Plant Soil (2012) 357:161–172
DOI 10.1007/s11104-012-1135-5

REGULAR ARTICLE

Spatial variation in root system activity of tomato (*Solanum lycopersicum* L.) in response to short and long-term waterlogging as determined by ^{15}N uptake

Dorte Bodin Dresbøll · Kristian Thorup-Kristensen

Received: 16 August 2011 / Accepted: 10 January 2012 / Published online: 23 February 2012

Abstract
Background and aim Root system activity is affected by abiotic stresses, which often creates spatial differences in root conditions. This is expected to influence plants ability to cope with suboptimal conditions.
Methods Changes in root system activity were determined as ^{15}N root uptake in top and bottom layers of potted tomato plants (*Solanum lycopersicum* L.), after waterlogging the bottom layer for 24 h or 5 d. The plants were grown in peat-based media; non-compacted or highly-compacted, resulting in differences in gas diffusion, air permeability and oxygen availability.
Results The roots were affected by short-term waterlogging (24 h) by decreasing uptake in the bottom layer and increasing uptake in the pot top layer. Long-term waterlogging (5 d) decreased the ^{15}N root uptake more in both layers. Root uptake recovered fast (within 6 h) after short-term waterlogging, whereas recovery of long-term waterlogged roots took more than 24 h, suggesting production of new root biomass. Despite affecting physical properties, medium compaction did not affect root uptake. Aboveground biomass was affected by waterlogging by increasing the dry matter percentage, decreasing nitrogen (N) percentage and increasing starch content.
Conclusions The results confirmed that root uptake in different layers of small pots could be distinguished by the ^{15}N technique, which was applicable under potentially denitrifying conditions. The results demonstrated that during short-term stress in part of the root system plants increased uptake from the non-affected parts of the root system, probably as compensation for suboptimal conditions.

Keywords Peat · Oxygen · Isotope · Anoxia · Gas diffusivity

Responsible Editor: Michael Denis Cramer.

D. B. Dresbøll
Department of Food Science,
Faculty of Science and Technology, Aarhus University,
Kirstinebjergvej 10,
DK-5792 Aarslev, Denmark

D. B. Dresbøll (✉) · K. Thorup-Kristensen
Department of Agriculture and Ecology,
Faculty of Life Sciences, University of Copenhagen,
Højbakkegård Allé 13,
DK- 2630 Taastrup, Denmark
e-mail: dbdr@life.ku.dk

Introduction

Root system activity can be negatively affected by suboptimal physical properties of the rhizosphere and by abiotic stresses such as salinity, drought or waterlogging (e.g. Morard et al. 2000). Although such stresses may only directly affect a part of the root system, the remaining roots are likely to be affected indirectly by the suboptimal conditions. When plants are grown in small pots with restricted space, variation in rhizosphere properties occur within short distances,

influencing root growth and activity and hence the overall growth and survival of the plant. In potted plants, water is generally supplied from beneath by ebb/flood irrigation, where the lower part of the pot is submerged in water for several minutes before the water is drained again. During shady days with low water uptake or if water is retained for too long a wet zone can be created in the bottom of the pot. This will lead to anoxic conditions in the lower part of the pot and a risk of drying out of the upper part of the medium if media physical properties are suboptimal. Therefore, the spatial distribution of roots in potted media is important for plants which are exposed to abiotic stresses within a part of the growing medium.

Waterlogging leads to a decreased oxygen availability which affects the physiological processes of root growth and uptake and consequently plant performance (Drew 1997). The effect of waterlogging on root system activity will depend on the duration of waterlogging, severity of the waterlogging and the physical properties of the surrounding environment. Pore characteristics determine the ability to retain water and the efficiency of gas diffusion (Nkongolo and Caron 1999) and changes in these, such as compaction of the soil or growing media, are expected to affect the physical properties and thus the influence of waterlogging on plant physiology.

The function of roots of potted plants in situ is sparsely studied. Root studies are complex as the majority of potted plants are grown in organic growing media in small containers. Generally, root function studies are conducted in hydroponic systems where biotic and abiotic parameters are more easily controlled (e.g. Kläring and Zude 2009). However, in hydroponic studies roots are surrounded by uniform conditions, and the spatial differences within a solid growing media affecting the root system differently will not be revealed. In addition, these systems do not provide information on the interactions between roots and solid growing media. Significant soil environment-genotype interactions have been shown when comparing root lengths in non-solid media with soil media (Gregory et al. 2009; Wojciechowski et al. 2009) and this might apply to root activity as well. In situ methods are expected to identify such interactions and will provide more detailed knowledge on root system activity under heterogeneous conditions.

The use of stable isotopes to determine root nutrient uptake as a measure of root system activity is an applicable in situ method that is expected to determine differences in root function even within the restricted space of small pots. When determining plant root nitrogen uptake under various conditions, from studies of deep root nitrogen uptake in the field (Kristensen and Thorup-Kristensen 2004) to nitrogen uptake rate on single roots (Volder et al. 2009), ^{15}N has been shown to be a useful measure of root activity. In the majority of studies using ^{15}N, plants are given several days to take up and translocate the ^{15}N to aboveground biomass (Kristensen and Thorup-Kristensen 2004; Zanne et al. 2006). In this study, ^{15}N was used to elucidate short-term effects of waterlogging on root uptake by determining root nitrogen uptake before, during and after short and long-term waterlogging in the bottom part of small pots.

The objective was to study the effect of waterlogging on plant quality and the ability of plant root systems to overcome spatial abiotic stress. Root system activity was defined as total ^{15}N uptake caused by changes in root growth and uptake rates. It was hypothesized that 1) root system activity differs within a pot, with a greater activity in the bottom of the pot where the youngest roots are expected to be, 2) that plants are able to cope with waterlogging for a period of time, compensating by increasing growth and/or activity in the roots located above the water level, and 3) that differences in the degree of compaction will alter the physical properties of the medium affecting the water retention and gas diffusion, and hence the oxygen availability resulting in differences in root response to waterlogging. Finally, the aim was also to examine whether the ^{15}N technique was able to determine spatial differences in root uptake across small distances within the pot and to reveal changes over a short period of time.

