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The Mineral Composition of Wild-Type and Cultivated Varieties of Pasture Species

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Abstract: Mineral deficiencies in livestock are often prevented by using prophylactic supplementation, which is imprecise and inefficient. Instead, the trend for increased species diversity in swards is an opportunity to improve mineral concentrations in the basal diet. Currently, there are limited data on the mineral concentrations of different species and botanical groups, particularly for I and Se, which are among the most deficient minerals in livestock diets. We grew 21 pasture species, including some cultivar/wild type comparisons, of grasses, legumes and forbs, as single species stands in a pot study in a standard growth medium. Herbage concentrations of Co, Cu, I, Mn, Se, Zn, S, Mo and Fe showed no consistent differences between the wild and cultivated types. There were significant differences between botanical groups for many minerals tested. Forbs were highest in I and Se, grasses in Mn and legumes in Cu, Co, Zn and Fe. Comparing species concentrations to recommended livestock intakes, the forbs *Achillea millefolium*, *Cichorium intybus* and *Plantago lanceolata*, and the legumes *Medicago lupulina*, *Trifolium hybridum* and *Lotus corniculatus*, appear to be good sources of Co, Cu, I, Se and Zn. Further work is required to ensure these results are consistent in multispecies mixtures, in different soil types and in field trials.

Keywords: micronutrients; trace elements; antagonism; livestock intake; multispecies; multifunctional; sward; forb; legume; grass

1. Introduction

Minerals are essential elements in the diets of livestock, which regulate metabolic processes and provide cellular structure. Inadequate provision may result in wide ranging symptoms including poor weight gain or weight loss, reproductive problems, heart disease, anaemia, joint problems and fragile bones [1]. These effects may be sub-clinical, meaning that outward signs are not obvious, but they may still lead to suboptimal livestock health and performance, the cause of which is difficult to diagnose [2]. Minerals of particular concern in UK ruminant systems are Co, Cu, I, Mn, Mo, Se and Zn [3].

Mineral intake may be via feed or supplementation by other means, such as boluses, licks, drenches or injections. The disadvantage of supplements is that they are an additional cost for the farmer, they are often used prophylactically, without proper assessment of their necessity and with most being in an inorganic form, are less bioavailable [4,5]. Furthermore, supplement manufacturers can try to make their products look more attractive by increasing the quantity of minerals in them, but there is then a risk of toxicity [6]. Some forms of supplementation, such as licks, also do not control mineral intake relative to bodyweight, whereas delivery in the basal diet means that minerals are in proportion to the dry matter (DM) intake of the animal. The basal diet of most ruminant livestock is forage due to the low unit cost and inherent ruminal health benefits [7]. Although supplemental feeding of concentrates (cereals and by-products) is used, especially for dairy cattle or the finishing of beef cattle, supplementation is less practiced for small ruminants, especially in temperate sheep systems. Therefore, by better understanding and controlling mineral levels in forage we can improve profitability and sustainability of ruminant livestock enterprises through increasing their reliance on forage.

Due to the wide variety of minerals required by livestock, as well as other nutrients such as vitamins, protein, fibre and metabolizable energy, it is unlikely that a single pasture species will provide livestock with a complete diet [8]. Improved grasslands tend to have low plant diversity, and *Lolium perenne*, *Trifolium pratense* and *Trifolium repens* typically provide the basis of most swards, with a focus on maintaining sufficient DM yield and protein production over a grazing season, or providing sufficient early season growth for a silage cut [9]. These species-poor plant communities bear little resemblance to the natural vegetation that the ancestors of our modern livestock breeds evolved to graze. Hence, there is a growing trend toward more diverse pastures that contain a variety of legumes, herbs and forbs in addition to grasses [10], which can potentially deliver a range of ecosystem services besides biomass production [11]; yet, the selection of species on any mineral benefits they might bring is still very niche [9].

Although pasture species have not typically been bred for their mineral content [12], breeding for other traits is likely to have affected their concentrations. For example, cultivated varieties of *Triticum aestivum* have both lower concentrations of Zn, and a narrower range of genetic variation, than wild-type wheat [13]. Within cultivated varieties of wheat, there is evidence of declining mineral concentrations, e.g., 33–49%, 25–39% and 20−27% decreases in Zn, Cu and Mg concentrations in grain in 2000−2005 relative to the long-term (1845–1967) mean [14]. The authors suggest that breeding has resulted in a dilution effect, whereby mineral concentrations are decreased due to the greater yield of the crop, particularly since the advent of semi-dwarf cultivars, and found that soil concentrations of potentially bioavailable (EDTA-extractable) minerals had tended to increase [14]. Similarly, nutrients (e.g., protein, Ca, P, Fe, riboflavin and ascorbic acid) in vegetable crops such as *Brassica oleracea* var. sabellica, *Brassica oleracea* var. italica and *Brassica oleracea* var gemmifera declined between 6 and 38% in the years from 1950 to 1999, which the authors explain as being largely due to cultivar choice [15]. However, there do not appear to be any similar studies in pasture species to ascertain whether cultivated varieties differ in their mineral content relative to their wild-type cousins.

Furthermore, there seems to be few published measurements of mineral concentrations in pasture species at all [3]. Apart from Mn, legumes, herbs and forbs typically have higher mineral concentrations than grasses [16–18]. However, this overlooks the variability between species within each of these botanical groups [3] and also differences between minerals; for example, legumes have higher Cu but lower Fe and Zn concentrations than forbs [19]. In addition, soil and environmental factors, alongside pasture management and interspecific plant competition, affect mineral uptake by species [3], influencing not only the concentration of minerals in the plants, but also potentially affecting the relative differences between species. For example, the effect of N fertilizer on mineral concentrations has a significant interaction with plant species [20], as well as further interactions with the application of Ca and Se fertilizers, the harvest number (primary growth versus secondary cuts) and location, presumably due to climatic and soil factors [21]. Therefore, it is difficult to make a comparison of mineral concentrations in plant species and botanical groups across different studies. Much of the available data on plant mineral concentrations are from Scandinavia [17,22,23], and it is unknown how applicable the data are elsewhere due to differences in plant cultivars, soils and climate. Furthermore, many of these studies overlook I and Se (despite these being some of the most deficient minerals in livestock systems), primarily due to the additional challenge of their more complex analysis compared to other minerals. In addition to solely evaluating mineral content in isolation, we also need to account for antagonism between them, which can reduce bioavailability at the plant or livestock level. The most common example of this at the livestock level is the reduction in Cu absorption caused by excess Mo, S and/or Fe in the rumen [24].

