



Stratified soilless substrates decrease the vertical gravitational water gradient altering *Helianthus* root morphology

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Abstract

Background and aims Containerized soilless substrates are highly porous to ensure adequate air storage to overcome the “container” effect- the lower part of the container nears saturation which can decrease root health and growth. Substrate porosity is dynamic, evolving over time. As roots fill pores, substrate decomposition and *in-situ* particle movement change the physical structure, shifting its storage properties and performance. Research is sparse in understanding how developing roots change their morphology throughout production (temporally) and while growing throughout the three-dimensional substrate matrix (spatially). Thus, it would be beneficial to understand how root development impacts container moisture characteristics. This study aimed to

quantify root morphological development and water storage (θ) spatiotemporally in conventional or engineered soilless substrate systems.

Methods *Helianthus annuus* ‘Rio Carnival’ was grown in 30.5 cm tall PVC columns in a conventional (non-stratified; 100% of the container is filled with a single composite) bark- or peat-based substrates or engineered (stratified; fine-bark atop coarse-bark; peatlite layered over pine bark) systems. Columns were frozen after roots were partially- (22 d) or fully-grown (43 d) and were separated in five vertical sections. Root morphology and θ were measured within each layer.

Results The results showed that stratified systems overall stored less water, especially in coarser substrates. Partially rooted columns generally stored more water and fully rooted columns drained more. Plants grown in stratified systems had greater fine root development than when grown conventionally.

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Conclusion Container-grown roots can be engineered to produce more fibrous root systems by spatially manipulating substrate θ .

Keywords Stratification · Frozen column · Moisture distribution

Abbreviations

DAI	Days after initiation
Ψ_m	Matric potential (-hPa)
Ψ_g	Gravitational potential (-hPa)
θ	Volumetric water content ($\text{cm}^3 \cdot \text{cm}^{-3}$)
PB	Non-stratified pine bark
Strat-PB	Stratified fine bark over coarse bark
PL	Non-stratified peatlite
Strat-PL	Stratified peatlite over pine bark

Introduction

Container substrates are comprised of either a standalone base-substrate or a blended composite that includes organic (e.g., Sphagnum peat; pine bark) or inorganic (e.g., perlite; sand) materials, or both, to mass produce specialty crops quickly and effectively (Raviv et al. 2019). Due to gravitational forces and the large porosity of soilless substrates, a vertical moisture content gradient exists in the container upon equilibration post irrigation (Bilderback and Fonteno 1987). The vertical gradient can be exacerbated by increasing container height (e.g., gravitational potential increases every $1 \text{ hPa} \cdot \text{cm}^{-1}$; Milks et al. 1989) or by modifying the matric potential of the substrates. Owen and Altland (2008) and Milks et al. (1989) showed that air space is greatest at the top while volumetric water content (θ) is greatest at the bottom of a column with bark or peat, respectively, highlighting that the vertical moisture gradient occurs irrespective of the substrate used. This vertical gradient requires frequent irrigation for establishment of small young plant liners or plugs that are planted in the top one-third of the container. Thus, the consistent irrigation during liner establishment results in a near saturated substrate in the lower portion of the container, hindering gas diffusivity and oxygen availability for root respiration, and possibly impeding healthy root development in this anaerobic zone (Cannavo et al. 2011).

Substrate stratification is a technique that involves vertically layering fine-textured or fibrous substrates

atop a relatively coarse substrate to reduce the aforementioned θ gradient (Fields et al. 2021). Placing a fine substrate in the top slows infiltration rates (Criscione et al. 2022) and increases matric tensions, ultimately retaining more available water in the top half of conventional container systems (Criscione et al. 2024). The coarser particles in lower portion of the container increase drainage and contribute to less stored water overall (Fields et al. 2024).

There is a dynamic relationship between the substrate physical structure and in situ container-grown roots. Proliferating roots can alter pore shape and diameter (Cannavo et al. 2011; Judd et al. 2015a). As a result, this can change gas diffusivity rates, especially in finer textured substrates. Hydraulic conductivity and tortuosity are believed to increase and decrease, respectively, as the substrate-root system evolves over time (Nkongolo and Caron 2006; Caron et al. 2010; Cannavo and Michel 2013). Concurrently, there is a shift in the air to water storage, where most substrate systems have decreased air space and increased water storage throughout production (Altland et al. 2011; Judd et al. 2015a). These changes can result in preferential flow and redirect infiltrating or draining water, with greater leaching volumes at faster rates (Hoskins et al. 2014; Criscione et al. 2022). Despite the strong influence roots have on substrate moisture dynamics, root development is not deeply explored in horticultural research, where root dry biomass alone is the most commonly reported root metric at the end of the experiment (Judd et al. 2015b; Criscione and Fields 2024). More intrinsic morphological features, including total root length as an estimator of the substrate volume exploited and overall root vigor, root surface area and diameter as an indicator of absorptive area, and the ratio of fine and coarse root proportions that can highlight absorptive and transport capacity, respectively (Fitter 1996). These collective root measures can shape our understating of the complex interwoven nature of how roots alter substrate hydraulic characteristics and how root morphology is concurrently affected by evolving substrate physical properties. Measuring root characteristics and substrate water content over time will provide deeper insights on substrate-root dynamics (Ostonen et al. 2007).

Advancements made in observing root growth within the previous century have been reviewed by Judd et al. (2015b). Methods included the standard

in which biomass or a subjective rating is reported after washing the roots. More complete measures include root tracing or digital imaging (WINRHIZO-Regents Instruments, Quebec City, Canada; Rhizo-Vision; Seethepalli et al. 2021) of washed roots or roots grown in traditional rhizotrons, three- or four-sectioned clear container termed mini-horhizotrons (Judd et al. 2014; Lesmes-Vesga et al. 2022), or clear porometer cores termed rhizometers (Judd et al. 2015b). A frozen column method may be adapted to understand (1) how water is distributed in stratified substrate systems and (2) how root growth occurs spatially and temporally. This method involves freezing a column of substrate, cutting the column at predetermined depths and measuring moisture content in each depth (Kritz and Khaled 1995; Owen and Altland 2008). Each layer of the columns allows for easy measures of θ in conventional and stratified systems and root morphological measurements using root diameter classifications (Liu et al. 2018) as it occurs *in-situ* throughout the container column. Thus, the objective of this experiment was to examine the interconnectivity between substrate θ and root exploration. *Helianthus annuus* was chosen for this substrate-root assessment study due to its rapid and sufficient rooting, manageable shoot development in the greenhouse, and related literature on the genera (Furr and Reeve 1945; Seiler 1994; Corti Monzón et al. 2012; Masalia et al. 2018). The authors hypothesize that (1) stratified-grown systems will have increased moisture uniformity across the vertical profile when compared to conventional systems and (2) stratified-grown plants will develop more root length within thinner diameter classifications in the top strata-layer.

Materials and methods

Substrate preparation

Aged loblolly pine (*Pinus taeda*) bark was amended with pulverized dolomitic limestone ($4.3 \text{ kg}\cdot\text{m}^{-3}$; Austinville Limestone Co; Austinville, VA, U.S.) and granulated micronutrients ($0.15 \text{ kg}\cdot\text{m}^{-3}$; A90505; Micromax, Tel Aviv, Israel) prior to particle fractionation to achieve a target pH of 6.5. A 6.3 mm screen aperture was used to partition fine ($<6.3 \text{ mm}$) and coarse ($\geq 6.3 \text{ mm}$) bark particles. Bark was screened at 62% moisture content. Approximately 0.03-, 0.03-,

and 0.06 m^3 of unscreened, fine, and coarse bark, respectfully, was collected. A “peatlite” substrate (0.04 m^3) was made by using Sphagnum peat (Pro Grade; Sun Gro Horticulture, Agawam, MA, U.S.) and perlite (Sunshine Perlite; Sun Gro Horticulture) mixed at a 7:3 peat:perlite (by vol.) while incorporating $4.7 \text{ kg}\cdot\text{m}^{-3}$ pulverized dolomitic limestone (Austinville Limestone Co.) to achieve a targeted pH of 6.5.