Materials and methods

Growing medium and plant material

The growing medium was based on a blonde peat moss, 65% Baltic peat and 35% Danish peat with particle sizes of 0–10 mm. Limestone, wetting agent (Fiba-Zorb, Turftech International, UK) and fertilizer were added to the medium. The medium was potted by hand into pots with an upper diameter of 110 mm, a lower diameter of 76 mm and a height of 80 mm, at two different degrees of compaction; a standard filled

pot (0.25 g cm^{-3}) and a highly compacted medium (0.31 g cm^{-3}). The pots were irrigated and tomato seeds (*Solanum lycopersicum* L.) were sown at the end of May 2010. Plants were grown in a greenhouse at the Department of Food Science, Aarhus University (10°27′E, 55°18′N), Denmark with a temperature set point of 18°C and windows opening at 22°C, 16 h of light and fertigated by ebb/flood irrigation whenever necessary with fertilizer containing 0.5 M $Ca(NO_3)_2$, 0.5 M KNO_3, 0.2 M $MgSO_4$, 0.1 M KH_2PO_4 as well as micronutrients.

Experimental setup

Experiments were conducted on 5-weeks-old tomato plants. Three different water availability treatments; 24-hours-waterlogging (24 h-WL), 5-days-waterlogging (5 d-WL), no waterlogging (control), were applied to plants grown in either non-compacted or highly compacted media. Three days before the initiation of the experiments, fertilizer application was stopped and plants were subsequently irrigated with tap water. This was done to deprive the plants of nitrogen in order to ensure uptake of the ^{15}N isotope. The plants in the 24 h-WL treatment were moved to a water filled table, so that the bottom 30 mm of the pots was covered by tap water for 24 h, before the water was drained from the table. The plants were left for up to 0.25 h in order to allow macropore water runoff, then without lifting the pots from the table, injection of ^{15}N was carried out in situ. In the 5 d-WL treatment, plants were moved to a water filled table for 5 d where the bottom 30 mm of the pots was covered with tap water. After 5 d the water was drained by the same procedure as for the 24 h-WL treatment. In the control treatment, irrigation was conducted whenever necessary, which corresponded to once a day.

A solution of double-labelled $^{15}NH_4{}^{15}NO_3$ containing 2 mg ^{15}N (98 atom%) in 5 ml water was injected through small holes with a diameter of 2 mm in the side of the pots, in either the top (15 mm below surface) or bottom (15 mm above bottom) layer, with a 70 mm long needle. The needle was inserted horizontally at three different angles into the medium through the hole in the pot and the ^{15}N solution was released while the needle was pulled out of the medium again, to apply the ^{15}N evenly in the specific layer. In the waterlogging treatments, injection was conducted in either of the two layers (five replicates) from both the non-compacted and compacted treatments at one of 3 injection times resulting in 60 treated plants. ^{15}N injection times were immediately after drainage of the water at 0 h (8:00), at 6 h (14:00) or at 12 h (20:00) after drainage in the 24 h-WL treatment. In the 5 d-WL treatment the injections were carried out immediately after drainage at 0 h (8:00), at 12 h (20:00) or at 24 h (8:00, + 1 d). Finally, in the control treatment, ^{15}N solution was also injected in 5 replicate pots, either in the top or bottom layer of the pot from both non-compacted and highly compacted media. Thus, a total of 20 plants from the control treatment were treated with ^{15}N. This control evaluation was carried out at two points; the same day as the 24 h-WL experiment, and again the following day when the 5 d-WL experiment was carried out.

Plants were left in the greenhouse for approximately 2 h after injection, before the aboveground biomass was harvested and fresh weight determined. The plant material was oven dried (70°C for 24 h), dry weight was determined, plant material was finely ground and 3 mg material was packed in tin capsules for ^{15}N isotope analysis at UC Davies Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The growing medium from the three water availability treatments were also oven dried (70°C until stable weight) immediately after cessation of waterlogging, packed in tin capsules containing 10 mg finely ground medium and analyzed for ^{15}N. Samples of growing media without any addition of ^{15}N were analyzed for natural abundance of ^{15}N. Finally, samples of growing media were analyzed, where plants were harvested immediately after injection, in media either waterlogged for 24 h or not waterlogged, to determine the ^{15}N recovery before plant uptake.

An additional experiment was conducted in order to examine differences in short-term uptake of $^{15}NH_4^+$ or $^{15}NO_3^-$. Uptake of ^{15}N was determined before and after 24 h of waterlogging by applying ^{15}N as either $^{15}NH_4NO_3$, $NH_4{}^{15}NO_3$ or $^{15}NH_4{}^{15}NO_3$. The plants were given 2 mg of N in total independent of labelling. This corresponded to 2 mg ^{15}N when given as double labelled NH_4NO_3 and 1 mg ^{15}N when given as single labelled NH_4NO_3. The ^{15}N was applied as described above into the top or bottom layer of non-compacted media and plants were harvested 2 h after injection. At harvest, the growing media injected with any of the

three differently labelled NH_4NO_3 before and after the 24 h of waterlogging were oven dried (70°C until stable weight) and analyzed for remaining ^{15}N.

Oxygen profiles

Oxygen profiles were determined from the top to the bottom layers of the growing media in all three treatments by the use of oxygen microsensors (Unisense A/S, Unisense Science, Aarhus, DK) (Dresbøll 2010). In short, two Clark-type oxygen sensors with a tip diameter of 100 μm, and with the long (70 mm) slender part reinforced by stainless steel were mounted on a motor, that controlled the movement of the sensors into the medium. Profiles were determined on three replicate pots of both non-compacted and highly compacted media. Oxygen content was determined at 2 mm intervals through the pot profile and average values for each centimetre was subsequently calculated. The pots were moved from the greenhouse to the laboratory immediately prior to measurement. In all treatments, the oxygen profiles were determined at the same time as ^{15}N was added to the media in the greenhouse.

