Nitrate reduction in leaves and roots of young pedunculate oaks (Quercus robur) growing on different nitrate concentrations

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Received 26 February 1999; received in revised form 29 July 1999; accepted 31 July 1999

Abstract

Against the background of high rates of nitrogen (N) input into forest ecosystems and, in part, high nitrate (NO₃⁻) concentrations in the soil solutions, NO₃⁻ reduction activity and N accumulation in leaves and roots of young pedunculate oaks (Quercus robur) were investigated. Seedlings with unrestricted root growth, and 2-year-old saplings with cut root-stocks, were grown hydroponically at different forms and concentrations of N. The nitrate reductase activity (NRA) of the leaves and fine roots was measured in vivo without addition (NRAH₂O) or with addition of exogenous NO₃⁻ (NRAKNO₃) to the incubation assay. The amounts of reduced NO₃⁻ as calculated with the NRAH₂O and NRAKNO₃ were compared with the uptake of ¹⁵NO₃⁻, and with root-to-shoot translocation of NO₃⁻ as determined by NO₃⁻ concentrations of the xylem sap and transpiration rates. Compared with the NRAH₂O, the NRAKNO₃ was higher by a factor of approximately 10 in the current year's fine roots, and by a factor of about 60 in the leaves. In only one case did increased NO₃⁻ concentrations of the nutrient solution result in an increase in NRA. In some cases, NRA was diminished in the presence of ammonium (NH₄⁺) in the root medium. The quantities of reduced NO₃⁻ as calculated on the basis of NRAH₂O agreed with the amounts of ¹⁵N accumulated in roots and leaves, and with the amounts of NO₃⁻ translocated from the roots to the shoots. The contribution of the leaves to the total plant's NO₃⁻ reduction as computed on the basis of NRAH₂O was 1–17% in the seedlings, but up to 86% in the saplings; here, it correlated significantly with the leaf:root ratios on a fresh-weight basis. Compared with the leaf:root ratios, the form and concentration of the supplied N had a much lower impact on the share of the leaves in NO₃⁻ reduction; and did not affect the foliar N concentrations. In the leaves as well as in the roots, the concentrations of soluble NO₃⁻ were very low (< 0.5 mg NO₃⁻·N g dry weight⁻¹). The results show that young pedunculate oaks have a low affinity for NO₃⁻—N, even in the case of high NO₃⁻ supply. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Ammonium; Leaf; Leaf:root ratio; ¹⁵Nitrogen; Nitrate; Nitrate translocation; Nitrate reductase activity; Nitrogen; Quercus robur; Root; Xylem sap

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1. Introduction

Nearly all higher plants can use nitrate (NO$_3^-$) as a source of nitrogen (N), and the majority of these species is capable of reducing NO$_3^-$ in both roots and shoots (Runge, 1983). This is also true for many woody plants. The extent of NO$_3^-$ reduction in roots and shoots is, inter alia, dependent upon the N form (solely NO$_3^-$; or both NO$_3^-$ and ammonium (NH$_4^+$)), the amount of NO$_3^-$ supplied, and the amount of radiation (cf. Blevins, 1989; Peuke and Kaiser, 1996). In the presence of NH$_4^+$ in the root medium of trees, NO$_3^-$ reduction can be suppressed (Bigg and Daniel, 1978; Titus and Kang, 1982; Yandow and Klein, 1986; Pietiläinen and Lähdesmäki, 1988). With increasing NO$_3^-$ concentration of the root medium, the contribution of the shoot to total N assimilation often increases, which has been explained by a saturation of the NO$_3^-$ reduction process in the root (Titus and Kang, 1982; Gojon et al., 1991).

In stands of pedunculate oak (Quercus robur) in northwestern Germany, the elevated N input due to atmospheric deposition of NH$_4^+$ and NO$_3^-$ led to high NO$_3^-$ concentrations in the soil solution and to increased N concentrations in the leaves, but a substantial accumulation of NO$_3^-$ in the leaves was not observed (Thomas and Kiehne, 1995; Thomas and Büttner, 1998a,b). Although the oak is second only to the beech (Fagus sylvatica) as the most important deciduous forest tree in Central Europe, investigations on the effects of the form and the quantity of the supplied N on the extent and partitioning of NO$_3^-$ reduction under controlled conditions are scarce. Stadler et al. (1993) determined the distribution of NO$_3^-$ reduction in young pedunculate oaks. However, the study was conducted with exogenous NO$_3^-$ added to the in vivo nitrate reductase activity (NRA) assay. This is believed to indicate, at endogenous amounts of reductant, the potential capacity of the tissue to reduce nitrate rather than the actual reduction rates. The in situ activity is commonly considered to be most closely approximated by an assay without exogenous NO$_3^-$ (Andrews, 1986). The objectives of the present investigations were to test, in young pedunculate oaks under controlled conditions, whether an increase of the NO$_3^-$ supply in the root medium leads to (1) an increase of the NRA in roots or leaves, (2) an increase of the contribution of the leaves to the total plant’s NO$_3^-$ reduction, or (3) to NO$_3^-$ accumulation in the tissue, particularly at ample NO$_3^-$ supply. The finding of reaction (1) or (3) would indicate that young oak trees exhibit a high affinity to NO$_3^-$ in stands subject to elevated NO$_3^-$ supply caused, for example, by atmospheric N deposition. To assess possible effects of the leaf/root ratio on the partitioning of the NO$_3^-$ reduction within the plants, the study was performed on seedlings with unrestricted root growth, and with 2-year-old saplings whose rootstocks had been cut back at the beginning of the cultivation, resulting in a low production of fine root biomass during the first 2 months of the investigation. In the saplings, effects of the presence of NH$_4^+$ in the root medium on NRA were also studied.

