ELSEVIER

Aquatic Botany 81 (2005) 326–342

Aquatic botany

www.elsevier.com/locate/aquabot

The effects of NH_4^+ and NO_3^- on growth, resource allocation and nitrogen uptake kinetics of *Phragmites australis* and *Glyceria maxima*

Edita Tylova-Munzarova [a,*], Bent Lorenzen [b], Hans Brix [b], Olga Votrubova [a]

[a] *Department of Plant Physiology, Faculty of Sciences, Charles University in Prague, Vinicna 5, 128 44 Prague 2, Czech Republic*

[b] *Department of Plant Ecology, University of Aarhus, Nordlandsvej 68, DK-8240 Risskov, Denmark*

Received 17 July 2003; received in revised form 18 January 2005; accepted 21 January 2005

Abstract

The effects of NH_4^+ or NO_3^- on growth, resource allocation and nitrogen (N) uptake kinetics of two common helophytes *Phragmites australis* (Cav.) Trin. ex Steudel and *Glyceria maxima* (Hartm.) Holmb. were studied in semi steady-state hydroponic cultures. At a steady-state nitrogen availability of 34 μM the growth rate of *Phragmites* was not affected by the N form (mean RGR = 35.4 mg g^{-1} d^{-1}), whereas the growth rate of *Glyceria* was 16% higher in NH_4^+-N cultures than in NO_3^--N cultures (mean = 66.7 and 57.4 mg g^{-1} d^{-1} of NH_4^+ and NO_3^- treated plants, respectively). *Phragmites* and *Glyceria* had higher S/R ratio in NH_4^+ cultures than in NO_3^- cultures, 123.5 and 129.7%, respectively.

Species differed in the nitrogen utilisation. In *Glyceria*, the relative tissue N content was higher than in *Phragmites* and was increased in NH_4^+ treated plants by 16%. The tissue NH_4^+ concentration (mean = 1.6 μmol g fresh wt^{-1}) was not affected by N treatment, whereas NO_3^- contents were higher in NO_3^- (mean = 1.5 μmol g fresh wt^{-1}) than in NH_4^+ (mean = 0.4 μmol g fresh wt^{-1}) treated plants. In *Phragmites*, NH_4^+ (mean = 1.6 μmol g fresh wt^{-1}) and NO_3^- (mean = 0.2 - μmol g fresh wt^{-1}) contents were not affected by the N regime. Species did not differ in NH_4^+ (mean = 56.5 μmol g^{-1} root dry wt h^{-1}) and NO_3^- (mean = 34.5 μmol g^{-1} root dry wt h^{-1}) maximum uptake rates (V_{max}), and V_{max} for NH_4^+ uptake was not affected by N treatment. The uptake rate of NO_3^- was low in NH_4^+ treated plants, and an induction phase for NO_3^- was observed

* Corresponding author. Tel.: +42 22195 1697; fax: +42 22195 1704.
E-mail address: edmunz@natur.cuni.cz (E. Tylova-Munzarova).

0304-3770/$ – see front matter
doi:10.1016/j.aquabot.2005.01.006

in NH_4^+ treated *Phragmites* but not in *Glyceria*. *Phragmites* had low K_m (mean = 4.5 μM) and high affinity (10.3 l g^{-1} root dry wt h^{-1}) for both ions compared to *Glyceria* (K_m = 6.3 μM, affinity = 8.0 l g^{-1} root dry wt h^{-1}). The results showed different plasticity of *Phragmites* and *Glyceria* toward N source. The positive response to NH_4^+-N source may participates in the observed success of *Glyceria* at NH_4^+ rich sites, although other factors have to be considered. Higher plasticity of *Phragmites* toward low nutrient availability may favour this species at oligotrophic sites.

Keywords: *Phragmites*; *Glyceria*; Eutrophication; Wetland plant; Nitrate nutrition; Ammonium nutrition; Nitrogen form; NO_3^- uptake; NH_4^+ uptake; Michaelis–Menten kinetic

1. Introduction

Wetland ecosystems exposed to anthropogenic eutrophication undergo changes in sediment characteristics and species performance worldwide. Different aspects of eutrophication have been elucidated (van der Putten, 1997), however, the effects of changes in plant available inorganic nitrogen source associated with eutrophication on the performance of wetland plants remain unclear.

Both NH_4^+ and NO_3^- are important nitrogen sources for plant growth (Marschner, 1995) and the preference for a particular ion is an important factor affecting plant community composition (Kronzucker et al., 1997). The NH_4^+ preference is common in plants occupying habitats with restricted nitrification, where NH_4^+-N prevails (Garnett et al., 2001; Kronzucker et al., 1997). Ammonium dominates in waterlogged sediments, since NO_3^- decrease rapidly with soil depth (Andersen and Hansen, 1982) and is limited to hypoxic top soil layer and oxidised patches around roots of emergent macrophytes. Nutrient enrichment enhances plant productivity, litter accumulation and microbial activities, facilitating oxygen demand and diminishing the NO_3^- availability in the sediment (Cizkova et al., 2001; Kuhl et al., 1997; Nijburg and Laanbroek, 1997).

Wetland plant species are supposed to favour NH_4^+ rather than NO_3^-. However, among species occupying waterlogged soils, the preferential uptake of NH_4^+ is documented for *Oryza sativa* only (Sasakawa and Yamamoto, 1978), and both its uptake and assimilation is enhanced by the presence of NO_3^- (Kronzucker et al., 1999). The assimilation of NH_4^+ has lower energy costs, however, many plant species show reduced growth under strict NH_4^+ nutrition and develop NH_4^+ toxicity syndrome, which is associated with an accumulation of NH_4^+ in tissues (Mehrer and Mohr, 1989) or a diminished cation (e.g., Mg^{2+} or Ca^{2+}) uptake. In contrast, NO_3^- can be stored in vacuoles without detrimental effects and the ion participates in osmoregulations (Marschner, 1995). Moreover, improved adenylate energy charge or lower ethanol and lactate production, were observed in *Glyceria maxima*, *Phalaris arundinacea* and *Carex pseudocyperus* when NO_3^- was used as nitrogen source compared to NH_4^+-fed plants (Brix et al., 1994; Muller et al., 1994).

To examine how wetland plants respond to NH_4^+ versus NO_3^- the growth, resource allocation and N uptake kinetics of two fast growing helophytes *Phragmites australis* (Cav.) Trin. ex Steudel and *G. maxima* (Hartm.) Holmb. were compared. Both species colonise preferentially habitats of higher fertility (Hejny and Husak, 1978) and are often used for sewage treatment in artificial wetlands. However, *Phragmites* appears to be

sensitive to the conditions of highly eutrophic sediments rich in organic matter (van der Putten, 1997; Cizkova et al., 1996), where *Glyceria* was observed to expand or even replace *Phragmites* (Hroudova and Zakravsky, 1999; Crawford and Brandle, 1996). To which extent the preferences for particular N source participate at these interspecific differences is unknown. *Phragmites* may be more sensitive to eutrophication due to NH_4^+-N as the sole nitrogen form in the eutrophic sediment.

Since plant sensitivity to nitrogen forms generally depends on the rhizosphere nitrogen availability and pH (Marschner, 1995), the misinterpretation of results may occur when unrealistic nitrogen concentrations are used. The study was therefore carried out at nitrogen concentration corresponding with average nitrogen availability recorded in pore waters of selected European wetlands colonised with *Phragmites* and *Glyceria* (Cizkova et al., 2001; Picek et al., 2000).

2. Methods

2.1. Plant material and growth conditions

The mother plants of *Phragmites* or *Glyceria* were obtained at natural littoral stands in the Trebon basin, Czech Republic and long-term vegetatively propagated in outdoor sand culture in the Institute of Botany, Academy of Sciences, Trebon, Czech Republic. Ten cm long rootless rhizome cuttings with a single shoot (15–20 cm) were produced for experimental purposes and cultivated for 3 weeks in vermiculite before they were transferred to hydroponic cultures.

The plantlets were cultivated in a controlled growth cabinet (Umwelttechnic, Lindenstruth, Germany) with four independent hydroponic units. The growth cabinet was operated with a 16 h:8 h day:night photoperiod, 22 °C:15 °C thermoperiod and an 85%:90% relative humidity day:night period. One-hour transition periods were used between light and dark for a gradual change in the climatic parameters. Each hydroponic growth unit was composed of one 30 l growth tank with 10 plants of each species, and a nutrient solution reservoir (140 l) through which the nutrient solution was recirculated at a rate of 3 l min^{-1} (Lorenzen et al., 2001). The chemical composition of a basic nutrient solution was as follows: PO_4^{3-} 16.35 μM; K^+ 86.96 μM; Ca^{2+} 3186.27 μM; Mg^{2+} 1686.55 μM; Na^+ 2174.86 μM; SO_4^{2-} 3060.59 μM; Cl^- 6093.09 μM; SiO_3^{2-} 12.50 μM; BO_3^{3-} 2.50 μM; Fe^{2+} 2.01 μM; Mn^{2+} 0.20 μM; Zn^{2+} 0.20 μM; Cu^{2+} 0.20 μM (Lorenzen et al., 2001). Two of the hydroponic units received 34 μM NO_3^--N (KNO_3) whereas the other two units received 34 μM NH_4^+-N ($(NH4)_2SO_4$). The pH of the solutions was adjusted to pH 6.5 using H_2SO_4 and NaOH. Conductivity, temperature, pH, NO_3^-, NH_4^+ and phosphorus concentrations were monitored daily and adjusted to the desired levels. The NH_4^+ level was adjusted by $(NH_4)_2SO_4$, NO_3^- level by KNO_3 and phosphorus level by KH_2PO_4 stock solutions. Nitrate was measured using the UV method (Huber and Frost, 1993), NH_4^+ was detected using a salicylate-based method (ammonia in waters 1981, London, Her Majesty's Stationary Office) and orthophosphate detection was based on the ascorbic acid method for orthophosphate detection (Method EPA-600/4-79-020, 1983, US Environmental Protection Agency). Since nitrate was added to the NO_3^- treatments as

KNO_3, the NH_4^+ treatments received an equivalent amount of K^+ supplied as K_2SO_4 in order to establish similar K conditions in the four nutrient supply units. The iron concentration was adjusted daily using $FeSO_4$ to compensate for precipitation. To minimise changes in ion composition and conductivity and to avoid accumulation of NO_3^- ions in the NH_4^+ treatment due to microbial activity, the solutions were periodically renewed when the NO_3^- concentrations of NH_4^+ treatment solutions reached 7.14 μM NO_3^--N or when conductivity exceeded 1.5 mS cm^{-1} (approximately once in a 72 h period). The 80 plants were allowed to acclimate to the experimental conditions in the hydroponic cultures during a 21-day period before the start of the experiment.

2.2. Growth experiment

The growth experiment was carried out with 40 plants of each species equally divided between the two N sources and between the growth units. At the start of the experiment, the plants were weighed according to a standardized weighing procedure, marked and initial biomass and biometric characteristics of plants were recorded. After 28 days of cultivation, four plants of each species and from each container (a total of eight plants per species and treatment) were randomly selected and harvested. Plants were harvested using the harvesting procedure described below and main biometric characteristics were estimated. The remaining plants were divided into halves and cultivated for further 28 days before they were used for uptake kinetics measurements.

2.3. Plant harvest and analyses

Harvested plants were fractionated into roots, rhizomes, leaf blades and stems including leaf sheaths. The numbers of shoots and leaves were recorded together with the lengths of shoots, roots and rhizomes. The rhizome fraction was further divided into young segments (segments neighbouring the young growing buds) and older segments (segments of rhizome branches below the oldest shoots, approximately 1.5-month old).

The samples were rinsed in distilled water and weighed, frozen in liquid nitrogen and freeze dried for dry weight (dry wt) determination. The plant material was ground and analysed for total C, N, NH_4^+ and NO_3^- contents. The total carbon and nitrogen contents were analysed using a CN analyser (Na2000, Carlo Erba, Italy). For NH_4^+ and NO_3^- determinations, the plant material (10 mg dry wt) was extracted with 10 ml of distilled water for 20 min at 80 °C in screw-cap tubes and filtered using washed 25 mm GF/C filters. The extracts were analysed by flow injection analyser (Lachat, Quick Chem Instruments). NH_4^+ was detected by the salicylate method (ammonia in waters 1981, London, Her Majesty's Stationary Office). NO_3^- was determined as NO_2^- after cadmium reduction (Methods for Chemical Analysis of Water and Wastes, Method 353.2, Storet No. Total 00630, U.S. Environmental Protection Agency). The nitrogen use efficiency (NUE) was calculated as the total dry plant biomass divided by total N content. Leaf NUE was calculated as leaf biomass divided by leaf N mass.

The plant fresh weights were used for the calculation of relative growth rate (RGR = (ln final weight − ln initial weight)/days). The biomass allocation was char-

acterised using the ratio of root-supported tissue (shoot, rhizomes) to root biomass (S/R) and the ratio of above ground (shoot) to below ground (roots and rhizomes) biomass (A/B) (Lorenzen et al., 2001). The allocations into leaves, stems + leaf sheaths, rhizomes and roots were calculated as the ratio between the biomass of the fraction and the total dry biomass of the plants. The following biometric characteristics were estimated: relative shoot growth rate = (ln final shoot length − ln initial shoot length)/days (mm cm^{-1} d^{-1}), relative increase in shoot number = (ln final shoot number − ln initial shoot number)/days (No. d^{-1}), leaf number per individual shoot, dead to living leaf ratio based on leaf numbers and rhizome length/shoot number ratio (mm $No.^{-1}$).

2.4. Uptake kinetics measurement

Plant NH_4^+ and NO_3^- uptake was measured by the rate of depletion from nutrient solutions using an automated flow injection analyser (Lachat Instruments, Milwaukee, WI, USA) using the salicylate method (ammonia in waters 1981, London, Her Majesty's Stationary Office) and an UV spectrometer using the UV method (Huber and Frost, 1993).

The uptake studies were conducted with individual plants placed in separate root vessels inside the growth cabinet. Nutrient uptake kinetics were measured in eight plants of each species and each treatment. Plants were selected equally from the four growth units, and the uptake kinetics of NH_4^+ and NO_3^- were estimated independently. A measuring series of a single plant included the following steps. The root system of an intact plant was carefully rinsed in nutrient solution and transferred to a 1200 ml root chamber 8 h prior the measurement to avoid stress effect of handling. The climatic parameters, basic nutrient solution composition and N source were the same as during the cultivation. The solution level in the root chamber was marked and the chamber was placed in a 10 l tank containing additional 7 l of nutrient solution inside the growth cabinet. Centrifugal pump recirculated water between the tank and the root chamber at a rate of 1 l min^{-1}. Magnetic stirrer insured mixing of the nutrient solution in the root vessel. Plant roots were protected against direct light. Immediately prior the start of the uptake measurement the recycling flow was stopped and the nutrient solution in the root chamber was renewed. The plant received the fresh basic nutrient solution containing the measured form of N in concentration of 43 μM. Distilled water, H_2SO_4 and NaOH were added to the root vessel if necessary to maintain the constant volume (1.5 l) and pH (6.5) within the depletion period.

During depletion the concentration of N ions in the solution was monitored every three minutes until no further depletion by plant uptake was registered. NH_4^+ detection was provided by the flow injection analyser (Lachat, Quick Chem Instruments) using the salicylate method (ammonia in waters 1981, London, Her Majesty's Stationary Office). NO_3^- was detected with UV light (210 nm) (Huber and Frost, 1993) by UV spectrophotometer (UV-1201 Schimadzu corporation). Possible interferences of organic material with NO_3^- estimation were checked using the cadmium reduction method (Methods for Chemical Analysis of Water and Wastes, Method 353.2, Storet No. Total 00630, U.S. Environmental Protection Agency) and NO_3^- concentrations were corrected if necessary.

The uptake rates were calculated by fitting three to five consecutive samples of concentrations measured during depletions by linear regression analysis. The slopes of the regression lines were corrected for the loss, due to sampling by the flow injection analyser, and divided by root dry weight to obtain the nutrient uptake rate (μmol N g^{-1} root dry wt h^{-1}). The uptake kinetic parameters were calculated using a modified Michaelis–Menten equation (BARBER 69). Data were processed with Statgraphics ver. 3, Manugistics Inc., Maryland, USA using non-linear regression analysis. The calculated kinetic parameters were: the average maximum uptake rate at maximum nutrient levels, when uptake appeared to be saturated—V_{max} (μmol N g^{-1} root dry wt h^{-1}), half-saturation constant—K_m (μg N l^{-1}) and affinity—α (l g^{-1} root dry wt h^{-1}):

$$V = V_{max}C/(K_m + C), \qquad \alpha = V_{max}/K_m$$

The shifts of N form in the nutrient solutions were often followed by a period of very low N uptake called the induction phase. The induction phase was described using a polynomial ($y = ax^2 + bx + c$) curve fitting procedures. The local maximum of the function was calculated and considered as the end of the induction phase. The slope of the first order derivative equation was used as a measure of the induction rate.

2.5. Statistical evaluation

Statistical evaluation of the data was performed using NCSS 6.0.21 software. Means and standard errors (S.E.s) were calculated and the effects of treatments, plant species and interactions were analysed using analysis of variance (ANOVA). The experiment was considered as a randomised block design, the data from both units receiving the same treatment were pooled for the analysis. The residuals of the ANOVA analyses were tested for normality and transformed if necessary.

3. Results

At the beginning of the experimental period, the plants of the two species were very similar in size and there was no difference between plants from the two nitrogen treatments ($P > 0.05$). *Phragmites* and *Glyceria* plants had an average fresh wt of 12.79 ± 1.02 and 19.75 ± 1.23 g (mean ± S.E. ($n = 20$)), an average shoot length of 318.7 ± 10.60 and 414.51 ± 13.69 mm and an average shoot number of 5.00 ± 0.17 and 4.05 ± 0.22, respectively ($P > 0.05$).

3.1. Plant growth

On average, *Glyceria* had a 75% higher relative growth rate (RGR) than *Phragmites* (Tables 1 and 2) and the species responded differently to the source of nitrogen (NH_4^+ or NO_3^-). *Glyceria* had 16% higher RGR in the NH_4^+ treatment than in the NO_3^- treatment. The RGR of *Phragmites* was unaffected by the nitrogen treatments, on average the RGR was 35.4 mg g^{-1} d^{-1} (Tables 1 and 2).

Table 1
Relative growth rate, biomass allocation and biometric characteristics of *P. australis* and *G. maxima* grown with NH_4^+-N or NO_3^--N source (34 μM)

	P. australis		*G. maxima*	
	NH_4^+	NO_3^-	NH_4^+	NO_3^-
RGR (mg g^{-1} d^{-1})	34 ± 1.8	36 ± 1.8	66 ± 1.9	57 ± 2.0
Biomass allocation				
A/B ratio	1.2 ± 0.09	1.2 ± 0.10	2.6 ± 0.11	2.3 ± 0.10
S/R ratio	6.5 ± 0.30	5.2 ± 0.32	7.0 ± 0.38	5.4 ± 0.32
Leaves percentage[a]	19 ± 0.95	2.1 ± 1.0	33 ± 1.2	31 ± 1.0
Stems percentage[a]	34 ± 0.85	32 ± 0.91	38 ± 1.1	37 ± 0.91
Rhizomes percentage[a]	32 ± 1.1	30 ± 1.2	15 ± 1.5	15 ± 1.2
Roots percentage[a]	13 ± 0.58	17 ± 0.62	13 ± 0.73	16 ± 0.62
Dead material percentage[a]	1.9 ± 0.30	0.50 ± 0.32	0.48 ± 0.38	1.5 ± 0.32
Biometric characteristics				
Relative rate of shoot growth (mm cm^{-1} d^{-1})	0.30 ± 0.02	0.32 ± 0.02	0.50 ± 0.02	0.40 ± 0.02
Relative increase in shoot number (No. d^{-1})	0.02 ± 0.002	0.02 ± 0.002	0.04 ± 0.003	0.03 ± 0.003
Dead/living leaves ratio	0.08 ± 0.01	0.03 ± 0.01	0.08 ± 0.01	0.06 ± 0.01
Leaf number per shoot (No. $shoot^{-1}$)	6.8 ± 0.19	7.2 ± 0.19	4.4 ± 0.20	4.9 ± 0.21
Rhizome length/shoot number (mm $No.^{-1}$)	183 ± 29	184 ± 29	199 ± 26	282 ± 29

Values given are mean ± S.E. (n = 5–8).
[a] % total dry wt.

3.2. Biomass allocation and biometric characteristics

Plants in the NH_4^+ treatment had high S/R ratio compared to NO_3^- treated plants, whereas the A/B ratio was unaffected by the treatments indicating different aspects of root

Table 2
Statistical evaluation of main biomass and biometric characteristics using analysis of variance (ANOVA)

Variable	Source of variability		
	Species	Treatment	Interaction
RGR	**0.000**	**0.042**	**0.004**
A/B ratio	**0.000**	0.142	0.208
S/R ratio	0.323	**0.000**	0.584
Leaves percentage	**0.000**	0.972	0.096
Stems percentage	**0.000**	0.203	0.839
Rhizomes percentage	**0.000**	0.311	0.767
Roots percentage	0.547	**0.000**	0.965
Dead material percentage	0.552	0.603	**0.001**
Relative rate of shoot growth	**0.000**	0.053	**0.003**
Relative increase in shoot number	**0.000**	**0.008**	**0.023**
Dead/living leaves ratio	0.366	**0.008**	0.362
Leaf number per shoot	**0.000**	**0.013**	0.896
Rhizome length/shoot number	0.056	0.151	0.159

Effects of plant species, N treatments and interactions (species × treatment) are expressed by P-values. Numbers in bold indicate P-values < 0.05.

versus rhizome growth regulations (Tables 1 and 2). *Glyceria* had a two-folds higher A/B ratio than *Phragmites* but similar S/R ratio. The N treatments did not affect the biomass allocated to leaves, rhizomes and stems (including leaf sheaths) but *Phragmites* had more biomass allocated into rhizomes and less into leaves than *Glyceria* (Tables 1 and 2). Dead biomass constituted to less than 2% of the biomass. The relative amount of dead plant biomass increased in NH_4^+ treated *Phragmites*, whereas *Glyceria* showed the opposite tendency (Tables 1 and 2).

The N source affected the shoot growth in *Glyceria*. Ammonium treated *Glyceria* had high rate of shoot growth, a tendency to produce high number of individual shoots and a low rhizome length to shoot number ratio (e.g., proportion between above and below ground parts of stem) compared to NO_3^- treated plants. A similar response to NH_4^+ was not observed in *Phragmites* (Tables 1 and 2). The NH_4^+ treated plants of both *Phragmites* and *Glyceria* had lower numbers of leaves per individual shoots than NO_3^- treated plants, which was accompanied by a higher percentage of dead leaves (Tables 1 and 2).

3.3. Nitrogen utilisation and nitrogen use efficiency (NUE)

The nitrogen source affected the utilisation of nitrogen by *Glyceria* but not *Phragmites*. In *Phragmites*, there was no significant difference in tissue nitrogen content and C/N ratio between NH_4^+ and NO_3^- treated plants. Similarly, the distribution of nitrogen among individual plant parts of *Phragmites* remained unaffected by the nitrogen source (Fig. 1A

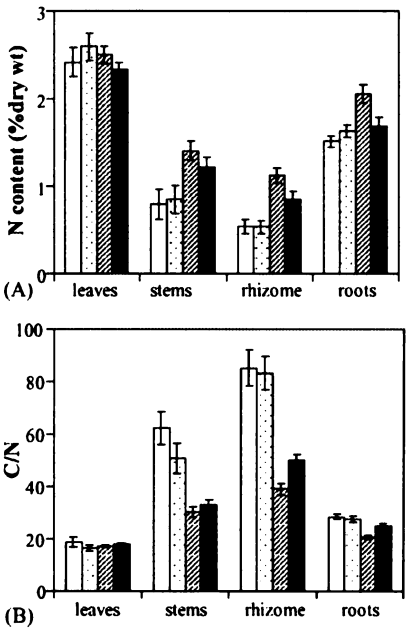


Fig. 1. Tissue relative N content (A) and C/N ratio (B) of *Phragmites australis* and *Glyceria maxima* grown with NH_4^+-N or NO_3^--N source. Values given are mean ± S.E. (n = 5–8). Results of *Phragmites* are illustrated with open (NH_4^+) and dotted (NO_3^-) columns, results of *Glyceria* with dashed (NH_4^+) and solid (NO_3^-) columns.

Table 3
Statistical evaluation of main characteristics of plant nitrogen utilisation using analysis of variance (ANOVA)

Variable	Source of variability		
	Species	Treatment	Interaction
Tissue N content (% dry wt)			
Leaves	0.546	0.974	0.250
Stems	**0.000**	0.476	0.190
Rhizome	**0.000**	**0.022**	**0.027**
Roots	**0.001**	0.146	**0.007**
Tissue C content (% dry wt)			
Leaves	0.423	**0.006**	0.259
Stems	**0.000**	0.234	0.056
Rhizome	**0.000**	0.907	0.717
Roots	**0.000**	**0.043**	**0.032**
C/N ratio			
Leaves	0.944	0.455	0.150
Stems	**0.000**	0.346	0.127
Rhizome	**0.000**	0.234	0.089
Roots	**0.000**	0.144	**0.025**
Nitrogen use efficiency (g dry wt g^{-1} N)			
Whole plant basis	**0.000**	0.317	0.081
Leaf basis	0.609	0.477	0.169
Tissue NH_4^+ content (μmol g^{-1} fresh wt)			
Leaves	**0.000**	0.746	0.167
Stems	0.182	0.945	0.912
Rhizome (young internodes)	**0.003**	0.343	0.704
Rhizome (old internodes)	**0.005**	0.186	**0.029**
Roots	0.621	**0.001**	0.765
Tissue NO_3^- content (μmol g^{-1} fresh wt)			
Leaves	0.223	**0.006**	**0.000**
Stems	0.826	**0.043**	**0.024**
Rhizome (young internodes)	**0.000**	**0.000**	**0.022**
Rhizome (old internodes)	0.111	0.915	0.715
Roots	**0.000**	0.916	**0.000**

Effects of plant species, N treatments and interactions (species × treatment) are expressed by *P*-values, Numbers in bold indicate *P*-values < 0.05.

and B and Table 3). In contrast, NH_4^+ treated *Glyceria* had higher nitrogen content and a lower C/N ratio compared to NO_3^- treated plants. Especially the below ground biomass of NH_4^+ treated plants had high N content compared to roots and rhizomes of NO_3^- treated plants (Table 3).

The nitrogen use efficiency (NUE) calculated on the whole plant basis differed between *Phragmites* and *Glyceria*, being higher in *Glyceria* compared to *Phragmites*. This parameter was not affected by the nitrogen source in *Glyceria*. In *Phragmites* plants receiving NH_4^+ showed considerably higher NUE than NO_3^- fed plants. NUE calculated on the leaf basis did not differ among species and treatments (Fig. 2 and Table 3).

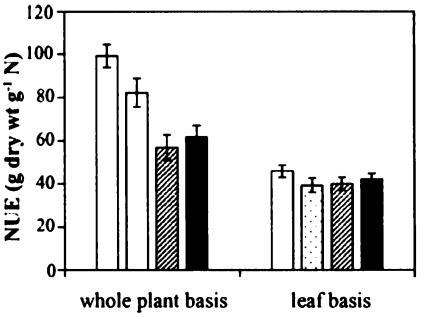


Fig. 2. Nitrogen use efficiency (NUE) of *P. australis* and *G. maxima* grown with NH_4^+-N or NO_3^--N source, calculated on the whole plant basis and leaf basis. Values given are mean ± S.E. (n = 5–8). Results of *Phragmites* are illustrated with open (NH_4^+) and dotted (NO_3^-) columns, results of *Glyceria* with dashed (NH_4^+) and solid (NO_3^-) columns.

3.4. Inorganic nitrogen ions in plant tissues

The tissue concentrations of NH_4^+ and NO_3^- ions generally differed between *Phragmites* and *Glyceria* and the interspecific differences were observed in the response to N treatments (Fig. 3A and B and Table 3).

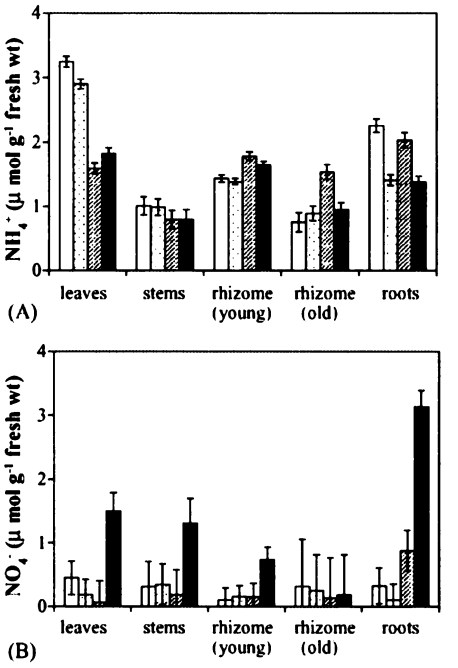


Fig. 3. Concentrations of NH_4^+ (A) and NO_3^- (B) in tissues *P. australis* and *G. maxima* grown with NH_4^+-N or NO_3^--N source. Values given are mean ± S.E. (n = 5–8). Results of *Phragmites* are illustrated with open (NH_4^+) and dotted (NO_3^-) columns, results of *Glyceria* with dashed (NH_4^+) and solid (NO_3^-) columns.

The NH_4^+ concentrations in plants were only slightly affected by the N source. The contents of NH_4^+ ions in NH_4^+ fed *Glyceria* plants were high in roots and rhizomes compared to NO_3^- treated plants, whereas the NH_4^+ concentrations in above-ground biomass (leaves and stems) were unaffected by the N treatments. In *Phragmites*, the NH_4^+ nutrition resulted into an increase in NH_4^+ contents in roots only. *Phragmites* leaves contained high NH_4^+ contents compared to *Glyceria* regardless to the N source. The effect of organ age was observed in rhizomes of both *Phragmites* and *Glyceria*. Both species contained low NH_4^+ levels in older rhizome parts compared to young growing buds (Fig. 3A and Table 3).

The NO_3^- contents in *Glyceria* tissues were considerably affected by the N source. NO_3^- nutrition caused significant increase of NO_3^- concentrations in all the plant parts of *Glyceria* and especially in roots. The tissue NO_3^- was high compared to NO_3^- fed plants of *Phragmites*, which implied the tendency of *Glyceria* to accumulate NO_3^- in non-reduced form. In *Phragmites*, there were no differences in NO_3^- contents between NH_4^+ and NO_3^- fed plants (Fig. 3B and Table 3).

3.5. Nitrogen uptake kinetics

NH_4^+ ions were taken up with a higher average maximum uptake rate (V_{max}) than NO_3^- in both species and treatments, indicating generally high uptake capacity for this ion in wetland plants. The NH_4^+ uptake rate did not differ between species and was not affected by the form of N supplied during the cultivation. In contrast, the uptake rate (V_{max}) of NO_3^- was low in NO_3^- deprived plants compared to V_{max} of NO_3^- acclimated plants, however, no differences were observed between species (Fig. 4A, Table 4).

Both NH_4^+ and NO_3^- acclimated *Phragmites* had low half-saturation constant (K_m) and high affinity compared to *Glyceria* plants (Fig. 4B and C, Table 4). The nitrogen regime of cultivation affected K_m and affinity in both species, but NO_3^- uptake was influenced less compared to the uptake of NH_4^+. Plants acclimated to NH_4^+ had considerably higher affinity for NH_4^+ ions than plants treated with NO_3^- (147 and 163% of NO_3^- acclimated plants in *Phragmites* and *Glyceria*, respectively). The affinity for NH_4^+ in these plants was also significantly higher than affinity for NO_3^-. In contrast, acclimation to NO_3^- increased the affinity for this particular N ion only slightly (Fig. 4C, Table 4). Similarly, the half-saturation constants (K_m) of NH_4^+ uptake decreased considerably in plants acclimated to NH_4^+-N source in both species. The half-saturation constants (K_m) of NO_3^- uptake were not affected by the N regime of cultivation in *Glyceria*, in *Phragmites* lower K_m of NO_3^- uptake was observed in NO_3^- starved plants (Fig. 4B, Table 4).

An induction phase for NO_3^- uptake in NH_4^+ acclimated plants was observed in *Phragmites* but not *Glyceria*, indicating different aspects of NO_3^- uptake regulation between species. The actual NO_3^- uptake rate of NH_4^+ acclimated *Phragmites* immediately after NO_3^- addition was low compared to the NO_3^- uptake rate of *Glyceria*. However, NO_3^- uptake rate of NH_4^+ acclimated *Phragmites* was gradually increasing during the first 80 min after the switch in N regime. This induction phase resulted in diminution of the uptake rate differences between species, although the uptake rate was still low compared to NO_3^- acclimated plants. The induction phase was insignificant in

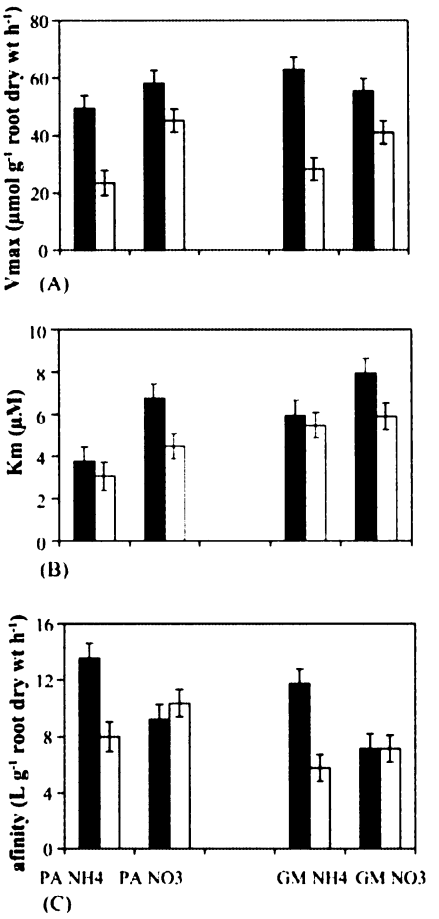


Fig. 4. Average maximum uptake rate per root dry wt (V_{max}) (A), half saturation constant (apparent K_m) (B) and affinity (C) of *P. australis* (PA) and *G. maxima* (GM) acclimated to NH_4^+-N (PA NH4, GM NH4) or NO_3^--N (PA NO3, GM NO3) source. Values given are mean ± S.E. (n = 4–6). Results of NH_4^+ uptake are illustrated with solid columns, results of NO_3^- uptake with open columns.

