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Root porosities and radial oxygen losses of *Rumex* and *Plantago* species as influenced by soil pore diameter and soil aeration

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**SUMMARY**

The effects of hypoxia in ballotini and quartz sand with respectively large and small soil pore diameters on root porosity was studied for *Rumex palustris* Sm., *Rumex acetosa* L. and *Plantago major* L. ssp. major. Under hypoxic conditions *R. palustris* produced large root pores when large soil pores were present. Absence of such large soil pores resulted in the collapse of the root structure and a reduced root growth. Increases in intercellular spaces in *P. major* roots seemed to result in a higher root porosity upon hypoxia, a small soil pore diameter or a combination of both but this was not significant. Only a limited number of roots with large root pores was produced. The morphological structure of *P. major* roots without large root pores remained intact also in soils with a small soil pore diameter. *R. acetosa* only slightly increased its root porosity upon hypoxia and its morphological structure also remained intact when soil pore diameter was small. Radial oxygen loss (ROL) was found in *R. palustris* roots when grown in waterlogged, uncompacted soils. *P. major* had some roots with ROL in all treatments except in waterlogged, compacted soil. *R. acetosa* did not produce any roots showing ROL. Under field conditions aerenchyma is of no use in water saturated, compacted soils. This explains why a species like *R. palustris* only grows on hypoxic soil when it is not compacted, in contrast to *P. major* which will grow on moist, compacted soils. *R. acetosa* cannot be found on either very moist or heavily compacted soil, since it does not produce a healthy root system under these conditions.

Key words: Soil aeration, soil pore diameter, root porosities, radial oxygen loss.

**INTRODUCTION**

The root pattern of a plant strongly affects its possibilities to explore the surrounding substratum for water and nutrients (Fitter *et al.*, 1991). The pattern may differ between individuals of one species as a result of physical soil parameters, which hence influence the development of the entire plant (Engelaar, Jacobs & Blom, 1993). The root pattern itself is the result of topology, branching characteristics and elongation rates of individual roots (Fitter, 1987). Elongation of the roots is strongly affected by two physical soil parameters, oxygen availability and mechanical resistance (Tackett & Pearson, 1964), and their interaction (Bengough & Mullins, 1990).

Under field conditions these soil characteristics are not always favourable for root elongation. In river floodplains soils may be flooded regularly or irregularly (Voesenek, Blom & Pouwels, 1989), inhibiting gas exchange between the atmosphere and the soil pores (Ponnamperuma, 1984). As the oxygen diffusivity decreases and the remaining oxygen is consumed by plant roots and soil organisms the soil becomes anoxic (Armstrong, 1979). Many wetland species overcome these difficulties by the formation of aerenchymatous roots (Jackson & Drew, 1984; Armstrong *et al.*, 1991) often in combination with shoot elongation to restore its contact with the atmosphere (Voesenek *et al.*, 1992). These roots do not depend on external oxygen for their respiration, but on atmospheric oxygen that reaches the root tip via the aerenchyma (Armstrong, 1979; Laan *et al.*, 1990). Some of this oxygen may be lost to the rhizosphere by radial oxygen loss (Laan *et al.*, 1989). So, the amount of oxygen available to the root tip in anoxic soils depends on: the root porosity, the radial oxygen loss and the amount that is consumed in the more basal parts of the root (Armstrong & Beckett, 1987). Another problem roots may face in the river floodplains is a high mechanical resistance of the soil to penetration as a result of trampling (Liddle & Greig-Smith, 1975) or repeated dry/wet cycles.
(Hadas, 1990). When soil pore diameters are smaller than the root diameter, roots will experience difficulty in penetrating the soil. The force needed by the root to penetrate the soil increases with increasing resistance of the soil particles to displacement (Wiersum, 1957). Elongation of the roots is inhibited and the root expands radially (Mackie-Dawson, 1989; Bengough & Mullins, 1991). This radial expansion is believed to deform the soil in front of the tip so that penetration becomes possible (Abdalla & Hettiaratchi, 1969; Hettiaratchi, 1990). Therefore, the radial pressures the root can exert on the soil might be essential for its penetrating abilities.