Substrate physical properties

A 7.6 cm tall, 347.5 cm^3 porometer (Fonteno and Harden 1995) was used to measure the static hydraulic properties of the raw substrates composites as previously described (e.g., unscreened bark, fine bark, coarse bark, peatlite) including the minimum volume of air-filled pores post gravitational drainage termed air space, maximum water storage post gravitational drainage termed container capacity, total porosity, and bulk density. Briefly, a core was filled with substrate material and was slowly bottom-up saturated. Thereafter, the core was drained, leachate measured, and the sample was weighed and dried at $105 \text{ }^\circ\text{C}$ for 48 h to determine container capacity and bulk density. A $\sim 120 \text{ cm}^3$ subsample was placed in an oven and dried ($105 \text{ }^\circ\text{C}$ for 48 h). The sample was separated for 5-min using a nest of sieves placed on oscillating, tapping shaker (Rx-29; W.S. Tyler, Mentor, OH, U.S.). The particle size distribution was characterized into four groups, including extra-large ($\geq 6.3 \text{ mm}$), large ($6.3 - 2.0 \text{ mm}$), medium ($2.0 - 0.7 \text{ mm}$) and fines ($<0.71 \text{ mm}$).

Experimental design

There were four substrate treatments (1) a non-stratified system that consisted of unscreened pine bark (PB), (2) a stratified system that consisted of coarse bark layered in the lower 50% of the substrate and fine bark layered above in the upper 50% (Strat-PB), (3) a non-stratified system that consisted of peatlite (PL), and (4) a stratified system that consisted of unscreened bark layered in the lower 50% of the substrate and peatlite layered above in the upper 50% (Strat-PL). Additionally, there were three rooting level treatments (i) a fallow system that contained no plants (ii) a partially rooted treatment and (iii) a fully rooted treatment. Thus, four substrate treatments were

analyzed at three rooting levels (0-, 22-, and 43 d of root growth) using non-repeated measures. Fallow treatments contained three replicates per substrate treatment. Partially and fully rooted treatments contained six replicates per substrate treatment. An illustration of the treatment diagram is in (Fig. 1).

A standard 7.6 cm (dia.) polyvinyl chloride (PVC) column was used to mimic a vertical container profile. The PVC column was cut to 38.1 cm in height; however, only the upper 30.5 cm was used for root and substrate θ measurements. The lower 7.6 cm was used to secure the PVC column into the frozen

column apparatus and create a water table to establish Z_0 . A screen was securely fastened at the bottom to contain the substrate in the PVC volume. The non-stratified treatments were filled similarly to a conventional nursery or greenhouse container, where the substrate was lightly filled to the surface of the PVC column and then gently tapped three times before being refilled to be level with the surface of the PVC column. The stratified treatments were first filled with the lower strata substrate (either coarse bark in Strat-PB or unscreened pine bark in Strat-PL) in the bottom 22.8 cm. Thereafter, the remaining 50% of the upper

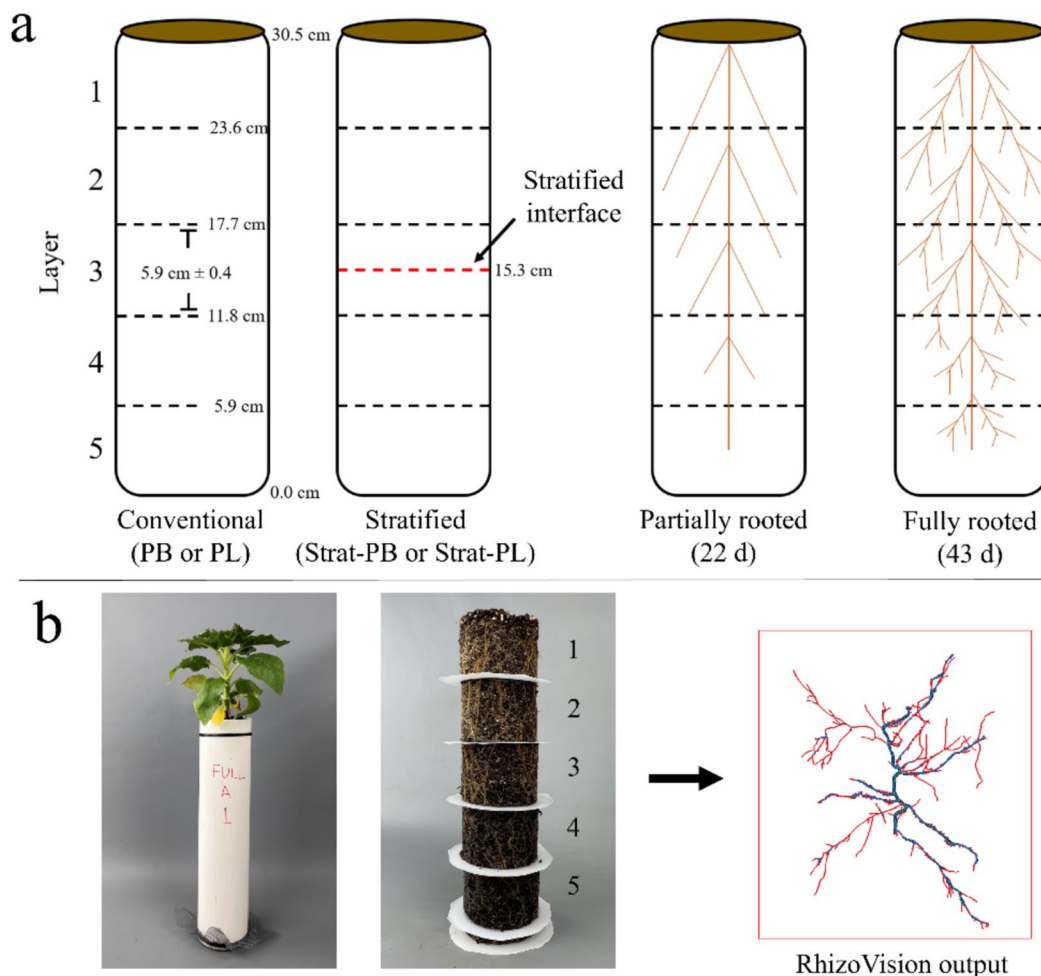


Fig. 1 **a** Illustration of the treatments. Numbers denote layers examined within column. Average caliper height of each layer was $5.9 \text{ cm} \pm 0.4$ ($\mu \pm \text{SD}$). The entire profile height examined was approx. 30.5 cm. Non-stratified represents 100% of the container is filled with a substrate and a stratified system represents two substrates stacked atop each other (50% on the top

and 50% on the bottom). Partially rooted treatments are plants grown for 22 d and fully rooted treatments are rooted profiles with plants grown for 43 d. **b** Example of pre-cut profile and layered profile. Root image is an example of RhizoVision (Seethepalli et al. 2021) output to analyze root morphological features

strata (remaining 15.3 cm) was filled with either fine bark or PL and gently tapped three times. More substrate was added to ensure the top strata was flush with the top of the container. All substrate profiles were thoroughly hand-watered.

Helianthus annuus ‘Rio Carnival’ sunflower seeds were sown on different dates. This plant was chosen as a representative crop for its rapid and deep rooting, with sufficient tap root and fibrous growth, to explore the entirety of a substrate profile while being a dwarf variety to maintain manageable growth in the greenhouse. On 18 November 2022, all fully rooted treatment seeds were sown. Three seeds were sown in the center of the substrate column approx. 2.5 cm deep and were gently misted. Germination began 5 d after seeding, where two-thirds of the seedlings were immediately severed at the shoot base. On 13 December 2022, the partially rooted treatment seeds were sown and were gently misted. Germination began 5 d after seeding and the same seedling measures were conducted. Three additional columns of each substrate were filled and left fallow.

Greenhouse conditions and fertigation

All columns were placed in a controlled greenhouse environment. The atmospheric conditions were set to $21.6\text{ }^{\circ}\text{C} \pm 3.2$, 33.7 relative humidity ± 9.1 , and 193 PAR ± 59.2 . Fertigation solution delivered 100 mg L^{-1} N using a 20 N-20 P₂O₅-20 K₂O water soluble fertilizer (Jack’s Peatlite; Allentown, PA, U.S.). Plants were irrigated for 45 s at a rate of 146 mL min^{-1} twice daily beginning five days after germination for rooted treatments. Fallow and unseeded columns (until seeds were sown) were only irrigated on 30 November 2022 to prevent excess moisture.

Harvests

Fully rooted plants were grown for 43 d. On 3 January 2023, representative photos were taken of the fully rooted treatment. On 4 January 2023, all fully rooted shoots were severed at the base of the stem. Photos of partially rooted sunflower were taken on 7 January 2023 (22 days after initiation; DAI). On 8 January 2023, all shoots were severed at the base of the stem. All shoot biomasses were dried for 7 d at $68\text{ }^{\circ}\text{C}$. After the harvest, columns were placed in the frozen water column apparatus.

Frozen water column

A modified frozen column apparatus as described by Owen and Altland (2008) was used. The fully and partially rooted treatments harvest were divided into two. For example, the PB and Strat-PB were harvested 40 h prior to PL and Strat-PL. Two mesh screens were placed on the bottom of the column and a PVC rubber union was attached. Thereafter, the columns were placed on the frozen water column apparatus and were leveled. Flexible pneumatic tubing was connected to a vertical clear tube that was open to atmospheric pressure. An irrigation hose was connected to parallel vertical PVC pipe that flowed into a clear tube to identify water table levels.