Table 4
Statistical evaluation of uptake kinetic parameters using analysis of variance (ANOVA)

Variable	Source of variability						
	Species	Treatment	Ion	Species × treatment	Species × ion	Treatment × ion	Species × treatment × ion
V_{max}	0.354	**0.005**	**0.000**	**0.042**	0.405	**0.009**	0.553
K_m	**0.000**	**0.001**	**0.005**	0.292	0.793	0.105	0.987
Affinity	**0.003**	0.099	**0.001**	0.740	0.675	**0.000**	0.885
Duration	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
Slope	**0.002**	**0.023**	**0.000**	0.094	**0.002**	**0.023**	0.094

Effects of plant species, N treatments, receiving N ion and interactions are expressed by P-values. Numbers in bold indicate P-values < 0.05.

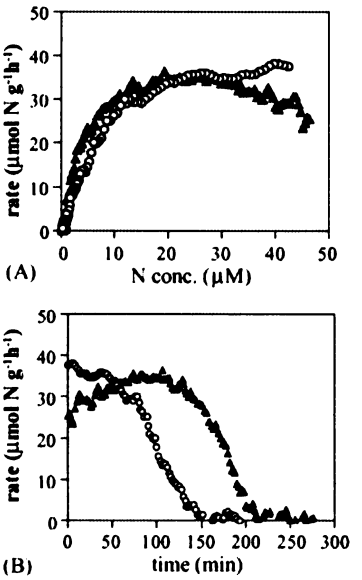


Fig. 5. Typical NO_3^- uptake curves of NO_3^- deprived *P. australis* and *G. maxima* plants, expressed as the dependence of uptake rate per root dry wt on ion concentration (A) and time (B). Results of *Phragmites* are illustrated with solid triangles, results of *Glyceria* with open circles.

Glyceria, both in duration and intensity (Figs. 5 and 6, Table 4). There was no induction phase for NH_4^+ uptake in either species.

4. Discussion

The study showed different response of *Phragmites* and *Glyceria* to N source. At the selected nitrogen concentration (34 μM), the growth of *Glyceria* was higher in NH_4^+-N treatment than in NO_3^--N (RGR was 116% of NO_3^- fed plants), whereas the growth of *Phragmites* was unaffected. The growth enhancement of *Glyceria* was reflected in an increased shoot growth, tillering and favoured production of aboveground organs, as similarly observed in NH_4^+ fed maize (Teyker and Hobbs, 1992), and may be related to higher tissue nitrogen content of NH_4^+ fed plants (Saarinen and Haansuu, 2000).

Both species had reduced root growth in the NH_4^+-N treatment. This phenomenon might be interpreted as the NH_4^+ toxicity syndrome (Vollbrecht and Kasemir, 1992; Teyker and Hobbs, 1992), if it is associated with other negative effects on the whole plant performance. However, no reduction of plant growth or visible symptoms of roots damage in NH_4^+ treated *Phragmites* and *Glyceria* were observed, although *Phragmites* RGR was slightly decreased. The root growth reduction is rather the result of a shift in the biomass distribution, which reflects the balance between plants demands and environmental conditions. The investment into organs that acquire the limiting resource is generally favoured (Chapin, 1980). Thus, the high tissue N content of NH_4^+ compared to NO_3^-

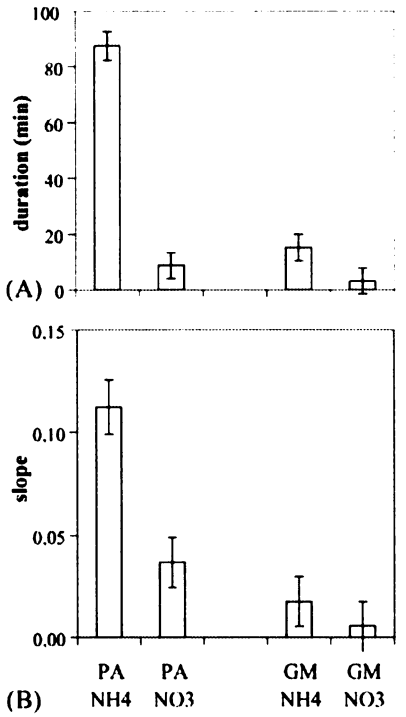


Fig. 6. The duration (A) and intensity (slope) (B) of induction phase of NO_3^- *P australis* (PA) and *G. maxima* (GM) acclimated to NH_4^+-N (PA NH4, GM NH4) or NO_3^--N (PA NO3, GM NO3) source. Values given are mean ± S.E. ($n = 4$–6).

treated plants observed in *Glyceria* might caused the down-regulation of the biomass investments into roots. In *Phragmites* other factors are involved, since no differences in total N contents between treatments were observed. The high proportion of dead biomass and senescent leaves in NH_4^+ fed *Phragmites* might imply N deficiency (Marschner, 1995), but this is not supported by the detected tissue N contents of this species. The leaf N of *Phragmites* (2.5% dry wt) corresponds to values reported for plants from very eutrophic habitats of south Bohemian fishpond littorals (Dykyjova, 1979). Also the foliar accumulation of NH_4^+, described as a factor associated with accelerated leaf senescence under NH_4^+ nutrition (in *O. sativa* (Chen et al., 1997)) and occurring in plants under stress (Barker, 1999; Mehrer and Mohr, 1989), was not confirmed. The tissue NH_4^+ levels did not exceed 3.5 and 2.0 μmol g^{-1} fresh wt in *Phragmites* and *Glyceria*, respectively. With roots as the only exception, the source of N did not affect NH_4^+ concentrations in plants. Although the discrepancy in methods for NH_4^+ detection (Husted et al., 2000) and species specific differences have to be considered, observed tissue NH_4^+ concentrations were below the levels usually described as necessary to induce symptoms of NH_4^+ toxicity (Barker, 1999; Vollbrecht and Kasemir, 1992).

The study of N uptake kinetics revealed the differences in uptake of NH_4^+ versus NO_3^- in both species as well as species-specific differences between *Phragmites* and *Glyceria*.

At the low rhizosphere N availabilities used in the present study the uptake of both inorganic ions is an active process provided by the saturable high-affinity transport system (HATS) ((Forde and Clarkson, 1999) and citations therein), which responses strongly to changes in external and plant internal N concentrations. Plants also significantly differ in HATS kinetics with correspondence to long-term nutritional status of their habitats. Species of low-fertility environments display generally lower absorption capacity (V_{max}), but a higher affinity and a lower half-saturation constant (Km) compared to fast-growing species of fertile habitats (Chapin, 1980). Moreover, the high-affinity uptake of NO_3^- is regulated differently than the uptake of NH_4^+. While the diminution of NO_3^- in the rhizosphere down-regulates the potential for its uptake, the absence of NH_4^+ causes an increase of its uptake capacity (for review see Forde and Clarkson, 1999).

The present study showed high uptake capacity (V_{max}) for NH_4^+ compared to NO_3^- in both species, even in fully induced NO_3^- fed plants. Also the affinity for NH_4^+ tended to be higher than those for NO_3^-, especially in plants acclimated to NH_4^+-N. These results indicate that wetland species may share characteristics of plats colonising habitats with restricted nitrification, where the preference for NH_4^+ over NO_3^- is described (Kronzucker et al., 1997).

The capacity of root transport systems (V_{max}) for both NH_4^+ and NO_3^- did not showed species-specific differences, even though *Glyceria* showed generally higher RGR and tissue N contents compared to *Phragmites*. On the contrary, plants differed in affinity and half saturation constant (K_m). Although previous NH_4^+ uptake studies (Romero et al., 1999) have indicated that *Phragmites* is adapted to fertile habitats, this study described features advantageous under nutrient limitation (low K_m and high affinity) in this species. This finding indicates a higher physiological plasticity for N uptake at low nutrient availabilities in *Phragmites* than in *Glyceria*, which agrees with field observations describing the ability of *Phragmites* to colonise oligotrophic systems, where *Glyceria* is not present (Brandle et al., 1996). Moreover, *Phragmites* had a high nitrogen use efficiency (NUE), compared to *Glyceria*, a feature favourable under nutrient limitation (Chapin, 1980). The lower A/B ratio (e.g., reduced investments to N rich tissues) participated at high NUE of *Phragmites*, but species also differed in the accumulation of NO_3^- in non-reduced form. *Glyceria* contained high concentrations of NO_3^- compared to *Phragmites* and this tendency was confirmed by additional experimental data, when plants were treated with NH_4^+ and NO_3^- at higher availability (Munzarova et al., in press). *Glyceria* was already shown to have generally high capacity for mineral ions accumulation compared to *Phragmites* (Dykyjova, 1979).

The species also differed in the NO_3^- uptake characteristics of NH_4^+ acclimated (NO_3^- deprived) plants. These results indicate differences in participation and regulation of inducible (iHATS) and constitutive (cHATS) transport.

In conclusion, the observed differences in the growth plasticity, N uptake and responses to N forms between *Phragmites* and *Glyceria* may help to explain the differences in species performance at natural habitats. *Glyceria* responded positively to NH_4^+-N treatment, although the species has a superficial root system and occupies slightly flooded littoral zones (Brandle et al., 1996; Buttery and Lambert, 1965), where NO_3^- availabilities are generally higher. No similar response was observed in *Phragmites*, which is the species rooting deeply (Buttery and Lambert, 1965) in NH_4^+ dominating layers of sediment and the

species with high tolerance to anoxia (Crawford and Brandle, 1996; Barclay and Crawford, 1982). This may favour *Glyceria* at NH_4^+-N rich sites, but the results must be taken with care and caution, since plant response to N source may considerably differ with respect to total N availability (Munzarova et al., in press). Moreover, other environmental factors negatively affecting plant performance at natural stands have to be considered. Litter accumulation, decomposition and the presence of toxic compounds in sediment have been described as some of the factors interfering with growth of *Phragmites* (van der Putten, 1997; Cizkova et al., 1996; Armstrong et al., 1996a,b) but comparable data are not available for *Glyceria*.

Acknowledgement

Authors would like to thank Hana Cizkova, Institute of Landscape Ecology of the Academy of Sciences of the Czech Republic, Trebon. The project was supported by the grant nos. 113100003 and 113100004 of the Ministry of Education, Youth and Sports of the Czech Republic.

References

Andersen, F.O., Hansen, J.I., 1982. Nitrogen cycling and microbial decomposition in sediments with *Phragmites australis* (*Poaceae*). Hydrobiol. B. 16, 11–19.

Armstrong, J., AfreenZobayed, F., Armstrong, W., 1996a. *Phragmites* die-back: sulphide- and acetic acid-induced bud and root death, lignifications, and blockages within aeration and vascular systems. New Phytol. 134, 601–614.

Armstrong, J., Armstrong, W., Wu, Z.B., AfreenZobayed, F., 1996b. A role for phytotoxins in the *Phragmites* die-back syndrome? Folia Geobot. Phytotax. 31, 127–142.

Barclay, A.M., Crawford, R.M.M., 1982. Plant growth and survival under strict anaerobiosis. J. Exp. Bot. 33, 541–549.

Barker, A.V., 1999. Foliar ammonium accumulation as an index of stress in plants. Commun. Soil Sci. Plant Anal. 30, 167–174.

Brandle, R., Pokorny, J., Kvet, J., Cizkova, H., 1996. Wetland plants as a subject of interdisciplinary research. Folia Geobot. Phytotax. 31, 1–6.

Brix, H., Lorenzen, B., Morris, J.T., Schierup, H.H., Sorrell, B.K., 1994. Effects of oxygen and nitrate on ammonium uptake kinetics and adenylate pools in *Phalaris arundinacea* L. and *Glyceria maxima* (Hartm.) Holmb. Proc. R. Soc. Edin. 102B, 333–342.

Buttery, B.R., Lambert, J.M., 1965. Competition between *Glyceria maxima* and *Phragmites communis* in the region of Surlingham broad. 1. The competition mechanism. J. Ecol. 53, 163–181.

Chapin, F.S., 1980. The mineral-nutrition of wild plants. Annu. Rev. Ecol. Syst. 11, 233–260.

Chen, S.J., Hung, K.T., Kao, C.H., 1997. Ammonium accumulation is associated with senescence of rice leaves. Plant Growth Regul. 21, 195–201.

Cizkova, H., Pechar, L., Husak, S., Kvet, J., Bauer, V., Radochova, J., Edwards, K., 2001. Chemical characteristics of soils and pore waters of three wetland sites dominated by *Phragmites australis*: relation to vegetation composition and reed performance. Aquat. Bot. 69, 235–249.

Cizkova, H., Strand, J.A., Lukavska, J., 1996. Factors associated with reed decline in a eutrophic fishpond, Rozmberk (South Bohemia, Czech Republic). Folia Geobot. Phytotax. 31, 73–84.

Crawford, R.M.M., Brandle, R., 1996. Oxygen deprivation stress in a changing environment. J. Exp. Bot. 47, 145–159.

Dykyjova, D., 1979. Selective uptake of mineral ions and their concentration factors in aquatic higher plants. Folia Geobot. Phytotax. 14, 267–325.

Forde, B.G., Clarkson, D.T., 1999. Nitrate and ammonium nutrition of plants: physiological and molecular perspectives. Adv. Bot. Res. 30, 1–90.

Garnett, T.P., Shabala, S.N., Smethurst, P.J., Newman, I.A., 2001. Simultaneous measurement of ammonium, nitrate and proton fluxes along the length of eucalypt roots. Plant Soil 236, 55–62.

Hejny, S., Husak, S., 1978. Higher plant communities. In: Dykyjova, D., Kvet, J. (Eds.), Ponds Littoral Ecosystems. Springer-Verlag, Berlin, pp. 23–64.

Hroudova, Z., Zakravsky, P., 1999. Vegetation dynamics in a fishpond littoral related to human impact. Hydrobiologia 415, 139–145.

Huber, E., Frost, M., 1993. Absorption measurements in the UV range: continuous recording of nitrate in water. Process. Contr. Qual. 4, 279–289.

Husted, S., Hebbern, C.A., Mattsson, M., Schjoerring, J.K., 2000. A critical experimental evaluation of methods for determination of NH_4^+ in plant tissue, xylem sap and apoplastic fluid. Physiol. Plant. 109, 167–179.

Kronzucker, H.J., Siddiqi, M.Y., Glass, A.D.M., 1997. Conifer root discrimination against soil nitrate and the ecology of forest succession. Nature 385, 59–61.

Kronzucker, H.J., Siddiqi, M.Y., Glass, A.D.M., Kirk, G.J.D., 1999. Nitrate-ammonium synergism in rice. A subcellular flux analysis. Plant Physiol. 119, 1041–1046.

Kuhl, H., Woitke, P., Kohl, J.G., 1997. Strategies of nitrogen cycling of *Phragmites australis* at two sites differing in nutrient availability. Int. Rev. Ges. Hydrobiol. 82, 57–66.

Lorenzen, B., Brix, H., Mendelssohn, I.A., McKee, K.L., Miao, S.L., 2001. Growth, biomass allocation and nutrient use efficiency in *Cladium jamaicense* and *Typha domingensis* as affected by phosphorus and oxygen availability. Aquat. Bot. 1482, 1–17.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. Academic Press, London.

Mehrer, I., Mohr, H., 1989. Ammonium toxicity—description of the syndrome in *Synapis alba* and the search for its causation. Physiol. Plant. 77, 545–554.

Muller, E., Alberts, B.P., Janiesch, P., 1994. Influence of NO_3^- and NH_4^+ nutrition on fermentation, nitrate reductase activity and adenylate energy charge of roots of *Carex pseudocyperus* L. and *Carex sylvatica* Huds. exposed to anaerobic nutrient solution. Plant Soil 166, 221–230.

Munzarova, E., Lorenzen, B., Brix, H., Vojtiskova, L., Votrubova, O. in press. Effect of NH_4^+/NO_3^- availability on nitrate reductase activity and nitrogen accumulation in wetland helophytes *Phragmites australis* and *Glyceria maxima*. Environ. Exp. Bot.

Nijburg, J.W., Laanbroek, H.J., 1997. The fate of N-15-nitrate in healthy and declining *Phragmites australis* stands. Microbial Ecol. 34, 254–262.

Picek, T., Lusby, F., Cizkova, H., Suckova, H., Simek, H., Kvet, J., Pechar, L., 2000. Microbial activities in soils of a healthy and a declining reed stand. Hydrobiologia 418, 45–55.

Romero, J.A., Brix, H., Comin, F.A., 1999. Interactive effects of N and P on growth, nutrient allocation and NH_4^+ uptake kinetics by *Phragmites australis*. Aquat. Bot. 64, 369–380.

Saarinen, T., Haansuu, P., 2000. Shoot density of *Carex rostrata* stokes in relation to internal carbon: nitrogen balance. Oecologia 122, 29–35.

Sasakawa, H., Yamamoto, Y., 1978. Comparison of the uptake of nitrate and ammonium by rice seedlings. Plant Physiol. 62, 665–669.

Teyker, R.H., Hobbs, D.C., 1992. Growth and root morphology of corn as influenced by nitrogen form. Agron. J. 84, 694–700.

van der Putten, W.H., 1997. Die-back of *Phragmites australis* in European wetlands: an overview of the European Research Programme on reed die-back and progression (1993–1994). Aquat. Bot. 59, 263–275.

Vollbrecht, P., Kasemir, H.I., 1992. Effects of exogenously supplied ammonium on root development of scots pine (*Pinus sylvestris* L.) seedlings. Bot. Acta 105, 306–312.

Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Environmental and Experimental Botany 55 (2006) 49–60
Environmental and Experimental Botany
www.elsevier.com/locate/envexpbot

Effect of NH_4^+/NO_3^- availability on nitrate reductase activity and nitrogen accumulation in wetland helophytes *Phragmites australis* and *Glyceria maxima*

Edita Munzarova[a,*], Bent Lorenzen[b], Hans Brix[b], Lenka Vojtiskova[a], Olga Votrubova[a]

[a] *Department of Plant Physiology, Faculty of Sciences, Charles University in Prague, Vinicna 5, 128 44 Prague 2, Czech Republic*
[b] *Department of Plant Ecology, University of Aarhus, Nordlandsvej 68, DK-8240 Risskov, Denmark*

Accepted 28 September 2004

Abstract

The effect of NH_4^+/NO_3^- availability on nitrate reductase (NR) activity in *Phragmites australis* and *Glyceria maxima* was studied in sand and water cultures with the goal to characterise the relationship between NR activity and NO_3^- availability in the rhizosphere and to describe the extent to which NH_4^+ suppresses the utilization of NO_3^- in wetland plants.

The NR activity data showed that both wetland helophytes are able to utilize NO_3^-. This finding was further supported by sufficient growth observed under the strict NO_3^- nutrition. The distribution of NR activity within plant tissues differed between species. *Phragmites* was proved to be preferential leaf NO_3^- reducer with high NR activity in leaves (NR_{max} > 6.5 μmol NO_2^- g dry wt^{-1} h^{-1}) under all N treatments, and therefore *Phragmites* seems to be good indicator of NO_3^- availability in flooded sediment. In the case of *Glyceria* the contribution of roots to plant NO_3^- reduction was higher, especially in sand culture. *Glyceria* also tended to accumulate NO_3^- in non-reduced form, showing generally lower leaf NR activity levels. Thus, the NR activity does not necessarily correspond with plant ability to take up NO_3^- and grow under NO_3^--N source. Moreover, the species differed significantly in the content of compounds interfering with NR activity estimation. *Glyceria*, but not *Phragmites*, contained cyanogenic glycosides releasing cyanide, the potent NR inhibitor. It clearly shows that the use of NR activity as a marker of NO_3^- utilization in individual plant species is impossible without the precise method optimisation.

Keywords: Phragmites; Glyceria; Wetland plants; Nitrate nutrition; NR activity; Nitrate reductase; Cyanogenic glycosides

1. Introduction

Plant preferences of N sources are one of the nutritional aspects affecting plant community establishment at sites where the NH_4^+/NO_3^- substrate ratio changes during succession or due to anthropogenic impact (Kronzucker et al., 1997). Among NH_4^+ dominating

* Corresponding author. Tel.: +420 22195 1697; fax: +420 22195 1704.
E-mail address: edmunz@natur.cuni.cz (E. Munzarova).

0098-8472/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.envexpbot.2004.09.011

habitats, wetlands are unique ecosystems colonised by species capable of rhizosphere oxidising (Armstrong and Armstrong, 1988), which maintain nitrifying activity in the rhizosphere (Both et al., 1992; Engelaar et al., 1995). Plant mediated denitrification is also responsible for the improvement of the N removal efficiency in artificial wetlands (Cottingham et al., 1999). However, the importance of NO_3^- in the mineral nutrition of wetland plants or the possible preference for NH_4^+ over NO_3^-, is not exactly known. Engelaar et al. (1995) described utilization of NO_3^- in *Rumex palustris* by detecting a high level of leaf nitrate reductase (NR) activity. The general importance of NO_3^- in wetland plants was, however, questioned by Cedergreen and Madsen (2003) because ambient NR activities were low in different submerged macrophytes although NO_3^- was available to plants. It is hypothesed that NH_4^+ suppresses NO_3^- uptake and assimilation due to a preferential uptake of NH_4^+. The preferential uptake of NH_4^+ over NO_3^- was observed in some wetland and marine species (Sasakawa and Yamamoto, 1978; Ingemarsson et al., 1984; Thursby and Harlin, 1984) as well as in plants colonising terrestrial habitats with the prevailing NH_4^+-N form (Kronzucker et al., 1997; Min et al., 2000; Garnett et al., 2001). The inhibition of NO_3^- uptake by NH_4^+ in plants growing typically in NO_3^- dominated soils (e.g. barley) was observed in some studies (Kronzucker et al., 1999a), but not in others (Samuelson et al., 1995). Because the importance of NO_3^- as a source of nitrogen in wetland plants is not clear, the present study is focused on its characterisation in two model species—common reed (*Phragmites australis* (Cav.) Trin. ex Steudel) and reed sweetgrass (*Glyceria maxima* (Hartm.) Holmb.). The NR activity was used as a marker because it corresponds not only to NO_3^- availability in the rhizosphere, but it also characterises the extent to which plants utilise NO_3^- as a source of nitrogen (Stewart et al., 1993).

P. australis and *G. maxima* are fast growing graminaceous species and their terrestrial relatives are typically shoot NO_3^- reducers at $NO_3^- > 0.5$ mM. If the NO_3^- availability is lower, the root importance increases (Andrews et al., 1992). However, this general pattern of NR activity distribution may not occur in wetland plants with extensive rhizome and root systems growing in reduced sediments. Although shoot NR activity is energetically advantageous due to the direct coupling with photosynthesis, high NR activity of roots compared to shoots was observed in different submerged wetland species (Cedergreen and Madsen, 2003) or plants colonising acid soils with prevailing NH_4^+-N source (Claussen and Lenz, 1999). In spite of the fact that root NO_3^- reduction may depend on anaerobic metabolism of carbohydrates, oxygen shortage induced root NR activity (Garcia-Novo and Crawford, 1973; Muller et al., 1994; de la Haba et al., 2001) or increased the relative importance of root NR activity to whole plant NR activity (Jiang and Hull, 1999). Moreover, NO_3^- nutrition had a positive effect on metabolic responses of root tissues to anaerobiosis, e.g., improved adenylate energy charge or lower ethanol and lactate production in *G. maxima, Phalaris arundinacea* and *Carex pseudocyperus* (Brix et al., 1994; Muller et al. 1994). It is supposed that the positive effect is related to the outflow of reduced cofactors which attenuates the adverse effect of hypoxia via lowering cell fermentation rates (Muller et al., 1994). Because no effect of NR activity on cell fermentations has been observed (e.g. Botrel and Kaiser, 1997) the mechanism of this process is still being discussed. Thus, it is still not clear whether wetland emergent helophytes, species colonising NH_4^+ dominated habitats with generally low oxygen availability, prefer roots as a site of NO_3^- reduction or favour energetically advantageous leaves. Erdei et al. (2001) detected leaf NR activity in *P. australis* during period of vegetative growth, but supplied no information on NR activity in roots and sediment characteristics. No data on *Glyceria* were published.

The major goals of the present study were the following: to characterise the relationship between NR activity and NO_3^- external availability in *P. australis* and *G. maxima*, to describe possible inhibitory effects of NH_4^+ and to compare these results with plant growth and N accumulation in plant biomass. Because it is not clear if wetland helophytes mimic the NR activity distribution pattern typical for their terrestrial relatives, the study also follows the distribution of NR activity among plant organs.

2. Materials and methods

2.1. Plant material

The parent plants used in the experiments came originally from natural littoral stands in the Trebon

basin, Czech Republic, and were propagated vegetatively in outdoor sand culture in the Institute of Botany, Academy of Sciences, for a long time. Rootless rhizome cuttings (10 cm) with one shoot (15–20 cm) were cultivated three weeks in coarse sand prior to their transfer into the experimental cultures.

2.2. Experimental set-up and growth conditions

Plants were cultivated in water and sand cultures. The short-term water culture experiment carried out in controlled growth cabinet with rhizostats was designed to study NR activity under the defined nutrient and climatic regimes. The long-term outdoor sand culture simulated the conditions of natural habitats.

The water culture experiment was run for two months. *Phragmites* and *Glyceria* were cultivated in a controlled growth cabinet (Umwelttechnic, Lindenstruth, Germany) with two hydroponic units. The growth cabinet was operated with a 16:8 day: night photoperiod, 22:15 °C thermo period and an 85:90% relative humidity day: night period. The irradiance was 400 μmol m^{-2} s^{-1}. Each hydroponic growth unit was comprised of two 30 L growth tanks with 10 plants of each species (*Phragmites* and *Glyceria* together), and a nutrient solution reservoir (140 L) through which the nutrient solution was recirculated at a rate 3 L min^{-1} (Lorenzen et al., 2001). The chemical composition of a basic nutrient solution was the following (μM): PO_4^{3-} 16.35; K^+ 86.96; Ca^{2+} 3186.27; Mg^{2+} 1686.55; Na^+ 2174.86; SO_4^{2-} 3060.59; Cl^- 6093.09; SiO_3^{2-} 12.50; BO_3^{3-} 2.50; Fe^{2+} 2.01; Mn^{2+} 0.20; Zn^{2+} 0.20; Cu^{2-} 0.20 (Lorenzen et al., 2001). The pH was adjusted to pH 6.5 and the conductivity was maintained below 1.5 mS cm^{-1}. The treatments differ only in the source of nitrogen. One hydroponic unit received 179 μM NO_3^--N (KNO_3) (NO_3^- treatment), and the other unit received 179 μM NH_4^+-N ($(NH4)_2SO_4$) (NH_4^+ treatment). The pH of the treatment solution and NO_3^-, NH_4^+ and PO_4^{3-} concentrations were monitored daily and, together with K^+ and Fe^{2+}, adjusted to the levels desired. As the microbial activity in aerated solution may convert NH_4^+ to NO_3^-, the NH_4^+ treatment solutions were renewed every time the NO_3^- concentrations increased above 7 μM NO_3^--N. The actual NH_4^+ and NO_3^- levels in the culture solutions during the experimental periods are shown in Fig. 1A and B.

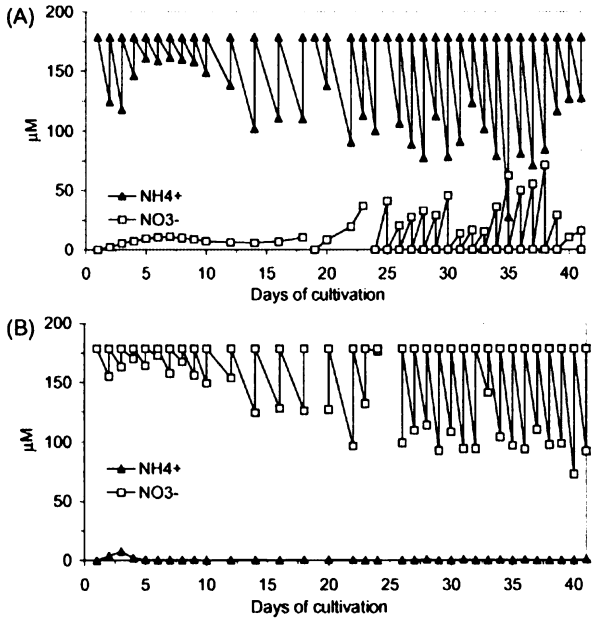


Fig. 1. Concentrations of NH_4^+ and NO_3^- ions in cultivation media of (A) NH_4^+ and (B) NO_3^- treatment of the water culture during the cultivation.

Sand culture was carried out for four months (May–August). Plants were cultivated outdoors and protected against the rainfall with a transparent plastic roof. Each cutting was planted in a 15 L plastic pot filled with coarse sand. Eight pots per treatment were placed in a plastic container with 130 L of the nutrient solution. The solution was changed every 3 weeks; the pH was adjusted to 6.8 using HCl. The water level was kept at the surface of sand.

The basic nutrient solution had the following chemical composition (μM): PO_4^{3-} 99.92; K^+ 104.92; Mg^{2+} 390.67; SO_4^{2-} 390.67; BO_3^{3-} 0.23; Fe^{2+} 20.42; Mn^{2+} 0.71; Zn^{2+} 0.01; Cu^{2-} 0.003; $Mo_7O_{24}^{2-}$ 0.001. Three N treatments were defined to simulate the trophic conditions of south Bohemia fishpond littorals with low (oligotrophic treatment) and high (eutrophic treatment) fertility and the trophic conditions of constructed wetlands (hypertrophic treatment). The treatments differed in chemical composition, total N level and NH_4^+: NO_3^- ratio. Along with the basic nutrients, the oligotrophic treatment contained also the following chemicals (μM): NH_4^+ 5.23; NO_3^- 20.96 (NH_4^+: NO_3^- ratio 1:4); Ca^{2+} 1102.87 and Cl^- 2190.03, in addition to basic chemical solution. Eutrophic treatment received

(μM): NH_4^+ 1317.85; NO_3^- 1317.92 (NH_4^+: NO_3^- ratio 1:1); Ca^{2+} 1099.19 and Cl^- 2198.32, and hypertrophic treatment contained (μM): NH_4^+ 7631.48; NO_3^- 1908.35 (NH_4^+: NO_3^- ratio 4:1); Ca^{2+} 1097.28 and Cl^- 323.59.

2.3. Plant growth, biomass sampling, C/N and NO_3^- analyses

The experimental plants were always harvested during light periods and plants were weighted to determine the relative growth rate (RGR = (ln final weight – ln initial weight)/days) Small subsamples of roots, rhizomes, leaf blades and stem bases were randomly collected from each plant. The rhizomes of the long-term sand culture were further divided into young parts (segments of young growing buds) and older parts (segments of rhizome branches below the original shoot of the cuttings; approximately 3.5 month old). All samples were washed with distilled water and stored in liquid nitrogen for NR activity measurements.

The remaining plant biomass was fractionated into leaves, stems, rhizomes and roots, washed with distilled water, weighted, frozen in liquid nitrogen and freeze dried. The dry weight (dry weight) was estimated, and the material was ground and analysed for total C, N, and NO_3^- contents. The total carbon and nitrogen contents were analysed using a CN analyser (Na2000, Carlo Erba, Italy) and C/N atomic ratio was calculated. NO_3^- was determined by flow injection analyser (Lachat, Quick Chem Instruments) using the cadmium reduction method (Methods for Chemical Analysis of Water and Wastes, Method 353.2, Storet No. Total 00630, U.S. Environmental Protection Agency).

2.4. NR activity estimation

NR activity was detected using an in vitro method modified according to Scheible et al. (1997). Plant material was ground to a fine powder in a mortar precooled with the liquid nitrogen and mixed with ice-cold extraction buffer. To reduce the loss of enzyme activity during extract preparation, the composition of the extraction buffer was modified for different plant material. The loss of activity was tested by addition of the commercial NR enzyme (NR from corn seedlings, Sigma) into the extracts and calculation the recovery of its activity. The recovery was calculated from the enzyme activity measured in assay mixtures containing commercial enzyme only, plant extract only and both commercial enzyme and plant extract together. With the exception of *Glyceria* leaves (recovery less than 20%), the recovery varied between 60–80% in extracts of all studied tissues of both species.

To improve enzyme stability in *Glyceria* leaf extracts, the following modifications were tested: addition of 3% (w/v) casein, 25 μM leupeptine, 0.5 μM phenylmethylsulphonyl fluoride (PMSF), 5 mM *o*-phenanthrolin, 1 μM pepstatine into the extraction buffer, but no positive effect was recorded. A significant improvement was achieved by addition of 5 mM $NiCl_2$ (agents preventing negative effect of cyanogenic glycosides on NR activity), which increased the enzyme recovery in *Glyceria* leaf extract up to 63% ($P < 0.05$). A positive effect of Ni^{2+} was observed in other tissues of *Glyceria*, but the effect was lower (the recovery was increased by 4% in stems ($P > 0.05$), 8% in rhizomes ($P < 0.05$) and 27% in roots ($P < 0.05$). In *Phragmites* no positive effect of Ni^{2+} application was detected ($P > 0.05$).

