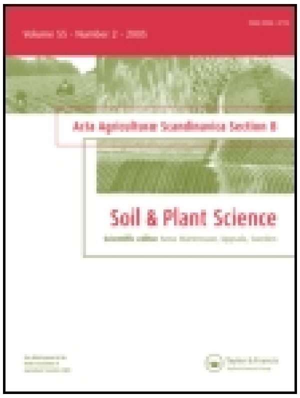

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Henning Høgh-Jensen [a] & Karen Søegaard [b]

[a] Aarhus University, Department of Environmental Science, Frederiksborgvej 399, Roskilde, 4000, Denmark

[b] Aarhus University, Faculty of Agricultural Sciences, Research Center Foulum, Blichers Alle 20, Tjele, 8830, Denmark

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ORIGINAL ARTICLE

Robustness in the mineral supply from temporary grasslands

HENNING HØGH-JENSEN[1] & KAREN SØEGAARD[2]

[1]*Aarhus University, Department of Environmental Science, Frederiksborgvej 399, Roskilde, 4000 Denmark,* [2]*Aarhus University, Faculty of Agricultural Sciences, Research Center Foulum, Blichers Alle 20, Tjele, 8830 Denmark*

Abstract

The current study examined the capacity of different temporary grassland legume–grass mixtures under different N supply levels to supply similar amounts of elements in systems where the herbage is cut for feed. Mixtures showed a good robustness in supplying equal amounts of mineral elements in the combined herbage as well as equal concentrations in dry matter of mineral elements compared with the same species in monocultures. The reasons for the mixed systems to be able to buffer differences in N supply levels as well as different compositions of the mixtures were that legume leaves and stems had similar concentrations of mineral elements, whether in monocultures or in mixtures with grasses. Grasses in mixture with legumes had however higher N, Ca, S, Zn, Cu and tended to have higher Mg concentration, both in stems and leaves, while Mn were less concentrated in mixtures' dry matter. Further, the mixtures doubled their dry matter accumulation in the two weeks just around grass heading. The systems partly buffered the time-wise differences in the sense that the P accumulation paralleled dry matter but the N was diluted. This was mirrored in a decrease in N concentration and maintenance of the concentration level of P and other elements. As the stem–leaf ratio was higher ($p < 0.05$) in festulolium than in ryegrass and as the stems of festulolium have lower concentrations of N, K, Ca, S, Mg, Fe and Cu than leaves, the mixtures including festulolium had a rapidly declining proportion of these elements in the combined mixtures' dry matter. Management options in improving the mineral supplies are thus to choose species when establishing the temporary grasslands according to functionality, to manipulate the content of legumes by the N supply level, and to time the harvest of the herbage.

Keywords: *Concentration in plant organs, elements, ecosystems services, grasses, herbs, legumes, minerals, mixtures.*

Introduction

Until around the mid-20th century, agriculture was generally of low intensity but since then widespread loss of biodiversity, as well as other environmental problems, has followed agricultural intensification (Hopkins & Holz, 2006, Erisman et al., 2008). In many areas, the increased supplies of in particular nitrogen (N) but also phosphorus (P) have led to dominance of just a few plant species; the enhancement of forage production and quality has been to the cost of sward biodiversity, as noted as early as 1900 (Lawes & Gilbert, 1900).

Grazed grassland may contain species of different growth stages, reflecting differences in content of carbohydrates, protein, fibre, minerals and secondary metabolites. Implications of such diversity in systems where the herbage is cut for feed are at present poorly understood. Buffering capacity, i.e. robustness, in this ecosystem provisioning (Millennium Ecosystem Assessment, 2005) is however required in modern animal production systems using forage crops as the sole diet for ruminants.

Many competition indices compare the performance of plants in mixture with their performance in pure stands (Weigelt & Jolliffe, 2003). Such comparisons are relevant to evaluate through yield density functions or with-and-without individual species in grassland (Goldberg & Scheiner, 1993; Sackville Hamilton, 2001). Evaluating measurements of the species in mixtures represent difficulties as the individual indices of performances may not be independent (Pearce & Gilliver, 1978; Høgh-Jensen & Schjoerring, 1997; Høgh-Jensen & Schjoerring, 2010).

Correspondence: Henning Høgh-Jensen, Aarhus University, Department of Enviromental Science, Frederiksborgvej 399, Roskilde, 4000 Denmark. Email: hhj@dmu.dk

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Grassland performance is traditionally measured in terms of dry matter, organic digestibility and protein; parameters important for the users', normally ruminants, performance. However, many pasture plants supply other elements that influence the performance of those ingesting the herbage.

Belesky et al. (2001) investigated mineral composition in pure stand chicory and Whitehead et al. (1978) in pure stand ryegrass but studies in mixtures have also been reported (Whitehead et al., 1983, Lawes & Gilbert, 1990, Hopkins et al., 1994). Hopkins et al. (1994) described the variation in selected macro- and micro-mineral concentrations in *Lolium perenne*/*Trifolium repens*-dominated swards over seasons and over years (Hopkins et al., 1994). Høgh-Jensen et al. (2006) investigated the development in the content of mineral elements in a mixture of chicory and ryegrass, and Høgh-Jensen et al. (2001) reported on the distribution of macrominerals in organs in a *Lolium perenne*/*Trifolium repens* mixture. In a review, Whitehead (2000) concluded that grasses in mixture with legumes often contain higher concentrations of P than the legumes and that white clover generally has higher concentrations of P than red clover and lucerne. More knowledge is needed regarding the variation in nutrient elements in individual species and species' organs in multi-species grassland (but see Whitehead et al. 1978) and how they interact under changing nutrient supply level and mixtures composition as these are expected to be the dominant factors that challenge the buffering capacity of the systems.