When all columns were placed on the frozen water column apparatus, the columns were slowly saturated from the bottom up over a 1.5 h period. Once columns were fully saturated, the valves were closed, and they were allowed to remain saturated for 30 min. Columns were then drained to Z_0 occurring 7.6 cm from the bottom or 30.5 cm from the top of the column. A rotating laser was used to identify and mark Z_0 . Extenders were attached to the columns to ensure they could be secured in a $-20\text{ }^{\circ}\text{C}$ freezer (Standex; Model no: NSPF331WWW/0; Salem, NH, U.S.) with built in rack to ensure the columns remained vertically plum while freezing. The freezer was set to $-10\text{ }^{\circ}\text{C}$. The columns remained in the freezer for 40 h. After 40 h, the PVC columns were removed from the freezer, extenders removed, and immediately placed on a Ridgid compound miter saw (Skilsaw; Naperville, IL, U.S.) equipped with a 0.2-cm thick, 25-cm diameter saw blade with 200 teeth (TX-60, Torrance, CA, U.S.). Each PVC column was cut four times to have five layers (Fig. 1). Average caliper height of each layer was $5.9\text{ cm} \pm 0.4$ ($\mu \pm \text{SD}$). Immediately after the column was cut, each layer was weighed and thawed for 15 h.

Half of each substrate treatment in each rooting stage ($n=3$) were evaluated for root morphological features, while the other half were only evaluated for volumetric water content (θ) measurements. Root morphology measurements are described in the subsequent section. The layers used to calculate θ were placed in an oven at $68\text{ }^{\circ}\text{C}$ for > 15 h, PVC rings were removed, and then the substrate was dried again at $110\text{ }^{\circ}\text{C}$ for 4 h. The PVC rings were weighed and measured the height of four cardinal directions with a

caliper to calculate vol. for θ measurements. Average vol. of each layer was $270 \text{ cm}^3 \pm 17.3 (\mu \pm \text{SD})$. These processes were assessed for each substrate and rooting treatment.

Root morphology measurements

Immediately after all roots were washed free of substrate and separated, they were placed inside a deionized water filled (400 mL) square container ($8,244 \text{ cm}^3$) on standard white printer paper (602.6 cm^2). A black out chamber with a hole at the top for camera placement was constructed to ensure each photo was nearly identical (i.e., same height and lighting).

Roots were carefully spread apart with the goal to minimize overlapping root tissues. Root images were recorded using an iPhone 13 Pro (Apple; Cupertino, CA, U.S.). After all the images were captured, digital images were scanned with TurboScan (Piksoft Inc; Piedmont, CA, U.S.) and converted to a JPG format. This technique was validated by using differently gauged wire of known lengths and diameters and plotting a regression against true values versus scanned length ($R^2=0.9991$; $m=1.05$; $b=1.70$) and diameter ($R^2=0.9344$; $m=0.93$; $b=0.13$). Rhizo-Vision (Seethepalli et al. 2021) batch analysis was employed and extensive root systems requiring multiple images were summed (besides average diameter). Root morphology measurements included total root length (cm), average diameter (mm), root volume (cm^3), and surface area (cm^2). Additionally, root length (cm) under different diameter classifications following criteria applied by Caruso et al. (2021) that included (i) very fine; <0.5 mm, (ii) fine; $0.5 - 1.0$ mm, (iii) large; $1.0 - 2.0$ mm, and added a last category (iv) very large; >2.0 mm. Fresh root weights were measured and roots were dried for 7 d at 68°C and reweighed. Specific root length ($\text{cm}\cdot\text{g}^{-1}$) and root tissue density ($\text{g}\cdot\text{cm}^{-3}$) values were calculated using dry biomass values.

Data analysis

The data presented in tables and figures with associated statistics were subjected to a one-way analysis of variance (ANOVA) and t-tests to identify any significant statistical differences across the means using JMP Pro (17.0; SAS Institute, Inc., Raleigh, NC, U.S.). If significant, Tukey's Honestly Significant

Difference ($\alpha=0.05$) post-hoc test was used to separate means across treatments for substrate hydraulic measurements and root morphological features. Pearson Correlation Coefficients levels were defined as weak negative / positive relationships ranging from (0 to -0.3 ; 0 to 0.3), moderate negative / positive (-0.3 to -0.7 ; 0.3 to 0.7), and strong negative / positive (-0.7 to -1 ; 0.7 to 1; Ratner 2009).

Results

Substrate physical properties

Decreasing mean particle diameter increased substrate container capacity by 12% in fine bark ($p<0.0001$; Table 1). Peatlite had similar container capacity values as fine bark (Table 1). Removing fine bark particles via screening increased total porosity values by 3% in coarse bark (Table 1). Differences in air space were observed across fine, coarse, and unscreened bark (Table 1). Coarse bark had $0.09 \text{ cm}^3 \cdot \text{cm}^{-3}$ greater air space than unscreened bark ($p<0.0001$; Table 1). Fine bark had the greatest bulk density ($0.21 \text{ g}\cdot\text{cm}^{-3}$); while, PL had the lowest ($0.11 \text{ g}\cdot\text{cm}^{-3}$; $p<0.0001$; Table 1).

The gradation of particle diameters across all individual substrates is illustrated in Fig. 2. Coarse bark contained the greatest proportion of extra-large particles ($p<0.0001$) and the least proportion of fine particles ($p<0.0001$; Table 1). In contrast, PL contained the least proportion of extra-large particles ($p<0.0001$) and the greatest proportion of fine particles ($p<0.0001$; Table 1). Fine bark had the greatest proportion of medium particles ($p<0.0001$; Table 1).

Temporal and spatial evolution of water storage

The expected vertical θ gradient existed across all substrate and rooting treatments (Table 2). In bark substrate profiles, the θ differential from the top surface to the container base decreased as rooting levels increased (Table 2). Statistical differences of θ were only detected in the upper and lower strata of fallow Strat-PB profiles; however, numerically, there is a 14- and 22% θ gradient reduction in stratified systems across the entire profile (Table 2). Similar results were seen vertically across the upper strata of fully rooted Strat-PB profiles. Fallow

Table 1 Static physical properties^a and particle size distribution^b of four individual substrate composites including a peatlite blend (7:3; peat:perlite), unscreened bark, screened fine bark particles (<6.3 mm) and screened coarse bark particles (>6.3 mm)

Substrate Treatment	Static physical properties				Particle size distribution			
	Air-filled porosity (cm ³ ·cm ⁻³)	Container capacity (cm ³ ·cm ⁻³)	Total porosity (cm ³ ·cm ⁻³)	Bulk density (g·cm ⁻³)	Extra-large (>6.3 mm; %)	Large (6.3—2.0 mm; %)	Medium (2.0—0.7 mm; %)	Fines (<0.7 mm; %)
Peatlite	0.18 cc	0.62 a	0.80 b	0.11 c	2.00 b	25.00 a	18.00 c	56.00 a
Unscreened bark	0.29 b	0.47 b	0.77 c	0.18 b	14.00 b	33.00 a	29.00 b	24.00 c
Fine bark	0.20 c	0.60 a	0.81 ab	0.21 a	0.00 b	31.00 a	39.00 a	31.00 b
Coarse bark	0.44 a	0.38 c	0.82 a	0.17 b	60.00 a	31.00 a	1.00 d	8.00 d
<i>p</i> -value ^d	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.6206	<0.0001	<0.0001

^aMeasured via pomometer analysis (Fonteno and Harden 1995). Total porosity = minimum air space (minimum air-filled porosity after free drainage) + maximum water holding (water storage maximum water holding capacity after free drainage)

^bPercent of particle dry weight in different diameter classifications

^cLetters denote detected differences amongst means utilizing Tukey’s HSD ($\alpha=0.05$)

^dMeasures of overall treatment effects utilizing one-way ANOVA analysis with a significance value of ($\alpha=0.05$)

