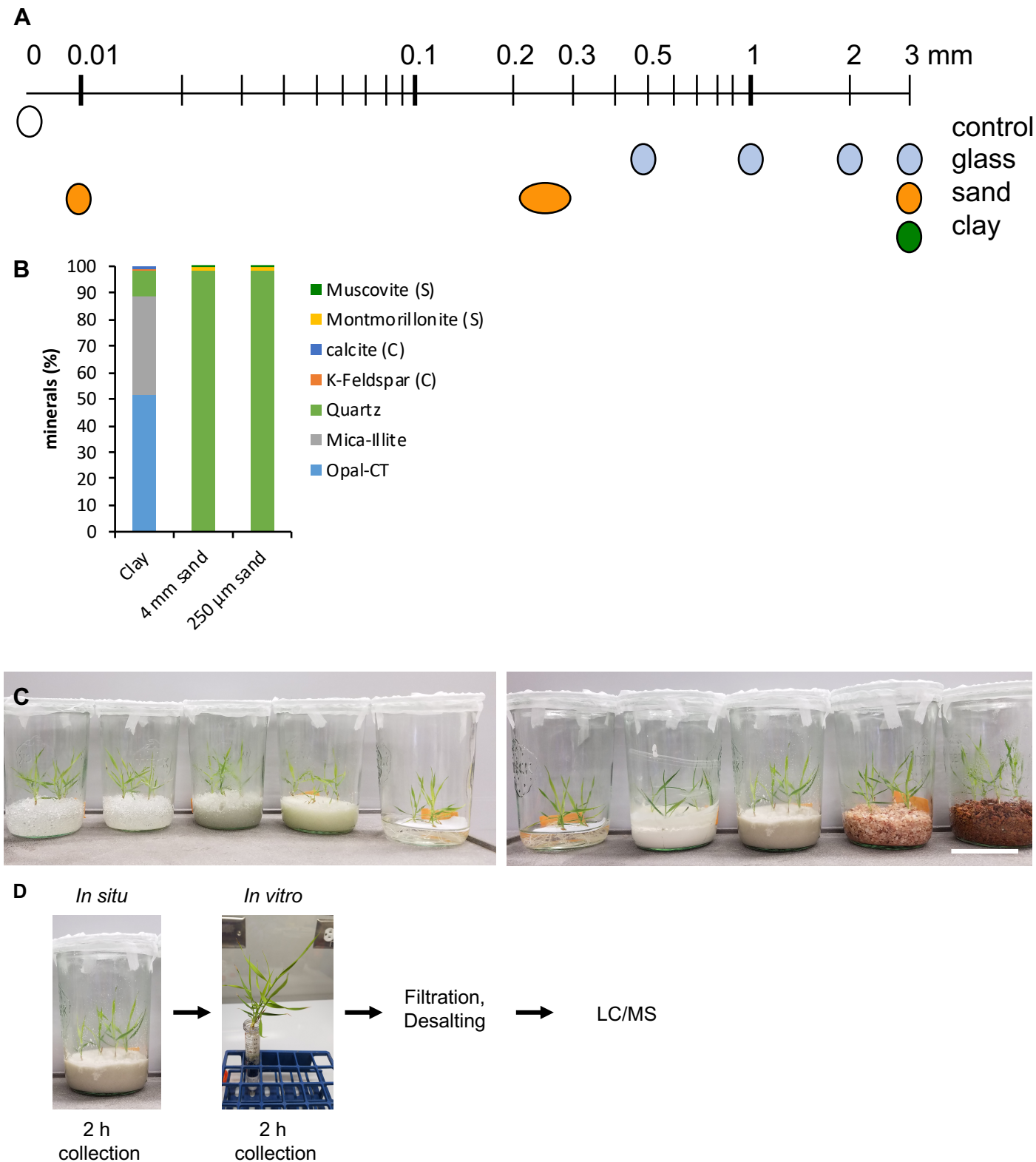