In the temperate and boreal regions of the northern hemisphere, ectomycorrhizas play an important role in the supply of forest trees with water and nutrients, particularly with respect to phosphorus and nitrogen. This is also true for the species of the Fagaceae (for example, Marschner, 1995). In the case of supraoptimal N supply, however, the number of mycorrhizas, related to root dry matter, decreases distinctly, due to changes in the root system (Kottke, 1995). A complete fertilization of pedunculate oak seedlings resulted in a very low extent of mycorrhization, caused by a large reduction in the number of finest roots (Herrmann et al., 1992). In those plants, it is improbable that mycorrhizas exert a great influence on nutrient relations. Since the present study aimed to reveal possible changes in NO$_3^-$ metabolism under continuously high NO$_3^-$ supply, non-mycorrhizal plants were used.

The pedunculate oak belongs to the deciduous tree species with a rhythmic growth pattern. In the seedlings, the shoot exhibits an enhanced growth during about 2 weeks in spring and early summer, and, eventually, a small additional growth peak in late summer. Apart from these growth phases, the growth rate is only low (Hoffmann, 1972; Alatou et al., 1989; Thomas, 1991). To test the capability of the pedunculate oak for
NO₃⁻ uptake and NO₃⁻ reduction also in periods of relatively low shoot growth rates, the investigations were carried out between the growth phases. The measurements of NRA were performed in vivo with and without addition of NO₃⁻ to the assay. To confirm the results of the NRA measurements, they were compared with data obtained by ¹⁵N tracer studies, and by estimations of root-to-shoot allocation of NO₃⁻ on the basis of transpiration and xylem sap concentration.

2. Materials and methods

2.1. Plant cultivation

At the end of February, acorns of pedunculate oak (Q. robur L.; provenance forest district Duingerwald, growth district Weserbergland, Lower Saxony, northern Germany), derived from the Forest Seed Centre in Oerrel (Lower Saxony), were sown in quartz sand. In mid-May, when the shoots were about 15 cm high, five seedlings each were transferred into 12-l culture vessels, according to Ahr (cf. Baumeister and Ernst, 1978), which contained tap water. To prevent anaerobiosis, the water was constantly aerated. After a few days, the tap water was replaced with nutrient solution containing 1 mM NO₃⁻ as the N source. The combination of other nutrients was slightly modified after Thomas and Gehlen (1997). For the duration of the entire experiment, the nutrient solutions were replaced with fresh solutions at weekly intervals. The pH (5.2) was adjusted 3–4 days after replacement. The plants were cultivated in a greenhouse at 20°C during the day (6.00–20.00 h) and 15°C at night with 60–90% relative humidity. During daytime, additional light was given (Osram HQJE, 400 W) with a photon flux density of 120–200 µmol m⁻² s⁻¹ at the level of the central part of the shoot.

At the end of April, 2-year-old saplings of pedunculate oak, which had been obtained from a tree nursery (provenance lowlands of northwestern Germany), were taken into cultivation. The root-stocks were cut back by approximately one-third of their length. The preparation and conditioning of the plants for the culture in nutrient solution were then carried out as described previously (Thomas and Gehlen, 1997). At the end of May, when the buds began to open, the plants were supplied with nutrient solutions containing 1 mM N, provided in the form of either solely NO₃⁻ or 0.5 mM NH₄⁺ + 0.5 mM NO₃⁻. Light, temperature and relative humidity were the same as for the seedlings.

During June, the N concentrations of the nutrient solutions of seedlings and saplings were successively increased until the desired final concentrations were reached. In the case of the seedlings, these were 1, 2, 4 and 8 mM NO₃⁻, and in the saplings, 2, 4 and 8 mM N, given in the form of only NO₃⁻, or as equimolar NH₄⁺ + NO₃⁻. The concentrations of other macronutrients (except for P) were also increased to give constant ratios to N. For 1 day prior to the experiments, the plants were placed into tap water (NO₃⁻ concentration < 0.1 mM) to increase their NO₃⁻ uptake capacity and to rinse NO₃⁻ from the roots. At the beginning of the experiments, Mikropur (Katadyn, Wallisellen, Switzerland) was added to the solutions to suppress microbial activity.

2.2. N-labelling experiment

In early August and early September, six seedlings, which were between two growth phases of the shoot (leaf expansion had ceased, and new buds had not yet opened; Alatou et al., 1989), were selected from each NO₃⁻ treatment. These plants were placed into fresh nutrient solutions. In each three plants per NO₃⁻ treatment, NO₃⁻ was given, in the respective concentrations of 1, 2, 4 or 8 mM, in the form of Ca(¹⁵NO₃)₂ with 10.8 atom% ¹⁵N. The other three plants of each NO₃⁻ treatment were supplied with unlabelled NO₃⁻. After 3 days, the plants were harvested, and the fresh weight of leaves and fine roots (diameter ≤ 2 mm) was determined. The material was dried, pulverized and analyzed for total N and ¹⁵N with a C–N-analyzer (NA 1500; Carlo Erba, Rodano/Milan, Italy) coupled to an isotope mass spectrometer (MAT 251; Finnigan, Bremen, Germany). The N standard was acetanilide. From the differences in the ¹⁵N concentrations between labelled and unlabelled plants, the ¹⁵N accumulation for the experimental period was calculated.
2.3. Determination of NO$_3^-$ translocation from roots to shoot