The objectives of the current studies were to study the combined capacity in the temporary grassland systems to supply similar amounts of elements, i.e. robustness, to (1) including various species in the herbage under two N supply levels, and to (2) sampling time around grass heading date on the content of minerals in individual species and organs.

Materials and methods

Experimental area

On a sandy clayey soil at Research Centre Foulum in Denmark (56°29′39 N, 9°34′41 E) *Trifolium repens* (white clover, c. Milo), *Trifolium pratense* (red clover, c. Rajah), *Medicago sativa* (lucerne, c. Pondus), *Lolium perenne* (perennial ryegrass, c. Mikado) and *Festulolium braunii* [(K. Richter) A. Camus, c. Perun] were established with two replicates in spring 2005; undersown a *Hordeum vulgare* (spring barley). The soil is classified as a Typic Hapludult with 8.0% clay and 1.6% C in the 0.15 m topsoil.

Experimental design and sampling

The pastures were managed with four cuts. The current study included spring growth of each year targeting the period around grass heading. The dynamic development was investigated by sampling at the normally expected optimum around grass heading date $\pm$one week. Sampling time took place later in 2006 than in 2007 due to later onset of the growth period. Sampling took place on 18 May, 24 May and 1 June and 2 May, 9 May and 16 May in 2006 and 2007, respectively. Yields were determined by harvesting by a Haldrup plot harvester at 7 cm stubble height. The mean temperatures of the two sampling years were similar.

The species were either grown in pure stand or in mixtures with one grass and one legume; in total 11 combinations, termed 'systems', under four N supply levels, with two replicates. The seeding rate for pure lucerne and grasses was 25 and for clovers 10 kg ha^{-1}. The seeding rate for lucerne/grass was 15/10 and for clover/grass 5/20 kg ha^{-1}. Each system were represented at two N supply levels as indicated in Table I; pure stand legumes at 0 or 120 kg N ha^{-1} yr^{-1}, pure stand grasses at 240 or 360 kg N ha^{-1} yr^{-1}, and the mixtures at 120 or 240 kg N ha^{-1} yr^{-1}. The N supply was distributed over season proportionally 35:25:20:20, so that 35% of 120 kg was supply at spring growth, 25% after first cut, etc. All plots were fertilized with 200 kg K ha^{-1} yr^{-1} as KCl, with 120 kg K applied in spring. Nitrogen fertilizer was applied as a NS 24-7 fertilizer compound, which means that S was applied at rates of 0, 12, 25 and 37 kg ha^{-1} yr^{-1} at the four N-levels, respectively. No other fertilizers were added.

Sampling took place in subsamples of *c.* 250 g fresh weight. Botanical composition was determined after the species were separated by hand into leaves, including laminae and petiole, and stems, including floral parts. The various fractions were then dried at 80 °C to constant weight and ground in a titanium-coated mill (SuperMill 1500, Newport Scientific Europe Ltd., Macclesfield, UK).

Subsamples of the plant materials was digested in an open vessel system using 70-mL HD polyethylene vials (Capitol Vial, Fulton Ville, NY, USA) and a graphite-heating block (Mod Block, CPI International, Amsterdam, Holland). In brief, the plant materials were first digested for 15 min in 35% HNO_3 at 95 °C. After cooling additional 70% HNO_3 was added together with H_2O_2. During digestion, the vials were covered with HD polyethylene watch glasses. Samples were cooled overnight and diluted to 50 mL with ultra pure water. After appropriate dilution, the samples were analysed for

Ca, Cu, Fe, K, Mg, Mn, P, S and Zn by ICP-MS (Agilent 7500c, Agilent Technologies, Manchester, UK) (Rodushkin et al. 1999).

The content of total N was analysed using an ANCA-SL Elemental Analyser coupled to a 20-20 Tracermass Mass Spectrometer (SerCon Ltd., Crewe, UK) using the Dumas dry-combustion method.

Calculations and statistical analysis

The dataset was not complete as an analysing strategy was pursued which enabled us to compare mono vs. mixtures of the single species in data from 2007 using respectively legumes at 120N and grasses at 240N. To identify the dynamic development in the proportions of nutrient elements in leaf and stem and DM-weighted means at the same N supply level, data from the 240N treatment from 2006 and 2007 were used. To analyse the effect of the N supply level, data from the third cut from 2006 and 2007 were used.

Statistical analyses performed using a general analysis of variance (PROC GLM) and estimated LS-means for each plant species (SAS Institute Inc. 1999). Significant difference is estimated on a 5% level if nothing else is indicated. Comparison of the means for the individual systems was based on a Duncan *t*-test with a significance level at 5%.

Results

Overall the various combinations of grassland species, called the systems, demonstrated very different ($p = 0.0001$) abilities to accumulate dry matter (Table I). However, the capacities of these systems to adapt to changing external supplies of N were substantial as the N level had no effect ($p = 0.19$) on dry matter accumulation between systems. The ratio between the organs leaf and stem was for the single species not influenced by the N level ($p > 0.05$) nor by the systems ($p > 0.05$). When the effect of variance among the cropping systems is taken out statistically, the N supply level had no effect ($p > 0.05$) on the DM-weighted mean concentrations of Ca, Mg, Zn or Mn (data not shown).

Compared with ryegrass, festulolium accumulated more dry matter and the proportion of stem versus leaf was much higher ($p < 0.05$) (Table I). White clover was not in the reproductive phase, red clover had a relative low proportion of stem and lucerne had a considerable high proportion of stem (Table I). Thus, the temporary grassland systems' main adaptive mechanism was the relation between grass and legumes in the mixtures; shown as proportion of legume in Table I.

Effect of cropping in pure stands versus mixtures on the combined concentration of elements

The mean content of minerals in the herbage, weighted by DM accumulated in each organ, is one important element of the quality concept, particular in cut systems. In an overall analysis there was a significant effect of defoliation time (data not shown) and system on the weighted mean content of N, P, K, Ca, S, Mg, Fe and Mn (Table II).

