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ACCUMULATION OF N-NH_4 AND N-NO_3 IN PINE UNDER CONDITIONS OF NITROGEN STRESS

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Abstract: This paper discusses the results of a study on the impact of nitrogen stress on the accumulation of various N forms in young pines and in needles of mature pine trees. With the increase in N content in the growth environment (fertilization), a several fold increase in total N in plants was found while N-NH_4 increased tens of times as compared to the treatment without fertilization. At a decreased biomass yield by ca. 50 % the proportion of N-NH_4 in the total N pool fluctuated from 10 to 15 %, or even more. The share of N-NO_3 in the total N amount was several fold lower than that of N-NH_4. In needles of trees growing under the impact of N deposition, the observed share of N-NH_4 in the total N pool was at a level of ca. 3 %, but the total amount of soluble non-protein N compounds constituted ca. 26 %. In needles of trees under low N deposition only trace amounts of mineral N were detected. Thus it can be suggested that the ratio of total N to soluble non-protein N compounds as well as the total N to mineral N ratio may be used as indices of N stress for pine.

Keywords: Pine, N total, N-NH_4, N-NO_3

INTRODUCTION

The effect of N deposition on the forest environment has been dealt with by numerous authors (Gundersen, 1995; Schneider *et al.*, 1996; Bergmann *et al.*, 1999; Fitzhugh *et al.*, 2003; Augustin *et al.*, 2005; Herrmann *et al.*, 2005; Elvir *et al.*, 2006; Ladanai *et al.*, 2007; Sirulnik *et al.*, 2007; van der Salm *et al.*, 2007).

These studies concentrate on both direct and indirect effects of uncontrolled N deposition input to soils and plants. Atmospheric N is deposited mainly in the form of N-NH_4 and is either directly bound by plants or migrates to the soil. Bergmann *et al.* (1999) showed that the N influx enters the soil organic matter thus accelerating its mineralization. It was demonstrated that oxides (e.g. NO_2) from the deposition may be bound to the soil organic matter (Fitzhugh *et al.*, 2003). Salm *et al.* (2007) found increased concentrations of N compounds in surface waters which were ascribed to the increased leaching out of these compounds from the soil. Ladanai *et al.* (2007) also observed that the increase in the N input to pine and spruce stands resulted in the increased outflow of N from the soil.

The increased input of deposition was found to account for increased flux of N from the soils due to accelerated mineralization of litter and soil organic matter as well as for the intensification of N cycling in the forest environment (Gundersen, 1995; Fitzhugh *et al.*, 2003; Herrmann *et al.*, 2005; van der Salm *et al.*, 2007; Sirulnik *et al.*, 2007). Some of these authors paid special attention to the direct effect of N deposition on forest stands, which is held responsible for accelerated defoliation and, as a consequence, for deterioration of the tree. Accelerated defoliation was observed in numerous studies (Forest Condition in Europe 1998, 2000; The Condition of

Forests in Europe2006). However, direct relationships between the accelerated defoliation of forests and N deposition are difficult to establish therefore, more attention has been paid to the relationship between N supply to plants including that from atmospheric deposition and N transformations within the plant.

In the literature on the subject an ever increasing number of papers deal with the accumulation of non-protein N compounds in plants including trees (Gezelius & Näsholm, 1993; Schneider *et al.*, 1996; Geßler *et al.*, 1998b; Fotelli *et al.*, 2002; Collier *et al.*, 2003; Elvir *et al.*, 2006; Nahm *et al.*, 2006). To this group of such compounds belong mostly amino acids but also NH_4^+ and NO_3^- ions.

In our study, conducted in the seventies of the last century, it was found that, under conditions of high N contents in the growth environment (fertilization, deposition), significant amounts of N mineral were accumulating both in young pine trees and in needles of mature stands (Ostrowska, 1974). At that time however, the N-NH_4 accumulation in plants was not a subject of study in general. The increase in N deposition, including the increase in the N-NH_4 share in the deposition, has, to some extent, activated interest in solving the problem of accumulation of soluble non-protein N compounds including N-NH_4 and N-NO_3 in plants.