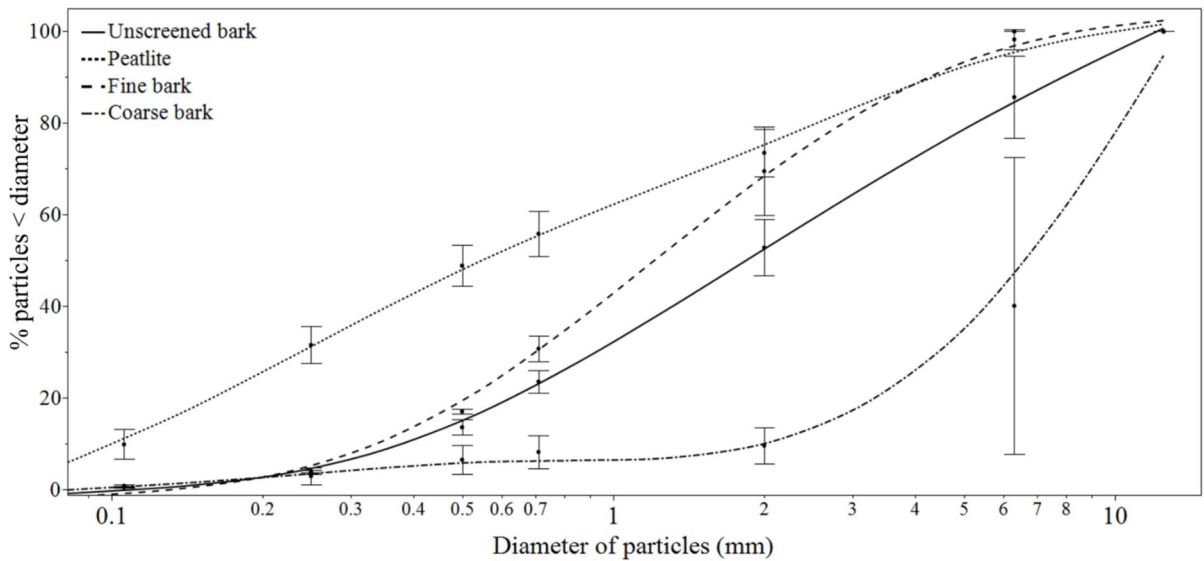


Fig. 2 Particle size distribution curve of unscreeded pine bark, screened fine bark particles (<6.3 mm), screened coarse bark particles (>6.3 mm), and a peatlite (7:3 peat:perlite) sub-

strates. Each error bar is constructed using a 95% confidence interval estimate of the mean

Strat-PB had decreased θ resulting in increased drainage at the stratified interface and in the immediate sublayers [column layers three ($p < 0.05$) and four ($p < 0.0001$), respectively; Table 2]. There were similar results in a partially rooted system (layer four; $p < 0.0010$), but not in a fully rooted profile (Table 2). Fully rooted Strat-PB treatments had 16- and 22% less of a moisture gradient from the top of

the column to the base and in the upper strata than in PB, respectively (Table 2). There was a general observation that a partially rooted system increased θ when compared to a fallow profile and a fully rooted system decreased θ relative to a partially rooted system (Table 3; Fig. 3). In partially rooted Strat-PB, a 7-, 5-, and 3% increase in θ occurred in layer one, three, and five, respectively, whereas,

Table 2 Vertical moisture distribution comparison across two substrate treatments including a (i) conventional substrate profile (i.e., PB and PL) and (ii) a stratified profile that contains layered substrates atop each other (i.e., Strat-PB and Strat-PL).

Layers denote height of column, where the top of layers 1, 2, 3, 4, and 5 are approx. 29.5-, 23.6-, 17.7-, 11.8-, and 5.9 cm \pm 0.4 from the bottom of layer 5

	Conventional	Stratified (Strat)
	θ - Volumetric water content ($\text{cm}^3 \cdot \text{cm}^{-3}$)	
Pine bark substrate (PB) ^f		
Layer		
Fallow profile ^a		
1	0.36 e ^d	0.34 d
2	0.43 d	0.43 c
3	0.53 c	0.47 b * ^c
4	0.62 b	0.50 b ***
5	0.75 a	0.72 a
Profile θ differential ^h	0.39	0.38
Upper strata θ differential ⁱ	0.17	0.13 *
Lower strata θ differential ^j	0.21	0.25 **
p-value ^c (fallow)	<0.0001	<0.0001
Partially rooted (22 DAI ^b)		
1	0.39 e	0.39 d
2	0.46 d	0.45 c
3	0.54 c	0.52 b
4	0.65 b	0.55 b **
5	0.76 a	0.77 a
Profile θ differential	0.37	0.39
Upper strata θ differential	0.15	0.13
Lower strata θ differential	0.22	0.26
p-value (partially rooted)	<0.0001	<0.0001
Fully rooted (43 DAI)		
1	0.35 c	0.35 c
2	0.40 bc	0.39 bc
3	0.49 bc	0.43 bc
4	0.54 ab	0.48 b
5	0.68 a	0.59 a
Profile θ differential	0.33	0.25
Upper strata θ differential	0.14	0.09
Lower strata θ differential	0.20	0.16
p-value (fully rooted)	0.0004	0.0001

Table 2 (continued)

	Conventional	Stratified (Strat)
Peatlite substrate (PL) ^g		
Fallow profile		
1	0.54 c	0.50 c
2	0.64 b	0.52 c **
3	0.69 ab	0.57 b **
4	0.72 a	0.59 b ***
5	0.73 a	0.72 a **
Profile θ differential	0.19	0.22
Upper strata θ differential	0.15	0.07 **
Lower strata θ differential	0.04	0.15 **
p-value (fallow)	<0.0001	<0.0001
Partially rooted (22 DAI)		
1	0.58 d	0.54 d *
2	0.66 c	0.55 d ***
3	0.72 b	0.59 c ***
4	0.75 a	0.63 b ***
5	0.77 a	0.72 a ***
Profile θ differential	0.19	0.17
Upper strata θ differential	0.14	0.04 ***
Lower strata θ differential	0.05	0.13 ***
p-value (partially rooted)	<0.0001	<0.0001
Fully rooted (43 DAI)		
1	0.55 d	0.50 b **
2	0.63 c	0.52 b ***
3	0.67 b	0.56 b **
4	0.70 a	0.55 b ***
5	0.70 a	0.66 a
Profile θ differential	0.15	0.15
Upper strata θ differential	0.12	0.06 **
Lower strata θ differential	0.03	0.10 **
p-value (fully rooted)	<0.0001	0.0001

^aFallow profiles contain no plants

^bDays of growth after experimental initiation

^cMeasures of overall treatment effects utilizing one-way ANOVA analysis with a significance value of ($\alpha=0.05$)

^dLetters denote detected differences amongst means utilizing Tukey's HSD ($\alpha=0.05$) in a vertical column

^eAsterisks denote either non-significance (no asterisk), or *, **, *** values represent significant at $P \leq 0.05$, 0.01, or 0.001, respectively, across stratified and conventional profiles utilizing a t-test

^fBark-based substrates include a non-stratified (100% container filled with unscreened pine bark) or stratified [the top 50% of the container volume is filled with screened fine bark particles (<6.3 mm) and the bottom 50% of the container volume is filled with screened coarse bark particles (>6.3 mm)]

^gPeat-based substrates include a non-stratified (100% container filled with peatlite at a 7:3 blend) or stratified [the top 50% of the container volume is filled with peatlite (7:3 peat:perlite) and the bottom 50% of the container volume is filled with unscreened bark particles]

^hMoisture content gradient occurring from the bottom to the top of the container (i.e., layer 5 – layer 1)

ⁱMoisture content gradient occurring in the upper strata (i.e., layer 3 – layer 1)

^jMoisture content gradient occurring in the lower strata (i.e., layer 5 – layer 3)

only a 2% increase occurred in layer four of PB (Table 3).

In both peat-based substrates systems, irrespective of conventional or stratified, the existing moisture gradient decreased as root systems grew (Table 2). When fully rooted, a Strat-PL stored less water than PL, with exception of the bottom layer (layer five; Table 2). Stratified profiles had less of a change in θ in the upper strata (top of profile to half the column height), while the lower strata had a greater difference in θ (half the column height to profile base; Table 2). In most instances across PL and Strat-PL profiles, a partially rooted system increased θ when compared to a fully rooted system (Table 3). For example, in Strat-PL, a partially rooted profile increased θ in the top (when compared to a fallow profile) and bottom (when compared to a fully rooted profile) layers (Table 3). In

the bottom three layers in PL systems, fully rooted profiles decreased θ in every case across rooting levels (Table 3).

Crop growth and development

Partially rooted plants grown in Strat-PB were shorter ($p=0.0308$; Supplemental Fig. 1) than when grown in other substrate treatments. However, there were no differences in partially rooted shoot dry biomass values ($p=0.0717$; Supplemental Fig. 2) across substrates. At study completion, there were no differences in fully rooted plant height ($p=0.4694$) or dry biomass ($p=0.0864$; Supplemental Figs. 1 and 2).