At an N supply of 120 kg N ha^{-1} yr^{-1}, the pure stand of the single legumes accumulated more ($p < 0.05$) N, Ca, S and Mg than the mixtures. At an N supply of 240 kg N ha^{-1} yr^{-1}, the mixtures accumulated more ($p < 0.05$) N, P, K, Ca, S, Mg, Fe, Zn and Cu than the pure stand grasses (partly shown in Table II).

Table I. Accumulations of DM (kg ha^{-1}) in white clover (WC), red clover (RC), lucerne (LU), ryegrass (RYE) and festulolium (FEST), proportion of legumes (% of DM) in the combined herbage plus the proportion of stem (% stem of DM) in grass/legumes in the first cut over two seasons under varying N supply levels (kg N ha^{-1} yr^{-1}), each year averaged over three sampling times. Mean, n = 12. Different letters indicate statistical difference between systems following a student *t*-test.

	N levels	WC	WC+ RYE	WC+ FEST	RC	RC+ RYE	RC+ FEST	LU	LU+ RYE	LU+ FEST	RYE	FEST
DM	0N	1417a	–	–	2642b	–	–	2617b	–	–	–	–
	120N	1592c	2124bc	3044a	2574ab	2704ab	3467a	3057a	3111a	3398a	–	–
	240N	–	2464c	3444ab	–	2809bc	3691ab	–	3088abc	3808a	2413c	3298abc
	360N	–	–	–	–	–	–	–	–	–	2730a	3805b
%leg	0N	100	–	–	100	–	–	100	–	–	–	–
	120N	100	25.4	6.6	100	68.3	34.6	100	66.3	33.0	–	–
	240N	–	12.5	3.1	–	60.9	18.8	–	54.3	22.4	0	0
	360N	–	–	–	–	–	–	–	–	–	0	0
%stem grass/legume	0N	–/0	–	–	–/14	–	–	–/47	–	–	–	–
	120N	–/0	29/0	47/0	–/14	32/16	44/18	–/48	39/46	49/46	–	–
	240N	–	31/0	48/0	–	33/14	46/18	–	39/45	48/47	30/–	47/–
	360N	–	–	–	–	–	–	–	–	–	34/–	48/–

Table II. Accumulations of macro (kg ha $^{-1}$) and micro (g ha $^{-1}$) elements in the combined herbage of white clover (WC), red clover (RC), lucerne (LU), ryegrass (RYE) and festulolium (FEST) in the first cut over two seasons under varying N supply levels (kg N ha $^{-1}$ yr $^{-1}$), each year averaged over three sampling times. Mean, n = 12. Different letters indicate statistical difference between systems following a student *t*-test.

	N level	WC	WC+RYE	WC+FEST	RC	RC+RYE	RC+FEST	LU	LU+RYE	LU+FEST	RYE	FEST
N	0N	60.5b	–	–	108.5a	–	–	95.6ab	–	–	–	–
	120N	69bc	57c	58c	94ab	86abc	87abc	107a	94ab	82abc	–	–
	240N	–	71cd	77bc	–	103a	93ab	–	101a	101a	55d	62cd
	360N	–	–	–	–	–	–	–	–	–	73a	88b
P	0N	5.0b	–	–	9.5a	–	–	9.1ab	–	–	–	–
	120N	6.0b	6.3ab	7.2ab	9.5ab	8.5ab	9.7ab	9.9a	10.0a	10.0a	–	–
	240N	–	8.8ab	9.2ab	–	11.0ab	10.9ab	–	11.0ab	11.9a	7.5b	8.2ab
	360N	–	–	–	–	–	–	–	–	–	9.6a	11.aa
K	0N	49.6a	–	–	94.7b	–	–	97.0b	–	–	–	–
	120N	60b	61b	83ab	91ab	77ab	106a	98ab	97ab	106a	–	–
	240N	–	81bc	112ab	–	103abc	124a	–	109ab	128a	72c	91abc
	360N	–	–	–	–	–	–	–	–	–	93a	131b
Ca	0N	29.0a	–	–	42.3a	–	–	37.2a	–	–	–	–
	120N	28abcd	16.4cd	14.0d	40.9a	29.4abc	25.1bcd	41.0a	31.2ab	23.2bcd	–	–
	240N	–	16.9cde	16.0de	–	30.9a	22.4bcd	–	28.8ab	23.9abc	11.1e	12.0e
	360N	–	–	–	–	–	–	–	–	–	14.6a	16.8a
S	0N	3.53a	–	–	6.23a	–	–	6.52a	–	–	–	–
	120N	5.32ab	4.84b	5.11b	8.05ab	5.65ab	6.31ab	8.93a	6.81ab	6.67ab	–	–
	240N	–	7.9a	9.1a	–	9.6a	10.8a	–	10.0a	10.0a	5.1a	5.4a
	360N	–	–	–	–	–	–	–	–	–	9.9a	10.8a
Mg	0N	2.86a	–	–	7.16b	–	–	5.51b	–	–	–	–
	120N	3.13cd	2.14d	2.61cd	7.03a	4.86abc	5.00abc	6.00ab	4.74abc	4.11bcd	–	–
	240N	–	3.0c	3.8bc	–	5.8a	5.2ab	–	4.9ab	5.2ab	2.0c	2.6c
	360N	–	–	–	–	–	–	–	–	–	3.2a	4.3a
Fe	0N	166a	–	–	204a	–	–	175a	–	–	–	–
	120N	154a	154a	156a	185a	207a	190a	178a	270a	187a	–	–
	240N	–	177abc	176abc	–	200ab	206a	–	184abc	209a	153bc	147c
	360N	–	–	–	–	–	–	–	–	–	172a	190a
Zn	0N	39a	–	–	92b	–	–	93b	–	–	–	–
	120N	54b	51b	66b	141a	86b	80b	106ab	86b	80b	–	–
	240N	–	98abc	116ab	–	125a	143a	–	125a	143a	62c	69bc
	360N	–	–	–	–	–	–	–	–	–	117a	128a
Mn	0N	140a	–	–	156a	–	–	90a	–	–	–	–
	120N	122ab	131ab	154ab	157ab	173ab	183a	105b	123ab	157ab	–	–

Table II. (*Continued*).