The objective of this study was to assess the relative ability of different pasture species to take up those minerals most limiting in UK pastures Co, Cu, I, Mn, Se and Zn [25], and to compare the concentrations to recommended levels for pastures. The main antagonistic minerals (Mo, S and Fe) were also measured, although these too have vital nutritional roles. Plants were grown in monocultures in a controlled pot study to test the following hypotheses: (i) botanical groups can be separated on the basis of their mineral composition, and there is greater variability between botanical groups, than within a botanical group, and (ii) wild-type species have greater mineral concentrations than their cultivated relatives. Using these data, we aimed to identify species that could make a beneficial contribution to the mineral content of a pasture sward, which could be tested further at a field scale.

2. Materials and Methods

A total of 21 temperate pasture species common to the UK were grown and were selected on the basis that they are commonly included in herbal ley mixtures within agricultural and research settings. Where available, both a wild-type and cultivated species were selected (Table 1). All seeds were purchased from seed companies, including wild-type seeds, which were collected from wild parent samples. The species represent three botanical groups: grasses (10 species, 5 wild-type and 8 cultivated), legumes (6 species, 5 wild-type and 6 cultivated) and non-leguminous forbs (5 species, 4 wild-type and 4 cultivated) (Table 1). *T. repens*, a common pasture species, was originally included in the experiment but failed to germinate in sufficient numbers. There was no more than one wild-type variety of each species grown, and only for *T. pratense* was more than one cultivar grown. The two cultivars were selected because one was early flowering (var. Merula), and the other late flowering (var. Altaswede). A randomised complete block design with a nested treatment structure was used with 4 replicates. The main treatment was plant species, and these were categorised by botanical group (grass, legume or forb), and type (wild or cultivated) for statistical analysis.

Plant species were grown in single species stands in a standard growing medium. This medium, rather than a soil, was selected, for several reasons. Firstly, due to the many thousands of plant species and cultivars available, it would not be feasible to screen them all in a single study. The use of a growing medium therefore allows further plant species to be subsequently screened, without significant spatial and temporal variability present in soil properties. The growing medium was a standard Rothamsted prescription soil (pH 6.5) supplied by Petersfield Growing Mediums (Leicester, UK) containing 80% sterilised loam, 15% 2EW sand and 5% lime free grit (5 mm). The growing medium had optimal levels of P, Mg, Cu and Zn, and optimal CEC and pH values (Table S1). Selenium levels were in the 25th percentile of those in English and Welsh topsoils. Although this does not confer a measure of deficiency, given livestock generally are often deficient in Se, we can therefore infer that the growing medium is low in Se. The medium was allowed in K compared to the RB209 fertilizer recommendations for the UK [26], although not low enough that there was likely to be a growth response to K fertilizer.

For each stand, a 4.5 L pot (21 cm max. diameter, 18.3 cm height) and corresponding saucer was used. No additional fertiliser was added and none of the species showed any signs of nutrient deficiency throughout the experiment. These pots were in an outdoor cage at Rothamsted Research (Harpenden, UK). The cage area had a concrete floor surrounded by netting on all sides to protect plants from herbivory and was covered by a roof to protect from frost and to prevent the plants from receiving rainfall. Seeds were initially broadcast-sown on 25th April 2018. After germination, the number of plants was thinned to give 10 evenly spaced plants per pot. Pots were kept well-watered with de-ionised water and weeded to remove any volunteer species.

Table 1. Species of grasses, legumes and forbs grown in the pot experiment. A cultivar name is given if one was available. Additionally shown is whether the species is commercially available or wild-type.

Latin Name	Commercial	Wild
Grasses		
Alopecurus geniculatus *		Wild
Alopecurus pratensis *	Zuberska	
Anthoxanthum odoratum *	Commercial	Wild
Dactylis glomerata *	Sparta	Wild
Festuca arundinacea	Debussy	
Festulolium loliaceum	AberNiche	
Holcus lanatus		Wild
Lolium perenne *	Nifty	Wild
Phleum pratense	Promesse	
Poa trivialis	Dasas	
Legumes		
Lotus corniculatus *	Leo	Wild
Medicago lupulina *	Virgo	Wild
Melilotus officinalis *	Commercial	Wild
Trifolium dubium		Wild
Trifolium hybridum	Dawn	
Trifolium pratense *	Merula & Altaswede	Essex
Forbs		
Achillea millefolium *	Commercial	Wild
Centaurea nigra		Wild
Cichorium intybus	Puna II	
Plantago lanceolata *	Endurance	Wild
Sanguisorba minor *	Commercial	Wild

* Species that have both a wild and cultivated version. Note that Alopecurus geniculatus and Alopecurus pratensis are considered wild-type/cultivated versions of the same plant for the purposes of this experiment.

2.1. Sample Collection and Analysis

On the 29 June 2018, 65 days after seeds were sown, the plant biomass was cut to 2 cm above the soil surface to obtain whole shoot concentrations while avoiding soil contamination. A second cut was taken after a further 75 days, on 12 September 2018. Only the second cut was analysed for biomass and mineral content, as it was considered to be more typical of that consumed by livestock, and only vegetative material was included in the samples (excluding inflorescences). Herbage samples were dried at 40 °C for 48 h, milled and then split into two. One half was sent for I analysis and the second half was re-dried at 80 °C for a further 24 h for a full mineral analysis. Subsamples were extracted using a perchloloric acid digest and analysed for a suite of micro- and macrominerals using inductively coupled plasma atomic emission spectroscopy (ICP-AES) and ICP mass spectrometry (ICP-MS). Samples were also analysed for iodine by NUVetNA (University of Nottingham, UK) using a 25% tetramethylammonium hydroxide (TMAH) extraction for 4 hrs and analysis by ICP-MS. The total concentration of minerals in the growing medium (prior to plant growth) was assessed using aqua regia digest and ICP-AES and ICP-MS. Ammonium nitrate or Mehlich III extracts of soils were used to give a measure of the availability of the minerals (NRM laboratories, Bracknell, UK).

2.2. Statistical Analysis

Statistical analysis was performed using Genstat (VSN International Ltd., version 20.1.0.23823). Plant yield, as dry matter (DM), and concentrations of I, Cu, Co, Se, Zn, Mn, Fe, Mo and S were $\log_{10}$ transformed to satisfy the normality and homogeneity of variance assumptions of the analysis. For DM and each mineral in turn, a linear mixed model (LMM, using the REML directive in Genstat) was used to assess the statistical significance of type (wild/cultivated), botanical group (forb/grass/legume), the interaction between type and botanical group, and the species within a type-botanical group (i.e., the species within each of the cultivated forbs [CF], cultivated legumes [CL], cultivated grasses [CG], wild forbs [WF], wild legumes [WL] and wild grasses [WG]). The fixed structure of the linear mixed model was (Type*Botanical_group) / (nameFC + nameFW + nameGC + nameGW + nameLC + nameLW), where, for example, 'nameFC' refers to the names of all species falling into the grouping FC. The random structure was block. In addition to testing the significance of plant type with all plants included, an LMM with the same fixed and random structures was fitted with only those species that had both a wild and cultivated version (Table 1). This was to check that the plant type comparison was not biased by presence of species with higher mean mineral concentrations in one of these groups and not the other. In each case, subsequent to fitting the LMM, differences within the type, within the botanical group, and within the type-botanical group interaction were determined using the means and least significant differences. Post-hoc comparisons to determine whether there were differences between species within the type-botanical group were not made between all species, in order to reduce the risk of Type I errors where multiple comparisons can increase the likelihood of 'false positives' in statistical outcomes. Instead, means and least significant differences were only used to compare whether the species with the highest concentration of a given mineral had a significantly greater concentration than the species with the second highest concentration. Likewise, for the difference between the species with the two lowest concentrations. Significance is defined at the 95% level ($p < 0.05$). Finally, the differences between the trade-offs and synergies in mineral concentrations were visualised using a Principal Components Analysis in CANOCO5 [27].