Based on the results above, the extraction buffer used for the analyses of *Phragmites* tissues extracts contained 100 mM HEPES-KOH (pH 7.5), 5 mM Mg acetate, 1 mM EDTA, 10% (v/v) glycerol, 0.1% Triton X-100, 5 mM DTT, 1% (w/v) BSA and 1% (w/v) PVPP, 20 μM FAD and 5 μM Na_2MoO_4, 3% (w/v) casein, 25 μM leupeptine, 0.5 μM phenylmethylsulphonyl fluoride and 5 mM *o*-phenanthrolin. The extraction buffer used for *Glyceria* tissues was further modified by adding of 5 mM $NiCl_2$.

NR activity was determined as the rate of NO_2^- accumulation. Fifty microliter of the plant extract was mixed with 250 μl of the assay buffer, prewarmed to 25 °C and incubated for 2, 5, 10 and 30 min at 25 °C. The linearity of the assay was checked and the optimal duration of the incubation time was set for each species and plant fraction. The assay buffer contained 100 mM HEPES-KOH (pH 7.5), 5 mM KNO_3, 0.25 mM NADH and 5 mM EDTA (for the determination of maximal NR activity (NR_{max})) or 10 mM Mg acetate (for the determination of actual NR activity (NR_{act})) (Aguera et al., 1999). The reaction was stopped with 25 μl of 0.6 mM Zn acetate and 75 μl 0.15 mM phenazine methosulphate and samples were kept 15 min at the room temperature prior to the further processing. NR activity was

estimated as the rate of NO_2^- accumulation, and NO_2^- was determined colorimetrically after adding 300 μl of 1% (w/v) sulphanilamide in 3 M HCl and 300 μl of 0.02% (w/v) *N*-(1-naphthyl)-ethylenediamine in distilled H_2O. The colour developed during 20 min at room temperature and the absorbance was measured at 540 nm (Spectrophotometr UNICAM Helios α 9423 UVA) after centrifugation at 14 × 000 *g* (Heraeus Sepatech Biofuge 13). Enzyme assay mixtures containing Zn acetate prior to the plant extracts were used as the time zero controls.

The detected NR_{max} ($-Mg^{2+}$) and NR_{act} ($+Mg^{2+}$) were expressed on the units of dry weight. NR_{max} data were further used for the calculations of the whole plant NO_3^- reduction capacity and the relative contribution (%) of individual plant fraction to the total plant reduction capacity. This approximation was done by multiplying NR_{max} by the dry wt of the given plant fraction. The whole plant NO_3^- reduction capacity was calculated as the sum of NR activities of the different plant fractions divided by the whole plant dry wt.

2.5. Statistical evaluation

Statistical evaluation was performed using NCSS 2000 and PASS 2000 software. The method optimisation data were analysed with Kruskal-Wallis one-way ANOVA test, and the multiple comparisons were calculated using Kruskal-Wallis Z test. NR activity data were analysed and means and standard errors (S.E.) were calculated with analysis of variance (ANOVA). The normality distribution of residuals was tested using the Multiple Regression analysis of NCSS software and data were transformed when necessary.

3. Results

3.1. Plant growth

The relative growth rate (RGR) of plants in the water culture was higher in *Glyceria* than in *Phragmites* ($P<0.05$). In *Phragmites*, NH_4^+ and NO_3^- treated plants had similar RGR 24.87 ± 8.63 and 28.90 ± 8.63 mg g^{-1} d^{-1} ($P>0.05$), respectively. In *Glyceria*, RGR of NO_3^- grown plants was 53.46 ± 7.15 mg g^{-1} d^{-1}, which was significantly higher than the RGR of NH_4^+ treated plants (44.78 ± 5.73 mg g^{-1} d^{-1}) ($P<0.05$).

In contrast to the water culture experiment, no differences in RGR between *Phragmites* and *Glyceria* were observed in the sand culture ($P>0.05$). Moreover, both species responded similarly to N treatments. RGR was considerably lower in plants cultivated in oligotrophic treatment (mean ± S.E., $n=8$): 23.67 ± 1.18 and 28.51 ± 1.18 mg g^{-1} d^{-1} in *Phragmites* and *Glyceria*, respectively, than in plants from the eutrophic (40.37 ± 1.18 and 39.59 ± 1.18 mg g^{-1} d^{-1}) and hypertrophic (40.00 ± 1.18 and 39.21 ± 1.26 mg g^{-1} d^{-1}) treatments ($P<0.001$).

3.2. Tissue N and NO_3^- concentrations

Plants in the water culture had a high concentration of N in the tissues, comparable only to plants in the hypertrophic treatment of the sand culture (compare Tables 1 and 2). N concentration measured in different tissues differed between species ($P<0.05$) and between organs ($P<0.001$). The tissue N concentrations were affected by N treatments but, interestingly, N treatment affected the species differently (sp. x tr. interactions: $P<0.05$). In *Phragmites*, NH_4^+ treated plants had high tissues N concentration while in *Glyceria* the highest N tissue concentration was found in NO_3^- treated plants (Table 1). In the NO_3^- treatment, *Glyceria* had three (roots, rhizome, stems) and six (leaves) fold higher tissue NO_3^- concentrations than *Phragmites* (Table 1).

Tissue N concentrations of sand culture plants of both species increased with N availability (from oligotrophic to hypertrophic treatment) ($P<0.001$), but the response was species specific (sp. x tr. interaction: $P<0.001$). *Phragmites* accumulated more N in the oligotrophic treatment and *Glyceria* accumulated more N in the eutrophic and hypertrophic treatments (Table 2). In both species the difference in tissue N concentration between eutrophic and hypertrophic treatments did not correlate with the increase of RGR or NO_3^- tissue concentration. Plants cultivated in the eutrophic treatment contained higher amounts of NO_3^- in their tissues compared with hypertrophic treatment, although it was still far less than in the NO_3^- fed plants from the water culture (Table 2). Thus, the tissue NO_3^- contents mimicked the NO_3^-/NH_4^+ ratio applied in the cultivation media.

Table 1
Total tissue N and N-NO_3^- contents of *Phragmites* and *Glyceria* grown with NH_4^+-N or NO_3^--N source in the water culture

	Phragmites australis		*Glyceria maxima*		Source of variability		
	NH_4^+	NO_3^-	NH_4^+	NO_3^-	sp.	tr.	sp. x tr.
Tissue total N content (% dry wt)							
L	4.85 ± 0.09	3.90 ± 0.10	3.59 ± 0.09	3.77 ± 0.09	0.000	0.001	0.000
St	3.12 ± 0.18	2.59 ± 0.18	2.64 ± 0.18	3.01 ± 0.18	0.888	0.645	0.028
Rh	1.53 ± 0.12	1.50 ± 0.11	2.43 ± 0.11	2.46 ± 0.12	0.000	0.979	0.795
R	2.19 ± 0.15	2.39 ± 0.15	2.32 ± 0.15	3.24 ± 0.17	0.008	0.004	0.035
Tissue N-NO_3^- content (mg g dry wt^{-1})							
L	0.01 ± 0.44	0.70 ± 0.44	0.25 ± 0.44	4.75 ± 0.44	0.000	0.000	0.000
St	0.10 ± 0.62	3.30 ± 0.62	0.39 ± 0.62	9.64 ± 0.44	0.000	0.000	0.000
Rh	0.03 ± 0.47	1.14 ± 0.47	0.16 ± 0.47	3.71 ± 0.47	0.001	0.000	0.029
R	0.09 ± 0.39	2.64 ± 0.31	0.38 ± 0.31	8.59 ± 0.22	0.000	0.000	0.000

The statistical evaluation using analysis of variance (ANOVA). L, leaves; St, stems; Rh, rhizomes; R, roots. *Notes*: values given are mean ± S.E., $n = 4$. Effects of plant species (sp.), N treatments (tr.) and interactions (species x treatment) are expressed by *P*-values. Data were square root transformed for the statistical analysis.

3.3. Nitrate reductase activity

In the water culture experiment, nitrate reductase activity was detected in all tissues of *Phragmites* and *Glyceria* in both N treatments. Leaves and roots had higher NR activity than stems and rhizomes ($P < 0.001$) (Fig. 2). Although the NR_{max} indicated NR in the different tissue types, activated NR activity (NR_{act}) was observed only in leaves of *Phragmites* and roots of *Glyceria*. The other tissue types had the NR_{act} lower than 0.5 μmol NO_2^- g dry wt^{-1} h^{-1} (Fig. 2A and B). Both NR_{max} and NR_{act} levels were affected by NO_3^- availability ($P < 0.001$). The NR_{max} and NR_{act} of leaves, roots and stems were higher in NO_3^- treated plants than in NH_4^+ treated ones (Table 3). Although the positive effect of NO_3^- in the culture solutions was more pronounced in *Phragmites*, the differences between species were not significant (sp. x tr. interactions: $P > 0.05$).

The whole plant capacity for NO_3^- reduction did not differ between species ($P = 0.089$). *Glyceria* allocated more biomass to leaves (47.8%) compared to *Phragmites* (34.7%), which compensated for a lower leaf NR activity level found in *Glyc-*

Table 2
Tissue total N and N-NO_3^- contents of *Phragmites* and *Glyceria* grown in oligotrophic (OL), eutrophic (EU) and hypertrophic (HYP) treatments in the sand culture

	Phragmites australis			*Glyceria maxima*			Source of variability		
	OL	EU	HYP	OL	EU	HYP	sp.	tr.	sp. x tr.
Tissue total N content (% dry wt)									
L	2.57 ± 0.05	2.97 ± 0.05	3.61 ± 0.05	1.35 ± 0.05	2.46 ± 0.05	3.81±0.05	0.000	0.000	0.000
St	–	–	–	–	–	–	–	–	–
Rh	0.43 ± 0.04	1.05 ± 0.04	1.21 ± 0.04	0.40 ± 0.04	1.09 ± 0.04	1.87 ± 0.04	0.000	0.000	0.000
R	1.13 ± 0.08	1.77 ± 0.08	2.18 ± 0.08	0.60 ± 0.08	1.77 ± 0.08	2.82 ± 0.08	0.590	0.000	0.000
Tissue N-NO_3^- content (mg g dry wt^{-1})									
L	0.05 ± 0.04	0.19 ± 0.04	0.09 ± 0.04	0.02 ± 0.04	0.32 ± 0.04	0.30 ± 0.04	0.014	0.001	0.051
St	–	–	–	–	–	–	–	–	–
Rh	0.01 ± 0.06	0.04 ± 0.06	0.05 ± 0.06	0.02 ± 0.06	0.57 ± 0.06	0.51 ± 0.06	0.000	0.001	0.003
R	0.09 ± 0.08	0.81 ± 0.08	0.48 ± 0.08	0.12 ± 0.08	1.38 ± 0.08	0.87 ± 0.08	0.000	0.000	0.022

The statistical evaluation using analysis of variance (ANOVA). L, leaves; St, stems; Rh, rhizomes; R, roots. *Notes*: values given are mean ± S.E., $n = 3$. Effects of plant species (sp.), N treatments (tr.) and interactions (species x treatment) are expressed by *P*-values.

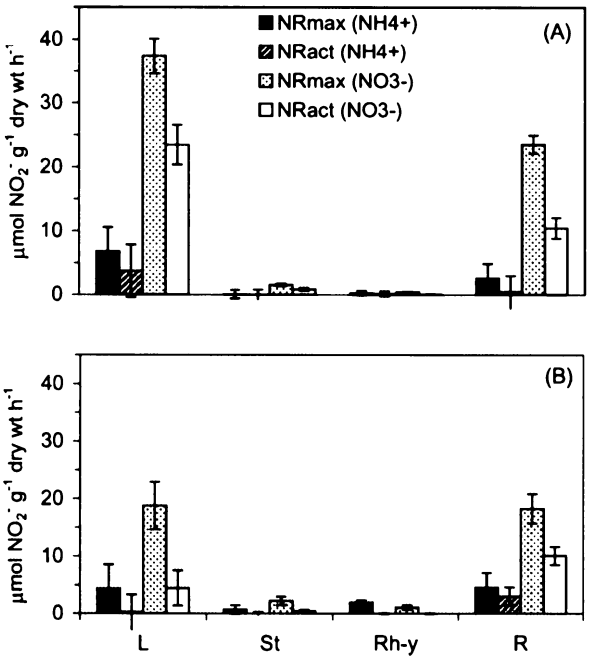


Fig. 2. NR activity in tissues of (A) *Phragmites australis* and (B) *Glyceria maxima* grown with NH_4^+-N or NO_3^--N source (178 μM) in the water culture. Values given are means ± S.E. (n = 4–5). L, leaves; St, stems; Rh-y, young rhizomes; R, roots.

eria. The NR capacity was strongly affected by N treatment ($P < 0.001$). NR_{max} in NH_4^+ treated *Phragmites* and *Glyceria* was 2.91 ± 1.66 and 3.26 ± 1.85 (mean ± S.E., n = 4–5) and in NO_3^- treated plants raised to 18.69±1.65 (*Phragmites*) and 11.92±1.85 (*Glyceria*) μmol NO_2^- g dry wt^{-1} h^{-1}. Thus, the NO_3^- treatment increased whole plant NR_{max} 6.42 times in *Phragmites* and 3.66 times in *Glyceria*. However, the difference in response to N treatments between species was not statistically significant (sp. x tr. interactions: $P = 0.062$).

The distribution of whole plant NR_{max} did not differ between species and was not affected by N treatments (see tr. and sp. x tr. interactions in Table 4). The majority of the NO_3^- reduction capacity of both species (70–80% of the whole plant NR_{max}) was localized in leaves ($P < 0.001$). Roots contributed to the total NR_{max} only with 10–20% (Fig. 3A and B).

In the sand culture experiment nitrate reductase activity was generally low compared with NR activity of plants in the water culture experiment ($P < 0.001$). Interestingly, the NR activity was detected in *Phragmites* in all N treatments, but was negligible in the oligotrophically treated plants of *Glyceria*. Even in the oligotrophic treatment, leaf tissues of *Phragmites* had the highest NR_{max} and NR_{act} levels (NR_{max} > 7 μmol NO_2^- g dry wt^{-1} h^{-1} and NR_{act} > 4 μmol NO_2^- g dry wt^{-1} h^{-1}), followed by roots with considerably lower activities. Rhizome NR activity was negligible in both young (growing) and old branches. In contrast, *Glyceria* had the highest levels of NR_{max} in root tissues, which were also the only tissues with detectable NR_{act}. Unlike *Phrag-*

Table 3
Statistical evaluation of NR_{max} ($-Mg^{2+}$) and NR_{act} ($+Mg^{2+}$) tissues levels in plants grown in the water and sand cultures using analysis of variance (ANOVA)

Variable	Water culture			Sand culture		
	Species	Treatment	sp. x tr.	Species	Treatment	sp. x tr.
NR_{max} ($-Mg^{2+}$)						
L	0.032	0.000	0.096	0.000	0.003	0.508
St	0.369	0.069	0.932	0.000	0.036	0.023
Rh-y	0.012	0.381	0.210	0.116	0.614	0.676
Rh-o	–	–	–	0.008	0.101	0.117
R	0.317	0.000	0.091	0.000	0.000	0.002
NR_{act} ($+Mg^{2+}$)						
L	0.001	0.002	0.243	0.000	0.003	0.009
St	0.081	0.012	0.282	0.346	0.975	0.549
Rh-y	0.025	0.508	0.508	0.248	0.265	0.265
Rh-o	–	–	–	0.068	0.042	0.042
R	0.061	0.000	0.029	0.000	0.000	0.003

L, leaves; St, stems; Rh-y, young rhizomes; Rh-o, older rhizomes; R, roots. *Notes*: effects of plant species (sp.), N treatments (tr.) and interactions (species x treatment) are expressed by P-values. NR_{act} data were square-root transformed for the analysis.

Table 4
Statistical evaluation of the relative contribution (%) of individual plant organs in the whole plant reduction of NO_3^- (expressed on dry wt basis) in plants grown in the water and sand cultures using analysis of variance (ANOVA)

Variable	Water culture			Sand culture		
	Species	Treatment	sp. x tr.	Species	Treatment	sp. x tr.
L	0.103	0.765	0.304	0.000	0.744	0.859
St	0.307	0.407	0.962	0.001	0.559	0.086
Rh	0.073	0.005	0.073	0.090	0.619	0.410
R	0.647	0.223	0.326	0.005	0.594	0.512

Data based on NR_{max} ($-Mg^{2+}$) are shown. L, leaves; St, stems; Rh, rhizomes; R, roots. *Notes*: effects of plant species (sp.), N treatments (tr.) and interactions (species x treatment) are expressed by *P*-values.

mites, NR_{max} of oligotrophic grown *Glyceria* plants did not exceed 2 μmol NO_2^- g dry wt^{-1} h^{-1} and NR_{act} was undetectable (Fig. 4). The activity correlated positively with total N availability in the cultivation media, increasing from oligotrophic to hypertrophic treatment in both species (sp. x tr. interactions: $P > 0.05$, Table 3).

The whole plant capacity for the NO_3^- reduction (NR_{max} data) did not differ between species ($P = 0.084$), but was strongly affected by the N treatment ($P < 0.001$) being 2.02 ± 0.63 and 1.81 ± 0.63 (mean ± S.E., $n = 3$–5) in oligotrophic, 3.15 ± 0.72 and 3.78 ± 0.56 in eutrophic, and 4.13 ± 0.72 and 6.73 ± 0.72 μmol NO_2^- g dry wt^{-1} h^{-1} in hypertrophic *Phragmites* and *Glyceria*, respectively. Similarly to the results observed in the water culture experiment, the majority of the NO_3^- reduction capacity of *Phragmites* was localized in leaves (more than 80%, $P < 0.001$). N treatments did not affect the distribution of whole plant NR activity (Table 4). Roots contributed to the whole plant NO_3^- reduction with 10–20% of the plant NR activity; rhizomes, despite representing up to 41% of plant biomass, contributed less than 2% and the contribution of stem was even insignificant. Low NR activity levels were detected in

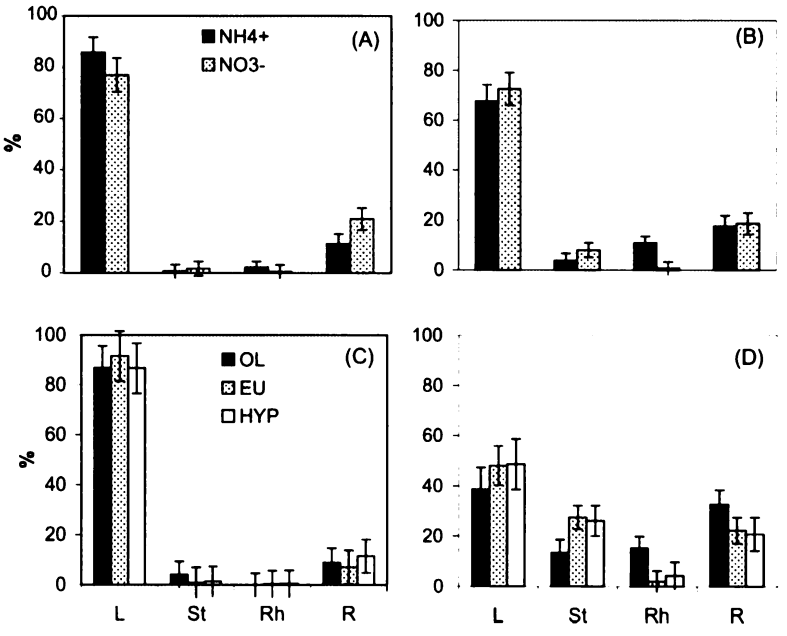


Fig. 3. Relative contribution (%) of individual plant organs in the whole plant reduction of NO_3^- (expressed on dry wt basis) in (A, C) *Phragmites australis* and (B, D) *Glyceria maxima* grown in the (A, B) water and (C, D) sand cultures. Data based on NR_{max} are shown. Values given are means ± S.E. ($n = 3$–5). L, leaves; St, stems; Rh, rhizomes; R, roots.

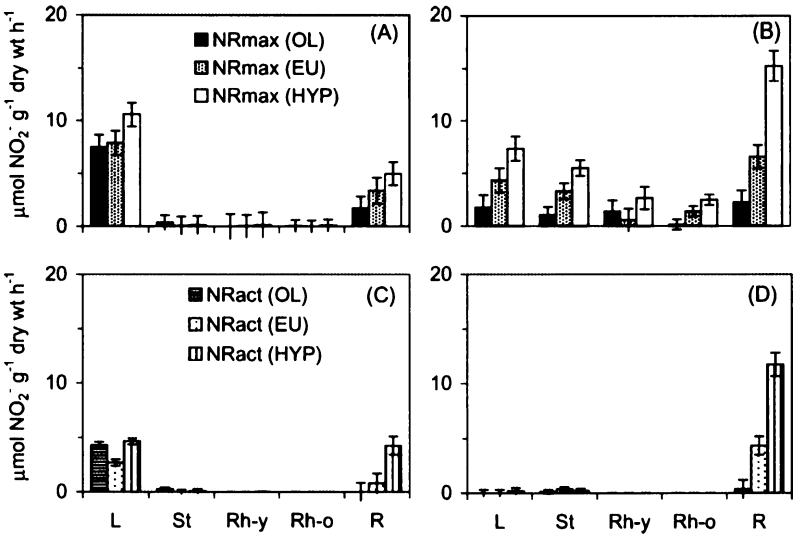


Fig. 4. NR activity in tissues of (A, C) *Phragmites australis* and (B, D) *Glyceria maxima* grown in oligotrophic, eutrophic and hypertrophic treatments in the sand culture. Values given are means ± S.E. ($n = 4$–5). Results of (A, B) NR_{max} and (C, D) NR_{act} are shown. L, leaves; St, stems; Rh-y, young rhizomes; Rh-o, older rhizomes; R, roots.

both young growing buds and older rhizome branches. In *Glyceria*, the contribution of roots and stems to the whole plant NO_3^- reduction was higher than what was observed in *Phragmites*. The contribution of leaves to total plant NR activity did not exceed 50% (Fig. 3C and D). If grown in oligotrophic conditions, rhizomes of *Glyceria* plants contributed to the total NR activity with 15%. The contribution of rhizomes in other treatments was less than 5%, although they represent up to 19% of the total plant biomass.

4. Discussion

Both species were able to maintain growth and N uptake under NO_3^- nutrition. This was pronounced mainly in *Glyceria*, which had high RGR and relative tissue N concentration in NO_3^- treated plants. Continuous daily monitoring of NH_4^+ and NO_3^- concentrations in the water culture solutions allowed comparison of NR activity data with the real occurrence of N forms in the cultivation media. NR activity of NH_4^+ fed plants was 16 and 27% of fully induced NO_3^- grown *Phragmites* and *Glyceria*, respectively, and NO_3^- represented approximately 13% of total N in the growth media of NH_4^+ treatment, oscillating around 20 μM (as seen in the Fig. 1A). Thus, it seems that *Phragmites* and *Glyceria* had a high ability to utilise NO_3^- in spite of the presence of NH_4^+. This is in contrast to the submerged species examined by Cedergreen and Madsen (2003), as well as with the finding of Muller et al. (1994) who observed inhibition of *Carex pseudocyperus* NR activity by 60–70% and documented NH_4^+/NO_3^- ratio 1:2. Both species also showed high sensitivity to low amounts of external NO_3^- even NH_4^+ predominance in the cultivation media, as was similarly observed in terrestrial species, e.g. in barley (Samuelson et al., 1995). Since co-provision of both N forms was shown to enhance growth or N acquisition in many species in contrast to provision of NH_4^+ or NO_3^- alone, e.g. in rice (Kronzucker et al., 1999b) or wheat (Chen et al., 1998), both species may benefit from apparent ability to utilise simultaneously both N forms to high extent. *Glyceria*, which does not tolerate water depth above 0.7 m (Hejny and Husak, 1978) and produces a superficial root system in slightly flooded zones (Buttery and Lambert, 1965; Brandle et al., 1996), may benefit from NO_3^- in the aerated water column or hypoxic sediment surface layers. *Phragmites* colonises habitats from shallow to very deep water (Hejny and Husak, 1978, Brandle et al., 1996). The rooting depth varies in correspondence with sediment quality (Fiala, 1976), but the species generally roots deeper than *Glyceria* (Buttery and Lambert, 1965). At shallow water or even terrestrial

stands, *Phragmites* may benefit from NO_3^- present in aerated sediment. Similarly, it may take advantage from the bulk water NO_3^- or oxidised surface sediment layers via intensively branched roots developing at stem nodes (Koncalova and Pazourek, 1988). Both species are also capable of rhizosphere oxidising (Armstrong and Armstrong, 1988; Both et al., 1992). Considering the fact that *Phragmites* and *Glyceria* are able to utilise NO_3^- under NH_4^+ predominance, it can be concluded that both species are apparently able to benefit from NO_3^- also via roots in the hypoxic sediment. The ability to utilise NO_3^- emerging due to rhizosphere oxidation was already shown in *Rumex palustris* (Engelaar et al., 1995).

The distribution of NR activity within plant tissues proved *Phragmites* to be a leaf NO_3^- reducer. In contrast to terrestrial graminaceous species subjected to <0.5 mM NO_3^-, the leaf NR activity level exceeded root NR activity even in nutrient stressed oligotrophic culture at 20 μM NO_3^-. Thus, the emergent helophytes seem not to prefer roots as the site of NO_3^- reduction, which was shown to be relatively common in submerged wetland species (Cedergreen and Madsen, 2003) or terrestrial species of NH_4^+ dominated habitats (Claussen and Lenz, 1999). Since the relative importance of roots might increase under low oxygen supply (Jiang and Hull, 1999), the ability of efficient transport of atmospheric oxygen into roots in the emerging species may be responsible for the observed differences.

Keeping high leaf NR activity under all N regimes, *Phragmites* seems to be a useful indicator of NO_3^- presence in the waterlogged sediment. NR activity 1–2.5 μmol NO_2^- g dry wt^{-1} h^{-1} (recalculated using dry to fresh wt. ratio 0.33) detected in leaves of *Phragmites* at lake Balaton (Erdei et al., 2001) was comparable to NH_4^+ treated plants in the water culture. In contrast, root NR activity of *Glyceria* was high, especially in the sand culture. It is known, that relative importance of root NR activity generally increases under low NO_3^- supply (Andrews et al., 1992; Marschner, 1995). However, the experiment showed that root NR activity was important especially in eutrophic and hypertrophic treatments in spite of increasing NO_3^- availability and relative tissue N contents. Thus, it seems that the differences between cultivations are likely caused by other factors. Oxygen availability could be one of them. The importance of root, but not leaf NR activity, was already shown to increase during oxygen shortage in non-wetland graminaceae species *Poa pratensis* (Jiang and Hull, 1999). Although not measured, oxygen availability in continuously recirculated solution might be higher than in the sand culture, where the water level was stagnant and kept with the surface of sand. Since oxygen shortage was shown to cause dephosphorylation of NR (Botrel and Kaiser, 1997), the activation status (NR_{act}/NR_{max}) increased with declining oxygen availability (de la Haba et al., 2001). However, no clear differences between NR_{act}/NR_{max} of water and sand culture grown plants were found in the present study. The NR_{act}/NR_{max} in roots of *Glyceria* in the water cultures was not generally higher than in the sand cultures, where oxygen availability was presumably lower. The correlation of NR_{act}/NR_{max} in roots of *Glyceria* with nutrient availability was rather observed in the sand culture, where NR_{act}/NR_{max} increased from oligotrophic (16%), eutrophic (66%) to hypertrophic (77%) treatment.

The apparent difference in growing season might be another factor responsible for the differences between cultivations. Water culture experiment ran under constant conditions of long days, but plants form the sand culture were cultivated outdoors and harvested at the end of August. Erdei et al. (2001) detected higher NR activity in *P. australis* during period of vegetative growth and very low activity later in the season. Similarly, the decline of NR activity in leaves in June–August was observed in *Coffea arabica* (Da Matta et al., 1999). Therefore, it seems that the low leaf NR activity in sand culture grown *Glyceria* plants might also be attributed also to seasonal effect.

Rhizomes and their older branches, in particular, are known to be primarily storage organs (Klimes et al., 1999). The study proved that rhizomes contribute to the NO_3^- reduction of both plant species differently. While they were not involved in NO_3^- reduction in *Phragmites*, their contribution to NO_3^- reduction in *Glyceria* cultivated under low NO_3^- availability was relatively high. This feature was not that pronounced under high NO_3^- supply. These results correspond with the already demonstrated fact that N uptake of rhizomes is of minor importance (for review see Touchette and Burkholder, 2000), but the importance might raise under low N availability (Brooker et al., 1999). The involvement of rhizomes in N assimilation is not clear, but it is generally considered to be relatively low (for re-

view see Touchette and Burkholder, 2000). That agrees also with our finding of low NR activity in both young growing buds and older rhizome branches. Although rhizome metabolic activity was shown to change during the growing season (Steinmann and Brandle, 1984), metabolic differences among branches of different age were not well characterised yet. The respiration rate was shown to be higher in young than older branches (Cizkova and Bauer, 1998), but additional data are lacking.

Our results clearly showed that the use of NR activity as the marker of rhizosphere NO_3^- and plant preferences must always be done with care and caution. *Phragmites* had high leaf NR activity in spite of low RGR and tissue NO_3^- contents, while *Glyceria* responded to NO_3^-, but accumulated NO_3^- in unreduced form. High NO_3^- content, especially in leaves, may indicate NO_3^- luxury consumption (Chapin, 1980). Glyceria was also shown to have a higher capacity for mineral accumulation (e.g. high concentration factors for main nutrients (N, P, K, Ca, Mg)) than *Phragmites* (Dykyjova, 1979).

The second reason for the caution when interpreting data of NR activity is the fact that it seems to depend on the methodology used. The reliable use of NR activity as a marker of NO_3^- utilization in individual plant species is impossible without the method optimisation, which is, unfortunately, not often taken into account in ecological studies. Plant species and even organs may significantly differ in the content of chemical compounds interfering with nitrate reductase activity estimation (e.g. phenolics, enzymes inactivating NR, glycosides etc.). *Glyceria*, but not *Phragmites*, contains cyanogenic glycosides releasing CN^- during homogenisation. Since they are very potent inhibitors of NR activity (Maranville, 1970), their release leads to serious underestimation of NR activity when ignored. Moreover, the differences between in vitro and in vivo method of NR activity estimation have to be kept in mind. In vitro activity is detected under saturating availability of all necessary substrates and thus it is usually higher than activity detected in vivo as summarised by Cedergreen and Madsen (2003).

Acknowledgements

Authors would like to thank Dr. Hana Cizkova (Institute of Botany of the Academy of Sciences of the Czech Republic, Trebon) for general help and Dr. James Seago (SUNY Coll Oswego, Department of Biology, Oswego, USA) for language correction of the manuscript. The project was supported by the grant GAAV CR no. B6111306—Nitrate reductase activity in wetland plants.

References

Aguera, E., Poblete, L., de la Haba, P., Maldonado, J.M., 1999. Light modulation and in vitro effects of adenine nucleotides on leaf nitrate reductase activity in cucumber (*Cucumis sativus*). Physiol. Plant. 105, 218–223.

Andrews, M., Morton, J.D., Lieffering, M., Bisset, L., 1992. The partitioning of nitrate assimilation between root and shoot of a range of temperate cereals and pasture grasses. Ann. Bot. 70, 271–276.

Armstrong, J., Armstrong, W., 1988. *Phragmites australis*—a preliminary study of soil-oxidizing sites and internal gas transport pathways. New Phytol. 108, 373–382.

Both, G.J., Gerards, S., Laanbroek, H.J., 1992. The occurrence of chemolitho-autotrophic nitrifiers in water-saturated grassland soils. Microbial. Ecol. 23, 15–26.

Botrel, A., Kaiser, W.M., 1997. Nitrate reductase activation state in barley roots in relation to the energy and carbohydrate status. Planta 201, 496–501.

Brandle, R., Pokorny, J., Kvet, J., Cizkova, H., 1996. Wetland plants as a subject of interdisciplinary research. Folia Geobot. Phytotx. 31, 1–6.

Brix, H., Lorenzen, B., Morris, J.T., Schierup, H.H., Sorrell, B.K., 1994. Effects of oxygen and nitrate on ammonium uptake kinetics and adenylate pools in *Phalaris arundinacea* L. and *Glyceria maxima* (Hartm.) Holmb. P. R. Soc. Edinb. B 102, 333–342.

Brooker, R.W., Callaghan, T.V., Jonasson, S., 1999. Nitrogen uptake by rhizomes of the clonal sedge Carex bigelowii: a previously overlooked nutritional benefit of rhizomatous growth. N. Phytol. 142, 35–48.

Buttery, B.R., Lambert, J.M., 1965. Competition between *Glyceria maxima* and *Phragmites communis* in the region of Surlingham broad. 1. The competition mechanism. J. Ecol. 53, 163–181.

Cedergreen, N., Madsen, T.V., 2003. Nitrate reductase activity in roots and shoots of aquatic macrophytes. Aquat. Bot. 76, 203–212.

Chapin, F.S., 1980. The mineral-nutrition of wild plants. Annu. Rev. Ecol. Syst. 11, 233–260.

Chen, J.G., Cheng, S.H., Cao, W.X., Zhou, X., 1998. Involvement of endogenous plant hormones in the effect of mixed nitrogen source on growth and tillering of wheat. J. Plant Nutr. 21, 87–97.

Cizkova, H., Bauer, V., 1998. Rhizome respiration of *Phragmites australis*: effect of rhizome age, temperature, and nutrient status of the habitat. Aquat. Bot. 61, 239–253.

Claussen, W., Lenz, F., 1999. Effect of ammonium or nitrate nutrition on net photosynthesis, growth, and activity of the enzymes nitrate

reductase and glutamine synthetase in blueberry, raspberry and strawberry. Plant Soil. 208, 95–102.

Cottingham, P.D., Davies, T.H., Hart, B.T., 1999. Aeration to promote nitrification in constructed wetlands. Environ. Technol. 20, 69–75.

