Contents, uptake rates and reduction of nitrate of *Rumex palustris* and *Plantago major* spp. *major* grown on compacted soil

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Summary

1. Net nitrate-uptake rates, nitrogen contents and nitrate-reduction rates of *Rumex palustris* and *Plantago major* spp. *major* plants grown on loose or compacted soil were studied.
2. Compaction lead to a slower growth rate for both species. Accumulation of nitrate caused increased internal nitrogen concentrations in *P. major* when grown on compacted soil.
3. After transfer to a nutrient solution plants of both species grown on compacted soil showed faster nitrate-uptake rates, when expressed per unit lateral-root length or lateral-root surface, than plants grown on loose soil. For *Plantago*, rates also increased when expressed per unit shoot or root dry weight.
4. Based on measured nitrate-reductase rates, only *R. palustris* should be able to reduce the largest part of the amount of nitrate taken up from the nitrate solution in the time period it was exposed to this solution.
5. Nitrate-reductase activities of *R. palustris* and the shoot of *P. major* correlated well with the internal nitrate concentrations. Roots of *P. major* showed no correlation at all. A possible explanation for this is compartment of nitrate and nitrate-reductase activities in these roots.
6. The accumulation of nitrate in roots of *P. major*, occurring on compacted soils, may be beneficial for maintaining the osmotic potential needed to penetrate soils with a high mechanical resistance. *Rumex palustris*, a species not occurring on compacted soils, does not show such an accumulation.

Key-words: Active nitrate uptake, compaction, niche difference, nitrate-reductase activity, osmotic potential


Introduction

Soil compaction can be caused by trampling or by fluctuating water levels. The contribution of large pores to the total-pore volume decreases (Coulon & Bruand 1989) and the bulk density and resistance of the soil to root penetration increase (Vomocil & Flocker 1961; Kamaruzaman 1988).

The growth of roots is impeded at large bulk densities (Veen & Boone 1990; Bengough & Mullins 1991) and is restricted to a smaller soil volume (Blom 1978; Engelaar, Jacobs & Blom 1993). Plant roots may respond to soil compaction in several ways. When the compaction is local they may concentrate at less compacted sites (Garcia, Cruse & Blackmer 1988). When a compacted area in the soil can not be evaded, many roots can extend themselves by alternating radial and longitudinal expansion (Hettiaratchi 1990). The osmotic potential of root cells must generate sufficient turgor to overcome the external pressures inhibiting growth. Sprent & Thomas (1984) argued that nitrate may possibly play an important role in the osmotic driving force for leaf expansion. Veen & Kleinendorst (1986) found a similar role for nitrate in the osmoregulation of Italian Ryegrass. Because the availability of nitrate is closely linked with the soil water flow (Habib & Lafolie 1991), nitrate will be readily available to roots even in compacted soils, as long as the water flow in the soil is not inhibited.

Final contents of nitrate in roots will depend on net uptake rates, reduction of nitrate and transport to the shoots. These processes are variable within different plant species and between individuals of one species. Nitrate could be important in regulating osmotic potentials of root cells and the penetrating abilities of the roots.
In this paper, compaction-induced changes in internal concentrations, nitrate-uptake rates and nitrate-reductase activities of two river foreland species, which grow on sites with different levels of soil compaction were compared. The ecological importance of these changes with respect to the occurrence of the species on compacted or non-compacted soils is evaluated. Species were chosen on the basis of their occurrence in the field: Rumex palustris Sm. from wet, untrampled sites (Blom et al. 1994) and Plantago major spp. major L. growing on relatively dry places, on and directly along heavily trampled paths (Haeck 1992).