Physical properties of media

Samples for determination of physical properties were taken directly from the pots after harvest of the plants and stored at 4°C until analysis. Duplicate pots were oven dried at 70°C until weight was stable and water content was determined as g cm^{-3}. Samples were taken by the use of 100 cm^3 soil cores inserted into the middle of the pot. The peat above and below the core was cut off and samples from both compacted and non-compacted media were taken. The small peat cores were placed on top of a sandbox (08.01 Sandbox, Eijkelkamp Agrisearch Equipment, NL) and saturated with water from beneath (Topp and Zebchuk 1979). The peat-water characteristics was determined by draining the peat samples to matric water potentials of −4, −6.4, −10, −16, −30, −50, −100, −160 and −500 hPa. The sandbox was used for matric water potentials from −4 to −100 hPa and a ceramic pressure plate (Soilmoisture Equipment Corp., Santa Barbara, CA) for matric water potentials from −160 to −500 hPa.

Matric water potentials corresponding to the water content at harvest was determined from the water release curve. Peat cores were adjusted to the different matric water potentials, −36, −40 or −91 hPa for highly compacted media and −37, −48 or −208 hPa for the non-compacted media on a sandbox or on a ceramic plate for potentials above −100 hPa. This corresponded to water contents determined at cessation of the 5 d-WL treatment, the 24 h-WL treatment, and the control treatment respectively. In addition peat cores were adjusted to −100 hPa. The peat cores were analyzed for volumetric water content, air diffusivity, air permeability and air filled pore space. Air diffusivity was analyzed by a non-steady state method (Taylor 1949). In short, the samples were placed in an air-tight chamber that was flushed with N_2 gas to remove all O_2. Then O_2 from the outside atmosphere was allowed to re-enter the chamber below the sample. During a 0.5–2 h period O_2 diffusing through the sample was frequently determined as the O_2 concentration in the chamber above the sample (Schjønning 1985). Subsequently, air permeability was determined by introducing a convective transport of pressured air (approximately 4 hPa) through the sample (Grover 1955). The effective air-connected porosity, ε_{eff} (m^3 m^{-3}) was determined at the same four matric potentials for the highly compacted and non-compacted media by an air-pycnometer constructed according to Flint and Flint (2002).

Plant parameters

Fresh weight of the plants was determined at harvest and plant material was oven dried (70°C for 24 h) for determination of dry weight. Starch content was determined on the dried and ground plant material. The soluble sugars (glucose, fructose and sucrose) were extracted with 80% ethanol and 5 mM HEPES (4-(2-Hydroxyethyl)piprazine-1-ethanesulfonic acid) until the leaf material appeared decolourised. The extracted and decolourised leaf material was prepared for starch analysis by gelatinization in an autoclave at 120°C for 90 min and incubated with Na-acetate buffer containing amyloglucosidase and α-amylase for 16 h. The starch concentration was determined as glucose equivalents by ion chromatography using a pulsed amperometic detector (PAD) with a gold electrode (Dionex, ICS 3000, Sunnywale, Canada), using 200 mM NaOH as eluent.

Statistical analysis

Statistical significance of differences in ^{15}N uptake, plant parameters and peat physical properties were

averaged over 5 plants and tested by analysis of variance (one-way ANOVA, F-test). Multiple comparisons were based on values of least significant difference (LSD) derived from analyses of variance (Proc GLM, SAS Institute Inc., Cary, NC). In assessing differences between results, tests with $P<0.05$ were considered statistically significant.

Results

^{15}N uptake

Within 2 h of injection up to 25% of the applied ^{15}N was recovered in the plants. Under standard conditions where plants were irrigated as necessary, plants had a significantly ($p<0.05$) greater uptake of ^{15}N from the bottom layer of the pot compared to the top layer (Fig. 1). The uptake was approximately three times greater in the bottom layers of the non-compacted media while uptake in the compacted media were approximately 30% greater in the bottom than in the top layer.

After 24 h of waterlogging the ^{15}N uptake from the bottom layer, which had been under water decreased (Fig. 1a), this was especially the case when the medium was compacted. In the non-compacted media the ^{15}N uptake in the top layer increased significantly ($p<0.05$) when measured immediately after the bottom layers had been waterlogged for 24 h. In the compacted media there was a greater initial uptake in the top layer, and this remained elevated after the period of waterlogging. Six hours after the water had been drained away, the uptake in the bottom layer of both compacted and non-compacted media had increased significantly ($p<0.05$), it exceeded the uptake in the control treatments, while the uptake in the top layer remained at the same level as immediately after drainage. When water had been drained for 12 h the ^{15}N uptake decreased in both layers of the pots in both the compacted and the non-compacted medium. Under these conditions, significantly less ^{15}N was taken up in the top layer compared to the bottom layer ($p<0.05$).

After 5 d of waterlogging the ^{15}N uptake decreased in the entire pot (Fig. 1). In contrast to the 24 h waterlogging period no increase was seen in the top layers after 5 d of waterlogging, although there was a tendency towards a greater uptake in the this layer compared to the bottom layer. No significant differences were observed between the compacted and non-compacted media. When the media had been drained for 24 h after the waterlogging period in the non-compacted media there was a tendency towards an increase in the uptake in the bottom layer. However, the results showed that 24 h was insufficient time for recovery of the roots after long-term waterlogging.

