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Water supply and not nitrate concentration determines primary root growth in *Arabidopsis*

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ABSTRACT

Understanding how root system architecture (RSA) adapts to changing nitrogen and water availability is important for improving acquisition. A sand rhizotron system was developed to study RSA in a porous substrate under tightly regulated nutrient supply. The RSA of *Arabidopsis* seedlings under differing nitrate (NO_3^-) and water supplies in agar and sand was described. The hydraulic conductivity of the root environment was manipulated by using altered sand particle size and matric potentials. Ion-selective microelectrodes were used to quantify NO_3^- at the surface of growing primary roots in sands of different particle sizes. Differences in RSA were observed between seedlings grown on agar and sand, and the influence of NO_3^- (0.1–10.0 mM) and water on RSA was determined. Primary root length (PRL) was a function of water flux and independent of NO_3^-. The percentage of roots with laterals correlated with water flux, whereas NO_3^- supply was important for basal root (BR) growth. In agar and sand, the NO_3^- activities at the root surface were higher than those supplied in the nutrient solution. The sand rhizotron system is a useful tool for the study of RSA, providing a porous growth environment that can be used to simulate the effects of hydraulic conductivity on growth.

Key-words: *Arabidopsis* root; hydraulic conductivity; matric potential; nitrate; sand; water potential.

INTRODUCTION

The supply of nitrogen (N) and water to plant roots is affected by spatial and temporal heterogeneity in soil, and this is complicated by microbially mediated cycling between the different chemical forms of N (Ellis, Dendooven & Goulding 1996; Miller *et al*. 2007). For many plants, nitrate (NO_3^-) is the primary source of N because it is more mobile in solution than ammonium and often found at higher concentrations. The root is the main organ for nutrient acquisition, and understanding how root growth adapts to changing NO_3^- and water availability is important for improving nutrient acquisition (reviewed by Miller & Cramer 2005; Kant, Bi & Rothstein 2011). Root growth has a high degree of plasticity that is partly regulated by water and N supply, and this is sometimes described as a foraging response to seek nutrients within the growth environment (reviewed by De Kroon *et al*. 2009). Such adaptations can be quantified by measuring the root system architecture (RSA), which is defined by changes in the growth rate and the spatial distribution of the root system (Malamy 2005).

In soil, the delivery of water to the root is largely driven by water potential differences within the root–soil system. These differences in water potential for a given hydraulic conductivity of the soil and transpirational demand from the shoots determine the flux of water that can be supported through the plant–soil (Sperry *et al*. 1998; Cramer, Hawkins & Verboom 2009). Water potential (Ψ_t) is the sum of the osmotic potential of water (Ψ_o) caused by dissolved solutes and the matric potential (Ψ_m) caused by the capillary pressure of water held between substrate particles. The relationships between soil hydraulic conductivity (its conductance to water) and saturation are established, and robust empirical relationships exist between soil water content, matric potential and hydraulic conductivity (van Genuchten 1980).

The effect of soil hydraulic conductivity on root development has long been recognized (Passioura 1991), but it has received little attention. In particular, the direct influence of hydraulic conductivity of the substrate on RSA has been neglected. The effects of water stress on plant growth have been studied using model systems such as an osmoticum (PEG) or equilibrated vermiculite (Verslues, Ober & Sharp 1998), where plant growth is related to the water potentials of the growth environment and typically the effects of water potential. It has been shown that primary root length (PRL) is stimulated at small negative water potentials, although the rate of PR growth decreases with increasing water stress (Sharp, Silk & Hsiao 1988; Wiegers, Cheer & Silk 2009). In addition, the formation of lateral roots (LRs) increased with water supply on agar plates (Deak & Malamy 2005), and LRs are known to proliferate in wetter soil zones (Greacen & Oh 1972).

NO_3^- supply influences individual RSA characteristics differently. PRL has been shown not to be sensitive to NO_3^- concentration (Zhang & Forde 1998; Linkohr *et al*. 2002), although PR growth of at least one *Arabidopsis* accession (No-0) has been shown to be sensitive to low NO_3^-

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concentrations (Walch-Liu & Forde 2008). In contrast, LR development and elongation are stimulated by local NO_3^- application in numerous plant species (Zhang & Forde 1998; Zhang *et al*. 1999; Casimiro *et al*. 2003; Visser *et al*. 2008), and this result was first demonstrated in sand culture (Drew & Saker 1975). *Arabidopsis* vertical agar plate experiments showed an increased LR density in response to local high-NO_3^- patches (Zhang & Forde 1998; Remans *et al*. 2006a,b). Conversely, seedlings growing on uniformly high-NO_3^- supply exhibit suppressed LR development (Zhang *et al*. 1999). Much less is known about other root parameters, such as basal root (BR) growth, in response to both NO_3^- and water supply.

The RSA responses of the model plant *Arabidopsis* to water and NO_3^- availability tend to be studied using agar and hydroponic culture methods (Zhang & Forde 1998; Casson & Lindsey 2003; Little *et al*. 2005; Orsel *et al*. 2006; Remans *et al*. 2006a; Chopin *et al*. 2007). However, the root growth environment of these laboratory techniques is very different from that encountered by a growing root in the field. Physical parameters, such as Ψ_t and hydraulic conductivity, facilitate the delivery of N and water to the root and may be limiting in the field; therefore, becoming important regulators of RSA. When different root growth systems are compared, they have often given very different results (Hargreaves, Gregory & Bengough 2009; Wojciechowski *et al*. 2009).