Da Matta, F.M., do Amaral, J.A.T., Rena, A.B., 1999. Growth periodicity in trees of Coffea arabica L. in relation to nitrogen supply and nitrate reductase activity. Field Crop Res. 60, 223–229.

de la Haba, P., Aguera, E., Benitez, L., Maldonado, J.M., 2001. Modulation of nitrate reductase activity in cucumber (*Cucumis sativus*) roots. Plant Sci. 161, 231–237.

Dykyjova, D., 1979. Selective uptake of mineral ions and their concentration factors in aquatic higher plants. Folia Geobot. Phytotx. 14, 267–325.

Engelaar, W.M.G.H., Symens, J.C., Laanbroek, H.J., Blom, C.W.P.M., 1995. Preservation of nitrifying capacity and nitrate availability in waterlogged soils by radial oxygen loss from roots of wetland plants. Biol. Fert. Soils 20, 243–248.

Erdei, L., Horvath, F., Tari, I., Pecsvaradi, A., Szegletes, Z., Dulai, S., 2001. Differences in photorespiration, glutamine synthetase and polyamines between fragmented and closed stands of *Phragmites australis*. Aquat. Bot. 69, 165–176.

Fiala, K., 1976. Underground organs of *Phragmites communis*, their growth, biomass and net production. Folia Geobot. Phytotx. 11, 113–224.

Garcia-Novo, F., Crawford, R.M.M., 1973. Soil aeration, nitrate reduction and flooding tolerance in higher plants. N. Phytol. 72, 1031–1039.

Garnett, T.P., Shabala, S.N., Smethurst, P.J., Newman, I.A., 2001. Simultaneous measurement of ammonium, nitrate and proton fluxes along the length of eucalypt roots. Plant Soil. 236, 55–62.

Hejny, S., Husak, S., 1978. Higher plant communities. In: Dykyjova, D., Kvet, J. (Eds.), Pond Littoral Ecosystems. Structure and Functioning. Springer-Verlag, Berlin, pp. 23–64.

Ingemarsson, B., Johansson, L., Larsson, C.M., 1984. Photosynthesis and nitrogen utilization in exponentially growing nitrogen-limited cultures of *Lemna gibba*. Physiol. Plant 62, 363–369.

Jiang, Z.C., Hull, R.J., 1999. Partitioning of nitrate assimilation between shoots and roots of Kentucky bluegrass. Crop Sci. 39, 746–754.

Klimes, L., Klimesova, J., Cizkova, H., 1999. Carbohydrate storage in rhizomes of *Phragmites australis*: the effects of altitude and rhizome age. Aquat. Bot. 64, 105–110.

Koncalova, H., Pazourek, J., 1988. Root dimorphism in flooded *Carex gracilis* Curt. Acta U. Carol. Biol. 31, 43–50.

Kronzucker, H.J., Siddiqi, M.Y., Glass, A.D.M., 1997. Conifer root discrimination against soil nitrate and the ecology of forest succession. Nature 385, 59–61.

Kronzucker, H.J., Glass, A.D.M., Siddiqi, M.Y., 1999a. Inhibition of nitrate uptake by ammonium in barley. Analysis of component fluxes. Plant Physiol. 120, 283–291.

Kronzucker, H.J., Siddiqi, M.Y., Glass, A.D.M., Kirk, G.J.D., 1999b. Nitrate-ammonium synergism in rice. A subcellular flux analysis. Plant Physiol. 119, 1041–1046.

Lorenzen, B., Brix, H., Mendelssohn, I.A., McKee, K.L., Miao, S.L., 2001. Growth, biomass allocation and nutrient use efficiency in *Cladium jamaicense* and *Typha domingensis* as affected by phosphorus and oxygen availability. Aquat. Bot. 1482, 1–17.

Maranville, J.W., 1970. Influence of nickel on the detection of nitrate reductase activity in *Sorghum* extracts. Plant Physiol. 45, 591–593.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. Academic Press, London.

Min, X.J., Siddiqi, M.Y., Guy, R.D., Glass, A.D.M., Kronzucker, H.J., 2000. A comparative kinetic analysis of nitrate and ammonium influx in two early-successional tree species of temperate and boreal forest ecosystems. Plant Cell Environ. 23, 321–328.

Muller, E., Alberts, B.P., Janiesch, P., 1994. Influence of NO_3^- and NH_4^+ nutrition on fermentation, nitrate reductase activity and adenylate energy charge of roots of *Carex pseudocyperus* L. and *Carex sylvatica* Huds. exposed to anaerobic nutrient solution. Plant Soil. 166, 221–230.

Samuelson, M.E., Ohlen, E., Lind, M., Larsson, C.M., 1995. Nitrate regulation of nitrate uptake and nitrate reductase expression in barley grown at different nitrate:ammonium ratios at constant relative nitrogen addition rate. Physiol. Plant 94, 254–260.

Sasakawa, H., Yamamoto, Y., 1978. Comparison of the uptake of nitrate and ammonium by rice seedlings. Plant Physiol. 62, 665–669.

Scheible, W.R., Lauerer, M., Schulze, E.D., Caboche, M., Stitt, M., 1997. Accumulation of nitrate in the shoot acts as a signal to regulate shoot-root allocation in tobacco. Plant J. 11, 671–691.

Steinmann, F., Brandle, R., 1984. Carbohydrate and protein metabolism in the rhizomes of the bulrush (*Schoenoplectus lacustris* (L.)) Palla in relation to natural development of the whole plant. Aquat. Bot. 19, 53–63.

Stewart, G.R., Pate, J.S., Unkovich, M., 1993. Characteristics of inorganic nitrogen assimilation of plants in fire-prone mediterranean-type vegetation. Plant Cell Environ. 16, 351–363.

Thursby, G.B., Harlin, M.M., 1984. Interaction of leaves and roots of *Ruppia maritima* in the uptake of phosphate, ammonia and nitrate. Marine Biol. 83, 61–67.

Touchette, B.W., Burkholder, J.M., 2000. Review of nitrogen and phosphorus metabolism in seagrasses. J. Exp. Marine Biol. Ecol. 250, 133–167.

Different sensitivity of *Phragmites australis* and *Glyceria maxima* to high availability of ammonium-N.

Edita Tylová[a*], Lenka Steinbachová[a], Olga Votrubová[a], Bent Lorenzen[b], Hans Brix[b]

a) *Department of Plant Physiology, Faculty of Science, Charles University in Prague, Viničná 5, 128 44 Prague 2, Czech Republic*
b) *Department of Biological Sciences, Plant Biology, Aarhus University, Ole Worms Allé Building 1135, 8000 Aarhus C, Denmark*

*Corresponding author. Tel.: +420 22195-1697; fax: +42-22195-1704.
E-mail address: edmunz@natur.cuni.cz (E. Tylová)

Complete correspondence address to which the proofs should be sent:
Edita Tylová, Department of Plant Physiology, Faculty of Science, Charles University in Prague, Viničná 5, 128 44 Prague 2, Czech Republic

Abstract

The ability to cope with NH_4^+-N was studied in the littoral helophytes *Phragmites australis* and *Glyceria maxima*, species commonly occupying fertile habitats rich in NH_4^+ and often used in artificial wetlands. In the present study, *Glyceria* growth rate was reduced by 16% at 179 μM NH_4^+-N, and the biomass production was reduced by 47% at 3700 μM NH_4^+-N compared to NO_3^--N. Similar responses were not found in *Phragmites*. The amounts (mg g^{-1} dry wt) of starch and total non-structural carbohydrates (TNC) in rhizomes were significantly lower in NH_4^+ (8.9; 12.2 starch; 20.1; 41.9 TNC) compared to NO_3^- treated plants (28.0; 15.6 starch; 58.5; 56.3 TNC) in *Phragmites* and *Glyceria*, respectively. In addition, *Glyceria* showed lower amounts (mg g^{-1} dry wt) of soluble sugars, TNC, K^+, and Mg^{2+} in roots under NH_4^+ (5.6; 14.3; 20.6; 1.9) compared to NO_3^- nutrition (11.6; 19.9; 37.9; 2.9, for soluble sugars, TNC, K^+, and Mg^{2+}, respectively), while root internal levels of NH_4^+ and Ca^{2+} (0.29; 4.6 mg g^{-1} dry wt, mean of both treatments) were only slightly affected. In *Phragmites*, no changes in soluble sugars, TNC, Ca^{2+}, K^+, and Mg^{2+} contents of roots (7.3; 14.9; 5.1; 17.3; 2.6 mg g^{-1} dry wt, means of both treatments) were found in response to treatments. The results, therefore, indicate a more pronounced tolerance towards high NH_4^+ supply in *Phragmites* compared to *Glyceria*, although the former may be susceptible to starch exhaustion in NH_4^+-N nutrition. In contrast, *Glyceria's* ability to colonise fertile habitats rich in NH_4^+ is probably related to the avoidance strategy due to shallow rooting or to the previously described ability to cope with high NH_4^+ levels when P availability is high and NO_3^- is also provided.

Keywords: *Phragmites*; *Glyceria*; Wetland plants; Nitrate; Eutrophication; High nitrogen load; Wastewater treatment, NH_4^+/NO_3^- ratio; Ammonium toxicity

1. Introduction

Although NH_4^+ generally prevails in wetland soils, significant amounts of NO_3^- may occur in the bulk water, top sediment layers, or rhizosphere of emergent macrophytes, which support nitrifying activity at their root surfaces (Engelaar et al., 1995). Capability of NO_3^- utilization is documented in wetland plants (Engelaar et al., 1995; Munzarová et al., 2006), but NH_4^+ preference is often suggested (e.g. Cedergreen and Madsen, 2003) as the feature of plants adapted to habitats in which NH_4^+ is prevalent (Britto and Kronzucker, 2002). In agreement with this, our previous study showed a higher growth rate of *Glyceria maxima* with NH_4^+-N than with NO_3^--N (Tylová-Munzarová et al., 2005), when supplied at levels (34 μM) corresponding with average N concentrations in the pore waters of wetlands in the Třeboň basin, Czech Republic (Čížková et al., 2001). Here *Glyceria* commonly occupies very eutrophic littoral zones, being more tolerant to severe eutrophication compared to *Phragmites australis*, the dominant emergent helophyte in this area (Hroudová and Zákravský, 1999). As the NH_4^+/NO_3^- ratio of heavily eutrophic sites is often shifted in favour of NH_4^+ (Kühl and Kohl, 1992; Čížková et al., 2001), the preference for NH_4^+-N might improve the competitive potential of *Glyceria*. The link is, however, not simple, since *Glyceria* responds negatively to NH_4^+-N (Munzarová et al., 2006), when applied at levels (179 μM) occurring in eutrophic wetland habitats (Kühl and Kohl, 1992; Čížková et al., 2001).

Sensitivity to NH_4^+-N alone is a widespread phenomenon (for summary see Britto and Kronzucker, 2002), but pronounced tolerance is common in plants at sites where NH_4^+-N is the dominant form of N (Britto et al., 2001; Britto and Kronzucker, 2002). A positive response to NH_4^+ (100 μM) was found in, e.g., *Typha latifolia* (Brix et al., 2002), but the ability to cope with large amounts of NH_4^+ as sole N source seems to be limited even in wetland plants. The symptoms of NH_4^+ toxicity were described in rice under K^+ shortage (Britto and Kronzucker, 2002 and citations therein); high NH_4^+ supply negatively affected the growth of *Acorus calamus* (Vojtíšková et al., 2004), *Zostera marina* (for summary see Touchette and Burkholder (2000)), and *Stratiotes aloides* (Smolders et al., 1996). In *Phragmites*, NH_4^+ dominance in the sediment was shown to correlate with low carbohydrate levels in rhizomes (Čížková et al., 1996; Kubín and Melzer, 1996).

The ability to cope with high NH_4^+-N is of special importance in considering plant usage in constructed wetlands, as NH_4^+-N commonly prevails in the wastewater (see e.g. Cottingham et al., 1999). Since both *Phragmites* and *Glyceria* are used for wastewater

treatment, an understanding the responses to NH_4^+-N might help to improve their performance in these systems. Therefore, the present study was designed (i): to describe the extent to which morphology and metabolic relations of wetland plants under high N load are modified by the form of supplied N; and (ii): to analyse factors that might explain differences in the growth response of *Phragmites* and *Glyceria* to NH_4^+ versus NO_3^- described by Munzarová et al. (2006). In particular, the existence of NH_4^+ toxicity symptoms (e.g. root growth suppression, accumulation of NH_4^+ in plant tissues, diminution of essential cations and carbohydrate exhaustion) was assessed at 179 μM N, pH 6.5. The sensitivity of *Phragmites* and *Glyceria* to NH_4^+ was further tested at high N (3700 μM), which simulated wastewater N concentrations (see e.g. Cottingham et al., 1999) and lower pH (5.0).

2. Materials and methods

2.1. Plant material and experimental set-up

The plants of *Phragmites australis* (Cav.) Trin. ex Steudel and *Glyceria maxima* (Hartm.) Holmb. came from littoral stands in the Třeboň basin, Czech Republic, that have been propagated long-term in outdoor sand cultures at the Institute of Botany, Academy of Sciences. Rootless rhizome cuttings (10 cm) with one shoot (15-20 cm) were cultivated for three weeks in coarse sand (irrigated with tap water) prior to their transfer into the experimental cultures.

Effects of NH_4^+-N versus NO_3^--N were followed in two indoor water culture experiments: experiment 1 simulated N availability of eutrophic wetland habitats (179 μM N, pH 6.5); experiment 2 simulated N levels of constructed wetlands (3700 μM N, pH 5.0; see e.g. Cottingham et al., 1999). In experiment 2, only growth characteristics were followed.

Experiment 1 was performed as described in Munzarová et al. (2006), with similar growth conditions. Each growth unit was comprised of two 30 L growth tanks with 10 plants of each species (*Phragmites* and *Glyceria* together); 170 L of the nutrient solution was recirculated at a rate 3 L min^{-1} through each tank. The composition of nutrient solution (μM) was: PO_4^{3-} 16; K^+ 87; Ca^{2+} 3186; Mg^{2+} 1687; Na^+ 2175; SO_4^{2-} 3061; Cl^- 6093; SiO_3^{2-} 13; BO_3^{3-} 2.5; Fe^{2+} 2.0; Mn^{2+} 0.2; Zn^{2+} 0.2; Cu^{2+} 0.2; and N 179 added as sole NH_4^+ (NH_4^+ treatment) or NO_3^- (NO_3^- treatment). The pH (6.5), NO_3^-, NH_4^+, and PO_4^{3-} levels were

adjusted daily, and the solutions were renewed every time NO_3^- accumulating in NH_4^+ treatment due to microbial activity exceeded 7 μM.

Experiment 2 was performed in a room with constant growth conditions: 16/8 day/night regime (irradiance 300 μmol m^{-2} s^{-1}), 21/15 day/night thermoperiod, rel. humidity 75-85%. Individual plants were cultivated for one month in 0.7 L glass containers (attached to a small glass plate with a rubber band) covered with an opaque foil and black polypropylene granules to prevent algal growth and exposure of belowground organs to the light. The cultivation solution was a modified quarter-strength Hoagland 3 nutrient solution with the following basic composition (μM): PO_4^{3-} 254; Mg^{2+} 250; SO_4^{2-} 250; BO_3^{3-} 0.03; Fe^{2+} 5.1; Mn^{2+} 0.18; Zn^{2+} 0.002; Cu^{2+} 0.001; $Mo_7O_{24}^{2-}$ 0.0002. Along with the basic nutrients, the NH_4^+ treatment contained (μM): NH_4^+ 3701; Ca^{2+} 1252; K^+ 1567 and Cl^- 7519; and NO_3^- treatment contained (μM): NO_3^- 3743; Ca^{2+} 1248 and K^+ 1500. The nutrient solution was renewed every second day and pH was adjusted to 5.0 using HCl.

2.2. Plant harvest, biomass sampling, chemical analyses, and statistical evaluation

Plants were harvested as described in Tylová-Munzarová et al. (2005). S/R (shoots plus rhizomes/roots) and A/B (shoots/roots plus rhizomes) ratios, and the relative allocation of biomass into leaves, stems, rhizomes, or roots were calculated based on dry weight.

Plants in experiment 1 were analysed for total C, N, NH_4^+, Ca^{2+}, Mg^{2+}, K^+ contents, and the contents of non-structural carbohydrates. The contents of C, N, Ca^{2+}, Mg^{2+} and K^+ were analysed in plant dry matter ground to fine powder (homogenizer Retsch, MM301, Germany). C and N were determined with the CN analyser (Na2000, Carlo Erba, Italy) and the C/N atomic ratio was calculated. Ca^{2+}, Mg^{2+} and K^+ contents were analysed by ICP-OES (Thermo Jarrell Ash, USA) after microwave digestion in HNO_3.

Carbohydrates and NH_4^+ were analysed on subsamples of each plant part, which were taken prior to the fractionation, frozen in liquid nitrogen, freeze-dried, and ground in a Retsch homogenizer (see above). NH_4^+ was detected using the salicylate method as described in Tylová-Munzarová et al. (2005). The analysis of non-structural carbohydrates involved HPLC detection of soluble carbohydrates (sucrose, glucose, fructose), and detection of starch (as glucose after a hydrolysis with α-amylase and amyloglucosidase); both methods according to Steinbachová-Vojtíšková et al. (2006). TNC (total non-structural carbohydrates - sum of

starch and soluble carbohydrates, mg g^{-1} dry wt); ratio of hexoses (glucose and fructose) to sucrose), and starch/ soluble carbohydrates ratio were calculated.

Statistical evaluation was performed using NCSS 2000 and PASS 2000 software (Jerry Hintze, Kaysville, Utah). Growth and metabolic characteristics estimated in each experiment were subjected to Two-way analysis of variance (ANOVA); the differences between plant species, treatments and interactions (species x treatment) were analysed for each variable.

3. Results

At 179 μM total N, *Glyceria* relative growth rate was a 16% lower under sole NH_4^+ (44.8 mg g^{-1} d^{-1}) compared to NO_3^- (53.5 mg g^{-1} d^{-1}) N source, while *Phragmites* showed no significant response to the form of supplied N (24.87±8.63 and 28.90±8.63 mg g^{-1} d^{-1} in NH_4^+ and NO_3^- fed plants, respectively; P>0.05). The negative response of *Glyceria* to NH_4^+-N was pronounced at 3700 μM N and pH 5.0; the total biomass production was 47% lower in NH_4^+ fed plants; 1.8±0.2 and 3.4±0.5 g dry wt per plant in NH_4^+ and NO_3^- treatment, respectively (P<0.05). In contrast, *Phragmites* reached 2.6±0.9 and 2.3±0.5 g dry wt per plant in NH_4^+ and NO_3^- treatment (P>0.05). *Glyceria*, but not *Phragmites*, also tended to reduce the allocation of biomass to roots (by 14%) under NH_4^+-N source at 3700 μM N (Table 1); similar symptoms were not found at 179 μM total N (Table 1).

Internal NH_4^+ levels were high in roots, stems (P<0.05), and particularly rhizomes (P<0.01) of NH_4^+ treated plants at 179 μM N (Fig. 1), but both species responded similarly (sp. x tr. interactions: P>0.05). Rhizomes of NH_4^+ treated plants exhibited lower content of TNC (by 54 and 26 % in *Phragmites* and *Glyceria*, respectively), particularly of starch (by 68 and 22%), while amounts of soluble carbohydrates (sucrose, glucose, fructose) (Fig. 1; P>0.05), ratio of hexoses (glucose, fructose) to sucrose or C/N ratio remained unaffected (Table 2). The starch diminution was more conspicuous in *Phragmites*, but the differences between species were not significant (Table 2). *Phragmites* (not *Glyceria*) showed low starch/soluble carbohydrates ratio in rhizome (Table 2).

In contrast to rhizomes, roots of NH_4^+ treated plants did not show lower starch amounts (Table 2). *Glyceria*, however, exhibited significantly lower content of TNC (by 28%; Table 2) and, particularly, of soluble carbohydrates (by 54%; Fig. 1) in NH_4^+ treated plants. A similar response was not observed in *Phragmites* (sp. x tr. interactions: P<0.05). Roots of

NH_4^+ treated *Glyceria* therefore possessed a 2.4 times higher ratio of starch to soluble carbohydrates compared to NO_3^- fed plants (Table 2).

The contents of Ca^{2+}, Mg^{2+}, and K^+ in leaves or roots of *Phragmites* did not change in response to treatments (Fig. 1; $P>0.05$). In *Glyceria*, however, 35% lower Mg^{2+} and 46% lower K^+ levels were found in roots of NH_4^+ treated plants (Fig. 1; $P<0.05$;). The response therefore significantly differed between the species (sp. x tr. interactions: $P=0.084$ (Mg^{2+}); $P<0.001$ (K^+)).

4. Discussion

The present study shows lower growth rate of *Glyceria* but not *Phragmites* under sole NH_4^+ compared to NO_3^- at both 179 and 3700 μM total N levels. The sensitivity of *Glyceria* to NH_4^+-N was rather unexpected, since the positive response to NH_4^+ was found at 34 μM total N availability in this species (Tylová-Munzarová, 2005). We therefore focused on mechanisms underlying the response to N source at 179 μM total N level in more detail and showed differences in C/N balance, carbohydrate status, and tissue levels of essential cations between NH_4^+ and NO_3^- fed plants.

Symptoms of NH_4^+ toxicity are often correlated with increased concentrations of NH_4^+ in plant tissues (Hecht and Mohr, 1990). In agreement, higher contents of NH_4^+ were detected in NH_4^+ compared to NO_3^- fed plants of both species. The trend was, however, even more pronounced in *Phragmites* and therefore seemed not being the cause of the negative growth response to NH_4^+ observed in *Glyceria*. The form of supplied N affected also the carbohydrate status of belowground organs, which is one of the important prerequisites for wintering and spring outgrowth in perennial helophytes. Particularly in *Phragmites*, lower amounts of starch were found in rhizomes of NH_4^+ fed plants. Similar adverse effect of NH_4^+ on rhizome starch contents was indicated in this species by field observations of Kubín and Melzer (1996). Since starch is considered a more efficient C storage form compared to soluble carbohydrates (Kubín and Melzer, 1996), NH_4^+-N source obviously reduces the C storage capacity of *Phragmites*. Carbohydrate exhaustion, one of the important factors underlying *Phragmites* die-back in eutrophic habitats (Čížková et al., 1996), may therefore be worsened by the increase of NH_4^+/NO_3^- pore water ratio.

In contrast, *Glyceria* responded to NH_4^+-N by significant decrease of contents of soluble carbohydrates in roots. This raises the question whether energy wasteful NH_4^+

transmembrane cycling (as described by Britto et al, 2001) occurs in *Glyceria*, contributing to a negative growth response to excessive NH_4^+ levels. This speculation, however, cannot be confirmed without the measurements of membrane NH_4^+ fluxes and root energy status, which were not examined in the present study. Brix et al. (1994) described positive effects of NO_3^- addition to growth media on energy status of NH_4^+ fed *Glyceria* roots, but the extrapolation of results to conditions of excessive NH_4^+ levels is difficult, because plants were cultivated at low (10μM) NH_4^+-N and NO_3^- apparently acted as an additional N source.

Sensitivity to excessive NH_4^+ is furthermore attributed to induced deficiency of essential cations (Britto and Kronzucker, 2002). In the present study, the lower contents of Mg^{2+} and K^+ levels were found *Glyceria* but not *Phragmites*. *Glyceria* might therefore be more susceptible to nutrient deficiencies induced by NH_4^+. Our findings are in agreement with Čížková-Končalová et al. (1996), who found low K^+ contents in rhizomes of *Glyceria* under excessive sewage dose, while *Phragmites* showed rather the opposite tendency. Čížková and Lukavská (1999) also found no consistent difference in *Phragmites* K^+ content between vigorous and die-back sites, and Dinka and Szeglet (1999) detected even higher K^+ content in reed rhizome at hypertrophic die-back sites compared to stabilized mesotrophic sites. It is therefore possible that the negative response to high NH_4^+ levels found in *Glyceria* is related to induced K^+ shortage, as insufficient K^+ supply was shown to exacerbate adverse effects of excessive NH_4^+ in different species, even in highly NH_4^+ tolerant *Oryza sativa* (for summary see Britto and Kronzucker, 2002). Because K^+ performs irreplaceable functions in plants (Marschner, 1995), its shortage impairs important plant processes (including enzyme activation, proteosynthesis, photosynthesis, and osmoregulations) and increases plant susceptibility to various stresses (Cakmak, 2005). K^+ deficiency was also shown to impair export of photosynthates into roots (Cakmak, 2005), which may help to explain low levels of soluble sugars in roots of NH_4^+ fed *Glyceria*.

In summary, our results indicate that *Phragmites* is more tolerant than *Glyceria* of high external NH_4^+ levels. These findings are in agreement with the overall growth strategy of the two species, as *Phragmites* is generally rooted deeper than *Glyceria* into hypoxic sediments (Buttery and Lambert, 1965), where NH_4^+ dominates. Moreover, the sensitivity of *Phragmites* to highly eutrophic sediments is obviously not related to the direct adverse effect of high NH_4^+ availability. Other factors (e.g. sensitivity to litter accumulation) are apparently more important factors triggering *Phragmites* die back, but *Phragmites* may be weakened due to reduction of carbohydrate storages under NH_4^+ predominance and may thus be more susceptible to occasional stresses (e.g. high water level, especially in the spring, mechanical

damage, mowing, grazing). In contrast, *Glyceria's* ability to colonise fertile habitats rich in NH_4^+ (Hroudová and Zákravský, 1999) is related to (i): the avoidance strategy due to shallow rooting or formation of floating mats (Buttery and Lambert, 1965); and (ii): the ability to cope with high NH_4^+ levels when enough P is available and NO_3^- is co-provided (Hroudová and Zákravský, 1999; Tylová - unpublished results).

Acknowledgement

The authors thank Prof. James L. Seago (Oswego State University, USA) for the kind help with language corrections, Dr. Helena Lipavská for the help with the analysis of non-structural carbohydrates; Dr. Hana Čížková for the valuable discussion, and Drahomíra Bartáková for the technical assistance. The work was supported by the MŠMT ČR (Research Intention: Signalization and molecular mechanisms of cell response, MSM 0021620858).

References

Britto, D.T., Kronzucker, H.J., 2002. NH_4^+ toxicity in higher plants: a critical review. J. Plant. Physiol. 159, 567-584.

Britto, D.T., Siddiqi, M.Y., Glass, A.D.M., Kronzucker, H.J., 2001. Futile transmembrane NH_4^+ cycling: A cellular hypothesis to explain ammonium toxicity in plants. Proc. Natl. Acad. Sci. USA 98, 4255-4258.

Brix, H., Dyhr-Jensen, K., Lorenzen, B., 2002. Root-zone acidity and nitrogen source affects *Typha latifolia* L. growth and uptake kinetics of ammonium and nitrate. J. Exp. Bot. 53, 2441-2450.

Brix, H., Lorenzen, B., Morris, J.T., Schierup, H.H., Sorrell, B.K., 1994. Effects of oxygen and nitrate on ammonium uptake kinetics and adenylate pools in *Phalaris arundinacea* L. and *Glyceria maxima* (Hartm.) Holmb. P. Roy. Soc. Edinb. B. 102, 333-342.

Buttery, B.R., Lambert, J.M., 1965. Competition between *Glyceria maxima* and *Phragmites communis* in the region of Surlingham broad. 1. The competition mechanism. J. Ecol. 53, 163-181.

Cakmak, I., 2005. The role of potassium in alleviating detrimental effects of abiotic stresses in plants. J. Plant Nutr. Soil Sci. 168, 521-530.

Cedergreen, N., Madsen, T.V., 2003. Nitrate reductase activity in roots and shoots of aquatic macrophytes. Aquat. Bot. 76, 203-212.

Čížková, H., Lukavská, J., 1999. Rhizome age structure of three populations of *Phragmites australis* (CAV.) TRIN. ex STEUD.: Biomass and mineral nutrient concentrations. Folia Geobot. 34, 209-220.

Čížková, H., Lukavská, J., Pribáň, K., Kopecký, J., Brabcová, H., 1996. Carbohydrate levels in rhizomes of *Phragmites australis* at an oligotrophic and a eutrophic site: A preliminary study. Folia Geobot. Phytotx. 31, 111-118.

Čížková, H., Pechar, L., Husák, S., Květ, J., Bauer, V., Radochová, J., Edwards, K., 2001. Chemical characteristics of soils and pore waters of three wetland sites dominated by *Phragmites australis*: relation to vegetation composition and reed performance. Aquat. Bot. 69, 235-249.

Čížková-Končalová, H., Květ, J., Lukavská, J., 1996. Response of *Phragmites australis*, *Glyceria maxima*, and *Typha latifolia* to additions of piggery sewage in a flooded sand culture. Wetlands Ecol. Manage. 4, 43-50.

Cottingham, P.D., Davies, T.H., Hart, B.T., 1999. Aeration to promote nitrification in constructed wetlands. Environ.Technol. 20, 69-75.

Dinka, M., Szeglet, P., 1999. Carbohydrate and nutrient content in rhizomes of *Phragmites australis* from different habitats of lake Ferto/Neusiedlersee. Limnologica 29, 47-59.

Engelaar, W.M.G.H., Symens, J.C., Laanbroek, H.J., Blom, C.W.P.M., 1995. Preservation of nitrifying capacity and nitrate availability in waterlogged soils by radial oxygen loss from roots of wetland plants . Biol. Fert. Soils 20, 243-248.

Hecht, U., Mohr, H., 1990. Factors controlling nitrate and ammonium accumulation in mustard (*Sinapis-alba*) seedlings . Physiol. Plantarum 78, 379-387.

Hroudová, Z., Zákravský, P., 1999. Vegetation dynamics in a fishpond littoral related to human impact. Hydrobiologia 415, 139-145.

Kubín, P., Melzer, A., 1996. Does ammonium affect accumulation of starch in rhizomes of *Phragmites australis* (Cav) Trin ex Steud? Folia Geobot. Phytotx. 31, 99-109.

Kühl, H., Kohl, J.G., 1992. Nitrogen accumulation, productivity and stability of reed stands (*Phragmites australis* (Cav.) TRIN. ex Steudel) at different lakes and sites of the lake districts of Uckermark and Mark Brandenburg (Germany). Int. Rev. Ges. Hydrobio. 77, 85-107.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. Academic Press. London.

Munzarová, E., Lorenzen, B., Brix, H., Vojtíšková, L., Votrubová, O., 2006. Effect of NH_4^+/NO_3^- availability on nitrate reductase activity and nitrogen accumulation in wetland helophytes *Phragmites australis* and *Glyceria maxima*. Environ. Exp. Bot. 55, 49-60.

Smolders, A.J.P., Roelofs, J.G.M., den Hartog, C., 1996. Possible causes for the decline of the water soldier (*Stratiotes aloides* L) in the Netherlands. Arch. Hydrobiol. 136, 327-342.

Steinbachová-Vojtíšková, L., Tylová, E., Soukup, A., Novická, H., Votrubová, O., Lipavská, H., Čížková, H., 2006. Influence of nutrient supply on growth, carbohydrate, and nitrogen metabolic relationsin *Typha angustifolia*. Environ. Exp. Bot. 57, 246-257.

Touchette, B.W., Burkholder, J.M., 2000. Review of nitrogen and phosphorus metabolism in seagrasses. J. Exp. Mar. Biol. Ecol. 250, 133-167.

Tylová-Munzarová, E., Lorenzen, B., Brix, H., Votrubová, O., 2005. The effects of NH_4^+ and NO_3^- on growth, resource allocation and nitrogen uptake kinetics of *Phragmites australis* and *Glyceria maxima*. Aquat. Bot. 81, 326-342.

Vojtíšková, L., Munzarová, E., Votrubová, O., Říhová, A., Juřicová, B., 2004. Growth and biomass allocation of sweet flag (*Acorus calamus* L.) under different nutrient conditions. Hydrobiologia 518, 9-22.

Table 1

Biomass allocation of *Phragmites australis* and *Glyceria maxima* grown with either NH_4^+-N or NO_3^--N at a solution concentration of 179 µM and 3700 µM. Values given are means ± STD (n = 6-19). A/B – ratio of aboveground (shoot) to belowground (roots and rhizomes) biomass, S/R – ratio of root-supported tissue (shoot, rhizomes) to root biomass, Sh - shoot, Rh – rhizome, R – roots. Figures in bold indicate $P<0.05$ (ANOVA); [a] % total dry wt.