In the present study we have also returned to the problem of the dependence of N mineral accumulation in pine on plant growth conditions, taking into account the results of studies conducted over the last few years. The objective of the study was to evaluate the possibility of N fixation by pine under conditions of N excess in the growth environment.

MATERIALS AND METHODS

The experiment was conducted under green-house conditions using one-year-old pine seedlings. The plants (five per pot) were planted in pots filled in 17 kg with quartz sand or soil taken from the fresh conifer forest site. The following basic fertilization was applied, in mg/pot: P - 120 as KH_2PO_4, K – 360 as KH_2PO_4 + KCl, Mg – 100 as $MgSO_4$, Ca – 100 as $CaCO_3$ and microelements – 1.8L/pot as Swam nutrient solution (Steward, 1963).

The soil was taken from 0-30 cm layer, mixed thoroughly and put into pots together with the basic fertilization. Nitrogen in the form of $(NH_4)_2SO_4$ or NH_4NO_3 was mixed accurately with sand and soil prior to filling in the pots. Pine seeds were sown into the uniform substrate in the hotbed in spring a year before the experiment (using soil of 0-30 cm layer sampled from a fresh conifer forest and mixed). In the spring of the following year, fairly similar seedlings were taken for planting in the pots, five seedlings per pot. The pots were watered every day while maintaining the field moisture capacity at a level of ca. 10 % of the substrate weight. The experiment was conducted in four replications.

Following the ending of the growing season, plants were removed from pots and their roots were rinsed on sieves with deionized water. The fresh plant mass was then determined. Afterwards, two of the plants (immediately after removing them from pots) were homogenized (needles, shoots and roots separately) in 0.03 M solution of CH_3COOH (mass:volume 1:10). The homogenate was then centrifuged and the content of N-NH_4 and N-NO_3 was determined in the solution by the Bremner micromethod on alkalizing the solution with MgO roasted at 700 °C and on distilling N-NH_4 off to the boric acid solution. The nitrates remaining in the solution were then reduced with Dewarda alloy and N-NH_4 was distilled off as previously. In the three remaining plants the water content was determined (plants were dried up at 105 °C) and, subsequently, the dry biomass harvest from each of the pots. In the dry mass (needles, shoots and roots) the total N content was determined using Kjeldahl method. In parallel, under the same conditions, a kit of aminoacids (kat. Flug No 09425) was distilled and, separately, also Arginine, Glutamine and Leucine. It turned out that N from aminoacids did not migrate to the solution.

Current year needles were sampled from the third whorl of a tree crown of pine stands (about 60 year-old) growing on the fresh conifer forest site within the low and high N deposition impact sites (Kozienice Coal Burning Power Plant and Nitrogen Plant at Puławy) during autumn (October). At Puławy needles were taken from three stands (Puławy I, II, III) around the Nitrogen Plant. Needles sampled from each of the trees were divided into two parts: one was dried at 60 ^{o}C and ground while the other one was homogenated as previously described. Total N was determined in the dry material while in the fresh one – the mineral N content in the form of N-NH_4 and N-NO_3 was determined. In addition, the needles sampled in trees remaining within high N deposition were analyzed for non-protein soluble N compounds (SNN). After centrifuging, the homogenized material was divided into two parts: one was analyzed for N-NH_4 and N-NO_3 by direct distillation of the homogenate while the other was mineralized (H_2SO_4+H_2O_2) and then distilled. The amount of N determined by the direct distillation of homogenate was subtracted from the amount of N determined after the homogenate mineralization. The difference obtained corresponds to the amount of unidentified non-protein soluble N compounds.