In this paper, we describe the RSA of *Arabidopsis thaliana* (Ws) wild-type seedlings subjected to different NO_3^- and water supplies. Significant differences in RSA were measured under different water and NO_3^- treatments, and between seedlings grown on agar and sand. In combination with RSA measurements in these different growth substrates, we quantified the NO_3^- activity at the surface of a growing root in order to determine whether PRL was more sensitive to water or NO_3^- supply.

MATERIALS AND METHODS

Experimental approach

Experiments were conducted to study root architectural responses to altered N and water supply in a sand rhizotron system. The RSA was compared for *Arabidopsis* seedlings growing in sand and agar culture systems (see below for details) under different N and water supplies. The concentration of NO_3^- supplied was changed and seedlings were grown in sands with different particle sizes and at different water matric potentials. The NO_3^- available at the surface of primary roots grown in sands of different particle sizes was quantified using ion-selective microelectrodes. For all experiments, the same nutrient solution was used (as described in Orsel *et al*. 2006) with final concentrations of each component: 0.5 mM $CaSO_4$, 0.5 mM $MgCl_2$, 1 mM KH_2PO_4, 10 μM $MnSO_4{\cdot}7H_2O$, 24 μM H_3BO_3, 3 μM $ZnSO_4{\cdot}7H_2O$, 0.9 μM $CuSO_4{\cdot}5H_2O$, 0.04 μM $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 72 μM Fe sequestrene. For the different NO_3^- supply experiments, KNO_3 was added at final concentrations of 0.1, 1 and 10 mM. The K^+ concentration was kept constant by the addition of K_2SO_4 to the 0.1 and 1 mM KNO_3 solutions. For all experiments, final volume was made up using distilled H_2O, adjusted to pH 5.7 and buffered with 1 mM MES.

Agar culture

Arabidopsis seeds were grown on agar Petri dishes as described previously (Zhang & Forde 1998). The method was modified as agar was added to the nutrient solution at a final strength of 2%. This concentration was higher than that used previously by some authors, because lower percentages failed to give full agar setting with this nutrient solution. Square plates (120 × 120 mm) were set at an angle of 60°, and four seedlings per plate were grown under the same environmental conditions as described for all the experiments. The plants were orientated such that only the roots were in contact with the agar.

Sand rhizotron system

Acid-washed Redhill T sand (Sibelco UK Ltd, Sandbach, UK) was used for each sand experiment. To set up the experimental system (Fig. 1), a 5 L filter funnel (20 cm diameter) was vacuum-saturated with nutrient solution in order to support a defined water tension. Rhizotrons were constructed using modified clear jewel CD cases (142 × 124 × 10 mm) and positioned on a layer of sand to enable a continuous connection of solution from the aspirator to the seedling. Orientation was at an angle of 60° to encourage the roots to grow near to the surface for easy access. This orientation was found from trial experiments, at steeper angles roots grew along the sand/plastic interface (data not shown). Each experimental unit was filled with sand and saturated with nutrient solution before four seedlings were transferred to the growth media on the exposed surface. The system was covered with opaque sheeting, apart from the exposed seedlings that were covered with a transparent polythene sheet, to minimize evaporative losses and algal growth. Different particle sizes were obtained from Redhill T sand by sieving into the following fractions: <250, 250–425 and >425 μm. Matric potential was adjusted by altering the difference in height between the sand culture and the level of the nutrient solution reservoir in the aspirator bottle (see Fig. 1). We used three matric potentials of –1.5, –3.0 and –4.5 kPa (indicated as arrows in Supporting Information Fig. S1a).

Physical characterization of growth substrates

The water potential (Ψ_t) of sand samples was determined using a WP4 Dewpoint PoteniaMeter (Decagon Devices, Inc, Pullman, WA, USA). The water release characteristics for the sands, which is the relationship between matric potential and water content, were determined using the burette method (Marshall, Holmes & Rose 1996). Saturated hydraulic conductivity K_{sat} was measured using the constant head permeability test (Marshall *et al*. 1996). Water release

