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RESPONSE OF TWO WETLAND GRAMINOIDS TO N:K SUPPLY RATIOS IN A TWO-YEAR GROWTH EXPERIMENT

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Abstract

Changes in nutrient availability in wetlands have been observed during the recent years, mostly due to human pressure. A shift from N limitation to P or K limitation causes changes in plant species composition, nutrient use efficiency, plant growth, interspecific competition and plant species performance. Several studies have shown that stoichiometry indices such as N:P and N:K ratios in plant biomass can be a good indicator of nutrient limitation. However, the implications of an N:K ratio for wetland vegetation have hardly been investigated.

In order to estimate a critical N:K ratio that can indicate the type of nutrient limitations, a greenhouse experiment has been established. The response of two grass species: *Holcus lanatus* and *Molinia caerulea*, to the range of N and K supply was analysed for two years. The effect of six combinations of N:K supply ratios (from 0.5 to 225), combined with two levels of fertility in a factorial design, on aerial biomass production, nutrient concentrations and nutrient resorption efficiency was tested.

The aerial biomass increased with an increasing N:K supply ratio during both vegetation seasons at the low level of supply. Significant differences were observed not only between species but also between the N:K ratios during the two years. In the first year, the optimal N:K supply ratio was 4.5 for *Holcus lanatus* and 225 for *Molinia careluea* at the high fertility level. In 2010, the optimal N:K supply ratio was similar for both grasses. At the high fertility level, the shoot biomass was the highest at an N:K supply ratio of 13.5; at the low level, shoot productivity reached the peak at a 225 N:K supply ratio. Moreover, both plant species showed the same pattern of aerial biomass production to N:K supply ratios at both fertility levels, but differences in the N:K biomass ratios make it impossible to determine a critical N:K ratio. The N:K nutrient supply ratio was a better indicator of plant performance than the N:K biomass ratio of the analysed species. The tested graminoids did not show a similar response to N:K supply ratios at the high and low levels of supply, indicating that nitrogen was the most important factor limiting the plant growth during the two years, and that these plant species were less sensitive to K shortage than to N deficiency.

Key words: N:K ratio, fertilisation, growth experiment, stoichiometry indices, wetlands.

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REAKCJA DWÓCH GATUNKÓW TRAW Z SIEDLISK PODMOK£YCH NA ZRÓ¯NICOWANE DAWKI N:K W DWULETNIM DOŒWIADCZENIU KONTROLOWANYM

Abstrakt

W ostatnich latach w wyniku antropopresji na obszarach wodno-b³otnych obserwuje siê zmiany dostêpnoœci zwi¹zków biogennych. Zmiana czynnika limituj¹cego, w szczególnoœci azotu, na rzecz fosforu i potasu wp³ywa na zmiany sk³adu gatunkowego roœlinnoœci, efektywnoœæ poboru zwi¹zków biogennych przez roœliny, wzrost roœlin, konkurencjê miêdzygatunkow¹ oraz inne ich parametry. Badania wskazuj¹, ¿e wskaŸniki stechiometryczne zawartoœci biogenów w roœlinach, takie jak N:P i N:K, mog¹ byæ dobrymi indykatorami czynnika limituj¹cego wzrost roœlin na mokrad³ach. Jednak zastosowanie stosunku N:K w odniesieniu do roœlinnoœci mokrade³ jest dot¹d w niewielkim stopniu rozpoznane.

W celu wyznaczenia krytycznej wartoœci stosunku zawartoœci azotu do potasu (N:K) w biomasie roœlin, wskazuj¹cego, który z biogenów jest czynnikiem limituj¹cym wzrost roœlin na obszarach podmok³ych, przeprowadzono doœwiadczenie kontrolowane w warunkach szklarniowych. Reakcja dwóch gatunków roœlin *Holcus lanatus* i *Molinia caerulea* na nawo¿enie zró¿nicowanymi dawkami azotu i potasu by³a badana w okresie dwóch lat. Wp³yw nawo¿enia N i K na produkcjê nadziemnej biomasy roœlin, zawartoœæ zwi¹zków biogennych oraz resorpcjê biogenów badano w szeœciu kombinacjach stosunku N do K (od 0.5 do 225) na dwóch poziomach nawo¿enia – niskim i wysokim (3 x niski). Dawki azotu przy niskim poziomie nawo¿enia waha³y siê od 9.30 mg do 146.69 mg wazon^{-1} rok^{-1}, a potasu od 18.53 do 0.65 mg wazon^{-1} rok^{-1}. Nadziemna biomasa roœlin zwiêksza³a siê wraz ze wzrostem stosunku N:K w przypadku niskiego poziomu nawo¿enia w ci¹gu dwóch lat. Statystycznie istotne ró¿nice stwierdzono nie tylko miêdzy badanymi gatunkami traw, ale tak¿e miêdzy analizowanymi latami. W pierwszym roku badañ najwiêksz¹ biomasê wytworzy³ *Holcus lanatus,* gdy stosunek N:K wynosi³ 4,5, natomiast *Molinia caerulea,* gdy stosunek N:K wynosi³ 225. W drugim roku wegetacji najwiêksz¹ biomasê wytworzy³y obydwa gatunki w przypadku nawo¿enia N:K równego 13,5, ale tylko przy wysokim poziomie nawo¿enia. Mimo i¿ reakcja badanych traw na nawo¿enie by³a analogiczna, to ró¿nice w zawartoœci N i K w komórkach tych roœlin uniemo¿liwiaj¹ wyznaczenie granicznej wartoœci N:K, która wskaza³aby sk³adnik limituj¹cy wzrost roœlin. Wykazano, ¿e stosunek N:K w aplikowanych nawozach by³ lepszym wskaŸnikiem oceny reakcji roœlin na zmianê dostêpnoœci zwi¹zków biogennych ni¿ stosunek N:K wyznaczony na postawie koncentracji N i K w roœlinach. Zró¿nicowane reakcje badanych traw w przypadku wysokiego i niskiego poziomu nawo¿enia wykaza³y, ¿e azot by³ g³ównym czynnikiem limituj¹cym wzrost roœlin. Analizowane trawy by³y mniej wra¿liwe na niedobory potasu ni¿ na niedobór azotu. Niedobór potasu by³ obserwowany tylko wówczas, gdy dawki nawo¿enia azotowego by³y bardzo wysokie.