	N level	WC	WC+RYE	WC+FEST	RC	RC+RYE	RC+FEST	LU	LU+RYE	LU+FEST	RYE	FEST
	240N	–	149a	192a	–	185a	207a	–	145a	196a	163a	183a
	360N	–	–	–	–	–	–	–	–	–	202a	224a
Cu	0N	11a	–	–	28b	–	–	21ab	–	–	–	–
	120N	14b	12b	13b	32a	32a	22ab	25ab	21ab	19ab	–	–
	240N	–	18bc	19bc	–	32a	28a	–	25ab	26ab	13c	14c
	360N	–	–	–	–	–	–	–	–	–	21a	25a

The robustness in supplying elements was high as the mean proportion of elements in legumes, weighted by dry-matter of each organ, was generally not affected by the system in which the legumes were cultivated, whether in pure stand or in association with perennial ryegrass or festulolium (data not shown). Similarly, the proportion of elements in grasses was generally not affected by the system in which they were cultivated, whether in pure stand or in association with white clover, red clover or lucerne (data not shown). Co-cultivation of grasses with legumes did however stimulate ($p < 0.05$) the N content of the grasses.

Effect of including various species in swards on the concentration of minerals in plant organs

The concentration of elements (Table III) in the leaves of legumes in pure stand did differ from leaves of legumes in mixture in the case of N, P (lucerne higher than white clover), K (white clover higher than lucerne), S (lucerne higher than white and red clover), Mg (red clover higher than white clover), Fe (white clover higher than lucerne and red clover), Zn (red clover higher than white clover and lucerne), Mn (white clover higher than red clover) and Cu (all three differed). The concentration of elements in the stems of pure stand red clover and lucerne differed from those of mixtures ($p < 0.05$) in the case of N, P, K, S, Mg, Mn and Cu (Table III).

The concentrations of elements in the leaves and stems of grasses were generally higher when cultivated in mixture with legumes compared to being in pure stands with Mn as the only exception (Table IV). In particular the concentration of N were higher ($p < 0.05$), which may facilitate the higher concentrations of the other elements, as described in the previous section in relation to Table II. The two tested grasses differ however morphologically as stem constituted very different proportions of the herbage (Table I). The concentration of elements (Table IV) in the leaves of grasses in pure stand did not differ ($p > 0.05$) except for Zn and Mn. In the stems, they only differed in the case of S. These differences are important when considering the dynamic development around the time of grass heading.

Timing the defoliation to optimize combined herbage quantity and quality

Timing of the defoliation in systems where the herbage is being cut for feed is very important for both quantity and quality. Postponing the harvest by 2 weeks just around grass heading doubled the dry matter accumulation in the herbage (data not shown) following a fairly linear trend. This increase

Table III. Concentrations of elements in leaves and stems of legumes cultivated in pure stands (pure) or in mixtures (mix) with grasses at an N supply of 120 kg N ha^{-1} yr^{-1}. Mean values, n = 6–12. Units for macro elements are% and ‰ for micro elements. Different letters indicate statistical difference between systems for each legume species organ following a student *t*-test.

	White Clover		Red Clover				Lucerne			
	Leaf		Leaf		Stem		Leaf		stem	
	Pure	Mix	Pure	Mix	Pure	Mix	Pure	Mix	Pure	Mix
N	4.16	4.30	4.31	4.43	3.42	3.45	4.64	4.46	2.38	2.43
P	0.276	0.313	0.294	0.309	0.325	0.369	0.328	0.321	0.251a	0.305b
K	3.05	3.07	2.66	2.63	3.56	4.01	2.31	2.42	3.54	3.71
Ca	1.95a	1.64b	1.71	1.58	1.10	1.00	1.94	1.71	0.726	0.778
S	0.216	0.224	0.207	0.209	0.157	0.172	0.293	0.286	0.105a	0.125b
Mg	0.189	0.204	0.232	0.232	0.303	0.330	0.202	0.206	0.171	0.195
Fe	10.92	10.14	6.80	7.62	3.68	3.77	7.58	8.64	2.61	3.49
Zn	3.28	2.27	5.36a	3.00b	3.96	2.86	3.24	2.87	3.00	2.66
Mn	9.26a	7.07b	8.26	7.29	2.67	2.39	5.77	5.16	1.19	1.20
Cu	0.687	0.810	0.986	1.052	0.884	0.858	0.816	0.855	0.629	0.628

in dry matter accumulation was mirrored in an increase in N accumulation in the second year but the in first year the optimum in N accumulation in all systems were at the second sampling occasion (Figure 1). The accumulation of P follows a similar linear trend (Figure 2).

The proportion of N, per unit of combined herbage dry matter, decreased over the 2-weeks period the second year, except in the combined red clover/ryegrass mixture, but the trend the first year indicated an optimum in the middle of the period (Figure 3). Phosphorus concentration (Figure 4) as well as S, Mn, Fe, Mg, Zn and Cu (data not shown) also differed between the two years; with an increase over period the first year. This indicates a decoupling of the N concentration and the other elements just around grass heading.

