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Nitrogen accumulation and efficiency in herbage depending on legume species in grassland sward

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The objectives of the experiment were to quantify the effect of different perennial legume species on N accumulation in harvested herbage, to assess the possible effect on nitrogen dynamics in grasses grown in mixture with legumes, to identify more suitable legume-based mixtures for efficient N use within perennial swards. Red clover (*Trifolium pratense* L.) and lucerne (*Medicago sativa* L.) were sown in monoculture or in mixtures with white clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.) and timothy (*Phleum pratense* L.) for cutting. White clover was sown both in monoculture and with grasses. The grasses were also sown without legumes and either fertilized with 240 kg N ha^{-1} $year^{-1}$ (N_{240}) or not (N_0). The N concentration, accumulation and use efficiency were estimated during three years of swards use. Nitrogen concentration in herbage and accumulation per unit of area varied in relation to legume species, year of sward use and cuts within year. All legumes had a positive effect on the improvement of N use efficiency.

Key words: nitrogen accumulation, nitrogen concentration, clover, lucerne, grasses, herbage

INTRODUCTION

Nowadays the quality of crop products, the environmental impact of crops and cropping systems, the low costs of production are important and multiple targets. Understanding the processes that govern N fluxes, particularly N accumulation and distribution in crops, a higher N use efficiency is of major importance with respect to both environmental concerns and the quality of crop products [1–3]. The efficiency of N accumulation depends on many factors, such as temperature, light, soil pH, mineral macro- and micro-nutrient availability, well adapted cultivars, rapid and effective nodulation [4, 5]. In perennial plants, the selection of species can also influence the productivity and efficiency of N cycling. Not all legumes had similar impacts on nitrogen accumulation and biomass production, therefore N uptake in mixed stands strongly depended on the legume species [6, 7].

A key issue in all systems is synchrony of N supply with plant demand. In legume/grass pasture systems, there is much less potential to alter the synchrony of N supply and demand. Can we usefully improve the efficiency of N cycling in these farming systems through manipulation of these processes by species selection, modification of plant tissue quality or management practices [8]? Experiments conducted on loamy *Cambisols* in Lithuania cannot yet answer these questions.

The objectives of the present experiment were to quantify the effect of different legume species on N accumulation in harvested herbage, to assess the possible effect on nitrogen dynamics in grasses grown in mixture with legumes as compared with grasses alone, to identify more suitable legume-based mixtures for efficient N use in perennial swards.

MATERIALS AND METHODS

Field studies were conducted on a loamy *Endocalcari-Epihypogleyic Cambisol* in Dotnuva (55°24'N). Soil pH varied between 6.5–7.0, humus content was 2.5–4.0%, available P 50–80 mg and K 100–150 mg kg^{-1}. Legumes and grasses were sown with a cover crop of barley for grain in 1999. Red clover (*Trifolium pratense* L.) cv. 'Arimaičiai' and lucerne (*Medicago sativa* L.) cv. 'Birutė' were sown in monocultures or in mixtures with white clover (*Trifolium repens* L.*) cv.* 'Atoliai' and perennial ryegrass (*Lolium perenne* L.) cv. 'Sodrė' and timothy (*Phleum pratense* L.) cv. 'Gintaras II' for 3-years use for cutting. The legume/grass ratio in the mixtures was 60:40. The grasses (mixture of perennial ryegrass and timothy) were also sown without legumes and either fertilized with 240 kg N ha^{-1} $year^{-1}$ (N_{240}) or not (N_0). The experiments had a randomised block design with four replicates. The net plot size was 2.5 × 12.5 m. P and K were applied according to the need based on soil analysis. Plants were sampled in 2000, 2001 and 2002. The yield of swards was taken at the flowering stage of legumes. The swards were cut 3 times per year in the first and second year and twice in the third year ley. N concentration was determined by the Kjeldahl method. The nitrogen nutrition index (NNI) was calculated as NNI = Nact/Nc [9], i.e. the ratio of the measured N concentration in cuts (Nact) and the critical N concentration (Nc) of the same sward in the dry matter (DM). The critical N concentration was calculated according to the relation Nc (%) = 4.8 $W^{-0.33}$ [10], where W represents crop biomass per unit of ground area. The yield data were statistically processed using analysis of variance and regression analysis.