3. Results

3.1. Plant Yield

There was an interaction between plant type and botanical group on the mean DM yield of the five replicates of each species. The wild forbs had a higher DM than the other plant types and botanical groups, at 8.37 g DM per pot. The yield of the cultivated grasses, wild grasses and cultivated forbs did not differ from one another, at 6.78, 6.71 and 6.61 g DM, respectively. The cultivated and wild legumes had significantly lower yields than the other plant types and botanical groups, at 5.04 and 4.87 g DM, respectively. Within a plant type and botanical group, the species differed from one another significantly (Figure 1), except for within the wild forbs.

3.2. Mineral Composition: Comparison of Botanical Groups

Botanical group significantly affected plant concentrations of minerals (Table 2), although there was an interaction between plant type and botanical group for most of the minerals (Cu, Co, Se, Mo, Zn, Mn and S). However, this interaction did not affect the pattern between the botanical groups for most of the minerals, but did affect whether differences between any two groups were significant.

For both cultivated and wild species, legumes contained significantly greater mean concentrations of Cu (12.4 mg kg^{-1} DM and 10.3, respectively, Table 3) than the forbs (8.10 and 6.68 mg kg^{-1} DM, respectively) and grasses (6.02 and 5.30 mg kg^{-1} DM, respectively). Legumes also had higher concentrations of Co and Zn than the other botanical groups; this difference was significant for the cultivated species but not always for the wild species. For example, Co concentrations were 0.223 mg kg^{-1} DM in cultivated legumes, significantly greater than both the cultivated grasses and forbs (0.120 and 0.160 mg kg^{-1} DM, respectively). In the wild species, Co concentrations were 0.179 mg kg^{-1} DM in legumes, significantly greater than the 0.130 mg kg^{-1} DM in grasses, but not significantly greater than the 0.162 mg kg^{-1} DM in forbs. For Fe, there was no significant interaction with plant type, and legumes had significantly greater concentrations (136 mg kg^{-1} DM) than either the forbs or the grasses (92.3 and 59.0 mg kg^{-1} DM, respectively).

Table 2. Summary of linear mixed model (LMM) analysis showing whether there is a statistically significant interaction between plant type (wild/cultivated) and botanical group (forb/legume/grass) for the concentration of 9 minerals in plant biomass. Where the interaction is not significant ($p > 0.05$), the significance of the individual effects is reported.

	Plant Type	**Botanical Group**	**Type−Botanical Group Interaction**
I	0.804	<0.001	0.051
Cu			0.001
Co			0.005
Se			0.029
Mo			0.003
Zn			<0.001
Mn			<0.001
Fe	0.389	<0.001	0.219
S			<0.001

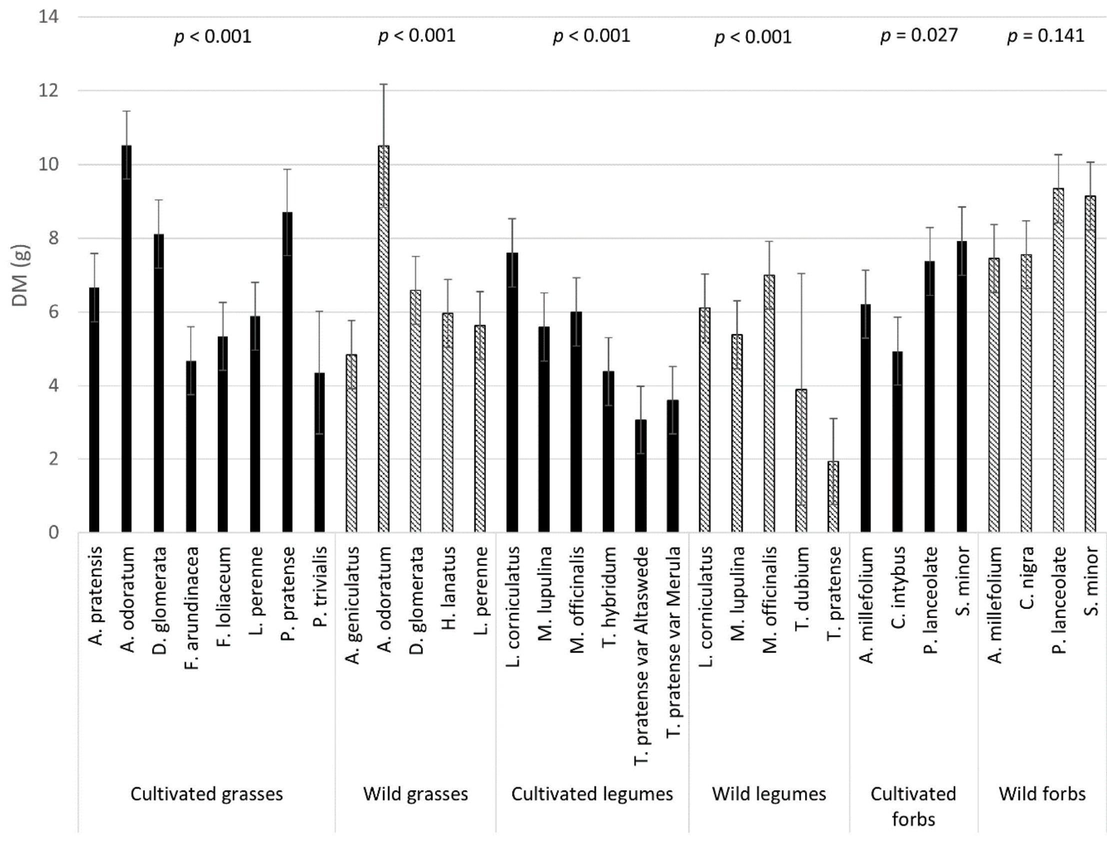

Figure 1. Barchart of means and confidence intervals of the dry matter (DM) yield of pasture species, in mass per pot, split by botanical group and plant type. Error bars show the confidence interval over the five replicates, as given by the LMM analysis. The p-value shows the significance of the difference between species, within plant type and botanical group.