Discussion

Robustness of temporary grassland (see definition in Allen et al. 2011) in quantity and quality terms was reported more than a century ago and differences in responses to manures and balances between grasses and legumes were noted (Lawes & Gilbert, 1900). In recent years, the issue of robustness to maintain functionality when disturbed has emerged out of diverse research fields, from engineering, computer science to ecology, and of the complex adaptive systems approach (see e.g. Webb 2007, Levin & Lubchenco, 2008). Positive feedback mechanisms are increasingly recognized as an important component of ecosystem dynamics (e.g. Perry et al. 1989). The feedback mechanisms contribute to performance robustness on a system level when agroecosystems are exposed to stresses and disturbances by management operations or climatic stresses. One of the objectives of the current study were thus to examine the combined capacity of the temporary grassland systems to supply similar amounts of elements when including various species in the grassland sward on the combined accumulation of minerals in the herbage under various N supply levels.

Stresses and disturbances are common for all temporary grassland systems as the whole idea is to harvest biomass or other services. The current study indicates that plant diversity stabilizes the output services from such systems (Tables I and III and IV), partly by changes in the grass–legume proportion. This feature of ley mixtures has been reported multiple times (e.g. Haynes, 1980).

Managers are faced with the complex decision of timing the defoliation when harvesting the leys for hay or silage around an optimum, decisions that must consider the combined quality and yield, which

Table IV. Concentrations of elements in leaves and stems of grasses in pure stands (pure) or in mixtures (mix) with legumes at an N supply of 120 kg N ha^{-1} yr^{-1}. Mean values, n = 6–12. Units for macro elements are% and ‰ for micro elements. Different letters indicate statistical difference between systems for each grass species organ following a student *t*-test.

	Perennial ryegrass				Festulolium			
	Leaf		Stem		leaf		stem	
	Pure	Mix	Pure	Mix	Pure	Mix	Pure	Mix
N	2.80a	4.13b	1.35a	2.35b	2.67b	3.14a	1.17	1.58
P	0.305	0.332	0.268a	0.316b	0.253	0.228	0.244	0.234
K	3.15a	3.86b	2.42a	3.53b	3.36	3.35	2.43	2.63
Ca	0.543	0.567	0.204a	0.305a	0.569	0.606	0.181	0.220
S	0.208a	0.288b	0.108a	0.155b	0.189	0.212	0.089	0.105
Mg	0.107	0.111	0.071a	0.097b	0.113	0.117	0.076	0.088
Fe	7.26a	8.87b	2.21a	3.94b	6.24	7.48	1.67	2.43
Zn	2.49a	3.88b	2.24a	3.94b	1.83	2.69	1.99	2.99
Mn	8.43a	7.30b	6.19	6.43	7.02a	6.21b	5.62	5.50
Cu	0.578a	0.832b	0.319a	0.501b	0.541b	0.858a	0.331	0.384

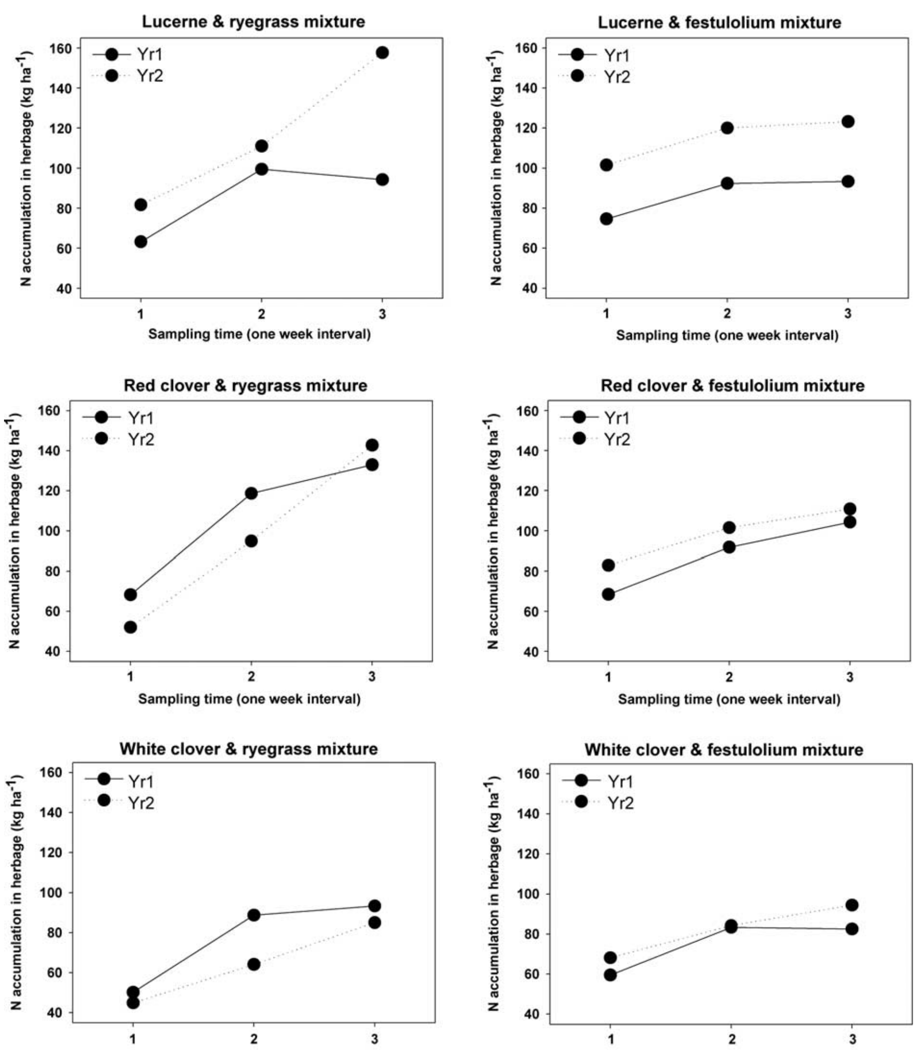

Figure 1. Dynamic developments in nitrogen accumulation around optimal defoliation time in six grass–legume mixtures with one N supply level (240 kg N ha^{-1} yr^{-1}) for two consecutive years.

