Nitrogen supply through transpiration mass flow can limit nitrogen nutrition of plants

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ABSTRACT

Pea (*Pisum sativum* L.), sunflower (*Helianthus annuus* L.) and maize (*Zea mays* L.) plants were cultivated for 10 days in hydroponics at 1mM and 7mM nitrate or ammonium concentrations at regulated pH 6 and ambient CO_2 level. Plant growth, content of total N and both ions in plant tissues, uptake of water and both N ions were evaluated, N uptake related to transpiration mass flow and to diffusion supply was calculated. Pea and sunflower preferred nitrate nutrition while maize plants used both N ions. The content of total N as well as of both N ions in plant tissues increased with N level with some exceptions. The uptake of both N ions related to transpiration mass flow was dependent on transpiration rate and N ion concentration. At a 1mM N concentration the uptake of N ions related to transpiration mass flow was low and reached in maize up to 16 times, in sunflower 11 times and in pea 2–3 times lower values in comparison with diffusion supply. At a 7mM N concentration N uptake in pea plants was totally supplied by transpiration mass flow, in sunflower plants the ratio of N supply related to transpiration mass flow amounted to 50% and in maize plants N supply through diffusion prevailed, amounting to 70–80%. These results explicate N starvation at low N supply that can intensify at elevated CO_2 causing decreased stomatal diffusion.

Keywords: pea; sunflower; maize; NO_3^-, NH_4^+ uptake; transpiration mass flow; diffusion supply; N starvation; plant acclimation

The acquisition of inorganic nitrogen is considered as one of the most important aspects of plant mineral nutrition. Plants take up nitrogen mainly in the form of nitrate and ammonium. Differences in the uptake and assimilation of both these N ions in different kinds of plants were the topic of numerous papers published in the past (Haynes and Goh 1978) as well as recently (Matt et al. 2001). The occurrence of both N ions in plant stands depends on natural processes taking place in soils and on different anthropogenic activities as well, especially agricultural ones with the aim to intensify plant production.

In the last decade numerous studies were devoted to the clarification of plant behaviour (Drake et al. 1997) and to the description of interactions between photosynthesis, metabolism, growth and nitrogen supply at elevated CO_2 (Geiger et al. 1999, Stitt and Krapp 1999, Maroco et al. 2002).

The main goal of this study is to compare the uptake of NO_3^- and NH_4^+ at different N levels and constant pH value of nutrient solution in pea, sunflower and maize. Plant growth, N content, N uptake and N supply to roots by diffusion and transpiration mass flow were investigated. The acquired results are discussed also with respect to N supply at elevated CO_2.

MATERIAL AND METHODS

Three plant species were used for the experiments: pea (*Pisum sativum* L.) cv. Liliput, sunflower (*Helianthus annuus* L.) cv. Albena and maize (*Zea mays* L.) hybrid CE 205. Plant seeds were germinated for three days in vermiculite moistened with distilled water at 25°C before being placed to hydroponics.

The uptake of NO_3^- and NH_4^+ by experimental plants was measured in water cultures during 10-day growth period at two different concentrations, 1mM and 7mM respectively, in a growth chamber at 25/20°C, 60% RH, 16/8h photoperiod and ambient CO_2 level. Average irradiance was 310 µmol/s/m² PPFD at the top of the plants at a 30cm distance from the light panel.

Nutrient solution of the following composition was used: KH_2PO_4 3.6mM, $CaCl_2.6\ H_2O$ 1.7mM, $MgSO_4$ 2.1mM, KCl 3.5mM, Fe-chelatone 170µM; the following microelements were added: H_3BO_3 46µM, $MnCl_2.4\ H_2O$ 7.4µM, $ZnSO_4.7\ H_2O$ 0.5µM, $CuSO_4$ 0.23µM and $H_2MoO_4.H_2O$ 0.11µM. Nitrogen was used in the form of KNO_3 or $(NH_4)_2SO_4$.

Each treatment consisted of 32 plants and each treatment was placed in a container with 4 l of nutrient solution. The nutrient solution was aerated by a membrane pump with the intensity of 750 ml/h per container and with simultaneous moderate circulation of the solution.

The growth of plants was evaluated by determination of fresh matter using non-destructive techniques. The weight of 32 plants of each treatment placed on a portable panel was determined. Fresh and dry matter of single plants was determined at the end of the experiment.

