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Physiological responses of basil to low oxygen availability in soilless culture

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ABSTRACT

Soilless cultivation systems in controlled environment agriculture create varying rootzone oxygen conditions, which can impact biomass production. The present study investigates the effect of mild hypoxia and its effect on shoot biomass production, root anatomy and architecture of basil (*Ocimum basilicum L.*) in ebb-flood, deep-water, and aeroponic systems in a greenhouse. After 4–5 weeks, aeroponically grown plants produced greater shoot biomass than those in the ebb-flood system, although root dry weights did not differ significantly. Using optical coherence tomography (OCT), we non-destructively quantified aerenchyma formation, confirming the suitability of OCT for imaging living root tissues. Aerenchyma developed 14–21 days after transplanting and were more extensive in unaerated deep-water systems than in aerated or aeroponic systems. Ethylene emission 20 days after transplanting did not differ between treatments. Thus, basil shows adaptative responses at mild hypoxia, which can lead to yield losses. This emphasises the need to define rootzone oxygen thresholds.

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KEYWORDS
Aeroponics; hydroponics; controlled environment agriculture; optical coherence tomography; aerenchyma

Introduction

Basil (*Ocimum basilicum L.*) is often cultivated in controlled environment agriculture (CEA) such as greenhouses and vertical farms, to promote season extension, enable pot-grown product, or improve productivity relative to resource input. In commercial CEA operations, the manipulation of environmental factors is commonly conducted to improve crop yield, quality, and health. This can include different factors such as light intensity and wavelength, air temperature, carbon dioxide (CO_2), airflow, humidity, nutrient concentration and availability, pH and electrical conductivity of the nutrient solution and root zone oxygen (Blok et al., 2017; Kaiser et al., 2024). This list illustrates that CEA facilities often have more options to adjust the shoot environment than the root environment. However, the root provides the shoot with water and nutrients, hence optimal root function is crucial for optimal crop performance (Tumber-Dávila et al., 2022).

Rootzone conditions can be (semi-)controlled in hydroponic and aeroponic systems. Hydroponic systems, predominantly ebb-flood or deep-water culture, are used to irrigate the crops with nutrient-rich solution throughout their lifecycle (Eldridge et al., 2020). In ebb-flood systems, plants are regularly flooded with nutrient solution until the growing substrate becomes saturated. The substrate slowly dries until the next irrigation cycle, whilst the plant takes up water, which enables air pockets to emerge within the micropore structure of the substrate (e.g. coir, peat, rockwool) (Allaire et al., 2005).

In deep-water culture systems, the roots remain constantly submerged in nutrient solution. The nutrient solution is often enriched with oxygen by pumping air (or gaseous oxygen) into the water column and mixing the solution well throughout (Eldridge et al., 2020). This serves to prevent root respiration depleting the available oxygen within the solution. This aeration of the rootzone helps to improve plant biomass production (Mobini et al., 2015; Zeroni et al., 1983).

In aeroponic systems, a thin substrate can be used for sowing, because the roots are subsequently growing freely into an air cavity filled with aerosol. The aerosol consists of aerosolised nutrient solution of diameter 1–100 µm, which dynamically deposits into a thin film of nutrient solution around the roots (Eldridge et al., 2020). The aerosol is either created by spraying the nutrient solution through very fine nozzles or by using ultrasound to cavitate a solution into an aerosol. The small droplet size in the aerosol and the microfilm around the roots can allow for optimal diffusion of oxygen into or across the solution (Eldridge et al., 2020). Hence, it is widely assumed that the better oxygen availability in the rootzone of

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aeroponic systems improves biomass production compared to hydroponic systems.

Each system is different but all aim to achieve the same objective: delivering the nutrient solution and oxygen required to develop a healthy root stock for the crop of choice. However, few studies have made direct comparisons between these systems and their results are conflicting. In some studies, plant performance was better in the aeroponic system. For example, potatoes produced more tubers and higher total yield per plant (Ritter et al., 2001) while chrysanthemum cuttings produced a higher biomass in an aeroponic system than in a deep-water system (Blok et al., 2017). In contrast, lettuce produced a lower yield when plants were grown in aeroponic systems compared to a hydroponic system (El-Ssawy et al., 2020). Other studies could not confirm differences in plant performance between the systems. For example, basil grown in an aeroponic system and a deep-water system did not differ in shoot biomass, but plants in both systems produced a higher fresh weight than in drip irrigated peatmoss slabs (Khater et al., 2021). The different response could be a result of different growing conditions or different plant species. However, none of these studies mechanistically discusses the impact of the root environment on plant productivity.

In the roots, energy is provided through oxygen consuming respiratory processes or through glycolysis and fermentation processes when oxygen availability is limited (Voesenek et al., 2006). Diffusion and solubility of oxygen in water are much lower than in air (Jackson et al., 1985). Oxygen saturated water has a dissolved oxygen concentration of around 250 µmol L^{-1} (or 8.6 ppm) at 23°C (Chun & Takakura, 1994). In comparison, air with 21% oxygen has a concentration of 9300 µmol L^{-1} (or 206,152 ppm) at 20°C. The oxygen concentration in well-aerated soil or growing substrate will average between those values depending on the amount and size of air-filled spaces. Thus, roots in flooded soil or growing systems with continuously submerged roots are subjected to lower oxygen environments than in most other systems and may suffer from hypoxic conditions (Lin et al., 2021). However, it is unclear what oxygen concentrations in the rootzone ensure optimal root functioning and plant growth.