S³owa kluczowe: stosunek N:K, nawo¿enie, eksperyment kontrolowany, wskaŸniki stechiometryczne, mokrad³a.

INTRODUCTION

Human activities such as farming, flood control, mowing, grazing, natural environment conservation and management have impacts on natural or semi-natural vegetation. Global environmental changes, e.g. anthropogenic eutrophication, high atmospheric N deposition or acidification, have resulted in changes in nutrient availability (VITOUSEK, HOWARTH 1991, VERHOEVEN et al.

1996a,b, BOBBINK et al. 1998, RUSTAD et al. 2001). Nutrient enrichment of an ecosystem causes changes of plant species composition, primary productivity, nutrient use efficiency, plant growth, interspecific competition and plant species performance (VERHOEVEN et al. 1996a,b, GÜSEWELL et al. 2005). It is usually associated with an increase in biomass productivity and a decrease in the diversity of plant communities in all the succession stages and with shifts in the dominance of species (OLDE VENTERINK et al. 2003, ROEM, BERENDSE 2000, GÜSEWELL et al. 2005).

The biomass production by wetland plants is most commonly limited by the availability of N, P and/or K. Fertilization experiments have shown that biomass production is generally limited by N in young fens, whereas old fens, which are mown and harvested for over 40 years, tend to be limited by P and/or by K (OOMES et al. 1996, OLDE VENTERINK et al. 2003, OLDE VENTERINK et al. 2009). It has been suggested that addition of a limiting nutrient enhances the biomass production but supplementation with a non-limiting nutrient will have little or no effect (VITOUSEK, HOWARTH 1991, VERHOEVEN et al. 1996b). However, simultaneous limitation by two nutrients might also occur. Besides, different species may show different responses to various nutrients, depending on their ability to take up and use a certain nutrient, their nutrient requirements or on their responsiveness to enhanced nutrient supply (PEREZ-CORONA, VERHOEVEN 1999, EL-KAHLOUN et al. 2000, GÜSEWELL et al. 2003b).

The relative availability of N and P in soil is reflected in the concentrations of these nutrients in plant tissues (VERHOEVEN et al. 1996a, GÜSEWELL, KOERSELMAN 2002). Several fertilization studies carried out on wetlands have shown that N:P ratios in plant biomass are a good indicator of nutrient limitation (KOERSELMAN, MEULEMAN 1996, GÜSEWELL et al. 2003a). Some studies have suggested that a decreased species diversity, changes in nutrient use efficiency, plants growth, interspecific competition and plant species performance are associated with increasing N:P or N:K ratios (VERHOEVEN et al. 1996a, ROEM, BERENDSE 2000, SEASTEDT, VACCARO 2001) but these mechanisms are not well understood. The N:K and P:K ratios are less frequently used as indicators due to shortage of information (HOOSBEEK et al. 2002, OLDE VENTERINK et al. 2003, LAWNICZAK et al. 2009). Therefore, the recognition of a N:K ratio in wetland plant tissues can be a useful method for diagnosing nutrient limitation, which can be a helpful tool for wetland protection (OLDE VENTERINK et al. 2009).

According to field studies, a critical N:K biomass ratio ranges from 1.75 to 2.1 (DE WIT, 1963, OLDE VENTERINK et al. 2003). HOOSBEEK et al. (2002) suggested a critical N:K ratio of 1.2 and 1.4 for bog vegetation, indicating that either N or K can be a limiting factor. The N:K ratio < 1.2 indicated N-limitation and sites with N:K > 1.4 were characterised by K-limitation, whereas the ones with N:K between 1.2 and 1.4 were co-limited by N and K. In fertilisation experiments, limitation by K was observed only where the

N:K ratio for whole vegetation was higher than 2.1 (Olde Venterink et al. 2003). However, a study of Lawniczak et al. (2009) in a controlled experiment showed an increase in biomass production following K fertilisation at a biomass N:K ratio around 4.0. However, that experiment was conducted only for one year. As suggested by a study of Güsewell et al. (2003b), a similar response of the shoot biomass N:P ratio to N and P nutrient supply was observed after many years, as determined in field fertilisation experiments. This raised the need to study the N:K ratio over more than one vegetation season (Lawniczak et al. 2009) in order to recognise whether it can be used as an indicator of nutrient limitation in wetlands.

In order to estimate a critical N:K ratio which can indicate the type of nutrient limitations, a greenhouse experiment has been established. Another aim of the study has been to test the response of two grass species to the range of N and K supply within two years.