The nitrate concentration in nutrient solution or in tissue extracts was estimated by means of a selective liquid electrode. 0.05M solution of aluminium sulphate and cop-

per sulphate at a 2:1 ratio was used for NO_3^- extraction from fresh plant tissues.

The ammonium content in plant organs was determined after homogenisation of fresh tissue and extraction with acidified (pH 3) 80% ethanol. The supernatant received after centrifugation was evaporated at 40°C and the rest was dissolved in distilled water. The concentration of NH_4^+ in water extracts or nutrient solutions was determined by micro-Kjehldal techniques.

The volume of water taken up by plants for the growth process and transpiration was measured. Actual concentrations of NO_3^- and increasing concentration of NO_3^- ions were determined in initial solutions and every day afterwards during the plant growth together with pH estimation, and after pH adjustment, the volumes and N ion concentrations were adjusted to original ones with distilled water and N ion solutions. The pH value of nutrient solutions was adjusted to 6 by adding 0.1N H_2SO_4 or 0.1N NaOH.

Total NO_3^- and NH_4^+ uptake was determined in nutrient solution, N ion uptake related to diffusion and transpiration mass flow was calculated in the following way:

$$N_t = N_d + N_{tr}$$

where: N_t = total N uptake (determined in solution)
N_{tr} = N uptake related to transpiration mass flow
N_d = N uptake related to diffusion (concentration gradient)

The amount of nitrogen decrease from the original concentration in nutrient solution was related to the plant water uptake during the same time interval, and it was considered as N uptake supplied to the roots by transpiration mass flow – N_{tr}. This amount was subtracted from the amount of nitrogen decrease and the rest was considered as N uptake supplied to the roots by diffusion – N_d. N uptake was related to fresh weight of a single plant.

Total nitrogen content in plant tissues was determined after their mineralisation in concentrated sulphuric acid with addition of hydroperoxide as a catalytic agent by Kjehldal techniques.

The results were statistically processed by standard error calculation. Differences were evaluated by *t*-test ($p < 0.05$) and by Duncan's multiple range test. All analyses were repeated 3–5 times or more and the number of repetitions is mentioned.

RESULTS

Growth of experimental plants and N content changes

The growth of plants (estimated from dry weight) was influenced both by N forms and by their concentrations. The increasing concentration of NO_3^- tended to stimulate the shoot growth of pea plants. On the contrary, ammonium nutrition inhibited the growth of pea plants

significantly and the inhibition increased with increasing NH_4^+ concentration (Figure 1).

The fastest growth of sunflower plants was determined at 7mM NO_3^- concentration and the slowest at 7mM NH_4^+ concentration. The growth stimulation by 7mM NO_3^- nutrition was reflected in shoots while 7mM NH_4^+ nutrition decreased the growth of both plant organs. The growth of sunflower plants at 1mM NO_3^- or NH_4^+ was comparable (Figure 1). The growth of maize plants at NO_3^- and NH_4^+ nutrition differed slightly in comparison with pea and sunflower plants. There was no difference in growth at the lower (1mM) concentrations of both
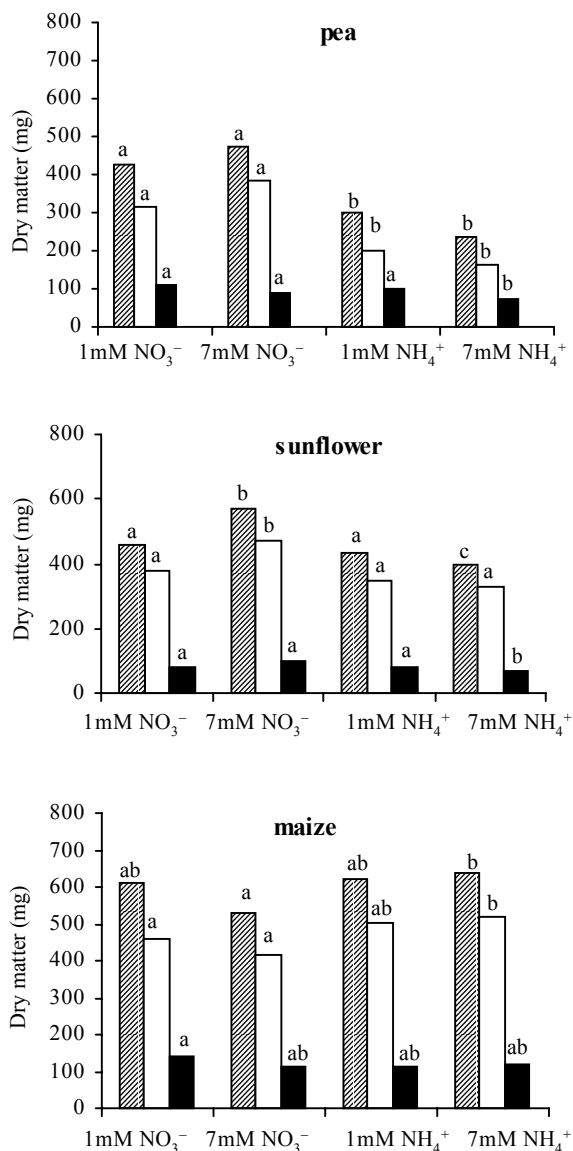