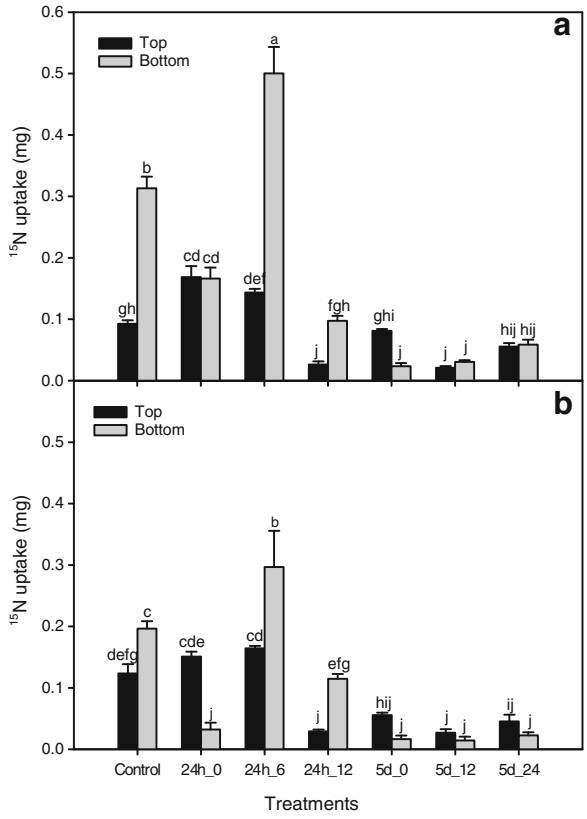

Fig. 1 Plant uptake of double labelled ammonium nitrate ($^{15}NH_4{}^{15}NO_3$) before, during and after waterlogging (**a**) non-compacted (0.25 g cm^{-3}) and (**b**) highly compacted media (0.31 g cm^{-3}), where tomato plants were either irrigated regularly (control), waterlogged for 24 h (24 h-WL) or waterlogged for 5 days (5 d-WL). Root ^{15}N uptake was determined right after cessation of the waterlogging (24h_0 and 5d_0) and after 6 (24h_6) and 12 (24h_12) hours in the 24 h-WL treatment and after 12 (5d_12) and 24 (5d_24) hours in the 5 d-WL treatment. Results are means of 5 replicates and bars show standard error. Statistics are made across **a** and **b** and bars with different letters are significant different ($p<0.05$)

The addition of single labelled NH_4NO_3 ($^{15}NH_4NO_3$ or $NH_4{}^{15}NO_3$), revealed that 2 h after injection more NO_3^- uptake was detected compared to NH_4^+ uptake (Fig. 2). When added as double labelled $^{15}NH_4{}^{15}NO_3$ significantly more ^{15}N was detected in the plants. The

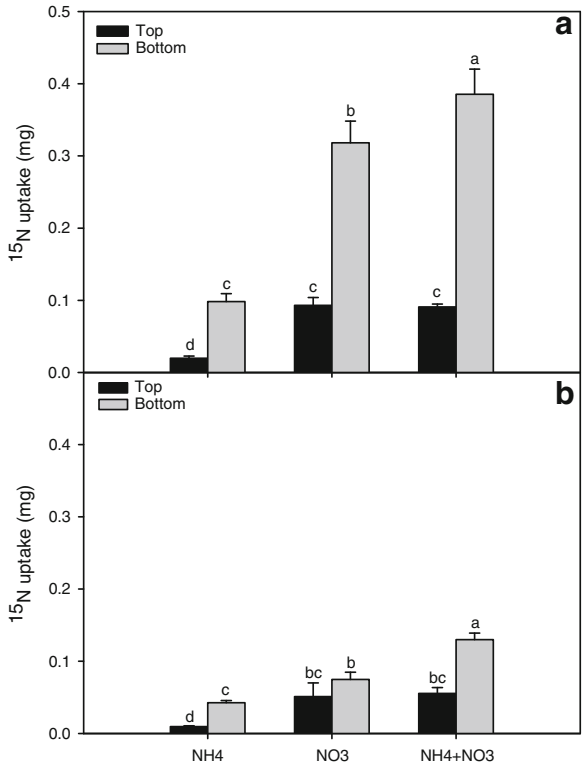

Fig. 2 Plant uptake of differently labelled NH_4NO_3, ammonium ($^{15}NH_4NO_3$), nitrate ($NH_4{}^{15}NO_3$) or ammonium and nitrate ($^{15}NH_4{}^{15}NO_3$) in plants irrigated regularly (control) (**a**) and plants waterlogged for 24 h (24 h-WL) (**b**) when N was given either in the top or bottom of the pot. Results are means of 5 replicates and bars show standard error. Bars with different letters are significantly different ($p<0.05$)

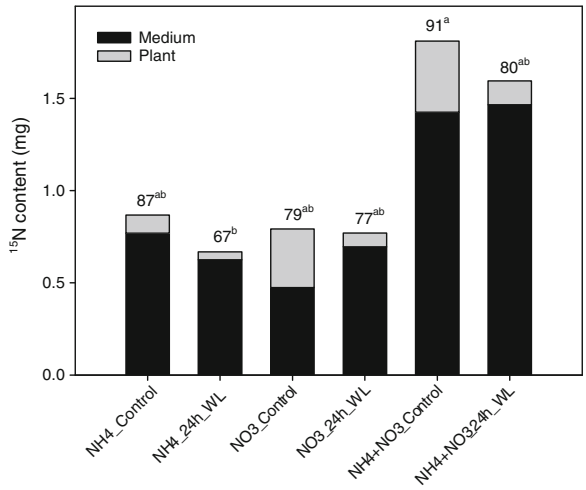

Fig. 3 Plant uptake and ^{15}N remaining in medium after uptake of differently labelled NH_4NO_3 where either ammonium ($^{15}NH_4NO_3$), nitrate ($NH_4{}^{15}NO_3$) or ammonium and nitrate ($^{15}NH_4{}^{15}NO_3$) was labelled, in plants irrigated regularly (control) and plants waterlogged for 24 h (24 h-WL) are shown. Results are means of 5 replicates. Numbers above bars represent total percentage ^{15}N recovery in plant and growing media and different letters express significant differences ($p<0.05$)

recovery of ^{15}N from the differently labelled nitrogen sources varied in plant and medium. Significantly more ^{15}N was recovered when added as double labelled $^{15}NH_4{}^{15}NO_3$ in the control treatment compared to when added as $^{15}NH_4NO_3$ after 24 h of waterlogging (Fig. 3).

Oxygen profiles

In the control treatment, oxygen concentrations corresponded to ambient oxygen concentration throughout the entire profile and were independent of the degree of compaction. When measured immediately after cessation of the 24 h-WL treatment and drainage of the pots, the oxygen concentrations in the bottom 20 mm of the non-compacted media had decreased to around 20% of ambient levels (Fig. 4a). In the compacted media the zone of low oxygen were deeper with the bottom 30 mm having lower oxygen concentrations resulting in almost anoxic conditions at the bottom of the pots (Fig. 4b). After 6 and 12 h oxygen was present throughout the profile corresponding to concentrations determined in the control treatment. A similar pattern was seen after 5 d of waterlogging although the oxygen concentrations found were lower than after 24 h of waterlogging (Fig. 4c and d). In the non-compacted media the concentrations decreased significantly in the bottom 30 mm of the pots, with concentrations around 10% of ambient oxygen content in the bottom 10 mm (Fig. 4c). After 12 and 24 h oxygen had re-entered the medium but concentrations were still lower compared to the control and the 24 h-WL treatment. In the compacted media, anoxic conditions were determined in the bottom 10 mm of the pot, and decreased availability of oxygen was seen in the bottom 40 mm (Fig. 4d). After 12 h, concentrations were still low whereas ambient oxygen conditions were found after 24 h.