Materials and methods

PREPARATIONS

Seeds of R. palustris and P. major were collected in the Rhine delta area, the Netherlands. They were germinated on moist filter paper in Petri dishes at a temperature of 10°C 12h/25°C 12h. Seedlings with two fully developed leaves were transferred to pots, each with a volume of 1-7 litres, filled with calcareous river sand. The soils of half the pots were compacted by hand after saturating the soil with water. Excess water was removed by leakage and evapotranspiration. The bulk densities of the uncompacted and compacted pots ranged from 1-02 to 1-18 g cm⁻³ and from 1-34 to 1-41 g cm⁻³ respectively. Total pore volumes were 55–61% and 46–49% for loose and compacted soils respectively. At the start of the experiment soils were moistened with tap water to 60% of their water holding capacity. In the uncompacted series 38–46% of the total soil volume and in the compacted series 22–28% was occupied by gas-filled pores. A lid was placed on all the pots, with a hole for the plants in the centre, confining the sand. The pots with plants were placed randomly in a greenhouse. Additional Photo-synthetically Active Radiation (PAR) of 150 μmol m⁻² s⁻¹ was given during a 16 h day period by sodium (Philips Son-T 400 W, Eindhoven, the Netherlands) and mercury lamps (Philips HG, Eindhoven, the Netherlands) with day and night temperatures of 21–24°C and 18°C respectively. Water losses owing to evapotranspiration were compensated for by adding nutrient solutions to a predetermined weight every day. This solution which contained 0.5 mM Mg²⁺, 3.0 mM K⁺, 2.0 mM Ca²⁺, 4.0 mM NO₃⁻, 0.5 mM H₂PO₄⁻, 1.75 mM SO₄²⁻ and traces of FeEDTA, Cl⁻, B³⁺, Mn²⁺, Cu²⁺ and Mo⁶⁺ was applied to the bottom of the pot by means of a syringe.

SAMPLING

Measurements started 9 weeks after potting. The aim was to measure plants of comparable size and age. Therefore, every day one plant, the largest at that time independent of age, was sampled 2-5 h after start of the day period. Plants and soil were removed carefully from the pots and the roots were rinsed with tap water for 3–5 min to remove all the soil. A preliminary experiment showed that this treatment did not significantly influence the nitrate-uptake rate by intact plants. Hereafter, the plants were transferred to the plant compartment of an uptake measurement system, consisting of two independent closed circuits. The first had a total internal volume of 600 ml and consisted of a plant root cuvette, an aeration cuvette and an electrode cuvette through which a nutrient solution was pumped, at a rate of 1800 ml min⁻¹. The second circuit contained a thermostatic water-bath (Haake G, Mess-Technik GmbH, Karlsruhe, Germany) keeping the temperature of the nutrient solution at 25°C. The nutrient solution consisted of 5 mM MES-buffer with the trace elements in the same concentration as during the growth period, while the concentration of the macro nutrients was decreased to one tenth of the concentration applied to the pots. At this nitrate concentration (400 μM), the uptake of nitrate is an active process (Glass et al. 1990) occurring at a saturated rate, Vₘₐₓ (Doddema & Telkamp 1979; De Willigen & Van Noordwijk 1987). The fact that uptake was an active process was checked by supplying KCN (final concentration 1 mM) to additional plants and comparing the uptake rates before and after this addition. The solution was brought to a pH of 5.6 with Tris-buffer. Net nitrate consumption by the plant was continuously measured with a nitrate-specific electrode (Philips, IS 561–NO₃–, Philips Scientific, Cambridge, UK) in combination with a reference electrode (Yokogawa SR20/AP24, Electrofact B.V., Amersfoort, the Netherlands), for a period of 120–160 min after transfer of the plants. The pH was monitored by a pH electrode (Hanna Instruments, H1911, Braunschwig, Amsterdam, the Netherlands) in combination with a Methrom 654 pH-meter. The nutrient solution and a Perspex cylinder placed over the shoot were flushed with moistened air preventing excessive evaporation. Four TL-lamps (Philips TLD 18W/84) provided a PAR of 100 μmol m⁻² s⁻¹.