Figure 1. Dry matter of one plant of pea, sunflower and maize growing 10 days in water cultures with 1 and 7mM NO_3^- or NH_4^+ at pH 6

The values indexed with different letters (in comparison of single organs) are significantly different ($n = 10$)

x axis: N treatments
y axis: dry matter weight of one plant or plant organ
□ whole plant   ▨ aboveground part   ■ root

Table 1. Total nitrogen content in plant tissues of pea, sunflower and maize growing 10 days in water cultures with 1 and 7mM NO_3^- or NH_4^+ at pH 6

N treatment		Total N mg/g dry matter					
		pea		sunflower		maize	
		shoot	root	shoot	root	shoot	root
NO_3^-	1mM	38.6a	27.1b	38.5a	26.6b	37.1a	30.4b
	7mM	39.1a	32.0a	37.8a	32.2a	39.8a	33.9a
NH_4^+	1mM	41.0a	27.3b	41.6a	33.9a	39.1a	33.8a
	7mM	39.8a	33.1a	45.1b	41.1c	42.3ab	32.6ab

The values indexed with different letters are significantly different ($n = 3$)

Table 2. Nitrate and ammonium content in plant tissues of pea, sunflower and maize growing 10 days in water cultures with 1 and 7mM NO_3^- or NH_4^+ at pH 6

N treatment		μmol NO_3^- or NH_4^+/g dry matter			
		NO_3^-		NH_4^+	
		shoot	root	shoot	root
		Pea			
NO_3^-	1mM	6.9a	177a		
	7mM	81.7b	197b		
NH_4^+	1mM			48.2a	66.1a
	7mM			57.0b	189.0b
		Sunflower			
NO_3^-	1mM	357a	728a		
	7mM	841b	1946b		
NH_4^+	1mM			23.2a	57.1a
	7mM			32.1b	77.5b
		Maize			
NO_3^-	1mM	315a	652a		
	7mM	373b	1008b		
NH_4^+	1mM			25.9a	46.3a
	7mM			27.7a	44.0a

The values indexed with different letters are significantly different ($n = 3$)

N sources, the difference appeared at 7mM concentrations when NO_3^- nutrition significantly decreased and 7mM NH_4^+ nutrition significantly increased the growth of maize plants if only these treatments were compared. The growth changes were evident only in shoots (Figure 1).

Total N content had an increasing tendency with increasing N nutrition with some exceptions caused probably by differences in regulation and utilisation of the uptake of both N ions by experimental plants in both plant organs. The increase was mostly significant in the roots for both N sources (Table 1). The content of NO_3^- in plant organs increased markedly with NO_3^- concentration. The highest increase was observed in sunflower plants, less intensive in maize plants and the lowest in pea plants. The increase in all three crops was 2–3 times higher in the roots than in the aboveground parts. NH_4^+ content increased considerably less than NO_3^- content. The increase was marked only in pea and sunflower, but not in maize plants (Table 2).