When roots are flooded, root internal oxygen concentrations decline with declining external oxygen availability. Concurrently, ethylene is trapped inside the root because the diffusion rate of ethylene in water is orders of magnitude lower than in air (Vandenbussche et al., 2012). This can lead to changes in root system architecture (growth angle, lateral root formation), aerenchyma formation, as well as reduced root and shoot growth (Daniel & Hartman, 2024; Voesenek & Sasidharan, 2013). However, most of these studies have been conducted at severely hypoxic conditions to induce clear responses. In horticultural production, environmental conditions are controlled to various degrees in order to optimise yield and quality and to balance resource input with monetary value of the crop. Hence, CEA growers need to know thresholds when conditions noticeably affect the crop. For example, mature lettuce plants (26 days after transplanting) seem to downregulate root respiration at dissolved oxygen concentrations below 78 µmol L^{-1} (Chun & Takakura, 1994), suggesting that root function is also impeded under these conditions. Therefore, it is important to investigate plant responses to mild hypoxia, while using the knowledge of responses under severely hypoxic conditions, in order to understand how much oxygen in the rootzone is needed for optimal plant growth at different stages of development.

In addition, aerenchyma formation is usually determined by taking root cross sections (Vidoz et al., 2016), which is a destructive method, limits the field of view to the one cell layer in the cross section, and does not allow for longitudinal studies. Instead of taking physical root cross-sections, we use optical coherence tomography (OCT) to non-invasively obtain microscopic 3D images of the root internal structure. OCT imaging is a technique initially developed for human medicine and its application in plant sciences has so far been limited. However, it is seen as a promising tool for various applications in plant science, horticulture and postharvest quality (Li et al., 2019; Saleah et al., 2024). It can provide a 3D view of the internal plant tissue morphology through multiple cell layers of the root without cutting the root tissue and without embedding it in another material, thereby saving preparation time and allowing for longitudinal studies. The capability of 3D imaging allows for inspection of larger regions than a randomly chosen cross-section and enables tissue thickness and cell size quantification (de Wit et al., 2020). Therefore, OCT imaging is a useful tool to non-destructively quantify aerenchyma formation by determining the number and the length of the air-filled pore spaces in a specific section of the root, delivering data that is impossible to obtain by traditional cross-section microscopy.

In addition to rootzone oxygen, flooding influences a number of factors in the rootzone on the physical, chemical and biological level and the role of some of these factors in flooding responses are not yet understood (Daniel & Hartman, 2024). In CEA, many of these factors can be controlled or mitigated. Therefore, research in CEA improves understanding of crop performance and helps growers to optimise growing conditions and it provides useful insight into mechanisms that are difficult to study in the field due to confounding factors.

The aim of the present study is to understand the effect of oxygen availability in the rootzone as an individual factor on shoot biomass production, root anatomy and architecture using soilless growing systems and to get a better understanding of aerenchyma formation and development under low rootzone oxygen conditions using OCT imaging.

Material and methods

Two experiments were conducted in a 64 m^2 greenhouse compartment at Wageningen University, located in the Netherlands (51°59'12.8"N 5° 39'38.0"E). Climate conditions in both experiments were 26/ 20°C (day/night temperature respectively), 70% relative humidity and additional lighting with high-pressure sodium lamps was supplied when the outer solar radiation decreased below 250 W m^{-2} s^{-1} within the 18 h daytime period. Fertigation (EC 1.7, pH 6, for detailed composition see Supplementary Table S1) was conducted from a central tank (water temperature 20–26 °C), with solution not recirculated for the sake of consistency.

Experiment 1: comparison of three soilless growing systems

This experiment evaluated relative performance and plant physiology between ultrasonic aeroponic, deep-water culture hydroponic, and ebb-flood hydroponic methods. Sweet basil (*O. basilicum* L.) 'Salvo F1' (CN Seed, Ely, UK) was sown using two sowing methods per irrigation system to boost commercial relevance of the trial, with basil either placed at 7 plants per bunch into rockwool plugs (Grodan, Roermond, NL) or onto jute fibre mats (HollandBioProducts, Elst, NL) with a final density of 1021 plants per m^2 or broadcast evenly onto jute fibre mats at a density of about 1000 seeds per m^2. Each system (aeroponic, deep water, ebb-flood) provided 11.6 m^2 growing area, with trays of 0.24 m^2 placed onto the surface to hold the growing media, namely jute fibre mats for the aeroponic beds and deep-water beds and broadcast sowing for the ebb-flood table and rockwool cubes for the ebb-flood system for sowing in bunches (7.5 × 7.5 cm; Grodan, Roermond, the Netherlands). In the ebb-flood system tables were flooded every 1–2 days for 10 minutes depending on plant size. The deep-water system consisted of individual boxes with 50 L nutrient solution for each tray as a proxy for commercial production in large water basins. Three litres of nutrient solution were replaced in the individual 50 L tanks every day. In the aeroponic system, fresh nutrient solution was provided every hour, and the atomisers were set to 4/ 4 min (duration on/off respectively) (8 ultrasonic atomisers per m^2 bed).

