Nitrogen acquisition in wetland plants

(nutritional aspects of plant performance under eutrophication)

PhD thesis

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Abreviations:

A/B ratio - ratio of aboveground (shoots) to belowground (rhizomes, roots) biomass
EC - European Commission
EEA - European Environmental Agency
FAA - free amino acids
FAO - Food and Agriculture Organisation of the United Nations
HATS - high affinity transport system
LATS - low affinity transport system
N - nitrogen
NR - nitrate reductase
P - phosphorus
ROL - radial oxygen loss
S/R ratio - ratio of root-supported tissue (shoot, rhizomes) to root biomass

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The table of contents:

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*. Aquatic Botany 81: 326-342.

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*. Environmental and Experimental Botany 55: 49-60.

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Tylová, E., Steinbachová, L., Soukup, A., Votrubová, O., Gloser, V. Performance of *Phragmites australis* and *Glyceria maxima* under changing nitrogen and phosphorus availability. I. Plant growth, resource allocation and C/N balance.

Hereby I confirm, that the PhD thesis was created by my own under supervision of Dr. Olga Votrubová and was not submitted at any other school to get any other academical degree.

Prague 29.7.2007

Edita Tylova

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Introduction and aims of the study:

Human activities including agriculture, industry, and households alter global N, P cycles towards higher availability and mobility of nutrients worldwide (Smith et al. 1996, Vitousek et al. 1997, FAO 2004, Khan and Ansari 2005). In wetlands, nutrient enrichment and subsequent eutrophication are forces seriously affecting their functions, stability (EEA 1999), diversity, and the rate of natural succession (Khan and Ansari 2005). The addition of N generally leads to the stimulation of overall biomass productivity, but losses in diversity (Vitousek et al. 1997, Güsewell and Bollens 2003). Massive growth of algae and duckweeds triggers a decline of submerged macrophytes (e.g. isoetids, characeans) due to shading effect (Khan and Ansari 2005). Moreover, a retreat of species using efficient N utilization as the competitive advantage occurs in favour of highly productive species; together with the loss of rare and uncommon species (Verhoeven et al. 1996, Vitousek et al. 1997, Levine et al. 1998, Bedford et al. 1999, Sand-Jensen et al. 2000, Hejný et al. 2002, Güsewell and Bollens 2003, Khan and Ansari 2005). At many localities, anthropogenic impact is related also to the invasion of non-native plant dominants (Bedford et al. 1999, Cronk and Fennessy 2001). The spreading of *Typha domingensis* (Vaithiyanathan and Richardson 1999, Lorenzen et al. 2001, Cronk and Fennessy 2001) or non-native clones of *Phragmites australis* (Saltonstall 2002, Tewksbury et al. 2002) in North American wetlands, positively related to N, P enrichment, are some of the typical examples.

In Europe, vice versa, wetlands instability and changes in sediment properties caused by eutrophication resulted in a considerable retreat of *Phragmites australis* during last decades (Ostendorp 1989, Kühl and Kohl 1992, van der Putten 1997, Kubín and Melzer 1997, Fogli et al. 2002). This large-scale reed die-back occurring at eutrophic habitats shows us that even fast-growing dominant species may suffer from the altered growth conditions. The loss of *Phragmites* in Europe was even so serious, that the species gained the global interest (Ostendorp 1989, van der Putten 1997, Brix 1999). This effort, integrated particularly in EUREED I and II projects of the European Union (1993-1994 and 1996-1998; for further details see Brix (1999)), brought about the knowledge of many theoretical and practical aspects of wetland plant performance, identifying several mechanisms of eutrophication impact. Among others, litter accumulation and phytotoxicity of its degradation products, high oxygen demand in the sediment, mechanical damage caused by biotic or abiotic factors, delayed translocation of storage compounds, disturbance of carbon balance, and/or changes in

plant morphology and anatomy induced by high nutrient availability can be mentioned (for summary see e.g. (Ostendorp 1989)).

However, it seems to us, that less attention was generally paid to the "**primary nutritional aspects of N addition**", it means to general characteristics of N acquisition in wetland plants. In terrestrial habitats, not only the availability of N *per-se*, but also the form of available N (NH_4^+ versus NO_3^-), and the ratio to other nutrients (particularly N/P) affect plant performance and community composition considerably (Chapin et al. 1986, Marschner 1995, Troelstra et al. 1995a, Kronzucker et al. 1997, Nordin et al. 2001). Similar mechanisms may act in wetlands, but the extent to which they really affect performance and competition ability of individual species is not completely clear. Among others, Güsewell and co-workers showed the importance of N/P ratio, especially under N or P limiting conditions (Güsewell and Bollens 2003, Güsewell et al. 2003). Similarly, the observations of Smolders et al. (1996), Dorland et al. (2003), Paulissen et al. (2004), and Paulissen et al. (2005) indicate that the shift in NH_4^+/NO_3^- ratio affect the composition of wetland community. Especially some aquatic communities (e.g. isoetids of soft-water lakes) seem very sensitive towards high relative abundance of NH_4^+ (Bobbink et al. 1998). Obviously, the interspecific differences in NH_4^+ tolerance may exist in relation to the conditions of microhabitat, as NH_4^+/NO_3^- ratio differs among individual wetlands as well as within the wetland. NH_4^+/NO_3^- ratio also increases along with the eutrophication, as the litter accumulation increases microbial activities and oxygen demand, which leads to the diminution of NO_3^- from the sediment (Kühl and Kohl 1992, Čížková et al. 2001b). Furthermore, NH_4^+ is the substantial form of N in wastewater (see e.g. Cottingham et al. (1999), Szogi et al. (2003), Edwards et al. (2006); for further details see Tab. 1), which is commonly treated with the help of wetland or aquatic plants (Gopal 1999, Květ et al. 1999, Vymazal 2002).

Therefore, **the general aim of this thesis** is to follow <u>basic aspects of N acquisition and utilization in wetland plants, and/or their growth and metabolic responses to N level, N/P ratio, and NH_4^+/NO_3^- ratio.</u> Simplified water or sand cultivations (Fig. 2) are used to separate the nutritional aspects of plant performance from other potentially interfering factors acting at natural habitats. The study is a part of broader physiological effort of the team of Dr. Olga Votrubová (Department of Plant Physiology, Charles University in Prague), focused on different aspects of wetland plant performance (see diploma and PhD theses: Soukup (1997), Vojtíšková (1999), Erhart (2000), Říhová (2000), Munzarová (2000), Raimanová (2001), Lichtenberková (2002), Jirovská (2003), Soukup (2005), Steinbachová (2006), and the publications therein). The study was realised in the collaboration with the Department of

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Biological Sciences, Aarhus University (the team of Prof. Hans Brix; http://person.au.dk/en/hans.brix@biology.au.dk) and with the Department of Plant Physiology and Anatomy, Faculty of Science of Masaryk University in Brno (the team of Dr. Vít Gloser; http://www.muni.cz/people/2691). The study focuses particularly on two dominants of our littoral vegetation: *Phragmites australis* (Cav.) Trin. Ex Steud. and *Glyceria maxima* (Hartm.) Holmb., which occupy similar habitats, but differ in the growth strategy (e.g. dormancy requirement, rooting depth), and sensitivity to eutrophication. While *Phragmites* seems positively responding to early stages of eutrophication, *Glyceria* is more tolerant to highly eutrophic sediments rich in organic matter (Ozimek and Klekot 1979, Crawford and Brändle 1996, Hroudová and Zákravský 1999, Hroudová and Zákravský 2002), and its spread following reed retreat was repeatedly documented (e.g. Hroudová and Zákravský (1999), Hroudová and Zákravský (2002)); for further characteristics of selected species see Fig. 1). Therefore, they represent useful model species for understanding the mechanisms underlying competition ability in the changing environment. Both species are also commonly used in wastewater-treating systems (Květ et al. 1999, Vymazal 2002).

The original findings of the thesis are summarised in the following original papers (or submitted manuscripts), and are confronted with the current general view on the topics, given as the review.

Included articles:

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*. Aquatic Botany 81: 326-342.
(article 1)

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*. Environmental and Experimental Botany 55: 49-60.
(article 2)

Tylová E., Steinbachová L., Votrubová O., Lorenzen B., Brix H. Different sensitivity of *Phragmites australis* and *Glyceria maxima* to high availability of ammonium-N. Accepted in Aquatic Botany.
(manuscript 1)

Tylová E., Steinbachová L., Votrubová O., Gloser V. Phenology and autumnal accumulation of N reserves in belowground organs of wetland helophytes *Phragmites australis* and *Glyceria maxima* affected by nutrient surplus. Submitted to Environmental and Experimental Botany.
(manuscript 2)

Tylová E., Steinbachová L., Soukup A., Votrubová O., Gloser V. Performance of *Phragmites australis* and *Glyceria maxima* under changing nitrogen and phosphorus availability. I. Plant growth, resource allocation and C/N balance. Submitted to Environmental and Experimental Botany.
(manuscript 3)

Other articles of the author, related to the topics:

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.

Vojtíšková L., **Munzarová E.**, Votrubová O., Čížková H., Lipavská H. 2006. The influence of nitrogen nutrition on the carbohydrate and nitrogen status of emergent macrophyte *Acorus calamus* L. Hydrobiologia 563:73-85.

Steinbachová-Vojtíšková L., **Tylová E.**, Novická H., Soukup A., Votrubová O. 2006. Lipavská H., Čížková H. Influence of nutrient supply on growth, carbohydrate and nitrogen metabolic relations in *Typha angustifolia*. Environmental and Experimental Botany 57: 246-257.

Steinbachová L., Lipavská H. **Tylová E.**, Votrubová O., Čížková H. Performance of *Phragmites australis* and *Glyceria maxima* under changing nitrogen and phosphorus availability. II. Carbohydrate metabolism. Submitted to Environmental and Experimental Botany.

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Fig. 1: Brief characteristic of the selected species.

Phragmites australis (**Cav.**) **Trin. Ex Steud.** (common reed; *Poaceae*) is a cosmopolitan perennial emergent grass with broad ecologic amplitude, which is native in freshwater marshes and littorals of Eurasia, Australia and America (Mal and Narine 2004), but genotypes introduced to North America from Europe exhibit high invasiveness displacing native reed populations and other species during last decades (Cronk and Fennessy 2001, Saltonstall 2002, Tewksbury et al. 2002, Mal and Narine 2004). The species possesses extensive rhizome system penetrating deeply into the flooded sediment (Buttery and Lambert 1965, Ennabili et al. 1998, Mal and Narine 2004); the depth of penetration is related to the sediment properties and water depth (Fiala 1976).

    Phragmites is adapted to grow in nutrient rich habitats (Romero et al. 1999), but is able to colonise also oligotrophic sites (Romero et al. 1999), where *Glyceria* does not occur (Brändle et al. 1996). Enormous biomass production, accumulation of considerable amounts of N and P in the tissues (Tanner 1996), and the substantial ability of rhizosphere oxidation (Armstrong et al. 1992) predetermine *Phragmites* for the usage in wastewater treating systems, where the species is widely (and even dominantly) used (Vymazal 1995, Tanner 1996, Ennabili et al. 1998, Gopal 1999). Although positively affected by early stages of eutrophication, *Phragmites* shows sensitivity to highly eutrophic sediments rich in organic matter (den Hartog et al. 1989, Brändle et al. 1996, Čížková et al. 1996, van der Putten et al. 1997, Gopal 1999, Hroudová and Zákravský 1999), which is widely described in European reed populations (Gopal 1999, Cronk and Fennessy 2001). *Phragmites* is especially sensitive to litter accumulation (van der Putten et al. 1997), phytotoxins occurrence (Armstrong and Armstrong 1999, Armstrong and Armstrong 2001a), and suffers from high sulphide levels (Crawford and Brändle 1996, Armstrong et al. 1996a). *Phragmites* growth is maximal in pH range 5.5-8.1, but the species is able to survive even highly acidified habitats (pH 2.9) (for summary see Mal and Narine (2004)).

Glyceria maxima (**Hartm.**) **Holmb.** (reed sweet grass; *Poaceae*) is a perennial emergent littoral grass native to Eurasia, which naturalised also in North America, New Zealand and southern Australia (Clarke et al. 2004, Wei and Chow-Fraser 2006). Similarly to *Phragmites*, the spread of *Glyceria* in North America correlates with the increasing nutrient availability resulting from urbanization (Wei and Chow-Fraser 2006). *Glyceria* is a shallow rooting species (Buttery and Lambert 1965, Tanner 1996) sensitive to high water levels (Crawford and Brändle 1996, Wei and Chow-Fraser 2006), which forms even floating mats in sheltered deeper-water stands (Tanner 1996). It remains partially active during winter period (Dykyjová 1978, Tanner 1996) and starts growing early in the spring (Buttery and Lambert 1965, Dykyjová 1978, Crawford and Brändle 1996). The potential of early-spring establishment of vegetative cover favours *Glyceria* compared to late-emerging *Phragmites*, species with strong winter dormancy (Buttery and Lambert 1965, Cronk and Fennessy 2001).