RESULTS AND DISCUSSION

Total dry matter yield

There were significant differences in the DM yield among the various legume species and their mixtures with grasses in each year of ley use (Table 1). Lucerne and lucerne/grass swards were the highest yielding in all three years and varied less between years compared with the other swards. The DM yields of red clover alone or with grasses were by 20–30% lower in the first and second years and over 3 times lower in the third year. White clover and white clover/grass swards were the poorest yielding. All legume and legume/grass swards exceeded the yield of grasses without fertilizer N, and lucerne-based swards surpassed the N_{240} fertilized grasses. In most countries, legume–grass mixtures gave generally a higher yield than grass swards without N fertilizer [11].

Nitrogen concentration

The N content/unit biomass (N% in DM) is frequently used as an indication of how adequate N supply is for crop growth. Plant contents can range between 1% and 5% N on dry weight basis, with distinct annual patterns of decreasing contents with maturity of the sward: in general, N uptake, for which the capacity in grasses is high, mirrors the growth pattern [12]. Differences in N concentration in the organic matter of cut herbage were observed among different mixtures in each cut and in all three years of swards use (Fig. 1). In 2000, N concentration in all mixtures increased from the first to the second cut. N concentration in the red clover/grass mixture was the highest among mixed swards in the first cut. In white clover/lucerne/grass and lucerne/grass swards the concentration was slightly lower than in red clover/grass. In white clover/grass herbage N concentration was lower than in red clover- or lucerne-based mixtures, therefore it tended to have an only slightly lower concentration than in grass fertilized with nitrogen. The

Table 1. **Effect of different legume and legume/grass swards on the productivity of ley over three years of use**

Treatment (sward composition)	DM kg ha^{-1} year^{-1}		
	1st (2000)	2nd (2001)	3rd (2002)
White clover	3580	4122	1645
Red clover	6550	8488	1580
Lucerne	8998	10562	4782
W. clover/r. clover	6138	7302	1125
W. clover/lucerne	8680	10640	5575
W. clover/grasses	4560	3850	1487
R. clover/grasses	7225	8800	1605
Lucerne/grasses	9492	11112	5655
W. clover/r. clover/ grasses	6872	7840	1575
W. clover/lucerne/ grasses	8770	10292	4950
Grasses, N_{240}	9295	6360	2050
Grasses, N_0	3012	1922	770
$LSD_{.05}$	461.0	699.0	634.0