Seeds were germinated in the dark for 4 days at 100% humidity, 23–24°C. Four replicates in time were grown successively between July and November 2022. A replicate is a batch of plants grown at the same time. All treatments have been grown in each replicate batch. Each replicate in time was harvested once all systems reached a stem length of approximately 20 cm, which was achieved 4–5 weeks (28 or 35 days) after transplanting them (or moving the trays) into the greenhouse. Three trays per treatment were harvested (25 × 45 cm per tray, Figure 1) and number of plants, leaf area (LA), shoot fresh weight, shoot dry weight, and root dry weight were determined.

Experiment 2: effect of oxygen availability in the rootzone on root anatomy and physiology

Experiment 2 evaluated the differences in plant physiology between basil plants grown in the

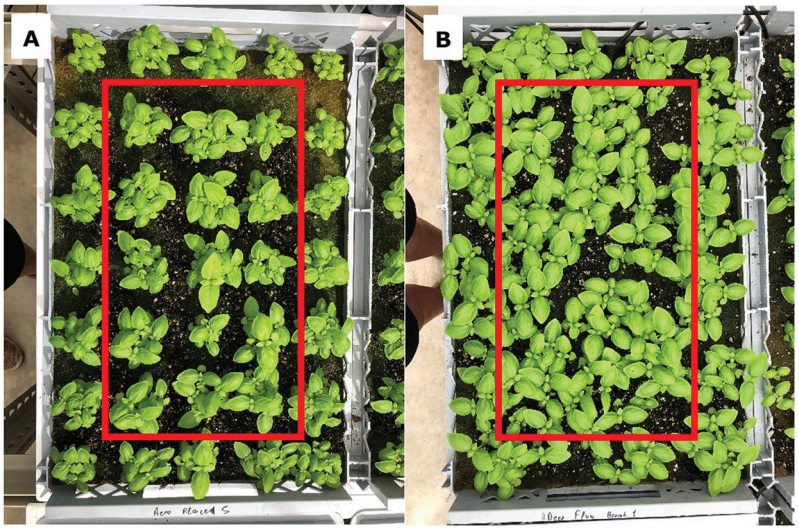


Figure 1. Growing trays with basil in placed bunches (A) and broadcast sowing (B). Red squares indicate the harvest area.

aeroponic system and the deep-water system with or without aeration (3 treatments). The experiment was conducted in the same greenhouse compartment as experiment 1, only in the middle row of the compartment. Treatments were applied per bed (4 trays per bed) and fully randomised within one replicate in time. Sweet basil (*O. basilicum* L.) 'Salvo F1' (CN Seed, Ely, UK) seeds were sown onto jute fibre matting (BlueMat 400, HollandBioProducts, Elst, NL) in a grid of 7×10 plants per tray (292 plants per m^2) and germinated for 4 days at 100% humidity, 23–24°C. Individual replicates in time were sown at one-week-intervals. The trays were then moved into the greenhouse, which is considered as timepoint of transplanting. In contrast to experiment 1, the same type of bed was used for the aeroponic system, and the hydroponic systems. These beds accommodate 4 trays in a row on the same bed with a total rootzone volume of 40 L per bed. The nutrient solution was completely exchanged every hour in the aeroponic system and once a day in the aerated deep-water system. In the unaerated deep-water system, 3 L were exchanged manually on a daily basis with normally oxygenised nutrient solution to maintain EC and pH levels similar to the other treatments. Oxygen concentration in the rootzone was tracked using optical sensors (MiniOptode, Unisense, DK). Representative data in Figure 2 shows that in the unaerated deep-water culture, oxygen concentrations fluctuated over the course of a day. However, over several days the oxygen concentration decreased, despite the daily partial exchange of nutrient solution.

In each replicate in time, individual plants from three different trays were harvested at 14 days and 21 days after transplanting. Shoot fresh and dry weights were measured at every harvest. Root architecture and traits like total root length and root thickness were analysed using 2D rootscans of the whole root system of one plant and the RhizoVision Software. To measure aerenchyma presence and length, 3D rootscans of the main root were performed on a 4-mm-long section of the root located 2–3 cm from the base of the root. This ensures that data from both harvest dates is in a comparable area of the root that is not growing in length any longer. The 3D OCT scans were taken using a Ganymede II HR OCT setup (Thorlabs, Dortmund, Germany) with a central wavelength of 900 nm, operating at an A-line rate of 36 kHz, and using the OCT-LK3-BB scan lens. The resolution of the system was around 5.6 µm in the lateral and 2.1 µm in the axial direction (in tissue). The root was immersed in water and covered by a coverslip to reduce the refractive index contrast of the root with its direct surroundings. The 2D cross-sectional images were obtained along the root over a length of 4 mm with 1024 A-lines (spacing of 3.9 µm) and 3D images were taken over an en-face area of 2 mm ×4 mm along the root with 256×512 A-lines (7.8 µm spacing). Each A-line is repeated 4 times and averaged to increase the signal-to-noise ratio. The 3D images covered approximately the full upper side of the root, while the bottom side was not imaged due to limited imaging depth. Image acquisition was done with the ThorImage 5.2 software. Aerenchyma length and number were obtained from the measurement on 2D images in ThorImage. Length is the longest observed distance between two edges of the aerenchyma along the root direction.