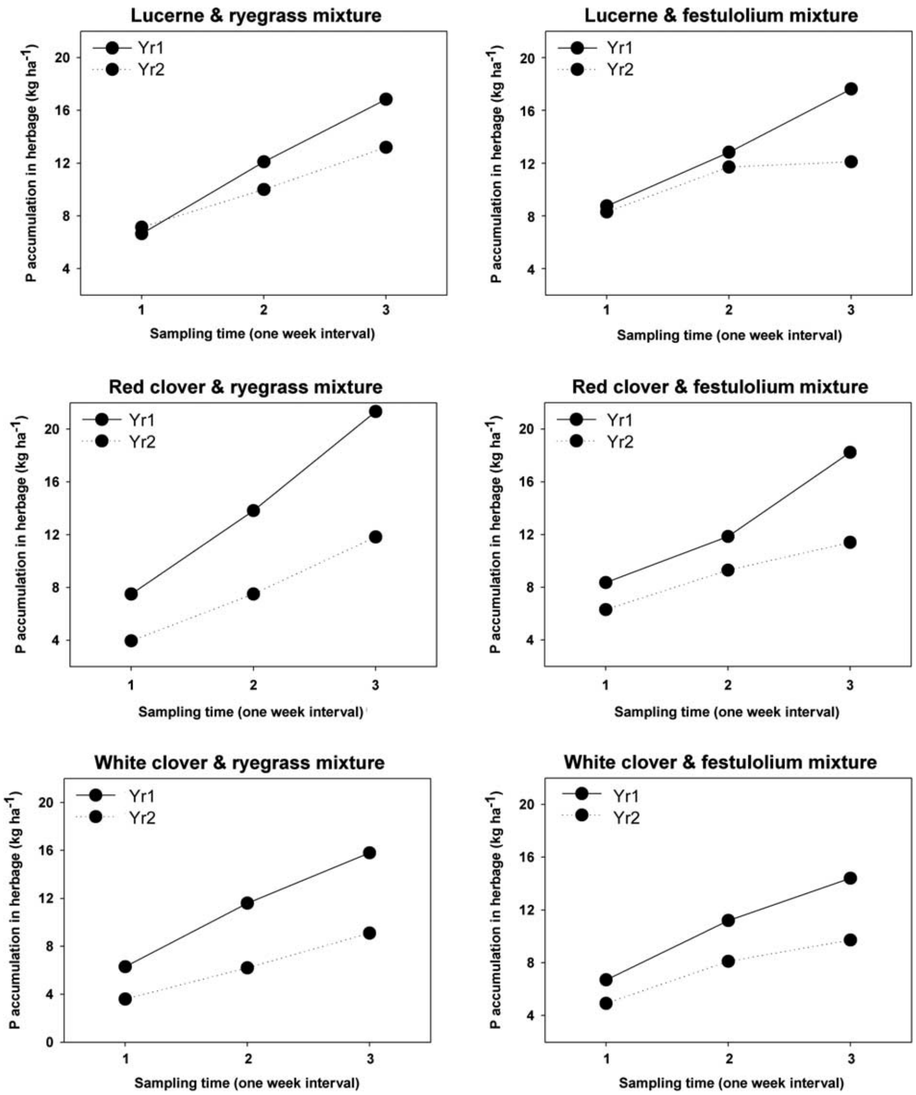

Figure 2. Dynamic developments in phosphorus accumulation around optimal defoliation time in six grass-legume mixtures with one N supply level (240 kg N ha^{-1} yr^{-1}) for two consecutive years.

may be difficult in multispecies mixtures where individual species have different phenological development stages. Thus, another objective of the current study was to determine the effect of foliar age on the content of minerals in individual species and organs around the time of grass heading in spring.

Whitehead (2000) showed how the critical concentration of both N and P can decline with increasing maturity in pastures. The current study confirms this as the N content, although it continues to accumulate (Figure 1), is expected decrease after grass heading per unit dry matter (Figure 3). The N concentration was positively correlated with the concentration of P (Figure 4) across all systems ($r = 0.43$). This does agree with Whitehead (2000), p. 140) which underlines that although a combined herbage of mixtures does not necessarily exhibit the same behavioural pattern as herbage from monocultures, mixtures may demonstrate a much more robust supply pattern in their combined herbage. We did however observe an apparent decoupling of the N concentration and the other elements just around grass heading.

The relative concentrations or the ratios of certain important minerals in the diet are often a matter of concern to managers. The N supply to temporary grasslands may cause an excess K accumulation and in the current study the K/(Mg+Ca) ratio exceeded in most cases the 2.2 critical limit for lactating dairy cattle (Grunes & Welch, 1989), which may lead to grass tetany. The observed K levels around