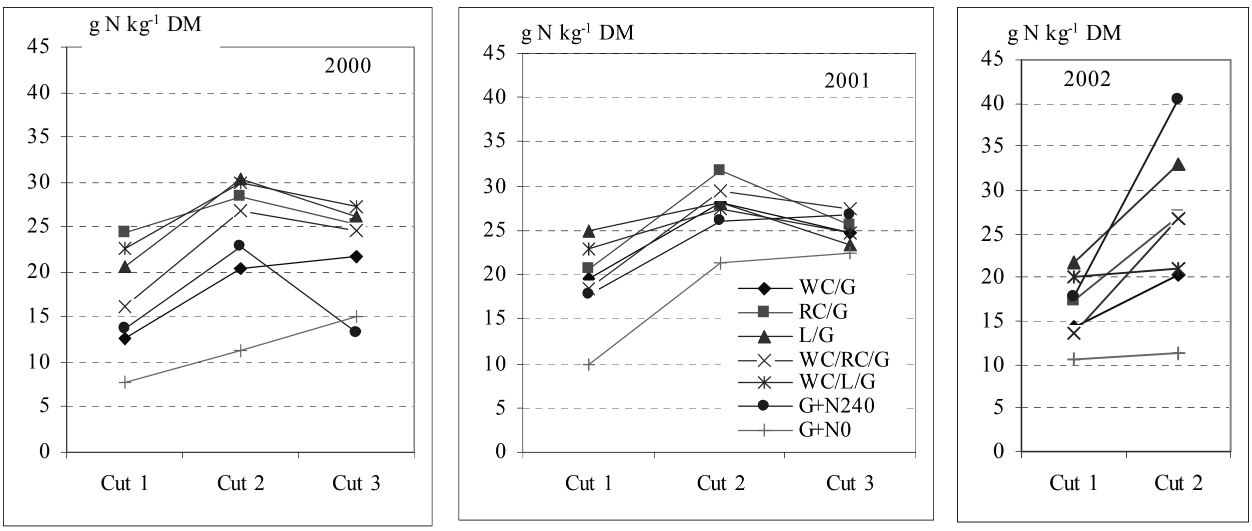

Fig. 1. N concentration (g N kg^{-1} DM) in the harvested biomass of legume/grass mixtures and monocultures of grass fertilized with nitrogen and without nitrogen at each cut

Abbreviations: WC/G – white clover/grasses, RC/G – red clover/grasses, L/G – lucerne/grasses, WC/RC/G – white clover/red clover/grasses, WC/L/G – white clover/lucerne/grasses, G+N_{240} – grasses, N_{240}, G+N_0 – grasses, N_0.

highest N concentration was found in lucerne-based swards in the second cut. In the third cut, N concentration slightly decreased in red clover/grass, lucerne/grass, white clover/red clover/grass and white clover/lucerne/grass mixtures and markedly decreased in a grass mixture fertilized with nitrogen. However, N concentration in the third cut of white clover/grass mixtures and grass without nitrogen fertilization increased. In 2001, N concentration increased in herbage from the first to the second cut in all swards and slightly declined in the third cut, except for the grass sward without nitrogen. The level and pattern of N concentration in all swards were similar to those in 2000, with very small variations between swards within cuts. A similar trend from the first cut to the second was observed in 2002, however, the level of N concentration was lower than in the previous year, except for the lucerne/grass and grass swards fertilized with nitrogen in the second cut.

N concentration is not a fixed value and decreases as the crop develops and its structure and biochemical composition change. Leaves, with their high content of protein, etc. are formed in early growth, while the supporting stems, with more cellulose and lignin and little protein, develop later. So N content (N%) decreases with age and also depends on the environment [13].