    Glyceria is less sensitive to litter accumulation compared to *Phragmites* (van der Putten et al. 1997) and can successfully cope with very high nutrient levels in the sediment (Ozimek and Klekot 1979, Crawford and Brändle 1996, Hroudová and Zákravský 1999, Hroudová and Zákravský 2002, Lucassen et al. 2006). The spread of *Glyceria* is observed on highly eutrophic habitats rich in organic matter (Hroudová and Zákravský 1999, Hroudová and Zákravský 2002), where *Phragmites* retreat occurs (Hroudová and Zákravský 1999). At these sites, *Glyceria* forms monodominant stands very poor in species (Hroudová and Zákravský 2002). *Glyceria* performance is not negatively affected by absence of oxygen in the rhizosphere, but shows sensitivity to reduced conditions (Brix and Sorrell 1996). Similarly to *Phragmites*, the species affects rhizosphere characteristics by releasing considerable amounts of oxygen and thus stimulating activity of nitrifiers at the roots and rhizomes surfaces (Nijburg et al. 1997, Ottová et al. 1997). *Glyceria* is used in wastewater treatment as well, but the usage is less common compared to *Phragmites* (Ozimek and Klekot 1979, Vymazal 1995, Tanner 1996).

Fig. 2: Plant cultivations.

The outdoor sand cultures unprotected against the rainfall:

The outdoor sand cultures protected against the rainfall:
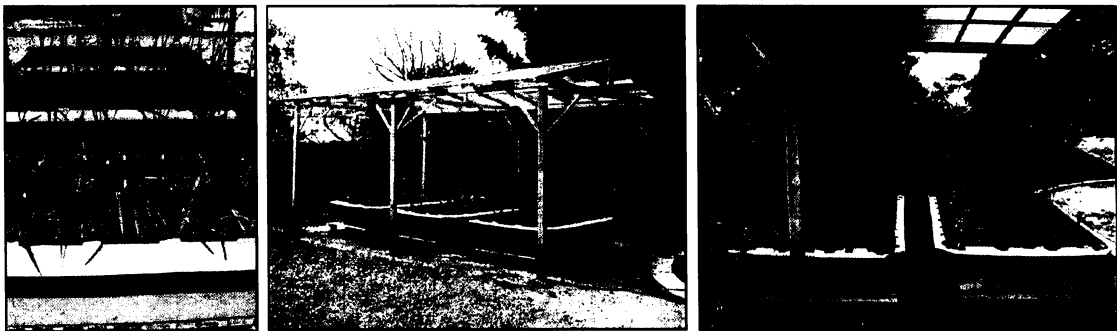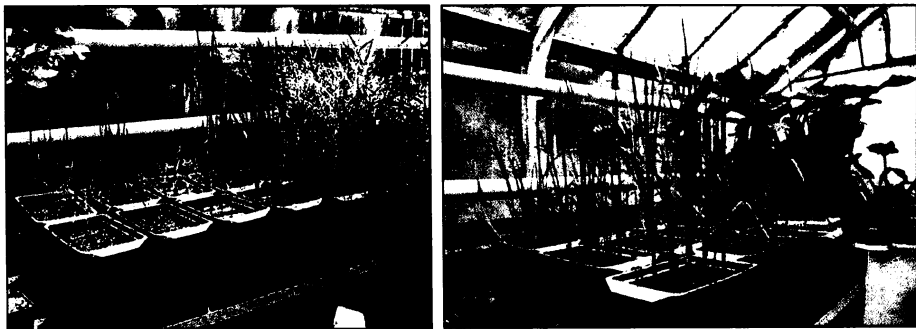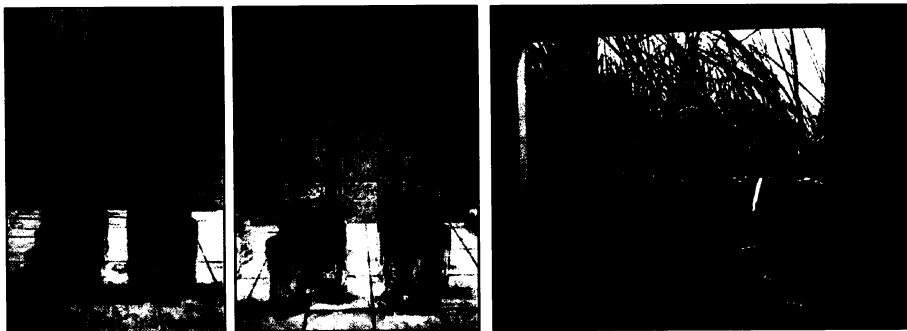

The water cultures in the greenhouse:

The water cultures in the room with constant growth conditions and in the controlled growth cabinet:

The flooded sediment is highly specific substrate for plant growth, as low oxygen diffusion rate in the water (10^4 lower compared to air) induces establishment of reduced soil conditions and transformation of nutrients. Along with gradually decreasing soil redox potential, NO_3^- disappears from the sediment being denitrified to gaseous N compounds (NOx, N_2) or ammonified to NH_4^+. Subsequently, iron, manganese, and sulphur are reduced to highly soluble reduced forms (Fe^{2+}, Mn^{2+}, S^{2-}) (for summary see e.g. Ponnamperuma (1984), Jackson et al. (1991), Cronk and Fennessy (2001)), carrying potential risk of phytotoxicity (Crawford 1994). Even nutrients, which are not directly subjected to reduction are affected, e.g. PO_4^{3-} is released from iron oxohydroxides in consequence of Fe^{3+} to Fe^{2+} reduction, and the availability of positively charged cations (K^+, Mg^{2+}, Ca^{2+}) increases (Cronk and Fennessy 2001).

The presence of wetland plants interferes with the availability of nutrients and the occurrence of their chemical forms in the flooded sediment significantly. Particularly helophytes, rooting in the flooded sediment and carrying photosynthetically active organs above the water level, are important. These species posses the system of internal air spaces supplying oxygen to belowground organs buried in the hypoxic/anoxic sediment (Armstrong and Armstrong 1988, Armstrong 1989, Končalová 1990, Armstrong and Armstrong 1990, Armstrong et al. 1991, Armstrong et al. 1996d), and the protective surface layers in belowground organs, which prevent penetration of toxic compounds into plant tissues and loss of oxygen (Soukup et al. 2002). In root apical parts or lateral roots, where this protection is less conspicuous or not fully differentiated, oxygen is released into the rhizosphere (radial oxygen loss; ROL) (Armstrong and Armstrong 1988, Armstrong et al. 1992, Armstrong et al. 2000). Subsequently, reduced compounds (particularly Fe^{2+}, Mn^{2+}) are oxidised back to less soluble ones, and the sediment phytotoxicity is alleviated (for summary see Crawford (1994), Cronk and Fennessy (2001)). ROL also influences N transformations in wetlands, as it allows preservation of nitrifying activity at the roots surfaces in spite of overall hypoxic/anoxic conditions (Engelaar et al. 1995, Nijburg et al. 1997, Ottová et al. 1997). The importance of NO_3^- emerging in consequence of ROL for mineral nutrition of wetland plants was long-term marginalized, but results of Kirk (2001) or Engelaar et al. (1995) indicate, that might be significant.

Fig. 3: Anthropogenic N, P pollution of wetlands.

The sources of nutrients for aquatic ecosystems involve groundwater, fluvial and atmospheric inputs, coming from both point and non-point sources (Smith et al. 1996, Daniel et al. 1998, Correll 1999). The most important N inputs come from the agriculture. The global consumption of mineral fertilizers exceeded 80 and 30 million tons of nitrogen (N) and phosphorus (P_2O_5) fertilizers per year; 57% of N amount is consumed in Asia, 18% in North America and 17% in Europe (FAO 1999). The further N is supplied as organic fertilizers (manures), fixed with nitrogen-fixing crops and mobilized in consequence of land clearing and wetland draining (Vitousek et al. 1997). In addition, above 20 million tons of N is released into atmosphere with fossil fuel combustion yearly, N is emitted also due to agriculture activities and transport (Vitousek et al. 1997, EC 2002a). Consequent atmospheric N deposition present another N input into wetlands (important especially in midwestern and eastern USA, northern Europe), being 10-100 times higher compared to natural background (Smith et al. 1996, Vitousek et al. 1997, Tracy et al. 2003).

In **Europe**, the agricultural run-off is the main source of **N pollution** (both NO_3^- and NH_4^+), followed by human wastewater (EEA 1999, Sand-Jensen et al. 2000). From 1950 until 2000, the usage of mineral N fertilizers in 15 EU member states increased from 1 to 10 million tons, although the decreasing trend occurs from 1988 (EC 2002b). In 1991, Nitrates directive (91/676/EEC) was accepted to reduce water pollution caused by NO_3^- from agricultural sources, but until 1998 no significant decrease in NO_3^- concentrations of surface and ground waters occurred. High NO_3^- concentrations are still present especially in rivers of Western Europe (EC 2002b, EEA 2003). In contrast to NO_3^-, NH_4^+ concentrations in rivers decreased, which indicates reduced pollution with organic substances (coming from human wastewater, industrial effluents and agriculture run-off (manure and slurry)). The improvement was achieved as a result of Urban wastewater treatment directive (91/271/EEC) accepted in 1991 (and amended in 1998 - directive 98/15/EC). The directive forces EU member states to increase proportion of wastewater subjected to either primary, secondary and tertiary treatment, but in many large cities sufficient wastewater facility were still lacking in 1998 (Bedford et al. 1999, EC 2002a, EEA 2003).

Anthropogenic **P pollution** comes preferentially from households and industry; detergents comprising polyphosphate salts are the main sources (EEA 1999, Hejný et al. 2002, EEA 2003, Khan and Ansari 2005). Large amounts of P are supplied also with mineral fertilizers and manures (Smith et al. 1996). Currently, the water quality of European water bodies is improving; in period 1990-1998 P concentrations in rivers of EU member states decreased by 30-40%, as a result of reduced P emissions achieved by improvements in industry, wastewater treatment, usage of phosphate-free detergents and decreased consumption of phosphate fertilisers (EC 1999, Hejný et al. 2002, EEA 2003). However, P concentrations in European rivers still remain high compared to "natural background", especially in Western Europe. Eutrophication of European lakes is also decreasing, but high P levels still occur especially in EU accessing countries (EEA 2003).

As summarised by (Smith et al. 1996), trophic state of water body (oligotrophic, mesotrophic, eutrophic, and hypertrophic) corresponds to low, intermediate, high and greatly excessive input of nutrients, respectively. Considering lakes, oligotrophic bodies contain less than 350 µg L^{-1} of N and 10 µg L^{-1} of P in water; mesotrophic lakes 350-650 µg L^{-1} of N and 10-30 µg L^{-1} of P; eutrophic 650-1200 µg L^{-1} of N and 30-100 µg L^{-1} of P and hypertrophic above 1200 µg L^{-1} of N and 100 µg L^{-1} of P (Smith et al. 1996, Sand-Jensen et al. 2000). Similarly, Khan and Ansari (2005) describes oligotrophic state of a water body by N and P levels below 250-600 and 5-10 µg L^{-1}, respectively, with primary productivity 50-300 mg C m^{-2} day^{-1}. In moderately eutrophic water, the reported levels are 500-1100; 10-30 µg L^{-1} for N; P, respectively, and primary productivity above 1 g C m^{-2} day^{-1}.

Anthropogenic forces are further important factors interfering with nutrient cycles in wetlands. The enrichment of N and P (for details see Fig. 3), which are the principal nutrients for plant growth (Marschner 1995) and usually limiting nutrients in "undisturbed" wetland ecosystems (Smith et al. 1996, Verhoeven et al. 1996, Vitousek et al. 1997, Carr and Chambers 1998, Bedford et al. 1999, Correll 1999), leads to the stimulation of plant growth. Subsequent litter accumulation enhances microbial activity, oxygen demand (Čížková et al. 1996, Rolletschek 1997), and shifts soil redox potential to lower values, increasing risk of

Table 1: Examples of N, P levels detected in pore waters of natural wetlands of different trophic status and in wastewater entering (and leaving) artificial wetlands.