Physical properties

All physical properties determined; air filled pore space, gas diffusion and air permeability, were significantly greater ($p<0.05$) in the non-compacted media compared

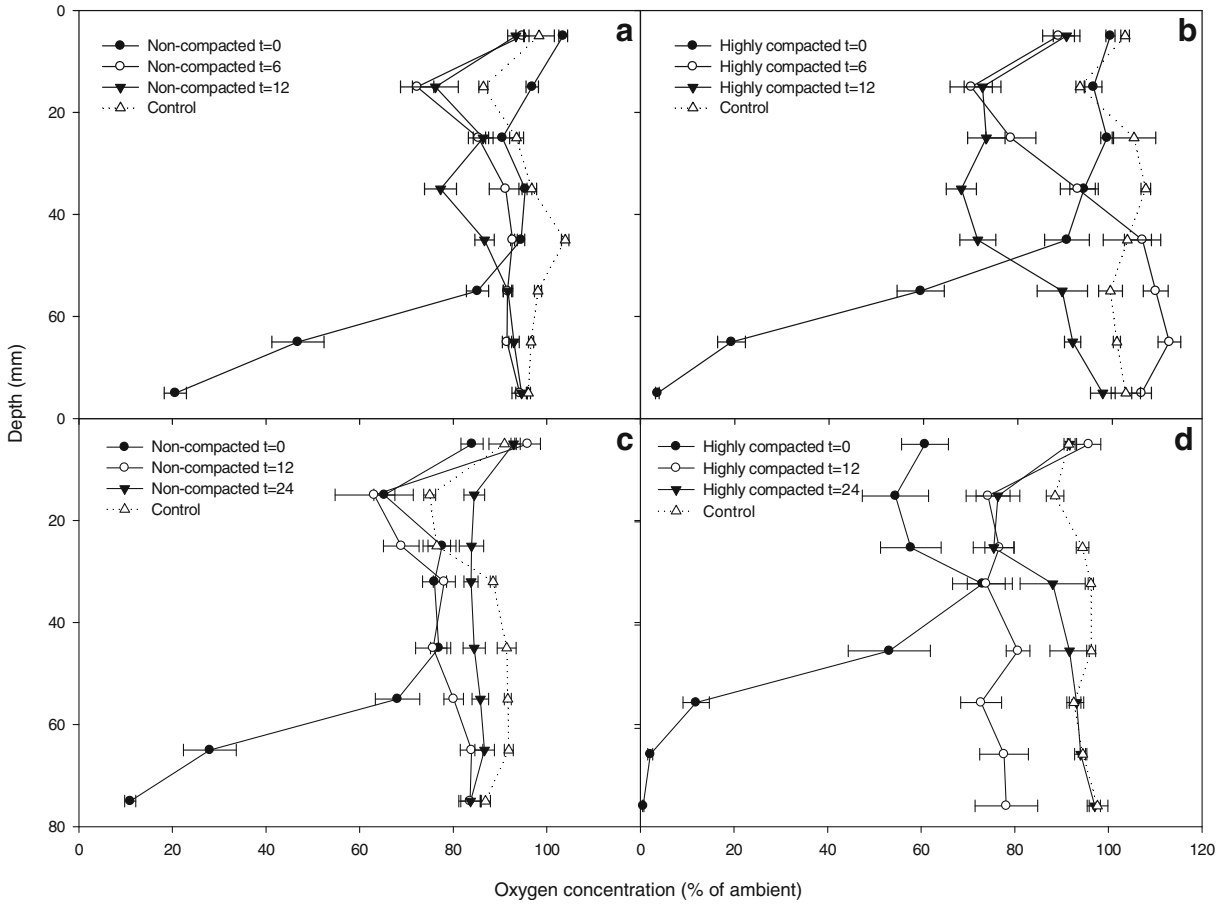

Fig. 4 Oxygen profiles of either non-compacted (**a** and **c**) or highly compacted (**b** and **d**) growing media of 5 week old tomato plants. (**a** and **b**) waterlogged for 24 h (24 h-WL treatment); immediately after cessation of waterlogging (t=0) and 6 and 12 h after water was removed (t=6 and t=12) and control treatment irrigated regularly (c and d) waterlogged for 5 days (5 d-WL treatment); immediately after cessation of waterlogging (t=0) and 6 and 12 h after water was removed (t=6 and t=12) and control treatment irrigated regularly. Results are means of two simultaneous measurements in 3 replicate pots and error bars represent the standard error

to the highly compacted when determined at three different volumetric water contents, 0.57, 0.56 and 0.47 m^3 m^{-3} for the highly compacted media and 0.52, 0.51 and 0.34 m^3 m^{-3} for the non-compacted media. These water contents corresponded to the water contents immediately after cessation of the 5 d-WL treatment, the 24 h-WL treatment and the control treatment respectively. Gas diffusion and air permeability was approximately twice as high in the non-compacted media (Table 1). Despite the differences in air filled pore space, gas diffusion and air permeability the water release curves determined on either the non-compacted or highly compacted media did not differ significantly (data not shown). The physical properties did not differ considerably between the two waterlogging treatments whereas the control treatment had lower volumetric water content and thus an increased gas diffusion and air permeability.