Expected differences in root morphological features existed across the partially and fully rooted PB and Strat-PB profiles illustrating root growth

Table 3 Statistical analysis representing changing in volumetric water content with differing root development of plants grown in two substrate treatments including a (i) conventional substrate profile (i.e., PB and PL) and (ii) a stratified profile

that contains layered media atop each other (i.e., Strat-PB and Strat-PL). Layers denote the height of column, where the top of layers 1, 2, 3, 4, and 5 are approx. 29.5-, 23.6-, 17.7-, 11.8-, and 5.9 cm \pm 0.4 from the bottom of layer 5

			Layer				
			1	2	3	4	5
Pine bark substrate (PB)	Conventional	Fallow ^a	b ^e	–	–	ab	–
		Partially rooted ^b	a	–	–	a	–
		Fully rooted ^c	ab	–	–	b	–
		p-value ^d	0.0369	NS	NS	0.0274	NS
	Stratified (Strat)	Fallow	–	–	ab		a
		Partially rooted	–	–	a		a
		Fully rooted	–	–	b		b
		p-value	NS	NS	0.0157	NS	0.0023
Peatlite substrate (PL)	Conventional	Fallow	–	–	ab	b	b
		Partially rooted	–	–	a	a	a
		Fully rooted	–	–	b	c	c
		p-value	NS	NS	0.0376	0.0002	0.0011
	Stratified (Strat)	Fallow	a	b	–	ab	a
		Partially rooted	b	a	–	a	a
		Fully rooted	b	b	–	b	b
		p-value	0.0036	0.0178	NS	0.0040	0.0177

^aFallow profiles contain no plants

^bPartially rooted plants were grown for 22 days after experimental initiation

^cFully rooted plants were grown for 43 days after experimental initiation

^dMeasures of overall treatment effects vertically utilizing one-way ANOVA analysis with a significance value of ($\alpha=0.05$). NS denotes non-significance ($\alpha > 0.05$)

^eLowercase letters denote detected differences amongst means utilizing Tukey's HSD ($\alpha=0.05$) vertically across rooting level treatments. Larger letters denote larger values

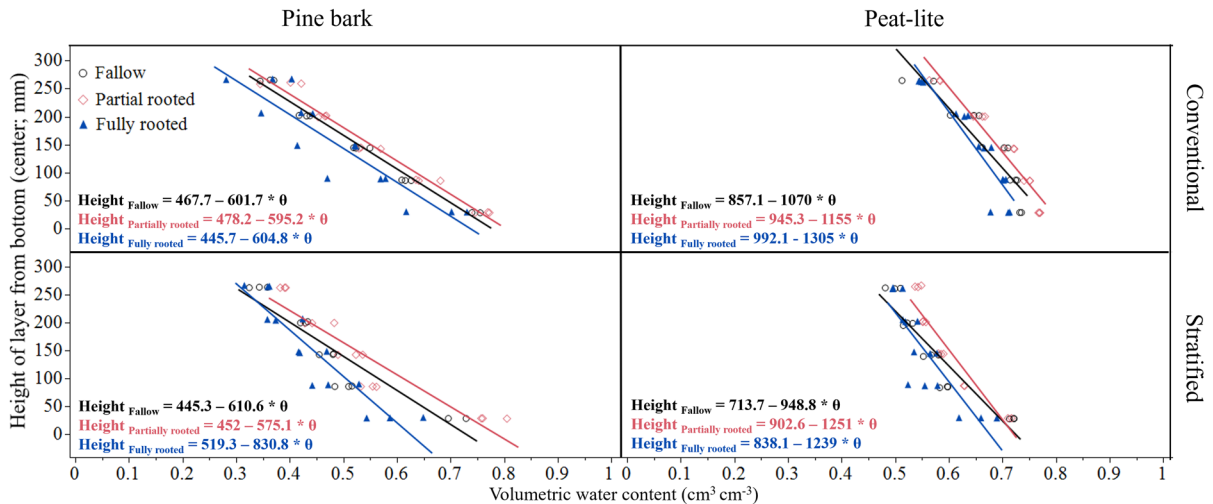


Fig. 3 Visual representation of data within Table 2 that shows the moisture content depth distribution curves of four substrate treatments including a (1) bark-based non-stratified (100% container filled with unscreened pine bark; PB), (2) bark-based stratified [the top 50% of the container volume is filled with screened fine bark particles (<6.3 mm) and the bottom 50% of the container volume is filled with screened coarse bark particles (>6.3 mm); Strat-PB], (3) peat-based non-stratified (100%

container filled with peatlite at a 7:3 blend; PL), or (4) peat-based stratified [the top 50% of the container volume is filled with peatlite (7:3 peat:perlite) and the bottom 50% of the container volume is filled with unscreened bark particles; Strat-PL]. Root levels include a fallow (no roots), partially rooted system (22 d of growth after experiment initiation) and fully rooted system (43 d of growth after experiment initiation). Equations are linear regression fit to moisture data

and development over time (Table 4). There were more pronounced differences of root traits in the top versus bottom layers of Strat-PB when compared to PB (Table 4): (1) root dry biomass and density were greater in the top layer across rooting levels (Supplemental Table 1); (2) average root diameter ($p=0.0184$) increased with downward root development in a partially rooted system (Table 4); and (3) there were smaller diameter roots in the top layers (layers one and two) and larger diameter roots in the bottom layers (layers four and five; $p=0.0015$) of partially rooted plants. Root length was greater in the top layer of a partially rooted Strat-PB than PB (Table 4). Once fully rooted, Strat-PB plants had 1.5× total root length in the bottom layer than in non-stratified profiles (Table 4). Though not statistically significant, fully rooted Strat-PB grown plants had numerically greater vol. (13%) and surface area (17%) when compared to PB grown plants (Table 4).

Plants grown in partially rooted Strat-PB profiles had 19% more very fine roots (<0.5 mm in dia.) than in PB. When fully rooted, Strat-PB profile had nearly 26 m more very fine roots throughout the whole

container than in PB (Table 5). Despite no statistical significance ($p>0.05$), fully rooted plants grown in Strat-PB had nearly 25 m more fine roots (0.5 – 1 mm) throughout the whole container and 7 m more ‘large roots’ (> 1 mm) than PB (Table 5).

Differences were detected across partially and fully rooted peat-based systems highlighting continuous root growth and development similar to PB-based systems (Table 6). The top layer of Strat-PL had the greatest dry root biomass which decreased with downward root growth ($p=0.0072$; Supplemental Table 1). Average root diameter was greatest in the top layer of PL profiles then continuously decreased towards the container base. However, the average diameter of roots in Strat-PL decreased towards the stratified interface (layer three) before increasing diameter near the container base ($p=0.0251$; Supplemental Table 1). Fully rooted Strat-PL grown plants had thinner average diameter roots when compared to PL-grown plants throughout the whole vertical profile (Table 6).

Fully rooted plants grown in Strat-PL systems had a 48 m increase in total root length throughout the container when compared to PL (Table 6). There was 20 m (23%) more total root length in the top half

Table 4 Root morphological characteristics of plants grown in one of two substrate treatments including a (1) conventionally filled pine bark substrate (100% container filled with unscreened pine bark; PB) and (2) pine bark stratified substrate [the top 50% of the container volume is filled with screened

fine bark particles (<6.3 mm) and the bottom 50% of the container volume is filled with screened coarse bark particles (>6.3 mm); Strat-PB]. Layers denote the height of column, where the top of layers 1, 2, 3, 4, and 5 are approx. 29.5-, 23.6-, 17.7-, 11.8-, and 5.9 cm \pm 0.4 from the bottom of layer 5