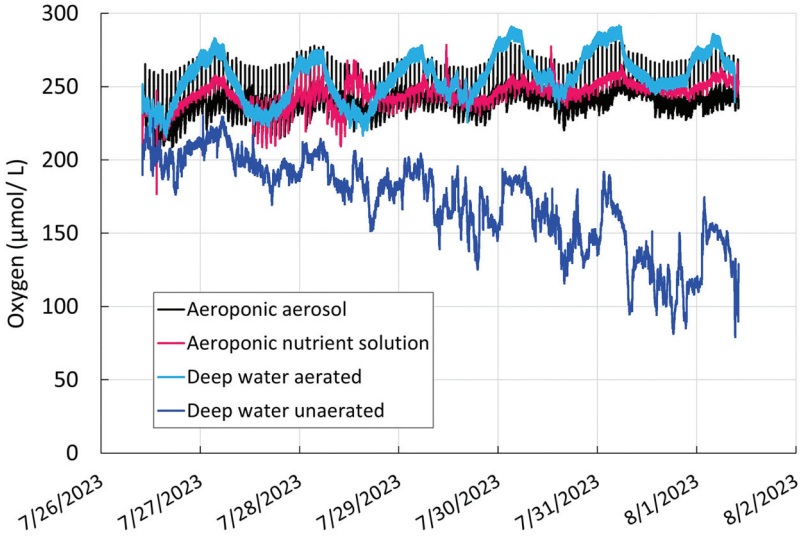


Figure 2. Oxygen concentration in the rootzone of the different growing systems in experiment 2. Oxygen concentrationwas measured in the nutrient solution of the deep flow systems (aerated: light blue, unaerated: dark blue) and the aeroponic systems (bottom of the bed, dark pink) and in the aerosol of the aeroponic system (black). Data is shown for replicate 1. For comparison, air with 21% oxygen has a concentration of 9300 µmol L^{-1} at 20°C.

Statistical analysis

Both experiments had a randomised block design, where replicates in time form the blocks and trays are pseudo replicates within a block.

In experiment 1, a 2-factorial ANOVA with Tukey post-hoc test ($p = 0.05$) was carried out with growing system and sowing method as factors for shoot fresh weight. For root dry weight, due to an incomplete factorial design (i.e. roots in rockwool blocks could not be measured), estimated marginal means were calculated using the emmeans package in R, and post-hoc pairwise comparisons were performed with Tukey's correction for multiple testing. Error bars presented in the figures are the pooled standard error from the ANOVA.

In experiment 2, a 1-way ANOVA and Tukey post-hoc test with treatment as the distinguishing factor was carried out for shoot fresh weight, root dry weight, total root length, root surface, total length of aerenchyma if present and ethylene in the sample headspace. For the percentage of aerenchyma, a Kruskal–Wallis test with Dunnet post-hoc test (Bonferroni adjustment) was carried out. All statistical tests were carried out using R (v4.3.2; R Core Team 2023).

Results

In experiment 1, where three growing systems were compared, shoot biomass per plant grown in the aeroponic system was on average 29% higher than in plants grown in the ebb-flood system, and 18% higher than in plants grown in the deep-water system. Plants sown as placed seeds in a bunch produced overall a 17% lower fresh weight than plants sown as broadcast, due to competition between individual plants in one bunch. However, the effect was not statistically significant within one growing system (Figure 3).

In experiment 2, where the supply of rootzone oxygen was varied, shoot biomass did not differ between plants from the aeroponic system and plants from the deep-water system (Figure 4). Interestingly, shoot biomass in the unaerated hydroponic growing system with low oxygen in the rootzone (Figure 2) was also not significantly different to the other two growing systems (Figure 4).

Root dry weight per plant was not significantly different between treatments in both experiments (Figures 5 and 6). Similarly, root-shoot-ratios did not significantly differ between treatments in both experiments (data not shown).

In experiment 1 and 2, different root architecture patterns were observed (Figure 7(a-e)). However, the differences could not be quantified by the overall root dry weight (Figure 6). Image analysis of root scans showed an average total root length of 2.98 ± 0.21 m with no differences between treatments on day 14 in the greenhouse (Figure 7d). However, on day 21 in the greenhouse, plants in the aeroponic system showed a 43% shorter total root length than plants in the two deep-water systems (Figure 7e). Similarly, the total root surface was not different between treatments on day 14 in the greenhouse, with an average of 25.93 ± 0.32 cm2, while the root surface of the aeroponic treatment was 45% lower than in the

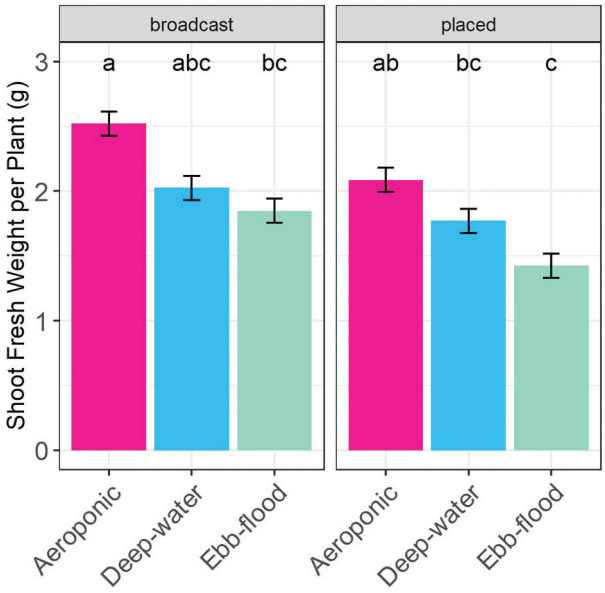


Figure 3. Fresh weight per plant of basil grown in three growing systems and two sowing strategies in each system in experiment 1. Means of four independent replicates in time with three trays per replicate ± standard error. Plants were grown to a minimum of 20 cm height in all replicates (28–35 days after transplanting).