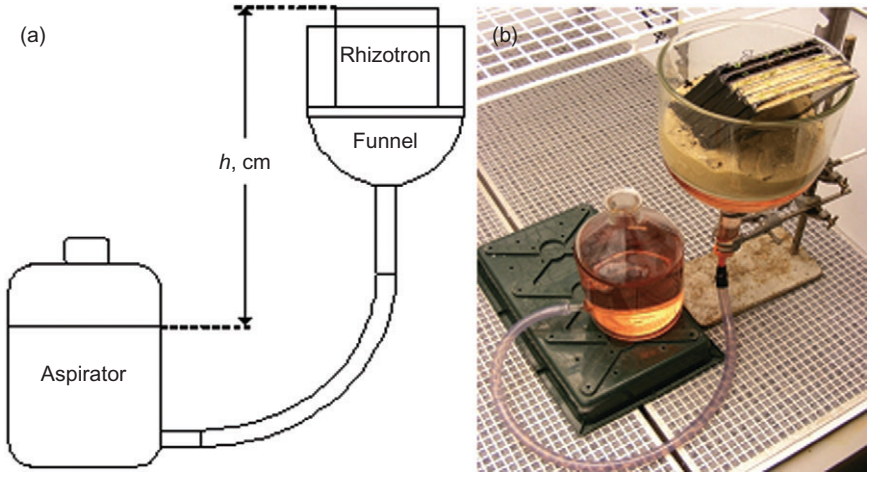

Figure 1. A schematic (a) and photograph (b) of the sand rhizotron system for growth of *Arabidopsis* seedlings in sand where tension height (*h*, cm) can be altered.

data were fitted to the Mualem van Genuchten models (Supporting Information Table S1; van Genuchten 1980), and unsaturated K_{unsat} was predicted (Supporting Information Fig. S1). The value of Ψ_m in the different grades of sand was adjusted by altering the water tension height, to give hydraulic conductivities of the sand to water between 0.2 and 1.4 m D^{-1} (Table 1).

Plant growth conditions

For both sand and agar experiments, *Arabidopsis thaliana* (Ws) seeds were sterilized, stratified and germinated on Whatman no. 2 filter paper (Whatman, Maidstone, UK) soaked in distilled water. At 5 d post-germination (dpg), seedlings were selected for similar size and PRL, and transferred to the respective experimental systems (four seedlings per experimental unit). At 12 dpg, the following RSA characteristics (see Zobel & Waisel 2010) were measured manually for each seedling: PRL, BR length (BRL), LR number (LRN) of length ≥1 mm and total LR length (TLRL). For all germination and physiology experiments, growth conditions were cycled: 16 h light (290 μmol m^{-2} s^{-3}), 8 h darkness, at 22 °C and 75% relative humidity. A minimum of two independent replicates were used for each experiment comparing different nitrate supplies or the effect of particle sizes, with three or four in all other treatments.

NO_3^--selective microelectrodes

Double-barrelled NO_3^--selective microelectrodes were prepared using filamented double-barrelled borosilicate glass (Miller & Zhen 1991). Microelectrodes were mounted on a micromanipulator (model NMN-21; Narashige, Tokyo, Japan). Both microelectrode reference barrels and reference electrodes were backfilled with 200 mM KCl. The experimental unit was secured to the stage of an Olympus microscope (model SZX9, Olympus, Southend-on-Sea, UK). Calibration curves were obtained using solutions of known NO_3^- activities (Miller & Zhen 1991). The NO_3^- activities at the surface of intact primary roots were measured at the root tip (RT) and at 2 mm back from the tip (RT – 2 mm), and only data with unaltered calibration and recalibration curves before and after each measurement were considered acceptable. Care was taken to minimize the area of sand exposed for the measurement to avoid drying, but steady recordings were obtained suggesting that evaporative losses were minimal (Supporting Information Fig. S2). For the analysis of agar, a scalpel was used to cut 0.2 g sections from the plate, and each was melted in 1.8 g of distilled water (1 in 10 dilution) by heating in a microwave oven. A minimum of at least three replicates were used for each measurement.

Statistical analyses

All statistical analyses were carried out using GenStat (12th edition; VSN International Ltd, Harpenden, UK). For root physiology experiments, means were plotted and general analysis of variance (ANOVA) was carried out followed by comparison of means using the LSD (5%). LSD and SED values are given in Supporting Information Table S2. Some data were transformed to a log scale to stabilize variance

Sand	Water potential (MPa)	Matric potential (kPa)	Calculated K_{unsat} (m d^{-1})
Redhill T	–0.11	–1.5	1.35
Redhill T	–0.33	–3.0	0.79
Redhill T	–0.40	–4.5	0.18
<250 μm	–0.06	–3.0	0.99
250–425 μm	–0.26	–3.0	0.36
>425 μm	–0.33	–3.0	0.17

Table 1. Measured values for total water and matric potentials, and calculated values of the hydraulic permeability (K_{unsat}), showing the ranges for the sands used in this study

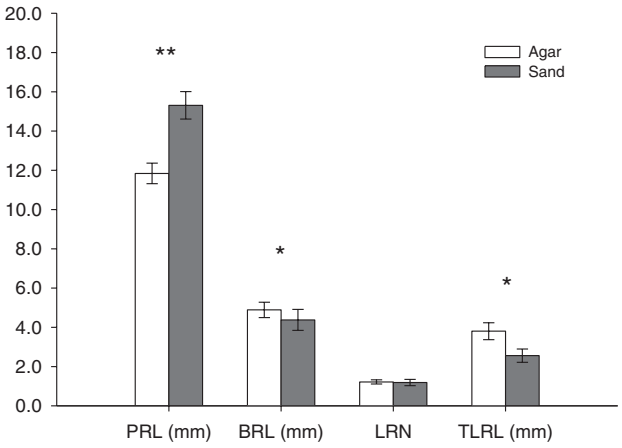

Figure 2. Comparison of the root architectural characteristics of *Arabidopsis* seedlings grown on agar and sand under the same nutrient supply. The average primary root length (PRL), basal root length (BRL), lateral root number (LRN) and total lateral root length (TLRL) were plotted for each treatment ($n = 120$; d.f. 57), and data were compared on the $\log_{10}$ scale to stabilize variance across treatments. Significance: $*P < 0.05$; $**P < 0.01$.

across treatments (figure legend will indicate). Graphs were made using SigmaPlot (version 11; Systat Software Inc, San Jose, CA, USA).