	Phragmites australis		*Glyceria maxima*		*P*-values		
	NH_4^+	NO_3^-	NH_4^+	NO_3^-	species	treatment	sp. x tr.
Solution N concentration 179 µM							
A/B-ratio	2.0±0.5	2.0±0.5	3.7±0.6	3.9±0.6	**<0.001**	0.674	0.309
S/R-ratio	6.8±1.6	6.3±1.6	8.0±1.6	7.9±0.8	**0.002**	0.527	0.586
SH-biomass (%)[a]	66.0±6.5	65.2±5.8	78.3±2.9	79.3±2.5	**<0.001**	0.933	0.507
Rh-biomass (%)[a]	20.6±4.2	20.5±4.0	10.2±1.8	9.4±2.7	**<0.001**	0.630	0.726
R-biomass (%)[a]	13.4±3.0	14.3±3.2	11.6±2.2	11.3±1.0	**0.002**	0.641	0.423
Solution N concentration 3700 µM							
A/B-ratio	2.6±0.6	3.0±1.0	2.0±0.2	1.9±0.3	**0.007**	0.509	0.349
S/R-ratio	6.8±2.1	7.9±1.9	4.8±0.8	3.9±0.3	**<0.001**	0.854	0.152
SH-biomass (%)[a]	71.4±4.8	74.0±5.1	66.8±1.8	65.7±3.4	**0.002**	0.697	0.311
Rh-biomass (%)[a]	14.9±5.0	14.2±5.5	15.6±3.6	14.0±2.9	0.892	0.575	0.801
R-biomass (%)[a]	13.7±3.1	11.8±2.3	17.5±2.2	20.3±1.2	**<0.001**	0.690	**0.032**

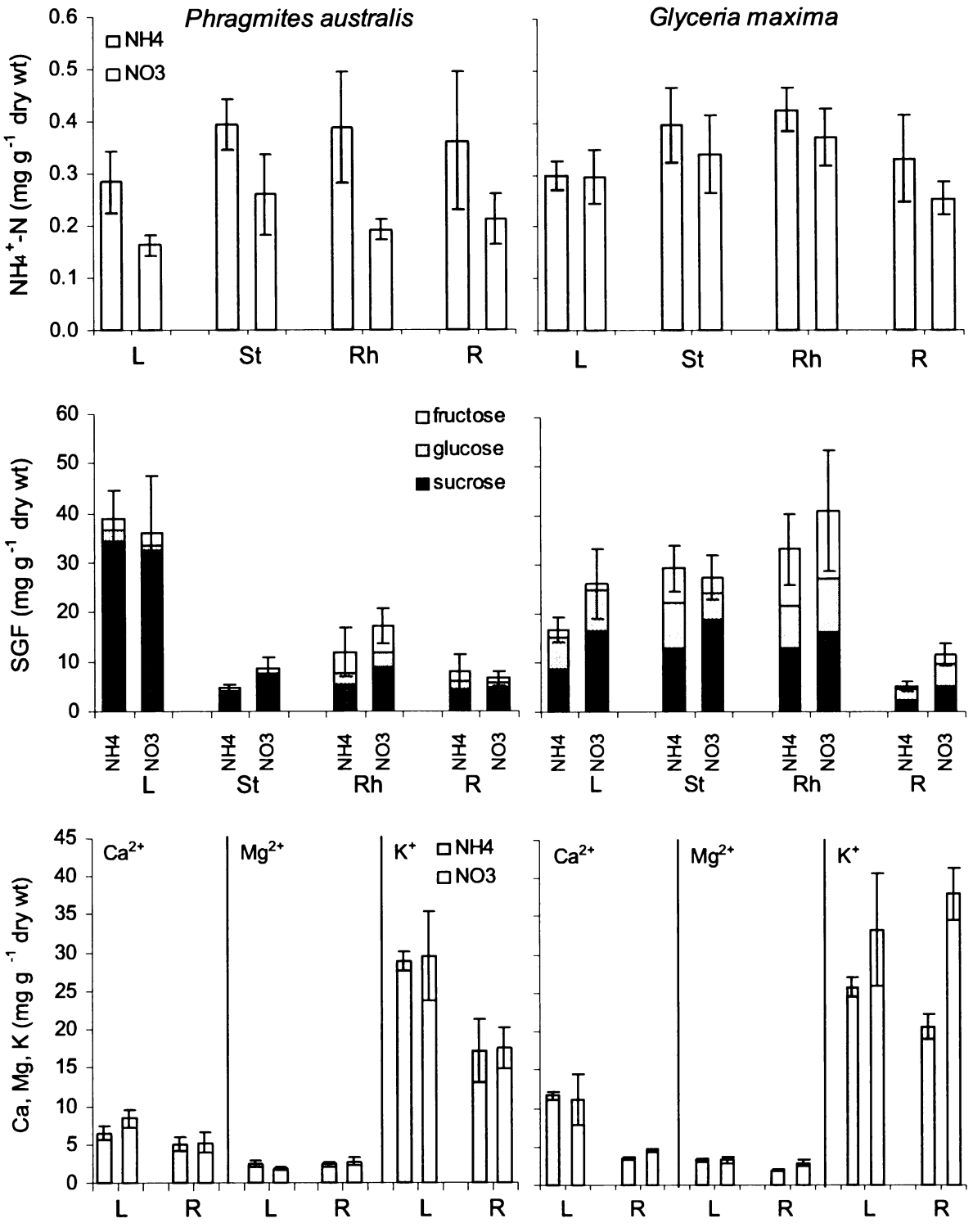
Table 2

Characteristics of C and N metabolism in *Phragmites australis* and *Glyceria maxima* grown with either NH_4^+-N or NO_3^--N at a solution concentration of 179 µM. Values given are means ± STD (n = 3-5). C/N – C/N atomic ratio; ratio of hexoses (glucose, fructose) to sucrose; TNC – total non-structural carbohydrates (sum of starch, glucose, fructose, sucrose; mg g^{-1} dry wt); starch (mg g^{-1} dry wt); ratio of starch to soluble carbohydrates (sum of glucose, fructose, sucrose). Figures in bold indicate $P<0.05$ (ANOVA).

		Phragmites australis		*Glyceria maxima*		*P*-values		
	Plant component	NH_4^+	NO_3^-	NH_4^+	NO_3^-	species	treatment	sp. x tr.
C/N	Leaf	9.0±0.3	10.7±0.7	12.0±0.7	11.1±0.6	**0.001**	0.242	**0.003**
	Stem	14.3±1.1	16.6±0.7	15.1±1.2	12.7±1.1	**0.030**	0.972	**0.002**
	Rhizome	31.0±4.0	31.4±5.0	18.2±0.8	17.0±1.1	**<0.001**	0.855	0.707
	Root	20.7±2.1	19.3±2.1	19.5±2.0	12.8±0.9	**0.007**	**0.005**	**0.045**
hexoses/sucrose	Leaf	0.1±0.0	0.1±0.0	1.0±0.2	0.6±0.1	**<0.001**	**0.016**	**0.023**
	Stem	0.3±0.1	0.2±0.3	1.3±0.2	0.5±0.1	**<0.001**	**0.002**	**0.004**
	Rhizome	1.1±0.7	1.0±0.3	1.6±0.5	1.5±0.4	0.098	0.670	0.893
	Root	0.7±0.2	0.4±0.1	1.4±0.3	1.3±0.2	**<0.001**	0.164	0.606
TNC	Rhizome	20.1±5.6	58.5±6.3	41.9±6.8	56.3±18.0	0.281	**0.014**	0.194
	Root	15.6±3.5	14.1±1.4	14.3±1.1	19.9±2.2	0.103	0.128	**0.018**
starch	Rhizome	8.9±0.7	28.0±14.3	12.2±1.4	15.6±4.9	0.245	**0.009**	0.055
	Root	7.6±0.8	7.4±0.1	9.0±1.7	8.4±0.6	0.071	0.501	0.722
starch/soluble carbohydrates	Rhizome	1.0±0.4	2.8±0.6	0.4±0.1	0.4±0.0	**<0.001**	**0.006**	**0.005**
	Roots	1.2±0.3	1.2±0.3	1.8±0.6	0.8±0.2	0.712	0.072	0.071

Legends to figures

Fig. 1. Contents of NH_4^+, soluble carbohydrates (glucose, fructose and sucrose), and Ca^{2+}, Mg^{2+}, K^+ in tissues of *Phragmites australis* and *Glyceria maxima* grown with NH_4^+-N or NO_3^--N at 179 μM total N level. Values given are means ± STD (n = 3-5). L – leaves, St – stems, Rh – rhizome, R – roots.



Tylová et al.
Figure

Phenology and autumnal accumulation of N reserves in belowground organs in two co-occurring helophytes with different dormancy requirement, *Phragmites australis* and *Glyceria maxima*, under nutrient surplus.

Edita Tylová[a*], Lenka Steinbachová[a], Olga Votrubová[a], Vít Gloser[b]

a) *Department of Plant Physiology, Faculty of Science, Charles University in Prague, Viničná 5, 128 44 Prague 2, Czech Republic*

b) *Department of Plant Physiology and Anatomy, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic*

*Corresponding author. Tel.: +420 22195-1697; fax: +42-22195-1704.
E-mail address: edmunz@natur.cuni.cz (E. Tylová)

Complete correspondence address to which the proofs should be sent:
Edita Tylová, Department of Plant Physiology, Faculty of Science, Charles University in Prague, Viničná 5, 128 44 Prague 2, Czech Republic

Abstract

Two co-occurring dominant wetland helophytes and potential competitors, *Phragmites australis* and *Glyceria maxima*, were cultivated under N, P availabilities simulating the trophic status of wetlands with different fertility (oligo- and eutrophic). The long-term outdoor cultivation was performed with the goal to characterise the extent to which the nutrient enrichment affects plant growth, phenology, and particularly, the accumulation of N storage compounds in belowground organs of wetland rhizomateous plants prior the onset of winter dormancy. Selected species differ in their dormancy requirement and sensitivity towards severe eutrophication, but in the present study, they responded similarly towards nutrient surplus. The enhanced growth, delayed shoot senescence, and delayed retranslocation of N into belowground organs were found in more fertile treatment. Furthermore, N levels remaining in dry leaves were proportionally related to those of living ones, being significantly higher in eutrophic treatment. The efficiency of N retranslocation from senescing leaves was 60% in both species and treatments. The formation of N reserves was, however, not disrupted in either species. Although plants in eutrophic treatment accumulated N in their belowground organs significantly later in the season (in period September-December), the amount of accumulated N was sufficient to reach high belowground N standing stock. Therefore, the lost of N with N-rich litterfall, observed repeatedly at eutrophic habitats, obviously does not increase risk of N shortage in over-wintering organs in these particular species, but rather endangers plants indirectly, as the decay of N-rich litter facilitates eutrophication of the given habitat.

Considering formation of N reserves, the differences in species response to treatments were negligible. *Phragmites* and *Glyceria* accumulated similar belowground N standing stock prior the winter. *Glyceria* may, however, additionally profit from N-standing stock of over-wintering green leaves and from the potential of growth and N assimilation during mild winter period, which is not possible in fully dormant *Phragmites*.

Keywords: Phragmites; *Glyceria*; Eutrophication; Wetlands; Phenology; Reserve Formation; Rhizome; Free Amino Acids, Retranslocation; Storages

1. Introduction

Wetlands, habitats serving irreplaceable ecological functions, are constantly exposed to anthropogenic press and undergo changes in species occurrence worldwide. Even dominant species are affected; common reed (*Phragmites australis* (Cav.) Trin. ex Steudel) declined at many European water bodies during last decades (for summary see e.g. Ostendorp, 1989; van der Putten, 1997; Brix, 1999), while non-native reed clones concurrently invaded North American wetlands (Chambers et al., 1999). The nutrient enrichment is the general triggering factor, but the underlying mechanisms are obviously various and mutually interacting (Ostendorp, 1989; van der Putten, 1997). Although the indirect effects of eutrophication (litter accumulation and phytotoxicity of its degradation products) are apparently main reasons of reed die-back (Brix, 1999), direct impacts on plant phenology, resource allocation and storage formation are repeatedly discussed (Čížková-Končalová et al., 1992; Kühl and Kohl, 1993; Woitke et al., 1997).

In perennial helophytes, the amount of C and N reserve substances in belowground organs is one of the critical factors, which determines the success of over-wintering, spring outgrowth and affects plant susceptibility to different stresses, e.g. floods or mechanical disturbances (Čížková-Končalová et al., 1992). Several studies dealing with *Phragmites* showed thinned C reserves (particularly carbohydrates) at eutrophic habitats (Kubín et al., 1994; Čížková et al., 1996; Čížková et al., 2001), caused by the decrease of actual carbohydrate levels in response to N surplus (Čížková-Končalová et al., 1996; Kohl et al., 1998) as well as by the prolonged vegetative growth and delayed autumnal translocation to belowground organs (Kohl et al., 1998).

Similarly to C reserves, the delayed translocation of N rich compounds into rhizome was observed at unstable *Phragmites* stands. Particularly the delayed translocation of free amino acids (FAA) a particularly of asparagine (Asn), which are the dominant N transport and storage compounds in *Phragmites* (Haldemann and Brändle, 1988; Woitke et al., 1997; Kohl et al., 1998), was commonly found at eutrophic sites (Kühl and Kohl, 1993; Lippert et al., 1999) and indicated also by our previous experiments dealing with *Phragmites* (Tylová et al., unpublished results). As the amount of winter N storages was shown to be important factor affecting e.g. rate of spring outgrowth and thus the success in the competition for space (Gloser, 2005), the disrupted accumulation of N in over-wintering organs may weaken plants at eutrophic habitats considerably. However, it is not clear, whether the observed delay in N retranslocation may really endanger N storage function of reed rhizomes, since plants

generally respond to N surplus by the shift in C/N balance in favour of N assimilation (Marschner, 1995), increasing N and FAA status of their tissues (in reed e.g. Kohl et al., 1998). This shift in internal C/N balance may compensate weak retranslocation of N under these conditions, which is indicated e.g. by studies of Woitke et al. (1997) or Lippert et al. (1999). These authors observed comparable autumnal N contents in reed at sites of different fertility and productivity, although the retranslocation of N was more conspicuous at less fertile habitats (Kühl and Kohl, 1993; Lippert et al., 1999). Dinka and Szeglet (1999) detected higher N levels in rhizomes at dieback compared to vigorous reed stands in autumn.

Autumnal formation of belowground N storages (and the effect of eutrophication on this process) might, however, be of different importance in species with dissimilar dormancy requirement. This situation occurs in *Phragmites* and the co-occurring helophyte *Glyceria maxima* Hartm. Holmb., which is the competitor of reed, particularly at eutrophic habitats. While *Phragmites* has strong dormancy requirement, *Glyceria* possesses only weak winter dormancy, its leaves can remain physiologically active during mild winter (Westlake, 1966; Dykyjová, 1978; Květ et al., 1999) and spring outgrowth is earlier compared to *Phragmites* (Buttery and Lambert, 1965; Dykyjová, 1978). The formation of storages thus might be less critical in *Glyceria*, which is also more tolerant to heavily loaded habitats compared to *Phragmites* (Brändle et al., 1996; Crawford and Brändle, 1996; Hroudová and Zákravský, 1999), and its spread following reed retreat was repeatedly documented (Ostendorp, 1989; van der Putten, 1997; Hroudová and Zákravský, 1999). It is possible, that the difference in timing or efficiency of autumnal N accumulation in storage organs affects the competition of these two species at eutrophic habitats, but the experimental data are lacking. Our study therefore focuses on the relationships among sediment fertility, plant growth, phenology, and internal N dynamics of *Phragmites* and *Glyceria* during late summer till early winter (August-December). Two major questions are followed in simplified long-term sand cultivation: i, does the nutrient surplus affect autumnal plant development and N storage capacity of *Phragmites* and *Glyceria*? ii, is there any difference in species response, which may affect the competition of selected species at eutrophic habitats?

2. Methods

2.1. Plant material

The mother plants came from the littoral stands in the Třeboň basin, Czech Republic, but they were long-term vegetatively propagated in outdoor sand culture in the Institute of Botany, Academy of Sciences, Třeboň, Czech Republic. Rootless rhizome cuttings (10 cm) with one shoot (15-20 cm) were cultivated three weeks in coarse sand and irrigated with tap water prior the transfer into the experimental culture.

2.2. Experimental setup and growth conditions

The long-term sand cultivation (May-December) was designed to study the effects of N plus P availabilities on plant performance, phenology, and belowground N storage formation. Plants were cultivated outdoors protected by a transparent plastic roof. The seasonal temperature course is given in Fig. 1; the data were obtained from Czech Hydrometeorological Institute (www.chmi.cz), station Prague-Karlov (located close to the experimental site).

Each cutting was planted individually in a 15 L plastic pot filled with pure coarse quartz sand. Twelve pots per treatment were placed in a plastic container with 185 L of the nutrient solution. The solution was renewed every 3 weeks; the pH was adjusted to 6.8 using 2M HCl. The water level was kept at the surface of sand. The surface of sand was covered by expanded ceramic aggregate (Keramzit) and the exposed solution by black non-woven textile to prevent algae growth.

Two treatments were defined to simulate trophic conditions in south Bohemia fishpond littorals with low (oligotrophic treatment) and high (eutrophic treatment) fertility. The treatments differed in chemical composition, especially in total N and P levels. The following chemical composition was identical in both treatments (μM): Mg^{2+} 390.67; SO_4^{2-} 390.67; BO_3^{3-} 0.23; Fe^{2+} 20.42; Mn^{2+} 0.71; Zn^{2+} 0.01; Cu^{2+} 0.003; $Mo_7O_{24}^{2-}$ 0.001. In addition to above mentioned chemicals, the oligotrophic treatment received (μM): NH_4^+ 4.30; NO_3^- 21.94; PO_4^{3-} 1.01; K^+ 499.72; Ca^{2+} 766.82 and Cl^- 2014.67; and eutrophic treatment received: NH_4^+ 443.86; NO_3^- 2193.69; PO_4^{3-} 99.92; K^+ 104.92; Ca^{2+} 1096.06; and Cl^- 443.55.

2.3. Plant growth and harvest

Plant growth was continuously monitored during the whole experimental period by shoot counting. The numbers of newly emerging tillers, well-developed shoots, and senescent shoots were counted weekly. The incidence of flowering was registered.

Plants were harvested three times during the experiment (5.8., 16.9., and 8.12.) to follow phenology. Plants were carefully taken out from the cultivation pots and washed. Immediately after, the samples (±1.5 g fresh wt) of living leaf blades, dead leaf blades (December harvest), rhizomes, and roots were taken for the detection of free amino acids (FAA) and total N contents. The samples were weighed, frozen in liquid nitrogen, and stored at –80°C prior the analyses. Remaining plant biomass was fractionated into leaf blades, stems plus leaf sheaths, rhizomes, and roots. The fractions were dried (at 80°C) for the dry weight (dry wt) determination.

The biomass allocation was characterised using the ratio of root-supported tissue (shoot, rhizomes) to root biomass (S/R) and the ratio of aboveground (shoot) to belowground (roots, rhizomes) biomass (A/B). The allocations into different organs (leaf blades, stems plus leaf sheaths, rhizomes, and roots) were calculated (in percentage) as the ratio between the biomass of a particular fraction and the total plant dry biomass.

2.3. Analyses of free amino acids (FAA) and total N contents

Frozen samples were freeze-dried and grounded to fine powder (homogeniser Retsch, MM301, Germany). The total N contents were analysed using a Leco CNS 2000 analyser. Leaf N contents were used to estimate the retranslocation efficiency: ((mature living leaf N content in September – dead leaf N content in December)/mature living leaf N content in September)*100 and the retranslocation proficiency: dead leaf N content in December.

FAA were extracted with 0.01 M HCl and analysed using HPLC (Waters Alliance 2690XE, Waters 474 fluorescence detector, Milford, USA) after derivatisation with 9-fluorenylmethyl formate (FMOC); for details see Gloser (2002). The method did not allow separating Gaba and Pro, the sum of both is therefore presented in the text.

2.4. Statistical evaluation

Statistical evaluation was performed using NCSS 2000 and PASS 2000 software. The general effects of treatment, date, and interactions were analysed with the analysis of variance (Two-Way ANOVA). Statistics not given in figures and tables are noted in the text.

3. Results

3.1. Growth response to nutrient surplus

At the beginning of cultivation (May), plants possessed similar ($P>0.05$) starting weights (0.95 g dry wt per plant, mean of both species). Until December, oligotrophically treated plants reached 7.1±2.6 and 8.1±3.3 g dry wt. per plant; the biomass production in eutrophic treatment was significantly higher ($P<0.001$, both species): 183±36 and 243±88 g dry wt. per plant (in *Phragmites* and *Glyceria*, respectively) (Fig 2). Species showed similar annual productivity ($P>0.05$, Fig 2) as well as similar response to the nutrient regime ($P>0.05$). Plants in more fertile treatment entered the reproductive phase. The flowering started at 22.8. and 22.7. in *Phragmites* and *Glyceria*, respectively, but it was infrequent (±1 panicle per plant). In contrast, no flowering occurred in oligotrophic treatment.

The nutrient surplus stimulated allocation of plant biomass to aboveground structures, increasing A/B and S/R ratios in both *Phragmites* and *Glyceria* (Table 1), but the species differed considerably in the distribution of biomass among belowground storage organs (rhizomes and roots). *Phragmites* generally allocated more biomass into rhizomes, which formed 16-49% of total plant dry wt. compared to 7-12% in *Glyceria* ($P<0.05$; Table 1), but this allocation considerably decreased in eutrophic treatment, particularly during period of intensive growth. In contrast, *Glyceria* allocated biomass preferentially into roots, particularly in oligotrophic treatment (Table 1). In both species and treatments, the relative proportion of belowground organs gradually increased during autumn, but this trend was more conspicuous in *Phragmites* (Table 1).

3.2. Onset of winter dormancy

In both species, nutrient surplus prolonged growth period (delayed shoot senescence), but the effect was more conspicuous in *Phragmites*. In this species, shoot senescence started in oligotrophic treatment in September, and the percentage of dead shoots reached 90% in November. In contrast, shoots of eutrophically treated plants remained green (<1% of dead shoots; calculated without tillers) until mid November ($P<0.05$), when the frost occurrence (Fig. 1) triggered massive and fast shoot senescence (Fig. 3). Surprisingly, similar frost occurrence in late October (Fig 1) did not affect plant growth considerably (Fig. 3). In *Phragmites*, shoot senescence almost proceeded until December in both treatments. The percentage of living shoots was 4 and 7% in December in oligotrophic and eutrophic treatment ($P>0.05$) (Fig. 3), which represented 4 and 14% of total shoot dry wt (Fig. 2),

respectively. The availability of nitrogen plus phosphorus affected also the ability to produce dormant aboveground tillers in *Phragmites* during late autumn ($P<0.05$). These tillers were found only in plants grown in eutrophic treatment and emerged in period September-October (Fig. 3).

In agreement with the general growth strategy, *Glyceria* exhibited less obvious transition into dormancy than *Phragmites*. Although the senescence of *Glyceria* shoots was again more pronounced in oligotrophic treatment ($P<0.01$), 62 and 85% shoots were still living in December in oligotrophic and eutrophic treatment, respectively (Fig. 3). The proportion of living shoot biomass thus represented 16 and 35% of total shoot dry wt., which was significantly higher compared to *Phragmites* ($P<0.001$, Fig. 2). In *Glyceria*, the frost occurrence in November did not accelerate shoot senescence markedly. *Glyceria* tillers emerging in late autumn in eutrophic treatment were not dormant, their growth continued till the end of the experiment in December (Fig. 3).

3.3. Autumnal N dynamics in leaves

Both species increased N status of living leaves in response to nutrient surplus; higher total N levels (Table 2) as well as levels of free amino acids (FAA; Fig. 4; Table 3) were found in eutrophic compared to oligotrophic treatment during the whole experimental period. During summer, leaf FAA levels were, however, low and FAA formed less than 2% of total N content in leaves in both species and treatments (Fig. 4; Table 3), with Glu as the most abundant leaf FAA in *Glyceria* (both treatments) and eutrophicaly treated *Phragmites*. Asp dominated in leaves of *Phragmites* in oligotrophic treatment (Table 4).

During autumn, the gradual accumulation of FAA, particularly of Asn and Gaba+Pro, occurred in living leaves (Fig. 4; Table 3), but the total N levels did not significantly change. Shoots also entered the senescence process, which was accompanied with the retranslocation of significant amounts of N-rich compounds. In leaves, the retranslocation efficiency varied around 60%, but did not differ between species ($P>0.05$) or in response to treatments ($P>0.05$). N levels remaining in dead leaves (the retranslocation proficiency) were therefore significantly higher in more fertile treatment in both species (Table 2). Considering the differences in biomass, the amount of N remaining in dead leaves (N standing stock) in December was 0.24 ± 0.02 and 0.79 ± 0.22 g N per plant in eutrophic treatment, but only 0.005 ± 0.0009 and 0.009 ± 0.003 g N per plant in oligotrophic treatment ($P<0.001$), in

Phragmites and *Glyceria*, respectively. Dead leaves contained also significant amounts of FAA (Fig. 4; Table 3), but the composition of FAA pool differed considerably from living ones; Gln was the predominant FAA in dead leaves of both species, particularly in eutrophic treatment (Table 4). The proportion of leaves, which remained living until December was low, especially in *Phragmites* (see Table 1), but these living leaves contained considerably higher FAA levels compared to summer ($P<0.05$) (Fig. 4; Table 3), with Asn as the predominant amino acid (Table 4).

3.3. Autumnal N dynamics in belowground organs

In both species, belowground organs (rhizomes and roots) accumulated N during autumn, but the accumulation was considerably more intensive in eutrophic compared to oligotrophic treatment (Table 2). While N status of belowground organs differed only slightly between treatments in summer, the intensive autumnal N accumulation resulted in significantly higher total N levels of eutrophically treated plants in December, particularly in *Phragmites* (Table 2). In *Glyceria*, similar trend was found only in roots but not rhizomes (Table 2). N standing stock of belowground organs prior the winter was therefore significantly higher in more fertile treatment ($P<0.001$), being 0.059±0.004 and 0.055±0.004 g N per plant in oligotrophic and 1.77±0.024 and 1.29±0.39 g N per plant in eutrophic treatment, in *Phragmites* and *Glyceria*, respectively. However, no statistically significant difference between species was found in this parameter in either treatment ($P>0.05$), the species only differed in the allocation of N between rhizomes and roots. While in *Phragmites*, 63 and 58% of belowground N standing stock was allocated in rhizomes in December; *Glyceria* rhizomes contained only 43 and 20%, in oligotrophic and eutrophic treatment, respectively.

The levels of FAA followed similar trends (Fig. 4; Table 3); FAA and particularly Asn gradually accumulated during autumn, and the accumulation was considerably more intensive in eutrophic treatment (Fig. 4; Table 3). Moreover, the changes in FAA status observed in belowground organs of both species indicated different timing of N storage formation between the treatments. Rhizomes and roots of oligotrophically treated plants contained high amounts of FAA already in August; FAA formed about 20% of rhizome N and 5-14% of root N pool in both species, and this proportion changed only slightly later in the season (Fig. 4; Table 3). In contrast, only 3-8% of N in rhizomes and less than 2% in roots were allocated in

FAA in eutrophic treatment in August (both species), but the allocation increased up to 16-25% and 13-16%, in rhizomes and roots, respectively, in December (Fig. 4; Table 3).

Asn was the predominant FAA in belowground organs of both species regardless the harvest date and treatment (Table 5); the changes in its absolute levels affected the overall FAA fluctuation of given tissues considerably. While in August, high Asn levels were found in belowground organs of oligotrophically treated plants ($P<0.05$), intensive Asn accumulation till the end of vegetation season occurring in eutrophic treatment reversed this trend (Fig. 4, Table 3). Asn was clearly the most important FAA accumulated prior the winter, carrying 12-18 and 8-9% of total N content of rhizomes and roots, respectively, in December; with no difference between species or treatments ($P>0.05$).

4. Discussion

Although differences in growth strategy, sensitivity to hyperfertilization, and intensity of winter dormancy were previously described between *Phragmites* and *Glyceria* (Buttery and Lambert, 1965; Brändle et al., 1996; Crawford and Brändle, 1996; Hroudová and Zákravský 1999), both species responded very similarly towards nutrient surplus in the present study. Eutrophic treatment enhanced biomass production, prolonged vegetative growth, and delayed autumnal translocation of N-rich compounds into belowground organs. This delay was clearly visible in changes of belowground N status occurring from late summer to early winter in both species. While roots and rhizomes of oligotrophically treated plants showed relatively high N status already in August-September, and this parameter changed only slightly later in the season, belowground organs in eutrophic treatment accumulated the majority of N in period September-December. The delayed translocation of N was visible also in the fluctuation of free amino acids (FAA) levels in plant tissues and coincided with the late onset of shoot senescence in eutrophic treatment.

Although other nitrogenous compounds (e.g. soluble proteins) are also involved (Haldemann and Brändle, 1986), FAA and particularly Asn are highly important N-rich compounds for transport and storage of N in *Phragmites* as well as other wetland species, e.g. *Typha angustifolia*, *Schoenoplectus lacustris* (Haldemann and Brändle, 1988; Woitke et al., 1997; Kohl et al., 1998). Asn and Gln are also the dominant products of protein breakdown in senescing leaves (Dangl et al., 2000). Therefore, the changes in FAA pool present good indicator of plant internal N status as well as N transport processes. In *Phragmites*, FAA accumulation in belowground organs starts in July-September (Fiala, 1976; Graneli et al.,

1992; Kühl and Kohl, 1993); at low fertile sites the process is terminated early (even within July), at more nutritive sites it was observed to continue until late autumn (Kohl et al., 1998). In agreement, oligotrophically treated plants contained high Asn levels in belowground organs already in August, and FAA formed significant portion of the belowground allocated N, with minor changes later in the season. Obviously, the retranslocation of N into belowground organs was almost finished in this treatment until September, which agreed with the simultaneous shoot senescence. In contrast, low FAA levels occurring in rhizomes and roots of eutrophically treated plants until September clearly indicate the delayed onset of N retranslocation process due to the prolonged vegetative growth, which was similarly observed at eutrophic wetland habitats e.g. by Kühl and Kohl (1992) or Lippert et al. (1999).

Besides the delayed translocation of N into belowground organs, eutrophically treated plants showed also higher N levels in dead leaves at the beginning of winter period, which resemble the observations from eutrophic reed habitats (Kühl and Kohl, 1993; Lippert et al., 1999). The levels were proportionally related to N status of living mature leaves, which was similarly found in other wetland graminoids by Miao (2004) or in large data set of different species by Kobe et al. (2005). As the content of N remaining in senesced leaves (N proficiency) is one of important indicators of N retranslocation success (Killingbeck, 1996; Kohl et al., 1998), eutrophically treated plants exhibited less efficient N conservation. They obviously did not realise their maximum (potential) resorption (Killingbeck, 1996) and leaved the nutrient cycle more open (Boar, 1996; Kohl et al., 1998; Lippert et al., 1999), as N allocated in the litter is temporarily lost until its decomposition (Boar, 1996). Probably even more important are the negative consequences to sediment properties. The decay of N-rich litter releases considerable amounts of N into wetland, facilitating eutrophication. The occurrence of different stressors, e.g. phytotoxic compounds or herbivores (Votrubová et al., 1997; Armstrong and Armstrong, 2001), might subsequently induce the damage of belowground organs and lost of reserves (more than 70% of rhizome biomass was found dead at die-back sites by Dinka and Szeglet (2001)).

In spite of all above-mentioned facts (the delayed retranslocation, higher N loses with litterfall), it was evident that belowground N reserves, one of the prerequisites of wintering and spring outgrowth in flooded habitats, were not shortened in response to nutrient enrichment. In both species, eutrophically treated plants reached significantly higher belowground N standing stock prior the winter. Obviously, although the majority of N was translocated to belowground organs very late in the season, the total amount of resorbed N was high due to high aboveground N standing stock accumulated during the period of growth,

as was similarly summarised for wooden plants by Aerts (1996). High external N availability during late autumn might also cooperate on the observed results, as N taken up from external solution in this period obviously contributes to the formation of N storages in grasses (Gloser, 2005), with the positive effect on plant development in the following season (Gloser, 2005).

The fraction of leaf N retranslocated during senescence was similar in both treatments (both species), in spite of different N status of plants. This finding agrees with general conclusions of Aerts (1996), who found resorption efficiency only weakly controlled by plant internal nutritional status, varying around 50% across different species or growth forms. The underlying physiological mechanisms of leaf senescence (e.g. ratio of soluble/insoluble N compounds (Pugnaire and Chapin, 2005), phloem transport (Chapin and Moilanen, 2005)) probably determine resorption efficiency rather than plant nutritional status *per se* (Aerts, 1996). The comparable extent of retranslocation (±60%) in both species and treatments in the present study also showed that earlier start of retranslocation, observed repeatedly at meso- compared to eutrophic habitats (Kühl and Kohl, 1993; Lippert et al., 1999), did not necessarily result in greater efficiency of this process. This was apparent namely in eutrophically treated *Phragmites*, in which 60% of N was retranslocated from shoots, although these shoots were dying off very sharply after the frost occurrence. The 60% efficiency is even higher compared to 0-50% already documented for shoots of *Phragmites* at natural habitats of different fertility (Boar, 1996; Kohl et al., 1998). The direct comparison is however inaccurate, since N retranslocation from leaf blades, calculated in the present study, is generally higher compared to those of whole shoots (Aerts, 1996).

Considering the formation of belowground N reserves in response to treatments, the differences between *Phragmites* and *Glyceria* were negligible, although the dormancy requirement is obviously stricter in *Phragmites* compared to *Glyceria* (Buttery and Lambert, 1965). Both species exhibited comparable annual productivity and accumulated similar belowground N standing stock prior the winter. *Glyceria*, however, may additionally profit from N-standing stock of over-wintering green leaves and from the potential of growth and N assimilation during mild winter period, which is not possible in fully dormant *Phragmites*. Furthermore, as *Glyceria* reaches the seasonal rhizome FAA maximum in February compared to October/November in *Phragmites* (Haldemann and Brändle, 1988), shoot senescence and N downward transport seem to gradually continue, improving N storages in this species during the winter period and, particularly, improving the supplementation of N resources for its early sprouting at the beginning of new vegetation season.

Species, however, differ considerably in the distribution of belowground stored N between rhizomes and roots. Although rhizome is generally considered as the principal storage organ in wetland clonal helophytes (Klimeš et al., 1999), which valids for *Phragmites* (Fiala, 1976), roots are of the particular importance in *Glyceria*. In this species, roots formed important portion of belowground biomass (see also Westlake (1966)), contained 57-80% of N allocated belowground and possessed even higher FAA levels compared to rhizomes (particularly in eutrophic treatment) at the beginning of winter period. High root N storage under eutrophic conditions thus apparently allows this species to compensate generally lower allocation of biomass into belowground structures. In contrast, *Phragmites* allocated only 37-42% of belowground N into roots, and according to Fiala (1976), roots growth is even restricted in period July-August, when rhizome is filled with the reserve material.

Acknowledgement

Authors would like to thank Dr. Hana Čížková (Institute of Landscape Ecology, Academy of Sciences of the Czech Republic, Třeboň) for the valuable discussion; and Drahomíra Bartáková (Department of Plant Physiology, Faculty of Science, Charles University in Prague) for the technical assistance.