Directly after the nitrate-uptake measurements nitrate-reductase activity in the roots and shoots of the plant were determined in duplicate or triplicate by a modification of the assay described by Jaworski (1971). For the shoot the youngest two or three fully developed leaves were cut into segments of 0.5×0.5 cm after removal of the veins. Between 100 and 250 mg fresh weight of these segments were put into 25 ml flasks wrapped with aluminium foil, containing 4 ml 0.25 M phosphate buffer (pH = 7.8) with chloramphenicol (0.5 mg ml⁻¹). After two 1 min periods of vacuum infiltration, 1 ml 0.2 M KNO₃ solution, containing 1-propanol (75 μl ml⁻¹) was added, and the flasks were closed with a rubber stopper. Samples of 0.4 ml were taken after 30 and 60 min incubation at 30°C in a shaker (60 rpm). NO₃⁻ accumulation was
Table 1. Free NH$_4^+$, NO$_3^-$, organic nitrogen and total nitrogen concentrations of shoots and roots (µmol g dry wt$^{-1}$) of Rumex palustris and Plantago major ssp. major plants grown on loose (L) or c compacted (C) soil. For each species and treatment three plants were combined into one sample.

<table>
<thead>
<tr>
<th></th>
<th>NH$_4^+$</th>
<th>NO$_3^-$</th>
<th>N organic</th>
<th>N total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R. palustris</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>shoots</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>16</td>
<td>34</td>
<td>1920</td>
<td>1970</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>19</td>
<td>1905</td>
<td>1934</td>
</tr>
<tr>
<td>lateral roots</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>8</td>
<td>53</td>
<td>1074</td>
<td>1132</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>80</td>
<td>1141</td>
<td>1231</td>
</tr>
<tr>
<td><strong>P. major</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>shoots</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>3</td>
<td>12</td>
<td>1234</td>
<td>1249</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>446</td>
<td>1775</td>
<td>2228</td>
</tr>
<tr>
<td>roots</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>1</td>
<td>53</td>
<td>771</td>
<td>825</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>536</td>
<td>850</td>
<td>1392</td>
</tr>
</tbody>
</table>

measured colorimetrically using a photospectrometer (Vitratron, Meyvis Co., Bergen op Zoom, the Netherlands). The same assay was used for 300–500 mg fresh weight of 1–0–1.5 cm long root segments but instead the flasks were flushed for 1 min with N$_2$-gas before each incubation period.

After determination of remaining lateral-root length (Comair rootscanner, Hawker de Havilland Victoria Ltd, Melbourne, Australia), lateral-root volume, tap-root fresh weight, lateral-root fresh weight and shoot fresh weight, the dry weight (24 h, 70 °C) of shoot, tap root and lateral roots were measured and lateral root surface area was calculated.

For each species and both treatments three additional plants were harvested and their nitrate-reductase activities were measured as described above. Hereafter, the three plants of one species and treatment were combined into one sample and the internal nutrient concentrations of these samples were determined according to Troelstra (1983).

**STATISTICAL ANALYSES**

Differences in plant-growth parameters, uptake rates and nitrate-reduction rates within one species between pots with either loose or compacted soil were analysed by Student’s t-tests. Growth parameters were log transformed and percentages arcsin transformed before analysis. Differences in growing period between the compacted and loose series of one species were tested with a Mann–Whitney U-test. Linear regression lines were calculated for the mean internal nitrate concentrations versus nitrate-reductase activities (Sokal & Rohlf 1981). All statistical analyses were made with the aid of the SAS statistical package (SAS Institute Inc, Cary, NC).

**Results**

Plantago plants on compacted soil produced less shoot dry weight than plants on loose soil (0.16–0.59 g in 97–103 days compared to 0.68–1.81 g in 64–81 days) and less lateral-root dry weight (0.08–0.30 g compared to 0.29–0.91 g). For R. palustris plants the growth period on compacted soil, compared to loose soil, was also significantly longer (87–97 days and 67–80 days, respectively) but final shoot dry weights (0.52–1.70 g and 0.90–1.61 g for compacted and loose soil, respectively) and lateral-root dry weights (0.13–0.47 g compared to 0.27–0.49 g) did not differ significantly. Tap-root dry weight of R. palustris decreased significantly when plants were grown on compacted soil: 0.16–0.73 g and 0.75–1.20 g for the compacted and the loose soil, respectively.