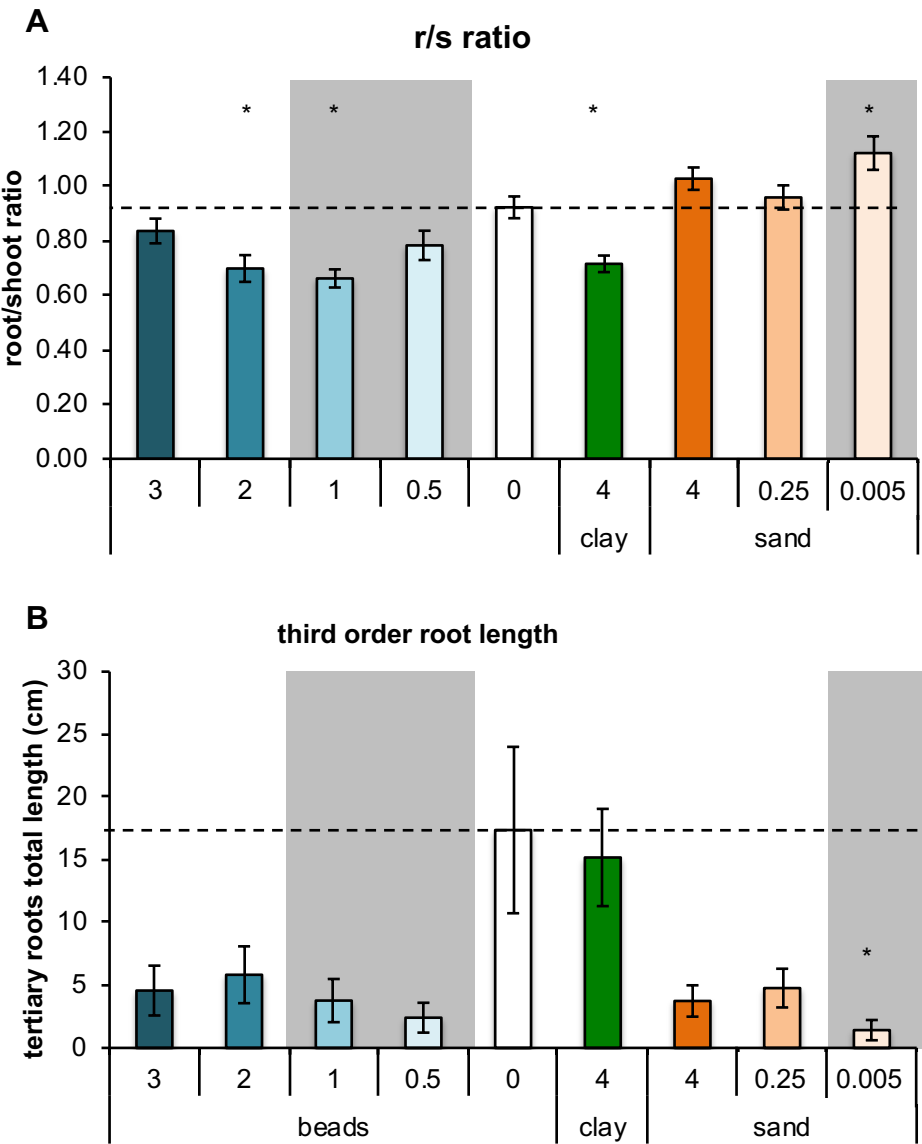