RESULTS

The increase in N fertilization added both as $(NH_4)_2SO_4$ and as NH_4NO_3 caused, at the highest dose, about 50 % reduction of yield in plants grown on the soil and an almost complete dieback of plants grown on sand under conditions of model experiments (Table 1). The content of N in plants increased with the increase in fertilization dose regardless of whether the plants were grown on soil or on sand and irrespective of the form of the fertilization applied. The total N in needles increased from ca. 12 mg/g d.w. (no-fertilization treatment) to ca. 56 mg/g d.w. at the highest fertilization dose. N-NH_4 increased correspondingly, from ca. 0.22 to 27 mg/g d.w., while N-NO_3 rose from ca. 0.16 to 0.72 mg/g d.w.

The contents of the N forms examined were lower in shoots than in needles and the lowest in roots, and they also increased with the increase in the fertilization dose (Table 1).

The correlation coefficients between total N and mineral N forms in needles and shoots was 0.79-0.90, and in roots - 0.18-0.68. The strongest relationship was noted between total N and N-NH_4 in needles and shoots (0.90 and 0.88 respectively), a slightly less strong relationship – in roots (0.68), and a lack of correlation (correlation coefficient 0.18) was found between total N and N-NO_3 in roots.

The N-total/N-NH_4 ratios in plants grown on sand attained 56.4 in needles, 11.4 in shoots and 22.8 in roots in the treatment without fertilization whereas at an optimal fertilization in the form of $(NH_4)_2SO_4$, i.e. at the highest yield, the respective ratios were: 25.7 (needles), 15.3 (shoots) and 14.6 (roots). At the highest N load, on the other hand, the N-total/N-NH_4 ratios were: 1.9 in needles, 2.9 in shoots, and 5.2 in roots. The results obtained were similar when NH_4NO_3 was applied while there was no higher accumulation of N-NO_3 in the plants. Largely similar results were obtained in pine grown on soil, whereas these plants were found to contain less N-NO_3 than those grown on sand.

The total N in needles sampled from the mature pine stands growing in the fresh conifer forest site located in the neighbourhood of the Kozienice Power Plant fluctuated around ca. 1.5 % - a figure which probably reflects the relatively low N abundance in the forest site. Under such conditions the accumulation of N-NH_4 and N-NO_3 was very low (Table 2), therefore, the ratio total N to N-NH_4+N-NO_3 in needles was 260. No significant correlations were found between total N and mineral N (correlation coefficients lower than 0.2). The results were compared with those on the contents of various N forms found in needles of trees subject to the heavy impact of N load (Puławy I, II and III). N-total in needles around the Puławy Nitrogen Plant fluctuated in the range of 2.4-3.1 %, N-NH_4 – from 0.22 to 0.77 mg/g d.w., while N-NO_3 - from 0.05 to 0.33 mg/g d.w. Mineral N forms in needles remaining under the impact of N deposition are highly variable which likewise results in a wide fluctuation of total N to mineral N ratio (37-109).

Table 1. Accumulation of total N, N-NH_4 and N-NO_3 in pine under the progressive level of N fertilization