Plant parameters

No significant differences were found in the dry weight between the three treatments. However, the dry matter percentage increased significantly ($p<$ 0.05) when plants were waterlogged for 5 d (Table 2). The degree of compaction did not affect the plant size or dry matter percentage. Due to the significant difference in dry matter percentage, starch content was

Table 1 Physical properties of non-compacted and highly compacted growing media immediately after cessation of the waterlogging treatments and in the control treatment. Values are compared to values determined at −100 hPa. Results are means of 10 replicates and brackets show standard error. Results with different letters within each parameter are significantly different ($p<0.05$)

	Highly compacted	Non-compacted
Porosity ($m^3\ m^{-3}$)		
	0.87 (0.002)^{b}	0.88 (0.002)^{a}
Air permeability (μm^2)		
5-days-waterlogged	58.9 (6)^{e}	134.0 (15)^{cd}
24-hours-waterlogged	62.1 (6)^{e}	146.6 (17)^{bc}
Control	82.9 (7)^{e}	302.0 (36)^{a}
−100 hPa	89.0 (7)^{de}	196.4 (24)^{b}
Gas diffusion ($m^2\ s^{-1}$)		
5-days-waterlogged	7×10^{-7} (5×10^{-8})^{d}	1.5×10^{-6} (1×10^{-7})^{c}
24-hours-waterlogged	8×10^{-7} (5×10^{-8})^{d}	1.6×10^{-6} (1×10^{-7})^{c}
Control	1.6×10^{-6} (7×10^{-8})^{c}	3.3×10^{-6} (7×10^{-8})^{a}
−100 hPa	1.7×10^{-6} (6×10^{-8})^{c}	2.3×10^{-6} (1×10^{-9})^{b}
Air filled pore space ($m^3\ m^{-3}$)		
5-days-waterlogged	0.3 (0.007)^{e}	0.4 (0.008)^{d}
24-hours-waterlogged	0.3 (0.007)^{e}	0.4 (0.007)^{d}
Control	0.4 (0.006)^{c}	0.5 (0.005)^{a}
−100 hPa	0.4 (0.006)^{c}	0.5 (0.006)^{b}

determined on the dried and ground material although this is not the optimal pre-treatment before starch analysis. However, it was shown that the starch content in leaves and stem increased with waterlogging (Table 2). For the highly compacted media the starch content was significantly higher ($p<0.05$) in the waterlogged treatments compared to the control, and the 5 d-WL plants had significantly higher ($p<0.05$) starch content than the 24 h-WL plants (Table 2). The leaves of the 5 d-WL plants showed physical signs of the higher starch content by having darker more coarse and crumpled leaves compared to the control and the 24 h-WL treatment.

Discussion

Root system activity was shown to differ within small distances of 40 mm between top and bottom layers of pots with 5 week old tomato plants. Under standard conditions with regular irrigation cycles the root system activity was greater in the bottom of the pots compared to the top. Root growth and uptake activity have previously been shown to be significantly greater in young roots (Volder et al. 2009) and as the bottom of the pots probably had the majority of new roots due to geotropism and higher nutrient and water levels, a higher growth rate and uptake activity was expected in

Table 2 The dry weight, dry matter content, nitrogen concentration and starch content of tomato plants irrigated regularly (control), waterlogged for 24 h (24 h-WL) or waterlogged for 5 days (5 d-WL). Results are means of 5 replicates and numbers in brackets show standard deviation. Results with different letters within each column are significantly different ($P<0.05$)

Compaction	Treatment	Dry weight (g)	Dry matter content (%)	Nitrogen (%)	Starch (μmol Glu/g dw)
Non-compacted	Control	8.3 (1.8)	23.3 (2.7)^{b}	3.5 (0.5)^{a}	694^{c} (58)
	24 h-WL	8.2 (1.6)	23.5 (1.3)^{b}	3.4 (0.5)^{a}	835^{bc} (79)
	5 d-WL	8.6 (1.8)	28.8 (2.3)^{a}	2.2 (0.3)^{b}	1414^{a} (91)
Highly compacted	Control	8.6 (2.3)	23.7 (2.3)^{b}	3.5 (0.5)^{a}	722^{c} (194)
	24 h-WL	8.5 (2.4)	24.1 (2.4)^{b}	3.4 (0.8)^{a}	971^{b} (144)
	5 d-WL	8.4 (2.6)	30.5 (2.6)^{a}	2.3 (0.5)^{b}	1322^{a} (106)

this layer. In addition, a larger total root length could be expected in the bottom layer leading to higher total uptake. However, in the highly compacted media a more similar root uptake in the top and bottom layer was observed compared to the non-compacted media. As compaction of peat based growing media increased pore continuity and capillarity and thus improved water and nutrient distribution in the medium (Dresbøll and Thorup-Kristensen 2011), this was expected to result in a more even distribution of roots in the medium, with a larger root density and activity in the top layers compared to top layers of non-compacted media. In addition, water content was lower in the non-compacted media and lowest in the top layer, which could result in a decrease in root system activity compared to the highly compacted media.

Short-term waterlogging (24 h) resulted in low oxygen concentrations in the bottom layer of the pots and root uptake decreased significantly. Plant roots are dependent on oxygen to maintain physiological processes such as nutrient uptake (Morard and Silvestre 1996). The duration of waterlogging is critical as the roots can either survive with decreased physiological activity or simply die if waterlogging conditions proceed (Morard and Silvestre 1996). During short-term waterlogging most roots would be able to survive in a standby state (Morard and Silvestre 1996), however, anoxic conditions for more than a few days would result in root damage. Under longer term waterlogging, roots would adapt to the conditions and produce new roots with increased aerenchyma formation (Drew 1997). However, when waterlogging only part of the root system the translocation of carbon from the shoot to the anoxic roots was shown to decrease while an increase of carbon translocation to the aerated roots was observed as compared to a fully aerated control (Schumacher and Smucker 1985). Thus, plants grown with heterogeneous soil conditions where parts of the root system experience suboptimal conditions are able to compensate through increased activity and growth in other parts of the root system where conditions are not limiting. The results from this study revealed an increased uptake in the growing media layer that was not directly affected by the water stress. This could be explained by tomato plants actively overcoming short-term waterlogging by compensating with greater activity per root length in non-affected roots as N demand was unaltered during short-term stress or by increasing root length and hence the total uptake. Alternatively, it could simply be explained by improved water conditions in the layer above waterlogging increasing root growth and activity. However, as control treatments were irrigated regularly the latter seems unlikely. N uptake was determined as ^{15}N translocated to shoots, hence, N might also have been taken up by roots but not translocated to the shoot due to lack of respiratory energy caused by the anoxic conditions.