# Figure S1: Experimental setup



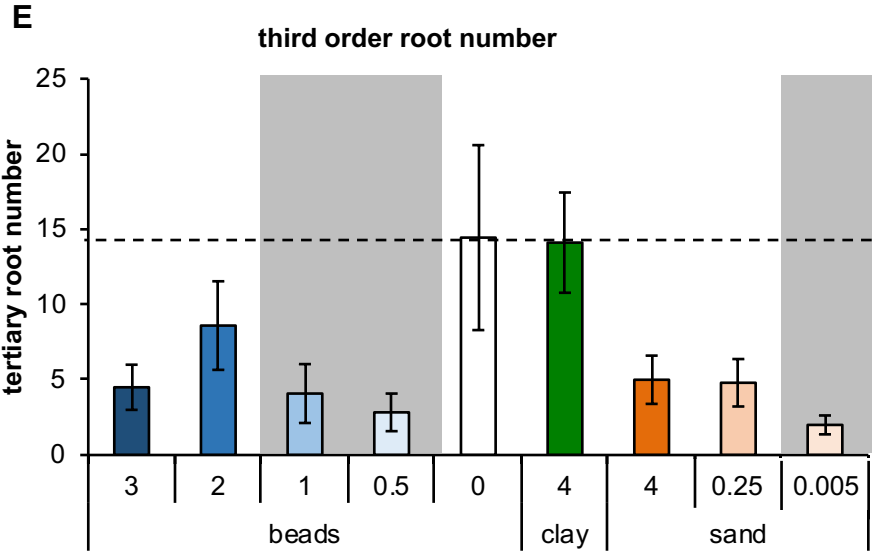
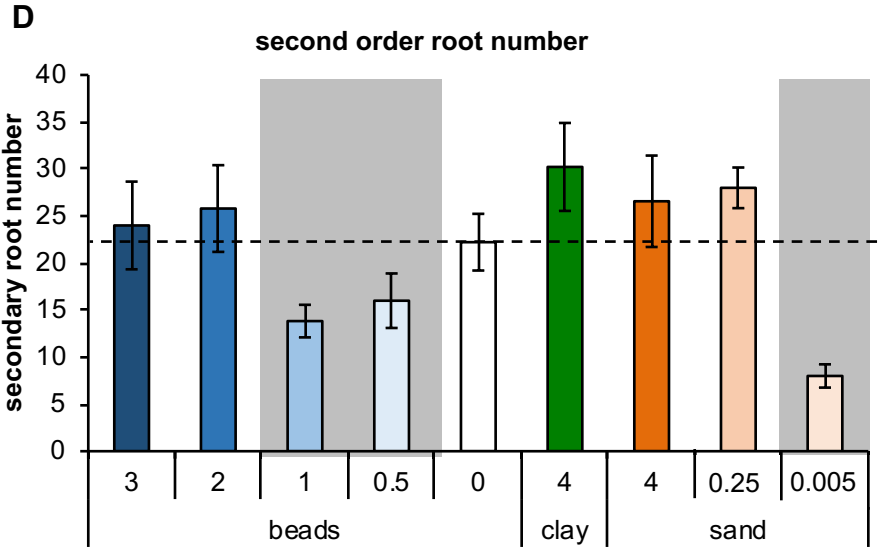
**Figure S1 | Experimental setup** (A) Illustration of particle sizes utilized, logarithmic scale. (B) X-ray diffraction analysis of 4 mm clay, 4 mm sand, and 250  $\mu$ m sand. Trace elements are indicated as being present in sand (S) or clay (C). (C) Jar setup, one jar per condition is depicted. Top panel, from left: 3 mm, 2 mm, 1 mm, 0.5 mm glass beads, hydroponic control. Bottom panel, from left: hydroponic control, 5  $\mu$ m sand, 250  $\mu$ m sand, 4 mm sand, 4 mm clay. Scale bar: 10 cm. (D) Root exudate collection procedure, the *in vitro* picture displayed is representative of the setup, but a larger tube (50 ml) was used for exudate collection.