Uptake relations between N ions and water

Large differences in water consumption between the experimental plants were determined. Pea plants consumed on average 3.3 times and sunflower 2.5 times high-

Table 3. Water consumption by pea, sunflower and maize plants growing 10 days in water cultures with 1 and 7mM NO_3^- or NH_4^+ at pH 6

N treatment		ml/g FM/day		
		pea	sunflower	maize
NO_3^-	1mM	33.7 ± 1.4a	24.6 ± 0.7a	9.45 ± 0.2a
	7mM	36.4 ± 1.6a	21.9 ± 0.6b	9.73 ± 0.2 a, b
NH_4^+	1mM	27.8 ± 1.2b	25.7 ± 0.8a, c	9.85 ± 0.2a, b
	7mM	35.2 ± 1.5a	28.5 ± 0.9c	10.7 ± 0.3b
Average ($n = 4$)		33.3 ± 3.3A	25.2 ± 2.38A	9.9 ± 0.55B

The values indexed with different letters are significantly different; (a, b) compared experimental variants of single plant species or (A, B) compared plant species ($n = 7–8$)

er amount of water per g of fresh matter production than maize plants (Table 3).

At low – 1mM N nutrition the amount of both N ions supplied to the roots by diffusion highly prevailed the amount related to consumed water. In maize plants the N amount related to transpiration mass flow was for NO_3^- approximately 16 times, for NH_4^+ 12 times, in sunflower plants for both ions 11 times, in pea 2–3 times lower than the supply related to diffusion. At 7mM N concentration N uptake in pea plants was totally supplied by transpiration mass flow, in sunflower plants the ratio of N supply related to transpiration mass flow reached 50% and in maize plants N supply through diffusion prevailed amounting to 70–80% (Figure 2). Very similar relations were estimated in both N ions. The differences determined between the experimental plants were connected with differences in the plant growth, namely with the amount of consumed water (Figures 2 and 3).

The values of total N uptake obtained in nonsterile water cultures are mostly influenced by N losses caused by microbial activities. Denitrification accounted for the highest percentage of nitrogen loss from these systems (Smart et al. 1998). Denitrifying bacteria have high carbon requirements and this can explain different colonization of rhizosphere in different plant species after their carbon availability. Nitrification processes at NH_4^+ nutrition together with NH_3^+ volatilisation from plant tissues can also cause N losses (Olsen et al. 1995 and others).
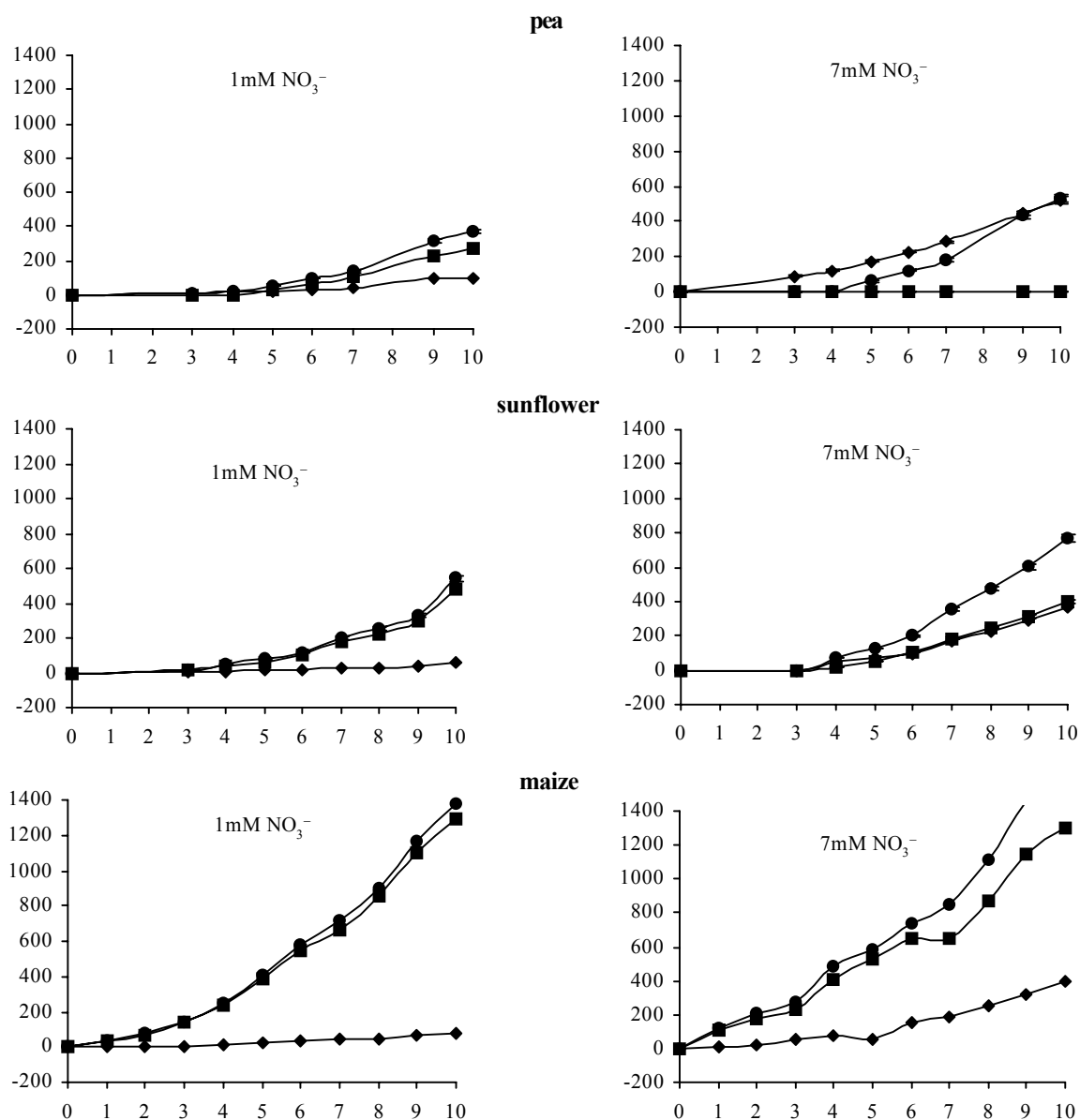