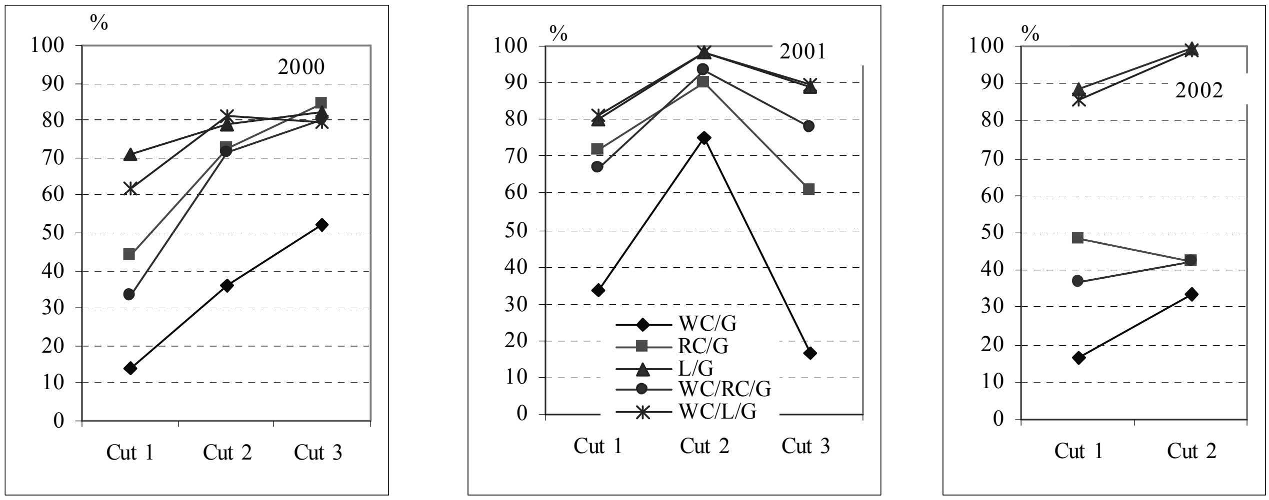

Fig. 2. Legume content (%) in the harvested DM at each cut during 2000–2002
Abbreviations: WC/G – white clover/grasses, RC/G – red clover/grasses, L/G – lucerne/grasses, WC/RC/G – white clover/red clover/grasses, WC/L/G – white clover/lucerne/grasses.

Seasonal changes in N concentrations in herbage can be explained by increasing the legume share in legume/grass swards from the first to the third cut (Fig. 2). Legume content in the mixtures fluctuated considerably between cuts, ley years and legume species in mixture; however, legumes accounted for the largest share in the herbage yield. In most situations lucerne had the highest percentage, red clover was the second ranking, and

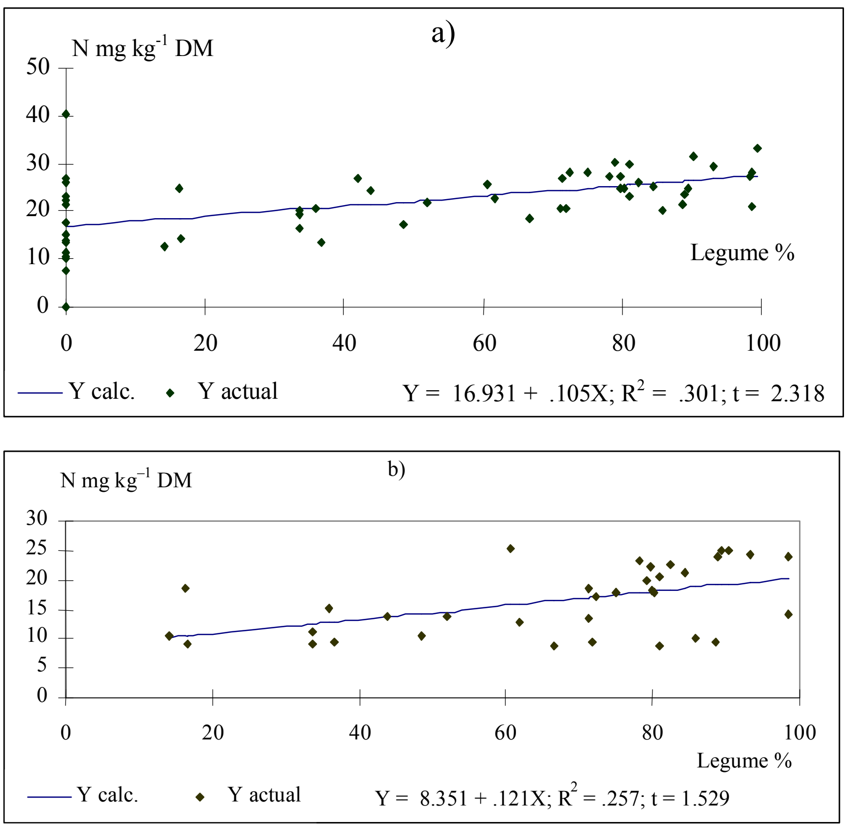

Fig. 3. Relationship between N concentration (N mg kg^{-1} DM) in all sward herbage and legume content (a) and N concentration in grass herbage from mixture with legumes and legume content (b)