	Layer	Dry root biomass (mg)	Rooting density ($\mu\text{g}\cdot\text{cm}^{-3}$)	Average root diameter (μm)	Total root length (cm)	Rooting volume (cm^3)	Root surface area (cm^2)	Specific root length ($\text{m}\cdot\text{g}^{-1}$)	
Conventional (PB)	Partially rooted ^a								
	Top (1,2)	53.7 a ^d	195.9 a	646.1 a	711.5	4.0 a	147.7	140.1 b	
	Interface (3)	13.5 b	51.6 b	548.9 b	377.4	1.2 b	66.3	288.5 a	
	Bottom (4,5)	21.8 ab	83.1 ab	580.7 b	584.5	1.9 ab	106.5	268.0 a	
	Whole	89.0	110.2	591.9	1,673.4	7.1	319.1	194.4	
	(t vs i vs b) ^c	0.0271	0.0300	0.0035	0.1919	0.0285	0.1072	0.0034	
	Fully rooted ^b								
	Top (1,2)	689.4	2,463.8	747.8	5,157.5	40.5	1,250.5	86.5	
	Interface (3)	152.4	580.6	675.5	2,422.0	12.8	535.2	176.9	
	Bottom (4,5)	219.2	817.6	672.7	4,986.6	24.7	1075.4	353.5	
	Whole	1,061.0	1,300.8	698.7	12,566.1	78.0	2,861.1	151.1	
	(t vs i vs b)	0.0867	0.1004	0.2935	0.1413	0.1551	0.1773	0.2413	
	(p v f; W) ^c	0.0623	0.0639	0.0336	0.0129	0.0348	0.0193	0.4192	
	Stratified (Strat-PB)	Partially rooted							
Top (1,2)		72.5 a	210.5 ab	577.5 b *	1,540.4 a **c	6.2 a *	283.1 a *	210.5 ab *	
Interface (3)		15.0 b	261.4 a	590.9 b	392.1 b	1.4 b	73.3 b	261.4 a	
Bottom (4,5)		21.9 b	193.6 b	707.3 a **	406.1 b	2.2 b	92.4 b	193.6 b *	
Whole		109.4	134.6	625.2	2,338.7	9.8	449.2	213.1	
(t vs i vs b)		0.0005	0.0394	0.0064	0.0025	0.0022	0.0041	0.0394	
Fully rooted									
Top (1,2)		691.1 a	2,563.3 a	721.0 a	6,270.7	42.8 a	1,438.6 ab	91.4 b	
Interface (3)		176.8 b	662.4 b	653.3 b	3,391.3	16.0 b	704.3 b	194.1 ab	
Bottom (4,5)		364.4 b	1,344.8 ab	676.1 ab	8,818.3	43.0 a	1,874.8 a	242.2 a	
Whole		1,232.3	1,516.5	683.5	18,480.3	101.7	4,017.1	150.8	
(t vs i vs b)		0.0079	0.0084	0.0184	0.0598	0.0349	0.0436	0.0167	
(p v f; W)		0.0043	0.0041	0.0315	0.0049	0.0046	0.0039	0.0165	

^aPartially rooted plants were grown for 22 days after experimental initiation

^bFully rooted plants were grown for 43 days after experimental initiation

^cMeasures of overall treatment effects vertically utilizing one-way ANOVA analysis (t vs i vs b) or t-test (p v f; W) with a significance value of ($\alpha=0.05$). NS denotes non-significance ($\alpha>0.05$). *T*Top layers (1 and 2), *I*Interface layer (3), *B*Bottom layers (4 and 5). *P*Partially rooted, *F*Fully rooted, *W*Whole container, where all layer values were summed

^dLetters denote detected differences amongst means utilizing Tukey's HSD ($\alpha=0.05$) vertically across rooting level treatments

^eAsterisks denote either non-significance (no asterisk), or *, **, *** values represent significant at $P\leq 0.05$, 0.01, or 0.001, respectively, across stratified and conventional profiles utilizing a t-test

(layers one and two) of fully rooted Strat-PL than in PL (Table 6). Fully rooted Strat-PL grown plants had 15% greater rooting volume than fully rooted PL plants (Table 6). Fully rooted Strat-PL plants had greater specific root length in the top layers (one and two) relative to PL grown plants (Table 6). Plants

grown in Strat-PL had 8.5 m greater very fine root length in the top half and 17 m more very fine root length throughout the whole container than PL treatments (Table 7). The Strat-PL grown plants contained more large and extra-large root length than PL-grown plants across rooting treatments (Table 7).

Table 5 Root morphological characteristics of plants grown in one of two substrate treatments including a (1) conventionally filled pine bark substrates (100% container filled with unscreened pine bark; PB) and (2) a pine bark stratified substrate [the top 50% of the container volume is filled with

screened fine bark particles (<6.3 mm) and the bottom 50% of the container volume is filled with screened coarse bark particles (>6.3 mm); Strat-PB]. Layers denote the height of column, where the top of layers 1, 2, 3, 4, and 5 are approx. 29.5-, 23.6-, 17.7-, 11.8-, and 5.9 cm \pm 0.4 from the bottom of layer 5

Layer	Conventional (PB)				Stratified (Strat-PB)			
	Root length in cm (very fine; <0.5 mm)	Root length in cm (fine; 0.5–1 mm)	Root length in cm (large; > 1 mm)	Root length in cm (very large; > 2 mm)	Root length in cm (very fine; <0.5 mm)	Root length in cm (fine; 0.5–1 mm)	Root length in cm (large; > 1 mm)	Root length in cm (very large; > 2 mm)
Partially rooted ^a								
Top (1, 2)	290.7	332.9	70.7 a ^d	17.2 a	691.0 a* ^e	733.8 a*	98.3 a*	17.2 a*
Interface (3)	175.0	175.7	25.5 ab	1.2 b	150.2 b	209.2 b	31.9 b	0.8 b
Bottom (4, 5)	248.0	292.9	41.2 b	2.3 b	115.7 b	215.7 b	69.1 b	5.7 b
Whole container	713.7	801.5	137.5	20.6	956.9	1,158.7	199.4	23.7
<i>p</i> -value (t vs i vs b) ^c	0.3014	0.1994	0.0539	0.0055	0.0006	0.0061	0.0827	0.0006
Fully rooted ^b								
Top (1, 2)	1,476.8	2,660.2	867.7	152.8	2,158.0	2,993.4 ab	942.7 ab	176.6
Interface (3)	808.4	1,193.6	373.8	46.2	1,286.7	1,616.2 b	435.1 b	53.3
Bottom (4, 5)	1,674.8	2,521.9	713.5	76.3	3,127.2	4,341.2 a	1,210.4 a	139.4
Whole container	3,959.9	6,375.7	1,955.1	275.4	6,571.9	8,950.9	2,588.2	369.3
<i>p</i> -value (t vs i vs b)	0.1093	0.1264	0.2830	0.1992	0.1195	0.0477	0.0190	0.0553
<i>p</i> -value (p v f; W) ^c	0.0102	0.0109	0.0339	0.0685	0.0098	0.0036	0.0019	0.0119

^aPartially rooted plants were grown for 22 days after experimental initiation

^bFully rooted plants were grown for 43 days after experimental initiation

^cMeasures of overall treatment effects vertically utilizing one-way ANOVA analysis (t vs i vs b) or t-test (p v f; W) with a significance value of ($\alpha=0.05$). NS denotes non-significance ($\alpha>0.05$). TTop layers (1 and 2), IInterface layer (3), BBottom layers (4 and 5). PPartially rooted. FFully rooted. WWhole container, where all layer values were summed

^dLetters denote detected differences amongst means utilizing Tukey's HSD ($\alpha=0.05$) vertically across rooting level treatments

^eAsterisks denote either non-significance (no asterisk), or *, **, *** values represent significant at $P\leq 0.05$, 0.01, or 0.001, respectively, across stratified and conventional profiles utilizing a t-test

Interrelationships across treatments

Layer height and θ ($r=-0.7834$) of both fallow and fully rooted treatments were highly correlated across substrates. A weaker relationship of layer height and θ occurred across partially rooted columns ($r=-0.6551$; Supplemental Table 2). All fully rooted systems had a stronger moderate negative relationship of θ with root biomass ($r=-0.5479$) and density ($r=-0.5306$) than in partially rooted systems (Supplemental Table 2). Dry root biomass was more strongly related with layer height than other root morphological features

across rooting levels and substrate (Supplemental Table 2). The change in θ in stratified systems [Strat-PB ($r=-0.8770$); Strat-PL ($r=-0.8679$)] was less affected by layer height than in conventional profiles [PB ($r=-0.9428$); PL ($r=-0.9039$)] across all substrates (Supplemental Table 2). Root dry biomass and θ ($r=-0.6978$), and specific root length and layer height ($r=-0.7299$), were more highly correlated in PL than other substrate treatments (Supplemental Table 2). Root diameter-based lengths and layer height was more important for partially rooted systems than fully rooted systems across substrates (Supplemental Table 2).