MATERIAL AND METHODS

The two plant species included in the experiment are common herbaceous grasses: *Holcus landaus* L. (Yorkshire-fog) and *Molinia caerulea* (L.) Moench, Meth. (Purple Moor Grass). These species occur at sites different in nutrient availability. *Holcus lanatus* prefers nutrient-rich wet meadows, in contrast to *Molinia caerulea*, which favours nutrient-poor sites with fluctuating groundwater levels (El-Kahloun et al. 2000).

The experiment was carried out in a greenhouse of the Department of Ecology and Environmental Protection, at the Poznan University of Life Sciences, from April 2009 to September 2010. In March 2009, seeds of two grasses were sown on wet substrate and germinated until a sufficient number of plants reached the height of 6 cm. Seeds were obtained from the Plant Breeding and Acclimatization Institute (IHAR) in Bydgoszcz. At the beginning of May, seedlings were transplanted to polyethylene pots of 15×15 cm width and 25 cm height. Pots were filled with pure quartz sand (source: Antoninek Glassworks, Poznañ) and placed in 4 l buckets filled with deionised water. Pots were regularly shifted around the greenhouse to prevent heterogeneity in light and temperature. In winter, the plants were stored outdoors. However, all the pots were isolated to prevent nutrient and water supply. In March, the plants were moved back to the greenhouse to continue the experiment. Some specimens died during the first vegetation season or did not survive the winter. Therefore, some combinations consisted of only three replicates.

The design of the experiment was based on some previous greenhouse investigations, which focused on the effect N:K and N:P supply ratios on plant growth (Güsewell et al. 2003b, Lawniczak et al. 2009). The treatments combined six N:K supply ratios with two nutrient levels of fertility (high and low). Each combination consisted of four replicates. In total, 48 pots of *Holcus lanatus* and 48 pots of *Molinia caerulea* were analysed.

Each plant received 5 ml of KCl and NH_4NO_3 solution and 2 ml of the appropriate solution weekly during 15 weeks in 2009 (June-September) and during 16 weeks in 2010 (May-August). The first application of nutrients began three weeks after transplanting seedlings into their pots. The fertility levels were calculated using the geometric mean of the total amount of N and K supplied per plant over the entire growth period (Table 1). Additionally, an N:K ratio supply of 225 was added. The high nutrient supply level was three-fold higher than the low one (Table 1). Furthermore, all other nutrients were supplied in non-limiting amounts (10 mg P, 52 mg Ca, 16 mg Mg, 15 mg S, 5 mg Fe, 0.03 mg Cu, 0.5 mg B, 0.24 mg Mn, 0.1 mg Mo, 0.12 mg Zn per pot). Stock solutions and water were prepared every two weeks. Every week, the applied solution was adjusted to pH of approximately 7.0, using diluted HCl, and added to pots using a pipette. Every three weeks, pots were leached with deionised water to prevent toxic effect of nutrient accumulation.

Table 1

Annual amounts of N and K supplied per pot at six N : K supply ratios and two fertility levels (high supply = 3 x low supply)

N : K supply ratio	Level of supply	mg N	mg K
0.5	high	27.90	55.60
1.5	high	48.30	32.10
4.5	high	83.50	18.60
13.5	high	144.50	10.70
40.5	high	250.00	6.17
225	high	440.07	1.96
0.5	low	9.30	18.53
1.5	low	16.10	10.70
4.5	low	27.83	6.20
13.5	low	48.17	3.57
40.5	low	83.33	2.06
225	low	146.69	0.65

Every year, plants were harvested after 3-4 months of growth, i.e. on 4^{th} September in the first year and on 5^{th} September in the second year. Shoots were clipped 1 cm above ground. The collected material was separated into living and senescent parts. During the growing seasons, senescent leaves were collected every two weeks, dried and weighed. All harvested material was dried for 48 h to constant mass and weighed. For further nutrient analyses, four replicates per species and treatment were ground and digested with a modified Kjeldahl procedure (1 h at 200°C and 340°C in a mixture of concentrated sulphuric acid, salicylic acid, copper, and selenium) (Bremner and

Mulvaney 1982). N concentrations in the diluted digested material were determined colorimetrically on a Srecord 40, and K concentrations were assessed with flame emission spectroscopy, on a Shewood Model 425.

Statistical analysis

All statistical analyses were performed using Statistica (StatSoft, Poland) software. Shoot biomass (living parts only), senescent shoot biomass, N and K concentrations and the N:K ratio of shoots and senescent leaves were square-transformed to assess the homogeneity of variance. Four-way interactions of the effect of species, N:K supply ratios, nutrient supply levels and time on the studied variables were tested with analyses of variance (ANOVA).

RESULTS

Nutrient supply influenced significantly all variables used to describe the plant growth of the analysed species (Table 2). In general, the shoot biomass depended more strongly on the fertility levels than on N:K supply ratios, whereas the N and K concentrations, as well as N:K biomass ratios were determined by the N:K supply ratios. Four-way differences between N:K supply ratios, fertility levels, plant species and studied years were significant with respect to N:K biomass ratios and senescent biomass. However, this effect was much weaker than the main effects (Table 2).

The aerial biomass increased with an increasing N:K ratio supply during the first vegetation season (Table 2). The shoot biomass ranged from 1.15 g to over 93.23 g during the first year and from 18.68 to 314.8 g during the second year (Figure 1). In 2009, the biomass was significantly lower than in 2010, particularly at the low fertility level. *Holcus lanatus* had higher shoot biomass than *Molinia caerulea* during both years. The maximum shoot biomass differed not only between the species but also among the N:K ratios. In 2009, the optimal N:K supply ratio was 4.5 for *Holcus lanatus* at the high fertility level. At the low fertility level for both species, as well as at the high fertility level for *Molinia caerulea*, the maximum shoot biomass occurred at the 13.5 supply ratio (Figure 1). In 2010, the optimal N:K supply ratio was similar for both grasses; at the high fertility level, the shoot biomass was the highest for the N:K supply ratio of 13.5; at the low level, the shoot productivity reached the peak at the 225 N:K supply ratio.