Site description	NH_4^+ (µmol/l)	NO_3^- (µmol/l)	total N (µmol/l)	PO_4^{3-} (µmol/l)
Natural wetlands:				
Phragmites littoral stands in the Třeboň basin, Czech Republic (Čížková et al. 2001b)				
	oligo to meso: **10.7**	oligo to meso: **1400**	n.e.	oligo to meso: **0.1**
	eu: **26.4**	eu: **6.4**		eu: **0.9**
	hyper: **129.3**	hyper: **7.14**		hyper: **3.2**
Phragmites littoral stands in the Třeboň basin, Czech Republic (Picek et al. 2000)				
	n.e.	meso: **14.2**	meso: **255**	meso: **2.2**
		hyper: **6.4**	hyper: **318.6**	hyper: **8.1**
Phragmites littoral stands in Uckermark and Mark Brandenburg, Germany (Kohl et al. 1998)				
	3-500 (no sig. difference among sites)	**1-140** (no sig. difference among sites)	n.e.	n.e.
Phragmites littoral stands in Uckermark and Mark Brandenburg, Germany (Kühl and Kohl 1992)				
	meso to eu: **84.3-223.6**	meso to eu: **25.7-44.3**	meso to eu: **128.6-249.3**	meso to eu: **18.1-29.4**
	eu: **202.1**	eu: **40**	eu: **242.1**	eu: **32.3**
	hyper: **171.4-255.7**	hyper: **30-40**	hyper: **211.4-285.7**	hyper: **35.5-38.7**
Phragmites stands in Osterseen lake complex, Bavaria, Germany (Kubín et al. 1994)				
	oligo: **3.2**	oligo: **3.8**	n.e.	oligo: **0.5**
	eu: **15.8**	eu: **2.0**		eu: **1.4**
Phragmites stands at intermittent lake Cerkniško jezero, Slovenia (Urbanc-Berčič and Gaberščik 2001)				
	up to **80.6**	up to **64.5**	n.e.	up to **22.1**
Constructed wetlands:				
Horizontal-flow wetland for treating primary domestic sewage in Victoria, Australia; planted with *Phragmites* (Cottingham et al. 1999)				
	influent: **3993**	influent: **<7.1**	influent: **5214**	n.e.
	effluent: **1279**	effluent: **1029**	effluent: **4293**	
Wetland for nitrate removal in California, USA; planted with *Typha* and *Scirpus* (Bachand and Horne 2000)				
	influent: **11.4**	influent: **662**	influent: **748**	n.e.
	effluent: **15**	effluent: **598**	effluent: **695**	
System for treating N-rich livestock wastewater in Duplin, Co., NC.; planted with various helophytes (Szogi et al. 2003)				
	influent: **2500-16071**	n.e.	n.e. but NH_4^+-N forms 95% of total N content	n.e.
	effluent: **143-4143**			
Horizontal sub-surface flow wetland for treating village wastewater near České Budějovice, Czech Republic; planted with *Phragmites* and *Phalaris* (Edwards et al. 2006)				
	influent: **916**	influent: **14.3**	n.e.	influent: **69.4**
	effluent: **201**	effluent: **8.6**		effluent: **31.0**

note: hyper-hypertrophic; eu-eutrophic; meso-mesotrophic; oligo-oligotrophic; n.e. - not estimated

oxygen shortage, sediment phytotoxicity (Armstrong et al. 1996a, Armstrong et al. 1996c, Čížková et al. 1999, Armstrong and Armstrong 1999, Armstrong and Armstrong 2001b), and damages of belowground organs (Votrubová et al. 1997). Furthermore, high oxygen demand of eutrophic sediment shifts the occurrence of N forms in the flooded sediment towards higher relative availability of NH_4^+ compared to NO_3^- (Kühl and Kohl 1992, Nijburg and Laanbroek 1997, Kühl et al. 1997, Čížková et al. 2001b, Sand-Jensen et al. 2005).

Integrating all above-mentioned facts, **N availability in wetland** shows significant spatial and temporal variability (Martin and Reddy 1997). NO_3^- is usually the dominant N form in the **bulk water** oxidised from the atmosphere (Úlehlová and Přibil 1978, Mengis et al. 1997, Cedergreen and Madsen 2003), although NH_4^+ may occur in significant amounts in stagnant waters colonised with floating macrophytes (Elster et al. 1995) or in fishponds in the spring period (Úlehlová and Přibil 1978). In the **sediment**, NO_3^- disappears rapidly with soil depth (Andersen and Hansen 1982, Rolletschek 1997), being almost limited to the hypoxic topsoil layers (oxidized from adjacent water column) or to the rhizosphere of emergent macrophytes (oxidized in consequence of ROL) (Engelaar et al. 1995, Nijburg et al. 1997, Ottová et al. 1997, Sand-Jensen et al. 2005). NH_4^+ seems therefore the dominant N form available for the rooting macrophytes (Cedergreen and Madsen 2003), as well as it is commonly the prevailing N form of wastewater (e.g. Wittgren and Tobiason (1995), Cottingham et al. (1999)). Some examples of NH_4^+, NO_3^-, and P availabilities in wetlands of different fertility as well as in wastewater treating wetlands are given in Tab. 1.

NH_4^+ versus NO_3^- preferences in plants, ecological consequences

Inorganic ions NH_4^+ and NO_3^- are the dominant N forms taken up by plants to meet their N requirements (Marschner 1995). Other nitrogenous compounds (low-molecular-weight dissolved organic N compounds, particularly amino acids) may also be important (Lipson and Nasholm 2001), especially under special ecological circumstances, e.g. extremely low N availability or impaired mineralization (Kielland 1994, Schiller et al. 1998, Raab et al. 1999). The majority of plant species show the highest growth potential on the mixture of both NH_4^+ and NO_3^- (Marschner 1995, Taiz and Zeiger 2002), but the preferences for particular N source may exit in individual plant species in response to long-term soil chemical conditions of their habitats (Kronzucker et al. 1997). Species colonising habitats with restricted nitrification (e.g. acid forest soils, heathland soils, soils of low temperature, waterlogged soils) frequently display preference for NH_4^+. Preferential NH_4^+ acquisition occurs also in plants forming

climax or stable communities, usually slow-growing perennials or woody species, e.g. late-succesional conifers (Kronzucker et al. 1997, Plhák 1998, Bijlsma et al. 2000, Bauer and Berntson 2001, Britto and Kronzucker 2002). Therefore, the NH_4^+/NO_3^- ratio of substrate seems to affect the composition of plant communities at different habitats considerably (Gigon and Rorison 1972, Stewart et al. 1993, Marschner 1995, Kronzucker et al. 1997, de Graaf et al. 1998, Garnett and Smethurst 1999, Forde and Clarkson 1999, Min et al. 1999, Olsson and Falkengren-Grerup 2000, Nordin et al. 2001, Garnett et al. 2001, Kreuzwieser et al. 2002, Britto and Kronzucker 2002, van den Berg et al. 2005).

In wetland plants, NH_4^+ preference is generally supposed (e.g. Ingemarsson et al. (1984), Brix et al. (2002), Cedergreen and Madsen (2003), Tylová-Munzarová et al. (2005)), particularly in species forming dense stands in nutrient-rich, acidic waters (Schuurkes et al. 1986). This assumption is based on general NH_4^+ predominance in the waterlogged sediment, and is supported by studies of NH_4^+ and NO_3^- uptake kinetics (see chapter Uptake of NH_4^+ and NO_3^- in wetland plants), or studies of nitrate reductase activity performed with different wetland species (see chapter NH_4^+ and NO_3^- assimilation, amino acids). In some cases, the positive growth responses to NH_4^+ were also found, e.g. in *Typha latifolia* (Brix et al. 2002) or *Glyceria maxima* (Tylová-Munzarová et al. 2005) at certain N level. Besides growth stimulation, *Typha latifolia* showed higher N, P, Ca, Fe, S status in response to NH_4^+-N under 100μM N availability (Brix et al. 2002), higher N contents were detected in NH_4^+ compared to NO_3^- fed *Glyceria maxima* under 34μM N availability (Tylová-Munzarová et al. 2005).

Wetland plants are also generally considered to be tolerant towards high NH_4^+ doses, which commonly trigger adverse effects in many NH_4^+ sensitive terrestrial species. This tolerance was particularly shown e.g. in *Oryza sativa* (Britto et al. 2001b), *Typha latifolia* (Brix et al. 2002), or *Phragmites australis* (Tylová et al. - manuscript 1). Although the majority of studies dealing with tolerance of NH_4^+-N nutrition is focused on crop species, it is obvious that even in wetland plants, this tolerance has its limitations, and individual species may differ considerably in their sensitivity to NH_4^+. Based on our own results, *Glyceria maxima* seems a good example. While the species grew better with NH_4^+ compared to NO_3^- N source at 34μM N level (Tylová-Munzarová et al. 2005), the response turned to opposite at 179 μM N level (Munzarová et al. 2006, Tylová et al. - manuscript 1). The symptoms of NH_4^+ toxicity were further observed e.g. in *Oryza sativa* under K^+ shortage (Liao et al. 1994), *Acorus calamus* (Vojtíšková et al. 2004), *Nymphaea caerulea* (Agami et al. 1976), seagrass

Zostera marina (van Katwijk et al. 1997, Touchette and Burkholder 2000), or wet heathland species *Cirsium dissectum* (de Graaf et al. 1998, Lucassen et al. 2003).

Besides individual plant species, the sensitivity towards high relative abundance of NH_4^+ was shown in some entire wetland communities (Bobbink et al. 1998). Elevated NH_4^+ levels seem to trigger the decline of isoetid communities in soft-water lakes in favour of species positively responding to NH_4^+ enrichment, e.g. of *Juncus bulbosus* (Roelofs et al. 1984). Increased NH_4^+ availability is considered as one of the important factors triggering the decline of *Stratiotes aloides* from its original littoral habitats (Smolders et al. 1996), as well as it is supposed to be involved in the decline of seagrasses community, since e.g. *Zostera marina* or *Ruppia dreparensis* display sensitivity towards NH_4^+ enrichment (for summary see Touchette and Burkholder (2000)).

On the other hand, the capability of NO_3^- uptake and utilization in wetland plants is obvious, particularly in emergent species with significant ROL (Ohlson and Hogbom 1993, Engelaar et al. 1995, Kronzucker et al. 2000, Erdei et al. 2001, Kirk 2001, Cedergreen and Madsen 2003, Kirk and Kronzucker 2005, Munzarová et al. 2006; for further see chapters Uptake of NH_4^+ and NO_3^- in wetland plants; NH_4^+ and NO_3^- assimilation, amino acids). Among them, the ability to sufficiently grow with sole NO_3^--N source was described e.g. in *Glyceria maxima* and *Phragmites australis* (Munzarová et al. 2006, Tylová-Munzarová et al. 2005, Tylová et al. - manuscript 1), or *Typha latifolia* (Brix et al. 2002). Similarly, species colonising NO_3^- dominated nitrogen-poor soft waters (Schuurkes et al. 1986) or species with roots (or leaves) subjected directly to NO_3^- rich bulk water, may take up considerable amounts of N as NO_3^-. The later includes not only various floating macrophytes (e.g. *Lemna minor* (Cedergreen and Madsen 2002, Cedergreen and Madsen 2004)), but also helophytes with root dimorphism, e.g. *Carex gracilis* or *Phragmites australis* (Končalová and Pazourek 1988, Kirk 2003). These species develop finely branched aquatic roots at the stem nodes, which allow acquiring nutrients directly from the bulk water or superficial oxidised sediment layers (Dykyjová and Hradecká 1976, Dykyjová 1978).

Uptake of NH_4^+ and NO_3^- in wetland plants

In emergent macrophytes, the majority of N is taken up from sediment or bulk water via soil or aquatic roots. The importance of aquatic roots is particularly high at nutrient poor sandy sediments (Dykyjová and Hradecká 1976, Dykyjová 1978). The foliar uptake of N may

also occur, e.g. when temporarily flooded, but the contribution to overall N acquisition is relatively low in contrast to submerged and floating plants, which take up considerable amounts of nutrients by both roots and shoots (Pedersen et al. 1997, Cedergreen and Madsen 2002). Roots of wetland plants typically possess protective gas-impermeable layers in the outer cortex, which differentiate constitutively, and very close to the root tip (Soukup et al. 2002). In general, the existence of such apoplastic barriers might conflict with the nutrient acquisition, as it considerably reduces the area of root plasmalemas directly subjected to external solution (Peterson 1988, Peterson 1989, Colmer and Bloom 1998, Enstone et al. 2002, Kirk 2003). The experiments evaluating the effect of these barriers on the root absorption capacity came, however, with inconsistent results. Among others, Colmer and Bloom (1998) observed the decline of NH_4^+ and NO_3^- uptake in basal root zones of *Oryza sativa*, but not in *Zea mays* (Bloom 1997, Colmer and Bloom 1998). In contrast, Rubinigg et al. (2002) did not found any correlation between the barrier formation and NO_3^- absorption capacity in adventitious roots of *Oryza sativa*. It seems in general, that the presence of the outer permeability barrier reduces but not eliminates ion uptake in the given root region (Enstone et al. 2002). This finding is supported by the following observations: i: symplastic transport pathway is functioning (Cholewa and Peterson 2004); ii: ions could be partially able to penetrate directly through Casparian bands (Ranathunge et al. 2005); and iii: substantial apoplastic ion flux occurs in regions where lateral roots penetrate the mother root. Furthermore, the permeability barrier is considerably less developed in fine lateral roots (Soukup et al. 2002), which therefore seem to provide the majority of nutrient uptake from the flooded soil (Kirk 2003).