In early August and early September, when the 2-year-old saplings had developed an appreciable mass of the current year’s fine roots, three plants, which were between two shoot growth phases (see earlier), were selected from each N-form treatment. The plants were placed into fresh solutions. On one of the two subsequent days, the transpiration of three leaves per plant was measured with a steady-state porometer (LI-1600; LI-COR, Lincoln, NE, USA). A previous study had shown that the gas exchange is relatively constant during the light period in pedunculate oak under greenhouse conditions (Thomas, 1991). All flushes were considered. After 2 days, the plants were harvested. The fresh weights of leaves and fine roots were measured. The leaves were subdivided into, at maximum, three size classes per plant. The fresh weight was determined from one representative leaf per size class, and the leaf area was measured with a Digital Image Analysing System (Delta-T Devices, Burwell, UK). From the relation of leaf area to fresh weight and the total leaf biomass, the total leaf area of the plant was calculated. The amount of water transpired during the 2-day experimental period was computed using the total leaf area and the transpiration rate.

After harvesting the leaves, the shoot was cut from the roots, and xylem sap was extracted from the shoot with a Scholander pressure chamber according to Berger et al. (1994). After the removal of the bark from the cut end of the shoot for a length of about 2.5 cm, a pressure of 0.2–0.3 MPa was applied for 10 min. The exuded xylem sap was collected with a pipette, and frozen at −18°C until analysis. From each plant, 0.1–0.5 ml xylem sap were collected.

The NO$_3^-$ concentrations of the xylem sap were measured with high-performance liquid chromatography (HPLC). After thawing, the samples were centrifuged (5000 × g; 10 min), filtered through Oasis HLB cartridges (Waters, Milford, MA, USA), and analyzed with UV-HPLC at 210 nm after separation with an anion exchange column filled with Partisil-10 SAX (Whatman, Maidstone, Kent, UK), according to Thayer and Huffaker (1980). The eluent was 30 mM KH$_2$PO$_4$ in distilled water (pH 3.0). From the NO$_3^-$ concentration of the xylem sap, the amount of NO$_3^-$ translocated from the roots to the shoot within 2 days was calculated by means of the measured transpiration rate, the duration of the daily light period (14 h), and the leaf area (see earlier).

2.4. Nitrate reductase activity

In the seedlings and saplings used for the $^{15}$N labelling or the NO$_3^-$ translocation experiments, the NRA of leaves and fine roots was measured according to Jaworski (1971). In addition to the saplings investigated in August and September, NRA was determined also in early July, when the saplings had developed only small biomasses of the current year’s fine roots, in plants treated in the same way as in the later investigation periods. In preliminary investigations, the NRA measurement had been optimized for the pedunculate oak with respect to the time course and the concentrations of buffer, propanol, NO$_3^-$ and H$^+$ in the assay. In these studies, it was also found that, under greenhouse conditions, NRA is relatively constant during the light period, and that the NRA of the lignified coarse roots is very low compared with fine roots. During the respective experimental periods, between 9.00 and 10.00 h Central European time, 100 mg of leaf material (pieces of ca. 3 mm$^2$ size from the intercostal area of the central leaf blade, which had been previously rinsed with deionized water) and 50 mg of the current year’s fine roots (pieces of ca. 2 mm length from the apical root sections without the root tip, after rinsing with deionized water) were taken from the plants. Until the start of the incubation, the samples were kept on ice and protected from light to prevent a premature onset of NO$_3^-$ reduction. They were infiltrated for 10 min under vacuum with 5 ml assay medium, consisting of 0.2 M KH$_2$PO$_4$ (pH 7.5) and 1.5% 1-propanol. The NR$_{A_{KNO_3}}$ (as a measure of NO$_3^-$ reduction capacity at non-limiting NO$_3^-$) was determined by adding 0.5 ml of 0.4 M KNO$_3$ to the assay; whereas, for the determination of the NR$_{A_{H_2O}}$ (as a measure of the actual NRA;
Keltjens and van Loenen, 1989) in parallel measurements, 0.5 ml deionized water was added instead. After vacuum infiltration, the samples were incubated at 30°C in the dark for 90 min. During that period, the NO$_2^-$ production was linear with time, as had been determined before. At 30 and 90 min after the start of the incubation, 1 ml assay was added to a mixture of 1 ml of 1% sulfanilamide in 3 M HCl, 1 ml aqueous 0.1% N-naphthylethylene diamine dihydrochloride, and 1 ml deionized water. After 20 min of incubation in the dark, absorbance was measured at 540 nm. The NRA (nmol NO$_2^-$ g fresh weight (FW)$^{-1}$ h$^{-1}$) was calculated from the difference between the values measured at 30 and 90 min. For leaves and fine roots of each plant, three parallel measurements of NRA$_{H_2O}$ and NRA$_{KNO_3}$ were performed. All flushes developed were considered. From the measured activities, the amounts of NO$_3^-$ reduced in the leaves and roots were calculated by means of the compartments’ biomasses and the length of the experimental periods. In the case of the leaves, only the duration of the daily light period (14 h) was considered, since NO$_3^-$ is assimilated only in light in the leaves (Riens and Heldt, 1992). For the roots, NO$_3^-$ reduction was computed on the basis of 24 h per day.