RESULTS

RSA was different between sand and agar

The RSA of seedlings grown on agar and sand under the same growth conditions was compared. The PRL of sand-grown seedlings were significantly longer ($P < 0.01$; d.f. 57) than that of agar-grown plants (Fig. 2). Whereas BRL and TLRL were significantly longer ($P < 0.05$; d.f. 57) in agar-grown seedlings when compared with those grown in sand. The LRN and LR density were not significantly different between treatments.

PRL was stimulated by changes in water flux to the root

The data in Fig. 3 clearly show that the PRL significantly increased with particle size (Fig. 3a, $P < 0.001$; d.f. 32) and tension (Fig. 3b, $P < 0.001$; d.f. 50), but the situation for other RSA characteristics was more complicated. Like PRL, BRL was significantly stimulated by sand particle size ($P < 0.01$; d.f. 32). In contrast to the primary root, the BRL showed no response to changes in matric potential (comparing Fig. 3a,b). The LRN and TLRL were not significantly different for roots growing in sand of different particle sizes (Fig. 3a), but when matric potential was increased both LRN and TLRL significantly decreased (Fig. 3b). The same response to matric potential was observed for LR density, but only for sand with a particle size <250 μm (Fig. 4).

The percentage of roots with laterals increased linearly with K_{unsat} ($r^2 = 0.93$; Fig. 5). Although PRL (negative, $r^2 = 0.66$) and LRN (positive, $r^2 = 0.94$) were correlated with K_{unsat}, the analysis of grouped data revealed that there was not a common curve that applied to both approaches to manipulate K_{unsat} (i.e. using different sand or adjusting matric potential). No clear relationship could be identified for BRL and TLRL plotted against K_{unsat}.

Root architectural responses to changes in nitrate supply are different for sand and agar

The RSA of seedlings grown on agar and sand was compared, but with the KNO_3 supply adjusted to give concentrations of 0.1, 1 and 10 mM (Fig. 6). There were some significant differences between the root growth patterns on agar and sand (Fig. 6a,b). The PRL was significantly longer

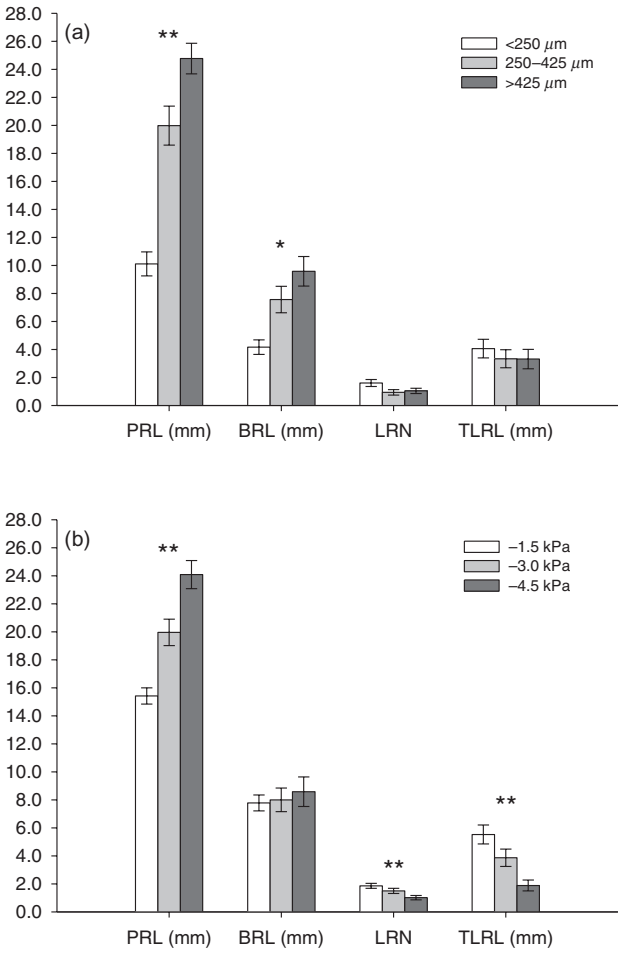

Figure 3. *Arabidopsis* root architectural characteristics of seedlings grown in sand with a constant N availability, but varying water delivery rates. Sands of three (a) particle sizes (>250, 250–425 and >425 μm) and (b) tension heights 15, 30 and 45 cm (1.5, 3.0 and 4.5 kPa) were used while keeping N availability constant across treatments. The average primary root length (PRL), basal root length (BRL), lateral root number (LRN) and total lateral root length (TLRL) were plotted for each treatment; (a) $n = 48$, d.f. 32; (b) $n = 72$, d.f. 50. Significance: $*P < 0.01$; $**P < 0.001$.