At root plasma membrane, the uptake of both NH_4^+ and NO_3^- is multiphasic, mediated by several transport systems in dependence on ion external availability. At low rhizosphere levels (<0.5mM), the uptake is active carrier-mediated process, provided by saturable **high-affinity transport system (HATS)**. HATS operates with Michaelis-Menten kinetics, displays sensitivity to metabolic inhibitors and to diminution of membrane H^+ gradient (Forde and Clarkson 1999, Howitt and Udvardi 2000, Crawford et al. 2000, Kochian 2000). In case of NO_3^-, HATS comprises two genetically distinct components with different pattern of regulation and affinity, constitutive (cHATS) and inducible (iHATS). Constitutive cHATS displays lower uptake capacity (lower Vmax), but higher affinity for NO_3^- than inducible iHATS. Molecular evidence showed two transporter gene families, *NRT1* and *NRT2*. NRT2 proteins are involved in inducible high-affinity uptake, NRT1 are transporters with low or

dual affinity (Forde and Clarkson 1999, Crawford et al. 2000, Liu and Tsay 2003). In contrast, NH_4^+ HATS shows less conspicuous inducible component (Forde and Clarkson 1999), although *AMT1* gene family of NH_4^+ transporters includes members with NH_4^+ inducible pattern of expression (Sonoda et al. 2003a, Sonoda et al. 2003b).

The regulation of HATS kinetics in response to nutrient availability is, however, different between NH_4^+ and NO_3^-. While the diminution of NO_3^- in the rhizosphere down-regulates plant capacity for NO_3^- uptake considerably (Forde and Clarkson 1999, Crawford et al. 2000, Tylová-Munzarová et al. 2005), plants deprived of NH_4^+ typically increase the capacity for NH_4^+ uptake (show higher V_{max} and lower K_m) (Wang et al. 1993a, Howitt and Udvardi 2000). The second mentioned pattern of regulation is generally more common, the up-regulation occurs in case of many nutrients, e.g. PO_4^- or K^+ (Liu et al. 1998, Raghothama 1999, Kochian 2000). The feed-back regulation of HATS in response to internal N status is also evident (Forde and Clarkson 1999, Walch-Liu et al. 2005). Among others, the levels of N metabolites, particularly of amino acids glutamine and asparagine (Forde and Clarkson 1999, Sonoda et al. 2003b), and of NH_4^+ itself (Wang et al. 1993b, Forde and Clarkson 1999) affect the activity of NH_4^+ transporters as well as the transporter genes expression considerably. Similarly, the internal levels of NO_3^-, NH_4^+, amino acids, and the activity of nitrate reductase determine the intensity of NO_3^- uptake (among others e.g. Forde and Clarkson (1999), Forde (2000), Walch-Liu et al. (2005)). The phloem-transported compounds (e.g. amino acids, carbohydrates) mediate the information of the actual whole-plant demands (Walch-Liu et al. 2005).

In higher concentration range (>0.5 mM), NH_4^+ and NO_3^- are taken up by **low-affinity transport systems (LATS)**; as a passive non-saturable influx through ion channels with the weak response to metabolic inhibitors (Wang et al. 1994, Forde and Clarkson 1999, Howitt and Udvardi 2000, Crawford et al. 2000). The responses of LATS to changes in N availability or internal N status are generally less pronounced compared to HATS (Forde and Clarkson 1999). In case of NH_4^+, its significant amounts can penetrate through K^+ channels because of similar radii (both bare and hydrated) of these two ions (Howitt and Udvardi 2000).

The concentrations exceeding 0.5 mM, however, rarely occur at natural stands, even at eutrophicated sites (Kohl et al. 1998, Picek et al. 2000, Čížková et al. 2001b); wastewater treating wetlands are the only exception. HATS is thus supposed to be responsible for the majority of N uptake *in situ*. The kinetic parameters of HATS differ among individual species in correspondence with long-term nutritional status of their habitats. Species adapted to low-fertility environments display lower root absorption capacity (lower maximum uptake rate;

V_{max}) but higher affinity (low half saturation constant; K_m). In contrast, fast growing species of fertile habitats possess higher absorption rates and display higher potential to facilitate the uptake capacity in response to increased nutrient availability, but the affinity is generally lower (Chapin 1980). The characteristics of HATS kinetics are therefore signs of plant ecological amplitude towards nutrient availability.

Previous studies dealing with **HATS kinetics in wetland plants** estimated maximum uptake rates and half saturation constants for NH_4^+ and NO_3^- in different emergent macrophytes (see Tab. 2). Comparing obtained results, *Phragmites* showed very high NH_4^+ uptake capacity (high V_{max}) compared to other species including *Glyceria*, which indicates its adaptation to fertile habitats (Romero et al. 1999). Such comparison, however, has to be done with care and caution, as the kinetic data obtained under different experimental conditions, particularly under different N levels applied prior the onset of measurements, may differ considerably. In our study (Tylová-Munzarová et al. 2005) we therefore compared NH_4^+ and NO_3^- uptake kinetics in *Phragmites* and *Glyceria* cultivated under similar growth conditions. Comparable V_{max} (μmol g^{-1} root d.wt. h^{-1}) for NH_4^+: 49.5- 58.2 and 55.4-62.9 as well as for NO_3^-: 23.5-45.2 and 28.3-41.0 in these two species (in *Phragmites* and *Glyceria*, respectively) were shown. Furthermore, our study showed higher uptake plasticity towards very low nutrient availability in *Phragmites* compared to *Glyceria* (Tylová-Munzarová et al. 2005), which corresponds with its ability to colonise oligotrophic habitats, where *Glyceria* occurs rarely (see Table 2 in Brändle et al. (1996)). In agreement, the ability to acclimate towards either very high or low NH_4^+ availability in *Phragmites* was indicated by Romero et al. (1999).

The studies of HATS kinetics also influenced the general view on the **NH_4^+ versus NO_3^- preferences in wetland plants**, indicating apparent but evidently non-strict preference for NH_4^+ (see e.g. Morris (1980), Kronzucker et al. (1999), Touchette and Burkholder (2000), Cedergreen and Madsen (2002), Tylová-Munzarová et al. (2005)). The preferential uptake of NH_4^+ over NO_3^- was observed in some wetland and marine species, e.g. in *Oryza sativa* (Sasakawa and Yamamoto 1978, Colmer and Bloom 1998, Kronzucker et al. 1999), *Lemna gibba* (Ingemarsson et al. 1984), *Ruppia maritima* (Thursby and Harlin 1984), *Juncus bulbosus or Sphagnum flexuosum* (Schuurkes et al. 1986). Higher maximum uptake rate for NH_4^+ compared to NO_3^- was found in littoral helophytes *Phragmites australis*, *Glyceria maxima* (Tylová-Munzarová et al. 2005) and *Spartina alterniflora* (Morris 1980), as well as in seagrasses (for review see (Touchette and Burkholder 2000)) or *Lemna minor* (Cedergreen

and Madsen 2002). In *Oryza sativa* (Kronzucker et al. 1999) and *Lemna gibba* (Ingemarsson et al. 1987), the presence of NH_4^+ was even shown to repress the uptake of NO_3^- considerably.

On the other hand, substantial uptake of NO_3^- in wetland plants was also documented in several studies. Kronzucker et al. (2000) showed significant uptake of NO_3^- in *Oryza sativa*, which even exceeded uptake of NH_4^+ at certain circumstances. Schuurkes et al. (1986) documented preferential uptake of NO_3^- in different submerged species of oligotrophic habitats, e.g. in *Littorella uniflora*, *Lobelia dortmanna*, *Luronium natans* or *Echinodorus ranunculoides*. In some wetland species, the positive effect of NO_3^- on the uptake of NH_4^+ was found, e.g. in *Oryza sativa* (Kronzucker et al. 1999, Kirk 2001), *Glyceria maxima* (Brix et al. 1994), or *Phalaris arundinacea* (Brix et al. 1994).

Table 2: Maximum uptake rates and half saturation constants for NH_4^+ and NO_3^- estimated in different emergent macrophytes.

V_{max} (μmol g^{-1} root dry wt. h^{-1})	K_m (μM)	
NH_4^+ uptake		
Phragmites australis		
151 under 15 μM PO_4^{3-} **229** under 50 μM PO_4^{3-} no clear effect of N availability	**21.8** tended to increase with increasing N, P levels (not significant)	(Romero et al. 1999)
49.5- 58.2 no significant difference between NH_4^+ and NO_3^- pre-cultivated plants	**3.8** in plants pre-cultivated with NH_4^+ **6.7** in plants pre-cultivated with NO_3^-	(Tylová- Munzarová et al. 2005)
Glyceria maxima		
4.6-10.3 no consistent effect of O_2, slightly higher Vmax in presence of NO_3^-	**1.6-3.0** no consistent effect of O_2, slightly lower Km in presence of NO_3^-	(Brix et al. 1994)
55.4-62.9 no significant difference between NH_4^+ and NO_3^- pre-cultivated plants	**6.0** in plants pre-cultivated with NH_4^+ **7.9** in plants pre-cultivated with NO_3^-	(Tylová- Munzarová et al. 2005)
Typha latifolia		
20.8-30.8 no effect of pH, but prolonged exposure to pH 3.5 almost completely stopped the uptake	**7.1** at pH 8 **17.4** at pH 5 **19.2** at pH 3.5	(Dhyr-Jensen and Brix 1996)
22.6 mean overall value at pH 3.5-7 **30.9** (highest value) at pH 6.5 **14.0** (lowest value) at pH 3.5	**2.9** mean overall value at pH 3.5-7 **1.3** (lowest value) at pH 7	(Brix et al. 2002)
Phalaris arundinacea		
24.7-29.6 no consistent effect of O_2, slightly higher Vmax in presence of NO_3^-	**3.1-6.2** no consistent effect of O_2, slightly lower Km in presence of NO_3^-	(Brix et al. 1994)
Spartina alterniflora		
12.9 under normoxia **8.1** under hypoxia **2.3** under 1 mM S$^-$	**1.1** under normoxia **2.5** under hypoxia **8.8** under 1mM S$^-$	(Bradley and Morris 1990)
4.2 mean V_{max} at salinity 0-50g L^{-1}; no effect of salinity observed	**2.7** under 5g L^{-1} salinity **17.6** under 50g L^{-1} salinity	(Bradley and Morris 1991)
9.7	**4.1**	(Morris 1980)

NO₃⁻ uptake		
Phragmites australis		
15.8 and 70.7* in two different clones	45.2 and 80.8 in two different clones	(Araki et al. 2005)
23.5 in plants pre-cultivated with NH_4^+	3.0 in plants pre-cultivated with NH_4^+	(Tylová-
45.5 in plants pre-cultivated with NO_3^-	4.5 in plants pre-cultivated with NO_3^-	Munzarová et al. 2005)
Glyceria maxima		
28.3 in plants pre-cultivated with NH_4^+	5.5-5.9 no significant difference	(Tylová-
41.0 in plants pre-cultivated with NO_3^-	between NH_4^+ and NO_3^- pre-cultivated plants	Munzarová et al. 2005)
Typha latifolia		
20.3 mean overall value at pH 3.5-7	9.0 mean overall value at pH 3.5-7	(Brix et al. 2002)
31.7 (highest value) at pH 5	1.7 (lowest value) at pH 5	
4.7 (lowest value)at pH 3.5		
Spartina alterniflora		
7.7	8.9	(Morris 1980)

Note: * the results of Araki et al. were recalculated using dry to fresh weight ratio 0.15 based of our own estimation of root water contents in this species.

Furthermore, the studies dealing with different emergent macrophytes showed that NH_4^+ and NO_3^- uptake kinetics might significantly differ in response to P availability (Romero et al. 1999), salinity (Bradley and Morris 1991, Chambers et al. 1998), S^{2-} availability (Chambers et al. 1998), pH (Brix et al. 2002), and/or oxygen shortage (Sasakawa and Yamamoto 1978). The adverse effects of oxygen shortage are documented among flood-sensitive species (see e.g. Kreuzwieser et al. (2002)), but even in wetland plants it may negatively affect the uptake of NO_3^-. The significant decline of NO_3^- uptake (by 75%) was documented in *Oryza sativa* in spite of its efficient internal aeration (Sasakawa and Yamamoto 1978). The uptake of NH_4^+ by wetland plants seems less hypoxia-sensitive (Sasakawa and Yamamoto 1978, Brix et al. 1994), but its depression may also occur (as observed e.g. in *Spartina alterniflora* or *Oryza sativa* (Bradley and Morris 1990, Kronzucker et al. 1998)), particularly when the exposure is prolonged (Kronzucker et al. 1998). Moreover, considerable genetically based differences in NO_3^- maximum uptake rate were observed between two clones of *Phragmites australis* (Araki et al. 2005). The diversity within the world-wide *Phragmites* population may therefore allow selecting genotypes highly efficient in nutrient removal and thus more useful in wastewater treating process (Araki et al. 2005).