We hypothesize that when soil hypoxia is combined with a large mechanical resistance, the large air pores in roots of some species will not withstand the pressures exposed on them as a result of the radial expansion of the root. The aerenchymatous structure may distort, resulting in a weak aeration of the root tip, a decreased radial oxygen loss and an even more inhibited root growth.

This paper describes the testing of this hypothesis by exposing roots of Rumex palustris Sm., Rumex acetosa L. and Plantago major L. ssp. major to hypoxia, a small confined pore diameter and a combination of both, under otherwise favourable conditions. Effects of hypoxia as the result of waterlogging and increased bulk density on radial oxygen loss from the roots of these species were tested by a pot experiment. R. palustris was chosen as a representative of species occurring on low places in the river floodplains where plants frequently have to face flooding and the accompanying hypoxic soil conditions (Voeselek et al., 1989; Blom et al., 1990). P. major is generally found on and along paths (Kutschera, 1960; Blom, 1976; Blom, Husson & Westhoff, 1979) where the soil is compacted and mechanical resistance to root penetration will be high in combination with a small soil pore diameter. R. acetosa was chosen as a control species as it occurs on those places in the river floodplains which are neither frequently flooded nor regularly trampled.

MATERIALS AND METHODS

Experimental design

Two separate experiments were performed. A water culture experiment was carried out to test the effects of soil hypoxia, a small soil pore diameter and the combination of both on elongation and porosity of individual roots. The effects of waterlogging, increased bulk density and the combination of both, on growth parameters and radial oxygen loss was studied by means of a pot experiment.

Plant material

For both experiments Rumex palustris, Rumex acetosa and Plantago major ssp. major seeds were collected from river floodplains near Nijmegen, The Netherlands. Seeds germinated on moist filter paper in petri dishes at a temperature of 10 °C, 12 h/25 °C, 12 h. Seedlings having two fully developed leaves were used at the start of the experiments.

Effects of small pore diameter and hypoxia on individual roots

The seedlings were placed in four water culture systems (Fig. 1). Each system existed of two major components: the root compartment, in which the plants grew, and the nutrient compartment, containing 251 of a modified Hoagland solution (Hoagland & Arnon, 1950). The solution was pumped from the nutrient compartment to the bottom of the root compartment, at a rate of 100 ml min⁻¹. It was recollected in the nutrient compartment by a drainage tube at the top of the root compartment. The nutrient solution, which was refreshed twice a week, contained: 20 mM NO₃⁻, 0.125 mM NH₄⁺, 1.5 mM K⁺, 1 mM Ca²⁺, 0.188 mM Mg²⁺, 0.25 mM PO₄³⁻ and 0.625 mM SO₄²⁻. Additional trace quantities of B, Mn, Zn, Cu, Mo and Fe-EDTA were added. In the nutrient compartment the solution was flushed with air. From day 8 onwards two of the four systems were flushed with N₂. Oxygen concentrations in the nutrient solutions were determined in samples from the rhizosphere by means of a Winkler titration (Drew & Robertson, 1974). The oxygen concentrations in the nutrient solution ranged from 5.71 mg l⁻¹ at the start to 0.61 mg l⁻¹ at the end and 5.39 to 6.86 for the nitrogen and air flushed situations, respectively. The root compartment was filled with either glass ballotini (diam 5-6 mm, SEM 0.18 mm) or compressed quartz sand (diam 0.32 mm, SEM 0.04 mm). Soil-pore diameters, calculated for the mean particle size, were 1.74 mm and 0.10 mm for the ballotini and the quartz sand respectively. The lid of the root compartment had holes for plants. In the case of the quartz sand the lid was secured by screws, confining the sand to a fixed volume. The four treatments therefore represented a factorial combination of aerobic and hypoxic solutions with large and small pores. Three plants of each species were used per treatment, giving nine per system in total. The systems were placed in growth chambers with a day/night regime 16/8 h, 25/20 °C. Relative humidity was in the range 40–70% and the PAR (400–700 nm) on plant level was 100–120 μmol m⁻² s⁻¹.