2.5. Nitrogen concentrations of the plants

In dried and powdered root and leaf material, the total N concentration was measured with a C–N-analyzer (NA 1500; Carlo Erba). All developed flushes were considered. After aqueous extraction (45°C; 1 h) and centrifugation (2500 × g; 15 min) of dried and powdered material, the concentrations of soluble NO$_3^-$ in leaves and roots were routinely determined photometrically after nitrification of salicylic acid (Cataldo et al., 1975). Under the selected conditions, the determination threshold was ca. 0.5 mg NO$_3^-$–N g dry weight (DW)$^{-1}$. In about 15% of the plants, the foliar NO$_3^-$ concentration was additionally analyzed photometrically by the more laborious, but also more sensitive, method of NO$_3^-$ reduction with hydrazine sulfate, and subsequent reaction of the generated NO$_2^-$ with N-naphthylethylene diamine dihydrochloride (Kamphake et al., 1967). Here, the determination threshold was about 0.02 mg NO$_3^-$–N g DW$^{-1}$.

2.6. Statistics

The results are given as means with standard errors. Comparisons of two different treatments were performed with the Mann–Whitney Ranked Sum Test (U-Test). Comparisons of more than two treatments were carried out with the non-parametric Ranked Sum Test after Nemenyi (Sachs, 1984). The significance of the correlation coefficients calculated from linear regressions was tested against the distribution of t-values. The significance level was 5% ($P < 0.05$).

3. Results

3.1. Growth and biomass

In early August, the seedlings grown on the highest NO$_3^-$ concentration had produced a significantly greater leaf mass than the plants of the other treatments (Fig. 1a). This resulted in a significantly increased ratio of leaf to fine root biomass (0.51 on a fresh-weight basis; as opposed to 0.25–0.28 in the other treatments). In early September, the leaf:root ratios were 0.24–0.27, and no significant differences in these ratios or in biomass production between the treatments were found.

In early July, the biomass of the current year’s fine roots of the 2-year-old saplings was still small (Fig. 2a). In August, the root biomass had increased, particularly in the saplings grown with both NH$_4^+$ and NO$_3^-$. Compared with the first investigation period, this led to diminished leaf/root ratios (Fig. 2b). In September, root biomass had distinctly decreased in saplings grown on higher NH$_4^+$ concentrations, compared with the treatment with 2 mM N; thus, the leaf/root ratios had increased. In NO$_3^-$-grown plants, the root biomass remained at a low level. Combined with an increased leaf biomass, this resulted in higher leaf/root ratios than in saplings grown on NH$_4^+$ + NO$_3^-$. In general, in the saplings, the leaf biomass was higher in plants grown on NH$_4^+$ +
Fig. 1. (a) Biomass of leaves and fine roots (diameter ≤ 2 mm) of pedunculate oak seedlings, grown on different NO$_3^-$ concentrations, in early August and early September. (b) Amounts of NO$_3^-$ reduced in these compartments during a 3-day period as calculated from the nitrate reductase activities measured without the addition of NO$_3^-$ to the assay (NRA$_{H_2O}$). For the leaves, all flushes were combined. NO$_3^-$ treatments marked with different letters differ significantly.

3.2. Nitrogen concentrations of the plants

The mean foliar N concentrations of the various treatments were 17.5–30.0 mg N g DW$^{-1}$ in the seedlings and 19.3–27.5 mg N g DW$^{-1}$ in the saplings. In none of the age classes did the foliar N concentrations significantly differ among the various N compositions of the nutrient solutions. In the saplings grown with NH$_4^+$ + NO$_3^-$, the foliar C/N ratios decreased, in tendency, with increasing N concentration of the substrate. This tendency was less distinct in plants grown with only NO$_3^-$, and absent in the seedlings (data not shown).

In the roots of the seedlings, the N concentrations were 16.8–25.6 mg N g DW$^{-1}$, and in those of the saplings, 18.5–43.0 mg N g DW$^{-1}$. The seedlings grown with the highest N supply exhibited the highest root N concentrations. In the saplings, an increase in root N concentrations with increasing N supply was only observed in the plants nourished with NH$_4^+$ + NO$_3^-$. Distinctly increased root N concentrations were only found in plants with reduced root biomass.

In all leaf and root samples analyzed after Cataldo et al. (1975), the NO$_3^-$ concentration was below the threshold of detection (< 0.5 mg NO$_3^-$ –N g DW$^{-1}$). The foliar NO$_3^-$ concentrations determined after Kamphake et al. (1967) were
between 0.03 and 0.07 mg NO$_3^-$–N g DW$^{-1}$. Thus, the fraction of soluble NO$_3^-$–N in total N of the leaves was negligible.

3.3. Nitrate reductase activity

Neither NRA$_{H_2O}$ nor NRA$_{KNO_3}$ differed among the leaves of different flushes; thus, a mean value was calculated for the entire leaf compartment of a single plant. In every case, NRA$_{KNO_3}$ was higher than NRA$_{H_2O}$ measured in the same plant compartment (Table 1). On average, NRA$_{KNO_3}$ was higher by a factor of about 10 in fine roots, and by a factor of ca. 60 in the leaves.

In the roots of the seedlings, NRA tended to increase with increasing NO$_3^-$ concentrations of the nutrient solution, but only for NRA$_{H_2O}$ in early August, differences were significant. In the leaves of the seedlings, NRA$_{KNO_3}$ decreased, in tendency, with increasing NO$_3^-$ concentrations.