References

Aerts, R., 1996. Nutrient resorption from senescing leaves of perennials: are there general patterns? J. Ecol. 84, 597-608.

Armstrong, J., Armstrong, W., 2001. An overview of the effects of phytotoxins on *Phragmites australis* in relation to die-back. Aquat. Bot. 69, 251-268.

Boar, R.R., 1996. Temporal variations in the nitrogen content of *Phragmites australis* (Cav.) Trin. ex Steud. from a shallow fertile lake. Aquat. Bot. 55, 171-181.

Brändle, R., Pokorný, J., Květ, J., Čížková, H., 1996. Wetland plants as a subject of interdisciplinary research. Folia Geobot. Phytotx. 31, 1-6.

Brix, H., 1999. The European Research Project on Reed Die-Back and Progression (EUREED). Limnologica 29, 5-10.

Buttery, B.R., Lambert, J.M., 1965. Competition between *Glyceria maxima* and *Phragmites communis* in the region of Surlingham broad. 1. The competition mechanism. J. Ecol. 53, 163-181.

Chambers, R.M., Meyerson, L.A., Saltonstall, K., 1999. Expansion of *Phragmites australis* into tidal wetlands of North America. Aquat. Bot. 64, 261-273.

Chapin, F.S., Moilanen, L., 2005. Nutritional controls over nitrogen and phosphorus resorption from Alaskan birch leaves. Ecology 72, 709-715.

Čížková, H., Istvanovics, V., Bauer, V., Balazs, L., 2001. Low levels of reserve carbohydrates in reed (*Phragmites australis*) stands of Kis-Balaton, Hungary. Aquat. Bot. 69, 209-216.

Čížková, H., Lukavská, J., Přibáň, K., Kopecký, J., Brabcová, H., 1996. Carbohydrate levels in rhizomes of *Phragmites australis* at an oligotrophic and a eutrophic site: A preliminary study. Folia Geobot. Phytotx. 31, 111-118.

Čížková-Končalová, H., Květ, J., Lukavská, J., 1996. Response of *Phragmites australis*, *Glyceria maxima*, and *Typha latifolia* to additions of piggery sewage in a flooded sand culture. Wetlands Ecol. Manage. 4, 43-50.

Čížková-Končalová, H., Květ, J., Thompson, K., 1992. Carbon starvation - a key to reed decline in eutrophic lakes. Aquat. Bot. 43, 105-113.

Crawford, R.M.M., Brändle, R., 1996. Oxygen deprivation stress in a changing environment. J. Exp. Bot. 47, 145-159.

Dangl, J.L., Dietrich, R.A., Thomas H., 2000. Senescence and programmed cell death. In Buchanan, B., Gruissem, W., Jones, R. (Eds), Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists, pp. 1044-1100.

Dinka, M., Szeglet, P., 1999. Carbohydrate and nutrient content in rhizomes of *Phragmites australis* from different habitats of lake Ferto/Neusiedlersee. Limnologica 29, 47-59.

Dinka, M., Szeglet, P., 2001. Some characteristics of reed (*Phragmites australis /Cav./ Trin ex Steudel*) that indicate different health between vigorous and die-back stands. Verh. Internat. Verein. Limnol. 27, 3364-3369.

Dykyjová, D., 1978. Nutrient uptake by littoral cmmunities of helophytes. In Dykyjová, D., Květ, J. (Eds), Pond Littoral Ecosystems-Structure and Functioning. Springer-Verlag Berlin Heidelberg New York, pp. 257-277.

Fiala, K., 1976. Underground organs of *Phragmites communis*, their growth, biomass and net production. Folia Geobot. Phytotx. 11, 225-259.

Gloser, V., 2005. The consequences of lower nitrogen availability in autumn for internal nitrogen reserves and spring growth of *Calamagrostis epigejos*. Plant Ecol. 179, 119-126.

Graneli, W., Weisner, S.E.B., Sytsma, M.D., 1992. Rhizome dynamics and resource storage in *Phragmites australis*. Welants Ecol. Manage. 1, 239-247.

Haldemann, C., Brändle, R., 1986. Seasonal variations of reserves and of fermentation processes in wetland plant rhizomes at the natural site. Flora 178, 307-313.

Haldemann, C., Brändle, R., 1988. Amino-acid composition in rhizomes of wetland species in their natural habitat and under anoxia. Flora 180, 407-411.

Hroudová, Z., Zákravský, P., 1999. Vegetation dynamics in a fishpond littoral related to human impact. Hydrobiologia 415, 139-145.

Killingbeck, K.T., 1996. Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. Ecology 77, 1716-1727.

Klimeš, L., Klimešová, J., Čížková, H., 1999. Carbohydrate storage in rhizomes of *Phragmites australis*: the effects of altitude and rhizome age. Aquat. Bot. 64, 105-110.

Kobe, R.K., Lepczyk, C.A., Iyer, M., 2005. Resorption efficiency decreases with increasing green leaf nutrients in a global data set. Ecology 86, 2780-2792.

Kohl, J.G., Woitke, P., Kühl, H., Dewender, M., Konig, G., 1998. Seasonal changes in dissolved amino acids and sugars in basal culm internodes as physiological indicators of the C/N-balance of *Phragmites australis* at littoral sites of different trophic status. Aquat. Bot. 60, 221-240.

Kubín, P., Melzer, A., Čížková, H., 1994. The relationship between starch content in rhizomes of *Phragmites australis* (Cav) Trin ex Steud and trophic conditions of habitat. Proc. R. Soc. Edin. B 102, 433-438.

Kühl, H., Kohl, J.G., 1992. Nitrogen accumulation, productivity and stability of reed stands (*Phragmites australis* (Cav.) TRIN. ex Steudel) at different lakes and sites of the lake districts of Uckermark and Mark Brandenburg (Germany). Int. Rev. Ges. Hydrobiol. 77, 85-107.

Kühl, H., Kohl, J.G., 1993. Seasonal nitrogen dynamics in reed beds (*Phragmites australis* (Cav.) TRIN. ex Steudel) in relation to productivity . Hydrobiologia 251, 1-12.

Květ, J., Dušek, J., Husák, Š., 1999. Vascular plants suitable for wastewater treatment in temperate zones. In Vymazal, J. (Ed.), Nutrient Cycling and Retention in Natural and Constructed Wetlands. Backhuys Publishers, Leiden, The Netherlands, pp. 101-110.

Lippert, I., Rolletschek, H., Kühl, H., Kohl, J.G., 1999. Internal and external nutrient cycles in stands of *Phragmites australis* - a model for two ecotypes. Hydrobiologia 409, 343-348.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. Academic Press, London, pp 1-889.

Miao, S.L., 2004. Rhizome growth and nutrient resorption: mechanisms underlying the replacement of two clonal species in Florida Everglades. Aquat. Bot.78, 55-66.

Ostendorp, W., 1989. Dieback of reeds in Europe - a critical review of literature. Aquat. Bot. 35, 5-26.

Pugnaire, F.I., Chapin, F.S., 2005. Controls over nutrient resorption from leaves of evergreen mediterranean species. Ecology 74, 124-129.

Van der Putten, W.H., 1997. Die-back of *Phragmites australis* in European wetlands: an overview of the European Research Programme on Reed Die-Back and Progression (1993-1994). Aquat. Bot. 59, 263-275.

Votrubová, O., Soukup, A., Pecháčková, A., 1997. Anatomy of common reed and its importance for survival in eutrophic habitats. Acta U. Carol. Biol. 41, 233-244.

Westlake, D.F., 1966. The biomass and productivity of *Glyceria maxima* I. Seasonal changes in biomass. J. Ecol. 54, 745-753.

Woitke, P., Kiehl, A., Kühl, H., Kohl, J.G., 1997. Nitrogen and carbohydrate pools of two rhizome types of *Phragmites australis* (Cav) Trin ex Steudel. Int. Rev. Ges. Hydrobio. 82, 161-168.

Table1

Biomass allocation of *Phragmites australis* and *Glyceria maxima* grown in oligotrophic and eutrophic treatments. Values given are means ± STD (n = 4). Effects of N treatments (tr.), date of harvest (date) and interactions (treatment x date) are expressed by P-values (Two-Way ANOVA). Figures in bold indicate P<0.05. AB – A/B ratio, SR – S/R ratio, L-l – living leaves, L-d – dead leaves, St-l – living stems, St-d – dead stems, Rh – rhizome, R – roots; 5.8., 16.9., and 8.12. – dates of harvests.

	Oligotrophic			Eutrophic			Statistics		
	5.8.	16.9.	8.12.	5.8.	16.9.	8.12.	tr.	date	tr. x d.
Phragmites australis									
AB	0.99±0.20	0.68±0.06	0.52±0.06	2.9±0.15	1.4±0.03	0.58±0.05	**0.000**	**0.000**	**0.000**
SR	6.2±1.6	5.2±0.38	4.8±0.50	9.6±1.1	8.0±0.72	3.9±0.21	**0.000**	**0.000**	**0.001**
L-l[a]	12.6±0.88	7.9±0.77	0.01±0.01	38.3±0.81	27.3±0.11	0.17±0.05	**0.000**	**0.000**	**0.000**
L-d[a]	1.6±0.80	5.3±0.94	8.9±0.59	0.22±0.18	0.15±0.09	12.7±1.4	**0.032**	**0.000**	**0.000**
St-l[a]	31.6±3.1	23.0±1.6	1.3±0.73	34.5±0.91	29.4±0.52	4.9±0.68	**0.000**	**0.000**	0.156
St-d[a]	3.7±1.5	4.1±0.71	24.0±3.3	1.4±0.30	1.0±0.14	18.7±3.1	**0.001**	**0.000**	0.396
Rh[a]	36.0±4.0	43.4±1.1	48.6±2.0	16.4±1.4	31.0±1.4	43.2±2.4	**0.000**	**0.000**	**0.000**
R[a]	14.6±2.9	16.3±0.93	17.3±1.5	9.2±1.0	11.2±0.86	20.4±0.87	**0.003**	**0.000**	**0.000**
Glyceria maxima									
AB	1.6±0.12	1.3±0.08	1.1±0.16	3.7±0.82	5.1±0.68	2.7±0.51	**0.000**	**0.001**	**0.003**
SR	2.4±0.25	2.0±0.19	1.8±0.25	7.6±1.1	9.7±1.3	4.5±0.79	**0.000**	**0.000**	**0.000**
L-l[a]	16.3±1.1	13.6±1.2	3.1±0.38	39.2±3.1	33.0±4.5	10.4±1.1	**0.000**	**0.000**	**0.000**
L-d[a]	16.8±1.7	16.1±1.8	23.7±4.9	7.0±0.97	14.4±1.5	27.3±3.4	0.054	**0.000**	**0.002**
St-l[a]	12.7±0.34	13.8±1.6	4.7±1.7	28.4±5.4	28.7±4.1	14.6±1.1	**0.000**	**0.000**	0.208
St-d[a]	14.9±3.1	13.6±2.5	20.5±2.8	3.5±0.64	7.2±1.6	19.8±2.9	**0.000**	**0.000**	**0.005**
Rh[a]	9.4±3.1	9.0±2.2	12.0±4.9	10.0±2.6	7.1±1.1	9.6±1.5	0.370	0.270	0.618
R[a]	29.9±2.3	33.8±2.2	35.8±3.5	11.9±1.6	9.5±1.3	18.5±2.9	**0.000**	**0.000**	**0.040**

Notes. [a] % total dry wt

Table 2

Total N contents (% dry wt) of *Phragmites australis* and *Glyceria maxima* grown in oligotrophic and eutrophic treatments. Values given are means ± STD (n = 3) Effects of N treatments (tr.), date of harvest (date) and interactions (treatment x date) are expressed by P-values (Two-Way ANOVA). Figures in bold indicate P<0.05. L-l – living leaves, L-d – dead leaves, Rh – rhizome, R – roots; n.e.- not estimated; 5.8., 16.9., and 8.12. – dates of harvests.

	Oligotrophic			Eutrophic			Statistics		
	5.8.	16.9.	8.12.	5.8.	16.9.	8.12.	tr.	date	tr. x d.
Phragmites australis									
L-l	1.7±0.38	1.6±0.18	n.e.	3.4±0.03	3.3±0.05	3.0±0.93	**0.000***	0.160*	0.707*
L-d	n.e.	n.e.	0.63±0.02	n.e.	n.e.	1.2±0.06	**0.000**	n.e.	n.e.
Rh	0.51±0.05	0.67±0.10	0.91±0.13	0.76±0.22	0.81±0.05	1.5±0.06	**0.000**	**0.000**	**0.026**
R	1.3±0.09	1.2±0.03	1.5±0.06	1.7±0.04	1.8±0.07	2.3±0.13	**0.000**	**0.000**	**0.016**
Glyceria maxima									
L-l	1.8±0.13	1.6±0.17	1.4±0.21	3.0±0.19	3.1±0.29	3.4±0.20	**0.000**	0.913	**0.032**
L-d	n.e.	n.e.	0.57±0.17	n.e.	n.e.	1.4±0.13	**0.003**	n.e.	n.e.
Rh	1.4±0.01	1.4±0.11	1.3±0.08	0.53±0.12	0.95±0.07	1.5±0.30	**0.001**	**0.003**	**0.001**
R	1.7±0.09	1.4±0.12	1.5±0.30	2.2±0.11	2.6±0.34	3.1±0.17	**0.000**	0.059	**0.003**

Notes. * December harvest was excluded from the analysis (L-l were almost not present in oligotrophic treatment in *Phragmites*)

Table 3

Statistical evaluation of tissue total FAA levels and the relative contribution of FAA-N (%) to the total N content in individual plant organs using Two-Way ANOVA. Effects of N treatments (tr.), date (d.) and interactions (treatment x date) are expressed by P-values. Figures in bold indicate $P<0.05$; n.e. – not estimated; L-l – living leaves, L-d – dead leaves, Rh – rhizome, R – roots.

variable	*Phragmites australis*			*Glyceria maxima*		
	treatment	date	tr. x d.	treatment	date	tr. x d.
Total FAA contents (μmol g dry wt^{-1})						
L-l	**0.004***	0.408*	0.941*	**0.000**	**0.000**	**0.000**
L-d	**0.000**	n.e.	n.e.	0.880	n.e.	n.e.
Rh	**0.023**	**0.000**	**0.001**	**0.000**	**0.003**	**0.001**
R	0.120	**0.000**	**0.000**	0.777	**0.000**	**0.000**
Proportion of FAA-N in total tissue N (%)						
L-l	0.864*	0.261*	0.834*	0.593	**0.000**	0.304
L-d	**0.001**	n.e.	n.e.	0.632	n.e.	n.e.
Rh	**0.043**	**0.003**	**0.016**	**0.000**	**0.000**	**0.000**
R	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.004**

Notes. * December harvest was excluded from the analysis (L-l were almost not present in oligotrophic treatment in *Phragmites*)

Table 4

Relative contribution of individual amino acids to total FAA pool (%) in living and dead leaves of *Phragmites australis* and *Glyceria maxima* grown in oligotrophic and eutrophic treatments. Values given are means (n = 4). Amino acids of generally low levels (Met, Val, Phe Leu, Ile, Orn, Lys) are not shown. Effects of N treatments (tr.), date of harvest (date) and interactions (treatment x date) are expressed by P-values (Two-Way ANOVA). Figures in bold indicate P<0.05; underlined figures indicate participation >10%; n.e.- not estimated; 5.8, 16.9., and 8.12. – date of harvests.

	Leaves-living									Leaves-dead		
	Oligotrophic			Eutrophic			Statistics			Oligo	Eu	Stat.
	5.8.	16.9.	8.12.	5.8.	16.9.	8.12.	tr.	date	tr. x d.	8.12.	8.12.	tr.
Phragmites australis												
Asn	1.3	9.7	n.e.	1.1	12.7	38.8	0.655*	**0.005***	0.576*	21.7	6.8	**0.022**
Gln	3.9	14.6	n.e.	5.9	9.9	12.4	0.276*	**0.000***	**0.024***	11.6	40.2	**0.000**
Asp	29.7	16.7	n.e.	11.8	6.8	3.5	**0.000***	**0.000***	**0.002***	8.0	1.51	**0.000**
Ser	7.3	7.3	n.e.	12.6	14.9	7.7	**0.000***	0.111*	0.121*	9.2	11.4	**0.009**
Glu	16.3	19.4	n.e.	23.4	30.0	2.9	**0.000***	**0.017***	0.197*	4.8	2.1	**0.013**
Arg	14.6	5.0	n.e.	12.3	3.7	0.77	0.063*	**0.000***	0.509*	3.3	0.76	**0.001**
Gly	n.e	0	n.e.	0	0	2.9	n.e	n.e	n.e	2.0	0	**0.005**
Thr	12.4	4.8	n.e.	11.1	5.2	1.7	0.818*	**0.013***	0.607*	2.7	5.7	**0.000**
Ala	6.9	5.6	n.e.	15.6	9.9	5.3	**0.000***	**0.005***	**0.038***	11.6	6.5	**0.001**
Gaba+Pro	0.92	2.4	n.e.	0.67	3.0	20.2	0.059*	**0.000***	**0.001***	9.0	10.5	0.189
Glyceria maxima												
Asn	5.9	12.7	37.3	2.6	10.8	38.1	0.253	**0.000**	0.909	5.9	13.9	**0.003**
Gln	11.9	12.2	6.8	7.3	15.0	11.0	0.812	**0.007**	0.051	9.6	37.4	**0.011**
Asp	8.3	12.5	9.9	9.6	8.8	4.7	0.167	0.136	0.245	6.3	2.5	**0.002**
Ser	12.0	13.5	8.3	18.7	20.2	9.3	**0.005**	**0.001**	0.187	4.7	4.4	0.309
Glu	16.5	14.1	12.3	22.6	21.3	9.9	**0.045**	**0.008**	0.198	5.6	2.9	0.126
Arg	12.9	7.0	4.7	20.2	2.2	1.1	0.896	0.198	0.796	3.3	1.1	**0.017**
Gly	2.9	11.0	0	0	4.4	0.89	**0.008**	**0.000**	**0.024**	1.6	1.5	0.491

Thr	5.7	3.7	3.3	5.7	4.4	3.9	0.669	0.056	0.917	4.5	5.4	0.205
Ala	6.3	5.4	3.3	6.6	7.0	1.9	0.504	**0.008**	0.502	6.9	4.0	0.226
Gaba+Pro	0.74	0.75	9.1	0.79	1.1	10.7	0.677	**0.000**	0.967	11.3	10.3	0.717

Notes. * December harvest was excluded from the analysis (L-l were almost not present in oligotrophic treatment in *Phragmites*)

Table 5

Relative contribution of individual amino acids to total FAA pool (%) in rhizomes and roots of *Phragmites* and *Glyceria* grown in oligotrophic and eutrophic treatments. Values given are means (n = 4). Amino acids of generally low levels (Met, Val, Phe Leu, Ile, Orn, Lys) are not shown. Effects of N treatments (tr.), date of harvest (date) and interactions (treatment x date) are expressed by P-values (Two-Way ANOVA). Figures in bold indicate P<0.05; underlined figures indicate participation >10%; Rh – rhizome, R – roots; n.e.- not estimated; 5.8., 16.9., and 8.12. – date of harvests.

	Rhizome									Roots								
	Oligotrophic			Eutrophic			Statistics			Oligotrophic			Eutrophic			Statistics		
	5.8.	16.9.	8.12.	5.8.	16.9.	8.12.	tr.	date	tr. x d.	5.8.	16.9.	8.12.	5.8.	16.9.	8.12.	tr.	date	tr. x d.
Phragmites australis																		
Asn	64.7	73.5	68.3	66.5	64.2	64.6	0.137	0.377	0.502	71.9	76.4	74.3	29.0	50.3	62.7	**0.000**	**0.000**	**0.000**
Gln	7.4	8.4	7.9	5.0	9.6	11.1	0.495	0.050	0.101	3.6	6.0	8.6	11.2	12.9	10.7	**0.000**	**0.002**	**0.001**
Asp	8.7	6.3	3.8	3.8	4.1	2.9	**0.000**	**0.000**	**0.030**	8.3	6.8	5.3	9.0	6.3	2.6	**0.018**	**0.000**	**0.004**
Ser	3.2	2.9	4.9	5.9	5.6	4.0	**0.001**	0.484	**0.003**	1.7	2.3	3.8	8.9	5.8	4.7	**0.000**	**0.001**	**0.000**
Glu	2.1	2.2	1.3	2.5	3.1	1.6	0.106	**0.032**	0.778	3.1	2.8	1.7	11.0	6.3	1.6	**0.000**	**0.000**	**0.000**
Arg	3.2	1.3	2.9	2.1	1.7	1.6	0.152	0.137	0.281	2.9	1.7	1.5	5.4	2.5	7.7	**0.000**	**0.001**	**0.000**
Gly	0.70	0	1.9	1.2	0.81	0	0.991	0.501	0.468	0	0	0	2.4	4.1	0	**0.000**	**0.002**	**0.002**
Thr	3.7	1.7	1.4	2.8	2.3	1.2	0.939	0.325	0.851	3.0	0.8	0.90	5.0	2.0	1.1	**0.000**	**0.000**	**0.015**
Ala	2.1	0.95	0.82	4.4	1.7	1.6	**0.011**	**0.006**	0.380	1.8	1.2	0.60	7.1	4.6	0.50	**0.000**	**0.000**	**0.000**
Gaba+Pro	0	0.13	2.0	0.24	0.83	5.3	**0.000**	**0.000**	**0.003**	0.06	0.27	0.91	0.80	1.5	4.1	**0.000**	**0.000**	**0.000**
Glyceria maxima																		
Asn	69.0	73.3	74.0	43.2	48.8	76.1	**0.034**	0.101	0.195	67.3	73.0	72.1	40.8	45.6	66.7	**0.000**	**0.000**	**0.000**
Gln	13.8	7.8	6.4	15.9	19.2	4.0	0.262	**0.030**	0.142	9.8	6.6	7.7	14.5	17.8	7.8	**0.000**	**0.001**	**0.001**
Asp	3.8	5.7	3.3	5.4	4.0	1.7	0.359	0.072	0.193	3.7	4.2	3.8	5.7	3.1	1.7	0.640	**0.001**	**0.004**
Ser	4.5	3.3	3.7	11.2	9.1	4.9	**0.000**	**0.002**	**0.005**	2.7	3.3	3.6	8.5	9.5	5.4	**0.000**	**0.000**	**0.000**
Glu	1.2	1.1	1.5	4.8	3.4	1.6	**0.001**	0.059	**0.029**	4.8	1.1	1.7	7.4	4.5	1.8	**0.000**	**0.000**	**0.001**
Arg	0.50	1.1	2.3	1.8	0.97	2.7	0.184	0.373	0.389	2.8	2.3	3.0	2.4	1.4	1.8	**0.012**	0.072	0.637

Gly	0.89	3.4	2.8	5.0	4.2	2.2	0.419	0.464	0.698	0.00	5.2	2.5	2.5	3.8	2.6	0.294	**0.008**	0.168
Thr	1.4	1.6	2.1	2.8	1.7	1.6	0.417	0.590	0.444	1.3	1.2	2.1	2.1	2.4	2.4	**0.003**	0.235	0.532
Ala	0.52	0.17	0.26	2.8	1.6	0.55	**0.043**	0.163	0.260	2.9	0.56	0.43	7.4	3.3	0.53	**0.000**	**0.000**	**0.000**
Gaba+Pro	0.04	0.01	0.09	0.67	0.53	0.93	**0.001**	0.580	0.790	0.16	0.06	0.19	0.98	0.65	2.0	**0.000**	**0.000**	**0.000**

Legends to figures

Fig. 1. Daily minimum temperatures (°C) recorded at hydrometeorological station Prague-Karlov located close to the experimental site (data source: Czech Hydrometeorological Institute). The arrow indicates the frost occurrence, which triggered massive shoot senescence in eutrophically treated *Phragmites* (see section 3.2.). The date is given as months (May-December).

Fig. 2. Total biomass (g dry wt) of above- and belowground organs of *Phragmites australis* and *Glyceria maxima* grown in oligotrophic and eutrophic treatments. Values given are means (n = 4) at each harvests dates; STD for total plant biomass (n = 4) is shown. A, S, D - months of harvests (5.8.; 16.9.; 8.12.).

Fig. 3. Shoot numbers per plant of *Phragmites australis* (A, C) and *Glyceria maxima* (B, D) grown in oligotrophic (A, B) and eutrophic (C, D) treatments. Shoots were counted weekly during the whole experimental period. Values given are means (n = 4). The arrow indicates the onset of dormant aboveground tillers formation in eutrophically treated *Phragmites*. The date is given as months (May-December).

Fig. 4. Free amino acid (FAA) contents (μmol g^{-1} dry wt) in tissues of *Phragmites australis* (A, C) and Glyceria maxima (B, D) grown in oligotrophic (A, B) and eutrophic (C, D) treatments. Values given are means (n = 4) at each harvest dates; STD for total FAA content (n = 4) is shown. Numbers above each column indicate FAA-N relative contribution (%) to total N content (values given are means; n = 4). * – dead leaves; n.e. - not estimated; A, S, D - months of harvests (5.8.; 16.9.; 8.12.); others - sum of Arg, Gly, Thr, Met, Val, Phe, Leu, Ile, Orn, Lys.

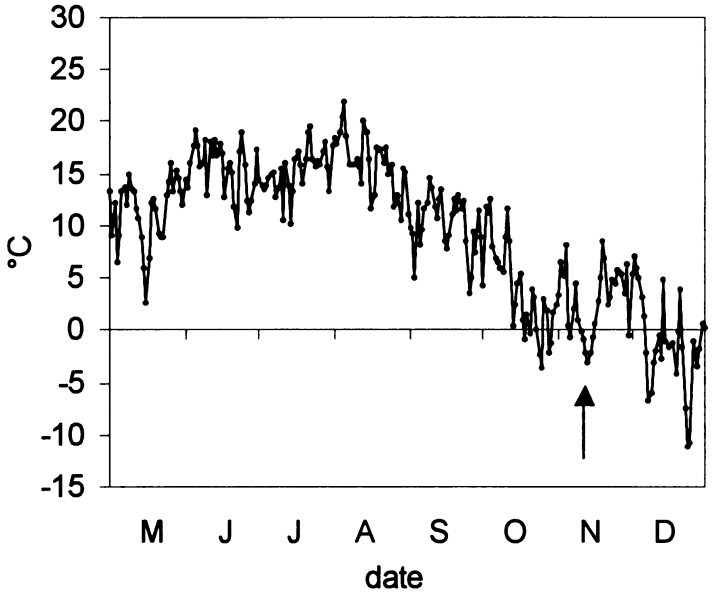


Fig 1 Tylová et al

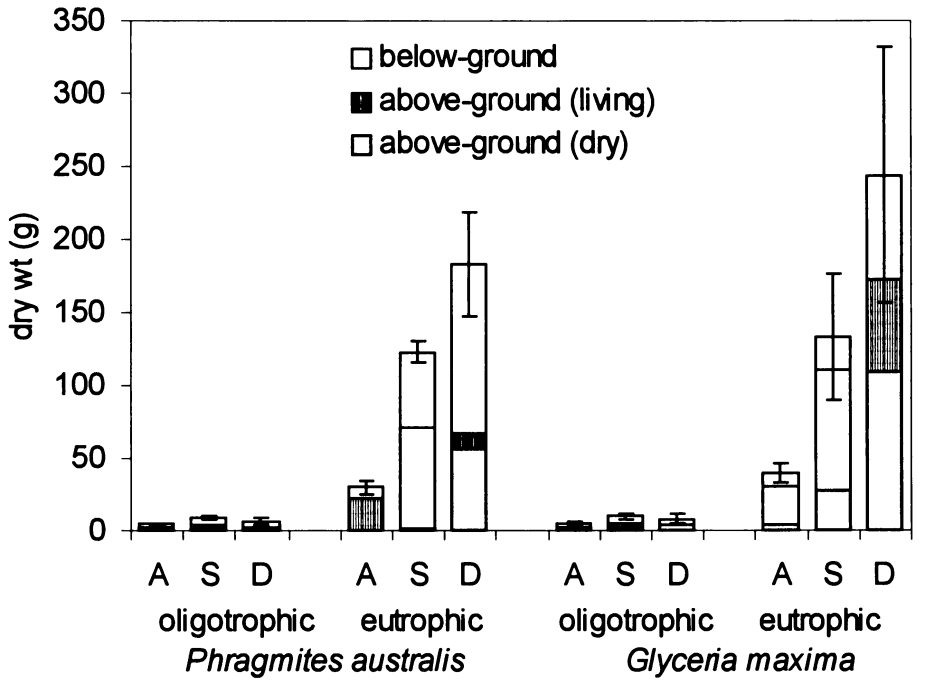


Fig. 2 Tylová et al

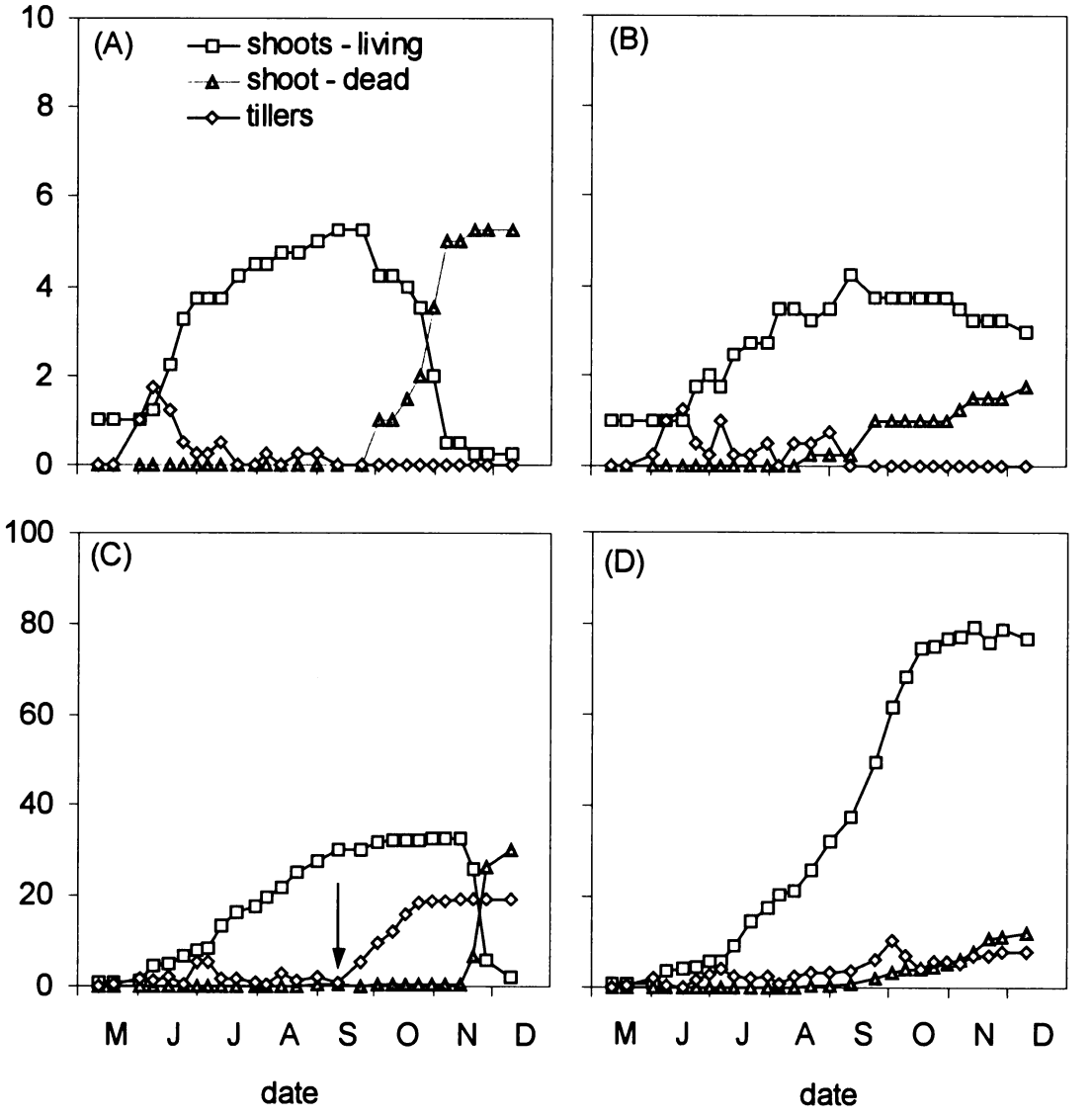


Fig. 3 Tylová et al

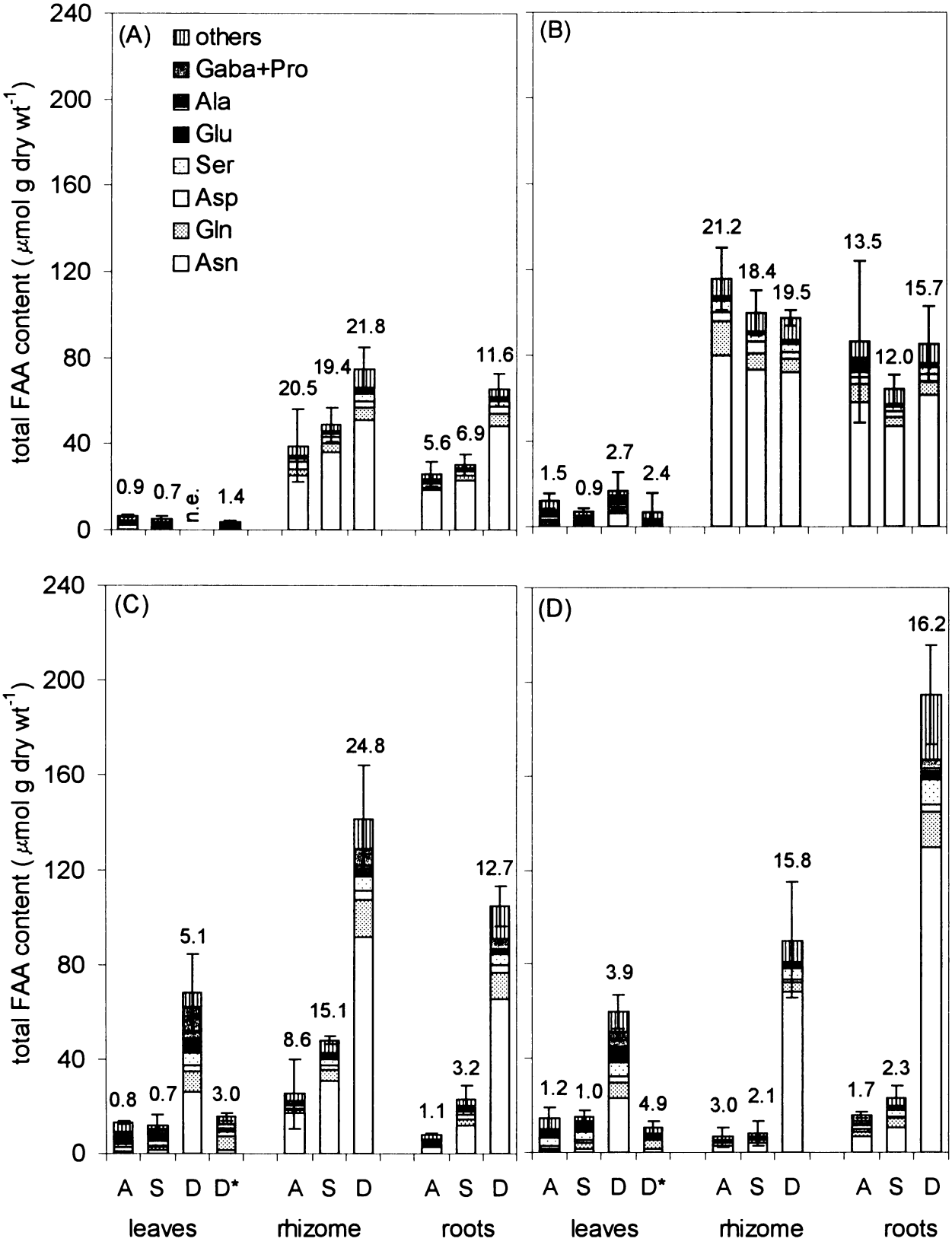


Fig. 4 Tylová et al

Performance of *Phragmites australis* and *Glyceria maxima* under changing nitrogen and phosphorus availability. I. Plant growth, resource allocation and C/N balance

Edita Tylová[a*], Lenka Steinbachová[a*], Aleš Soukup[a], Olga Votrubová[a], Vít Gloser[b]

[a] *Department of Plant Physiology, Faculty of Science, Charles University in Prague, Viničná 5, 128 44, Prague 2, Czech Republic*

[b] *Department of Plant Physiology and Anatomy, Faculty of Science, Masaryk University, Kotlařská 2, 611 37 Brno, Czech Republic*

[*] both of these authors contributed equally to this article

Corresponding author:
Edita Tylová
Department of Plant Physiology
Faculty of Science
Charles University in Prague
Viničná 5
128 44 Prague 2
Czech Republic
e-mail edmunz@natur.cuni.cz
tel. +420 22195 1697
fax +420 22195 1704

Abstract

Worldwide, freshwater wetlands undergo changes in species composition, being affected by eutrophication. The complexity of the process makes the importance of individual causal factors difficult to discern in nature. The present study follows the nutritional aspects of eutrophication in long-term sand cultures of two co-occurring littoral grasses. These grasses, *Phragmites australis* and *Glyceria maxima*, are fast-growing helophytes of fertile habitats, with different sensitivities towards highly nutrient-loaded habitats. Both species showed enhanced growth in response to eutrophic, and even hypertrophic, growth conditions (substrate N/P ratios 26/1 and 10/1, respectively), but displayed considerable sensitivity to an unbalanced high N load (substrate N/P ratio 100/1). The lower biomass production, along with the appearance of signs of stress, changes in resource allocation and morphology (e.g. high shoot density; low shoot diameters and heights; reduced root and rhizome growth) observed in this treatment, resemble those found at reed die-back sites. Changes in morphology and resource allocation were correlated with biomass C/N ratios, but low free amino acids levels in the belowground organs of eutrophically treated plants in late summer indicated a delayed accumulation of storage N compounds. The effects of nutrient availabilities on carbohydrate levels were followed, in detail, in a separate study (Steinbachová et al., submitted as Part II).