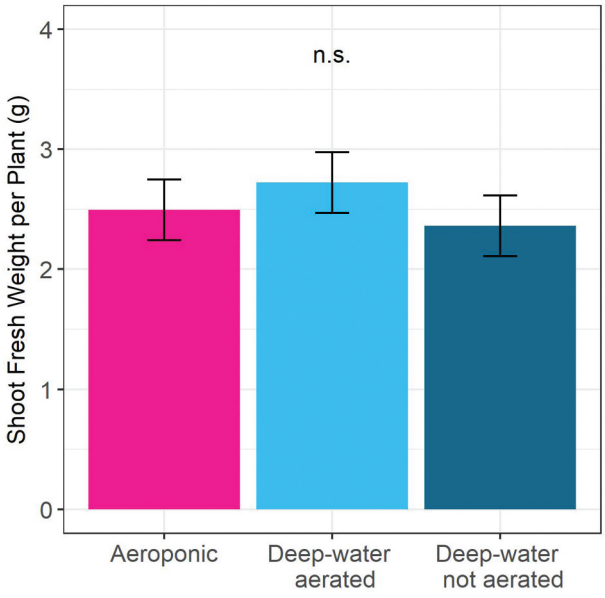


Figure 4. Fresh weight per plant of basil grown in different growing systems with high or low oxygen availability in the rootzone in experiment 2. Means of three independent replicates in time with three trays per replicate ± standard error. Plants were harvested 21 days after transplanting.

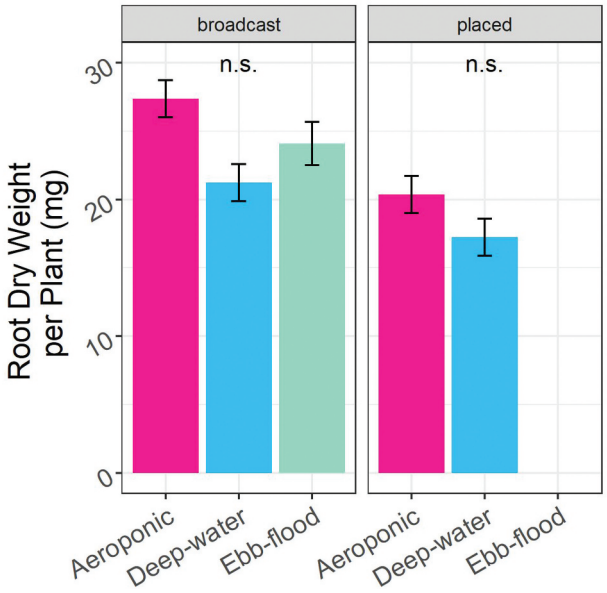


Figure 5. Root dry weight of plants in three different growing systems and two sowing strategies in each system in experiment 1. Each bar represents the mean of four independent replicates in time, but in the ebb-flood treatment the bar represents the mean of three independent replicates in time ± standard error. The placed treatment in the ebb-flood system was grown in rockwool, so roots could not be extracted to determine dry weight.

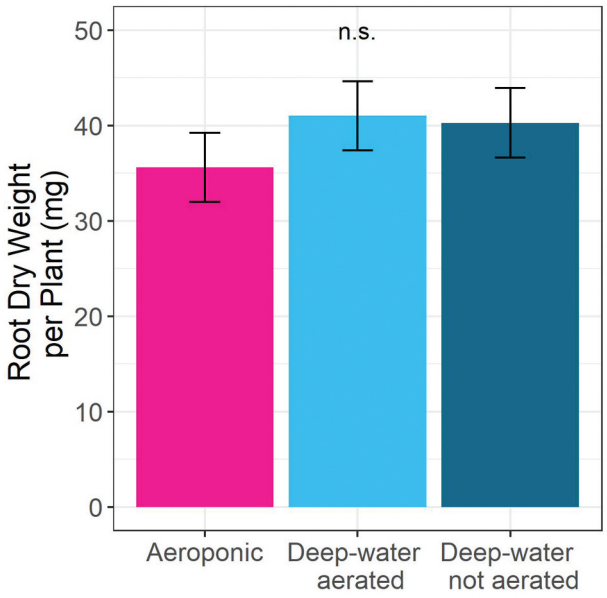


Figure 6. Root dry weight of plants in different growing systems in experiment 2. Each bar represents the mean of three independent replicates in time with three trays per replicate ± standard error.

hydroponic treatments on day 21 in the greenhouse (data not shown).