### Table 1
<table>
<thead>
<tr>
<th>Plants, compartments, N form, N concentration</th>
<th>Early July</th>
<th>Early August</th>
<th>Early September</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NRA$_{H_2O}$</td>
<td>NRA$_{KNO_3}$</td>
<td>NRA$_{H_2O}$</td>
</tr>
<tr>
<td><strong>Seedlings, NO$_3^-$</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fine roots</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mM</td>
<td>n.d.</td>
<td>27 ± 10 (a)</td>
<td>298 ± 98</td>
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<tr>
<td>2 mM</td>
<td>n.d.</td>
<td>38 ± 11 (a)</td>
<td>321 ± 77</td>
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<tr>
<td>4 mM</td>
<td>n.d.</td>
<td>84 ± 11 (b)</td>
<td>382 ± 92</td>
</tr>
<tr>
<td>8 mM</td>
<td>n.d.</td>
<td>95 ± 14 (b)</td>
<td>480 ± 50</td>
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<tr>
<td>Leaves</td>
<td>n.d.</td>
<td>13 ± 5</td>
<td>821 ± 163</td>
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<tr>
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<td>9 ± 7</td>
<td>751 ± 218</td>
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<td>n.d.</td>
<td>13 ± 6</td>
<td>660 ± 91</td>
</tr>
<tr>
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<td>n.d.</td>
<td>12 ± 4</td>
<td>733 ± 137</td>
</tr>
<tr>
<td>8 mM</td>
<td>n.d.</td>
<td>12 ± 4</td>
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<tr>
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<td>21 ± 2</td>
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<tr>
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<td>28 ± 16</td>
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<tr>
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<tr>
<td><strong>Saplings, NO$_3^-$ + NH$_4^+$</strong></td>
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<tr>
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<td>2 ± 1*</td>
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<tr>
<td>4 mM</td>
<td>11 ± 6</td>
<td>353 ± 71</td>
<td>22 ± 21</td>
</tr>
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</table>

* n.d., Not determined; *, significantly lower in comparison with plants grown with the same NO$_3^-$ concentration (2 mM NO$_3^-$ versus 4 mM [NH$_4^+$ + NO$_3^-$], and 4 mM NO$_3^-$ versus 8 mM [NH$_4^+$ + NO$_3^-$]). N-concentration treatments marked with different letters (a) and (b) differ significantly.
Fig. 3. Amounts of $^{15}$N, accumulated in fine roots and leaves of pedunculate oak seedlings, resulting from the uptake of $^{15}$NO$_3^-$ from the nutrient solution during 3 days, plotted against the amounts of NO$_3^-$ reduced in the respective compartments during the same time as calculated from the NRAH$_2$O. The regression was calculated for both compartments. The broken line indicates a 1:1 relation between measured $^{15}$N accumulation and calculated NO$_3^-$ reduction.

However, differences were not significant (Table 1).

In the fine roots of the 2-year-old saplings grown on NO$_3^-$, no distinct responses of NRA to increasing NO$_3^-$ concentrations of the substrate were found. This was also true for the fine root NRAH$_2$O of saplings supplied with NH$_4^+$ + NO$_3^-$. In the first two investigation periods, the fine root NRAKNO$_3$ of these plants tended to increase with increasing N supply, but in September, a significant decrease with increasing N concentration was found. At this time, a significant decrease in NRAKNO$_3$ was also detected in the leaves of the saplings grown on NO$_3^-$, whereas this trend was insignificant in the leaves of the saplings supplied with NH$_4^+$ + NO$_3^-$. Generally, from August to September, a decrease in foliar leaf NRAKNO$_3$ was observed in seedlings and saplings grown on high N concentrations (Table 1).

In the comparisons of saplings grown on NH$_4^+$ + NO$_3^-$ with NO$_3^-$-grown plants, statistical tests were performed with plants supplied with the same concentrations of NO$_3^-$ (2 mM NO$_3^-$ versus 4 mM [NH$_4^+$ + NO$_3^-$]), and 4 mM NO$_3^-$ versus 8 mM [NH$_4^+$ + NO$_3^-$]). Generally, NRAKNO$_3$ was higher in both leaves and roots of saplings grown with NO$_3^-$ as the only N source. In August (fine roots, 2 mM NO$_3^-$) and in September, the differences were significant (Table 1). In contrast, NRAH$_2$O was hardly affected by the presence of NH$_4^+$ in the nutrient solution, and a significant difference between the N-form treatments was found in only one case.

3.4. Correlation between NO$_3^-$ reduction and $^{15}$N accumulation

For the seedlings, the amount of NO$_3^-$ reduced in the roots or leaves as calculated on the basis of NRAH$_2$O was plotted against the amount of $^{15}$N accumulated in the same compartments during the 3-day investigation period (Fig. 3). Since the foliar NO$_3^-$ reduction was rather low, the respective values were pooled with the data obtained from the roots in calculating the regression. The correlation between both parameters was significant. Deviations from the 1:1 ratio occurred, but both parameters were of the same magnitude. A significant correlation ($r = 0.76$; $P < 0.0001$) was also obtained when the $^{15}$N accumulation was plotted against the NO$_3^-$ reduction computed on the basis of NRA KNO$_3$. However, the amounts of NO$_3^-$ calculated this way were much higher than the corresponding amounts of accumulated $^{15}$N, due to the fact that NRA KNO$_3$ of leaves and roots was much higher than NRAH$_2$O of these compartments (cf. Table 1).