Manganese concentrations were significantly higher in grasses than in either of the other botanical groups; 234, 127 and 126 mg kg^{-1} DM in cultivated grasses, forbs and legumes, respectively, and 403, 106 and 103 mg kg^{-1} DM, respectively in the wild botanical groups. Wild-type grasses had significantly greater concentrations of S, at 3240 mg kg^{-1}, than forbs and legumes, at 2300 and 2580 mg kg^{-1} DM, respectively, but there was no significant difference between the cultivated botanical groups. As presented above, grasses had significantly lower concentrations of Co, Cu and Fe than the other botanical groups, in both the cultivated and wild species.

Table 3. Concentrations (mg kg^{-1} DM) of minerals in plant material. Values are means and confidence intervals for the botanical groups (grass/legume/forb) across replicates and different species. Where there was a significant ($p < 0.05$) interaction between botanical group and plant type (wild/cultivated), values for plant type are presented separately. Superscript letters show significant differences within a mineral.

Mineral	Botanical Group	Cultivated	Confidence Interval	Wild	Confidence Interval
Cu	Forb	8.10 ^{c}	7.940–8.264	6.68 ^{d}	6.520–6.844
	Grass	6.02 ^{de}	5.924–6.116	5.30 ^{e}	5.152–5.452
	Legume	12.4 ^{a}	12.26–12.48	10.3 ^{b}	10.09–10.47
Co	Forb	0.160 ^{b}	0.1537–0.1656	0.162 ^{b}	0.1563–0.1684
	Grass	0.120 ^{c}	0.1174–0.1227	0.130 ^{c}	0.1256–0.1346
	Legume	0.223 ^{a}	0.2173–0.2286	0.179 ^{b}	0.1715–0.1873
Se	Forb	0.141 ^{a}	0.1362–0.1466	0.131 ^{ab}	0.1265–0.1362
	Grass	0.103 ^{c}	0.1003–0.1050	0.116 ^{bc}	0.1121–0.1196
	Legume	0.121 ^{b}	0.1182–0.1247	0.101 ^{c}	0.09787–0.1052
Mo	Forb	1.72 ^{b}	1.657–1.791	1.64 ^{b}	1.576–1.704
	Grass	4.62 ^{a}	4.524–4.709	5.25 ^{a}	5.079–5.435
	Legume	5.03 ^{a}	4.901–5.163	4.20 ^{a}	4.036–4.370
Zn	Forb	23.9 ^{c}	23.17–24.61	16.5 ^{d}	16.03–17.03
	Grass	34.0 ^{bc}	33.37–34.57	37.5 ^{bc}	36.46–38.56
	Legume	58.5 ^{a}	57.29–59.69	38.7 ^{b}	37.36–40.14
Mn	Forb	127 ^{c}	123.3–131.6	106 ^{d}	102.5–109.4
	Grass	234 ^{b}	229.2–238.7	403 ^{a}	390.7–415.1
	Legume	126 ^{c}	123.3–129.2	103 ^{d}	99.02–106.7
S	Forb	3410 ^{a}	3315–3512	2300 ^{b}	2236–2369
	Grass	3340 ^{a}	3282–3403	3240 ^{a}	3157–3332
	Legume	2400 ^{b}	2350–2449	2580 ^{b}	2498–2670
		Mean	Confidence interval		
I	Forb	2.03 ^{a}	1.985–2.068		
	Grass	0.630 ^{b}	0.6205–0.6390		
	Legume	0.621 ^{b}	0.6104–0.6321		
Fe	Forb	92.3 ^{b}	90.24–94.32		
	Grass	59.0 ^{c}	58.18–59.87		
	Legume	136 ^{a}	133.5–138.3		

Forbs had significantly higher concentrations of I than the other botanical groups, at 2.03 mg kg^{-1} DM versus 0.630 and 0.621 mg kg^{-1} DM for grasses and legumes, respectively. In cultivated species, forbs contained significantly higher concentrations of Se, at 0 mg kg^{-1} DM, than the grasses and legumes, at 0.103 and 0.121 mg kg^{-1} DM, respectively. In the wild species, forbs had the highest concentrations at 0.131 mg kg^{-1} DM, but this was only significantly greater than the legumes, at 0.101 mg kg^{-1} DM, and not the grasses, at 0.116 mg kg^{-1} DM. Although Mo concentrations did not differ significantly between grasses and legumes, both botanical groups had significantly higher concentrations than the forbs. For example, in the cultivated species, forbs contained 1.72 mg kg^{-1} DM Mo compared to 4.62 and 5.03 mg kg^{-1} DM in the grasses and legumes. Similarly, forbs contained significantly lower concentrations of Zn than the other botanical groups.

3.3. Mineral Composition: Comparison of Plant Type

There was no significant effect of plant type on Fe and I concentrations (Table 2), but for the remainder of the minerals there was a significant interaction with botanical group. Where there was a significant difference between the mean concentration of a mineral in the wild and cultivated plants of a given botanical group, it was almost always exclusively the cultivated species that had the higher concentration of the mineral (Table 3). This was seen for Cu, Co, Se, Mo, Zn and Mn in the legumes, and for Cu, Zn, Mn and S in the forbs. For example, the cultivated legumes contained 58.5 mg kg^{-1} DM Zn, but the wild legumes only 38.7 mg kg^{-1} DM. The exception to this trend was for Mn concentrations in grasses, where wild-types contained higher concentrations than cultivated types, at 403 and 234 mg kg^{-1} DM, respectively.

In the LMM analysis using only those species that had both a wild and cultivated cultivar (Table 1), plant type, or the interaction between plant type and botanical group, had a statistically significant effect on mineral concentrations for I, Cu, Co, Se, Mo, Zn and S (Table S2), but not for Mn or Fe. Significant differences between minerals generally reflected higher concentrations in cultivated species (Table S3). This was seen in legumes for I, Cu, Co, Mo and Zn; in grasses for I and Cu; and in forbs for S. The exception to this trend was for Co in forbs, where the wild-type species contained 0.162 mg kg^{-1} DM compared to 0.126 mg kg^{-1} DM in cultivated species. For Se, where only the plant type affected plant concentrations and there was no interaction with botanical group, wild plants contained higher concentrations than the cultivated plants (0.135 and 0.121 mg kg^{-1} DM, respectively).

3.4. Mineral Composition: Variation between Species

There was a significant difference between species in their concentration of any given mineral, within a plant type and botanical group, for almost all the minerals (Figures 2 and 3). The only exceptions were for the Se concentration of wild legumes, Mo concentrations of wild grasses and Fe concentrations of cultivated and wild grasses, wild legumes and cultivated forbs, where there was no significant difference between the species within those groups.