Optical coherence tomography scans (Figure 9) revealed that 14 days after transplanting, 17% of the plants in the non-aerated deep-water system showed aerenchyma, while there were no aerenchyma in roots grown in the aerated deep-water and aeroponic systems (data not shown). However, on day 21 after transplanting, aerenchyma could be observed in all treatments. Plants grown in a low oxygen root environment (not aerated deep-water system) showed in OCT images significantly more aerenchyma at 21 days after transplanting, than in the two systems with normal oxygen availability (aerated deep-water and aeroponic system) (Figure 8). However, when aerenchyma were present, there were no OCT measured differences in total length between the low oxygen and normal oxygen root environment (Figure 10). In addition, there were no differences in ethylene emission from excised roots after 20 days in the greenhouse (Figure 11).

Discussion

Basil produces higher biomass in the aeroponic system compared to the ebb-flood system

In experiment 1, sweet basil (*O. basilicum*) was grown to a marketable size and fresh mass produced was as expected for industrial production within 28 days in the greenhouse. Plants grown in the aeroponic system produced higher shoot fresh weight (Figure 3). The different growing systems may change availability of i) water, ii) nutrients and iii) oxygen in the rootzone due to the substrate structure and the different intervals and form of providing nutrients solution to the roots, as well as volume of the rootzone. In the ebb-flood system, the rockwool blocks were kept moist with nutrient solution, the aeroponic system was set in a way that assured moist roots at all times and in the deep-water system roots were submerged in nutrient solution. It can therefore be assumed that plants had sufficient access to water and nutrients throughout the experiment. This suggests that plant responses to rootzone oxygen concentrations induced a difference in shoot growth. However, root responses are difficult to measure in rockwool. The effect of rootzone oxygen was therefore investigated more closely in the second experiment in the aeroponic system and an aerated and unaerated deep-water system.

Dissolved oxygen in the unaerated deep-water system declines due to root respiration, but does not cause reduction in shoot growth in the first three weeks

Measuring oxygen in the rootzone in experiment 2 confirmed that the oxygen concentration in the unaerated deep-water system declined over about 5 days (Figure 2). In comparison, root respiration of lettuce plants in a different study with smaller rootzone volume decreased water oxygen concentrations from saturated water (250 µmol L^{-1}) to 78 µmol L^{-1}, which was suggested to be the limit for root

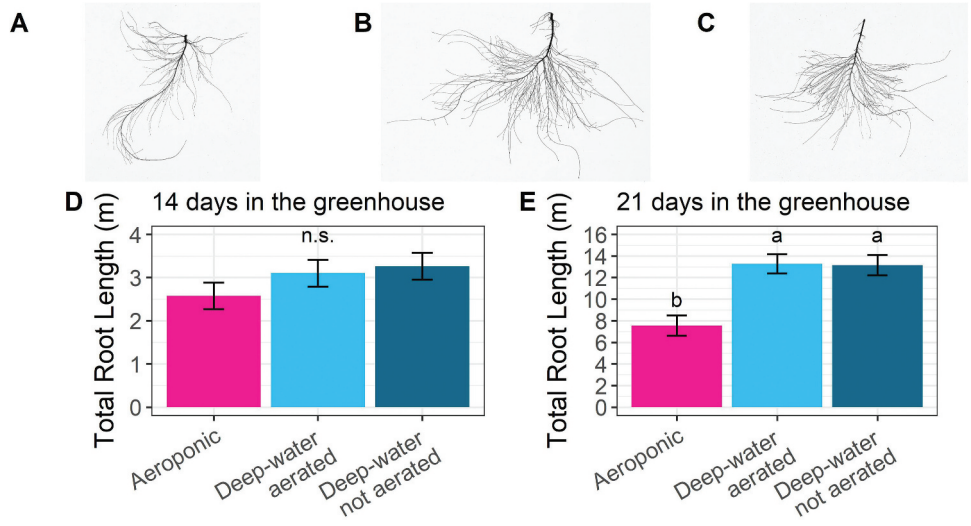


Figure 7. Photographs of root architecture of plants grown for 14 days in the aeroponic system (A), in the aerated deep water system (B) and in the unaerated deep water system (C) and total root length extracted from similar scans after 14 days in the respective system (D) and 21 days in the respective system (E). Each bar represents three independent replicates ± standard error. Bars with the same lower-case letter are not significantly different according to a one-way ANOVA.

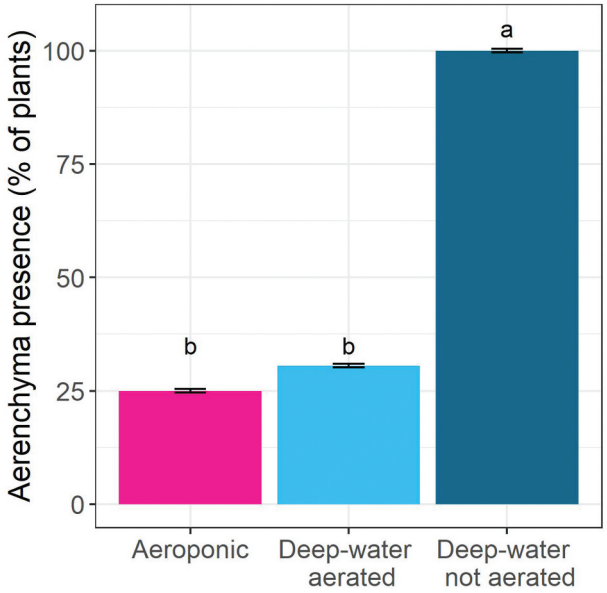


Figure 8. Percentage of aerenchyma found in a 3D scan of 4 mm sections of the main root, 2 cm below the root base at 21 days after transplanting. Each bar represents the mean number per root of three independent replicates in time and four plants per replicate ± standard error. Bars with the same lower-case letter are not significantly different according to a one-way ANOVA.