After 4 wk the shoots were separated from the roots. The roots were carefully removed from the substratum and rinsed with tap water. Per treatment and species one root system was photographed. Of each plant the three to five thickest roots were selected, and 0.5 cm long sections of these roots (0.5–1.0 cm behind the root tip) were fixed for 1 h
Figure 1. Waterculture system existing of a root compartment (A) filled with quartz sand, diam. 0.32 mm or glass ballotini, diam. 5-6 mm and a nutrient compartment (B) with: circular nutrient inlet (1), nutrient drainage (2), lid (3), nutrient pump (4), gas inlet for air or nitrogen gas (5) and one-way valve (6). In root compartments filled with sand the lid (3) was mounted tightly to the box by means of 6 rods in PVC-tubes (7), secured by a nut (8) on either side of the soil. The nutrient solution was pumped in the direction of the arrows.

Figure 2. System used for radial oxygen loss measurements existing of: 1, two glass plates, 2, inlet for N₂ and agar solution with leukomethylene blue (3), 4, N₂ inlet to solution stock, 5, bulk roots and 6 selected thick roots.
after vacuum infiltration with P-buffer (0·095 M) containing 1% glutaraldehyde. Fixation with glutaraldehyde gave the best reproducible results for our roots. Although some shrivelling of cortex cells may occur this was preferred over KMnO₄, which very likely leads to swelling of the cells. After rinsing twice with P-buffer and twice with demineralized water the sections were dehydrated in a series of increasing ethanol concentrations (30, 50, 70, 90, 100 and 100%, each step 10 min). After incubation with a Spurr (Spurr, 1969) embedding-resin/ethanol mixture (1:1, twice for 30 min) and twice in 100% Spurr resin (2 and 12 h), the sections were placed in Spurr which was allowed to polymerize for 24 h at 70°C. Cross-sectional couples of 100 μm thick were made with a glass-knife microtome (Ivan Sorvall Inc. Connecticut). Total-surface area and gas-space area of these coupes were measured on photographs, made through a dark field microscope, by means of an area metre (MOPsystem, Kontron, GMBH). Gas-space area was calculated as the fractional root porosity, i.e. the percentage of surface area of the cross section occupied by intercellular cavities and aerenchymatous spaces.

Effects of waterlogging and soil compaction on radial oxygen loss and growth parameters

For each species, twelve pots with volumes of 1·7 l were filled with calcareous river sand (FAO index: sand). In six pots bulk density was in the range 1·02-1·20 kg l⁻¹, and in the other six the sand was compacted to a bulk density of 1·35-1·52 kg l⁻¹. Total pore volume was 55-48% for the uncompacted and 41-35% for the compacted series respectively. At the start of the experiment the soils were moistened to 60% of the water holding capacity of the unconfined sand. Of the total pore volume 24-33% in the uncompacted series and 43-54% in the compacted series was occupied by water after filling the pots. A lid was placed on all pots, with a hole for the plants in the centre. For the compacted series the lid was mounted tightly to the pot with wire, confining the sand. After potting of the seedlings the pots were randomly placed in a greenhouse. Experimental conditions were: 8 h dark, 18°C and 16 h light, 21-24°C. A minimum PAR of 150 μmol m⁻² s⁻¹ (400-700 nm) was provided by sodium (Philips Son-T 400W) and mercury lamps (Philips HLRG). Water losses, due to evapotranspiration, were daily compensated for by adding nutrient solution to a predetermined weight. This solution was applied to the bottom of the pot with a syringe and contained: 0·5 mM Mg²⁺, 3·0 mM K⁺, 20 mM Ca²⁺, 40 mM NO₃⁻, 0·5 mM H₂PO₄⁻ and 1·75 mM SO₄²⁻. After 5 wk half the number of pots of both series were waterlogged by placing them in a container. Water temperature was 18-20°C. These pots received the same amount of nutrients as their drained counterparts. After three more weeks the plants were removed from the pots and, after rinsing with tap water, transferred to a glass plate (30 x 40 cm). The thickest roots (n = 3-4) were spread over the glass plate and their position was secured by small pieces of silicone tube. The rest of the roots was placed as a bulk just beneath and beside the shoot. A second plate was clamped on the first, separated by a silicone tube around the root system, thus creating a glass cuvette of 30 x 40 x 0·5 cm, with the shoot extruding at the top between the ends of the silicone tube (Fig. 2). This gap was closed with clay. The cuvette was flushed for 15 min with nitrogen gas through a union at the base of the second glass plate and a needle through the clay. It was then filled through the union with leucometethylene blue solution (30 mg l⁻¹) containing agar (1 g l⁻¹), titrated colourless with sodium dithionite solution. Blue coloration of the roots after 24 h was scaled from 1-5. A white root was given the value 1, a dark blue root the value 5. Radial oxygen loss from the thickest roots was monitored as blue coloration of the solution after 24 h. Afterwards shoot and root dry mass were measured and root length was determined using a Comair root scanner (Comair, Melbourne).