NH_4^+ and NO_3^- assimilation, amino acids

The **assimilation of NO_3^-** involves two-step reduction to NH_4^+, carried by two enzymes: nitrate (NR) and nitrite reductase (NiR). The activity of **nitrate reductase** is the critical step,

which integrates various regulatory signals, e.g. NO_3^- availability and uptake rate, light, carbohydrate availability, plant internal N status etc. (for review see e.g. Marschner (1995), Plhák (1998), Campbell (1999), Crawford et al. (2000), Tischner (2000), Campbell (2001), Foyer et al. (2003)). Because of its high importance in plant metabolism, NR is one of the most studied plant enzymes, which brought about considerable knowledge about its genetic basis, molecular structure, function, localization and/or regulatory mechanisms; reviewed repeatedly (see e.g. Rajasekhar and Oelmuller (1987), Kaiser and Huber (1994), Kaiser et al. (1999), Campbell (1999), Tischner (2000), Campbell (2001), Mackintosh and Meek (2001)). Similarly, the effects of different stress factors, e.g. drought (Foyer et al. 1998, Xu and Zhou 2005, Correia et al. 2005), salinity (Ashraf and Bashir 2003, Sharma and Dubey 2005, Carillo et al. 2005, Iqbal et al. 2006), aluminium (Balakumar et al. 1992, Dinev and Stancheva 1993, Lidon et al. 1998, Mistrik et al. 2000, Sharma and Dubey 2005), and/or heavy metals (Gouia et al. 2000, Chiraz et al. 2003, Shanker et al. 2005, Xiong et al. 2006) on its activity were studied extensively in different plant species.

In ecological studies, nitrate reductase activity is used as a marker of NO_3^- assimilation intensity, which allows characterisation of NO_3^- versus NH_4^+ importance in nutrition of different species under real ecological circumstances (Stewart et al. 1986, Hogbom and Ohlson 1991, Stewart et al. 1993, Ohlson and Hogbom 1993, Troelstra et al. 1995b, Cedergreen and Madsen 2003). Our study, however, showed that the interpretation of NR activity data must always be done with care and caution. Firstly, individual species might considerably differ in amounts of NO_3^- stored in plant tissues in unreduced form. In our study, *Phragmites* exhibited higher leaf NR activity compared to *Glyceria*, which might indicate more conspicuous ability of successful growth on NO_3^- N source in *Phragmites*. The overall growth response to NO_3^- was, however, more positive in *Glyceria*, in spite of significantly lower NR activity (Munzarová et al. 2006). *Glyceria* obviously tended to accumulate NO_3^- in unreduced form, without any negative consequences to N nutrition, but low NR activity might cause underestimation of NO_3^- importance in nutrition of this species.

Secondly, the reliable use of NR activity as a marker of NO_3^- utilization in individual plant species is impossible without the precise verification of the method 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.). In our study (Munzarová et al. 2006), *Glyceria* but not *Phragmites* appeared to contain cyanogenic glycosides, potent inhibitors of NR activity releasing CN^- during homogenisation (Maranville

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1970), which might lead 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 considered. *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).

Keeping these limitations in mind, NR activity approach is promising in characterisation of the real importance of NO_3^- in nutrition of wetland plants (Uhel et al. 1989, Ohlson and Hogbom 1993, Cedergreen and Madsen 2003, Kirk and Kronzucker 2005). This importance is generally questioned due to low NO_3^- availability in the waterlogged sediment. In agreement, Cedergreen and Madsen (2003) observed low ambient NR activity in different submerged and amphibious plants and attributed these results to i: general preferences for NH_4^+ in these plants, and ii: to NH_4^+ suppression of NR activity (Orebamjo and Stewart 1975). Similarly, NR activity detected in leaves of *Phragmites australis* at lake Balaton was relatively low, being present only in the period of maximal growth (Erdei et al. 2001). There are, however, also studies indicating importance of NO_3^- as a source of N in wetland plants at natural habitats, e.g. observations of substantial NR activity in different species of old-grown swamp forest (Ohlson and Hogbom 1993). In addition, different wetland species were shown to exhibit considerable NR activity in experimental cultures, e.g. *Phragmites australis*, *Glyceria maxima* (Munzarová et al. 2006), *Rumex palustris* (Engelaar et al. 1995), *Phalaris aquatica* (Sairam et al. 1995), or *Lemna minor* (Cedergreen and Madsen 2003).

Furthermore, NR activity in wetland plants is used as a marker in toxicity studies, particularly with respect to treatment of wastewater containing heavy metals or to phytoremediation of contaminated water bodies, which includes e.g. studies of Vajpayee and co-workers on *Nymphaea alba* (Vajpayee et al. 1999), *Vallisneria spiralis* (Vajpayee et al. 2001, Vajpayee et al. 2005), *Potamogeton crispus* (Ali et al. 2000), or *Nelumbo nucifera* (Vajpayee et al. 2000), or studies of Gupta and Chandra (Gupta and Chandra 1994, Gupta and Chandra 1998) on *Vallisneria spiralis* and *Hydrilla verticilata.*

Considering the waterlogging stress, NR activity is long-term questioned to be involved in alleviation of hypoxic/anoxic stress in plants due to consumption of reduced cofactors. Acting as an alternative electron acceptor for regeneration of NAD from NADH, NR activity may help to decrease intensity of cell fermentation rates, and consequently the risk of cytoplasmic acidosis (e.g. Roberts et al. (1984), Roberts et al. (1985), Perata and Alpi (1993), Müller et al. (1994)). This assumption is based on several studies, which documented positive effects of NO_3^- on tissues responses to anaerobiosis, e.g. in *Glyceria maxima,*

Phalaris arundinacea, Carex pseudocyperus (Müller et al. 1994, Brix et al. 1994), or *Oryza sativa* (Reggiani et al. 1985, Polyakova and Vartapetian 2003), as well as in non-wetland species as *Zea mays* (Roberts et al. 1985), *Solanum tuberosum* (Oberson et al. 1999), or *Triticum aestivum* (Polyakova and Vartapetian 2003). Furthermore, NR activity (or its activation state) often increases in response to anaerobiosis (Garcia-Novo and Crawford 1973, Müller et al. 1994, Botrel and Kaiser 1997, de la Haba et al. 2001). This hypothesis, however, possesses several weaknesses and thus the mechanisms is still the matter of discussion. Among others, Botrel and Kaiser (1997) did not found any effects of NR activity on cell fermentation rates in roots of *Hordeum vulgare*. Similarly, Stoimenova et al. (2003) showed, that the absence of NR activity in roots of transformed *Nicotiana tabacum* did not necessarily limit NADH recycling under anoxia. Furthermore, high energetic demand of NO_3^- reduction might even worsen metabolic relations of root cells under anoxia contrary to the suggested positive effect (Felle 2005).

In relation to above-mentioned discussion, the localisation of NR activity in wetland plants is not without interest. In plants, NR activity is generally localised in both shoots and roots in dependence on plant species, growth form, or NO_3^- availability (Andrews 1986, Andrews et al. 1992, Marschner 1995). In temperate perennial species, roots are supposed to be a preferential site of NO_3^- reduction under low NO_3^- availability (below 1mM), but shoot importance increases when NO_3^- availability rises (Andrews 1986). Shoot localisation is also energetically favourable due to direct coupling with the photosynthesis (Marschner 1995). In wetland plants, however, information of localisation of NR activity are scarce. One of few existing studies described high NR activity of roots compared to shoots in different submerged wetland species (Cedergreen and Madsen 2003). The localisation of NR activity was therefore studied in greater details in *Phragmites australis* and *Glyceria maxima* (Munzarová et al. 2006). Based on our results, root localization was shown to be relatively important in *Glyceria*. In contrast, *Phragmites* seemed to be the preferential leaf NO_3^- reducer, similarly to some terrestrial grasses studied by Scheurwater et al. (2002). Furthermore, low NR activity was generally found in rhizomes of *Phragmites*, while rhizome contribution to NO_3^- reduction was relatively high in *Glyceria* under low NO_3^- availability (Munzarová et al. 2006).

The **assimilation of NH_4^+** involves activity of glutamine synthetase (GS) and glutamate synthase (GOGAT), which incorporate NH_4^+ into amino acids (for review see e.g. Marschner (1995), Plhák (1998), Ireland and Lea (1999), Coruzzi and Last (2000)). GS has several

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isoforms with distinct functions; cytosolic GS1 isoenzymes dominate in roots, being involved in assimilation of NH_4^+ taken up from the external solution; chloroplast GS2 isoenzymes are predominant in leaves and function in primary NH_4^+ assimilation as well as in the reassimilation of NH_4^+ evolved during photorespiration (Cren and Hirel 1999, Coruzzi and Last 2000). The primary product of NH_4^+ assimilation is glutamine (Gln) and subsequently glutamate (Glu), which is the donor of amino group for synthesis of other amino acids in plant cells via action of various transaminases (Ireland and Lea 1999, Coruzzi and Last 2000). **Amino acids** are not only essential building compounds and metabolic substrates, but they represent important substances for transport and storage of N in plants (Marschner 1995, Peuke 2000). Among graminaceous plants, particularly glutamine and asparagine (Asn) are dominant low-molecular-weight N compounds transported via vascular tissues (Marschner 1995, Peuke 2000) as well as stored in large quantities in storage organs (together with e.g. storage proteins or NO_3^-) (Chapin et al. 1990, Gloser 2002).

The intensity of N assimilation processes is determined by overall N availability (Marschner 1995), and *vice versa* different steps of this process (N uptake rate, activity of N assimilating enzymes) are feedback regulated via downstream metabolites, which provides mechanism to optimise plant functions in response to needs (for review se e.g. Forde and Clarkson (1999), Coruzzi and Zhou (2001), Walch-Liu et al. (2005)). Different regulatory links also control plant morphological trays to optimally exploit available sources (Aiken and Smucker 1996, Scheible et al. 1997b, Forde and Lorenzo 2001), as well as different cross links co-ordinate C and N metabolic pathways (Foyer et al. 2003, Walch-Liu et al. 2005). Amino acids, particularly those transported in vascular tissues, are long-term supposed to be important long-distance **signals of plant N status** (Cooper and Clarkson 1989, Imsande and Touraine 1994, Coruzzi and Zhou 2001), although some contradictory evidences toward this hypothesis were found (as summarized by Forde and Lorenzo (2001).

Higher internal N status is the typical plant response to N surplus, which was repeatedly documented among wetland species (both at natural habitats and experimental cultures), e.g. in *Phragmites australis* (Dykyjová 1978, Dinka 1986, Bornkamm and Raghiatri 1986, Kühl and Kohl 1993, Kohl et al. 1998, Lippert et al. 1999, Dinka and Szeglet 1999b, Tylová et al. - manuscript 3), *Glyceria maxima* (Tylová et al. - manuscript 3), *Acorus calamus* (Vojtíšková et al. 2006), *Typha latifolia* (Dykyjová 1978, Dykyjová 1979), *Typha angustifolia* (Steinbachová-Vojtíšková et al. 2006), *Carex rostrata* (Saarinen 1998, Saarinen and Haansuu 2000). In agreement, high FAA content, typically with dominance of N-rich FAA (Asn, Gln,

Arg), was detected in *Phragmites australis*, *Glyceria maxima* or *Acorus calamus* under high N supply in our experimental cultures (Tylová et al. - manuscript 3, Vojtíšková et al. 2006).

In contrast to FAA, levels of total nonstructural carbohydrates (TNC) follow the opposite trend (Choo and Albert 1999), as was documented e.g. in *Phragmites australis* (Čížková-Končalová et al. 1996, Kohl et al. 1998, Steinbachová et al. - submited), *Glyceria maxima* (Steinbachová et al. - submited, Steinbachová 2006), *Acorus calamus* (Vojtíšková et al. 2006, Steinbachová 2006), or *Carex rostrata* (Saarinen 1998, Saarinen and Haansuu 2000). The TNC/FAA ratio therefore provides important measure of actual plant C/N balance with several regulatory consequences towards e.g. biomass partitioning, tillering, or root branching (Buysse et al. 1993, Saarinen 1998, Saarinen and Haansuu 2000, Forde and Lorenzo 2001). In wetland plants, low TNC/FAA ratios were found at more fertile sites e.g. in shoots of *Phragmites australis* (Kohl et al. 1998), or *Carex rostrata* (Saarinen 1998, Choo and Albert 1999, Saarinen and Haansuu 2000). It is, however, obvious that amino acids are not the only compounds involved in signalling related to plant internal N status. Among others, NO_3^- itself is an important signal, which triggers both systemic and localized responses (Scheible et al. 1997b, Zhang et al. 1999, Forde and Lorenzo 2001, Coruzzi and Zhou 2001, Forde 2002, Sakakibara et al. 2006) and co-ordinates carbon and nitrogen metabolism (Champigny and Foyer 1992, Scheible et al. 1997a). The close cooperation with the cytokinin action is also involved (Aiken and Smucker 1996, Takei et al. 2002, Sakakibara et al. 2006).

As mentioned above, amino acids represent important **storage** form **of N** (Haldemann and Brändle 1988, Chapin et al. 1990, Woitke et al. 1997, Kohl et al. 1998, Gloser 2002). The substantial accumulation of FAA, and particularly of Asn, in rhizomes prior the winter was described in several perennial helophytes, e.g. *Phragmites australis* (Haldemann and Brändle 1988, Woitke et al. 1997, Kohl et al. 1998, Tylová et al. - manuscript 2), *Glyceria maxima* (Tylová et al. - manuscript 2), or *Acorus calamus* (Weber and Brändle 1994). In *Phragmites*, FAA are even considered as the main N storage compounds (Haldemann and Brändle 1988, Woitke et al. 1997, Kohl et al. 1998). In *Acorus calamus* (*Araceae*) predominantly arginine (Arg), but also Asn and proteins are involved in the winter storages (Weber and Brändle 1994). The timing of FAA accumulation in belowground organs in autumn seems, however, be significantly affected by N status of habitat (see chapter Consequences of high N availability to wetland plant performance).