Figure 2. Time course of cumulative NO_3^--N uptake ●—● and supply to roots by diffusion ■—■ and transpiration mass flow ◆—◆ in nutrient solutions with 1 and 7mM NO_3^+ during 10-days growth of experimental plants

N concentrations in nutrient solutions were measured mostly every day in triplication with standard errors lower than 3% of the means

x axis: time in days, y axis: μmol N per plant

Different plant weight and different N amount available from seeds together with the above-mentioned nitrogen losses complicate the comparison of final nitrogen amount contained in standing biomass (Table 1) with total N uptake from the nutrient solution. However, all these factors did not influence the relations of N supply through diffusion and transpiration mass flow.

DISCUSSION

The uptake of water and mineral ions into root cells occurs separately by specialised permeases in the plasmalemma (Ruiz-Cristin and Briskin 1991, Johansson et al. 1998). It was also reflected in our results. When higher concentrations – 7mM NO_3^- or 7mM NH_4^+ were used, all N in intensively transpiring pea plants was supplied to the roots by means of transpiration mass flow and water uptake prevailed over N ion uptake at some time intervals (Figures 2 and 3). At low – 1mM concentrations the amounts of both N ions related to diffusion supply highly exceeded the supply related to transpiration mass flow in all experimental plants. According to Clement et al. (1978) at low N levels a high concentration gradient between the root surface and the nutrient solution is created by quick N ion transport through root cell membranes. They calculated that at 0.01 mg NO_3^-/l the concentration gradient through plasmalemma amounted to 70 000:1. In
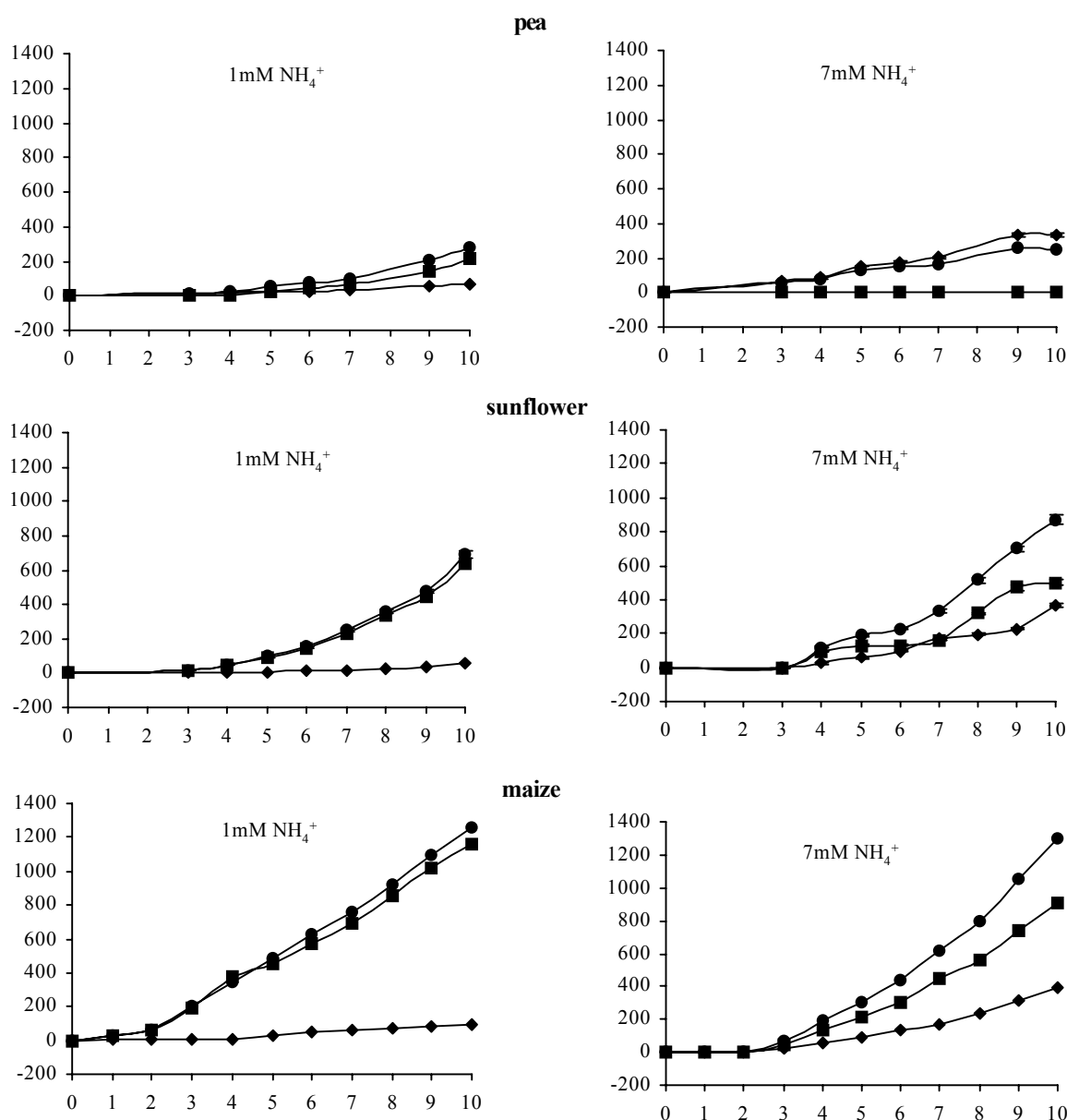

Figure 3. Time course of cumulative NH_4^+-N uptake ●—● and supply to roots by diffusion ■—■ and transpiration mass flow ◆—◆ in nutrient solutions with 1 and 7mM NH_4^+ during 10-days growth of experimental plants