**Figure S2: Additional root morphology data**

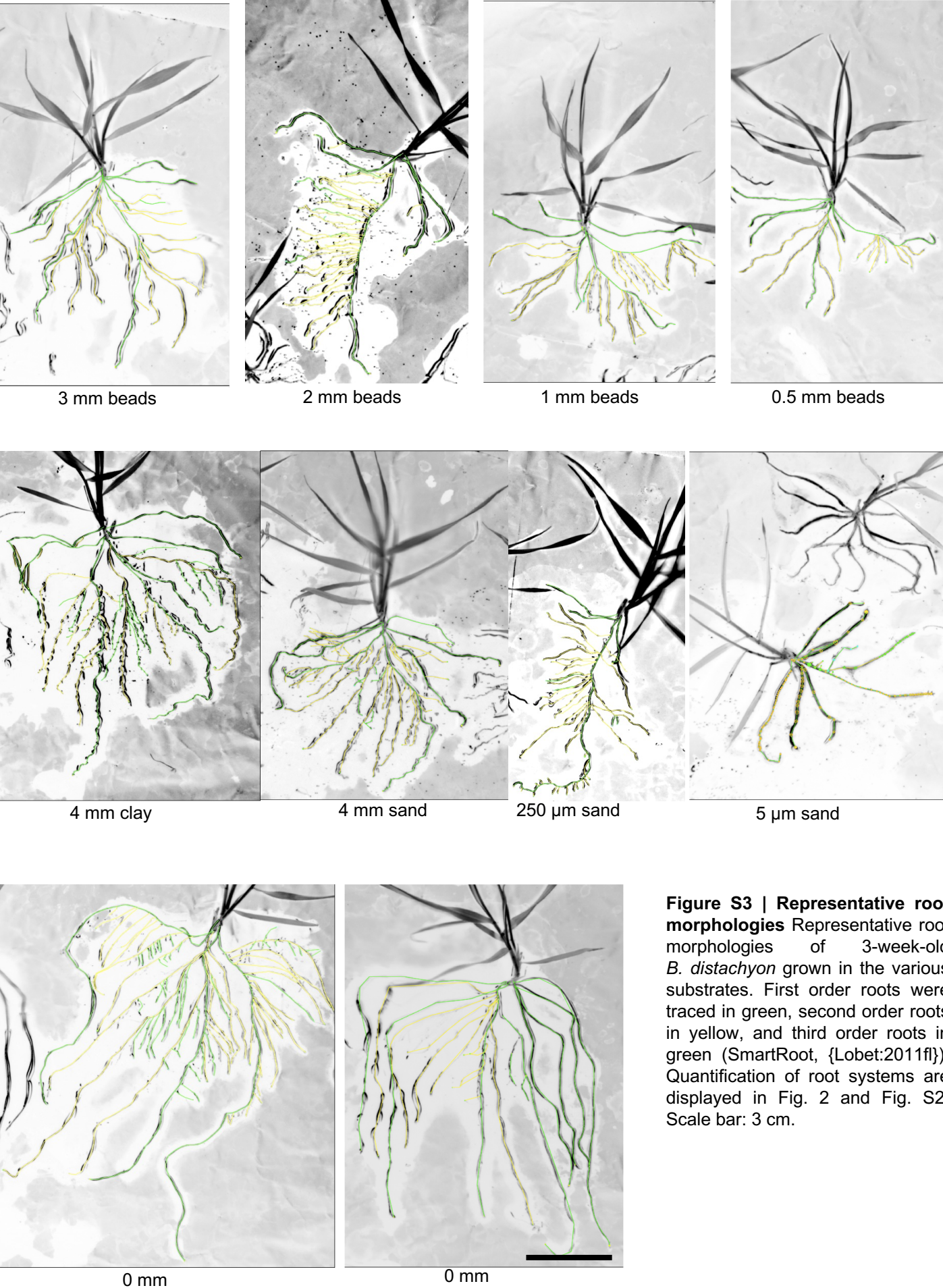


**Figure S2 | Additional root morphology** Root/shoot ratio (A), third order root length (B) first order (C), second order (D), and third order (E) root numbers. Data are means  $\pm$  SEM,  $n > 5$ . Significant differences are displayed as asterisks (\*,  $p = 0.05$ ) of substrates compared to hydroponic control (0, dashed line). Tissue weights, total root length and number, first and second order root lengths are given in Fig. 2. Grey areas: plants with weight and root morphology distinct from hydroponic controls.

**Figure S2: Additional root morphology data**



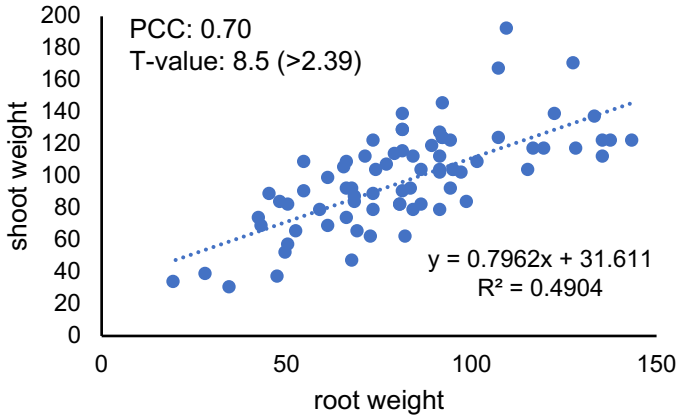
# Figure S3: representative root morphologies