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Furthermore, amino acid spectrum changes considerably under **oxygen shortage**. When subjected to anoxic/hypoxic conditions, substantial accumulation of particularly alanine (Ala) and γ-aminobutyric acid (Gaba) occurs in affected tissues in flood-intolerant (Good and Munch 1993, Kreuzwieser et al. 2002) as well as in flood-tolerant species (Haldemann and Brändle 1988, Fan et al. 1997, Reggiani et al. 2000, Reggiani and Bertani 2003), and also in algae (Vanlerberghe et al. 1991). Ala and Gaba are, together with e.g. ethanol, succinate, lactate, or acetaldehyde, the products of fermentation reactions involved in the energy production of anaerobic cell (Vartapetian 1991, Good and Munch 1993, Nilsen and Orcutt 1996, Drew 1997). Gaba synthesis, as a proton consuming process, might also help to ameliorate the cytosolic acidification associated with the hypoxic stress (Crawford et al. 1994). Furthermore, the accumulation of FAA seems to compensate loss of osmotically active compounds, particularly hexoses, which are intensively consumed (Menegus et al. 1984).

In wetland plants, the risk of oxygen shortage is particularly high in belowground organs during period of winter or early spring, when the supply of atmospheric oxygen via shoot aerenchymatous tissues is prevented. In agreement, high contents of Ala and Ser were detected in *Phragmites* basal culm internodes (Kohl et al. 1998) as well as rhizomes in the spring, particularly at more fertile site (Woitke et al. 1997). The Gaba or Ala accumulation was also observed in rhizomes of *Phragmites australis*, *Schoenoplectus lacustris*, or *Typha angustifolia* in winter (Haldemann and Brändle 1988), but the trend was relatively mild compared to experimental anoxic treatment. In *Phragmites*, the accumulation of Gaba and Ala was observed at sites with disrupted convective ventilation, e.g. at sites with lack of old (efflux) culms caused by mowing (Rolletschek et al. 1998), sites with low shoot densities (Rolletschek et al. 1999), sites with low shoot lengths and diameters (Rolletschek and Hartzendorf 2000), or sites affected by an extreme flood event (Koppitz et al. 2004, Koppitz 2004). Gas flow is reduced also in consequence of shoot or rhizome insect damage (Armstrong et al. 1996b), which may further increase Ala and Gaba contents in plants (Armstrong et al. 1996b, Koppitz et al. 2004). This insect pressure might be especially high at more fertile stands. The increase of herbivory in response to NPK fertilization was documented e.g. in red mangrove trees (Feller 1995). The general positive relationship between foliar N concentration and insect survivorship or reproductive rate was also summarized by e.g. Throop and Lerdau (2004).

Amino acid spectrum is altered also in response to **salinity**, which is one of important factors controlling species performance in many wetlands (Crawford 1994, Howard and Rafferty 2006, Sim et al. 2006), including e.g. broad coastal areas with saline and brackish

habitats (Howard and Rafferty 2006) or ephemeral wetlands in arid and semiarid climates (Deil 2005). Besides other responses, salinity triggers the accumulation of compatible solutes (amino acids and particularly proline, organic acids, polyols, or quaternary ammonium compounds) in plants to alleviate the osmotic stress (Cronk and Fennessy 2001). The substantial accumulation of proline (Pro) in response to saline stress was documented in different salt marsh species (e.g. *Juncus kraussii* (Naidoo and Kift 2006), or *Spartina anglica* (Mulholland and Otte 2001), and seagrassses (Vandiggelen et al. 1987, Adams and Bate 1994). Similar response occurs also in freshwater wetland species, e.g. in *Phragmites australis* (Rolletschek and Hartzendorf 2000, Hartzendorf and Rolletschek 2001), and *Phalaris arundinacea* (Maeda et al. 2006), particularly in clones or ecotypes with pronounced tolerance towards salinity (Zhu et al. 2003, Maeda et al. 2006).

NH_4^+ versus NO_3^- nutrition, NH_4^+ toxicity

The possibility to take up N as two chemically different forms brings several consequences to plant performance, which were summarized repeatedly (see e.g. Fernandes and Rossiello (1995), Marschner (1995), von Wiren et al. (2000), Britto and Kronzucker (2005)). The differences between NH_4^+ and NO_3^- acquisition are numerous. While the uptake of NH_4^+ is accompanied with the decrease of rhizosphere pH and reduced uptake of other cations (e.g. K^+, Mg^{2+}, Ca^{2+}), the opposite trends occur in NO_3^- fed plants. The majority of NH_4^+ is assimilated in roots and translocated to shoot in form of amino acids. NH_4^+ fed plants therefore show high FAA contents in roots and xylem (Chaillou et al. 1991, Cramer and Lewis 1993, Peuke and Jeschke 1995, Peuke et al. 1996, Bialczyk et al. 2004), but low contents of carbohydrates in roots, high root respiration rates, and/or high activity of PEP carboxylase in roots (for summary see Britto and Kronzucker (2002), Britto and Kronzucker (2005)). In contrast, long-distance NO_3^- transport is widely occurring, particularly under higher NO_3^- availability (Marschner 1995). NO_3^- is even important counter-ion for cations transported in xylem (Marschner 1995, von Wiren et al. 2000).

Although the assimilation of NH_4^+ has lower energy costs compared to NO_3^-, the exclusive NH_4^+ nutrition is potentially harmful to many plant species (Fernandes and Rossiello 1995, Marschner 1995, Gerendas et al. 1997, von Wiren et al. 2000). Sensitive plants even develop NH_4^+ **toxicity syndrome**, particularly when rhizosphere NH_4^+ levels exceed 100μM, and pH drops to low values (Marschner 1995, Britto and Kronzucker 2002). Certain sensitivity to excessive NH_4^+ supply occurs even in generally NH_4^+ tolerant wetland

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plants (see chapter NH_4^+ versus NO_3^- preferences in plants, ecological consequences). This growth and metabolic disorder includes the growth suppression (in some cases even higher mortality); low R/S ratio; accumulation of NH_4^+ in tissues; energy exhaustion; and/or the diminished uptake of essential cations (Mehrer and Mohr 1989, Marschner 1995, Gerendas et al. 1997, von Wiren et al. 2000, Britto et al. 2001b, Britto and Kronzucker 2002).

The risk of NH_4^+ toxicity is obviously related to excessive NH_4^+ uptake (Wang et al. 1993a, Britto and Kronzucker 2002). This leads to NH_4^+ over-accumulation in plant tissues (Hecht and Mohr 1990) or enhanced NH_4^+ extrusion, which results in energetically wasteful futile transmembrane NH_4^+ cycling (Britto et al. 2001b). As equilibrium partner of NH_4^+ (NH_3; pK_a 9.25) is highly phytotoxic in higher concentrations, plants are believed to keep low cytoplasmic NH_4^+ levels (in micromolar range) (Roberts and Pang 1992, Pearson and Stewart 1993, Weber and Brändle 1994, Marschner 1995, Howitt and Udvardi 2000). The accumulation of NH_4^+ is therefore suggested to be a part of the pathologic or stress responses (Mehrer and Mohr 1989, Hecht and Mohr 1990, Gerendas et al. 1997, Barker 1999). Some authors, however, detected millimolar NH_4^+ levels in cytoplasm (Wang et al. 1993b, Britto et al. 2001b) as well as xylem sap (Husted et al. 2000, Schjoerring et al. 2002) in plants showing no stress symptoms. These observations lead some authors to conclude that low NH_4^+ levels might not be an obligatory phenomenon in plants (Husted et al. 2000, Britto et al. 2001a, Britto and Kronzucker 2002, Schjoerring et al. 2002), and the opinions on the real behavior of NH_4^+ in plants are therefore still inconsistent (as an example see controversy between Britto et al. (2001a) and Howitt and Udvardi (2000)). One reason might be the methodological difficulty, since NH_4^+ detection shows high sensitivity to interference coming from various N-containing compounds (amino acids, amines, amides, proteins) (Gerendas et al. 1997, Husted et al. 2000, Schjoerring et al. 2002).

Besides excessive NH_4^+ uptake, the induced deficiency of essential cations (especially Ca^{2+}, Mg^{2+}, K^+) commonly occurs (Fernandes and Rossiello 1995, Marschner 1995, Britto and Kronzucker 2002), as their uptake and translocation to shoot are lower under NH_4^+ compared to NO_3^--N provosion in various plant species (e.g. Engels and Marschner (1993), Troelstra et al. (1995a), Peuke et al. (1996), Chance et al. (1999), Bar-Tal et al. (2001)). This phenomenon may accompany the sensitivity towards high levels of NH_4^+ even in wetland plants, as was shown in our study of *Glyceria maxima* and *Phragmites australis* (Tylová et al. - manuscript 1). In this study, *Glyceria* treated with 179μM N showed lower growth rate and lower contents of Mg^{2+} and K^+ in roots of NH_4^+ compared to NO_3^- fed plants, while no changes in either parameter occurred in *Phragmites* (Tylová et al. - manuscript 1). In

agreement, low K^+ contents in rhizomes of *Glyceria* but not *Phragmites* were found under excessive sewage dose (Čížková-Končalová et al. 1996). Similarly, NH_4^+ treated plants of *Cirsium dissectum* showed low K, Fe, Ca, Mg, Mn, and Zn status of roots, particularly at low pH (Lucassen et al. 2003).

In spite of these experimental evidences, the real importance of the cation deficiency in performance of wetland plants at natural NH_4^+-rich eutrophic stands is still not completely clear. Although several studies followed cation contents as well as their seasonal fluctuation in biomass (e.g. Dykyjová and Hradecká (1976), Dykyjová (1978)), the observations linking sediment characteristics with plant Ca^{2+}, Mg^{2+}, K^+ levels are scarce or came with inconsistent results. While low leaf Ca^{2+}, Mg^{2+}, K^+ contents were found in severely damaged *Phragmites* at stand located in close vicinity to discharge of raw piggery sewage (Čížková et al. 1996, Čížková-Končalová et al. 1996), no difference in rhizome K^+ content was later observed between vigorous and die-back sites in this species (Čížková and Lukavská 1999, Dinka and Szeglet 1999a). Furthermore, Dinka (1986) and Dinka and Szeglet (1999b) detected even higher K^+ content in *Phragmites* rhizomes at hypertrophic die-back site compared to stabilized mesotrophic site. Similarly, higher cation contents were found in *Phragmites* colonising site rich in nutrients and organic matter compared to site at eroded sandy bottom (Dykyjová and Hradecká 1976, Dykyjová 1979).

Besides the above-mentioned effects, which occur typically in response to high NH_4^+/NO_3^- ratio, numerous responses to high NH_4^+ supply seem very similar to those triggered by high availability of N *per se* (e.g. changes in biomass allocation or carbohydrate status). At natural wetland stands, where N enrichment and subsequent eutrophication are commonly accompanied with the increased NH_4^+ availability, the importance of these two factors is often difficult to distinguish. These responses are therefore summarised together in the following chapter (chapter Consequences of high N availability to wetland plant performance).

Consequences of high N availability to wetland plant performance

Increasing N availability triggers several responses in plants, including changes in growth rate, biomass distribution, morphology, chemical composition, phenology, and/or storage formation (Fernandes and Rossiello 1995, Marschner 1995). In wetland plants, all these responses obviously affect the susceptibility to different stresses, and thus competition ability at natural habitats. Furthermore, the shift between plant available N forms (NH_4^+

versus NO$_3^-$) commonly occurring along with the eutrophication has further consequences to wetland plant physiology.

Among general responses to N addition, enhanced **biomass production** and favoured allocation of biomass to aboveground structures (increased A/B and S/R ratios) are the most typical ones (Marschner 1995, Saarinen 1998), being conspicuous especially in fast growing species (Chapin 1980, Aerts et al. 1992). Such stimulatory effect is well documented in several wetland and aquatic plants, e.g. *Phragmites australis* (Dykyjová and Hradecká 1976, Dykyjová 1978, Bornkamm and Raghiatri 1986, Ulrich and Burton 1988, Kühl and Kohl 1992, Kühl and Kohl 1993, Votrubová and Pecháčková 1996, Čížková-Končalová et al. 1996, Clevering 1998, Romero et al. 1999, Lippert et al. 1999, Rickey and Anderson 2004, Tylová et al. - manuscript 3), *Glyceria maxima* (Čížková-Končalová et al. 1996, Tylová et al. - manuscript 3), *Acorus calamus* (Dykyjová 1978, Vojtíšková et al. 2004), *Typha angustifolia* (Ulrich and Burton 1988, Steinbachová-Vojtíšková et al. 2006), *T. domingensis* (Newman et al. 1996), *T. latifolia* (Dykyjová 1978, Ulrich and Burton 1988, Wetzel and van der Valk 1998), *Sparganium eurycarpum* (Ulrich and Burton 1988), *Carex stricta* (Wetzel and van der Valk 1998), *C. rostrata* (Aerts et al. 1992), *C. curta, C. elata, C. flacca, C. flava, C. panicea* (Güsewell 2005a), *Phalaris arundinacea* (Wetzel and van der Valk 1998), *Bolboschoenus maritimus* (Dykyjová 1978), *Vallisneria natans* (Xie et al. 2005), or *Potamogeton amplifolius* (Cronin and Lodge 2003).