The data show that *Alopecurus pratensis* had the highest Cu concentrations among the cultivated grasses (Figure 3). Of the wild grasses, *Holcus lanatus* had significantly higher Mn concentrations than the other species, but *L. perenne* the highest Co concentrations. Of the cultivated legumes, *T. hybridum* and *Medicago officinalis* had the greatest Cu and S concentrations, respectively (Figures 2 and 3), while among the wild-type legumes, *T. dubium* had the highest Cu concentration. Of the cultivated forbs, *C. intybus* contained the highest concentrations of Cu, Mn, Zn and Mo, and *A. millefolium* the highest concentration of I. In the wild forbs, *C. nigra* had the highest concentrations of Co, Mo and Fe, *A. millefolium* contained the highest concentrations of Cu, and *P. lanceolata* the highest concentrations of S. On the other hand, *Dactylis glomerata* and *Phleum pratense* had significantly lower concentration of I and of S and Mo, respectively, among the cultivated grasses. Of the cultivated forbs, *Sanguisorba minor, A. millefolium* and *P. lanceolate* contained the lowest concentrations of Co, S and Mn, respectively. Among the cultivated legumes, *T. pratense* var Altaswede and *M. lupulina* had the lowest concentrations of I and Cu, respectively.

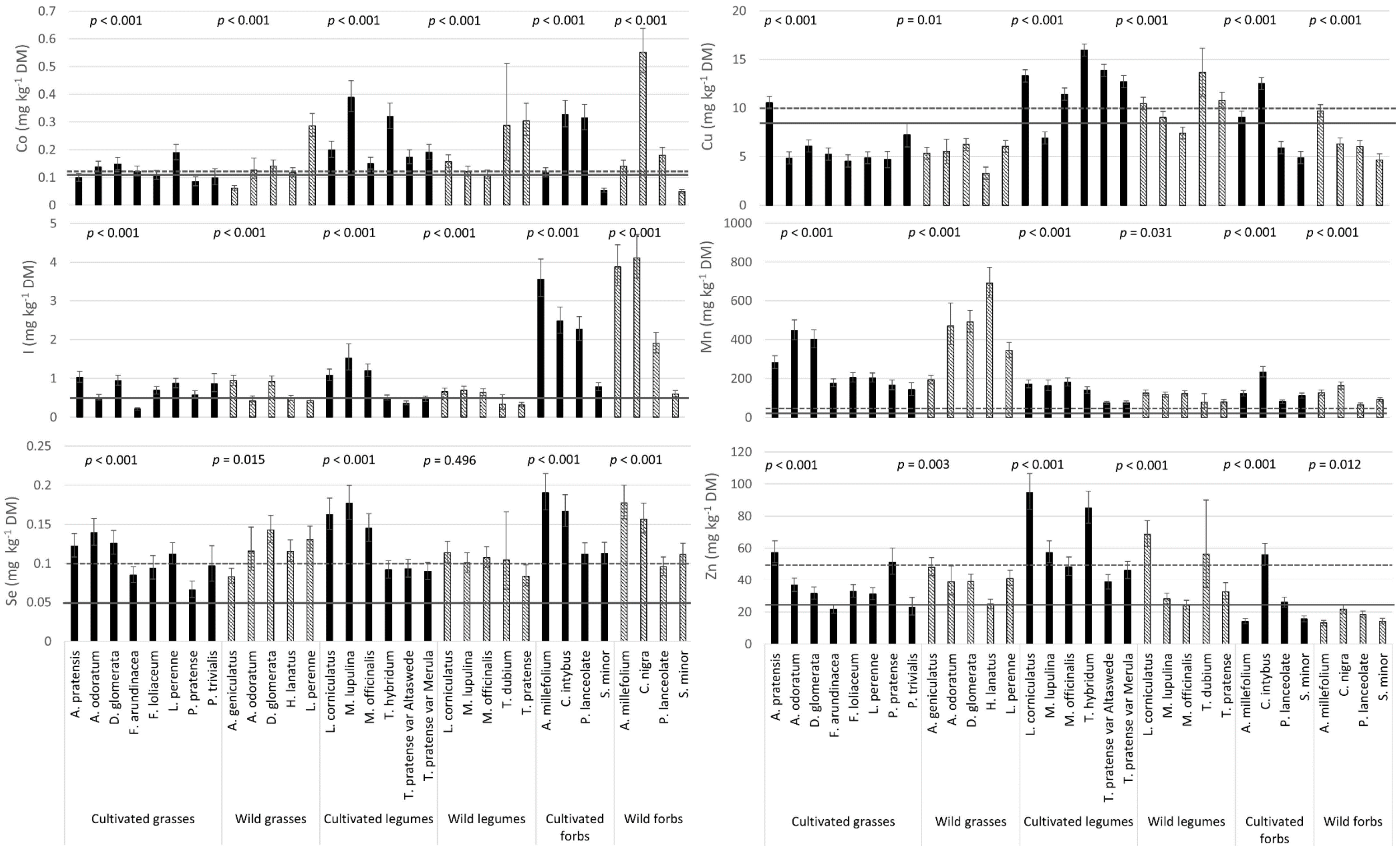

Figure 2. Barchart of means and confidence intervals of Co, Cu, I, Mn, Se and Zn in pasture species. Error bars show the confidence interval over the five replicates, as given by the LMM analysis. The *p*-value shows the significance of the difference between species, within a plant type and botanical group. — Indicates the recommended minimum level in pasture to prevent deficiency. Dashed lines (– –) indicate the recommended minimum levels in the total diet (AHDB, 2011).