The senescent biomass increased with increasing N:K supply ratios (Figure 2). It was significantly higher at the high fertility level and improved during the second vegetation season. *Holcus lanatus* was characterised by significantly faster senescence than *Molinia caerulea*, suggesting its higher biomass turnover.

Table 2

Results of the factorial four-way ANOVA testing of the effects of a N : K supply ratio, level of nutrient supply (low and high; high = 3 x low), time (two vegetation seasons) and plant species on aerial biomass, living shoot biomass, senescent shoot mass, nutrient concentrations (mg g^{-1}) and N : K ratios in shoot biomass. All variables were square-root transformed. Data are F-ratios and significance levels given as***.$p < 0.001$; ***.$p < 0.01$; *.$p < 0.05$; ns.$p \leq 0.05$

Effect	df	Aerial biomass		Shoot biomass		Senescent biomass		N con.		K con.		N : K	
		F	Sig	F	F	Sig.	F	F	Sig.	F	Sig.	F	Sig.
Species	1	57.41	***	54.99	***	501.20	***	0.12	ns	101.72	***	146.73	***
N : K supply ratio	5	31.90	***	31.70	***	58.26	***	489.19	***	292.05	***	420.38	***
Fertility level	1	210.96	***	210.64	***	31.92	***	298.63	***	16.44	***	73.25	***
Year	1	966.44	***	960.66	***	487.70	***	700.07	***	92.65	***	58.56	***
N : K × species	5	4.00	**	4.12	**	6.77	***	17.95	***	3.44	**	5.89	***
Species × level	1	0.09	ns	0.09	ns	1.49	ns	0.04	ns	35.99	***	1.34	ns
Species × year	1	293.31	***	294.66	***	6.34	*	163.02	***	293.16	***	135.95	***
N : K × level	5	21.71	***	21.66	***	7.77	***	100.42	***	23.24	***	35.84	***
N : K × year	5	4.22	**	4.26	**	3.49	**	3.62	**	13.36	***	21.81	***
Level × year	1	4.02	*	3.97	*	4.97	*	39.62	***	34.62	***	24.01	***
Species × N : K × level	5	10.71	***	10.73	***	9.83	***	2.97	*	7.95	***	6.06	***
Species × N : K × year	5	4.14	**	4.20	**	8.20	***	18.59	***	13.85	***	6.55	***
Species × level × year	1	0.04	ns	0.04	ns	3.84	ns	23.22	***	7.61	**	10.84	**
N : K × level × year	5	18.90	***	18.98	***	2.22	ns	21.72	***	2.97	*	7.78	***
Species × N : K × level × year	5	1.20	ns	1.21	ns	2.78	*	2.85	*	2.02	ns	6.43	***

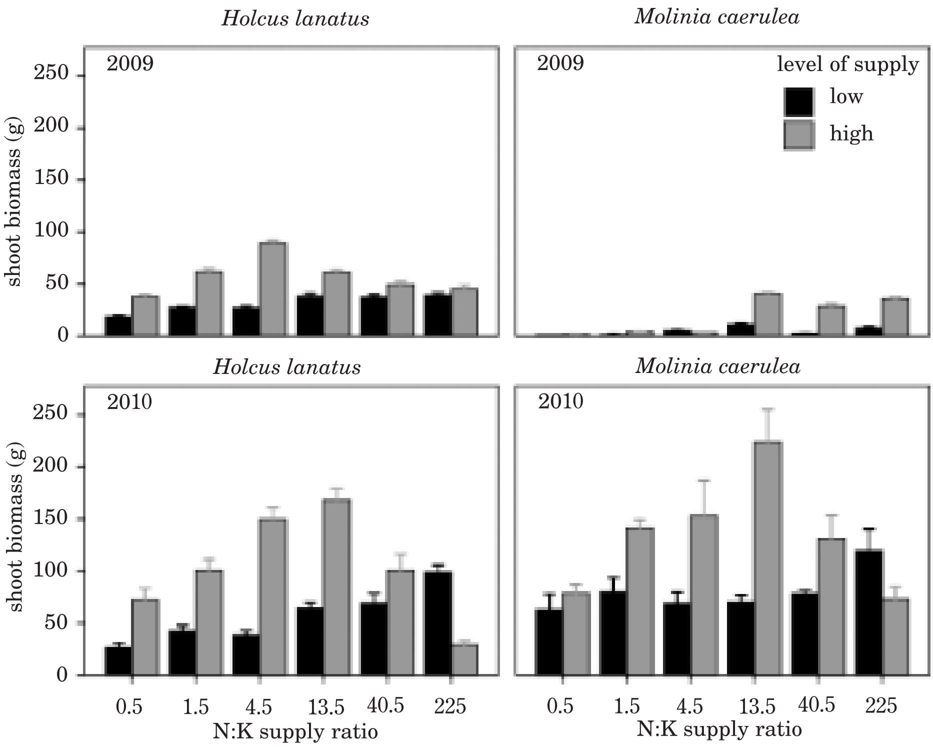

Fig. 1. Shoot biomass of two grass species grown for two years (2009 and 2010) at six N : K supply ratios and two levels of nutrient supply (low and high supply; high supply = 3 * low supply). Data are means ± se. n = 4