The enhanced growth obviously does not endanger plant directly, in fact it favours given plant in the competition for space, but brings about several side effects, which negatively affect long-term survival at waterlogged habitats. Among others, enhanced accumulation of litter, which seriously alter conditions of flooded substrate, was shown to strongly repress growth of some wetland plants. The sensitivity was documented e.g. in *Phragmites australis* (Čížková et al. 1996, van der Putten et al. 1997), *Acorus calamus* (Pai and McCarthy 2005), or *Lobelia dortmanna* (Sand-Jensen et al. 2005). Furthermore, delayed shoot senescence and thus accumulation of storage compounds in belowground organs, changes in resource allocation, delayed differentiation of internal structures (e.g. of protective surface layers or aerenchyma), etc. are usually correlated with the intensive growth under N surplus. These effects are discussed below.

When the availability of N is further elevated up to excessive levels, the positive growth response to N surplus turns to negative, which was experimentally proved e.g. in *Phragmites australis, Glyceria maxima, Typha angustifolia, Typha latifolia, Acorus calamus, Bolboschoenus maritimus, Sparganium eurycarpum,* or *Potamogeton alpinus* (Dykyjová

1978, Ulrich and Burton 1988, Čížková-Končalová et al. 1996, Vojtíšková et al. 2004, Boedeltje et al. 2005, Tylová et al. - manuscript 3, Steinbachová-Vojtíšková et al. 2006). Individual species, however, differ considerably in their sensitivity. This was nicely shown in experiments of Cronin and Lodge (2003), who observed that similar N availability, which stimulated growth of *Potamogeton amplifolius* was lethal for *Nuphar advena*. The adverse effect of excessive N dose is more serious under sub-optimal P availability, as was shown in *Phragmites australis* and *Glyceria maxima* (Ulrich and Burton 1988, Romero et al. 1999, Tylová et al. - manuscript 3), in *Typha latifolia, T. angustifolia*, and *Sparganium eurycarpum* (Ulrich and Burton 1988), *Sphagnum* sp. (Gunnarsson and Rydin 2000), *Sarracenia purpurea* (Gotelli and Ellison 2002), or seagrass *Zostera noltii* (Brun et al. 2002). In our experiments, the importance of P was especially visible in *Glyceria maxima*, which agrees with the field observations describing 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 *Glyceria* to expand into reed die-back sites in highly eutrophic areas may not necessarily be only due to the sensitivity of *Phragmites* to severe eutrophication (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 further common response to N addition is the preferential **allocation of biomass** to aboveground structures (e.g. Ulrich and Burton (1988), Maurer and Zedler (2002), Cronin and Lodge (2003), Vojtíšková et al. (2004), Xie et al. (2005), Steinbachová-Vojtíšková et al. (2006), Tylová et al. - manuscript 3). Mineral nutrients are not the limiting resources at the moment and the investment into roots is therefore down-regulated (Chapin 1980, Lambers et al. 1998, Saarinen and Haansuu 2000). This includes not only low relative proportion of root biomass, but also decreased lengths of individual roots, and suppressed branching. Such systemic responses were clearly demonstrated for high NO_3^- availability (Zhang et al. 1999, Zhang and Forde 2000, Forde and Lorenzo 2001, Forde 2002), but occurs also in case of other nutrients, particularly PO_4^{3-} (Lopez-Bucio et al. 2003). In wetland plants, several studies documented significant effect of overall sediment fertility (availability of both N and P) on root lengths. Low **root lengths** or rooting depth in response to N, P enrichment were observed e.g. in *Glyceria maxima* (Čížková-Končalová et al. 1996, Tylová et al. - manuscript 3), *Phragmites australis* (Votrubová and Pecháčková 1996, van der Putten 1997, Votrubová et al. 1997)), *Acorus calamus* (Vojtíšková et al. 2004), *Vallisneria natans* (Xie et al. 2005), *Elodea nuttallii, E. canadensis, Lagarosiphon major* (James et al. 2006). The importance of N versus

P is therefore difficult to distinguish, but e.g. in *Lemna minor* and *Spirodella polyrrhiza*, P seems more important nutrient controlling their root lengths (Elster et al. 1995).

Low root/shoot ratio (for summary see Marschner (1995), Britto and Kronzucker (2002)), and shorter, thicker roots (Anderson et al. 1991, Teyker and Hobbs 1992, Lang and Kaiser 1994, Cruz et al. 1997) occur also in many plant species in response to NH_4^+-N source. At lower NH_4^+ level, the effect mimic plant response to high N, as NH_4^+ fed plants commonly contained higher N levels in their tissues compared to NO_3^- supplied plants (Lucassen et al. 2003, Tylová-Munzarová et al. 2005). Under excessive NH_4^+ level, however, this response can be attributed to NH_4^+ toxicity. Retarded root growth was observed in various NH_4^+ sensitive species (Teyker et al. 1991, Teyker and Hobbs 1992, Vollbrecht and Kasemir 1992, Cao et al. 1993, Lang and Kaiser 1994), but occurs even in NH_4^+ tolerant wetland species, e.g. in *Cirsium dissectum* treated with NH_4^+ together with low pH (wet heathland species; Lucassen et al. (2003)), or *Glyceria maxima* under very high (3,7mM) NH_4^+ availability (Tylová et al. - manuscript 3). In addition, root morphological changes induced by NH_4^+ nutrition (shorter, thicker roots, higher frequency of lateral roots) might correspond to enhanced import of auxin into roots as a consequence of stimulated import of carbohydrates to meet carbon requirements for NH_4^+ assimilation in roots (Gerendas et al. 1997).

In water-logged sediment, the decreased root lengths under high fertility may also be related to oxygen supply (Čížková-Končalová et al. 1996, Sand-Jensen et al. 2005). Increased oxygen demand of eutrophic sediment (Čížková et al. 1996, Rolletschek 1997) as well as changes in internal root structure induced by N addition may induce oxygen shortage of root apical parts. The anatomical changes include particularly the decreased root porosity (documented e.g. in *Phragmites* (Votrubová and Pecháčková 1996, Čížková-Končalová et al. 1996) or wetland sedges (Končalová et al. 1993) and delayed differentiation of lignified protective layers in roots (Votrubová and Pecháčková 1996). As root apical meristem and the elongation zone have high oxygen requirements (Mancuso and Boselli 2002, Pang et al. 2006), root growth is suppressed under insufficient oxygen availability. Roots are also more susceptible to sediment phytotoxicity (Armstrong et al. 1996a, Armstrong et al. 1996c, Čížková et al. 1999, Armstrong and Armstrong 1999, Armstrong and Armstrong 2001b). The incidence of damaged root apical parts (or whole lateral roots) is therefore high in more fertile growth conditions, both in experimental cultures (Vojtíšková et al. 2004, Tylová et al. - manuscript 3) and at eutrophic habitats (van der Putten 1997, Votrubová et al. 1997). Oxygen shortage was also observed to trigger preferential formation of water roots at stem nodes compared to soil roots in *Phragmites australis* (Strand and Weisner 2002). Shallow rooting

might allow to overcome adverse conditions of deep, reduced sediment layers, but stands may be more susceptible to mechanical stress factors, e.g. wave action, fish grazing (Ostendorp 1989, Nilsen and Orcutt 1996) as the anchorage in the sediment is weakened.

Furthermore, plants under high nutrient availability showed higher incidence of young, short, unbranched, intensively growing roots (Vojtíšková et al. 2004, Steinbachová-Vojtíšková et al. 2006, Vojtíšková et al. 2006). Their incidence is obviously not related to systemic suppression of root branching in response to nutrient over-supply, but rather to higher growth rate and particularly **biomass turnover**, which compensate the loss of damaged biomass. In agreement, generally lower life span of organs at eutrophic die-back site was demonstrated e.g. for rhizomes of *Phragmites australis* (Čížková and Lukavská 1999, Dinka and Szeglet 1999b, Dinka and Szeglet 2001).

The **shoot morphology** is also affected, as indicated by studies including several wetland helophytes. In *Phragmites australis* or *Glyceria maxima*, moderate N addition was shown to trigger an increase in shoot heights and diameters (Kubín et al. 1994, Lippert et al. 1999, Tylová et al. - manuscript 3), but plants under excessive N dose usually possessed higher shoot numbers, but lower shoot lengths (Hardej and Ozimek 2002, Tylová et al. - manuscript 3). Low shoot lengths and low shoot diameters were also documented in *Phragmites* colonising severely eutrophic sites, where the species was obviously dying-back (Ostendorp 1989, Dinka and Szeglet 2001). The extensive study of Ostendorp et al. (2001), however, failed to prove clear relationship between trophic status of the habitat and the culm architecture in this species.

Shoot morphological trays are important determinants of convective ventilation efficiency (Rolletschek et al. 1999). Thin shoots generally posses lower flow rates, which can be compensated by high shoot density (Rolletschek et al. 1999). In addition, low shoot lengths may impair plant ability to support belowground parts with atmospheric oxygen under suddenly raised water level - and thus survive occasional floods. As indicated above (chapter NH_4^+ and NO_3^- assimilation, amino acids), enhanced tillering in response to N addition is triggered by high level of N rich compounds (particularly FAA) in plant tissues, which is the important signal regulating the emergence of new tillers (Saarinen and Haansuu 2000). In agreement, close correlation between tillering and leaf C/N ratio and FAA status was observed in *Phragmites* and *Glyceria* in our experiments (Tylová et al. - manuscript 3).

Besides morphological responses, high N availability triggers significant changes in **plant phenology**, particularly the delay in transition from vegetative to generative phase (Dykyjová 1978, Kühl and Kohl 1993, Marschner 1995) and the delay in autumnal shoot senescence (Hardej and Ozimek 2002, Tylová et al. - manuscript 2). The suppressed flowering under excessive N dose was demonstrated in experimental cultures of *Phragmites australis* and *Typha latifolia* (Dykyjová 1978), lower incidence of flowering (van der Putten 1997, Lippert et al. 1999) or the delayed onset of flowering (van der Putten 1997) were also observed at natural *Phragmites* stands of higher fertility. Such stands also commonly exhibit high incidence of shoot loss in early summer followed by the compensatory growth, which prolongs the vegetative phase of stand development (Kühl and Kohl 1992, Kühl and Kohl 1993).

Altered phenology has consequences to internal nutrient cycles, and particularly to **formation of storages** in over-wintering belowground organs during autumn. Prolonged vegetative growth results in delayed **translocation of carbohydrates** from shoots to belowground organs during autumn (Kohl et al. 1998, Steinbachová et al. - submited). The transition of belowground growing sinks into the storage sinks is also delayed (Steinbachová 2006), which prevents accumulation of storages in these organs. Low starch/soluble sugars ratio observed in high N treated plants is the sign of higher metabolic activity (Steinbachová et al. - submited, Vojtíšková et al. 2006). Soluble sugars are also less efficient storage compounds compared to starch (Kubín and Melzer 1996). In addition, our experiments with *Phragmites australis*, *Glyceria maxima* (Tylová et al. - manuscript 3), or *Typha angustifolia* (Steinbachová-Vojtíšková et al. 2006) showed lower relative allocation of biomass into rhizome under high N or N, P availability, which might further decrease plant storage capacity.

The carbohydrate levels in belowground organs may also be reduced in response to NH_4^+-N, as NH_4^+ assimilation presents important sink for carbon skeletons (for summary see Britto and Kronzucker (2002)). NH_4^+ stimulates starch degradation (Miyachi and Miyachi 1985), stimulates activity of starch degrading enzymes (Miyachi and Miyachi 1987), as well as down-regulates enzymes of starch synthesis (Matsumoto et al. 1968). In wetland plants, the adverse effect of excessive NH_4^+ on rhizome starch levels was indicated by field observations of *Phragmites australis* (Kubín and Melzer 1996). Its rhizomes growing in NH_4^+-rich sediments exhibited lower starch/soluble sugars ratio compared to plants from NO_3^- dominated sites. Low carbohydrate levels were also found in *Phragmites australis* and *Glyceria maxima* under high NH_4^+ availability in experimental cultures (Steinbachová et al.-

submited), as well as in *Zostera noltii* in response to winter NH_4^+ pulses (Brun et al. 2002). In all these above-mentioned studies, however, high NH_4^+ availability might mimic the overall availability of N. The effect of the form of supplied N is therefore difficult to distinguish. For this reason, our study involved cultivations of *Phragmites australis*, *Glyceria maxima* (Tylová et al. - manuscript 1), and *Acorus calamus* (Vojtíšková et al. 2006) under NH_4^+ versus NO_3^- nutrition, but receiving identical N levels. These experiments showed significantly lower starch content in rhizomes of NH_4^+ compared to NO_3^- fed plants in *Phragmites* (Tylová et al. - manuscript 1) as well as *Acorus* (Vojtíšková et al. 2006). In contrast, *Glyceria* reduced particularly the content of soluble sugars in roots in response to NH_4^+ nutrition (Tylová et al. - manuscript 1). From these results, we can conclude, that the shift in NH_4^+/NO_3^- pore water ratio in favour of NH_4^+ may *per se* participate on weakening of carbohydrate reserves in plants at eutrophic habitats. In addition, the effect is species specific, which may affect plant competition on the given habitat.