3.5. Correlation between NO$_3^-$ reduction and NO$_3^-$ translocated in the xylem

The average NO$_3^-$ concentration of the xylem sap obtained from the saplings was 96 ± 18 µM and did not exhibit distinct differences between plants grown on different forms and concentrations of N. The mean transpiration rate was $0.55 ± 0.07$ mmol H$_2$O m$^{-2}$ s$^{-1}$. Generally, no differences between the N treatments were found, but in September, the saplings grown on 8 mM (NO$_3^-$ + NH$_4^+$) showed diminished transpiration rates compared with all other treatments. The amount of NO$_3^-$ translocated from the roots to the shoot as computed from the NO$_3^-$ concentrations of the xylem sap, the transpiration rates, and the leaf area, correlated significantly with the amount of reduced NO$_3^-$, calculated on the basis
of NRA$_{H_2O}$ (Fig. 4). In the range of higher amounts of translocated and reduced NO$_3^-$, the calculated amounts of reduced NO$_3^-$ were lower than the corresponding amounts of translocated NO$_3^-$, but, as in the case of the $^{15}$N labelling experiment, the parameters were of the same magnitude. A significant correlation ($r = 0.58; P < 0.002$) was also obtained when the translocated NO$_3^-$ was plotted against the reduced NO$_3^-$ as computed on the basis of NRA$_{KNO_3}$. However, the values resulting from the calculation with the NRA$_{KNO_3}$ data were much higher than the corresponding amounts of translocated NO$_3^-$, since the NRA$_{KNO_3}$ in the leaves was much higher than NRA$_{H_2O}$ (cf. Table 1).

3.6. Reduction of NO$_3^-$ in leaves and roots

The quantities of NO$_3^-$ reduced in roots and leaves were calculated on the basis of NRA$_{H_2O}$. This could be done for three reasons. First, the amounts of soluble NO$_3^-$ accumulated in roots and leaves were negligible; thus, in the leaves of the seedlings, the $^{15}$N almost completely consisted of reduced N. Second, the quantities of reduced NO$_3^-$ as computed on the basis of NRA$_{H_2O}$ were in accordance with the magnitude of accumulated $^{15}$N in the case of the seedlings, and third, also in accordance with the amounts of NO$_3^-$ translocated from the roots to the shoots in the case of the saplings (Figs. 3 and 4).

In the seedlings, the roots contributed by far the largest fraction to NO$_3^-$ reduction (Fig. 1b). Under the assumption that the quantity of NO$_3^-$ reduction in the stem and in the petioles was negligible, the seedlings’ leaves contributed 4–14% to the total NO$_3^-$ reduction of the plant. Differences between the NO$_3^-$ treatments were not found. However, with regard to the NO$_3^-$ reduction per plant compartment determined on the basis of the NRA$_{H_2O}$ data from the experiment performed in August, the amount of NO$_3^-$ reduced in the roots was significantly higher in the 4 and 8 mM treatment, compared with the 1 and 2 mM treatment (Fig. 1b).

In comparison with the seedlings, much greater quantities of NO$_3^-$ were reduced in the leaves of the saplings. Here, the contribution of the leaves to the whole plant’s NO$_3^-$ reduction was particularly large in early July, when only small fine root biomasses had developed (Fig. 2a,c). It decreased from July to August; at this time, the biomasses of current year’s fine roots had distinctly increased. In September, more leaf biomass had been produced, and, in some plants, the generation of new fine roots could not compensate for the loss of fine roots produced in early summer. This resulted in higher leaf/root biomass ratios and, accordingly, a greater contribution of the leaves to NO$_3^-$ reduction (Fig. 2b,c). In the saplings, the ratio of leaves to fine roots on a fresh-weight basis correlated significantly with the contribution of the leaves to the NO$_3^-$ reduction of the total plant ($r = 0.43; P < 0.002$). Generally, the contribution of the leaves to the whole plant’s NO$_3^-$ reduction was larger in the saplings grown with NO$_3^-$ as the only N source; however, the differences to plants grown with NH$_4^+$ as NO$_3^-$ were insignificant in most cases.

A completely different pattern emerged when the contribution of the leaves to the capacity of the plant’s NO$_3^-$ reduction (on the basis of the NRA$_{KNO_3}$) was calculated. In the seedlings, the share of the leaves was about 20–50% (decreasing from August to September); and in the saplings, about 50–90%. In none of the age classes were

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**Fig. 4.** Amounts of NO$_3^-$ translocated from the roots to the shoot of 2-year-old saplings of pedunculate oak during 2 days as calculated from the NO$_3^-$ concentrations of the xylem sap and from the transpiration rates, plotted against the amounts of NO$_3^-$ reduced in the leaves during the same time as computed from the foliar NRA$_{H_2O}$. The broken line indicates a 1:1 relation between NO$_3^-$ translocation and NO$_3^-$ reduction.
differences in the foliar contributions among the N treatments found.

4. Discussion

4.1. Biomass, and nitrate reductase activity of roots and leaves

Like most terrestrial plants (Runge, 1983), the pedunculate oak produces the largest biomass when supplied with both N forms. The impairment of root growth in plants cultivated over prolonged periods with high NH$_4^+$ concentrations (Fig. 2a) was also found in a previous study on pedunculate oak (Thomas and Gehlen, 1997) as well as in an investigation on Douglas fir (Olsthoorn et al., 1991). This may be due to an increased demand of carbon skeletons for NH$_4^+$ assimilation, which results in reduced contents of carbohydrates in the roots (Marschner, 1995). However, detailed mechanisms of this NH$_4^+$-induced reaction are still unclear (Bloom, 1997). Ammonium-induced alterations of biomass production and metabolism may also be responsible for the distinct reduction in fine-root NRA and transpiration rates of saplings grown on high NH$_4^+$ concentrations and harvested in September (cf. Gerenda’s et al., 1997).