For R. palustris, N-concentrations of plants grown on loose or compacted soil did not differ (Table 1). For P. major, plants grown on compacted soil had a greater total N concentration than those grown on loose soil, which was the result of a bigger free NO$_3^-$ and organic N concentrations.

For R. palustris plants, soil compaction lead to a faster net uptake rate when expressed per unit lateral-root length or unit lateral-root surface area (Table 2). For P. major plants the uptake rates of plants grown on compacted soil were faster when expressed per unit shoot dry weight, lateral-root length, lateral-root dry weight or lateral-root surface area. Addition of KCN reduced the uptake rates to 0–5% of the initial.

**Table 2.** Mean nitrate uptake rates (µmol h$^{-1}$ ± 1 SEM) per plant, per g shoot dry weight (SDW), per m lateral-root length (LRL), per g lateral-root dry weight (LRDW) and per 1000 cm$^2$ lateral root surface area (LRSA) of Rumex palustris and Plantago major ssp. major plants in a nutrient solution after growing in loose (L) or compacted (C) soils. $n=4–5$

<table>
<thead>
<tr>
<th>Plant</th>
<th>SDW</th>
<th>LRL</th>
<th>LRDW</th>
<th>LRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R. palustris</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>24±5±3-6</td>
<td>20±0±3-2</td>
<td>0.35±0.04*</td>
<td>69.1±8.3</td>
</tr>
<tr>
<td>C</td>
<td>23±4±3-1</td>
<td>21±8±1-3</td>
<td>0.82±0.12</td>
<td>86.3±5.4</td>
</tr>
<tr>
<td><strong>P. major</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>24±2±3-3</td>
<td>20±1±2-0*</td>
<td>0.28±0.03*</td>
<td>49.3±7.0*</td>
</tr>
<tr>
<td>C</td>
<td>19±4±5-9</td>
<td>47±7±8-8</td>
<td>1.02±0.18</td>
<td>143.7±36.0</td>
</tr>
</tbody>
</table>

*P≤0.05.
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Discussion

Although the compaction caused a delay in growth, it is obvious from the nitrogen concentrations that the plants did not suffer from nitrogen deficiency (Table 1). Also for both species shoot/root ratio did not decrease as is often observed when plants become N-stressed (Hilbert 1990; Rufty, MacKown & Volk 1990).

The laterals of *R. palustris* grown on compacted soil had a lower specific root length (110-4 m of root per g dry weight ±12-0) than those grown on loose soil (200-9 ±10-8). This implies that these morphologically different roots were able to support the same amount of functional biomass with a less elongated system compared to plants grown on loose soil (Table 2). It was assumed that the tap root contributed far less to carbon or mineral nutrient acquisition, as it had a very small surface area and length compared to the lateral roots.

The measured uptake rates were the result of an active uptake process as may be concluded from the inhibition by KCN. The uptake rates remained constant with decreasing nitrate concentrations in the nutrient solution confirming that *V*<sub>max</sub> was measured at substrate concentrations well above the *K*<sub>m</sub> value. The fact that no changes in nitrate concentration of the nutrient solution were found after addition of KCN suggests no measurable nitrate efflux from or diffusion into the roots.

For *R. palustris*, efflux may have been prevented by the high percentage of nitrate reduction and nitrate transport to the shoot (Table 3) keeping internal nitrate concentrations relatively small. Indications for such a mechanism are the correlations in shoots and roots between nitrate-reductase activities and internal nitrate concentrations (Fig. 1), which were also found in other experiments for different *Rumex* species (Langelaan & Troelstra 1992). For *P. major*, the uptake rates seem even more independent from internal nitrate concentrations as the plants from compacted soil not only show the fastest uptake rates but also taken into account. There was a clear relation between the internal nitrate concentrations and nitrate-reductase activities for roots and shoots. In this figure plants that were not exposed to the nutrient solution were also taken into account. There was a clear relation between the internal nitrate concentrations and nitrate-reductase activities with the exception of *P. major* roots which showed no trend at all.