Nutrient concentrations in both species differed between the nutrient treatments (Table 2, Figs 3, 4). N concentrations generally increased with high N supply (Figure 3), whereas K concentrations increased with high K and declined with high N supply (Figure 4). In the first year, this effect was much stronger than in the second one. However, increased N concentrations were mostly observed at N:K supply ratios above 13.5 at the high fertilization level. During the second year, this effect was detected at 40.5 and 225 N:K supply ratios. At the low level, reduction of N was observed during both years, except for *Holcus lanatus*. K concentrations decreased twofold between the lowest and highest N:K supply ratios. The grasses responded quite similarly to the treatments. However, some differences between the species were observed. *Holcus lanatus* had higher K concentrations in the second year than *Molinia caerulea*.

The N:K ratio of shoot biomass increased eleven-fold across the range of N:K supply ratios (Figure 5). In the first year, the N:K ratios of *Holcus lanatus* and *Molinia caerulea* covered the same range of values in response to the N:K supply ratios. In the second year, the biggest changes were ob-

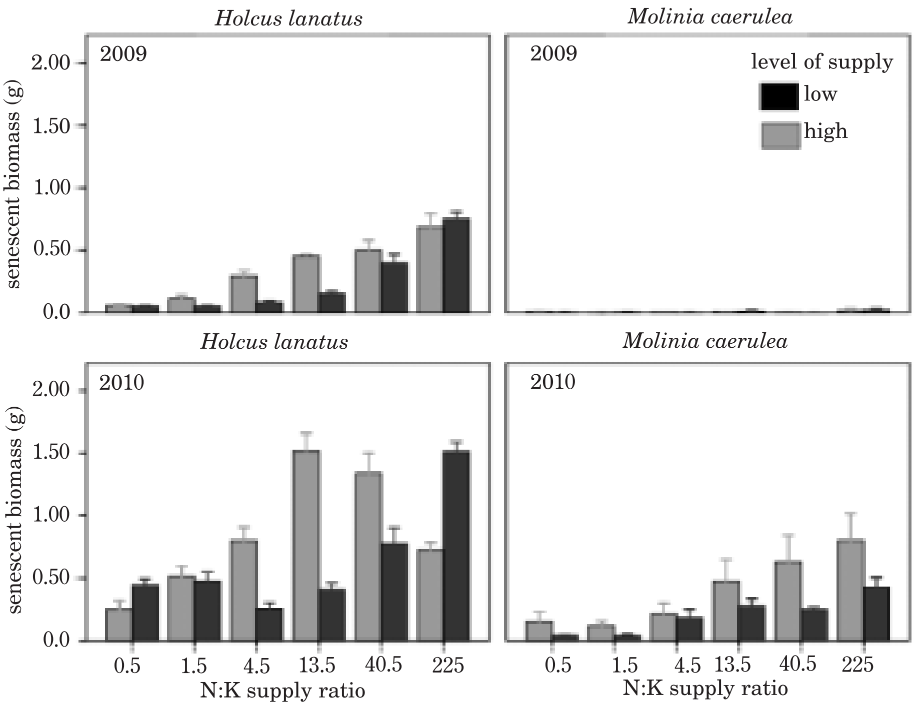

Fig. 2. Senescent biomass of two grass species grown for two years (2009 and 2010) at six N:K supply ratios and two levels of nutrient supply (low and high supply; high supply = 3 × low supply). Data are means ± se. *n* = 4

served in *Molinia caerulea*; its N:K biomass ratios increased twenty-fold at the high fertility level. At the N:K supply ratio of 13.5, the N:K biomass ratio was the highest and remained at that level even when N supplies increased. The N:K biomass ratios in *Holcus lanatus* increased with enhanced N:K supply ratios during both years.

Nutrient concentrations in senescent leaves, analysed only during the second year of growth, strongly depended on the nutrient treatments and varied considerably between the plant species. N concentrations during leaf senescence decreased by 5-65% (Figure 6a). The main differences appeared in *Holcus lanatus* at both fertilisation levels. K concentrations decreased by 25-98% during senescence (Figure 6b). Reduction of K was generally similar in both grass species, except *Holcus lanatus* at the 225 N:K supply ratio at the high fertility level. At the highest N:K supply ratios, K concentrations in senescent shoot parts were 77–97% lower than in living shoots. These differences were comparable to the results obtained in one-year fertilisation experiments (Lawniczak et al. 2009). Nutrient concentrations in senescent leaves indicate that substantial parts of K were withdrawn from leaves before senescence. This is one of the indicators of K deficiency, because leaching by rainfall or watering was excluded.