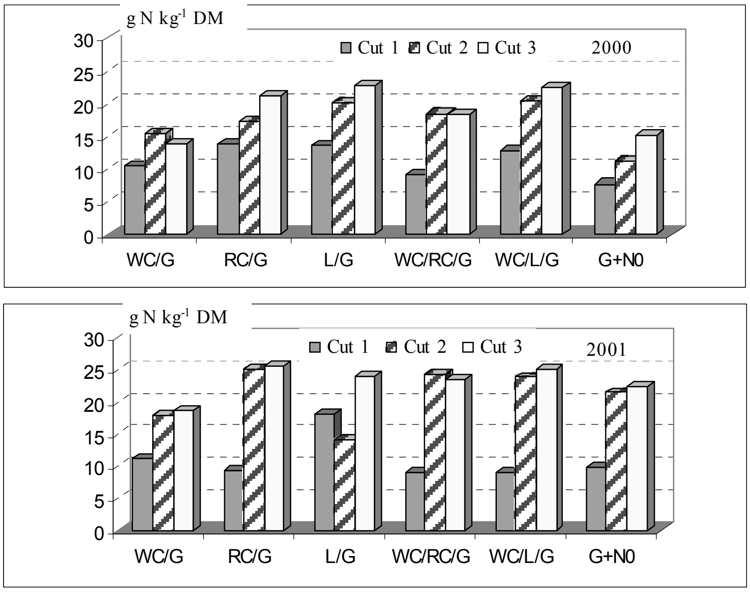

Fig. 4. N concentration (g N kg^{-1} DM) in the harvested biomass in grass grown in legume/grass mixtures and grass monoculture

Abbreviations: WC/G – white clover/grasses, RC/G – red clover/grasses, L/G – lucerne/grasses, WC/RC/G – white clover/red clover/grasses, WC/L/G – white clover/lucerne grasses, G+N$_0$ – grasses, N$_0$.

white clover had the lowest percentage relative to other legumes in all three years. Nevertheless, the relation between N concentration and legume proportion was significant, but the correlation was not very strong (Fig. 3a).

Biological nitrogen fixed by legume component in mixed swards can be transferred to grass component [8, 14, 15]. In our experiment, N concentration in grasses grown with lucerne or red clover was significantly higher than in grass herbage alone in 2000, but this N transfer effect was not confirmed in 2001, except for grasses in lucerne-based mixtures (Fig. 4). In most cases, N concentration in grasses from the second and third cuts was much higher than in those from the first cut because of the earlier grass vegetation stage and a larger part in swards compared with the first cut (Fig. 2). The obtained correlation suggests that legume proportion had a significant influence on N concentration in grasses from mixtures with legume, but the correlation was not strong