Statistical analyses

The results of the waterculture experiment were tested per species on main and interaction effects by means of a nested ANOVA procedure. Observations were nested per water-culture system. The parameters of the pot experiment were analyzed with a two-way ANOVA with compaction and waterlogging as main effects. Before testing growth parameters were log transformed and fractional root porosities were arcsin transformed (Sokal & Rohlf, 1981). When significant interaction effects were found a subsequent Tukey test procedure was used to interpret the main effects. All statistical analyses were made with the SAS statistical package (SAS Institute Inc. Cary NC).

RESULTS

Hypoxia in combination with large pores resulted in the formation of long, thick roots (Fig. 3). The proportion of these roots in the total root system was the largest for R. palustris followed by P. major and R. acetosa. Their contributions to the lateral root dry mass were 58% (SEM = 3·8), 37% (SEM = 6·4) and 10% (SEM = 2·3) respectively. Roots grown in quartz sand were shorter than roots grown in glass ballotini, especially in the two Rumex species. Thick roots produced in the two quartz sand treatments were more blunt-tipped and less elongated compared with those on the hypoxic, glass ballotini treatments (Fig. 3).
The fixation with glutaraldehyde caused some shrinkage, especially for both *Rumex* species. Maximal shrinkage, estimated by dividing the measured surface of a cell with its largest possible surface were 2, 7 and 11% in the ballotini/air treatment for *Plantago*, *R. acetosa* and *R. palustris* respectively. Cross-sectional coups show the aerenchyma formation by *R. palustris* in the hypoxic treatment with large pores and the total collapse of this structure when hypoxia was combined with a small confined pore diameter (Fig. 4b). The cell morphology and arrangement of both *P. major* and *R.*
Figure 4. Cross sections of the thickest roots of Plantago major (a), Rumex palustris (b) and Rumex acetosa (c) plants, grown on glass ballotini (diam. 5-6 mm) or quartz sand (diam. 0-32 mm) supplied with nutrient solution which was flushed with either air or nitrogen gas. (d) Root of Plantago major grown on glass ballotini supplied with nutrient solution flushed with nitrogen gas. Arrows indicate separations of cells resulting in large pores (indicated with *). Bar represents 250 μm in (a), (b) and (c).

Table 1. Mean root diameter 0.5–1.0 cm behind the root tip (μm), fractional root porosity (%) and largest cortical-cell diameter (μm), all ± 1 SEM, of the thickest roots of Plantago major, Rumex palustris and Rumex acetosa plants grown on glass ballotini (diam. 5-6 mm) or quartz sand (diam. 0-32 mm)