All these factors obviously reduce carbohydrate storage potential, which is important prerequisite for over-wintering and spring outgrowth of perennial wetland species (Čížková-Končalová et al. 1992, Čížková-Končalová et al. 1996, Steinbachová et al. - submited). The reduction seems, however, not enough conspicuous to endanger plants directly, but it increases plant susceptibility to occasional stresses e.g. flood event or mechanical disturbance (Čížková-Končalová et al. 1992, van der Putten 1997, Čížková et al. 2001a, Steinbachová et al. - submited, Steinbachová 2006).

Similarly to carbohydrates, the amount of N stored in over-wintering organs can significantly affect the growth of perennial plant species. In the spring, stored N is utilized in growing shoots (Granéli et al. 1992, Dinka and Szeglet 1999b) and positively affects plant development (e.g. number of tillers) (Gloser 2005). The autumnal formation of N storages in belowground organs depends highly on **retranslocation of N-rich compounds** from senescing shoots, but several studies indicated disruption of its effectiveness at eutrophic habitats. The delayed onset of FAA translocation into rhizomes was repeatedly observed in *Phragmites australis* affected by eutrophication (Kühl and Kohl 1993, Lippert et al. 1999). Similar response was also found in *Phragmites australis* and *Glyceria maxima* treated with high N, P availability in our experimental cultures (Tylová et al. - manuscript 3, Tylová et al. - manuscript 2). It is, however, not completely clear, whether this delay really disrupts rhizome N storage function, as plants generally respond to N enrichment by the increase of their N status (for references see chapter NH_4^+ and NO_3^- assimilation, amino acids), which may

compensate weak retranslocation under these conditions. In addition, N taken up from N rich external solution during late autumn may contribute to the formation of N storages (Gloser 2005).

The observations of autumnal N status of rhizomes coming from the natural habitats are contradictory. Čížková and Lukavská (1999) observed the lowest N rhizome contents in *Phragmites australis* at hypertrophic, compared to oligo- and mesotrophic stands. In contrast, Woitke et al. (1997) and Lippert et al. (1999) found comparable autumnal N contents in rhizomes of this species at sites of different fertility and productivity. And Dinka and Szeglet (1999b) detected even higher N levels in rhizomes of *Phragmites* at die-back compared to vigorous stands in autumn, but the N standing stock clearly followed the opposite trend due to low rhizome biomass. The formation of belowground N storages was therefore studied in more details in our experiments, using *Phragmites australis* and *Glyceria maxima* as the model species. The obtained data confirmed the delayed onset of retranslocation in more fertile conditions (Tylová et al. - manuscript 3, Tylová et al. - manuscript 2), as the majority of N was translocated to belowground organs very late in the season. The total amount of resorbed N was, however, high compared to plants in oligotrophic treatment due to high aboveground N standing stock accumulated during previous period of growth (Tylová et al. - manuscript 2). Similar relationship was found e.g. in woody plants (Aerts 1996). These results indicate that the delay is not critical feature, at least under normal circumstances. It may, however, cause a loss of aboveground N pool in situation of early coming winter. Furthermore, adverse conditions of eutrophic habitat cause damage of belowground organs (Votrubová et al. 1997, Armstrong and Armstrong 2001a) and thus loss of reserves. As an example, more than 70% of rhizome biomass was observed to be dead in *Phragmites australis* at die-back site (van der Putten 1997, Dinka and Szeglet 1999b).

Besides the timing, two other characteristics of retranslocation process determine the amount of N finally allocated into belowground organs prior the winter. **Retranslocation efficiency** (= the fraction of N primarily allocated in the given organ, which is retranslocated during the senescence process) and **retranslocation proficiency** (= the amount of N remaining in the senescent organ). The efficiency seems to be only weakly affected by nutrient availability or plant internal nutrient status (Chapin 1980, del Arco et al. 1991, Aerts 1996, Aerts and Chapin 2000), being determined rather by physiological mechanisms of leaf senescence (e.g. ratio of soluble/insoluble N compounds (Pugnaire and Chapin 2005) or phloem transport (Chapin and Moilanen 2005). In agreement, the efficiency of N retranslocation did not differ between oligotrophic and eutrophic growth conditions in

Phragmites australis or *Glyceria maxima* in our study in spite of the fact, that the process started significantly later in more nutritive treatment (Tylová et al. - manuscript 2). There are, however, also evidences indicating that dependence between N retranslocation efficiency and plant nutritional status may exist. Among others, Güsewell (2005a) found lower N retranslocation efficiency in several wetland species of *Carex* genus under higher N supply, particularly under very high N/P supply ratio. The observations from the natural habitats, however, did not reveal this relationship in the similar group of species (Güsewell 2005b).

In contrast, high N status of dead shoots (the proficiency) in more fertile conditions was repeatedly documented in different wetland species (Kühl and Kohl 1993, Lippert et al. 1999, Tylová et al. - manuscript 2). It generally shows close correlation with N status of living mature leaves (Miao 2004, Kobe et al. 2005, Güsewell 2005b, Tylová et al. - manuscript 2). From this point of view, N conservation is less efficient in plants at more fertile habitats (Boar 1996, Kohl et al. 1998, Lippert et al. 1999), as they do not realize their maximum (potential) resorption (Killingbeck 1996, Aerts and Chapin 2000), leaving N cycle more open (Boar 1996, Kohl et al. 1998, Lippert et al. 1999). Furthermore, N remaining in the **litter** affects its quality, **decomposability**, and thus nutrient cycles in wetlands (Aerts 1997, Aerts et al. 1999, Asaeda and Nam 2002, Asaeda et al. 2002, Güsewell and Verhoeven 2006). Although the climate has the major influence on decomposition rates, the chemical composition of litter: N, P levels, and lignin/N, C/N, and C/P ratios are important factors controlling the rate of litter decay, particularly during initial phase (first year) of the decomposition (Aerts 1997). The decay of N-rich litter facilitates eutrophication, being the substrate for intensive fungal and microbial colonization.

The summary of the original findings of the thesis:

This thesis followed basic aspects of N acquisition in two co-occurring helophytes, *Phragmites australis* and *Glyceria maxima*, which are understood as the model species. The main aim was to characterise the extent to which trays in N uptake, metabolism, and the response towards changes in N availability affect performance of wetland plants at eutrophic habitats. The particular interest was to separate the effects of nutrients *per-se* (and show their importance) from the complex of other factors related to eutrophication, using well-defined experimental cultures.

The study showed that overall biomass stimulation commonly found at eutrophic habitats, particularly in early stages of eutrophication, might closely be related to N:P supply ratio. While unbalanced high N load (N/P ratio 100/1) triggered negative growth responses in both *Phragmites* and *Glyceria*, similar N dose applied in N/P ratio 10/1 affected plant growth positively **(Tylová et al. - manuscript 3)**. The importance of P was especially visible in *Glyceria*, which agrees with the field observations describing well-developed *Glyceria* stands as indicators of local sources of pollutions with high P contents in the sediment (Hroudová and Zákravský 1999). Furthermore unbalanced high N load altered morphology of both species in the manner that could increase plant susceptibility to stresses (altering e.g. ventilation efficiency, ability to survive high water levels, plant anchorage, belowground storage capacity). These changes in biomass distribution, or shoot and root morphology resemble changes observed at stand affected by eutrophication. We can therefore conclude that not only secondary aspects of eutrophication (litter accumulation, sediment phytotoxicity) but also primary nutritional effects are important factors determining species performance and survival at the given habitat.

Besides morphological alterations, high N, P availability triggered the changes in plant phenology and formation of storages. The prolongation of vegetative phase (Dykyjová 1978, Kühl and Kohl 1993, Hardej and Ozimek 2002) as well as the weakening of carbohydrate storages (Čížková-Končalová et al. 1992, Steinbachová 2006) in response to N addition are well described symptoms. Less information is, however, available about formation of N reserves in wetland plants. Although the delay in translocation of N-rich compounds (particularly amino acids) was repeatedly observed at eutrophic stands, it was not clear if rhizome N storages are indeed adversely affected, or if the shift in C/N ratio in favour of N under N surplus may compensate weak retranslocation. Our study clearly showed, that in spite of the delayed shoot senescence, delayed retranslocation, and higher N levels remaining in

dead leaf biomass, both *Phragmites* and *Glyceria* accumulated higher N levels (and particularly higher N standing stock) in belowground organs prior the winter **(Tylová et al. - manuscript 2).**

The particular interest was also paid to plant response towards NH_4^+ versus NO_3^- N source, as the shift in NH_4^+/NO_3^- ratio in favour of NH_4^+ at eutrophic sites was indicated by several studies. Although (van der Putten 1997) summarised that the shift is probably not highly important factor in eutrophication impact, we observed different response of *Phragmites* and *Glyceria* to form of supplied N. The response was highly dependent on total N level. Higher growth rate under NH_4^+-N compared to NO_3^--N source was found in *Glyceria* at N level (34μM) corresponding with average N concentrations in the pore waters of wetlands in the Třeboň basin, Czech Republic **(Tylová-Munzarová et al. 2005)**. In contrast, the opposite trend occurred under 179μM N level simulating eutrophic wetland habitats **(Munzarová et al. 2006, Tylová et al. - manuscript 1)**, and particularly at extremely high N level (3700μM N) corresponding to constructed wetlands for wastewater treatment (Tylová et al. - manuscript 1). In *Phragmites*, however, no response to form of supplied N was found at either N level. These results obviously show, that commonly supposed high NH_4^+ tolerance of wetland plants as species colonising NH_4^+ dominated habitats, is not a completely general feature. Furthermore, even species with similar habitat preference may significantly differ in their response. *Phragmites* seems therefore more tolerant to high external NH_4^+ levels compared to *Glyceria*, which agrees with the overall growth strategy of the two species. *Phragmites* generally roots deeper than *Glyceria* into hypoxic NH_4^+ dominated sediment layers. It also indicates that the sensitivity of *Phragmites* to highly eutrophic sediments is not related to the direct adverse effect of high NH_4^+ availability. In contrast, *Glyceria's* ability to colonise fertile habitats rich in NH_4^+ is related to the avoidance strategy due to shallow rooting, or the ability to cope with high NH_4^+ levels when enough P is available or NO_3^- is co-provided. In addition, plants treated with NH_4^+-N showed lower carbohydrate levels (starch in rhizomes of *Phragmites*; soluble sugars in roots of *Glyceria*) compared to NO_3^- fed plants (Tylová et al. - manuscript 1). NH_4^+ prevalence may thus *per se* participate on carbohydrate shortage of plants at eutrophic habitats.

The importance of NH_4^+ versus NO_3^- as sources of N for wetland plant growth was further followed via the study of NH_4^+ and NO_3^- uptake kinetics and the study of nitrate reductase activity, the key enzyme of NO_3^- assimilation in plants. In both species, higher

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uptake capacities (Vmax) for NH_4^+ compared to NO_3^- in both species were found, even in fully induced NO_3^- fed plants. Similarly, 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 share characteristics of plants colonizing habitats with restricted nitrification, where the preference for NH_4^+ over NO_3^- is described (Kronzucker et al. 1997). The observed differences were, however, relatively small. Both species were also able to sufficiently grow with sole NO_3^--N source and displayed high NR activity (Munzarová et al. 2006). In littoral helophytes, therefore, the preference for NH_4^+ seems to be very weak or even absent in comparison with submerged wetland species (examined e.g. by Cedergreen and Madsen (2003)). This may correspond to the fact, that NH_4^+ prevalence in the sediment is not so strict (particularly in superficial sediment layers) and may change with water level fluctuation. Moreover, the existence of finely branched aquatic roots allows the uptake of NO_3^- from the oxidized water column.

The study of NH_4^+ and NO_3^- uptake also showed that both species possess high uptake capacity. This is the typical feature of fast growing species colonising preferentially stands of higher fertility. *Phragmites*, however, exhibited lower Km and higher affinity compared to *Glyceria*, characteristics advantageous under nutrient limitation. This finding indicates a higher physiological plasticity to adjust N uptake towards low nutrient availabilities. In agreement, field observations described the ability of *Phragmites* to colonise oligotrophic systems, where *Glyceria* is not present (Brändle et al. 1996).

In summary, the study showed that many trays of plant performance at natural stands and their responses towards eutrophication are underlined by physiological characteristics of N acquisition and utilization. Although secondary impacts of eutrophication (litter accumulation, phytotoxin occurrence) were repeatedly considered as more important factors triggering plant damage at eutrophic stands (e.g. (Ostendorp 1989, van der Putten 1997, Brix 1999)), the primary nutritional aspects of N addition and changes in NH_4^+ versus NO_3^- occurrence have to be taken into account.

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