Table 6 Root morphological characteristics of plants grown in one of two substrate treatments including a (1) conventionally filled peatlite substrate (100% container filled with peatlite at a 7:3 blend; PL) and a (5) peatlite stratified substrate [the top 50% of the container volume is filled with peatlite (7:3

peat:perlite) and the bottom 50% of the container volume is filled with unscreened bark particles; Strat-PL]. Layers denote the height of column, where the top of layers 1, 2, 3, 4, and 5 are approx. 29.5-, 23.6-, 17.7-, 11.8-, and 5.9 cm ± 0.4 from the bottom of layer 5

	Layer	Dry root biomass (mg)	Rooting density ($\mu\text{g}\cdot\text{cm}^{-3}$)	Average root diameter (μm)	Total root length (cm)	Rooting volume (cm^3)	Root surface area (cm^2)	Specific root length ($\text{m}\cdot\text{g}^{-1}$)	
Conventional (PL)	Partially rooted ^a								
	Top (1, 2)	72.8 a ^d	248.0 a	670.3 a	657.6 a	5.1	145.2	90.9 b	
	Interface (3)	10.3 b	39.7 b	568.6 b	192.0 b	0.6	34.7	190.2 a	
	Bottom (4, 5)	22.3 ab	85.0 ab	524.3 b	522.4 b	1.4	86.4	249.1 a	
	Whole	105.4	129.5	587.7	1,372.1	7.2	266.3	135.1	
	(t vs i vs b) ^c	0.0288	0.0316	0.0094	0.0660	0.0722	0.0670	0.0067	
	Fully rooted ^b								
	Top (1, 2)	632.8 a	2,301.2 a	855.9 a	3,532.0 a	35.6 a	967.1 a	55.7 b	
	Interface (3)	142.5 b	542.7 b	836.8 a	1,494.7 a	11.6 a	398.4 b	106.8 a	
	Bottom (4, 5)	181.0 b	688.8 b	749.0 b	2,719.1 b	16.2 b	642.4 ab	151.7 a	
	Whole	956.3	1,187.9	813.9	7,745.7	63.5	2,008.0	81.2	
	(t vs i vs b)	0.0002	0.0002	0.0181	0.0385	0.0389	0.0375	0.0035	
	(p v f; W) ^c	0.0014	0.0016	0.0004	0.0052	0.0097	0.0044	0.0122	
	Stratified (Strat-PL)	Partially rooted							
Top (1, 2)		82.9	284.1	644.2 a	1,928.0	11.5	386.5	186.9	
Interface (3)		12.7	48.8	545.0 b	370.1	1.2	65.2	307.7 *	
Bottom (4, 5)		18.1	69.4	592.2 ab **	544.0 *	1.9 *	102.7 *	322.2	
Whole		113.8	141.3	593.8	2,842.0	14.6	554.4	226.9 *	
(t vs i vs b)		0.1326	0.1486	0.0336	0.3552	0.1983	0.3088	0.0899	
Fully rooted									
Top (1,2)		684.8 a	2,486.3 a	755.3 *	5,593.3 *	46.7 *	1,372.9 *	82.6 *	
Interface (3)		140.1 b	531.6 b	709.7 *	2,756.2 *	15.4 *	627.0 *	247.6	
Bottom (4,5)		242.8 b	934.7 b	752.0	4,145.9	26.3	1,006.4	178.9	
Whole		1,067.7	1,337.7	739.0 *	12,495.4 *	88.5 *	3,006.3 *	123.2	
(t vs i vs b)		0.0158	0.0178	0.3048	0.1386	0.0572	0.1450	0.1903	
(p v f; W)		0.0210	0.0245	0.0027	0.0205	0.0209	0.0190	0.0257	

^aPartially rooted plants were grown for 22 days after experimental initiation

^bFully rooted plants were grown for 43 days after experimental initiation

^cMeasures of overall treatment effects vertically utilizing one-way ANOVA analysis (t vs i vs b) or t-test (p v f; W) with a significance value of ($\alpha=0.05$). NS denotes non-significance ($\alpha>0.05$). *T*Top layers (1 and 2), *I*Interface layer (3), *B*Bottom layers (4 and 5). *P*Partially rooted, *F*Fully rooted, *W*Whole container, where all layer values were summed

^dLetters denote detected differences amongst means utilizing Tukey's HSD ($\alpha=0.05$) vertically across rooting level treatments

^eAsterisks denote either non-significance (no asterisk), or *, **, *** values represent significant at $P\leq 0.05$, 0.01, or 0.001, respectively, across stratified and conventional profiles utilizing a t-test

Discussion

Moisture gradients in conventional and stratified substrate profiles

The height of the water column (Owen and Altland

2008) and pore diameter, determined by the particle size (Pustjarvi and Robertson 1975; Bartley et al. 2022), influences substrate matric tensions (Handreck and Black 2002). Consistent with previous findings (Milks et al. 1989; Owen and Altland 2008), a moisture gradient was observed in both pine bark or

Table 7 Root morphological characteristics of plants grown in one of two substrate treatments including a (1) conventionally filled peatlite substrate (100% container filled with peatlite at a 7:3 blend; PL) and a (5) peatlite stratified substrate [the top 50% of the container volume is filled with peatlite (7:3

peat:perlite) and the bottom 50% of the container volume is filled with unscreened bark particles; Strat-PL]. Layers denote the height of column, where the top of layers 1, 2, 3, 4, and 5 are approx. 29.5-, 23.6-, 17.7-, 11.8-, and 5.9 cm \pm 0.4 from the bottom of layer 5

Layer	Conventional (PL)				Stratified (Strat-PL)			
	Root length in cm (very fine; < 0.5 mm)	Root length in cm (fine; 0.5–1 mm)	Root length in cm (large; > 1 mm)	Root length in cm (very large; > 2 mm)	Root length in cm (very fine; < 0.5 mm)	Root length in cm (fine; 0.5–1 mm)	Root length in cm (large; > 1 mm)	Root length in cm (very large; > 2 mm)
Partially rooted ^a								
Top (1, 2)	259.1	313.1	64.3 a ^d	21.0 a	847.6	870.4	172.0	37.9
Interface (3)	84.3	92.2	14.9 b	0.7 b	174.4	167.0	27.6	1.1
Bottom (4, 5)	269.9	224.0	27.7 ab	0.8 b	209.3	287.3	45.7	1.7
Whole container	613.3	629.4	106.9	22.5	1,231.3	1,324.8	245.2	40.7
<i>p</i> -value (t vs i vs b) ^c	0.0405	0.0950	0.0298	0.0272	0.3777	0.3629	0.2732	0.0906
Fully rooted ^b								
Top (1, 2)	865.9	1,725.8 a	761.3 a	178.9 a	1721.3	2,708.1	967.7	196.2
Interface (3)	352.0	715.1 b	369.3 b	58.3 b	880.8 *** ^e	1,337.2 **	483.6	54.5
Bottom (4, 5)	795.7	1,317.5 ab	551.9 ab	54.0 b	1,149.4	2,039.2	850.3	107.1
Whole container	2,013.6	3,758.4	1,682.5	291.2	3,751.5	6,084.5	2,301.5	357.8
<i>p</i> -value (t vs i vs b)	0.0844	0.0214	0.0560	0.0297	0.0945	0.1243	0.2682	0.1170
<i>p</i> -value (p v f; W) ^c	0.0239	0.0029	0.0021	0.0110	0.0450	0.0150	0.0182	0.0454

^aPartially rooted plants were grown for 22 days after experimental initiation

^bFully rooted plants were grown for 43 days after experimental initiation

^cMeasures of overall treatment effects vertically utilizing one-way ANOVA analysis (t vs i vs b) or t-test (p v f; W) with a significance value of ($\alpha=0.05$). NS denotes non-significance ($\alpha>0.05$). TTop layers (1 and 2), IInterface layer (3), BBottom layers (4 and 5). PPartially rooted. FFully rooted. WWhole container, where all layer values were summed

^dLetters denote detected differences amongst means utilizing Tukey's HSD ($\alpha=0.05$) vertically across rooting level treatments

^eAsterisks denote either non-significance (no asterisk), or *, **, *** values represent significant at $P\leq 0.05$, 0.01, or 0.001, respectively, across stratified and conventional profiles utilizing a t-test

peat-based substrates and across levels of rooting. There were more significant changes of θ in the top layers of peat-based than pine bark-based stratified substrates when compared to conventional substrates. Less water was stored in stratified systems when compared to a conventional profile. Both Criscione et al. (2024) and Fields et al. (2024) demonstrated that stratified substrate systems store overall less water due to the coarse bark in the bottom strata increasing drainage (Milks et al. 1989; Bilderback and Fonteno 1987; Owen and Altland 2008), which can increase both oxygen supply and root growth (Fields and Criscione 2023). The finer-textured upper strata had

a more uniform θ across the vertical profile while the coarser sub-strata resulted in larger changes in θ due to increased drainage. These observed changes in the θ gradient validates our first hypothesis that stratified substrates improved vertical moisture distribution in containers.

A potential concern in stratified substrates is the formation of a perched water table at the stratified interface as in mineral soils (Miller and Gardner 1962), which could create anoxic conditions for roots. The continual decrease in moisture throughout the column height, with no notable variation in the center of the column, confirms there is no

perched water table in stratified substrate systems at the stratified interface post-gravitational drainage. However, the potential formation of a transient water table is considerable. Applicators may mistake the decreased infiltration rates of finer-textured soilless substrates (Bilderback and Jones 2001) when irrigating stratified systems (Criscione et al. 2022) as the presence of a perched water table. However, water likely infiltrates through the top strata in a ‘funnel flow’ pattern, slowing infiltration, then shifts to ‘macropore flow’ in the substrata, resulting in more transient flow (Selker 1996; Criscione et al. 2022).