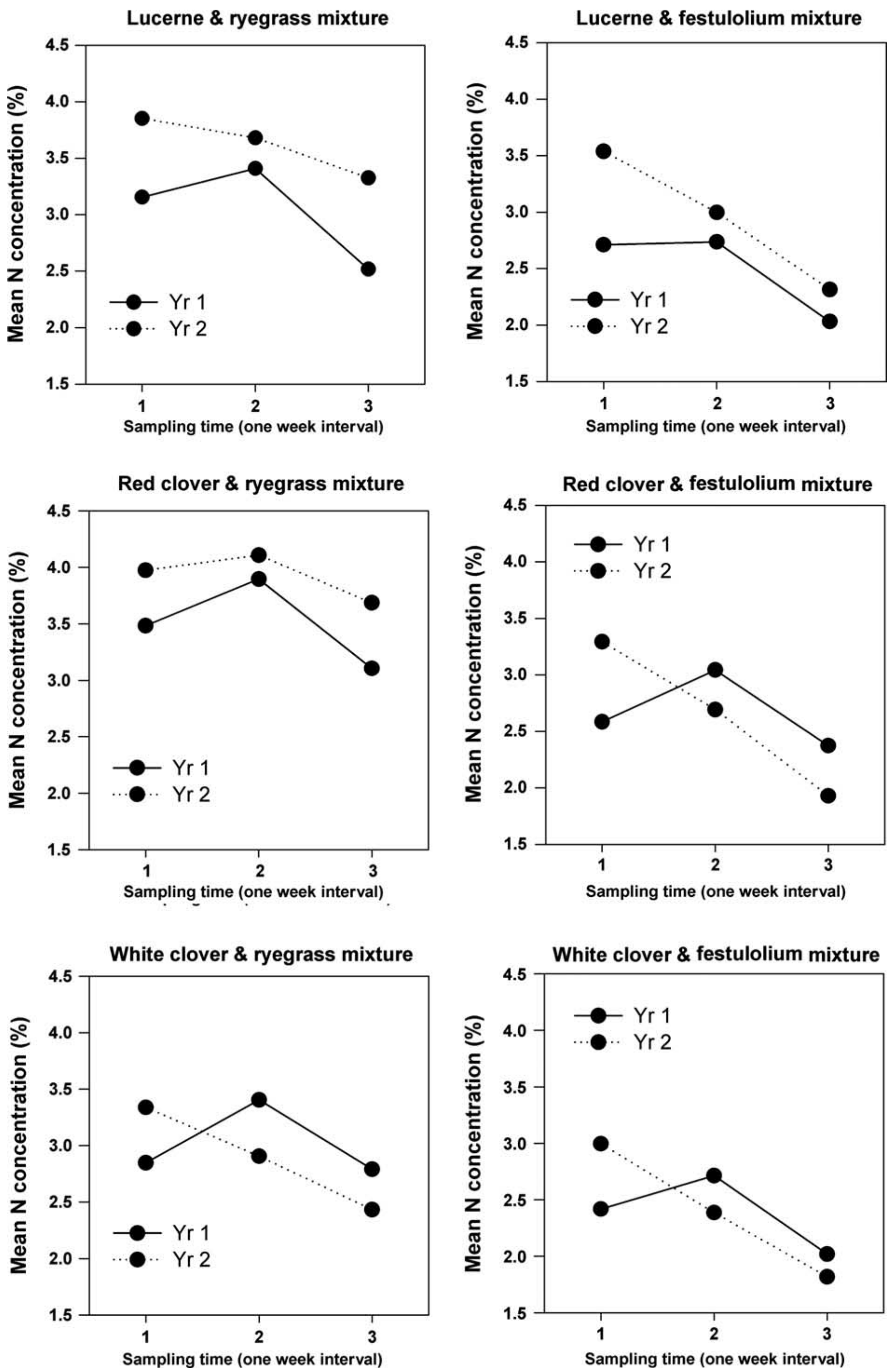

Figure 3. Dynamic developments in nitrogen concentration around optimal defoliation time in six grass–legume mixtures with one N supply level (240 kg N ha^{-1} yr^{-1}) for two consecutive years.

30 g kg^{-1} dry matter were however relatively normal as were the Ca levels. The system could however be limited with regards to Mg as the Mg level was lower than the dietary satisfactory levels of 0.2 g kg^{-1} dry matter (Spears, 1994; Wilkinson & Mayland, 1997). Mixtures may be much more robust in this regard as legumes under 120N supply levels tend to contains more mineral elements than grasses under 240N supply levels (Tables III and IV). Such robustness of mixtures may be important as the atmosphere becomes increasingly enriched in CO_2 (Lüscher et al. 1996).