Doze of N	Crop	Needles			Shoots			Roots		
mg/pot	g/pot	N-NH_4	N-NO_3	total N	N-NH_4	N-NO_3	total N	N-NH_4	N-NO_3	total N
			mg/g d.w.			mg/g d.w.			mg/g d.w.	
$(NH_4)_2SO_4$ - sand culture										
0	17.0	0.220	0.165	12.4	0.570	0.060	6.5	0.250	0.180	5.7
200	36.5	0.494 ± 0.153	0.188 ± 0.056	12.7 ± 1.7	0.808 ± 0.094	0.228 ± 0.056	12.4 ± 4.0	0.635 ± 0.139	0.565 ± 0.113	9.3 ± 0.8
400	36.0	1.141 ± 0.154	0.209 ± 0.029	18.5 ± 1.6	1.023 ± 0.247	0.175 ± 0.006	12.7 ± 1.8	0.983 ± 0.130	0.440 ± 0.093	13.0 ± 1.3
800	16.0	3.954 ± 0.985	0.344 ± 0.242	32.5 ± 4.1	1.240 ± 0.318	0.253 ± 0.056	19.6 ± 2.0	2.065 ± 0.941	0.315 ± 0.138	16.8 ± 1.5
1600	6.0	27.013 ± 6.411	0.721 ± 0.231	51.7 ± 4.5	16.125 ± 5.052	0.579 ± 0.104	46.8 ± 8.7	4.633 ± 1.799	0.585 ± 0.084	23.9 ± 2.0
NH_4NO_3 - sand culture										
200	30.0	0.332 ± 0.047	0.173 ± 0.026	14.3 ± 0.8	0.584 ± 0.155	0.254 ± 0.053	10.3 ± 1.6	0.904 ± 0.209	0.441 ± 0.055	8.7 ± 0.9
800	17.0	4.118 ± 0.758	0.481 ± 0.167	35.4 ± 11.4	1.921 ± 0.672	0.391 ± 0.074	18.0 ± 3.0	1.914 ± 0.293	0.418 ± 0.032	16.4 ± 1.4
1600	5.0	27.857 ± 1.437	0.537 ± 0.042	56.2 ± 8.4	18.746 ± 6.304	0.579 ± 0.109	49.8 ± 15.7	4.141 ± 0.590	0.462 ± 0.095	19.2 ± 2.2
$(NH_4)_2SO_4$ - soil culture										
0	28.0	0.115	0.086	11.3	0.316	0.030	7.5	0.402	0.029	5.7
200	55.6	0.416 ± 0.121	0.116 ± 0.022	14.2 ± 1.6	0.353 ± 0.055	0.102 ± 0.028	11.9 ± 1.8	0.121 ± 0.025	0.087 ± 0.024	9.2 ± 0.7
400	56.7	0.738 ± 0.096	0.135 ± 0.013	18.3 ± 2.0	0.484 ± 0.184	0.099 ± 0.015	12.7 ± 1.8	0.207 ± 0.077	0.098 ± 0.013	13.0 ± 1.3
800	33.2	2.574 ± 0.993	0.183 ± 0.052	27.5 ± 4.4	0.595 ± 0.167	0.141 ± 0.041	18.8 ± 2.1	0.417 ± 0.121	0.135 ± 0.041	17.4 ± 0.6
1600	24.7	22.140 ± 6.039	0.341 ± 0.110	54.9 ± 15.6	20.363 ± 6.140	0.252 ± 0.180	36.4 ± 5.8	1.505 ± 0.260	0.171 ± 0.022	22.7 ± 2.2

Essential fertilization mg/pot: P as KH_2PO_4 – 120, K as KH_2PO_4+KCl – 360, Mg as $MgSO_4$ – 100, Ca as $CaCO_3$ – 100
Microelements: as Swam nutrient solution – 1.8 L/pot

However, relatively high correlation coefficients were found (0.5-0.8) between total N and mineral N contents (Table 3). SNN (soluble non-protein N compounds) in the needles was about eight times higher than the content of N-NH$_4$ and N-NO$_3$ and constituted ca. 26 % of total N. The correlation coefficient between SNN and total N was 0.80 (Table 3).

Table 2. Accumulation of total N, N-NH$_4$, N-NO$_3$ and soluble nonprotein N-compounds (SNN) in pine needles

Set of stands	N-NH$_4$	N-NO$_3$	total N	SNN
	mg/g d.w.			
Puławy I	0.773 ± 1.550	0.331 ± 0.486	30.95 ± 5.86	7.99 ± 5.25
Puławy II	0.724 ± 1.254	0.220 ± 0.136	26.9 ± 6.1	n.d.
Puławy III	0.219 ± 0.229	0.051 ± 0.037	23.9 ± 4.6	n.d.
Kozienice	0.040 ± 0.018	0.017 ± 0.016	14.8 ± 1.2	n.d.