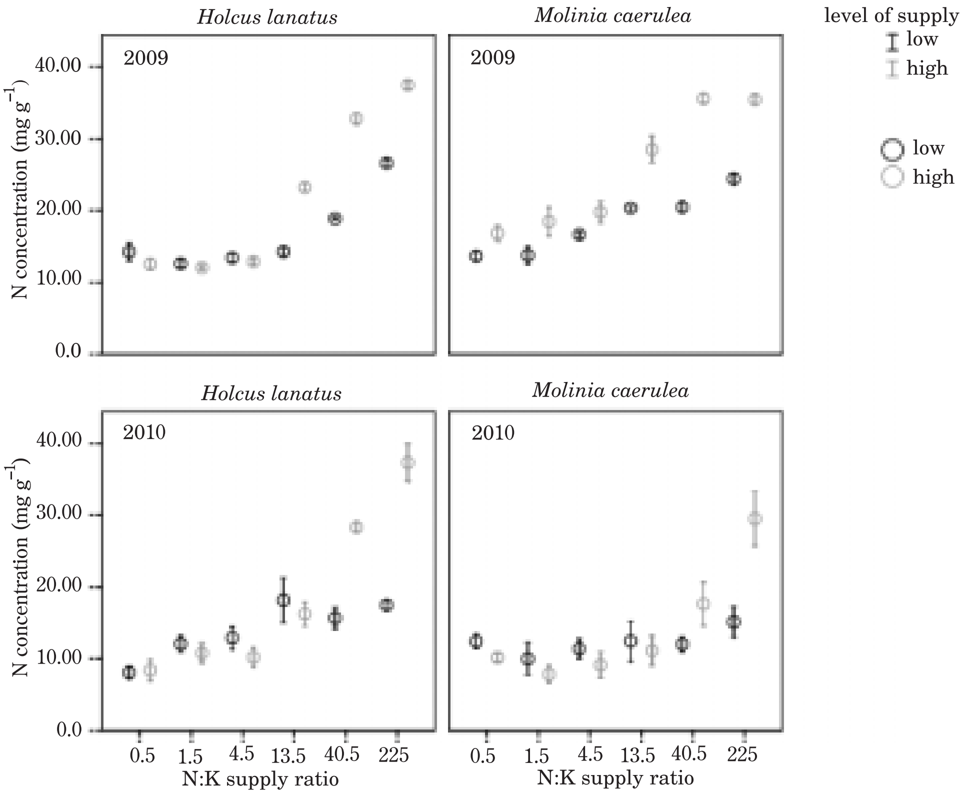

Fig. 3. Nitrogen concentrations in the living shoot parts of two grass species grown for two years at six N:K supply ratios and two levels of nutrient supply (high supply = 3 x low supply). Data are means ± se. n = 4

DISCUSSION

The results showed a significant effect of the N:K supply ratios on growth of the two grasses. During the first year, in both species, the biomass increased with an enhanced N:K supply ratio at the low fertility level and in *Molinia carulea* it also rose at the high fertility level, indicating that N was a limiting factor of the plant growth. At the high fertility level, *Holcus lantus* produced the highest biomass at the 4.5 N:K supply ratio, indicating the influence of K shortage on the plant growth. A shift from N-limited to K-limited growth was observed especially during the second vegetation season at the high level of nutrient supply. At the 13.5 N:K supply ratio and the high fertility level, the biomass production was the highest in both species over the entire range of N:K supply ratios. At the 40.5 and 225 N:K supply ratios, a decrease in the aerial biomass was observed, suggesting

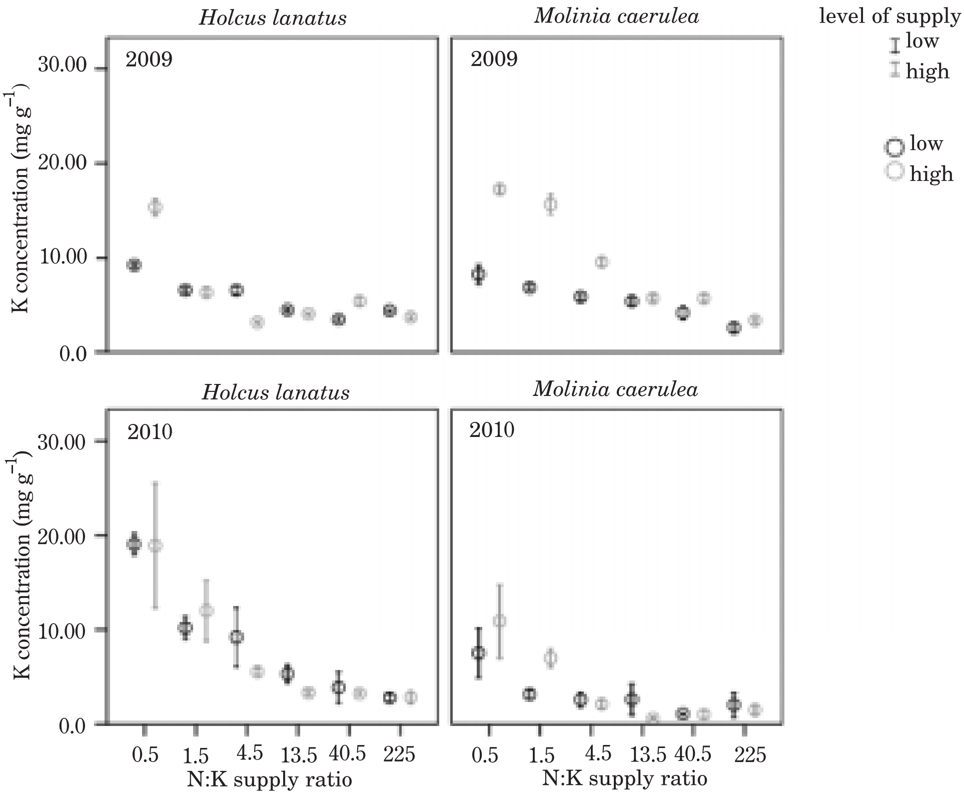

Fig. 4. Potassium concentrations in the living shoot parts of two grass species grown for two years at six N:K supply ratios and two levels of nutrient supply (high supply = 3 x low supply). Data are means ± se. n = 4

strong K deficiency. At the low nutrient supply level, during both years, a significant increase in biomass production between the N:K supply ratios above 13.5, 40.5 and 225 indicated that the plant growth at the 13.5 N:K supply continued to be N-limited.