<table>
<thead>
<tr>
<th></th>
<th>Air 5-6 mm</th>
<th>Air 0.32 mm</th>
<th>N₂ 5-6 mm</th>
<th>N₂ 0.32 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root diameter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. major</td>
<td>266 ± 16c</td>
<td>556 ± 5a</td>
<td>334 ± 18bc</td>
<td>418 ± 43ab</td>
</tr>
<tr>
<td>R. palustris</td>
<td>274 ± 27b</td>
<td>454 ± 67ab</td>
<td>726 ± 77a</td>
<td>354 ± 44b</td>
</tr>
<tr>
<td>R. acetosa</td>
<td>132 ± 13c</td>
<td>174 ± 46bc</td>
<td>356 ± 23a</td>
<td>283 ± 33ab</td>
</tr>
<tr>
<td>Fractional root porosity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. major</td>
<td>3.1 ± 1.1a</td>
<td>6.2 ± 2.1a</td>
<td>6.7 ± 0.68a</td>
<td>5.7 ± 0.06a</td>
</tr>
<tr>
<td>R. palustris</td>
<td>5.3 ± 1.1b</td>
<td>4.5 ± 0.65b</td>
<td>25 ± 1.1a</td>
<td>2.6 ± 1.3b</td>
</tr>
<tr>
<td>R. acetosa</td>
<td>0.33 ± 0.12b</td>
<td>0.53 ± 0.43b</td>
<td>2.5 ± 0.18a</td>
<td>0.61 ± 0.35b</td>
</tr>
<tr>
<td>Cell diameter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. major</td>
<td>21 ± 1.0b</td>
<td>32 ± 2.1a</td>
<td>23 ± 1.0b</td>
<td>30 ± 1.2a</td>
</tr>
<tr>
<td>R. palustris</td>
<td>27 ± 1.8b</td>
<td>42 ± 1.6a</td>
<td>32 ± 0.8ab</td>
<td>31 ± 3.1b</td>
</tr>
<tr>
<td>R. acetosa</td>
<td>10 ± 1.6c</td>
<td>21 ± 2.3b</td>
<td>27 ± 2.0ab</td>
<td>31 ± 1.8a</td>
</tr>
</tbody>
</table>

Plants were supplied with nutrient solution which was flushed with either air or nitrogen gas. n = 3–6. Different letters indicate significant differences between treatments within one species (P ≤ 0.05).
acutosa roots seemed to be less affected by treatments (Fig. 4a, c). Some of the couples of Plantago in the hypoxic, glass ballotini treatment and the aerobic, quartz sand treatment showed an irregular separation of some cell walls, mainly in a radial direction, connecting the intercellular spaces, thus creating large pores (Fig. 4d).

With the exception of the fractional root porosity for P. major there was an interaction between pore diameter and aeration for fractional root porosity, root diameter and cortical cell diameter for all three species. Root diameter increased as a result of hypoxia for both Rumex species (Table 1). This effect was partially or totally lost when hypoxia was combined with a small soil pore diameter. For P. major a small soil pore diameter resulted in an increase in root diameter. This increase also became less when a small soil pore diameter was combined with hypoxia. In both Rumex species hypoxia in combination with a large soil pore diameter increased the fractional root porosity. In P. major the fractional root porosity was not significantly affected by any of the treatments. The diameter of the largest cortical cells increased for P. major and R. palustris as a result of small pore diameter, and for R. acetosa as a result of small pore diameter and hypoxia. The interaction of the main effects meant an even higher increase when hypoxia and small soil pore diameter were combined for R. acetosa, whereas for R. palustris and P. major a combination of treatments resulted in a weakening of effect of the small soil pores.

In the pot experiment, all three species showed significant decreases in dry mass and root length as a result of a high bulk density under drained conditions (Fig. 5). R. palustris and P. major showed no interactions of compaction and waterlogging for dry mass parameters or total root length. For R. palustris there was a significant negative compaction effect for all parameters (Fig. 5, SDM P = 0.005, LDM P = 0.006, TDM P = 0.008, RL P = 0.003). Growth of R. acetosa was significantly reduced by compaction (SDM P = 0.018, LDM P = 0.016, TDM P < 0.001, RL P = 0.002), but not by waterlogging. In this species, waterlogging had no effect possibly because of the significant or nearly significant

Table 2. Coloration of the selected thick roots (TR), bulk roots (BR), number of thick roots showing radial oxygen loss (RR) plus number of selected roots (SR) and mean individual root length of the selected roots (IRL, cm ± 1 SEM, n = 7-12) of Plantago major, Rumex palustris and Rumex acetosa plants