Puławy I, II, III – stands under high N deposition; Kozienice – stand under low N deposition;
n.d. – not detected

Table 3. Correlation coefficient between N total, N-NH$_4$, N-NO$_3$ and soluble nonprotein N (SNN) for the Puławy and Kozienice Sites

Set of stand	Correlation coefficient	
Puławy I	total N - N-NH$_4$	0.487
n=38	total N - N-NO$_3$	0.556
	total N - (N-NH$_4$+N-NO$_3$)	0.512
	total N - SNN	0.802
Puławy II	total N - N-NH$_4$	0.674
n=44	total N - N-NO$_3$	0.561
	total N - (N-NH$_4$+N-NO$_3$)	0.686
Puławy III	total N - N-NH$_4$	0.804
n=10	total N - N-NO$_3$	0.596
	total N - (N-NH$_4$+N-NO$_3$)	0.812
Puławy I+II+III	total N - N-NH$_4$	0.553
n=92	total N - N-NO$_3$	0.512
	total N - (N-NH$_4$+N-NO$_3$)	0.567
Kozienice	total N - N-NH$_4$	0.120
n=29	total N - N-NO$_3$	0.153
	total N - (N-NH$_4$+N-NO$_3$)	0.147

DISCUSSION

Plants use N as both NH_4^+ and NO_3^-, depending on the current availability of these ions, which in the case of soil N is conditioned by biological processes in the soil. In the strongly acidic forest soils in Poland, mineral N occurs mainly in the form of N-NH$_4$ (Ostrowska, 1981). Rothstein & Cregg (2005) stated that shade-tolerant conifer species are generally thought to prefer NH_4^+ as a source of N. Availability of N-NH$_4$ in the soil is very low due to a soil pH > 5.5, which favours high nitrification rates (Fotelli *et al.*, 2000). In the experiments with pine presented in this paper, the substrate pH fluctuated around 7, but no increased utilisation of N was found from NH_4NO_3 as compared to that from $(NH_4)_2SO_4$.

In the N deposition, NH_4^+ ions dominate and may be easily taken up by plant leaves. The metabolic consequences of nitrogen influx via the leaves and its influence on the uptake of nitrogen by the roots is difficult to establish. Additional influx of N via the leaves may result in severe nutrient imbalances in forest stand exposed to atmospheric N and affect metabolic processes at the whole plant level (Rennenberg *et al.*, 1996). The disturbance of relationships between the elements in the pine needles exposed to atmospheric N was found by Ostrowska (1987; 1997). Collier *et al.* (2003) reported that NH_3 fumigation of the leaves of *Fagus silvatica* significantly increased the total cytokinins content of the roots, which can serve as shoot-to-root signal to regulate N uptake. Gezelius & Näsholm (1993) found that the accumulation of amino acids in young pines is conditioned by the N supply to plants. The shoot, needles and stem of low-N seedlings had a higher concentration of free amino acids and lower concentration of protein than those of high-N seedlings. Billow *et al.* (1994) stated that amino acid concentrations in foliage of Douglas-fir (*Pseudotsuga menziesii*) were significantly higher in fertilized trees than in control trees and the ratios of amino acid-N to total N were also significantly higher in fertilized trees. In both the deficient and excessive N supply to plants, some part of the absorbed N remains in the plant in the form of soluble non-protein nitrogen compounds (SNN). The content and dynamics of SNN in various species of trees was studied by Schneider *et al.*, (1996); Geßler *et al.*, (1998b); Weber *et al.*, (1998); Franco *et al.*, 2005; and Nahm *et al.*, 2006; 2007. In the total pool of soluble non-protein N compounds (TSNN), the amino acids, N-NH_4 and N-NO_3 were analysed. Geßler *et al.*, (1998b) found a seasonal maximum content of TSNN in fine roots of spruce (*Picea abies*) in July (32.7 µmol N/g f.wt.) and in the fine roots of beech (*Fagus silvatica*) in September (29 µmol N/g f. wt.). During the remaining months of the growing season these values were lower. In the total pool of TSNN, amino acids dominated, the N-NH_4 share fluctuated around ca. 7 %, while that of N-NO_3 was lower than 1 %. Fotelli *et al.*, (2000) reported that leaves of young beeches contained the highest levels of TSNN (4-7 □mol/g f.wt.) in May. Schneider *et al.*, (1996) determined that within current year's needles of spruce TSNN remained relatively constant during the entire vegetation period, but in the leaves of beech TSNN amounted to 5.6 µmol/g from April to July and increased to 9.5 µmol/g in September.