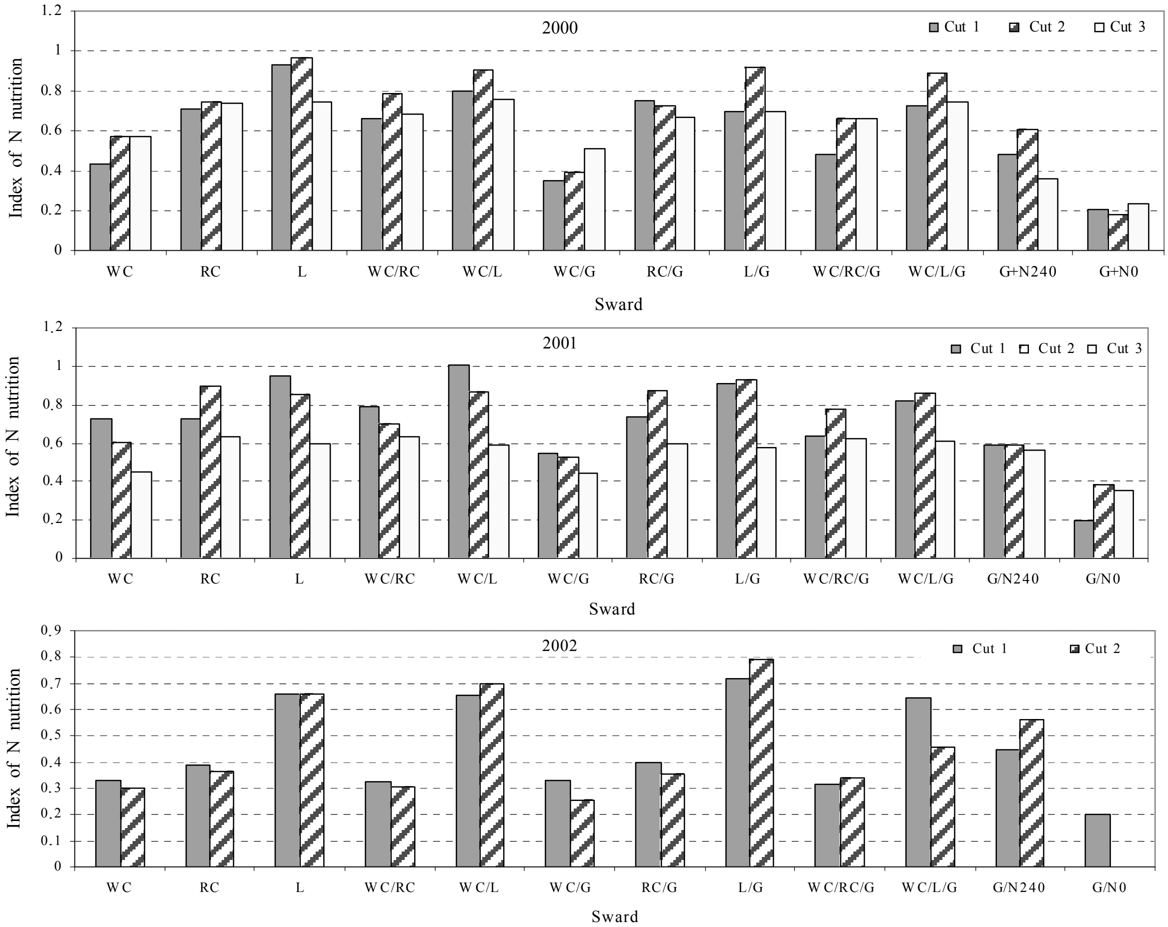

Fig. 5. N nutrition index of legume and legume/grass swards in different cuts

Abbreviations: WC – white clover, RC – red clover, L – lucerne, WC/RC – white clover/red clover, WC/L – white clover/lucerne, WC/G – white clover/grasses, RC/G – red clover/grasses, L/G – lucerne/grasses, WC/RC/G – white clover/red clover/grasses, WC/L/G – white clover/lucerne/grasses, G+N$_{240}$ – grasses, N$_{240}$, G+N$_0$ – grasses, N$_0$.

between N concentration in all sward herbage and legume proportion, either (Fig. 3b).

Nitrogen use efficiency

The N uptake rate of field-grown crops is regulated not only by soil N availability, but also by the crop growth rate. N uptake per unit biomass decreases as crop mass increases, suggesting that the dependence between N uptake and growth is complex. The concept of critical N concentration has been developed extensively in the last decade. It allows the N status of crops to be precisely and dynamically quantified over their development, and thus represents a unique tool for the study of the various agronomical and ecophysiological aspects underlying the N–growth relationship [2]. On the basis of the N_c concentration and N_{act} concentration ratio, the NNI has been proposed [9]. Values of the NNI greater than or equal to 1.0 indicate that the crop is in the situation of non-limited N supply. Values of NNI smaller than 1.0 indicate N deficiency. In our studies conducted on different legume, legume/grass and grass swards, the NNI was calculated at different times of the re-growth cycles (or cuts) (Fig. 5).

In all swards and in all cuts the NNI was lower than 1.0, indicating N deficiency, except for the