Our findings show that the response of littoral vegetation to eutrophication, considering nutritional aspects *per se*, is highly dependent on the sediment N/P ratio, and that the unbalanced high N load alters plant morphology and performance in a manner that could increase plant susceptibility to stresses (altering e.g. ventilation efficiency and ability to survive high water levels; plant anchorage and belowground storage capacity). As the positive effect of high P supply was especially visible in *Glyceria*, this species may be favoured in competition with *Phragmites* at highly fertile habitats rich in P. But generally, the ability of *G. maxima* to colonise heavily loaded sites is related to the avoidance strategy of shallow rooting, rather than to the tolerance of high N load, *per se*.

Keywords: Phragmites, Glyceria, Eutrophication, Wetlands, Nitrogen, Phosphorus, C/N ratio, N/P ratio, Free amino acids, NH_4^+

1. Introduction

Eutrophication, multilaterally affecting the survival of wetland plants, is considered the key factor inducing reed decline in European wetlands during the last decades (Ostendorp, 1989; Van der Putten, 1997; Kubín and Melzer, 1997). Data collected within the EUREED projects have shown the complex of external factors (e.g. litter accumulation, phytotoxins, direct mechanical destruction) leading to changes in plant performance, seasonal life cycles, and storage formation of to be the triggers of *Phragmites australis* die-back (for summary see Van der Putten, 1997; Brix, 1999). However, the complexity of inter-relationships within wetlands makes the importance of individual causal factors difficult to discern. Thus, it is still a matter open to discussion, to what extent changes in nutrient availability *per se* affect the competition ability of wetland plants and/or their susceptibility to additional stresses.

Locally increased shoot density (patchy organisation) and reduced stalk stability both have been described as factors accompanying the beginnings of the reed decline (Ostendorp, 1989). Eutrophication has been hypothesised as the key factor inducing these morphological changes, but the extensive study of *Phragmites* shoot characteristics (Ostendorp et al., 2001) in different European water bodies failed to prove a direct causality with the trophic status of the habitat. The majority of stem characteristics were instead correlated with geographic origin (Ostendorp et al., 2001), affected by the water level (Vretare et al., 2001), or showed clone-specific genetically based differences (Rolletschek et al., 1999b). Both a lowered stem diameter and mechanical stability of the reeds within a die-back site was also observed by Fogli et al. (2002), however in this case, the nutrient concentrations of sediment were even lower in the die-back site, as compared to the healthy site. The growth alterations of belowground organs (e.g. higher incidence of shorter roots, less developed laterals) commonly found in eutrophic habitats (Votrubová et al., 1997) seemed to be related to both phytotoxin occurrence (Armstrong and Armstrong, 2001) and nutrient availability, *per se* (Votrubová and Pecháčková, 1996; Votrubová et al., 1997). The high incidence of rhizome and root injuries (e.g. dead root and rhizome apices, damaged laterals) within damaged reed stands is generally attributed to the occurrence of phytotoxin (Armstrong and Armstrong, 2001), but nutrient over-load may participate in this damage by the induction of changes in root anatomy, e.g. the decrease of root porosity or delay in the differentiation of surface protective layers (Čížková-Končalová et al., 1996; Votrubová and Pecháčková, 1996).

Eutrophication is a result of N and/or P enrichment, but besides their absolute concentrations in pore water, the relative availabilities of both main nutrients (N/P ratio) was shown to affect wetland plant performance and relative participation of co-occurring species

(Güsewell and Bollens, 2003; Güsewell et al., 2003). However, the effect is visible, especially under N or P limiting conditions, being weak in other cases (e.g. Romero et al. (1999)). Also the form of N available to plants may modify plant growth response to nutrient availability, since the preference for a particular N source (NH_4^+ versus NO_3^-) was shown to be an important factor affecting plant community composition (Kronzucker et al., 1997). In wetlands, nutrient enrichment enhances plant productivity, litter accumulation and microbial activities. High oxygen demand then diminishes NO_3^- availability in the sediment, increasing the NH_4^+/NO_3^- ratio (Kühl et al., 1997; Čížková et al., 2001).

Several field studies have documented the spread of *Glyceria maxima* following reed retreat (Ostendorp, 1989; Van der Putten et al., 1997; Hroudová and Zákravský, 1999). Although it seems to be the result of changed conditions and interspecific competition, rather than a direct cause of reed die-back, both species obviously differ in their sensitivity to eutrophication (see tab. 2 in Brändle et al. (1996)). *Phragmites* growth is positively affected by the early stages of eutrophication, but showed sensitivity to highly eutrophic sediments rich in organic matter (Den Hartog et al., 1989; Brändle et al., 1996; Čížková et al., 1996b; Van der Putten et al., 1997; Hroudová and Zákravský, 1999). The comparison of the performance of these two species, under changing nutrient availability, thus presents a useful tool elucidating general physiological mechanisms of species' competition ability in the changing environment.

In this study, we focused on the following major goals: to characterise growth, morphology and the internal C/N balance of *Phragmites* and *Glyceria* under increasing N and P availabilities, as well as to find possible differences in species response to nutrients which may help to explain mechanisms of competition ability of these two species in eutrophic sites. To separate-out the effect of nutrients availabilities *per se*, plants were cultivated in sand cultures, under controlled conditions.

2. Materials and Methods

2.1. Plant material

Rootless rhizome cuttings (10 cm) with one shoot (15-20 cm) were cultivated for three weeks in coarse sand in a greenhouse, prior to their transfer into the experimental cultures. The cuttings were irrigated with tap water during this period. The mother plants were originally obtained from the natural littoral stands in the Třeboň basin, Czech Republic and then long-term vegetatively propagated in outdoor sand cultures in the Institute of Botany, Academy of Sciences, Třeboň, Czech Republic (http://www.butbn.cas.cz/coll_wet/angl.html).

2.2. Experimental set-up and growth conditions

Two long-term sand cultures were designed to study the effects of increaseing nitrogen plus phosphorus (experiment N plus P) and nitrogen *per se* (experiment N) availabilities. The experiments were carried out during a four month period (May-August); in an outdoor sand culture, protected via a transparent plastic roof. Each cutting was planted individually in a 15 L plastic pot, filled with pure coarse quartz sand. Eight pots per treatment were placed in a shared plastic container with 130 L of the nutrient solution. The solution was changed every 3 weeks; the pH was adjusted to 6.8 using 2M HCl. The water level was kept at the surface of the sand. The surface of the sand was covered by an expanded ceramic aggregate (Keramzit) and the exposed solution covered by a black non-woven textile, to prevent growth of algae.

In the experiment N plus P, three treatments of increasing N and P availabilities were defined to simulate the trophic conditions in the South Bohemia fishpond littorals, with the low (oligotrophic treatment) and high (eutrophic treatment) fertility as well as the trophic conditions commonly found in constructed wetlands (hypertrophic treatment). These N plus P treatments differed in chemical composition, total N and P levels, and NH_4^+/NO_3^- ratio. The following chemical composition was identical in all three treatments (μM): Mg^{2+} 390.67; SO_4^{2-} 390.67; BO_3^{3-} 0.23; Fe^{2+} 20.42; Mn^{2+} 0.71; Zn^{2+} 0.01; Cu^{2+} 0.003; $Mo_7O_{24}^{2-}$ 0.001. In addition to the above mentioned chemicals, the oligotrophic treatment received (μM): NH_4^+ 4.30; NO_3^- 21.94 (NH_4^+/NO_3^- ratio = 0.2); PO_4^{3-} 1.01; K^+ 499.72; Ca^{2+} 766.82; and Cl^- 2014.67; eutrophic treatment: NH_4^+ 443.86; NO_3^- 2193.69 (NH_4^+/NO_3^- ratio = 0.2); PO_4^{3-} 99.92; K^+ 104.92; Ca^{2+} 1096.06; and Cl^- 443.55; and hypertrophic treatment: NH_4^+ 4447.23; NO_3^- 5098.89 (NH_4^+/NO_3^- ratio = 0.9); PO_4^{3-} 999.24; K^+ 1049.19; Ca^{2+} 1096.06; and Cl^- 18.69.

In the experiment N, three treatments were defined, to study the effects of increasing the availability of N *per se*: low N, medium N, and high N treatment. The composition of nutrient solution was derived from the eutrophic treatment of the experiment N plus P, and the treatments were defined in order to differ in their total N level and NH_4^+/NO_3^- ratio. The following chemical composition was identical in all three treatments (μM): PO_4^{3-} 99.92; K^+ 104.92; Mg^{2+} 390.67; SO_4^{2-} 390.67; BO_3^{3-} 0.23; Fe^{2+} 20.42; Mn^{2+} 0.71; Zn^{2+} 0.01; Cu^{2+} 0.003; $Mo_7O_{24}^{2-}$ 0.001. In addition to the above mentioned chemicals, the low N treatment received (μM): NH_4^+ 5.23; NO_3^- 20.96 (NH_4^+/NO_3^- ratio = 0.2); Ca^{2+} 1102.87; and Cl^- 2190.03;

medium N treatment: NH_4^+ 1317.85; NO_3^- 1317.92 (NH_4^+/NO_3^- ratio = 1); Ca^{2+} 1099.19; and Cl^- 2198.32; and high N treatment: NH_4^+ 7631.48; NO_3^- 1908.35 (NH_4^+/NO_3^- ratio = 4); Ca^{2+} 1097.28; and Cl^- 323.59. The total N contents in the low N, medium N, and high N treatment of the experiment N corresponded to oligotrophic, eutrophic, and hypertrophic treatments of the experiment N plus P, respectively.

2.3. Plant harvest

After 4 months of cultivation, plants were carefully taken out of the cultivation pots, washed with tap water, and divided into leaf blades, stems with leaf sheaths, rhizomes, and roots. These fractions were dried at 80 °C for the dry weight (dry wt.) determination.

The biomass allocation was characterised using the ratio of root-supported tissue (shoots and rhizomes) to root biomass (S/R), and the ratio of aboveground (shoots) to belowground (roots and rhizomes) biomass (A/B). The allocations into different organs (leaf blades, stems plus leaf sheaths, rhizomes, and roots) were calculated (in percentage) as the ratio between the biomass of the particular organ and that of the total dry biomass of the plant.

The following biometric characteristics of plants were estimated: number and length of shoots, width of stem basis, incidence of flowering, leaf number per shoot, the ratio of dead/living leaf numbers, rhizome length, rhizome length/shoot number ratio, number and length of roots, percentage of injured roots, branched/unbranched root ratio (branched roots possessed lateral roots and represented a more differentiated part of the root system, while unbranched roots have no visible lateral roots and represented a less differentiated part).

2.4. FAA, C, N, NH_4^+, and P analyses

Samples of the individual tissues (leaf blades, rhizomes and roots; ± 0.5 g fresh wt.) for the analysis of free amino acids (FAA) and NH_4^+ ions were taken from intact plants, washed in distilled water, dried, weighed, and immediately frozen in liquid nitrogen and stored in a freezer (–80 °C) until analysis. FAA and NH_4^+ were determined in freeze-dried material ground to fine powder (Retsch homogeniser, MM301, Germany) using HPLC (Waters Alliance 2690XE, Waters 474 fluorescence detector, Milford, USA), according to Gloser (2002).

The dry biomass of leaf blades, rhizomes, and roots were ground and analysed for total C, N, and P contents. The total C and N contents were analysed using a Leco CNS 2000 analyser and the C/N atomic ratio was calculated. The P content was determined with a Skalar

SAN Plus System flow analyser, after digestion with sulphuric acid at 300°C. The N/P atomic ratio was then calculated.

2.5. Statistical evaluation

Statistical evaluation was performed using NCSS 2000 and PASS 2000 software (Jerry Hintze, 1996. NCSS and PASS. Number Cruncher Statistical Systems. Kaysville, Utah). Data were analysed using analysis of variance (ANOVA). The normal distribution of residuals was tested using the Multiple Regression analysis of NCSS software and data were ln transformed when necessary. Correlations were analysed using the Correlation Matrix.

3. Results

3.1. Plant growth and biomass allocation

Both species reposnded to increasing availability of N plus P with the gradual increase of the biomass production from oligotrophic up to hypertrophic treatment (Fig.1, Table 1) and with the preferential allocation of biomass into shoots at the expense of roots and rhizomes (Table 2). In contrast ($P<0.001$), plants subjected to increasing availability of N *per se* responded positively to medium N treatment only; high N treatment was obviously supraoptimal and the biomass production was lower compared to medium N treatment, especially in rhizomes and roots (Fig.1, Table 1).

3.2. Plant biometric characteristics

The biometric characteristics of plants in the experiment N plus P corresponded with the overall growth response; shoot numbers, average shoot lengths, leaf numbers per shoot, length of rhizomes, and root numbers increased from oligotrophic up to hypertrophic treatment (Table 3). The relative occurrence of dead leaves were, however, unaffected by the treatments (Table 3). The preferential allocation of biomass to aboveground structures coincided with low length of rhizome per individual shoot (rhizome length/shoot number ratio) in hypertrophic compared to eutrophic treatment in both species (Table 3).

Shoot morphology was differently affected in the experiment N. Although shoot numbers gradually increased with increasing N availability up to high N treatment; the average length of shoots and leaf numbers were the highest in medium N treatment. High N treated plants produced a great number of shoots, but the shoots were much shorter, and with a lower width of stem basis (Table 3). The enhanced tillering of high N treated plants was also reflected in a low rhizome length/shoot number ratio, low total rhizome length and low

numbers of roots (Table 3). The negative effect of high N treatment on plant growth was demonstrated by an increased dead/living leaves ratio in both species (Table 3).

3.3. Root morphology

Increasing availability of N plus P caused a significant decrease of average root length in *Glyceria*. Plants in hypertrophic treatment had shorter roots, by 49% and 43% compared to plants in oligotrophic and eutrophic treatments, respectively (Table 3). In *Phragmites,* this trend was much less visible. Plants in eutrophic treatment had a 29% lower average root lengths compared to oligotrophic treatment, but the trend was not further extended to hypertrophic treatment. In contrast to root lengths, the occurrence of unbranched roots and of roots with the visible symptoms of injury were not affected by the treatments in this experiment (Table 3).

Similarly, decreased average roots lengths were recorded in *Glyceria* treated with high availability of N *per se*; high N treated plants had roots shorter by 30% compared to plants in both low and medium N treatments. In *Phragmites*, rather the opposite trend was noted; high N treated plants had 18% higher average root lengths than plants in other treatments (Table 3). In both species, the branched/unbranched root ratio increased with increasing N availability (Table 3). In *Glyceria,* but not in *Phragmites*, high incidence of injured roots was observed in high N treated plants; injured roots represented 83% of all the roots per plant compared to 44% in medium N treatment (Table 3).

3.4. C/N balance

Increasing N plus P availability shifted C/N balance in favour of N assimilation; C/N atomic ratio gradually decreased from the oligotrophic to hypertrophic treatment in all the organs of both species (Table 4), but especially in *Glyceria* (see the significant sp. x tr. interactions in Table 4). Similar shift was observed also under increasing availability of N *per se* (Table 4).

In both species and experiments, the C/N atomic ratios were highly correlated with the allocation of biomass to aboveground and belowground organs; C/N ratio of leaves, rhizomes and roots negatively correlated with A/B and S/R ratios (Pearson correlation coefficient 0.69-0.98, $P<0.05$). Similarly, A/B and S/R ratios were positively correlated with leaf total FAA contents (Pearson correlation coefficient 0.74-0.90, $P<0.05$) as well as with the relative occurrence of Asn+Gln+Arg in leaves (Pearson correlation coefficient 0.73-0.92, $P<0.05$) in

both experiments. Rhizome and root FAA contents were, however, correlated only in the experiment N (Pearson correlation coefficient 0.72-0.98, $P<0.05$), but not in the experiment N plus P ($P>0.05$).

3.5. FAA contents

In leaves, the total FAA contents as well as the relative occurrence (%) of N-rich amino acids (Asn+Gln+Arg) in FAA pool increased with increasing N plus P availability (Fig. 2, Table 1). Similarly, the relative amount of N allocated in FAA gradually increased; while FAA contained 2.0 and 2.4% of total leaf N content in oligotrophically treated plants, it was 4.8 and 14.4% in hypertrophic treatment, in *Phragmites* and *Glyceria*, respectively. Similar trends were observed in the experiment N (Fig. 2, Table 1). FAA contained 4.0 and 1.6% of total N content in low N treatment, and 6.7 and 10.8% in high N treatment, in *Phragmites* and *Glyceria*, respectively.

In contrast to leaves, FAA status of belowground organs (rhizomes, roots) showed different response to treatments between the experiments. While total FAA contents and the proportion (%) of Asn+Gln+Arg increased from low N up to high N treatment in experiment N, similar trend occurred only between eutrophic and hypertrophic treatment in experiment N plus P (Fig. 2, Table 1). Plants in the oligotrophic treatment contained surprisingly high FAA levels, particularly in rhizomes (Fig. 2, Table 1), which represented 59.3 and 67.4% of rhizome total N content compared to 15.4-18.8% and 7.0-29.1% in eutrophic and hypertrophic treatments, in *Phragmites* and *Glyceria*, respectively. In contrast, only 13% of N was allocated in FAA in rhizomes of low N treated plants.

Moreover, high N treated plants showed high relative occurence of Ala in roots (11.3% and 10.7% of total FAA content in *Phragmites* and *Glyceria*) compared to the low and medium N treatments (< 6% in both species) ($P<0.05$). A similar trend was not found in the experiment N plus P and was not observed in other plant organs ($P>0.05$).

3.6. NH_4^+ ions in plant tissues

Plants in the experiment N plus P showed low internal concentrations of free NH_4^+ (< 9.0 μmol g^{-1} dry wt. in both *Phragmites* and *Glyceria*) in all the organs and treatments. In both species, leaf NH_4^+ concentrations gradually increased from oligotrophic to hypertrophic treatment, while there were no differences between the oligotrophic and hypertrophic treatments in belowground organs (Fig. 3, Table 1).

In contrast, plants in the experiment N showed low internal concentrations of free NH_4^+ only in low and medium N treatments (< 20.5 μmol g^{-1} dry wt. in both *Phragmites* and *Glyceria*), but the concentrations were considerably higher in high N treatment (Fig. 3, Table 1). Similar trends were recorded in all plant organs of both species, but were more pronounced in the roots (organ x treatment interaction: $P<0.05$).

3.7. P contents and N/P ratios

The relative contents of P in the plant leaves gradually increased from the oligotrophic to hypertrophic treatments in both species; while N/P atomic ratios followed the opposite trend (Fig. 4, Table 1). *Glyceria*, however, contained considerably higher relative amounts of P in the hypertrophic treatment than did *Phragmites* (see significant interactions in Table 1). Moreover, *Glyceria* exhibited generally lower N/P ratios compared to *Phragmites* ($P<0.05$), which was especially visible in the oligotrophic treatment (species x treatment interaction: $P<0.05$) (Fig. 4, Table 1).

In contrast, plants in the experiment N showed stable leaf P contents, with no differences between species or treatments (Fig. 4, Table 1). The N/P atomic ratio, however, increased from low N to high N treatment in both species (Fig. 4, Table 1). This trend tended to be more pronounced in *Glyceria*, but the differences between the response of *Phragmites* and *Glyceria* were not statistically significant (see interactions in Table 1).

4. Discussion

Both experiments showed enhanced growth of *Phragmites* and *Glyceria* in medium compared to low nutrient availability. This was similarly observed under a moderate sewage dose, while an excessive sewage dosage caused the opposite effect (Čížková-Končalová et al., 1996). In contrast to sewage application, the effects of very high nutrient loads applied in the present study were not generally negative, but dependent on the N/P ratio applied. Unbalanced high N availability (N/P ratio = 96; on a molar basis) in high N treatment was obviously supraoptimal, while similar N concentration applied in the N/P ratio = 9.6 (hypertrophic treatment) influenced plant growth positively. Similarly, Romero et al., (1999) showed a positive response of *Phragmites* to a N/P ratio of up to 33 (on a molar basis), while any further increase of N availability (N/P ratio = 66) caused growth inhibition. High phosphate was also shown to alleviate the deleterious effects of high nitrate on *Phragmites* by Ulrich and Burton (1988).

In correspondence with the findings of Koerselman and Meuleman (1996), N/P atomic ratios >16 detected in the leaves of high N treated plants indicate P limitation in both species. Although significant differences in N/P ratios of high N treated *Phragmites* and *Glyceria* were not found, the importance of P was especially visible in *Glyceria.* This species produced twofold amount of biomass than did *Phragmites* in hypertrophic treatment (the experiment N plus P), but reached only a comparable growth rate in high N treatment (the experiment N). In agreement, field observations described well-developed *Glyceria* stands as indicators of local sources of pollutions with high P contents in the sediment (Hroudová and Zákravský, 1999). Thus, the ability of this species to colonise highly eutrophic sites may not necessarily be only due to the sensitivity of *Phragmites* to organic matter typically present at these sites (Čížková et al., 1996b; Van der Putten et al., 1997), but also due to the ability of *Glyceria* to grow faster than *Phragmites* under extreme nutrient load, if enough P is available.

The negative growth response of *Phragmites* and *Glyceria* to high N treatment in the present study may also be attributed to high NH_4^+/NO_3^- ratio applied in this treatment (4:1) compared to all other treatments (NH_4^+/NO_3^- ratio 1:1 or lower). The sensitivity of *Glyceria* to high availability of NH_4^+-N form has already been described by Munzarová et al. (2006), despite the fact that NH_4^+-N may affect this species positively when applied in lower dosages (Tylová-Munzarová et al., 2005). Moreover, high concentrations of free NH_4^+ ions in tissues, and enhanced leaf senescence in high N treated plants of both *Phragmites* and *Glyceria*, may indicate nutritional disorder. Foliar accumulation of NH_4^+ is considered as the stress symptom in NH_4^+ fed plants (Barker, 1999), which affects leaf senescence via an increasing tissue sensitivity to ethylene; a hormone participating in regulation of senescence (Chen et al., 1997). Although the direct comparison must be done with care and caution due to differences in NH_4^+ detection techniques and among species, foliar NH_4^+ levels detected in high N treated plants are close to the levels described as the critical concentrations triggering rapid ethylene production in *Lycopersicon esculentum* (Barker, 1999). The NH_4^+/NO_3^- ratio is typically increased in the pore waters of eutrophic habitats because of a low sediment redox potential (Kühl et al., 1997; Čížková et al., 2001). Although a negative growth response to the high N treatment was recorded in both species, the differences in the prevailing growth strategy may favour *Glyceria* in competition with deeply rooting *Phragmites.* Shallow rooting and colonisation of slightly flooded littoral habitats (Buttery and Lambert, 1965; Brändle et al., 1996) allow *Glyceria* to spatially avoid highly reduced NH_4^+ dominated sediment zones. Moreover, *Glyceria* was shown to decrease rooting depth in response to the addition of piggery sewage (Čížková-Končalová et al., 1996). A similar response in root vertical

distribution was not observed in *Phragmites* (Čížková-Končalová et al., 1996), although this species changes the rhizome vertical penetration in response to water level (Weisner and Strand, 1996). High nutrient availability also caused a significant decrease of the average root length in *Glyceria* in the present study. This was not found in *Phragmites*, although the presence of very short roots in response to N addition has previously been described in this species (Votrubová and Pecháčková, 1996), and observed in eutrophic habitats (Votrubová et al., 1997). Lower root lengths presumably correspond with the ability to provide adequate oxygen for the root growth (Čížková-Končalová et al., 1996). This is of special importance considering decreased root porosity, which was observed in *Phragmites* (Čížková-Končalová et al., 1996; Votrubová and Pecháčková, 1996) and other species (e.g. wetland sedges (Končalová et al., 1993)) under high nutrient load. A decrease of rooting depth may negatively affect plant anchorage in the flooded substrate, and stands may be more susceptible to mechanical stress factors, e.g. wave action, fish grazing (Ostendorp, 1989).

Plants grown under high availability of N *per se* also showed some changes in shoot morphology, which are typically observed in *Phragmites* die-back sites affected by eutrophication. Higher shoot numbers, but lower average shoot lengths and diameters are the features typical for the early stages of reed die-back (Ostendorp, 1989; Dinka and Szeglet, 2001), and also the features inducible by sewage application (Hardej and Ozimek, 2002). Similar changes in the biometric characteristics where not visible under high availability of N+P (hypertrophic treatment), highlighting again the importance of a balanced N/P supply ratio. Considering the plant's ability to survive in the eutrophic wetland habitat, shoot parameters are important determinants of convective ventilation efficiency (Rolletschek et al., 1999a), and shoots of lower diameter posses lower flow rates. Although lower diameters can be compensated by higher shoot density (Rolletschek et al., 1999a), lower shoot lengths may impair plant ability to support belowground parts with atmospheric oxygen under the sudden rise of water level - and thus survive occasional floods. The increased relative occurrence of alanine (Ala) in roots of high N treated plants observed in the present study may indicate less efficient aeration of roots, despite a high shoot density in this treatment. Ala content increases in correspondence with the involvement of fermentation processes, showing a strong negative correlation with oxygen supply (Haldemann and Brändle, 1988; Rolletschek et al., 1998). The observed increase was, however, relatively small (and restricted only to roots) compared to changes observed in reed early in the spring (Kohl et al., 1998) or in damaged reed stands with a lowered ventilation efficiency due to a flood event (Koppitz et al., 2004) or mowing (Rolletschek et al., 1998).

Increasing nutrient availability typically causes changes in biomass distribution, increasing the plant S/R ratio (Saarinen, 1998). The stimulatory effect of nutrients on the aboveground biomass production of *Phragmites* and *Glyceria* has been repeatedly described (Čížková-Končalová et al., 1996; Votrubová and Pecháčková, 1996; Clevering, 1998). However, in species possessing an extensive rhizome system, the balance in resource allocation is more complex. Although moderate N addition may enhance rhizome growth, as was shown in different *Phragmites* by Clevering (1998); very high N load may cause a negative effect. Our experiments showed a decreased relative allocation of biomass into the rhizome, and a decreased length of rhizome per individual shoot under increasing N availability. This shift may negatively affect long-term survival of plants, since the rhizome affects plant anchorage in the flooded substrate (Weisner and Strand, 1996) and determines plant storage potential, a critical prerequisite for over-wintering (Čížková-Končalová et al., 1992). Since carbon starvation has been shown as one aspect of reed die-back (Čížková-Končalová et al., 1992; Čížková et al., 1996a), the relative decrease in rhizome biomass may even worsen the negative effect of low rhizome carbohydrate levels, which were found in plants at eutrophic habitats (Kubín et al., 1994; Čížková et al., 1996a), as well as in experimental cultures (Čížková-Končalová et al., 1996). A lowered plant storage capacity may disadvantage *Phragmites* in competition with *Glyceria*; since *Glyceria* is supposed to partly overcome the need for carbohydrate storages by the presence of physiologically active over-wintering shoots (Čížková-Končalová et al., 1996). These are not present in *Phragmites*, a species with a strong winter dormancy.

The internal plant levels of N rich compounds are generally accepted as the signal affecting biomass partitioning and emergence of new tillers (Saarinen, 1998; Saarinen and Haansuu, 2000). Correspondingly, enhanced tillering at the expense of rhizome growth and the preferential allocation of biomass into aboveground structures under high nutrient availability in the present study displayed a close correlation with the low C/N atomic ratios observed in all the plant parts of both species. However, the correlation with the tissue FAA levels was not so clear, although the relative abundance of mobile N and C compounds is considered as an even more reliable indicator of C/N balance (Kohl et al., 1998; Saarinen, 1998; Saarinen and Haansuu, 2000). In correspondence with Saarinen (1998) and Kohl et al. (1998), a close correlation is found with leaf FAA levels, but the lack of correlation is visible in belowground organs in the experiment N plus P. Here, considerably high FAA contents and proportion of Asn were detected in both species in the oligotrophic treatment. This may indicate differences in the physiological state of oligotrophically treated plants compared to

plants in all other treatments at the time of harvest (August). Belowground parts and especially older rhizome branches are important storage organs, with FAA as the dominant winter N storage compounds in *Phragmites* (Haldemann and Brändle, 1988; Woitke et al., 1997). In natural habitats, the accumulation of N reserves starts in August-September (Kühl and Kohl, 1993), but may be significantly delayed in stands of higher fertility (Kühl and Kohl, 1993). Since Asn was shown to be the most important transport and storage amino acid in this species (Haldemann and Brändle, 1988; Kohl et al., 1998), high FAA and Asn contents in the belowground organs (but not in leaves) of oligotrophically treated plants may indicate a more advanced translocation of N reserves. This finding is also supported by the higher percentage of dead leaves in this treatment, indicating accelerated shoot senescence. Correspondingly, a prolonged vegetative phase and delayed accumulation of reserves is considered as one of the factors endangering long-term survival of wetland plants in eutrophic habitats (Čížková-Končalová et al., 1992; Kühl and Kohl, 1993), and thus participating in reed die-back.

Acknowledgements

Authors would like to thank Dr. Hana Čížková (Institute of Systems Biology and Ecology, Academy of Sciences of the Czech Republic, Třeboň) for valuable discussion, Drahomíra Bartáková (Department of Plant Physiology, Faculty of Science, Charles University in Prague) for technical assistance, and Mr. Peter Lemkin for revision of English.