respiration in lettuce (Chun & Takakura, 1994). In bamboo (*P. praecox*), rootzone oxygen concentrations of 125 µmol L^{-1} for 2 days lead to a reduction of root respiratory processes (Ma et al., 2022). Root respiration is likely to be the cause for the decline in rootzone oxygen concentrations in the present study. The slower decline is likely due to the large volume of the nutrient solution tank compared to the size of the seedlings and some gas exchange with the surrounding air. Thus, sweet basil plants in the present study in the low oxygen treatment were subjected to conditions that can limit root respiration from about day 4–5 after transplanting. Only an extremely low oxygen availability in the rootzone, achieved by introducing nitrogen gas, reduced shoot growth of lettuce (Yoshida et al., 1997), but 125 µmol L^{-1} for 12 days reduced biomass production in *Phyllostachis* plants (Ma et al., 2022). A comparison of different hydroponic systems for growing basil showed that the choice of cultivar has a bigger effect on shoot biomass production than the growing system (Walters & Currey, 2015). Likewise, shoot fresh mass production of sweet basil was similar to the present study for plants grown in the aeroponic system and deep-water culture, independent of the oxygen supply. Plants were harvested at 14 days or 21 days after transplanting in experiment 2 and were therefore smaller than in experiment 1 but followed the expected shoot growth pattern. This indicates that the observed low oxygen concentrations are not influencing shoot biomass production up to 21 days after transplanting. However, plants in the aeroponic system showed a tendency to produce higher biomass per stem (differences not statistically significant). Thus, different oxygen concentrations in the root environment may have an effect on shoot growth after a longer growing period.

Root anatomy changes with low rootzone oxygen concentration is higher, leading to a higher dry matter content per volume. Another explanation for lower dry matter in the same volume in plants grown in the hydroponic systems could be the higher number and length of aerenchyma (Figures 8 and 10) and therefore more air-filled spaces in these roots.

Aerenchyma formation helps to maintain shoot growth in suboptimal rootzone oxygen conditions

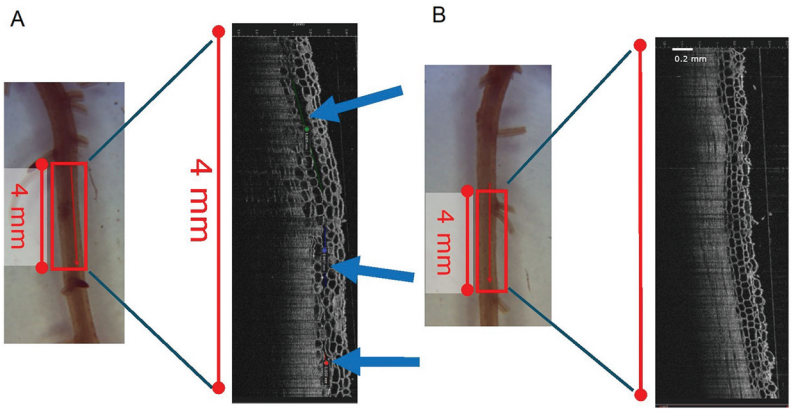


Figure 9. Example images from OCT applied on basil root A) plant grown in deep water culture showing aerenchyma and B) plant grown in the aeroponic system without aerenchyma. Reference brightfield microscope picture of the root section that was scanned (left). The red arrow indicates the position of the slice that is shown in the OCT image of a root section (right), blue arrows indicate aerenchyma.

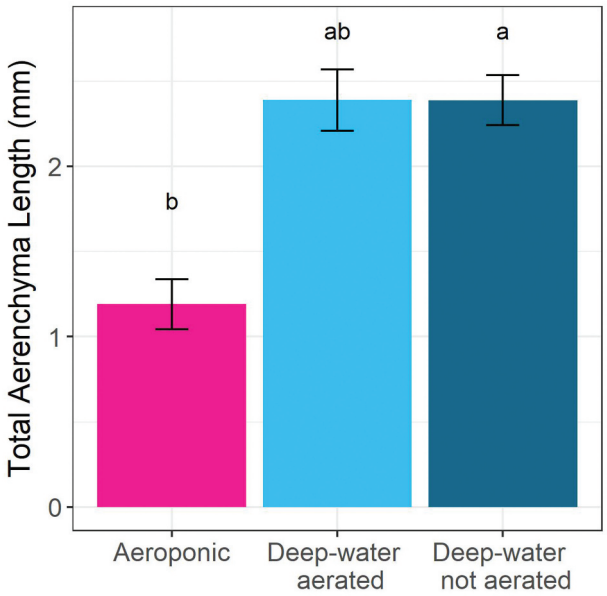


Figure 10. Total length of aerenchyma in plants grown in three different growing systems determined with OCT when aerenchyma where present on day 21 after transplanting in experiment 2. Each bar represents the mean of three independent replicates in time and four plants per replicate ± standard error. Bars with the same lower-case letter are not significantly different according to a one-way ANOVA.