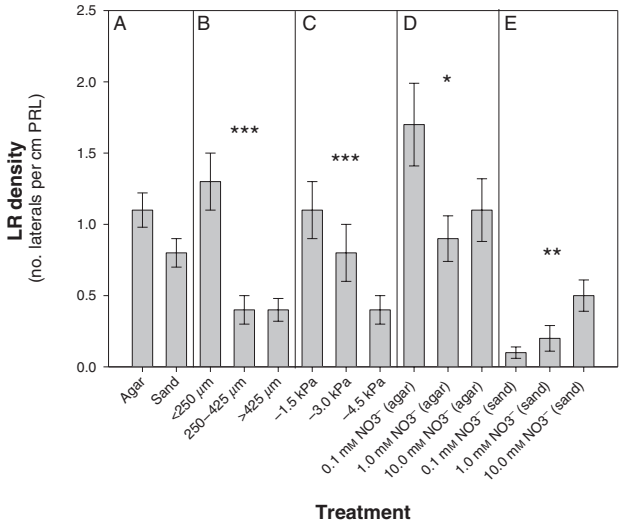

Figure 4. Lateral root (LR) density [number of LRs per cm primary root length (PRL)] was plotted for each treatment. Experiment A demonstrated no significant difference. For experiment B, d.f. 32, SED 0.021, LSD 0.042. For experiment D, d.f. 46, SED 0.033, LSD 0.066. Significance: $*P < 0.04$; $**P < 0.01$; $***P < 0.001$.

on sand when compared with agar at all three KNO_3 concentrations (consistent with Fig. 2), and there was no difference between treatments in sand, but on agar the PRL was greater ($P < 0.001$; d.f. 42) at 1 mM when compared with 10 mM KNO_3 supply (Fig. 6b). At the highest KNO_3 supply, LRN, TLRL and LR density are increased in sand, but this response was not observed in agar where LR density was significantly greater at 0.1 mM KNO_3 (Fig. 4). In the sand system, LRN, TLRL (Fig. 6) and LR density (Fig. 4) were significantly increased at 10 mM compared to 0.1 and 1.0 mM KNO_3 supplies.

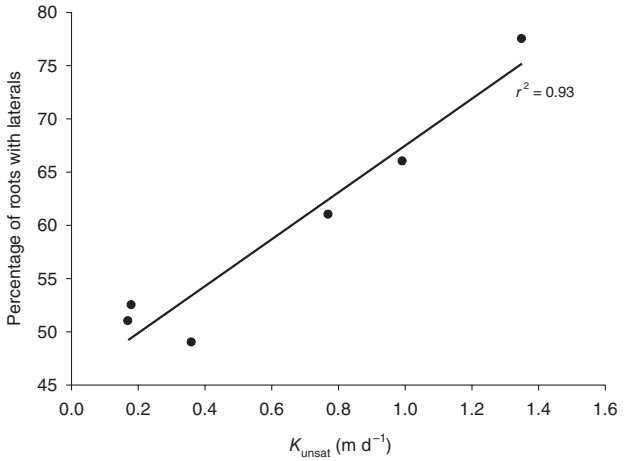

Figure 5. Plot of percentage of roots with laterals against K_{unsat} showing evidence of negative linear correlation. Linear regression line plotted ($r^2 = 0.93$).

PRL was stimulated by changes in water flux, but not root surface nitrate

In order to quantify the NO_3^- availability encountered by the sensing and uptake regions of the root, the activity was measured at the RT and 2 mm above the RT (RT – 2 mm) using NO_3^--selective microelectrodes. The NO_3^- activity reported by the microelectrodes on the surface of the root was greater than that supplied in the nutrient solution. However, the NO_3^- activity was not significantly different across particle size treatments or between locations on the root for seedlings supplied with 10.0 mM (Fig. 7a) and 0.1 mM (not shown). Therefore, PRL responses to water flux (Fig. 3) are independent of NO_3^- availability at the root surface.

In agar without plants, the NO_3^- activity was similar to the original 10 mM NO_3^- supply (Fig. 7b). However, when plants were introduced into the agar system, the NO_3^- activity