Nahm *et al.*, (2007) noted seasonal changes in various N forms in the leaves of beech in various stands. The maximum concentration of TSNN in leaves observed in May reached 32-36 µmol N/g f.wt, constituting ca. 0.28 % of N-total. The content of TSNN in leaves of *Araucaria angustifolia* was ca. 6 µmol/g f.wt., and in roots – ca. 10 µmol N/g f.wt., which, according to Franco *et al.*, (2005) corresponded to ca. 1-1.5 % of total N in foliage and ca. 2 % in roots. At the same time, in the TSNN pool of both the foliage and roots there dominated the amino acids while NH_4^+ and NO_3^- ions occurred only in trace amounts.

The results obtained in our study can not be used for direct comparison with the results obtained by other authors considering the various methods of extraction of SNN which were applied and, at the same time, the different study objective. This notwithstanding, in both our study and the works of other authors (Billow *et al.*, 1994; Gezelius & Näsholm, 1993; Weber *et al.*, 1998; Elvir *et al.*, 2006) it was stated that the increase in N level in the environment is accompanied by the increase in SNN in leaves and needles of tree species. In our study, the increase in N fertilization dose brought about a several fold increase in the total N content and many times higher increase in the N-NH_4 content in foliage of young pines which, in turn, was accompanied by a decrease in yields.

Schneider *et al.* (1996), Franco *et al.* (2005) and Nahm *et al.* (2006; 2007) found low levels of NH_4^+ and NO_3^- ions in the pool of soluble N compounds. Our results are similar for a low content of N in the growth environment of pine. At a high N level (fertilization), the quantity of N-NH_4 in the foliage of young plants took 10-15 % (and more) in the total N pool. In the needles of trees exposed to N deposition the share of N-NH$_4$ in the total N pool oscillated around ca. 3 %. The content of SNN in that foliage was ca. 9 mg/g d.w., i.e. about 26 % of total N. The share of N-NH$_4$ in TSNN was about 10 %, while N-NO$_3$ – about 4 %. Similar results were obtained in the spruce roots by Geßler *et al.* (1998b) despite the differences in the species examined and the methods applied.

Some authors (Fotelli *et al.*, 2002; Nahm *et al.*, 2006) are of the opinion that the accumulation of soluble non-protein N compounds may constitute a better indicator of N supply to plants than total N. In our study attention was paid to the relationships between total N and N-NH$_4$, N-NO$_3$ and SNN. With a sufficient N supply to pine trees no relationship was registered between total N and mineral N in the pine needles. On the other hand, a strong correlation (correlation coefficient > 0.8) was noted at the excess level of N in the environment. Thus, both the value of total N to TSNN ratio and the value of total N to mineral N (sum of N-NH$_4$ + N-NO$_3$) ratio in the foliage may be indicative of the N stress in tree species. In our experiments, the total N to mineral N ratio in pine needles ranged from between 30-50 for young foliage under conditions with no fertilization and between 2-10 at high doses of N fertilizer to about 260 in needles of pine stands growing on sites with a low N supply.

CONCLUSION

Pine under N stress conditions accumulates considerably larger quantities of N than pine growing under conditions of optimal N supply. Only trace levels of N-NH$_4$ and N-NO$_3$ were found in the total N pool in the foliage of mature pine stands at the low N level. Under conditions of high N deposition, N-NH$_4$ constituted ca. 3 % of N-total in the foliage while the content of TSNN constituted ca. 26 % of the total N pool. The share of N-NH$_4$ in the TSNN pool is about 10 %, while the share of N-NO$_3$ - about 4 %. Under conditions of N stress (fertilization), the content of N-NH$_4$ constituted 10-15 % (and more) of the total N pool in young pine plants.

Therefore we suggest that both the total N to TSNN ratio and the total N to mineral N (sum of N-NH$_4$ + N-NO$_3$) ratio in the foliage may be used as indices of N stress for pine.

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