However, a similar pattern of biomass production during the second year of growth occurred in both species across the range of N:K supply ratios but the N:K biomass ratios differed significantly. At the highest biomass, the N:K biomass ratio in *Holcus lanatus* was 4.5 while the N:K biomass ratio *in Molinia careulea* was very high, namely 58. These results seem to indicate that the species have different strategies of nutrient use, thus making it difficult to determine a critical N:K biomass ratio.

The shoot biomass of the tested grasses in the second year of the study was significantly higher, even at the high fertility level. This was in contrast to the N:P supply ratios, for which the biomass in the second year of experiment was higher at the low rather than high N:P supply ratios. This

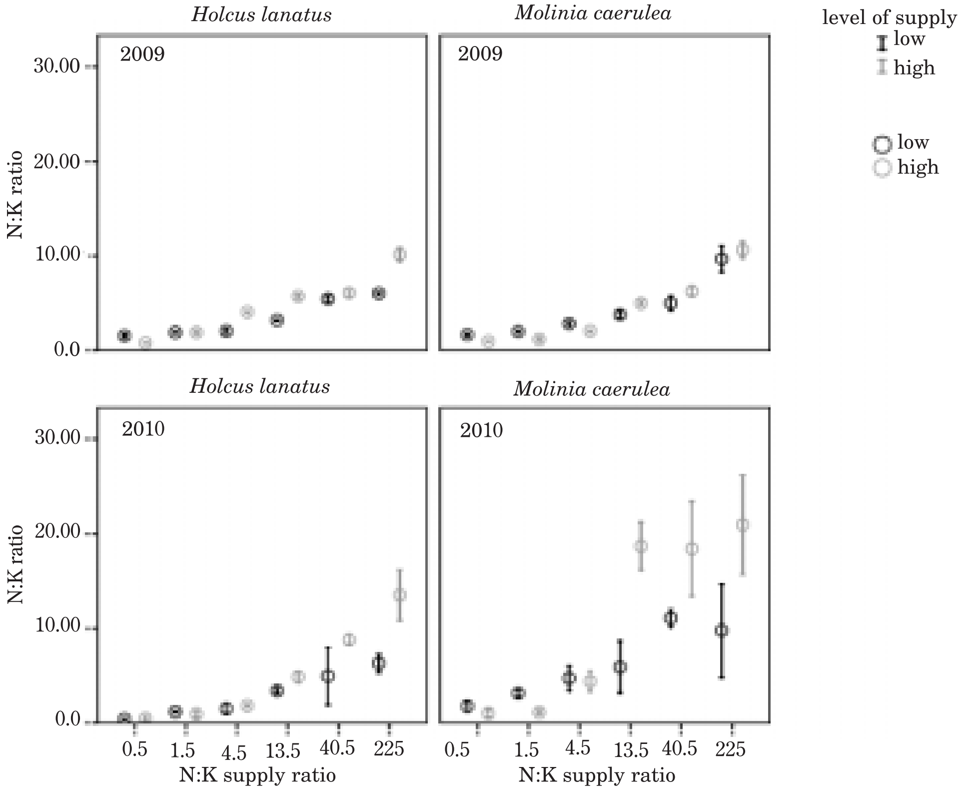

Fig. 5. The N:K biomass ratio of two grass species grown for two years at six N:K supply ratios and two levels of nutrient supply (high supply = 3 x low supply). Data are means ± se. n = 4

suggested that N was more important than K in limiting the plant growth between the two years.

Decreased biomass production at the 13.5 N:K supply ratio was observed in both years, indicating that strong K shortage may influence the plant growth. LAWNICZAK et al. (2009), in a similar type of experiment, did not observe a sharp decrease in shoot biomass between N:K supply ratios of 13.5 and 40.5, as recorded in this study. These variations may have occurred due to some specific differences between the species. On the other hand, SZCZEPANIAK (2004) suggested that the real effect of potassium fertilization on plant yields may be expected at low available K content in soil and under water stress during the plant growth.

On the other hand, at very high N:K supply ratios, a negative effect of NH_4^+ could have occurred due to some imbalance between Mg^{+2}, Ca^{+2} and other cations (KRUPA 2003). Although all pots were washed with deionised water every three weeks to prevent negative effect of NH_4^+ accumulation,