| Root coloration | Radial oxygen loss | | |
|-----------------|-------------------|---|---|---|
| | TR | BR | RR | SR | IRL |
| P. major | | | | | |
| DU | 3 | 1-2 | 2 | 11 | 190 ± 0.58a |
| DC | 4 | 2-3 | 1 | 10 | 103 ± 0.35b |
| WU | 3-4 | 2 | 2 | 7 | 190 ± 2.4a |
| WC | 2-3 | 1-2 | 0 | 8 | 185 ± 0.40a |
| R. palustris | | | | | |
| DU | 3 | 2-3 | 0 | 12 | 147 ± 0.69a |
| DC | 3 | 3-2 | 0 | 11 | 63 ± 0.35b |
| WU | 4-5 | 2-4 | 10 | 10 | 127 ± 0.35a |
| WC | 2-3 | 1-2 | 0 | 11 | 95 ± 2.7ab |
| R. acetosa | | | | | |
| DU | 1-2 | 1-2 | 0 | 10 | 217 ± 1.7a |
| DC | 1-2 | 1 | 0 | 8 | 57 ± 0.35c |
| WU | 2-3 | 1-2 | 0 | 7 | 87 ± 0.87b |
| WC | 1-2 | 1-2 | 0 | 8 | 47 ± 0.35c |

Plants were grown on drained uncompacted (DU), drained compacted (DC), waterlogged uncompacted (WU) and waterlogged compacted (WC) soil. Coloration is indicated by a figure of a scale, 1 meaning no blue coloration and 5 very dark coloration. Different letters indicate significant differences between treatments within species (P ≤ 0.05).
interaction between the waterlogging and compaction treatment. *P*-values for interaction effects were 0.07, 0.01 and 0.07 for shoot-dry mass, tap-root dry mass and total-root length respectively. For *P. major*, both waterlogging and compaction had negative effects on the shoots (*P* = 0.043 and 0.010 respectively).

The thickest roots from the plants were nearly always darker blue in colour after treatment with leucomethylene blue than the bulk roots which consisted of both thin and thick roots (Table 2). The colour of the thickest roots of *R. palustris* and *P. major* was affected by the treatment. Roots of *R. palustris* grown in waterlogged, uncompacted soil and roots of *P. major* grown on drained, compacted soil or waterlogged, uncompacted soil were darker than those grown on drained, uncompacted soil. Dark coloration was inhibited when the soil was waterlogged and compacted. *R. acetosa* roots only reacted with a weak coloration upon waterlogging. Radial oxygen loss was observed over the entire length of all investigated *R. palustris* roots grown on uncompacted waterlogged soil (Table 2). Occasionally when a root was accidentally folded during the preparations no ROL was observed from the damaged site down to the root tip. Radial oxygen loss was also observed in some roots of *P. major* grown on drained uncompacted, drained compacted and waterlogged uncompacted soil. None of the *R. acetosa* roots showed any ROL. The individual root length of *R. acetosa* was negatively affected by all treatments compared with the drained, uncompacted situation (Table 2). The length of individual *R. palustris* roots decreased only upon compaction. The combination of waterlogging and compaction resulted in a wide range of root lengths. The individual root length of *P. major* was only negatively affected by a high bulk density in a drained soil. All species showed an interaction effect between the compaction and waterlogging treatments for the individual root length.

**DISCUSSION**

The root systems as presented in Figure 3 can be explained as the result of aeration and penetrating capacities of the individual roots. As a result of hypoxia all three species produced new roots with an increased porosity, although not significantly for *P. major*. Gas diffusion from the atmosphere through the shoot to the root tip, e.g. oxygen, and in opposite direction, e.g. ethylene, is facilitated by such an increase. An increased porosity is known to promote the extension capacity of a root in a hypoxic surrounding (Laan et al., 1990, Armstrong & Beckett, 1987). The large increase in FRP of *R. palustris* was the result of the formation of shizogenous aerenchyma (Fig. 4b). *P. major* roots did not produce aerenchyma, with some individual exceptions as illustrated by Figure 4d. It is not clear why the reaction of individual roots on a plant was so different. However, there is another report on 'aerenchyma formation' for roots of *Plantago lanceolata* (Blaquiere, 1988), a species which was believed not to produce aerenchyma. The relatively small root system of *R. acetosa* under hypoxic conditions reflects its poor ability to produce new roots with an increased FRP (Fig. 3, Table 1). For both *Rumex* species the largest increase in root diameter was caused by hypoxia, for *P. major* it was due to a small soil pore diameter. The increase in root diameter is correlated with the increase in cortical-cell diameter (Table 1). Radial expansion of the roots in combination with a decreased longitudinal growth in response to a small soil pore diameter is well known (e.g. Moss, Hall & Jackson, 1988). From their results it is clear that the radial expansion starts not directly at the root tip but very near to it, as would be necessary for the soil-penetration mechanism proposed by Abdalla and Hettiaratchi (1969). In the roots shown by Moss et al. (1988), the external morphology behind the root tip looks damaged. This corresponds well with our results, especially for *R. palustris* where the root structure virtually collapsed when hypoxia and a small soil pore diameter were combined (Fig. 4b). In contrast, *P. major* roots grown under hypoxic conditions in quartz sand did not possess large pores. As a result its internal structure did not collapse and the individual root length decreased least of all species (Figs 3, 4a) when grown in hypoxic quartz sand.