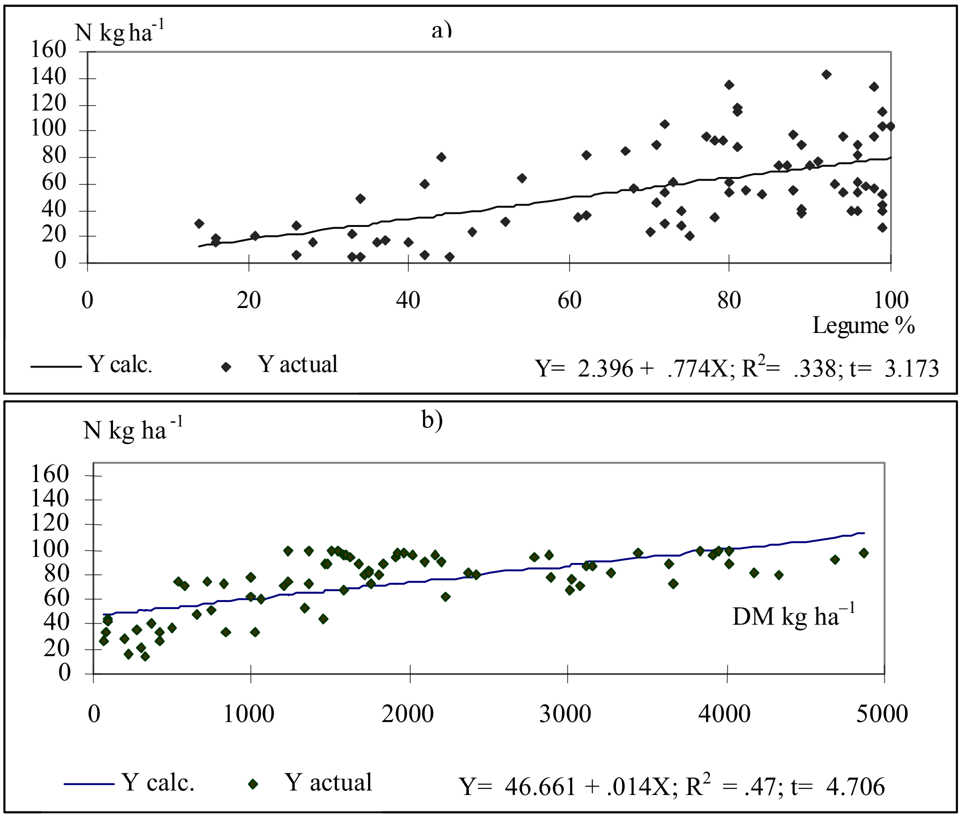

Fig. 6. Relationship between N accumulation and the percentage of legumes (a), and between N accumulation and the DM yield of swards (b)

Table 2. **Effect of different legume and legume/grass swards on N accumulation in the above-ground biomass at each cut and total per year**

Treatment	N kg ha-1										
	1st (2000)				2nd (2001)				3rd (2002)		
	Cut 1	Cut 2	Cut 3	Total	Cut 1	Cut 2	Cut 3	Total	Cut 1	Cut 2	Total
White clover	28.8	24.4	29.8	82.9	64.3	28.6	16.5	109.4	20.1	5.3	25.4
Red clover	61.2	54.6	61.1	177.0	96.8	77.4	39.8	214.0	21.6	7.4	29.0
Lucerne	113.8	95.8	57.0	266.7	133.4	103.0	39.8	276.2	73.5	40.4	113.9
W. clover/r. clover	55.6	54.3	54.1	164.0	95.4	54.6	35.8	185.8	14.7	5.1	19.8
W. clover/lucerne	92.2	90.2	57.6	240.0	143.0	102.9	40.0	285.9	80.6	44.4	125.0
W. clover/grasses	29.6	15.8	31.5	76.9	48.7	20.2	15.7	84.7	18.7	5.1	23.8
R. clover/grasses	80.1	52.9	52.0	185.2	105.4	73.7	35.2	214,3	23.5	6.1	29.6
Lucerne/grasses	89.2	93.3	54.9	237.3	134.7	114.7	38.4	287.8	88.9	51.2	140.1
W. clover/r. clover/ grasses	49.1	45.1	53.2	147.3	83.8	60.3	35.2	179.2	18.4	60.3	78.6
W. clover/lucerne/ grasses	81.6	87.6	61.3	230.5	117.6	95.7	41.0	254.3	74.2	26.8	101.0
Grasses, N_{240}	68.2	48.8	29.6	145.8	72.0	33.1	27.8	132.9	31.2	12.2	43.3
Grasses, N_0	16.6	5.3	5.9	27.8	8.6	13.1	9.9	31.7	8.0	-	8.0
$LSD_{.05}$	4.99	5.09	5.81	11.38	8.46	10.26	4.13	17.55	9.51	7.82	15.33