Table 3. Average nitrate reduction rates per plant (μmol h<sup>-1</sup>) of shoots (open symbols) and roots (closed symbols) of *Rumex palustris* and *Plantago major* ssp. major plants grown on loose or compacted soil versus their internal nitrate concentrations. Nitrate concentrations were determined on combined samples of three to five plants of the same species and treatment. Significant linear regressions between the two parameters are represented by the lines. *P* was 0.0004, 0.0005 and 0.022 for the shoots and roots of *R. palustris* and shoots of *P. major* respectively.

<table>
<thead>
<tr>
<th></th>
<th>N.R.A.</th>
<th>% roots</th>
<th>% shoots</th>
<th>% converted</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. palustris</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>18.8±1.13</td>
<td>11±1.7</td>
<td>89±1.7</td>
<td>82±8.4</td>
</tr>
<tr>
<td>C</td>
<td>20.3±3.79</td>
<td>12±1.9</td>
<td>88±1.9</td>
<td>78±2.2</td>
</tr>
<tr>
<td><em>P. major</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>5.34±1.34</td>
<td>57±10</td>
<td>43±10</td>
<td>26±6.5</td>
</tr>
<tr>
<td>C</td>
<td>1.69±0.51</td>
<td>53±5.9</td>
<td>47±5.9</td>
<td>9.3±2.0</td>
</tr>
</tbody>
</table>

Fig. 1. Nitrate-reductase activities (μmol NO<sub>3</sub>− g dry wt<sup>-1</sup> h<sup>-1</sup>) of shoots (open symbols) and roots (closed symbols) of *Rumex palustris* and *Plantago major* ssp. major plants grown on loose or compacted soil versus their internal nitrate concentrations. Nitrate concentrations were determined on combined samples of three to five plants of the same species and treatment. Significant linear regressions between the two parameters are represented by the lines. *P* was 0.0004, 0.0005 and 0.022 for the shoots and roots of *R. palustris* and shoots of *P. major* respectively.

rat., for both species. *Rumex palustris* shoots reduced the most of the nitrate taken up, based on the measured nitrate-reductase activities (Table 3). For *P. major*, the contributions of roots and shoots are approximately equal (Table 3). The relative contributions of roots and shoots, the total amount of nitrate being reduced and the percentage of nitrate being taken up which could have been reduced on basis of the nitrate-reductase activity measurements were not affected by growth on loose or compacted soil for either species. For *R. palustris*, about 80% of the net nitrate uptake could have been reduced with the measured nitrate-reductase activities; for *P. major* the activities only account for a minor part of the net nitrate uptake. Figure 1 gives the relation between the internal nitrate concentrations and nitrate-reductase activities for roots and shoots. In this figure plants that were not exposed to the nutrient solution were also taken into account. There was a clear relation between the internal nitrate concentrations and nitrate-reductase activities with the exception of *P. major* roots which showed no trend at all.
also the greatest internal concentrations (Tables 1 and 2). Compartment of the nitrate within the root as proposed by Siddiqi, Glass & Ruth (1991) could explain the independency of the nitrate-reductase activity of the internal nitrate concentration in the roots. A large part of the nitrate could be non-accessible to the root. Storage of nitrate in, for example, the vacuole would also reduce the efflux from the root.

Plantago major seedlings are very sensitive to a dry soil in their early growth stages (Blom 1976). Evapotranspiration dried the top soil in our pots quickly. In compacted soil with a greater penetration resistance, Plantago seedlings, which have a smaller root than R. palustris at the start, were probably not able to reach the deeper, wetter soil in a short time, which would explain their delay in growth.

Both species were able to increase their net nitrate-uptake rates in response to soil compaction, although the nitrate taken up was dealt with differently. Plasticity in uptake rates might be beneficial to the plant under circumstances in which the nitrate availability on the root-soil boundary is temporarily limiting (Kachi & Rorison 1990), resulting in depletion zones around the roots (Robinson 1991). The occurrence of P. major ssp. major is largely determined by its ability to grow in soils with a large mechanical resistance and its resistance to trampling by cattle or machinery (Haack 1992). The high nitrate content of the roots, resulting from a fast uptake rate in combination with a slow reduction rate, may be very important in maintaining the osmotic potential the cells need to expand.

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References


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