Six hours after the water had been removed, oxygen had re-entered the medium and root uptake in the bottom of the pots increased to levels well above what was determined prior to waterlogging. Roots may have accumulated sugars during the waterlogging, and when oxygen re-entered after drainage this could provide sufficient energy for the increased uptake. The low root system activity in the entire pot 12 h after the water was removed, was probably due to drying out of the medium. As experiments were carried out on warm sunny days and greenhouse temperatures increased up to 30°C, most of the water had been used or evaporated from the pots and in the evening the water content was low, which potentially resulted in a decrease in root system activity. Alternatively, the decrease could be a result of a diurnal pattern where nutrient uptake is lower late in the evening when photosynthesis decreases. Diurnal variation in net rate of NO_3^- uptake was determined on four different grass species showing that NO_3^- uptake was highest during the middle of the light period and lower during the dark period (Scheurwater et al. 1999). A circadian rhythm for root growth have also been shown in *Arabidopsis Thaliana* with lowest growth rates 10 h after initiation of the light period (Yazdanbakhsh and Fisahn 2011). Neither plants nor roots appeared to be affected by the short-term waterlogging more than a temporary decrease in root uptake and a temporary increase in root uptake above the waterlogged zone.

Long-term waterlogging (5 d), affected root system uptake more severely than the short-term waterlogging. Plant uptake decreased in the entire root system indicating that the prolonged waterlogged conditions had more than a direct effect of creating an anoxic zone in the bottom layer as shown by the oxygen measurements. During anoxia different phytotoxic compounds may be produced such as ethylene which decreases root growth (Visser et al. 1997). Production of phytotoxic compounds would probably affect the entire root system and not just the waterlogged roots. Twelve hours after the water had been removed the root system activity in

both the bottom and top layer was still low, and even after 24 h only a slight increase was observed. Under these conditions, a diurnal pattern could also affect root activity. However, then root N uptake would have been expected to increase again 24 h after the long-term waterlogging treatment, as root activity and growth was shown to increase after the dark period (Scheurwater et al. 1999; Yazdanbakhsh and Fisahn 2011). The medium did not dry out as fast after the 5 d-waterlogging compared to the 24 h-waterlogging, indicating lower water uptake by the plants, and therefore a drying out of the medium is unlikely to have caused the continuous low N uptake activity. This suggested that the roots had suffered from the long-term waterlogging and that new root growth in the previously waterlogged zone might be necessary to restore activity.

The degree of compaction did not affect the root system activity significantly although it showed lower activity in the bottom layer compared to the non-compacted media. Compaction likely lead to reduced oxygen diffusion and more frequent anoxic conditions in the bottom of the pots, thus slowing down root growth in the bottom leading to a more even root distribution compared to the uncompacted media. In peat based growing media compaction has previously been found to affect oxygen and water distribution without affecting overall plant quality (Dresbøll and Thorup-Kristensen 2011). Oxygen content, gas diffusion and air permeability was also shown to be affected by compaction in this study whereas the water holding capacity did not seem to be significantly influenced.

Short-term waterlogging showed no visible effect on the aboveground plant biomass, as roots in the upper layer probably compensated for the lower uptake in the bottom. Visible effects were observed in response to long-term waterlogging; the tomato plants showed signs of epinasty, they were darker and had coarse and crumpled leaves as compared to control plants probably due to ethylene production and the increased starch content. In general, the amount of soluble sugars and starch increases in leaves of waterlogged plants (Gonzalez et al. 2009). As the root growth and activity decrease, sink capacity for sugars produced in leaves becomes limited and plants will accumulate starch and soluble sugars in the leaves, leading to an increased dry matter percentage and decreased concentrations of N and other nutrients in the dry matter. After long-term waterlogging, the roots also showed visible signs of the suboptimal conditions being dark and shrivelled.

The use of stable isotopes (^{15}N) for determining root uptake was shown to be applicable even in small pots when short-term uptake was determined in a possible denitrifying environment. Two main concerns of using ^{15}N in these small waterlogged pots were the risk of diffusion of NO_3^- away from the injection depth and the risk of denitrification due to anoxic conditions in the lower layer of the pots. In order to diminish these risks and to ensure that oxygen levels were not significantly changed plants were harvested after just two hours of uptake. Average NO_3^- diffusion rates in soils have been estimated to be 5×10^{-11} m^2 s^{-1} corresponding to a movement in soil of 3 mm d^{-1} (Jungk 1991). Even though movement of NO_3^- was expected to be greater in the porous peat media with high water contents, it indicates that movement of NO_3^- by diffusion would not affect the results within the limited time provided for N uptake in these experiments and with a distance between injection depths of at least 40 mm. Direct measurements of diffusion or convection, by dividing the medium in two or more layers, were complicated due to the possibility of potentially recovering ^{15}N transported in roots, which had been taken up in another layer. Significant differences between the uptake in the top and bottom layers revealed that the method allowed distinction of uptake activity across a short distance. When conditions become anoxic, denitrification can occur in the medium where NO_3^- is reduced and lost as N_2. Potential denitrification rates in limed and fertilized peat based growing media were determined to be in the range from 200 to 500 μg L^{-1} dry peat h^{-1} (Amha and Bohne 2011). Thus, it was expected that a small amount of the applied N would be lost due to denitrification. However, with the amount of N provided and the short time span this was not expected to influence plant uptake, as confirmed by the results. When determining ^{15}N content in plant material as well as growing media, not all of the injected ^{15}N could be recovered. The amounts lost did not exceed expected losses due to handling and measurements, and were within the ranges reported in other studies (e.g. Blumfield and Xu 2006). In order to diminish the risk of denitrification, ^{15}N was applied as labelled NH_4^+. However, the use of either single or double labelled NH_4NO_3 revealed only few significant differences in subsequent ^{15}N recovery. In fact, the ^{15}N recovery was lowest in the treatment where NH_4^+ had been applied after 24 h of waterlogging, and

only small amounts of NH_4^+ were detectable in the plants compared to labelled NO_3^- or double labelled NH_4NO_3. Surprisingly, the NO_3^- uptake was proportionally more reduced by waterlogging than the NH_4^+ uptake. Tomato plants readily take up both NH_4^+ and NO_3^- (Evans et al. 1996). NH_4^+ assimilation typically occurs in the roots before translocation to above ground plant material whereas NO_3^- can be transported to the leaves before reduction and incorporation (Andrews 1986). This suggests that NO_3^- can be detected in the above ground biomass faster than NH_4^+, and due to the short time span here uptake of NH_4^+ alone would be too slow.