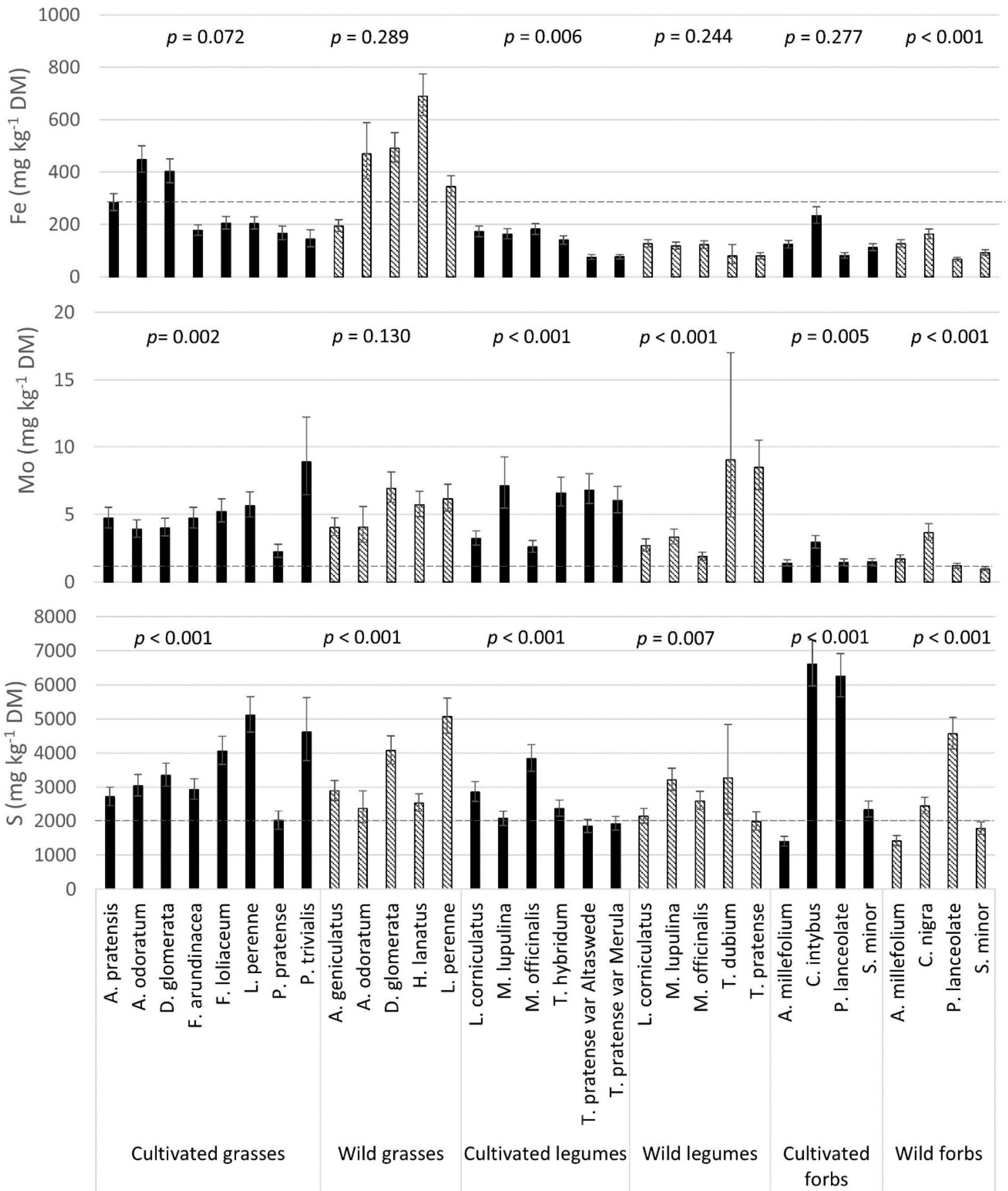

Figure 3. Barchart of means and confidence intervals of Mo, Fe and S in pasture species. Error bars show the confidence interval over the five replicates, as given by the LMM analysis. The *p*-value shows the significance of the difference between species, within a plant type and botanical group. Dashed lines (– –) indicate the maximum advised level in pasture to avoid deficiency of Cu (AHDB, 2011).

3.5. Plant Concentrations Relative to Recommended Levels for Livestock Intake

The UK Agriculture and Horticulture Development Board (AHDB) provide recommendations for the minimum concentration of beneficial minerals (Cu, Co, Se, I, Mn and Zn) in both pasture and the total diet to prevent livestock deficiency [25]. These recommendations also contain advised maximum levels of minerals that can affect the uptake of Cu (e.g., Mo, S and Fe). We used these values to give some context to the mineral concentrations measured in our plant species (Minimum and maximum advised concentrations in Figures 2 and 3, respectively). Where the recommendations advised a range of minimum pasture concentrations based on the livestock (sheep or cattle) and life-stage (growing or dry versus pregnant and lactating), we used the higher value.

Grown in the test medium, all of the species typically had concentrations indicative of sufficient Co, Se, Mn and I. The forbs had low Zn concentrations, with most species having less than the recommended minimum for pasture of 25 mg kg^{-1} DM, and across the grasses and legumes, species typically had Zn concentrations between the recommended pasture and total diet (50 mg kg^{-1} DM) values. Except for the cultivated grass, *A. pratensis*, all the grasses had Cu concentrations below the recommended minimum for pasture, of 8 mg kg^{-1} DM. The legumes and forbs each had species with a wide spread of Cu concentrations, but the legume species predominantly had Cu concentrations greater than the total diet minimum, while most of the forbs had Cu concentrations below the pasture minimum. All the species tested had concentrations below the maximum advised Fe concentration. Conversely, most species had concentrations above the advised maximum Mo and S concentrations advised. The forbs performed best for their Mo concentration, with *P. lanceolata*, *A. millefolium* and *S. minor* close to the advised maximum concentration. Sulphur concentrations in the cultivated grass *P. pratense*, and the cultivated and wild legumes *T. pratense* and *M. lupulina* were close to the maximum advised concentration, while *A. millefolium* and *S. minor* (across the cultivated and wild forbs) were lower.

4. Discussion

4.1. Impact of Plant Yield

There were significant differences in DM yield between botanical groups, and between species within a botanical group. Comparisons of mineral uptake on a total mass basis rather than a concentration basis shows fewer significant effects (Tables S4 and S5 and Figures S1 and S2), particularly with regard to whether there are potential differences between species within a botanical group and whether one species has a significantly higher or lower concentration than others in the group. This indicates that some of the observed differences may be due to a 'dilution effect', whereby the differences in plant biomass are greater than their ability to regulate (micro)mineral uptake, thereby resulting in concentration differences between species [28,29]. However, most significant differences between botanical group, between plant type and between species remain similar whether we consider them on a concentration or a mass basis. Therefore, we conclude that it is appropriate to compare element concentrations in botanical groups, plant types and species on a concentration basis. In our study, we controlled the growth of plants to 10 plants per pot. In a field environment, proportional species composition may vary due to competition between plants [30]. In this situation, the DM production of plants may become more important once more. For example, in the study of Lindström et al. [31], *C. intybus* had the highest and *P. pratense* the lowest mineral concentrations from field plots sown with multispecies swards, but total mineral offtake was smaller in the former due to it forming a smaller proportion of the total biomass (<10% versus <80%). Consequently, field studies of multispecies swards need to consider the yield of individual species in addition to their mineral concentration; this could further be manipulated by adjusting the seeding rates of species, and by other soil and management effects such as soil type, nutrient status, weather and seedbed preparation.