N concentrations in nutrient solutions were measured mostly every day in triplication with standard errors lower than 3% of the means

x axis: time in days, y axis: μmol N per plant

water cultures with aeration the diffusion occurs namely in the space near the root surface. In soils the distance for diffusive nutrient movement is limited in the range of 0.1 to 15 mm from the roots and the main diffusion distance is 0.32 mm (Barber 1995).

Plant roots are exposed to diverse forms of nitrogen in the soil solution. In warm aerobic agricultural soils, NO_3^- and NH_4^+ are at millimolar concentrations. Analyses of NO_3^- for agricultural soils are reported with an average value of about 6–7mM (Reisenauer 1966, Wolt 1994). The supply of NH_4^+ can be considered as equivalent to them even though the soil solution NH_4^+ concentration can represent only about 10% of that held by absorption on anionic soil colloids (Barber 1995). In natural soils the concentrations of NO_3^- and NH_4^+ are mostly low and similar and vary over three orders of magnitude (Jackson and Caldwell 1993).

N concentrations used in our experiments were in the range of concentrations estimated in agricultural soils even though the concentrations in soils and also in sand and vermiculite cultures cannot be simply compared with those used in hydroponic cultures. Stöhr (1999) considered in sand cultures a 2mM nitrate level as insufficient and 10mM nitrate as optimal for the growth of tobacco plants. In our experiments 7mM NO_3^- concentration increased the growth of sunflower and pea plants only, and the same concentration of NH_4^+ (except maize plants) decreased the growth. A progressive increase in nitrate content (lowest in pea) and except maize also a moderate increase in ammonium content at 7mM NO_3^- or NH_4^+ concentration (Table 2) documented the mostly superoptimum N level in hydroponics but not in sand cultures (Plhák 1994, 1996).