In conclusion, when tomato plants were subjected to short-term stress in part of the root system they were shown to increase uptake in roots above the waterlogged layer suggesting either increased activity in present roots or an increased root growth. However, when tomato plants were subjected to long-term waterlogging, root uptake was negatively affected in the entire medium and also the above ground plant parts showed a clear stress response. Root uptake recovered within 6 h after cessation of the short term waterlogging, but had not recovered 24 h after cessation of the long term waterlogging. Compaction of the medium influenced the oxygen availability as well as gas diffusion and permeability of the media while the effect on root uptake was limited. Finally, ^{15}N was shown to be an efficient tool in determining differences in root uptake within short distances in small pots and even when media were anoxic for a period of time.

Acknowledgements We thank Helle Kjærsgaard Sørensen and Ruth Nielsen for skilful technical assistance. In addition, we are grateful to Bo Vangsø Iversen for valuable discussions on determination of physical properties of growing media. Funding was provided from The Danish Council for Independent Research | Technology and Production Sciences (Ref. no. 09–061045).

References

Amha Y, Bohne H (2011) Denitrification from the horticultural peats: effects of pH, nitrogen, carbon, and moisture contents. Biol Fertil Soils 47:293–302

Andrews M (1986) The partioning of nitrate assimilation between root and shoot of higher plants. Plant Cell Environ 9:511–529

Blumfield TJ, Xu ZH (2006) *Araucaria cunninghamii* seedling response to different forms and rates of 15N-labelled fertilizer. Pedosphere 16:745–755

Dresbøll DB (2010) Effect of growing media composition, compaction and periods of anoxia on the quality and keeping quality of potted roses (Rosa sp.). Sci Hort 126:56–63

Dresbøll DB, Thorup-Kristensen K (2011) Spatial and temporal oxygen distribution measured with oxygen microsensors in growing media with different levels of compaction. Sci Hort 128:68–75

Drew MC (1997) Oxygen deficiency and root metabolism: Injury and acclimation under hypoxia and anoxia. Annu Rev Plant Physiol Plant Mol Biol 48:223–250

Evans RD, Bloom AJ, Sukrapanna SS, Ehleringer JR (1996) Nitrogen isotope composition of tomato (Lycopersicon esculentum Mill Cv. T 5) grown under ammonium or nitrate nutrition. Plant Cell Environ 19:1317–1323

Flint LE, Flint AL (2002) Porosity (2.3.2.3. Volumetric method with gas pycnometry). In: Dane JH, Topp GC (eds) Methods of soil analysis. Part 4. Physical methods. SSSA, Madison, pp 241–254

Gonzalez JA, Gallardo M, Hilal M, Rosa M, Prado FE (2009) Physiological responses of quinoa (*Chenopodium quinoa* Willd.) to drought and waterlogging stresses: dry matter partitioning. Bot Studies 50:35–42

Gregory PJ, Bengough AG, Grinev D, Schmidt S, Thomas WTB, Wojciechowski T, Young IM (2009) Root phenomics of crops: opportunities and challenges. Func Plant Biol 36:922–929

Grover BL (1955) Simplified air permeameters for soil in place. Soil Sci Soc Am J 19(4):414–418

Jungk A (1991) Dynamics of nutrient movement at the soil-root interface. In: Waisel J, Eshel A, Kafkafi U (eds) Plant roots, the hidden half. Marcel Dekker, New York, pp 455–481

Kläring HP, Zude M (2009) Sensing of tomato plant response to hypoxia in the root environment. Sci Hort 122:17–25

Kristensen HL, Thorup-Kristensen K (2004) Root growth and nitrate uptake of three different catch crops in deep soil layers. Soil Sci Soc Am J 68:529–537

Morard P, Silvestre J (1996) Plant injury due to oxygen deficiency in the root environment of soilless culture: a review. Plant Soil 184:243–254

Morard P, Lacoste L, Silvestre J (2000) Effect of oxygen deficiency on uptake of water and mineral nutrients by tomato plants in soilless culture. J Plant Nutr 23:1063–1078

Nkongolo NV, Caron J (1999) Bark particle sizes and the modification of the physical properties of peat substrates. Can J Soil Sci 79:111–116

Scheurwater I, Clarkson DT, Purves JV, Van Rijt G, Saker LR, Welschen R, Lambers H (1999) Relatively large nitrate efflux can account for the high specific respiratory costs for nitrate transport in slow-growing grass species. Plant Soil 215:123–134

Schjønning P (1985) A laboratory method for determination of gas diffusion in soil. The Danish Institute of Agricultural Sciences, Tjele, (In Danish with English summary) Rep. No. S1773, p 19

Schumacher TE, Smucker AJM (1985) Carbon transport and root respiration of split root systems of *Phaseolus vulgaris* subjected to short term localized anoxia. Plant Physiol 78:359–364

Taylor SA (1949) Oxygen diffusion as a measure of soil aeration. Soil Sci Soc Am Proc 44:55–60

Topp GC, Zebchuk W (1979) The determination of soil water desorption curve for soil cores. Can J Soil Sci 59:19–26

Visser EJW, Nabben RHM, Blom CWPM, Voesenek LACJ (1997) Elongation by primary lateral roots and adventitious roots during conditions of hypoxia and high ethylene concentrations. Plant Cell Environ 20:647–653

Volder A, Anderson LJ, Smart DR, Bloom AJ, Lakso AN, Eissenstat DM (2009) Estimating nitrogen uptake of individual roots in container- and field-grown plants using a ^{15}N-depletion approach. Func Plant Biol 36:621–628

Wojciechowski T, Gooding MJ, Ramsay L, Gregory PJ (2009) The effects of dwarfing genes on seedling root growth of wheat. J Exp Bot 60:2565–2573

Yazdanbakhsh N, Fisahn J (2011) Stable diurnal growth rhythms modulate root elongation of Arabidopsis thaliana. Plant Root 5:17–23

Zanne AE, Lower SS, Cardon ZG, Orians CM (2006) ^{15}N partitioning in tomato: vascular constraints versus tissue demand. Func Plant Biol 33:457–464