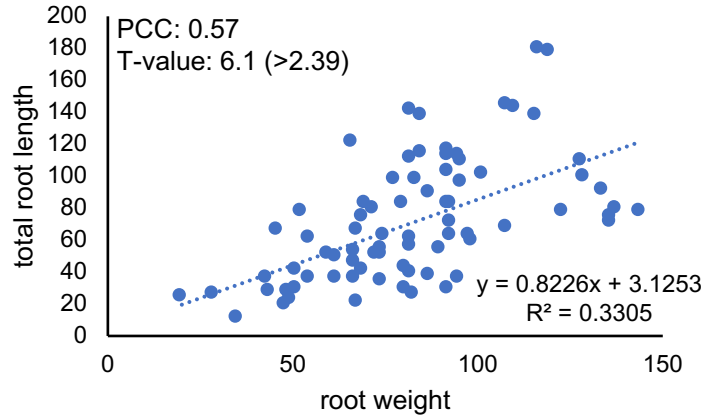
**Figure S3 | Representative root morphologies** Representative root morphologies of 3-week-old *B. distachyon* grown in the various substrates. First order roots were traced in green, second order roots in yellow, and third order roots in green (SmartRoot, {Lobet:2011fl}). Quantification of root systems are displayed in Fig. 2 and Fig. S2. Scale bar: 3 cm.