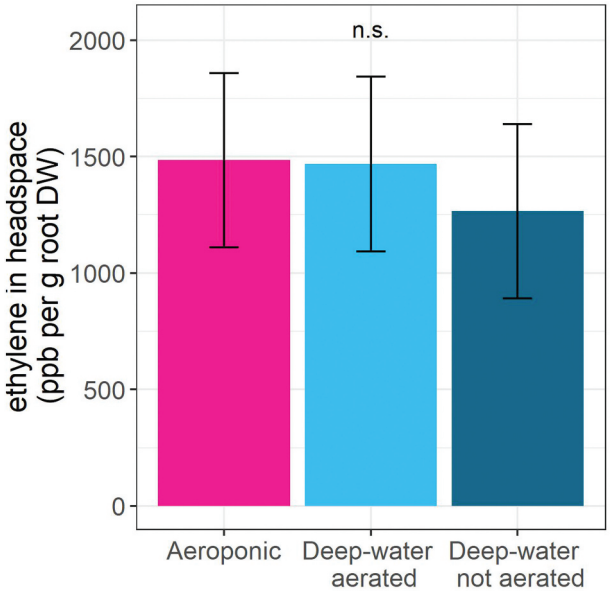


Figure 11. Ethylene concentrations in headspace of excised roots from three different growing systems. Measurements 30 minutes after excision. Data is normalised by root dry weight (DW). Values are means of three independent replicates in time and three plants per replicate ± standard error.

Many plant species form aerenchyma in response to flooding, which facilitates gas exchange between root and shoot (Voesenek & Sasidharan, 2013). In the present study, very few aerenchyma were formed in the growing systems with high oxygen availability in the rootzone, while plants the growing system with low oxygen availability showed a significant increase in number and length of aerenchyma between 14 days after transplanting and 21 days after transplanting (total aerenchyma length increased by 0.3 mm, 0.59 mm and 2.28 mm in plants grown in the aeroponic, aerated deep-water and non-aerated deep-water system respectively). In addition, total root length did not differ between plants grown in the aerated and non-aerated deep-water culture on both harvest timepoints (data not shown). Thus, the aerenchyma formation may have helped to maintain root growth in a low oxygen environment within the tested period of 21 days in the greenhouse. In another study, a rootzone oxygen concentration of 10 µmol L^{-1} reduced shoot biomass production in lettuce within 7 days of exposure (Yoshida et al., 1997). The rootzone oxygen concentrations in the present study were not as low but could become growth limiting after a longer period of exposure.

In submerged plant organs, ethylene is produced in response to lower oxygen concentrations and accumulates due to the slow diffusion rate of

ethylene in the surrounding water (Leeggangers et al., 2023). The concentration of ethylene in the tissue can remain very high for several days (Voesenek & Sasidharan, 2013). Ethylene accumulation and the formation of reactive oxygen species due to low oxygen concentration in the rootzone lead to cell death and hence aerenchyma formation in the root as an escape mechanism (Leeggangers et al., 2023). In the present study, ethylene release from excised roots 21 days after transplanting did not differ between roots grown in a high or low oxygen environment (Figure 11). This could be due to the aerenchyma formation between day 14 and day 21 after transplanting. The improved gas exchange through aerenchyma likely leads to improved diffusion of ethylene to the shoot, and therefore no accumulation could be measured in the roots (Visser & Pierik, 2007).

The use of OCT imaging helped to obtain more detailed information about the size and distribution of aerenchyma formation. With the appropriate image analysis, the volume of the air-filled pore space can be calculated from the same dataset. OCT is non-destructive and the roots can be submerged in water during imaging. It will be possible in future studies to image the same root multiple times throughout the development to determine when and where aerenchyma formation first occurs and how it develops over time. OCT is very promising tool in the phenotyping toolbox, and we encourage further exploration of this method in plant sciences.

This study examined physiological responses to mild rootzone oxygen depletion using novel technologies. In conclusion, the root environment can have an impact on shoot biomass production and should be considered in growing recommendations. The formation of aerenchyma helps basil to maintain biomass production under suboptimal rootzone oxygen concentrations in the first weeks after rootzone oxygen reduction. The formation of aerenchyma seems to be a universal response among different crops (Leeggangers et al., 2023). In the future, OCT imaging could be used for early detection of aerenchyma formation and for defining threshold concentrations for rootzone oxygen. This would help growers in CEA to further optimise crop growing conditions.

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Author contributions

CRediT: **Katharina Huntenburg:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing; **Jack Farmer:** Conceptualization, Resources, Writing – review & editing; **Jos de Wit:** Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing; **Jeroen Kalkman:** Data curation, Investigation, Methodology, Supervision, Writing – review & editing; **Leo F.M. Marcelis:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Disclosure statement

In accordance with Taylor & Francis policy and our ethical obligation as researchers, we are reporting that one author (JF) is involved with the company that funded the research leading to this article. We declare that the experimental setup and the analysis of the obtained data were carried out according to best scientific practice.

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Data availability statement

The data that support the findings of this study are openly available in 4TU.ResearchData at https://doi.org/10.4121/72f24086-fe15-42a0-830f-d87de252d902.v1.

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