Compared with woody pioneer species which tend to have high leaf nitrate reductase activities (Smirnoff et al., 1984; Smirnoff and Stewart, 1985), late-successional species such as pedunculate oak generally exhibit low foliar NRA. The range of the foliar NRA$_{KNO_3}$ (0.2–0.8 μmol NO$_2^-$ g FW$^{-1}$) was approximately the same as determined in field-grown pedunculate oaks (Al Gharbi and Hipkin, 1984; Gebauer and Schulze, 1997). Root NRA$_{H_2O}$ was normally higher than leaf NRA$_{H_2O}$ (Table 1). In contrast, the NRA$_{KNO_3}$ per unit of fresh weight was generally higher in leaves than in roots; but, on a dry-weight basis, these differences would vanish or even reverse in most cases, since the water content of fine roots (ca. 90%) is considerably higher than that of leaves (about 50%). However, the ratios of NRA$_{KNO_3}$ to NRA$_{H_2O}$ in the respective compartments show that the leaves have the potential to increase their NO$_3^-$ reduction rates to a greater extent than the roots. These findings are in accordance with the minute concentrations of NO$_3^-$ present in the leaves: in the case of increased NO$_3^-$ fluxes from the roots to the leaves, the leaves are able to readily reduce the NO$_3^-$, thereby avoiding its accumulation. In adult peach-trees, it was recently shown that NO$_3^-$ supplied to the leaves through the xylem induces a noticeable level of in situ NRA, even in the absence of tissue-accumulated NO$_3^-$ (Bussi et al., 1997).

4.2. Correlations between nitrate reductase activity, and uptake and translocation of nitrate

The good correlations between the amounts of reduced NO$_3^-$ calculated on the basis of NRA$_{H_2O}$, on the one hand, and the amounts of accumulated $^{15}$N in roots and leaves (Fig. 3) or the amounts of NO$_3^-$ translocated from the roots to the shoot (Fig. 4), on the other, provide evidence that NRA$_{H_2O}$ also gives a much closer approximation to the in situ NO$_3^-$ reduction than NRA$_{KNO_3}$ in pedunculate oak. In contrast, NRA$_{KNO_3}$ is rather a measure of the capacity of NO$_3^-$ reduction. Therefore, a calculation of the portions of the different compartments to the plant’s total NO$_3^-$ reduction on the basis of foliar NRA$_{KNO_3}$ will lead, at least in this species, to an overestimation of the contribution of the leaves (cf. Gebauer and Schulze, 1997).

Since the foliar NRA$_{H_2O}$ was rather low, the slope of the regression in Fig. 3 is determined by root NRA$_{H_2O}$ more strongly than by leaf NRA$_{H_2O}$. The finding that, in the range of higher amounts of NO$_3^-$ reduced, the computation of NO$_3^-$ reduction on the basis of NRA$_{H_2O}$ yields larger results than the calculation on the basis of $^{15}$N, can be easily explained by the translocation of reduced N compounds from the roots to the shoot. Within this range, the computation of NO$_3^-$ reduction on the basis of $^{15}$N would lead to an underestimation of root NRA (cf. Fig. 3). For the whole range, however, the correlation between the $^{15}$N values and the NO$_3^-$ reduction was rather good. Although the plants were, during the experimental periods, in an intermediate state between two growth phases, the shoot could have acted as
a sink for N compounds reduced in the roots. To estimate the fraction of reduced N in the leaves, which was translocated from the roots to the shoot during the 3-day experimental period, the differences between foliar $^{15}\text{N}$ and foliar NRA$_{\text{H}_2\text{O}}$ were calculated. This was feasible since: (1) the amounts of NO$_3^-$ accumulated in the leaves were only very small; (2) the NO$_3^-$ flux via the phloem is negligible (Peuke and Kaiser, 1996); and (3) the net phloem export of reduced N from non-senescent leaves to roots during few days represents only a very small fraction of the NO$_3^-$ assimilated in the shoot, even when the leaves contribute a large part to the entire plant’s N assimilation (Gojon et al., 1991). According to the calculation, 51–86% of the foliar reduced N had been translocated from the roots to the shoots. This finding agrees well with results obtained from peach-tree seedlings grown on different NO$_3^-$ concentrations (50–78%; Gojon et al., 1991). The portion of foliar reduced N originating from the roots increased with increasing NO$_3^-$ concentration of the nutrient solution during the first, but not during the second, experimental period (data not shown); thus reflecting the increase in the amount of NO$_3^-$ reduced in the roots (Fig. 1b).

In the 2-year-old saplings, the finding that the amounts of NO$_3^-$ translocated from the roots to the leaves were somewhat higher than the corresponding amounts of NO$_3^-$ reduced in the leaves as calculated on the basis of the NRA$_{\text{H}_2\text{O}}$ (Fig. 4) could be due to a slow, but noticeable decrease of gas exchange rates from midday to evening under greenhouse conditions (Thomas, 1991). Thus, the computation of the NO$_3^-$ flux by means of the transpiration rates may have resulted in an overestimation. Nevertheless, the correlation between NRA$_{\text{H}_2\text{O}}$ and NO$_3^-$ flux is satisfying also in the saplings.