Rooting influence on container water storage

In general, partially rooted profiles stored more water than substrate systems with fully rooted plants. The authors hypothesize that in a partially rooted system, the initial root migration either “clogged” the pores which created more microporosity (Scholl et al. 2014), or the displaced particles altered the pores arrangement with the substrate matrix. The reduced pore space (Altland et al. 2011) and subsequent increase in θ and decrease in air space (Judd et al. 2015a) is a result of decreased effective pore radius as roots proliferate (Lucas et al. 2019). Alternatively or occurring concurrently, root tips may have had increased mucilage or rhizodeposits to help facilitate proliferation through the unexplored particles and pores (Bengough et al. 2011; Holz et al. 2018) and could have contributed to the initial increased water storage (Young 1995; Carminati et al. 2010; Carminati 2013).

There could be multiple explanations for the decreased water storage from partial to fully rooted system across substrates. Several have reported that water traveling down root axes or bypassing restricted pores can increase infiltration rates, wetting-front uniformity, or drainage in both soilless substrates (Hoskins et al. 2014; Criscione et al. 2022) and mineral soils (Gish and Jury 1983). Roots can create a more homogeneous pore size distribution (Gish and Jury 1983) as proliferating root tissues fill pore voids and fragment particles (Scanlan 2009). The more homogenous pore size distribution straightens infiltration pathways (Jury and Horton 2004) and reduces substrate tortuosity (Cannavo et al. 2011) as a result of decreased pore-to-pore distances and increased particle connectivity. In

containerized systems, localized channeling along the container walls due to increased localized hydraulic conductivity (Nash and Laiche 1981) and changes in pore diameter distribution (Cannavo and Michel 2013) can lead to less water storage in fully rooted systems.

Root morphological development as a response to moisture

Conventionally grown plants had a relatively uniform growth rate with similar root growth in the top and bottom half. By comparison, stratified-grown plants grew in a more sequential habit in which the roots explored the upper strata longer. In similar time-frames, roots in stratified substrates had increased growth rates, greater biomass accumulation, and more fine root development localized in the top half. These results are similar to findings reported by Fields et al. (2022) and Fields and Criscione (2023). The authors hypothesize that these growth responses are due to (1) detections in a modified vertical water distribution across conventional versus stratified substrates, (2) finer particles installed in the top strata of the container, or (3) a combination of both. These responses are described in more detail below.

Differences in θ can influence root development and cause biochemical alterations in the root tip, often resulting in two primary responses: (1) a shift from lateral root development and investment in axile roots to grow more vertically (Koevoets et al. 2016) and (2) a hormone differential modulating growth orientation (Morohashi et al. 2017; Mizuno et al. 2002; Takahashi and Suge 1991; Strohm et al. 2012; Nakajima et al. 2017). It is possible that as the root tip proliferated in the top layers of a conventional substrate, the sharp 0.12 to $0.14 \text{ cm}^3 \cdot \text{cm}^{-3}$ vertical θ decrease in upper strata may have resulted in auxin to be redistributed to the root tip, causing more vertical root elongation. The spatial change in θ could have repressed shootward auxin transport (necessary for pericycle activation; Liu et al. 2015) and subsequent lateral root initiation (Bao et al. 2014). In turn, this could have resulted roots to exhibit a more downward bending nature (Koevoets et al. 2016) that induced deeper rooting (Masalia et al. 2018) early in the study when compared to stratified-grown plants.

The smaller change in θ in the top profile ($0.06 - 0.08 \text{ cm}^3 \cdot \text{cm}^{-3}$) of stratified grown plants likely

facilitated a hydrotropism response (Robbins and Dinneny 2015; Koevoets et al. 2016). Starch-filled amyloplasts would begin to be degraded and reduce gravisensing cell functions (Takahashi et al. 2003) while increasing more lateral root emergence (Bao et al. 2014; Robbins and Dinneny 2018). Concurrently, the pronounced growth in the top half in shorter timeframes may have been due to the increased water availability (Criscione et al. 2024), resulting in roots growing more horizontally towards areas with equal or higher water potential (Dietrich 2018; Masalia et al. 2018). An alternative possibility is that root development into the coarse bark sub-strata may have been momentarily restricted due to sensing of higher localized θ in the finer substrate above the stratified interface. Gravitropism responses still occurred in stratified-grown plants, reflected by biomass and root length values in the lower layers, it is just reduced by comparison.

The authors hypothesize that stratified-grown plants had greater fine root lengths in the top half partly due to the more uniform moisture profile. Cell elongation requires continuous supply of water that is dependent on the soil water availability (Robbins and Dinneny 2015). Fine root growth (Ji et al. 2023) and elongation rates (Bengough et al. 2011) can decrease in water stressed conditions, and although conventionally grown plants were unlikely to be severely water stressed, the increased water availability (Criscione et al. 2024) and moisture uniformity (Table 2) in stratified profiles likely facilitated continuous root elongation with less interruptions in growth. The continuous access to moisture possibly enabled greater production of low-resource-cost development of root tissues and decreased the need to radially thicken (shift to transport roots and undergo secondary development, i.e., loss of cortex, cork periderm growth, lignification; McCormack et al. 2015).

Textural classification differences in stratified substrate systems

The pores size and continuity of the substrate provide a framework that guides proliferating roots in the 3-dimensional substrate matrix. This matrix may predict root architecture. As roots elongate through the particle matrix, root tips can either (1) penetrate small

diameter pores to deform the substrate that creates new pore pathways, (2) elongate along an existing pore or particle surface before entering the pore or particle, or (3) both (Jin et al. 2013). Soilless-grown roots are likely to encounter more macropore space and rely on particles to grow alongside or inside particles (Pokorny and Weitzstein 1984) than in mineral soils.

Root tips will generally choose the path of least resistance to minimize metabolic resource use (Popova et al. 2016). Finer-textured particles and smaller-diameter pores generally provide lower mechanical resistance (Allaire et al. 1996) due to better pore distribution uniformity than unscreened pine bark (Fields et al. 2021). The finer texture matrix has an improved continuity of pores that support continuous root elongation due to reduced mechanical resistance and stress (Bennie 1991; Bengough et al. 2006; Colombi et al. 2017; Zhou et al. 2021). The finer textured particles also likely facilitate better root-particle contact (Schmidt 2011).

Conversely, roots may encounter more resistance in coarser-textured substrates, including more air-filled macropore spaces and flat plate-like geometric barriers. Larger diameter pores quickly release water and are immediately replaced by air (Fields et al. 2014). Xerobranching (i.e., refraining from lateral root emergence) can occur when a root approaches a localized air-filled macropore in soil to avoid desiccation (Orman-Ligeza et al. 2018). Lateral roots can still emerge due to moisture detection as roots grow alongside a macropore and particle contact is maintained. If no particle contact occurs in a macroscopic air space, axial root growth decreases with little to no lateral root emergence (Bao et al. 2014). This may explain why in conventional pine bark systems there was reduced root development (e.g., length) in the upper half when compared to stratified-grown plants. Additionally, stratified-grown plants may have had thicker roots in the coarse bark sub-strata due to increased particle obstruction (Singh and Sainju 1998). If particles are perpendicular to the elongating root axis, the root tip or statocytes detect the mechanical pressures and can result in a feedback loop that decreases elongation rates and increases radial expansion and thickening. When this occurs, the cortex and endodermis undergo more cellular division (Fitter 1996; Croser et al. 2000) to alleviate axial root cap stress (Bengough et al. 2006).

Conclusion

Descriptive research of root growth in soilless substrates is understudied relative to its shoot counterpart. This research explored using a frozen column method to evaluate root morphology over time in a three dimensional space, as well as study the impact of the root morphology on substrate water distribution through bark- and peat-based conventional and stratified substrate profiles.

The research highlights opportunities to spatially engineer where fibrous root systems develop within the container, ultimately allowing improving water and mineral nutrient uptake. Longer root systems in the upper layers work in concert with traditional top-down irrigation to immediately capture infiltrating water and solutes. Moreover, this improves plant access to water throughout the container if there are non-uniform wetting fronts or moisture is not uniformly distributed. Roots in stratified substrates fill the container volume quicker, which can lead to reduced production cycles and associated costs. Further research could investigate spatiotemporal root mucilage production of container grown roots or a shift in auxin and ABA hormone accumulation across root systems grown in different container layers.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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