Whenever mineral diffusion in soils is limited by insufficient soil water and low nutrient content, the supply of diluted minerals occurs mostly by transpiration mass flow causing N starvation. The increased elongation growth of roots at low nutrient and water content in soils is a common physiological reaction of plants. The increased number of root hairs and their length at water and nutrient deficit are documented as well (Barber 1995).

The sufficient nitrogen uptake seems to be very important in studies dealing with the effect of elevated CO_2 on photosynthesis, metabolism and growth of plants. Elevated CO_2 typically leads to a large increase of photosynthesis and biomass in nitrogen sufficient plants. In nitrogen limited plants the initial stimulation is followed by reversed inhibition called photosynthetic acclimation which is accompanied by starch and sugar allocation and by a decrease in nitrogen concentration in the plants (see overview in Stitt and Krapp 1999). In a study of 27 species, Porter et al. (1997) found the decrease in nitrates 22%, in total nitrogen 21%, and in leaf protein 18%. Photosynthetic acclimation is also accompanied at a strong N decrease by the decreased capacity for carboxylation (20–30% decrease of Rubisco activity, Geiger et al. 1999).

Photosynthetic changes at CO_2 elevation are followed by a partial closure of stomata, resulting in the lower transpiration water flow that could decrease the concentrations of soil-mobile nutrients including nitrates on the root surface (Conroy and Hocking 1993, Van Vuuren et al. 1997). For 41 observations covering 28 species, the average reduction of stomatal conductance was estimated to be about 20%. The reduction of evapotranspiration amounted to 17–22% in the C_3 plants including conifers and to 28–29% in the C_4 community, but some exceptions were also documented (Drake et al.1997, Pospíšilová and Čatský 1999). The decrease of nitrogenous compounds in plants growing at elevated CO_2 by about 20% correlates very well with a simultaneous decrease in evapotranspiration of approximately the same value.

Our results illustrate that N starvation can be expected in most cases in natural stands when water and N levels in soil are limited and N supply to the plant roots occurs predominantly by transpiration mass flow. N starvation can be intensified at elevated CO_2 while causing decreased stomatal diffusion. The results need not be accepted generally because there exists a diversity of plants with different strategies in mineral nutrition.

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ABSTRAKT

Přísun dusíku transpiračním hmotovým tokem může limitovat dusíkatou výživu rostlin

Rostliny hrachu, slunečnice a kukuřice byly pěstovány 10 dnů v 1mM a 7mM koncentraci NO_3^- a NH_4^+ ve vodní kultuře při stálém pH a okolní koncentraci CO_2. Byl sledován růst rostlin, obsah celkového N a obou N iontů v rostlinných tkáních, příjem vody a příjem obou N iontů a byl vypočítán jejich přísun k povrchu kořenů prostřednictvím transpiračního hmotového toku a difuze. Hrách a slunečnice preferovaly nitrátovou výživu, kukuřice využívala oba N ionty. Vyšší hladina N v živném roztoku zvyšovala s určitými výjimkami obsah celkového N a obou N iontů v rostlinných tkáních. Příjem obou N iontů přiváděných transpiračním hmotovým tokem byl závislý na intenzitě transpirace a na jejich koncentraci. Při 1mM koncentraci byl příjem obou N iontů prostřednictvím transpiračního hmotového toku malý a dosahoval u kukuřice až 16×, u slunečnice 11× a u hrachu 2–3× nižší hodnoty ve srovnání s příjmem N iontů prostřednictvím difuze. Při 7mM koncentraci příjem dosahoval u rostlin hrachu 100 %, u rostlin slunečnice kolem 50 % a u rostlin kukuřice 30 % celkového příjmu. Výsledky demonstrují přísun N živin ke kořenům při jejich různé hladině a mohou vysvětlit jejich snížený příjem vedoucí až k dusíkatému hladovění při nízké hladině. Snížení příjmu se může stupňovat při zvýšené koncentraci CO_2 vlivem snižující se evapotranspirace, což může být příčinou dusíkatého hladovění při tzv. aklimaci rostlin.

Klíčová slova: hrách; slunečnice; kukuřice; příjem NO_3^-, NH_4^+; transpirační hmotový tok; difuzní přísun; dusíkaté hladovění; aklimace rostlin

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