4.2. Differences between Wild and Cultivated Species

Our data indicate that we can reject the hypotheses that wild-type species will have greater mineral concentrations than cultivated species under our optimised conditions. Often, we found no significant difference between the two groups in their concentrations of the beneficial minerals (I, Se, Co, Cu, Mn and Zn) or the antagonistic minerals (S, Mo and Fe). Where significant differences were found, the cultivated species was just as likely, or more likely, to have the higher concentration. In wheat, fruits and vegetables, there have been a measured decline in macro- and micromineral concentrations over the long-term (50 years or greater) [14,15,32]. Comparisons of modern cultivars with historical or wild-type cultivars have indicated that there is often dilution, whereby the more modern cultivars with higher yields have consistently lower concentrations of beneficial minerals [13,32]. We are not aware of similar research in pasture systems, but the absence of this effect in our data indicates that the effect of plant breeding on mineral uptake differs between pasture species and arable and horticultural crops. This may be due to the location of the consumed minerals, being in the seeds of arable plants but foliar tissue in pasture plants. The regular cutting and grazing of pasture species mean that the need for minerals is more sustained than in arable and horticultural systems, where fertilizer applications can be timed to the needs of the crop more exactly. Therefore, it is possible that the development of cultivated pasture species may have been less likely to affect the root morphology and exudate release mechanisms by which they access minerals, as many of these same mechanisms apply to both macro- and micro-minerals [33,34].

Despite the lack of a consistent significant difference between wild and cultivated species at the level of the botanical group, it does not mean that plants could not be bred to take up higher concentrations of minerals. Where multiple cultivars of a single species of grass (*L. perenne*, *P. pratense*), legume (*T. pratense*, *T. repens*) or forb (*C. intybus*, *P. lanceolata*) have been analysed for their mineral content, differences between them have been found [17,35,36]. However, as in our results, comparisons between cultivars are significant for only a few of the minerals or species [17], inconsistent across minerals [36], or showed no consistent patterns across various sites, seasons and N fertilizer treatments [35]. Conversely, the recurrent selection of *Lolium multiflorum* cultivars for Mg uptake has been shown to increase Mg concentrations in the plant [12]. Although currently this cultivar has not been commercialised due to its slightly lower DM yield suggesting a physiological trade-off (76–93% of the highest yielding *Lolium multiflorum* cultivar, across different seasons and sites), the hope is that it can be used to identify the genetic markers responsible for Mg accumulation [12]. Therefore, while breeding may pave the way for increasing mineral concentrations of any given pasture species in the future, to optimise total pasture concentrations for livestock health, easier gains may be made by exploiting the differences between botanical groups and between species. The targeted selection of a combination of species could optimise the concentration of all minerals concurrently.

4.3. Inter- and Intra-Botanical Group Variability

Our data indicate that of the botanical groups, forbs were highest in I and Se and lowest in Mo and Zn; grasses were highest in Mn and lowest in Cu, Co and Fe; and legumes were highest in Cu, Co, Zn and Fe. It is difficult to compare mineral concentrations of plants across studies as they can vary widely, often spanning an order of magnitude even within a botanical group Tables 8 [3]. This variation comes from a host of factors including site differences, such as soil type and mineral concentrations, climate, interannual variation, plot management (such as the number and timing of herbage cutting or the application of slurry or mineral fertilizers), the part of the plant sampled, plant species and cultivar and potentially whether the experiment was conducted at a pot or field scale [3]. The relative concentrations of different botanical groups can be compared and the relative trends in our data are similar to other studies. For example, Lindström et al. [17] found Mn to also be highest in grasses and Co, Cu, Fe and Zn to be highest in legumes, while in the complete sward a decrease in the proportion of grass in favour of legumes and forbs is associated with an increase in mineral concentrations, with the exception of Mn [22,31,37]. These previous studies have predominantly been in Scandinavia or Australasia, and our data show that the same relationships between botanical groups occur, despite the use of different species and cultivars, and with different soils and climates. Previous studies also rarely measure Se or I concentrations [3]. Pastures often contain concentrations of these minerals that are insufficient for livestock health [25], and our data highlight that forbs may be valuable sources.

Mineral concentrations displayed a greater variability between botanical groups than within them. Furthermore, our data indicate that there are often several species with similar concentrations.

This indicates that there is some flexibility in species choice to improve the sward mineral composition, and that selection of the botanical group with the greatest concentrations of any given mineral could be an important first step. However, there are some caveats to this. Firstly, heterogeneity of the botanical groups does still exist, as also shown by Lindström et al. [17]. There was often a significant difference across all the species in a botanical group, and some species that contained a significantly lower concentration of a mineral than others in the botanical group. Therefore, selection of plant species using the botanical group alone is not sufficient. Furthermore, there may be occasions when selecting based on botanical group will miss a species with high concentrations of the mineral, if it appears as an outlier in its own botanical group. For example, although as a botanical group the forbs contained more Se than legumes, the legume species with the highest concentrations of Se, *M. lupulina* and *L. corniculatus*, had similar concentrations to the best performing forbs, *A. millefolium* and *C. intybus* (Figure 3).

4.4. Selecting Promising Species for Further Research

To assess the pasture species mineral concentrations, we have used recommended concentrations provided by the UK AHDB [25], and these are similar to values published elsewhere [7,19,38]. We have included recommended concentrations for the total diet in addition to pasture recommendations to account for systems that are solely grass-fed or could become so should the pasture mineral concentrations be sufficient to reduce the need for mineral supplementation. Determination of the required concentrations for livestock diets are not easy to measure experimentally, and will vary according to the breed, life stage and level of performance (milk yield, growth rate) [39]. Therefore, the cut off between deficient and sufficient concentrations of a mineral is not definitive, but this benchmark value is necessary to enable us to compare plant species. Livestock intake recommendations can be used to indicate which species could be used to increase the mineral concentrations of a sward and it is interesting to note that, for example, the grass species tended to have Cu concentrations that did not meet the recommended intake level despite optimal concentrations in the growing medium. Likewise, most species had sufficient I concentrations despite the growing medium having lower concentrations than 75% of soils in England and Wales (Table S1). We acknowledge that definitive decisions on species choice are not possible using data from a standard medium and growing conditions which will not be representative of all temperate soils and conditions. Instead, the aim was to provide an idea of potentially beneficial species that could be tested in further studies.

In a multispecies sward, it is probable that the majority of Mn would be from the grass component, as concentrations are higher than in legumes and forbs. However, under the conditions used in this study, it appears that the choice of species is not crucial, as all species tested had concentrations above the recommendations for the total diet. In a mixed sward, we need to consider that the minerals provided by any one species will only have a fractional effect on the total sward mineral content, but grasses are usually a major component of the total yield of a pasture [40]. Therefore, although *Anthoxanthum odoratum* and *D. glomerata* had the highest Mn concentrations, the actual choice of grass species is more likely to be driven by other factors, such as DM, energy, protein, sugar, digestibility, persistence, silage quality and season length [8]. For Co, Cu, I, Se and Zn, several legume and forb species appear to have among the highest concentrations. Under our conditions, *A. millefolium* (high in Cu, I, Se), *C. intybus* (high in Co, Cu, I, Se, Zn) and *P. lanceolata* (high in Co, I) are valuable forb species and *M. lupulina* (high in Co, I, Se), *T. hybridum* (high in Co, Cu, I, Zn) and *L. corniculatus* (high in Cu, Se, Zn) are valuable legume species for providing minerals. Due to the lack of consistent differences between the wild and cultivated types, the potential of these species is focused on the cultivated types. These legume and forb species are all commonly available, and are currently included in commercially available multispecies seed mixtures [40–42], although even in these mixes herbs and legumes typically form a minor component of the DM compared with grass (e.g., 16–32%, [40]).