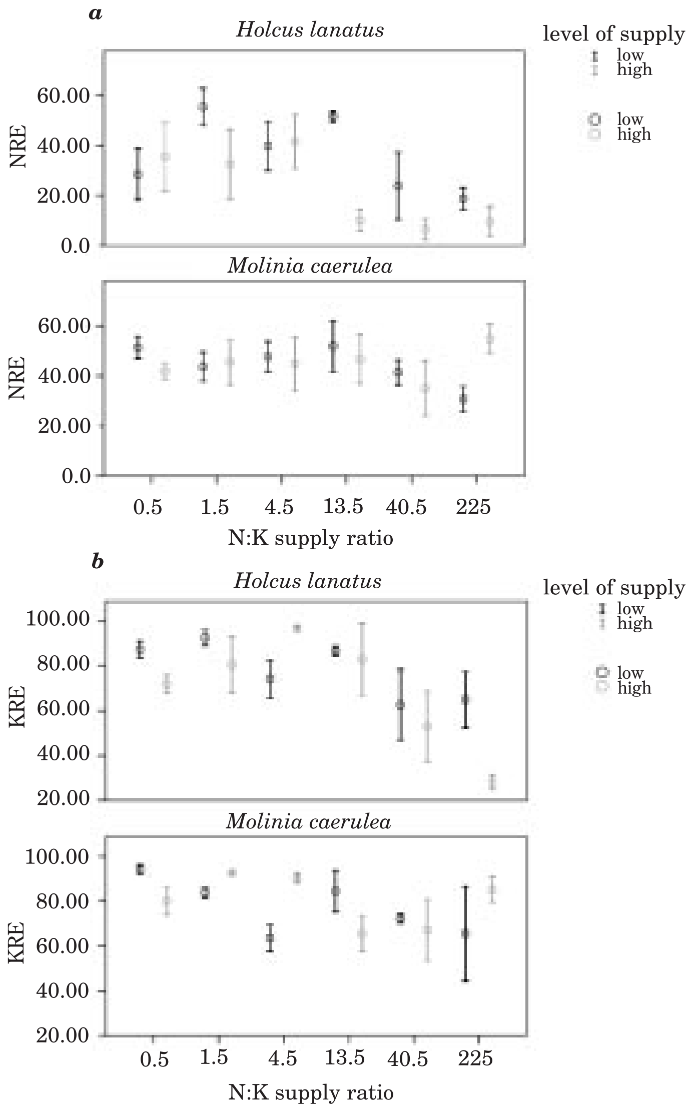

Fig. 6. Reduction in a) nitrogen and b) potassium concentrations during senescence for the two grass species in relation to the N:K supply ratios and levels of nutrient supply during the second vegetation season. Values represent the difference in nutrient concentrations between living and senesced shoot parts. as percentage of concentration in living shoot parts. Data are means ± se.

high amounts of nitrogen were added weakly. Compared to other growth experiments (Wassen et al. 1995), N concentrations of the analysed plants at the high N:K supply ratios such as 13.5 and 40.5 were generally low, with values below 20 mg g^{-1}, i.e. similar to the concentrations in the same species growing on a field (El Kahloun 2004). It was only at the high level of 225 N:K supply ratio that *Molinia caerulea* accumulated a high amount of N in plant tissues, suggesting some toxic effect of NH_4^+ accumulation. Alternatively, K concentrations were rather low, with values below 8 mg g^{-1}, which indicated strong K deficiency according to de Wit (1963) and Wassen et al. (1995). Cumulative effects of these factors could have caused decreased shoot biomass at the high level of nutrient supply.

Also, this diverse response of the studied graminoids to the N:K supply ratio may have resulted from some interspecific differences. *Holcus lanatus* is characteristic for moderately nutrient-rich sites and *Molina caerulea* grows on sites of lower fertility, particularly with strong P-limitation (El-Kahloun et al. 2000). According to Ellenberg et al. (1991), on a 1 to 9 scale, the nutrient indicator value of *H. lanatus* is 5 and that of *M. caerulea* just 1, indicating different preferences of these species to N availability in soil. In the first year of growth, these species positively correlated with Ellenberg's values; *Holcus* grew faster than *Molinia*. These results are also confirmed by the studies of Güsewell (2004) and Ryster, Lambers (1995), who observed stronger growth of species from nutrient-rich sites than from nutrient-poor ones despite low nutrient supply only in the first year of fertilisation. In the current study, this relationship disappeared in the second year only for *Molinia*, which suggested that it was less sensitive to K shortage than *Holcus*. *Molinia* was characterized by luxury consumption of the nutrient during the second year of growth. This species, at the low and high levels of nutrient supply, benefited more from available nutrients than *H. lanatus* by producing higher aerial biomass. My observations confirm the hypothesis stated by Güsewell et al. (2003b) that species from nutrient-poor sites react more readily to N supply than species from nutrient-rich sites. Also, Perez-Cordona, Verhoeven (1996) observed higher adaptation of species from poor-nutrient sites to enhanced nutrient availability.

Difficulties in estimating a critical N:K biomass ratio may have also been caused by different physiological roles of these nutrients. In an agricultural study, Marschner et al. (1996) suggested that nitrogen uptake is sufficient only at a certain amount of potassium. Gaj (2010) showed that it is important to analyse additionally Ca ions since, together with Mg and other solutes, they may partly replace K in some processes (Lindhauer et al. 1990).

Moreover, the current study did not show a similar response of the two grasses to the N:K supply ratio in the second year of growth. This response should be studied in a long-term experiment and include more species. In short-term experiments, plant species tend to produce very high biomass in a very short time (Ryster 1996, Güsewell 2004, 2005), but these differences may become smaller or disappear altogether with time (Elberse, Berendse 1993, Ryster 1996).

CONCLUSIONS

1. The response of the two grass species to the N:K supply ratios showed different patterns between the first and second years of the plant growth. During the second growing season, at the N:K supply ratio o 13.5 and the high fertility level as well as the 225 N:K supply ratio and the low fertility level, aerial biomass production was the highest in the both species.

2. The N:K biomass ratio differed between the two tested species at the maximum stand, which makes it impossible to determine a critical N:K ratio based on N and K concentrations in plant tissues.

3. The N:K nutrient supply ratio was a better indicator of plant performance than the N:K biomass ratio of the examined species.

4. The analysed graminoids did not show a similar response to N:K supply ratios at the high and low levels of supply, indicating that nitrogen was the most important factor limiting the plant growth during the two years, and that these plant species were less sensitive to K shortage than to N deficiency.

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