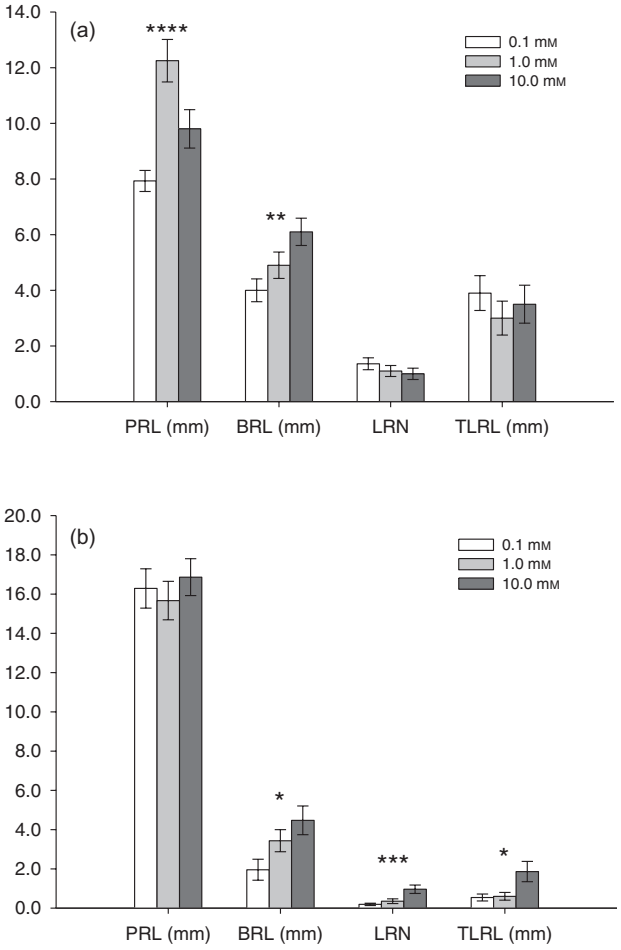

Figure 6. Root architectural characteristics of *Arabidopsis* seedlings grown under varying NO_3^- supply on either agar (a) or sand (b). Root system architecture (RSA) response to nitrate concentration was compared between agar (a) ($n = 24$; d.f. 42) and sand (b) ($n = 72$; d.f. 49). The average primary root length (PRL), basal root length (BRL), lateral root number (LRN) and total lateral root length (TLRL) were plotted for each treatment. Significance: $*P < 0.1$; $**P < 0.05$; $***P < 0.01$; $****P < 0.001$.

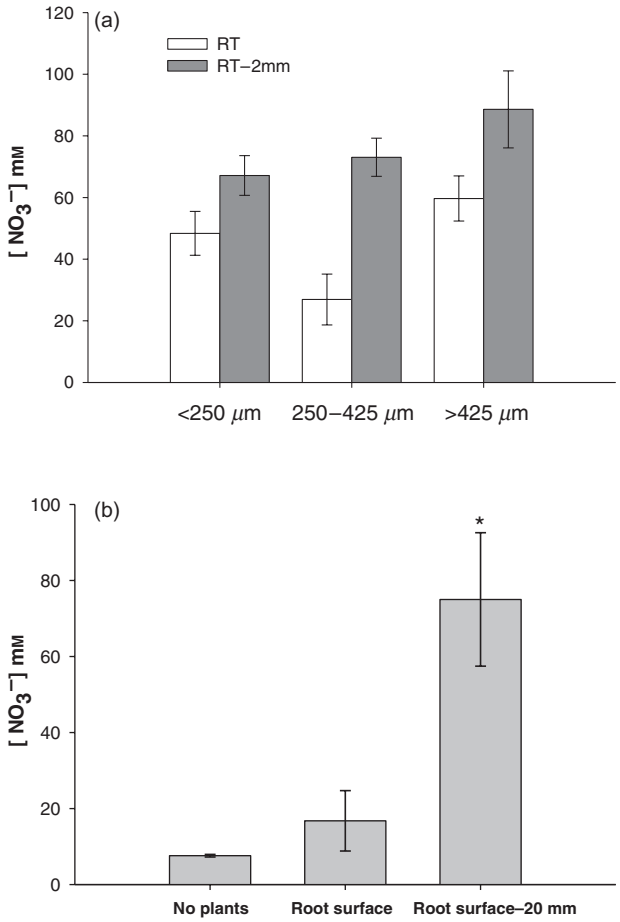

Figure 7. Ion-selective microelectrode measurements of NO_3^- activity: (a) at the root surface of *Arabidopsis* seedlings grown on sands of three particle sizes supplied with 10 mM KNO_3, and (b) in the agar around Petri dish-grown *Arabidopsis* seedlings. For the sand measurements, NO_3^- was measured at the root tip (RT) and 2 mm up from the tip (RT – 2 mm). For the agar measurements: NO_3^- was measured at the PR surface and 20 mm away from the PR; agar with no plants was used as a control; for each treatment $n = 3$, d.f. 6; significance: $^*P = 0.01$.

found at the root surface was significantly lower ($P < 0.01$; d.f. 6) than that observed 20 mm away from the root surface (Fig. 7b). In addition, the activities observed for the agar when plants were present were also greater than the initial 10 mM NO_3 added to the agar.

DISCUSSION

The use of sand to study root architecture

Sand culture is more similar to soil than agar or hydroponics (Table 2) in the sense that both sand and soil can be defined by comparable hydraulic parameters. Sand culture has minimal microbial impact on root nutrient acquisition relative to soil, but provides an easier environment than soil to study RSA in response to nutritional and physical (especially hydraulic) treatments.

In our sand rhizotron system, the hydraulic conductivity values obtained for the sands are similar to the saturated hydraulic conductivity ranges typically found for sandy and loamy soils (Campbell 1985; Marshall *et al*. 1996). When roots extract water, the value of the Ψ_m adjacent to the root becomes more negative (Carminati *et al*. 2010), so in our system tension height should be taken as an approximate estimate of the Ψ_m at the soil–root interface. However, because the hydraulic conductivities we use are at relatively high bulk matric potential (determined by the tension height), they are likely to be a reasonable estimate of matric potentials at the sand–root interface (Whalley *et al*. 2000). *Arabidopsis* has a relatively low transpiration demand (Christman *et al*. 2008), and the seedlings were grown in a covered system; therefore, it is unlikely that significant moisture gradients will develop near the root in this experimental system. The range in matric potential we used was very small (–1.5 to –4.5 kPa; Table 1), and water potential was dominated by the osmotic component. Thus, the effect of manipulating matric potential or the size of sand particles was primarily to change the hydraulic conductivity of the sand to water, and hence the flux of water to the root surface.

