu and Zn of the mineral recommendations (Agroscope, 2015a) of a lactating dairy cow (680 kg BW, 18 kg DM herbage intake and

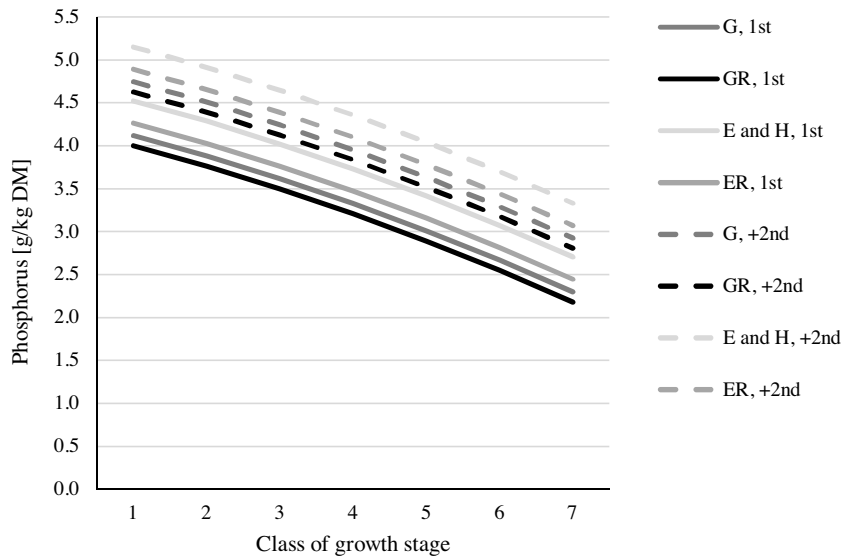


Fig. 2. Modelled response of P concentration to growth stage according to class of botanical composition and harvest in fresh herbage of mixed grassland.

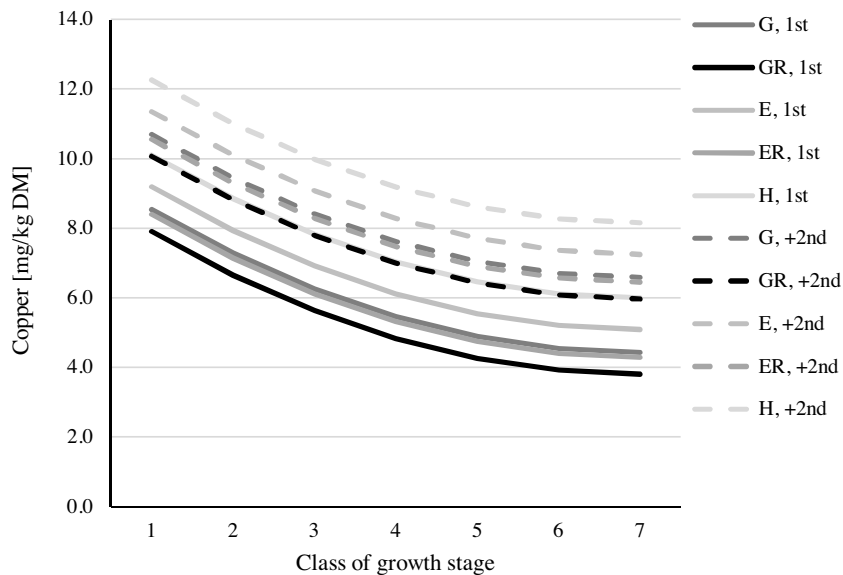


Fig. 3. Modelled response of Cu concentration to growth stage according to class of botanical composition and harvest in fresh herbage of mixed grassland.

35 kg milk production). The developed models contribute to assess reference values of fresh herbage from mixed grassland, as illustrated with P and Cu in Figs. 2 and 3. Validation studies of defined herbage from mixed grasslands are especially required in other major livestock production regions to consider regional differences including combined effects of soil, climate and altitude. In this respect, mineral concentrations of herbage (botanical composition of G and E, begin of heading, 1st harvest) originating from various sites in Switzerland were compared with the present modelled values (Schlegel et al., 2016). Whereas data from the Swiss plateau and the Jura Mountains were compatible with the present modelled values, data from the Alps were mostly underestimated.

5. Conclusions

The results of this study showed that macro and micro mineral concentrations were generally lowest in fresh mixed grassland herbage with high proportion of grasses, highest with high proportion of other broadleaved herbaceous plants, lower in the first harvest than in subsequent harvests and decreasing with increasing growth stage. The developed models

may be considered to assess reference values in order to take into account the native mineral concentration of fresh herbage from mixed grassland when optimizing herbivore diets in regards to a sustainable mineral supplementation.

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