white clover/lucerne sward in the first cut in 2001 (in the second year of ley use). The lowest NNI was calculated in grass swards without N fertilization. However, lucerne-based swards in some cuts reached indices close to 1.0, therefore we could presume that lucerne had the greatest positive influence on the improvement of N supply. All legumes had a positive effect on the improvement of N supply to swards, and in many cases in the first two years of use this effect was higher as compared with N_{240} application for grass sward.

Effect of legume species and mixture composition on N accumulation

Absolute amounts of harvested N from swards, calculated from harvested herbage concentration and swards DM yield, are shown in Table 2. N accumulation varied between legume species and cuts, and there were significant differences depending on legume species. The N accumulation identified in our study was similar to that determined by other researchers. Whitehead [12] has calculated that grass harvested either by cutting or grazing in a temperate environment and producing 8–15 t ha^{-1} DM usually contains between 200 and 350 kg N ha^{-1}.

N accumulation was significantly higher in lucerne-based swards in all cuts. The amount of nitrogen in red clover-based swards was lower than in lucerne, but higher than in grass herbage without N fertilization. White clover-based swards produced a lowest N yield (Table 2). Nitrogen uptake of field crops is highly variable within a single year, between years, between sites, and between crops, even when N supplies from both the soil and additional fertilizer inputs are plentiful. Nitrogen yield in mixtures should be greater than in monocultures, if nitrogen limits production and plants are complementary in their nitrogen use and the N amounts of ley are closely related to legume productivity and content in swards [2, 6, 16]. Nitrogen content in our study was related to the percentage of legumes in the sward and more strongly to the DM yield of legumes (Fig. 6). N content increased with increasing the proportion and yield of legumes in the herbage of the swards.

CONCLUSIONS

Nitrogen concentration in herbage and accumulation per unit area varied depending on legume species, year of sward use and cuts within year. The most abundant and stable nitrogen accumulation over the three years of use of the swards intended for cutting was recorded for lucerne, less abundant for red clover, and the lowest but more stable than red clover for white clover.

All legumes had a positive effect on the improvement of N use efficiency in swards, and in many cases this effect was higher compared with N_{240} application for grass sward.

In the companion grasses, N concentration was higher with the higher share of legumes in the legume/grass sward.

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SKIRTINGŲ ANKŠTINIŲ ŽOLIŲ RŪŠIŲ AZOTO KAUPIMAS IR JO EFEKTYVUMAS ŽOLYNUOSE

S a n t r a u k a

Tyrimo tikslas buvo įvertinti N susikaupimą skirtingų ankštinių rūšių žolėje, išsiaiškinti azoto kaitą varpinėse žolėse, augusiose žolyne su ankštinėmis arba be jų, nustatyti tinkamesnius ankštinių ir varpinių žolių mišinius, efektyviau išnaudojančius azotą daugiamečiuose žolynuose. Buvo tirti žolių mišiniai iš raudonųjų dobilų (*Trifolium pratense* L.), liucernų (*Medicago sativa* L.), baltųjų dobilų (*Trifolium repens* L.), daugiamečių svidrių (*Lolium perenne* L.) ir pašarinių motiejukų (*Phleum pratense* L.), taip pat tik ankštiniai arba tik varpiniai žolynai. Varpinių žolynai buvo tręšiami 240 kg N ha^{-1} (N_{240}) arba ne (N_0). Nustatyta azoto koncentracija žolėje, azoto išnaudojimo efektyvumas bei azoto sukaupimas ploto vienete per trejus žolynų naudojimo metus. Azoto koncentracija žolėje ir sukaupimas ploto vienete priklausė nuo ankštinių žolių rūšies, žolynų naudojimo metų ir pjūčių laiko. Visos tirtos ankštinės žolės N išnaudojo efektyviai.