There are a wide range of antagonisms between minerals which reduce bioavailability at the animal level [1,39]. The AHDB recommendations [25] highlight Mo, S and Fe as being important antagonists, especially for Cu absorption [24], but it is important to note that these minerals are also essential in the livestock diet [39]. There are also antagonistic relationships between minerals during uptake by plants e.g., Se and S [43], meaning that both absolute and relative concentrations of soil minerals could affect plant concentrations of minerals [44]. Likewise, in ruminants, minerals may become antagonists if present in too high a concentration [24]. Therefore, balancing the sufficiency of minerals in plant species with ensuring the correct balance between minerals is not a trivial task. It is difficult therefore to make recommendations for species selection based on the levels of antagonists in the plant material. Based on the AHDB UK recommendations, no species is of concern for its Fe concentration, but many of the legume, forb and grass species could be of concern for S and Mo concentrations. Low absorption and liver storage of Cu in livestock can be caused by antagonism with S, Mo and/or Fe. In a meta-analysis, it has been shown that S tends to have a more minor antagonistic effect than Mo [45], possibly due to the typical concentrations of the two minerals found in animal feedstuffs. There is also evidence that the antagonistic effect on Cu absorption of Mo and S combined is greater than the sum of the effects of each mineral individually [46]. Therefore, we suggest that concentrations of these antagonists in the complete sward needs to be the subject of further research and related to soil conditions and geochemistry.

4.5. Wider Considerations

Our data suggest some species that, if included in a sward, would increase the concentrations of minerals in plant foliar tissue which are known to be limiting for livestock health. However, there are many factors that still need to be tested. One of the main considerations is that this experiment was conducted on a single medium, in order that we could screen many plant species and types. In addition to the concentration of minerals differing between soils, there are many factors that would affect mineral bioavailability, including soil pH, organic matter content, texture and soil redox potential, affected by the moisture content of the soil and therefore its drainage [3]. Significant effects of soil type or field site on minerals have been measured, but differences in plant concentrations tend to be smaller than the differences in soil concentrations, due to the ability of plants to regulate their uptake [17,31]. Of more concern is whether the relative ranking of plants will be the same across different soil types. Indications from a pot trial [17] is that they do remain broadly the same, but at a field scale more variation can be seen [31]. The differences between these two studies may be due to different soils across studies, or because at a field scale the climate and weather can cause inter-annual differences in mineral uptake [23]. The scale of measurement may also affect mineral uptake, for example because the soil preparation for pot studies can affect the soil structure, macro- and microbiology and mycorrhizal associations; although, one comparison of the same soil type at the pot and field scale produced similar rankings between species [17]. There is a clear need for further data comparing the relative ranking of pasture species for their mineral concentration in different soils, both in controlled conditions of pot trials, and in field studies [18].

The application of our data also depends on how consistent mineral concentrations of species are in single species stands, compared with in multispecies swards. Often it is found that complementarity or facilitation between species leads to greater DM yields than in single species stands [47], and there are indications that legume mineral concentrations in mixed swards may remain similar to single species stands, whereas grass mineral concentrations may increase, depending on the mineral [22]. Longer-term trials are also important, as younger plants tend to have higher concentrations of minerals than older plants [29], and to assess temporal changes in total pasture mineral concentrations due to changes in proportional cover of species [48]. This may be intra-annual, as species differ in their heading date, or inter-annual, as some species are less persistent than others [49]. Grassland management, such as the application of lime or fertilizers, is also known to affect mineral concentrations of swards [23], and there is a lack of knowledge on mineral concentrations required in different soil types, and antagonistic effects between minerals [50]. It is also necessary to further investigate the effect of grazing management, which can affect the ratio of leaves and stems in the sward, the former being known to contain higher concentrations of minerals [51], and thus affect the mineral content of the sward. Finally, it is necessary to incorporate wider sward considerations, such as palatability, and other nutritional parameters, for instance crude protein, energy content and fibre content [18].

5. Conclusions

We found no consistent differences in mineral concentrations between wild and cultivated species. However, we found a significant difference between botanical groups for many of the minerals tested, and some broad trends emerged—forbs were highest in I and Se and lowest in Mo and Zn; grasses were highest in Mn and lowest in Cu, Co and Fe; and legumes were highest in Cu, Co, Zn and Fe. Intra-botanical group variability in mineral concentrations was smaller than inter-botanical group. Consequently, it appears that the botanical group can be used as a first step to selecting plants to improve the concentration of a given mineral. However, across a botanical group, plant species differ in their mineral concentrations and therefore selecting on the basis of the botanical group alone is not possible. In the growing medium tested, many of the grasses tested would supply sufficient Mn, and therefore grass species are more likely to be influenced by other sward requirements, e.g., digestibility. For Co, Cu, I, Se and Zn, the forbs *A. millefolium* (high in Cu, I, Se), *C. intybus* (Co, Cu, I, Se, Zn) and *P. lanceolata* (Co, I), and the legumes *M. lupulina* (Co, I, Se), *T. hybridum* (Co, Cu, I, Zn) and *L. corniculatus* (Cu, Se, Zn) appear to be good choices to increase foliar concentrations of these minerals.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/10/1463/s1, Figure S1. Barchart showing masses of Co, Cu, I, Mn, Se and Zn in pasture species; Figure S2. Barchart showing masses of Mo, Fe and S in pasture species; Table S1. Total (mg/kg) and extractable (mg/l) mineral concentrations in the growing medium prior to plant growth; Table S2. Summary of LMM analysis showing whether there is a statistically significant effect of plant type (wild/cultivated), botanical group (forb/grass/legume), and an interaction between type and botanical group, for the concentration of 9 elements in the plant biomass; Table S3. Concentrations (mg kg-1 DM) of elements in plant material for elements where plant type (wild/cultivated) significantly affected the concentration; Table S4. Summary of LMM analysis showing whether there is a statistically significant effect of plant type (wild/cultivated), botanical group (forb/grass/legume), and an interaction between type and botanical group, for the mass of 9 elements in the plant biomass; Table S5. Mass of elements in 10 plants.

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