4.3. Effects of form and concentration of N on nitrate reduction

Increased NO$_3^-$ concentrations of the nutrient solution caused an increase of NRA in only one case (NRA$_{\text{H}_2\text{O}}$ in the roots of the seedlings at early August; Table 1). A stimulation of NRA$_{K\text{NO}_3}$ by increasing NO$_3^-$ concentrations was also absent in *Acer saccharum* (Rothstein et al., 1996) and, in the case of foliar application of NO$_3^-$-containing mist, in *Q. robur* (Pearson and Soares, 1995), but was found in other forest tree species (*Picea abies*; Peuke and Tischner, 1991; *Acer rubrum*, *Pinus strobus*, *Pinus rigida*; Downs et al., 1993); whereas in *Pinus sylvestris*, an increase of substrate NO$_3^-$ had opposite effects on NRA of roots and needles (Pietiläinen and Lähdesmäki, 1988). In spite of a lack in an increase of NRA, NO$_3^-$ accumulation was found neither in the roots nor in the leaves. The same observations have been made in seedlings of *Pinus sylvestris* (Flaig and Mohr, 1992) and those of *Prunus persica* (Gojon et al., 1991) grown on high NO$_3^-$ concentrations. A lack of NO$_3^-$ accumulation in the tissue can be expected for periods with enhanced growth due to an increased demand of reduced N, but the same finding in periods with low growth rates points to an effective control of NO$_3^-$ uptake in the pedunculate oak (cf. Imsande and Touraine 1994). Corresponding results were obtained in field and laboratory experiments with *Picea abies* and *F. sylvestrica*, which took up only minute amounts of NO$_3^-$ from solutions containing high NO$_3^-$ concentrations (Geßler et al., 1998).

An inhibitory effect of NH$_4^+$ in the substrate on root or leaf NRA as was found, in some cases, in our study (Table 1) is a common observation in conifers (Bigg and Daniel, 1978; Yandow and Klein, 1986; Pietiläinen and Lähdesmäki, 1988; Peuke and Tischner, 1991) and has also been detected in some deciduous trees (*e.g.* *Malus domestica*; Titus and Kang, 1982); but not in young plants of *Fraxinus excelsior* and *Q. robur* (Stadler et al., 1993), possibly due to a different experimental design (shorter investigation period, N starvation before N treatment).

The findings that, at undisturbed root growth, the roots contributed by far the biggest part to NO$_3^-$ reduction, and that, at restricted root growth, the leaf/fine-root ratios correlated significantly with the portions of the leaves in NO$_3^-$ reduction, demonstrate that the biomass distribution between root and shoot plays a decisive role in the relative contribution of these compartments to NO$_3^-$ assimilation. This may be also due to the
fact that the roots are not capable of compensating a reduced biomass by increasing their in situ NRA. Compared with the leaf/root ratio, the form and concentration of N had a much lower impact on the foliar contribution to NO$_3^-$ reduction. In a previous study on 2-year-old $F$. excelsior and $Q$. robur grown on lower N concentrations (3 mM), effects of the N form and NO$_3^-$ concentration on the NO$_3^-$ reduction capacities of roots and leaves were not found either (Stadler et al., 1993).

4.4. Affinity of young pedunculate oaks to NO$_3^-$

On the basis of the following findings, it can be concluded that young pedunculate oaks are not effective sinks for N in stands subjected to elevated NO$_3^-$ supply resulting from, for example, atmospheric N deposition: (1) increased NO$_3^-$ concentrations of the substrate stimulated the biomass production and the presumed in situ NO$_3^-$ reduction in only one case (seedlings in early August); (2) a distinct increase in root N concentrations was only found when the root biomass was relatively low; (3) the foliar N concentrations of the plants were unaffected; and (4) NO$_3^-$ did not accumulate in the tissues. The same conclusion was drawn for seedlings and adult trees of $A$. saccharum (Rothstein et al. 1996), and might be a general feature of climax species of temperate deciduous forests. Within stands, it is also improbable that mycorrhizas associated with the pedunculate oak can act as an effective sink for N. The development of the extraradical mycelium as well as the mycorrhization of the roots is negatively affected by supraoptimal N availability (Wallenda and Kottke, 1998). Mycorrhizas are able to accumulate N in their mantle hyphae (Kottke et al., 1995), but, under controlled conditions, the total amount of N accumulated in the plant was found to be the same in mycorrhizal and non-mycorrhizal seedlings at low or high N addition rate (Colpaert et al., 1996). Additionally, the affinity of the plants to soil NO$_3^-$ might be reduced at high rates of NH$_3$ or NH$_4^+$ deposition caused by atmospheric pollution. Especially in the northwestern parts of Central Europe, the deposition of reduced N compounds reaches high levels (cf. Lövblad and Erisman, 1992). Evidence was provided that uptake of atmospheric NH$_3$ by the shoot results in a reduction of NO$_3^-$ uptake from the root medium (Clement et al., 1997). Model calculations showed that the adverse effects of NH$_3$ uptake by the leaves on NO$_3^-$ uptake by the roots can be relevant in plants with low relative growth rates, like late-successional forest trees such as oak, which are subjected to high atmospheric NH$_3$ concentrations (Stulen et al., 1998).

Acknowledgements

We thank Prof. Dr Rudolf Tischner and Dipl.-Biol. Sylke Siebrecht, Albrecht-von-Haller-Institut für Pflanzenwissenschaften, Universität Göttingen, for their valuable support in the analysis of xylem sap. The help of Dr August Reineking and Reinhard Langel, Isotopenlaboratorium für biologische und medizinische Forschung, Universität Göttingen, in the measurement of $^{15}$N is greatly appreciated.

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