Comparing our sand system with agar culture, we found significant reproducible differences in several aspects of RSA for *Arabidopsis* seedlings growing under complete nutrient supply (Fig. 3). This suggests that differences in the physical properties of the two culture systems (agar and sand) are influencing the response of RSA to water and NO_3^- treatments.

Why is RSA different between sand and agar?

Certain sands offer physical impedance to growth that is not found when roots grow on the surface of agar. Changes in RSA (e.g. a reduced PRL) have been described for mechanically impeded roots (reviewed by Clark, Whalley & Barraclough 2003). Pore size is related to particle size (Marshall *et al*. 1996), and the pore size of the sand used in this work is sufficient for the fine *Arabidopsis* roots (typical diameter 150 μm; Bowman 1994) to grow between particles and thus not be impeded. The physical structure of agar and the fact that the roots grow mostly over the surface mean that mechanical impedance effects can be excluded from these experiments. Therefore, physical impedance effects cannot explain the RSA differences observed in these experiments.

Root gas exchange may differ between sand and agar. The gaseous hormone ethylene is known to influence RSA (Ivanchenko, Muday & Dubrovsky 2008), and localized gradients may develop differently in sand and agar. Roots growing along an agar surface are in contact with the air, while those in wet sand may have a more restricted opportunity for gas exchange. However, the sand environments were unsaturated, except in one case (Table 1, row 1), so it is reasonable to assume that the roots were adequately aerated. Other work has concluded that in similar sand systems, oxygen availability does not limit growth (Whalley *et al*. 1999). Taken together, this information suggests that

Table 2. Relative differences between culture techniques used to study root architecture

Feature	Agar	Hydroponics	Sand	Soil
Matrix structure	Homogeneous	Homogeneous	Heterogeneous	Heterogeneous
Pores (air)	Moderate	Limited	Variable	Variable
Pores (water)	Moderate	Saturated	Variable	Variable
Impedance	Minimal	Minimal	Variable	Variable
Bacteria	Minimal	Minimal	Minimal	High
Light exposure	High	Minimal	Minimal	Minimal

Sand is more similar to the natural growth environment encountered in the soil than agar or hydroponics.

gas exchange properties are unlikely to explain the differences in RSA between sand and agar systems.

Light supply can be an important factor influencing root growth. On agar, the roots are not maintained in the dark, and light inhibition of root growth is ethylene and jasmonate mediated (Adams & Turner 2010). In sand, roots are likely to be exposed to less light, but PRL for agar and the smallest particle sand are similar (comparing Figs 2 & 3a). This result suggests that a difference in light interception by roots does not explain the RSA differences between sand- and agar-grown plants. When developing the method, we compared conventional agar-grown seedlings with those grown on agar with root light exposure limited, and found no significant difference in RSA (data not shown).

Unlike agar culture, the sand rhizotron system was not completely sterile. Although the sand was acid washed, contained no carbon source for microbial growth and the system was covered in opaque sheeting, when comparing agar and the sand culture data, we cannot exclude the possibility that there was some microbial growth in the sand system. However, during method development, we found that autoclaving the acid-washed sand had no effect on RSA when compared with unsterilized sand (data not shown). Therefore, we believe that microbial activity is not a significant contributor to the RSA of seedlings grown in our sand rhizotron system.

One of the biggest differences between the two systems is in the volume of nutrients supplied in each system. In the sand system, the volume of water and NO_3^- available to the plant is much greater than the finite volume supplied in agar plates. The impact on RSA of the volume of nutrients supplied between the two systems is further compounded by differences in the hydraulic properties which determine delivery of water and NO_3^- to the root. It is known that Ψ_t influences RSA (Sharp *et al*. 1988; Verslues *et al*. 1998), and our results demonstrate the influence of water flux on RSA. Therefore, differences in the volume of nutrients supplied and hydraulic parameters between sand and agar systems may explain the changes in RSA we have observed by affecting the delivery of water and NO_3^- to the root.

The effects of water and nitrate supply on root architecture

Several papers have reported the influence of water stress as Ψ_t on RSA (Sharp *et al*. 1988; Verslues *et al*. 1998; Wiegers *et al*. 2009). This is especially true for PRL which has been shown to decrease as water stress increases (Sharp *et al*. 1988). In our sand system, the opposite effects were obtained in response to the manipulation of substrate hydraulic conductivity: PRL increased with increasing particle size and water tension (Fig. 3). Nitrate is delivered to the root surface dissolved in water, but we have shown that the tight regulation of PRL by changes in water flux to the root was independent of NO_3^- activity at the root surface (Fig. 7).