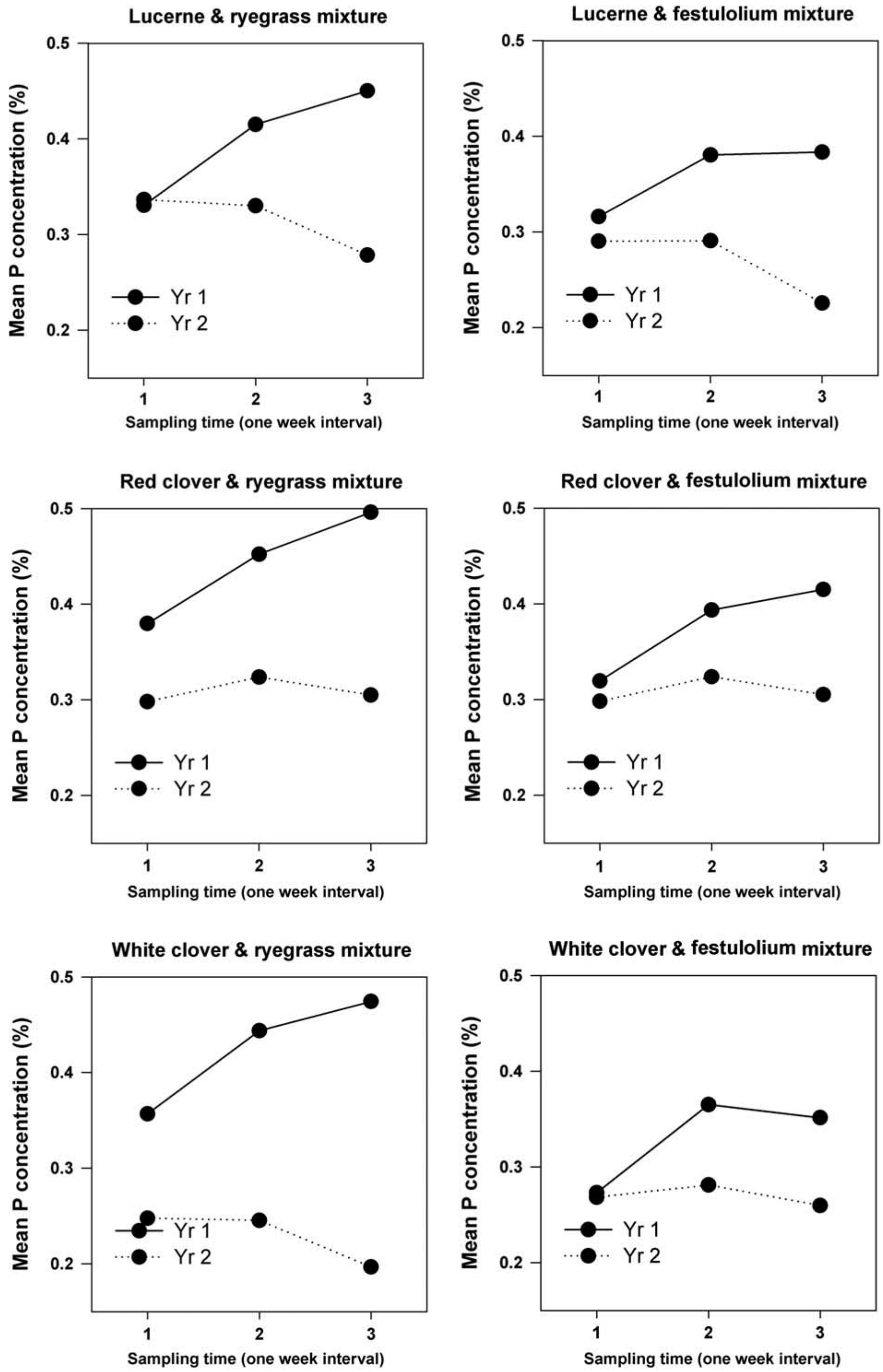

Figure 4. Dynamic developments in phosphorus concentration around optimal defoliation time in six grass-legume mixtures with one N supply level (240 kg N ha^{-1} yr^{-1}) for two consecutive years.

The systems' apparent robustness must however not be over-interpreted. The robustness in the combined supply of elements may contain critical interactions not yet uncovered. One of them may lie in the release of these nutrient elements from the various species and organs, not to mention cell fractions (Whitehead et al., 1985; Ibrahim et al., 1990). Another may lie in nutrient interactions, for example N, P and K interaction, which Høgh-Jensen and Schjoerring (2010) demonstrated to determine the competitive ability in a two-species mixture. A third may be that some systems favour productivity and resource-use efficiency, which may give lucerne, red clover and festulolium an advantage, while others may favour persistence to grazing which may give ryegrass and white clover some advantages.

Soil nutrient resources are generally heterogeneously distributed; a variation that is further enhanced via uneven manure applications or grazers' dung and urine distribution (Haynes & Williams, 1993). Generally, an enhanced N supply decreases the clover/grass ratio (Table I) and depresses the N_2 fixation rate (Høgh-Jensen & Schjoerring, 1994; Høgh-Jensen & Schjoerring, 1997; Eriksen et al., 2004). However, the N fertilizer effect is partly diminished assuming that legumes in pure stands generally are one N level lower than comparable mixtures (data partly shown in Table II).

The dynamic development in accumulation of dry matter (data not shown) and N were consistent over the two years for all mixtures of grasses and legumes (Figure 1). The accumulation of other elements were however less consistent as illustrated in the case of P (Figure 2). The consistency of N accumulation in the two production years (Figure 1) is underlined by a high consistency in the concentration of N (Figure 3). The systems are thus very robust in the supply of N. Similarly, the inconsistency of P accumulation (Figure 2) is associated with a high inconsistency in the concentration of P (Figure 4). There are no obvious explanations for this variation of P content over years. However, Høgh-Jensen et al. (2006) also observed significant changes over two production years in the nutrient element composition of ryegrass and chicory under constant conditions management wise. The overall impression is however that mixtures have a high degree of robustness in supplying elements (Table II).

The causal background for overall change in ecosystem function following changes in nutrient supplies or species composition is frequently difficult to predict. The variation in soil fertility is often related to below-ground species richness that may influence the function of ecosystems via nutrient supply (Naeem et al., 1995; Ekschmitt & Griffiths, 1998) which in turn influences the botanical composition (Høgh-Jensen & Schjoerring, 2010; Rasmussen et al., 2007). Increasing the supply of N to temporary grassland was shown by Mudd (1970) to decrease herbage Mn, which is confirmed in this study (data not shown). This is further underlined in the observed dilution of the Mn concentration in grasses coexisting with legumes.

Increasingly it is appreciated that additional species may profoundly affect ecosystem functions and provisional services (Naeem et al., 1995; Vitousek et al., 1997; Belesky et al., 2001; Høgh-Jensen et al., 2006; Gylfadóttir et al., 2007). Management optimization of temporary grassland systems, which are only kept for a few years (Allen et al. 2011) must take species traits and soil fertility into consideration (Whitehead et al., 1978; Hopkins et al., 1994).

In conclusion, the current study demonstrates that mixtures had a good robustness in supplying equal amounts of mineral elements in the combined herbage as well as equal concentrations in dry matter of mineral elements compared with the same species in monocultures. The reasons for the mixed systems to be able to buffer differences in N supply levels as well as different compositions of the mixtures were that legume leaves and stems had similar concentrations of mineral elements, whether in monocultures or in mixtures with grasses. Grasses in mixture with legumes had however higher N, Ca, S, Zn and Cu and tended to have higher Mg concentration, both in stems and leaves, while Mn were less concentrated in mixtures' dry matter. Further, the mixtures doubled their dry matter accumulation in the two weeks just around grass heading. The systems partly buffered the time-wise differences in the sense that the P accumulation paralleled dry matter but the N was diluted. This was mirrored in a decrease in N concentration and maintenance of the concentration level of P and other elements. Management options in improving the mineral supplies are thus to choose species when establishing the temporary grasslands according to functionality, to manipulate the content of legumes by the N supply level, and to time the harvest of the herbage.

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