The radial oxygen loss of roots from plants grown in the pot experiments reflected the internal structure of the roots very well. The only situation in which ROL was found for all roots was the waterlogged, uncompacted series of *R. palustris*. Assuming that an increased bulk density implies a rearrangement of the soil particles in a more dense way and thus a smaller mean soil pore diameter, the growth conditions of individual roots of this series can be compared with the *N₂*, glass ballotini series of the waterculture experiment, in which the roots produced aerenchymatous tissue. In the waterlogged, compacted series, which can then be compared with the *N₂*, quartz sand treatment, no ROL was observed, leading to the conclusion that here too the formation of aerenchyma was either inhibited or the aerenchyma collapsed as a result of radial expansion behind the root tip. The fact that in a few roots of *P. major* ROL was found in three different treatments strengthens the assumption that under certain conditions large pores may exist also in *P. major* roots. It is not quite clear which treatment triggers this phenomenon since it was also observed in the uncompacted, drained situation. The fact that for *P. major* and *R. palustris* the thickest roots always were darker coloured than the bulk roots emphasizes the
plasticity in the FRP of an individual plant. For *R. acetosa* this morphological plasticity was smaller, as may be concluded from Table 1 in combination with Table 2. In contrast to the results of the waterculture experiments where the combination of hypoxia and a small soil pore diameter had the greatest influence on growth of individual roots (Fig. 3), in the pot experiments this was achieved by an increased bulk density. This difference can be explained by the low moisture content of the top soil in the pots rather than by a difference in mechanical resistance between the two experiments. Due to evaporation the top soil was rather dry and the compaction delayed root penetration to moist soil. This is also reflected in the other growth parameters (Fig. 5). The interaction effect that was found for the individual root length of all species in the pot experiment should be interpreted as a smaller compaction effect when the soil was waterlogged compared with when the soil was drained (Table 2). All measured roots were long enough to reach the deeper moist soil layers. Therefore, for this parameter a partial relief of the mechanical pressure by the high water content in the waterlogged, compacted soils probably was of importance. *P. major* was much more affected by compaction in the pot experiments compared with the water-culture experiment as a result of a low moisture content during the early growth stage. The species is known to be very sensitive to the moisture conditions during establishment (Blom, 1976).

Combining the two experiments we may conclude that a small soil pore diameter indeed inhibits arechyma formation under hypoxic conditions for *R. palustris*, resulting in a poor aeration of the root tip and a decreased root extension and ROL. The higher fractional root porosity as a result of larger intercellular spaces in *P. major*, seems a less vulnerable solution for aerating the root in conditions of high mechanical resistance. Possible arechyma formation by this species was absent when the soil was hypoxic in combination with a high mechanical resistance. *R. acetosa* only increased its FRP slightly upon hypoxia. As a result of this the root did not collapse in the combination of hypoxia and a small, confined soil pore diameter. The results explain at least partially why *R. palustris* only occurs on wet sites which are not submitted to regular trampling or another treatment resulting in compaction. Arechyma, its adaptation to hypoxic soil conditions, is of no benefit in hypoxic, compacted soils. The results for *P. major* ssp. *major* indicate that in the established phase this species could occur on both drained and waterlogged soils independent of their bulk density. Therefore, the fact that it does not occur on low situated, wet places in the river floodplains has to be explained by restrictions during other life stages (Kuiper & Bos, 1992). Finally, *R. acetosa* cannot maintain a healthy root system when the soil is either hypoxic or compacted, explaining why in the field this species can only be found on raised, well drained, seldomly flooded places, which remain relatively undisturbed and uncompacted.

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