PR elongation was shown to be strongly dependent on inorganic N (both NO_3^- and NH_4^+) supply in tomato and maize, and this response was optimal at lower concentrations (Bloom, Jackson & Smart 1993; Bloom, Frensch & Taylor 2006). Conversely, *Arabidopsis* PR growth is arrested by NH_4^+ supply (Li *et al*. 2010) and is insensitive to NO_3^- supply across a large range from 0.01 to 100.0 mM (Zhang & Forde 1998; Linkohr *et al*. 2002; Orsel *et al*. 2006), although the latter response is known to be genotype dependent (Walch-Liu & Forde 2008). Here, we demonstrate an insensitivity of *Arabidopsis* Ws PRL to high (0.1–10.0 mM) NO_3^- supply (Fig. 6b), and while lower NO_3^- concentrations were not investigated, an insensitivity to low (0.05–1.0 mM) NO_3^- supply has been previously reported for Ws (Remans *et al*. 2006b).

The insensitivity of *A. thaliana* PRL to NO_3^- has previously been shown in agar culture experiments (Zhang & Forde 1998; Linkohr *et al*. 2002). However, our agar PRL results differed from those previously reported (Fig. 6a). This difference could be genotype dependent, and this has been demonstrated for No-0 plants that were sensitive to low NO_3^- concentrations compared to other genotypes (Walch-Liu & Forde 2008). Another possible explanation is that we used 2% agar, whereas 0.8% (Linkohr *et al*. 2002) and 1% (Zhang & Forde 1998) were used previously. It has been shown that a change of just 0.1% in gel concentration equates to a 1 to 2×10^{-4} MPa change in Ψ_m (Spomer & Smith 1996). We may assume that the Ψ_t experienced by roots in our agar system is approximately 20 times that of the lower concentration agar used in previous work. Ψ_t is known to influence PR growth (Sharp *et al*. 1988), and in gel substrates Ψ_m is a large component of Ψ_t. Therefore, the difference in Ψ_t is likely to account for the contrast in PRL we have observed compared to previous work.

A decrease in water availability has been shown to significantly repress the formation of LRs (Deak & Malamy 2005).

This result is confirmed for *Arabidopsis* roots growing in sand by the LRN (Fig. 3b), and the percentage of roots with no laterals increased as K_{unsat} decreased (Fig. 5). TLRL has been shown to respond to localized patches of high NO_3^- concentration (Zhang *et al*. 1999), and indeed the TLRL was greater for agar-grown seedlings compared to sand (Fig. 2). This suggests patchiness in NO_3^- concentration caused by a finite supply of water in agar culture which moves towards the plant, creating local patches of high NO_3^- concentration at the periphery of the agar plate, as confirmed by the NO_3^- microelectrode measurements (Fig. 7b).

While the water supply, determined by substrate hydraulic conductivity and gradient in water potential, is important for PRL, LRN and TLRL, changes in NO_3^- supply produce the same response in BRL (Fig. 6). BRL of sand-grown ($P < 0.1$; d.f. 49) and agar-grown seedlings ($P < 0.05$; d.f. 42) increased with increasing NO_3^- concentration. LRN and TLRL have been shown to respond to patches of high NO_3^- supply in agar (Zhang *et al*. 1999), and BRL could be responding in the same way here. Particularly as BRL increased at larger particle sizes, but was unaffected by matric potential, sands of a larger particle size have a larger pore size and poorer connectivity of solution, resulting in the occurrence of NO_3^- patches.

CONCLUSIONS

The sand rhizotron system is a step towards bridging the gap between the lab and the field, combining a porous growth environment with controlled water and nutrient delivery to give an additional tool to explore *Arabidopsis* RSA. The contrasting RSA responses in sand and agar may be explained by differences in the volume of nutrient supplied and the conductivity of each substrate to water. In the sand system, we have shown that changes in hydraulic conductivity over a narrow range of matric potentials can have large effects on RSA; the best example of this point was in the percentage of roots with laterals. In the case of PRL, water flux regulates growth independent of NO_3^- supplied across a range of 0.1–10 mM. In contrast, BRL is more closely regulated by NO_3^- supply and exhibits a similar response to that previously reported for LRs and high NO_3^- patches.

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SUPPORTING INFORMATION

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Figure S1. Water release characteristics of the sands used in these experiments. The water release characteristic was measured using the burette method. (a) Arrows indicate the tensions used during the water tension experiment, and K_{sat} was measured using the constant head permeability test (b). This information was computed using the van Genuchten model to calculate K_{unsat} (c).
Figure S2. Typical NO_3^- microelectrode recording obtained from the surface of an *Arabidopsis* root growing in sand and supplied with a full nutrient solution containing 0.1 mM NO_3^-. Stable measurements were recorded at the root tip (RT) and 2 mm up (RT – 2 mm) from the RT of an intact primary root. The recording shows the calibration of the microelectrode before (t = 0–10 min) and after (t = 25–32 min) the measurement with solutions of known NO_3^- activity (100, 10, 1, 0.1 and 0.01 mM).
Table S1. Sand physical properties including parameters used in the van Genuchten equation.
Table S2. Standard error differences (SEDs) and least square difference (LSD, 5%) values of root system architecture (RSA) characteristics for each physiology experiment (Exp).

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