References

Armstrong, J., Armstrong, W., 2001. An overview of the effects of phytotoxins on *Phragmites australis* in relation to die-back. Aquat. Bot. 69, 251-268.

Barker, A.V., 1999. Foliar ammonium accumulation as an index of stress in plants. Commun. Soil Sci. Plan. 30, 167-174.

Brändle, R., Pokorný, J., Květ, J., Čížková, H., 1996. Wetland plants as a subject of interdisciplinary research. Folia Geobot. Phytotx. 31, 1-6.

Brix, H., 1999. The European research project on reed die-back and progression (EUREED). Limnologica, 29, 5-10.

Buttery, B.R., Lambert, J.M., 1965. Competition between *Glyceria maxima* and *Phragmites communis* in the region of Surlingham broad. 1. The competition mechanism. J. Ecol. 53, 163-181.

Chen, S.J., Hung, K.T., Kao, C.H., 1997. Ammonium accumulation is associated with senescence of rice leaves. Plant Growth Regul. 21: 195-201.

Čížková-Končalová, H., Květ, J., Lukavská, J., 1996. Response of *Phragmites australis*, *Glyceria maxima*, and *Typha latifolia* to additions of piggery sewage in a flooded sand culture. Wetlands Ecol. Manage. 4, 43-50.

Čížková-Končalová, H., Květ, J., Thompson, K., 1992. Carbon Starvation - a key to reed decline in eutrophic lakes. Aquat. Bot. 43, 105-113.

Čížková, H., Lukavská, J., Pribáň, K., Kopecký, J., Brabcová, H., 1996a. Carbohydrate levels in rhizomes of *Phragmites australis* at an oligotrophic and a eutrophic site: A preliminary study. Folia Geobot. Phytotx. 31, 111-118

Čížková, H., Pechar, L., Husak, S., Květ, J., Bauer, V., Radochová, J., Edwards, K., 2001. Chemical characteristics of soils and pore waters of three wetland sites dominated by *Phragmites australis*: relation to vegetation composition and reed performance. Aquat. Bot. 69, 235-249.

Čížková, H., Strand, J.A., 'Lukavská, J., 1996b. Factors associated with reed decline in a eutrophic fishpond, Rozmberk (South Bohemia, Czech Republic). Folia Geobot. Phytotx. 31, 73-84.

Clevering, O.A., 1998. An investigation into the effects of nitrogen on growth and morphology of stable and die-back populations of *Phragmites australis*. Aquat. Bot. 60, 11-25.

Den Hartog, C., Květ, J., Sukopp, H., 1989. Reed - a common species in decline - introduction. Aquat. Bot. 35, 1-4.

Dinka, M., Szeglet, P., 2001. Some characteristics of reed (*Phragmites australis /Cav./ Trin ex Steudel*) that indicate different health between vigorous and die-back stands. Verh. Int. Ver. Theor. Angew. Limnol. 27, 3364-3369.

Fogli, S., Marchesini, R., Gerdol, R., 2002. Reed (*Phragmites australis*) decline in a brackish wetland in Italy. Mar. Environ. Res. 53, 465-479.

Gloser, V., 2002. Seasonal changes of nitrogen storage compounds in a rhizomatous grass *Calamagrostis epigeios*. Biol. Plant. 45, 563-568.

Güsewell, S., Bollens, U., 2003. Composition of plant species mixtures grown at various N:P ratios and levels of nutrient supply. Basic Appl. Ecol. 4, 453-466.

Güsewell, S., Bollens, U., Ryser, P., Klözli, F., 2003. Contrasting effects of nitrogen, phosphorus and water regime on first- and second-year growth of 16 wetland plant species. Funct. Ecol. 17, 754-765.

Haldemann, C., Brändle, R., 1988. Amino-acid composition in rhizomes of wetland species in their natural habitat and under anoxia. Flora 180, 407-411.

Hardej, M., Ozimek, T., 2002. The effect of sewage sludge flooding on growth and morphometric parameters of *Phragmites australis* (Cav.) Trin. ex Steudel. Ecol. Eng. 18, 343-350.

Hroudová, Z., Zákravský, P., 1999. Vegetation dynamics in a fishpond littoral related to human impact. Hydrobiologia, 415, 139-145.

Koerselman, W., Meuleman, A.F.M., 1996. The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. J. Appl. Ecol. 33, 1441-1450.

Kohl, J.G., Woitke, P., Kühl, H., Dewender, M., Konig, G., 1998. Seasonal changes in dissolved amino acids and sugars in basal culm internodes as physiological indicators of the C/N-balance of *Phragmites australis* at littoral sites of different trophic status. Aquat. Bot. 60, 221-240.

Končalová, H., Květ, J., Pokorný, J., Hauser, V., 1993. Effect of flooding with sewage water on three wetland sedges. Wetlands Ecol. Manage. 2, 199-211.

Koppitz, H., Dewender, M., Ostendorp, W., Schmieder, K., 2004. Amino acids as indicators of physiological stress in common reed *Phragmites australis* affected by an extreme flood. Aquat. Bot. 79, 277-294.

Kronzucker, H.J., Siddiqi, M.Y., Glass, A.D.M., 1997. Conifer root discrimination against soil nitrate and the ecology of forest succession. Nature 385, 59-61.

Kubín, P., Melzer, A., 1997. Chronological relationship between eutrophication and reed decline in three lakes of southern Germany. Folia Geobot. Phytotx. 32, 15-23.

Kubín, P., Melzer, A., Čížková, H., 1994. The relationship between starch content in rhizomes of *Phragmites australis* (Cav.) TRIN. ex STEUD. and trophic conditions of habitat. Proc. R. Soc. Edin. B. 102, 433-438.

Kühl, H., Kohl, J.G., 1993. Seasonal nitrogen dynamics in reed beds (*Phragmites australis* (Cav.) TRIN. ex Steudel) in relation to productivity. Hydrobiologia 251, 1-12.

Kühl, H., Woitke, P., Kohl, J.G., 1997. Strategies of nitrogen cycling of *Phragmites australis* at two sites differing in nutrient availability. Int. Rev. Ges. Hydrobio. 82, 57-66.

Munzarová, E., Lorenzen, B., Brix, H., Vojtíšková, L., Votrubová, O., 2006. Effect of NH_4^+/NO_3^- availability on nitrate reductase activity and nitrogen accumulation in wetland helophytes *Phragmites australis* and *Glyceria maxima*. Env. Exp. Bot. 55, 49-60.

Ostendorp, W., 1989. Dieback of reeds in Europe - a critical-review of literature. Aquat. Bot. 35, 5-26.

Ostendorp, W., Tiedge, E., Hille, S., 2001. Effect of eutrophication on culm architecture of lakeshore *Phragmites* reeds. Aquat. Bot. 69, 177-193.

Rolletschek, H., Bumiller, A., Henze, R., Kohl, J.G., 1998. Implications of missing efflux sites on convective ventilation and amino acid metabolism in *Phragmites australis*. New Phytol. 140, 211-217.

Rolletschek, H., Hartzendorf, T., Rolletschek, A., Kohl, J.G., 1999a. Biometric variation in *Phragmites australis* affecting convective ventilation and amino acid metabolism. Aquat. Bot. 64, 291-302.

Rolletschek, H., Rolletschek, A., Kühl, H., Kohl, J.G., 1999b. Clone specific differences in a *Phragmites australis* stand II. Seasonal development of morphological and physiological characteristics at the natural site and after transplantation. Aquat. Bot. 64, 247-260.

Romero, J.A., Brix, H., Comin, F.A., 1999. Interactive effects of N and P on growth, nutrient allocation and NH4 uptake kinetics by *Phragmites australis*. Aquat. Bot. 64, 369-380.

Saarinen, T., 1998. Internal C : N balance and biomass partitioning of *Carex rostrata* grown at three levels of nitrogen supply. Can. J. Bot. 76, 762-768.

Saarinen, T., Haansuu, P., 2000. Shoot density of *Carex rostrata* Stokes in relation to internal carbon : nitrogen balance. Oecologia 122, 29-35.

Steinbachová L., Votrubová O., Lipavská H., Tylová E., Čížková H. Performance of *Phragmites australis* and *Glyceria maxima* under changing nitrogen and phosphorus availability. II. Carbohydrate metabolism (submitted as Part I).

Tylová-Munzarová, E., Lorenzen, B., Brix, H., Votrubová, O., 2005. The effects of NH_4^+ and NO_3^- on growth, resource allocation and nitrogen uptake kinetics of *Phragmites australis* and *Glyceria maxima*. Aquat. Bot. 81, 326-342.

Ulrich, K.E., Burton, T.M., 1988. An experimental comparison of the dry-matter and nutrient distribution patterns of *Typha-latifolia* L, *Typha-angustifolia* L, *Sparganium-eurycarpum* Engelm and *Phragmites-australis* (Cav) Trin ex Steudel. Aquat. Bot. 32, 129-139.

Van der Putten, W.H., 1997. Die-back of *Phragmites australis* in European wetlands: an overview of the European Research Programme on Reed Die-Back and Progression (1993-1994). Aquat. Bot. 59, 263-275.

Van der Putten, W.H., Peters, B.A.M., Van den Berg, R.S., 1997. Effects of litter on substrate conditions and growth of emergent macrophytes. New Phytol. 135, 527-537.

Votrubová, O., Pecháčková, A., 1996. Effect of nitrogen over-supply on root structure of common reed. Folia Geobot. Phytotx. 31, 119-125.

Votrubová, O., Soukup, A., Pecháčková, A., 1997. Anatomy of common reed and its importance for survival in eutrophic habitats. Acta U. Carol. Biol. 41, 233-244.

Vretare, V., Weisner, S.E.B., Strand, J.A., Graneli, W., 2001. Phenotypic plasticity in *Phragmites australis* as a functional response to water depth. Aquat. Bot. 69, 127-145.

Weisner, S.E.B., Strand, J.A., 1996. Rhizome architecture in *Phragmites australis* in relation to water depth: Implications for within-plant oxygen transport distances. Folia Geobot. Phytotx. 31, 91-97.

Woitke, P., Kiehl, A., Kühl, H., Kohl, J.G., 1997. Nitrogen and carbohydrate pools of two rhizome types of *Phragmites australis* (Cav) Trin ex Steudel. Int. Rev. Ges. Hydrobio. 82, 161-168.

Table 1 Statistical evaluation of data shown in Figs. 1, 2, 3, and 4 using ANOVA. Effects of plant species (sp.), treatments (tr.) and their interactions (sp x tr) are expressed by *P*-values.

	Experiment N plus P			Experiment N		
	sp.	tr.	sp x tr	sp.	tr.	sp x tr
Biomass production (g)						
Leaves	0.000	0.000	0.166	0.000	0.000	0.000
Stems	0.000	0.000	0.149	0.015	0.000	0.001
Rhizomes	0.362	0.000	0.554	0.011	0.000	0.003
Roots	0.000	0.000	0.006	0.000	0.000	0.000
Total FAA content (μmol g^{-1} dry wt)						
Leaves	0.048	0.000	0.000	0.294	0.000	0.000
Stems	0.052	0.002	0.068	0.000	0.000	0.008
Rhizomes	0.006	0.000	0.159	0.721	0.000	0.498
Asn+Gln+Arg (% of total FAA content)						
Leaves	0.000	0.000	0.000	0.605	0.000	0.006
Stems	0.592	0.011	0.721	0.057	0.001	0.124
Rhizomes	0.014	0.000	0.587	0.017	0.000	0.067
NH_4^+ content (μmol g^{-1} dry wt)						
Leaves	0.067	0.000	0.003	0.244	0.000	0.230
Stems	0.943	0.023	0.842	0.168	0.000	0.015
Rhizomes	0.234	0.023	0.860	0.455	0.000	0.039
P content (% dry wt)						
Leaves	0.209	0.000	0.001	0.704	0.832	0.968
N/P atomic ratio						
Leaves	0.000	0.000	0.008	0.185	0.039	0.247

Table 2 Biomass allocation (means ± STD, n = 7-8) of *Phragmites australis* and *Glyceria maxima* grown in the experiment N plus P (oligo, eu, hyper) and the experiment N (low N, medium N, high N). Effects of plant species (sp.), treatments (tr.), and their interactions (sp x tr) are expressed by *P*-values using ANOVA. AB ratio – ratio of aboveground (shoots) to belowground (roots and rhizomes) biomass, SR ratio – ratio of root-supported tissue (shoots, rhizomes) to root biomass. Notes: [a] % total dry wt.

	Phragmites australis			*Glyceria maxima*			Statistics		
	Oligo	Eu	Hyper	Oligo	Eu	Hyper	sp.	tr.	sp x tr
AB ratio	1.14 ± 0.16	1.62 ± 0.25	2.31 ± 0.45	1.09 ± 0.18	2.84 ± 0.21	4.9 ± 0.27	0.000	0.000	0.000
SR ratio	5.31 ± 0.35	11.2 ± 0.85	12.7 ± 2.17	2.11 ± 1.14	6.34 ± 1.02	16.6 ± 0.79	0.000	0.000	0.000
leaves[a]	20.8 ± 1.26	30.3 ± 2.80	34.4 ± 1.63	27.1 ± 3.17	44.0 ± 2.03	51.1 ± 1.96	0.000	0.000	0.102
stems[a]	32.3 ± 3.17	31.4 ± 3.40	35.2 ± 1.27	24.9 ± 2.93	29.9 ± 2.35	31.9 ± 1.37	0.000	0.000	0.006
rhizomes[a]	30.7 ± 5.60	30.1 ± 2.03	23.0 ± 1.15	15.4 ± 4.61	12.4 ± 2.93	11.2 ± 2.19	0.000	0.006	0.466
roots[a]	16.3 ± 3.82	8.24 ± 1.59	7.3 ± 0.88	32.6 ± 2.67	13.8 ± 0.73	5.79 ± 0.43	0.000	0.000	0.000
	Low N	Medium N	High N	Low N	Medium N	High N			
AB ratio	0.91 ± 0.17	2.00 ± 0.46	3.81 ± 0.58	1.16 ± 0.21	3.54 ± 0.66	6.17 ± 0.67	0.000	0.000	0.030
SR ratio	7.80 ± 0.80	10.9 ± 3.06	11.8 ± 1.68	2.70 ± 0.51	7.85 ± 1.80	13.1 ± 1.80	0.000	0.000	0.000
leaves[a]	24.4 ± 2.72	33.4 ± 2.37	38.7 ± 0.99	30.8 ± 2.16	45.1 ± 5.86	48.0 ± 1.29	0.000	0.000	0.400
stems[a]	23.0 ± 3.39	32.6 ± 2.64	40.3 ± 1.72	22.6 ± 3.67	32.4 ± 4.96	38.0 ± 1.30	0.394	0.000	0.872
rhizomes[a]	41.2 ± 4.67	25.1 ± 2.87	13.2 ± 1.70	19.2 ± 4.24	10.7 ± 2.16	7.6 ± 1.17	0.000	0.000	0.040
roots[a]	11.4 ± 0.94	8.87 ± 2.29	7.90 ± 1.96	27.4 ± 3.72	11.8 ± 2.83	6.4 ± 0.73	0.000	0.000	0.000

Table 3 Biometric characteristics (means ± STD, n = 5-8) of *Phragmites australis* and *Glyceria maxima* grown in the experiment N plus P (oligotrophic, eutrophic, hypertrophic) and the experiment N (low N, medium N, high N). Effects of plant species (sp.), treatments (tr.), and their interactions (sp x tr) are expressed by *P*-values using ANOVA.

	Phragmites australis			*Glyceria maxima*			Statistics		
	Oligo	Eu	Hyper	Oligo	Eu	Hyper	sp.	tr.	sp x tr
shoot number (No. plant^{-1})	5.29 ± 0.95	21.0 ± 4.73	34.0 ± 12.3	5.00 ± 2.31	29.7 ± 7.83	92.0 ± 24.3	0.000	0.000	0.000
average length of shoot (mm)	441 ± 36.7	743 ± 64.8	923 ± 119	380 ± 96.3	808 ± 93.4	892 ± 87.8	0.344	0.000	0.061
leaf number (No. shoot^{-1})	11.2 ± 1.13	13.9 ± 1.10	14.2 ± 1.73	5.88 ± 1.60	7.56 ± 1.95	7.39 ± 0.34	0.000	0.000	0.947
dead/living leaves ratio	0.24 ± 0.13	0.07 ± 0.02	0.08 ± 0.01	0.68 ± 0.13	0.23 ± 0.09	0.39 ± 0.07	0.000	0.000	0.071
rhizome length (cm. plant^{-1})	60.3 ± 25.2	505 ± 179	694 ± 231	65.2 ± 36.0	712 ± 205.8	1514 ± 430	0.005	0.000	0.045
rhizome length/shoot number (mm No.$^{-1}$)	116 ± 44.4	236 ± 45.0	208 ± 35.8	155 ± 122	237 ± 38.5	164 ± 9.37	0.824	0.000	0.342
root number (No. plant^{-1})	61.3 ± 33.1	582 ± 265	833 ± 152	476 ± 185	3925 ± 979	9951 ± 5597	0.000	0.000	0.708
average length of root (mm)	131 ± 19.5	93.5 ± 6.0	116 ± 20.9	87.0 ± 4.54	76.3 ± 16.3	44.0 ± 8.61	0.000	0.003	0.003
branched/unbranched roots ratio	4.50 ± 1.66	4.86 ± 0.91	4.65 ± 0.56	7.98 ± 7.90	3.82 ± 2.25	3.42 ± 0.19	0.626	0.649	0.443
injured roots (% of total root number)	5.31 ± 4.79	16.9 ± 0.90	7.32 ± 3.60	12.5 ± 6.04	8.52 ± 6.71	14.9 ± 2.85	0.527	0.659	0.054
	Low N	Medium N	High N	Low N	Medium N	High N			
shoot number (No. plant^{-1})	12.9 ± 4.26	41.4 ± 12.3	54.9 ± 16.3	15.6 ± 6.00	36.6 ± 8.31	41.4 ± 17.3	0.379	0.000	0.165
average length of shoot (mm)	448 ± 168	895 ± 97.8	740 ± 61.6	609 ± 102	811 ± 83.6	710 ± 73.1	0.200	0.000	0.003
leaf number (No. shoot^{-1})	11.3 ± 2.06	14.8 ± 1.34	13.6 ± 1.39	7.13 ± 0.90	7.99 ± 1.02	8.33 ± 1.16	0.000	0.000	0.192

dead/living leaves ratio	0.10 ± 0.08	0.07 ± 0.02	0.25 ± 0.09	0.40 ± 0.13	0.37 ± 0.11	0.77 ± 0.18	0.000	0.000	0.330
rhizome length (cm. plant^{-1})	269 ± 128	842 ± 267	474 ± 137	457 ± 151	965 ± 251	635 ± 293	0.005	0.000	0.224
rhizome length/shoot number (mm No.$^{-1}$)	216 ± 98.7	205 ± 31.0	87.9 ± 17.9	329 ± 149	274 ± 102	157 ± 29.0	0.000	0.000	0.391
root number (No. plant^{-1})	267 ± 121	1012 ± 127	560 ± 102	1631 ± 730	4854 ± 808	3402 ±594	0.000	0.000	0.711
average length of root (mm)	118 ± 6.6	117 ± 7.50	140 ± 8.19	99.5 ± 22.4	100 ± 23.8	68.2 ± 2.78	0.000	0.447	0.010
branched/unbranched roots ratio	6.23 ± 1.44	10.5 ± 3.24	23.8 ± 6.70	4.76 ± 0.55	7.44 ± 0.86	16.6 ± 2.56	0.015	0.000	0.947
injured roots (% of total root number)	38.44±8.40	42.03±8.40	37.97±8.40	16.88±8.40	44.67±8.40	83.85±8.40	0.924	0.012	0.021

Table 4 C/N atomic ratios (means ± STD, n = 3) of leaves, rhizomes and roots of *Phragmites australis* and *Glyceria maxima* grown in the experiment N plus P (oligotrophic, eutrophic, hypertrophic) and the experiment N (low N, medium N, high N). Effects of plant species (sp.), treatments (tr.), and their interactions (sp x tr) are expressed by *P*-values using ANOVA.

	Phragmites australis			*Glyceria maxima*			Statistics		
	Oligo	Eu	Hyper	Oligo	Eu	Hyper	sp.	tr.	sp x tr
Leaves	22.4 ± 1.79	14.0 ± 0.97	12.5 ± 0.31	34.4 ± 2.10	19.5 ± 0.48	12.4 ± 0.43	0.000	0.000	0.000
Rhizomes	63.2 ± 3.27	44.1 ± 1.60	32.9 ± 2.58	44.7 ± 2.61	43.4 ± 3.66	18.8 ± 0.32	0.000	0.000	0.001
Roots	29.5 ± 1.30	22.8 ± 0.03	20.3 ± 1.27	36.1 ± 2.94	26.3 ± 2.64	13.9 ± 0.74	0.255	0.000	0.001
	Low N	Medium N	High N	Low N	Medium N	High N			
Leaves	16.5 ± 0.39	14.6 ± 0.03	12.7 ± 0.16	30.7 ± 0.67	17.4 ± 1.06	11.8 ± 0.04	0.000	0.000	0.000
Rhizomes	98.8 ± 13.9	40.9 ± 1.36	35.7 ± 0.79	100 ± 5.94	38.2 ± 2.35	23.1 ± 0.66	0.231	0.000	0.300
Roots	37.5 ± 4.89	23.4 ± 1.22	19.3 ± 0.61	68.3 ± 2.29	23.9 ± 2.58	15.2 ± 0.38	0.000	0.000	0.000

Legends to figures

Fig. 1 Biomass (mean ± SD, n = 5-8) of leaves (L), stems (ST), rhizomes (RH) and roots (R) of *Phragmites australis* (a, c) and *Glyceria maxima* (b, d) grown under increasing N plus P (a, b) and increasing N (c, d) supplies.

Fig. 2. Free amino acid contents (mean ± SD, n = 3) in leaves (L), rhizomes (RH) and roots (R) of *Phragmites australis* (a, c) and *Glyceria maxima* (b, d) grown under increasing N plus P (a, b) and increasing N (c, d) supplies. Numbers above each column indicate relative participation (%) of Asn + Gln + Arg to the total FAA content (means, n = 3).

Fig. 3. NH_4^+ contents (mean ± SD, n = 3) in leaves (L), rhizomes (RH) and roots (R) of *Phragmites australis* (a, c) and *Glyceria maxima* (b, d) grown under increasing N plus P (a, b) and increasing N (c, d) supplies.

Fig. 4. The relative contents of phosphorus (mean ± SD, n = 3) in leaves of *Phragmites australis* (a) and *Glyceria maxima* (b) grown under increasing N plus P (oligo, eu, hyper) and increasing N (low, medium, high N) supplies. Numbers above each column indicate atomic N:P ratios (means, n = 3).

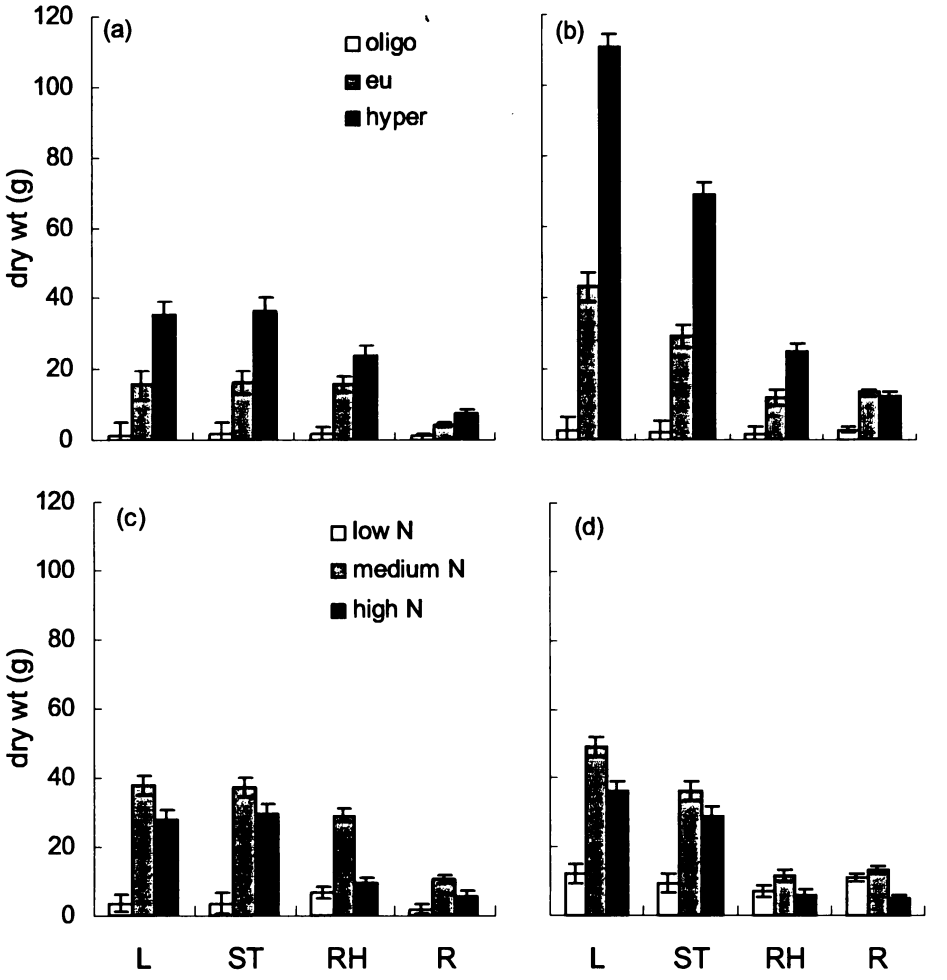


Fig. 1 Tylová et al.

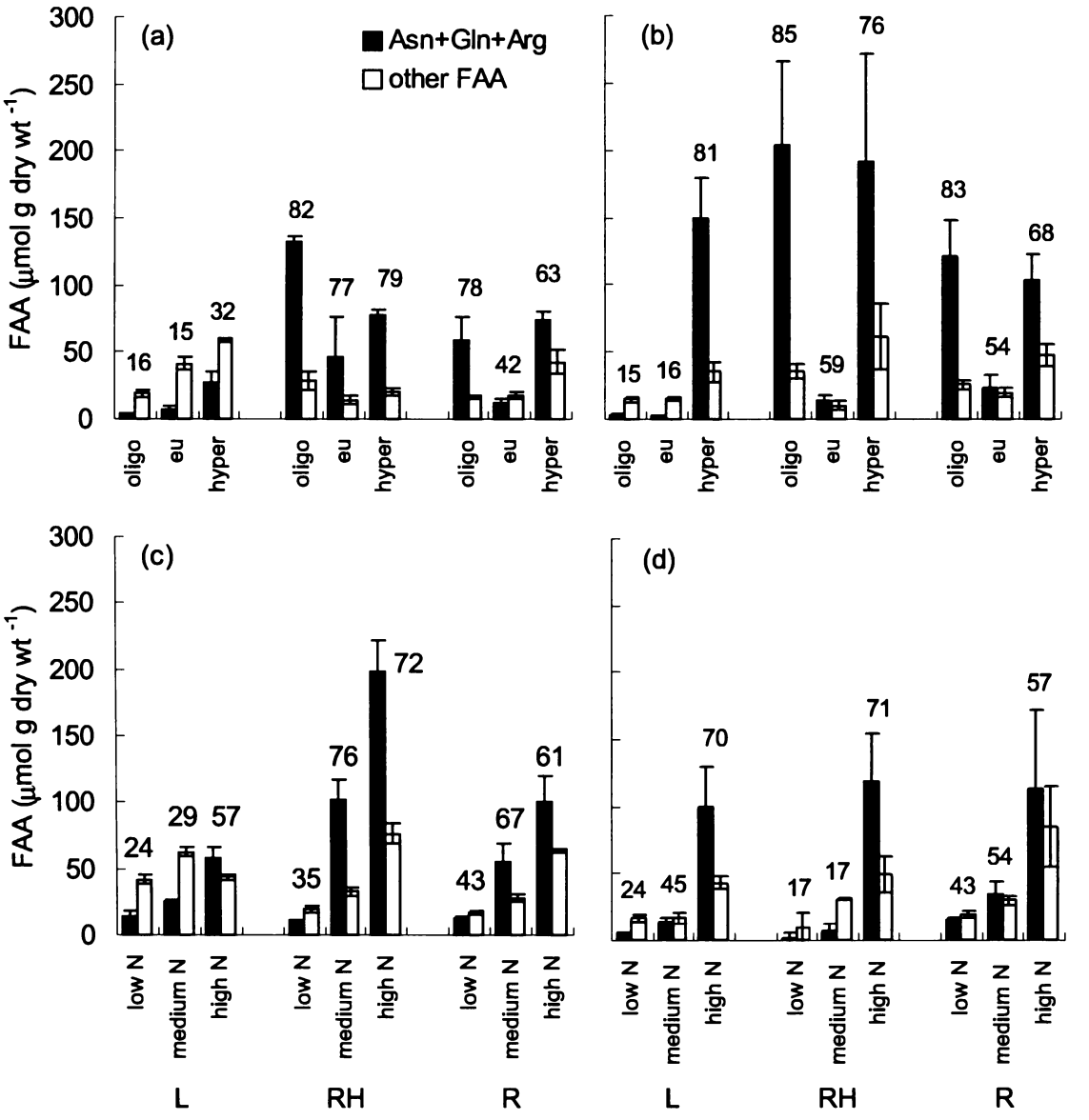


Fig.2 Tylová et al.

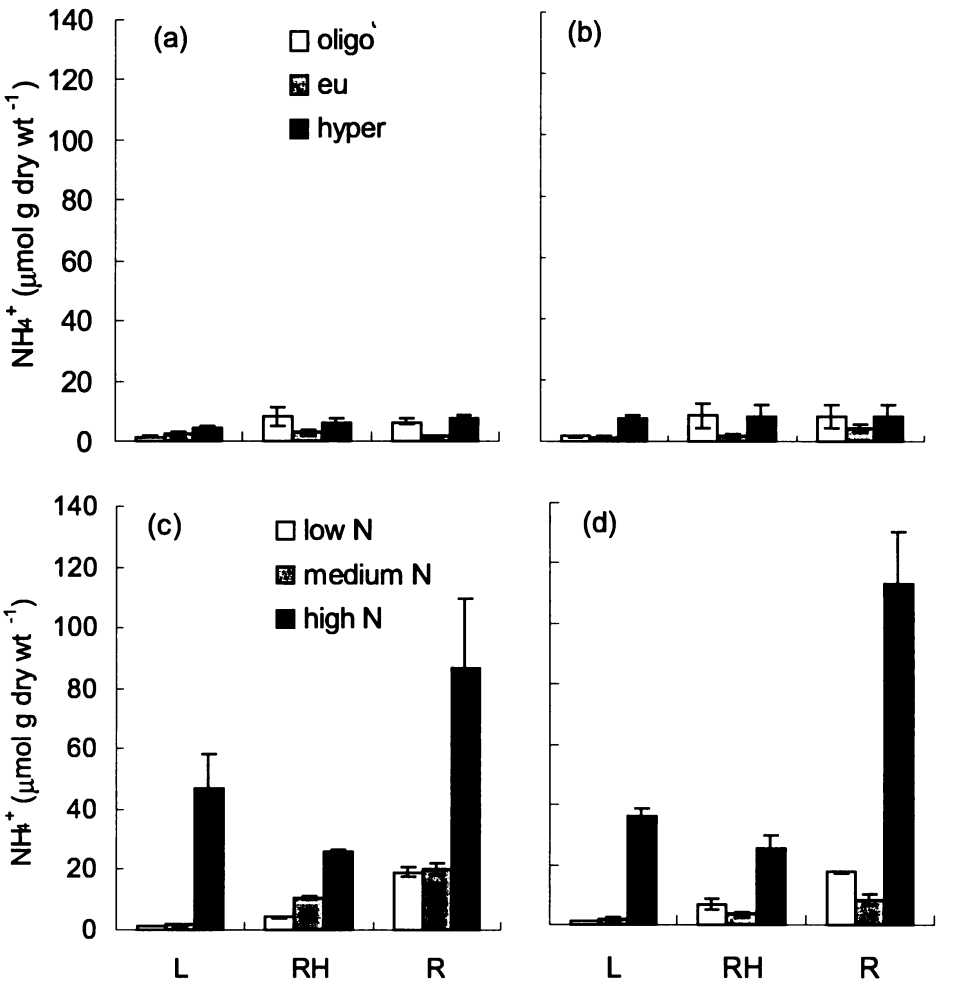


Fig. 3 Tylová et al.

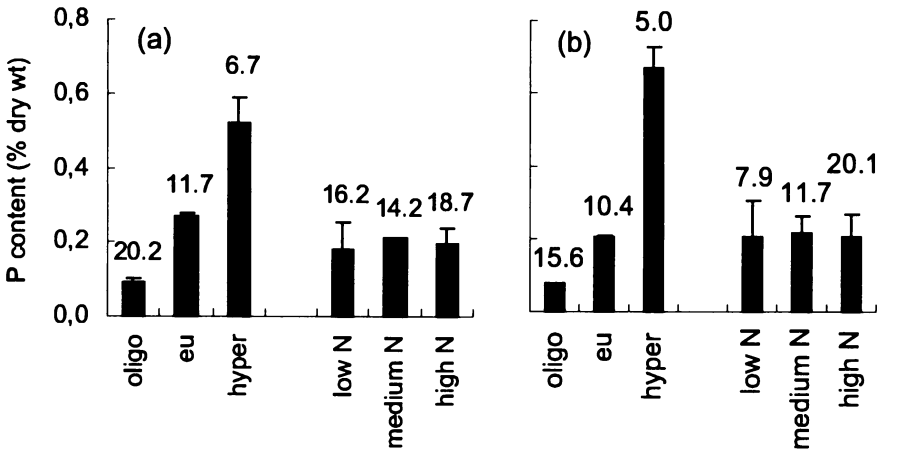


Fig. 4 Tylová et al.