**Figure S4: Correlation analysis of root parameters**

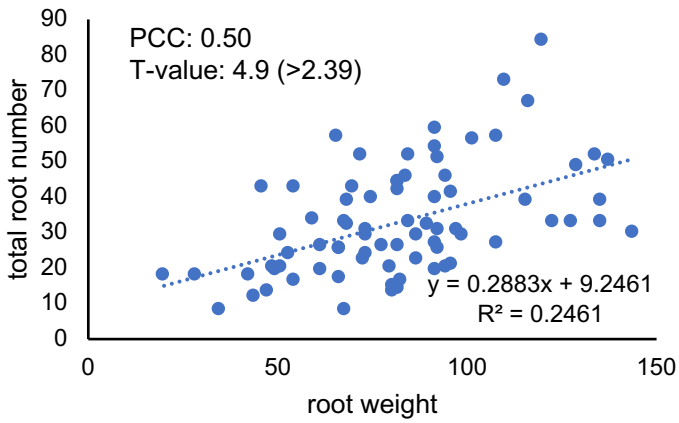
shoot - root weight



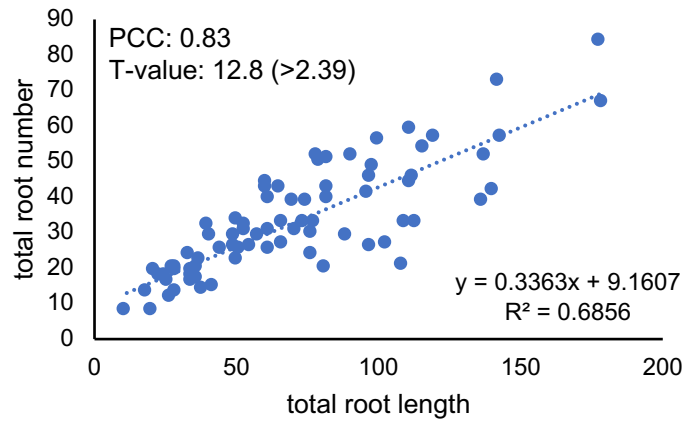
root length - root weight



root number - root weight

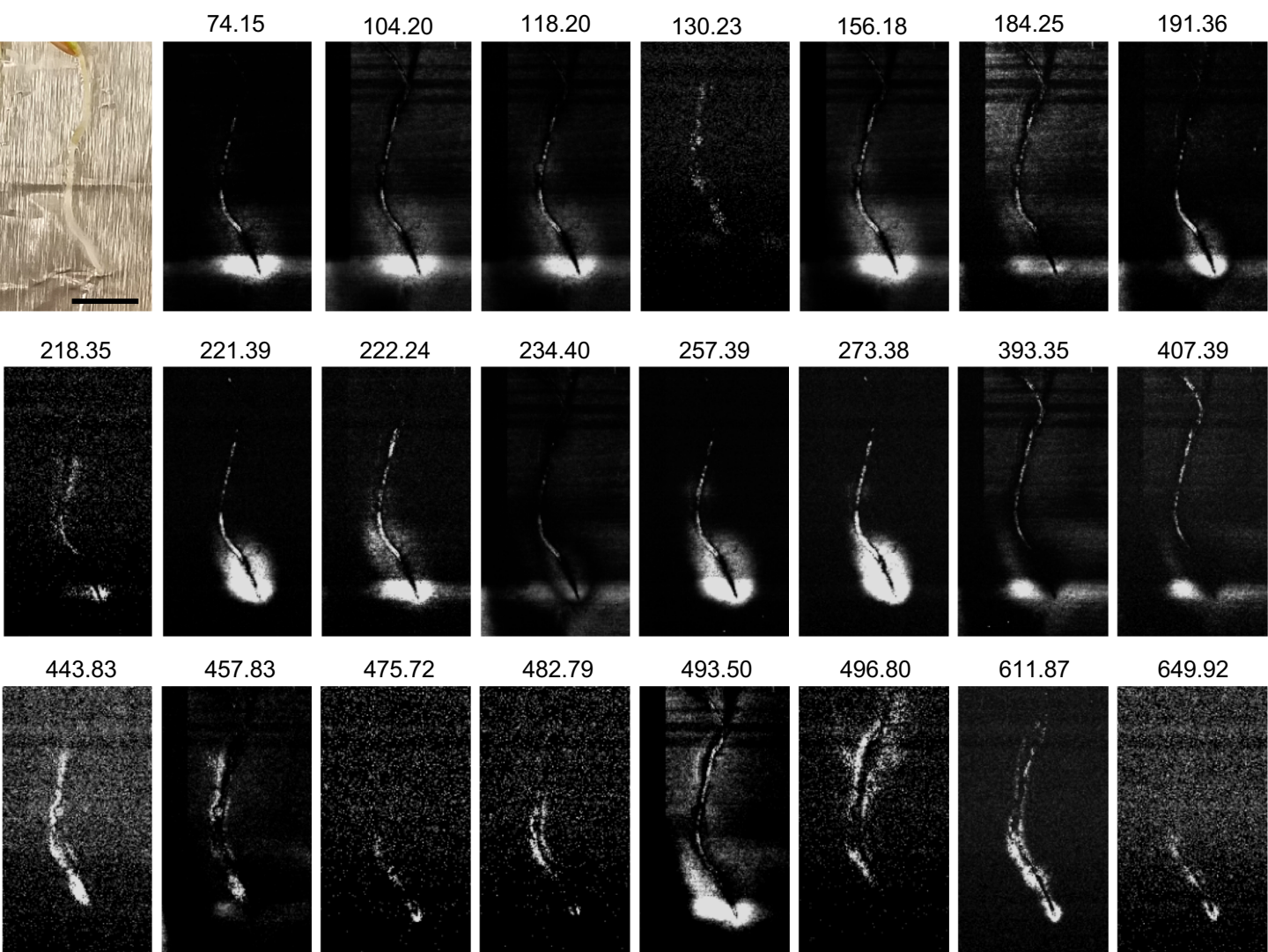


root number - root length



**Figure S4 | Correlation analysis of root parameters** Correlation plots of various root parameters. Dashed lines are linear regressions, equations and  $R^2$  values are given. Pearson correlation coefficients (PCC) were calculated, and the respective t-values are given.

**Figure S5: *B. distachyon* seedling exudation visualization**



**Figure S5 | Additional spatial exudation patterns** Plants were incubated for 6 h to allow for exudation. Distinct root-associated patterns of several ions were observed with Mass Spectrometry Imaging. The  $m/z$  of the different ions observed is indicated above the panels. Selected panels are presented in Fig. 3. Ion 156.18 is possibly a M+K+H adduct of 118.20. Scale bar: 1 cm.

# Figure S6: Distinct metabolites in various substrates

## A *In situ*

		Glass beads					Sand		
		0 mm	3 mm	2 mm	1 mm	0.5 mm	4 mm	250 $\mu$ m	5 $\mu$ m
Glass beads	3 mm	5							
	2 mm	8	4						
	1 mm	14	10	14					
	0.5 mm	10	5	5	5				
Sand	4 mm	8	5	7	4	2			
	250 $\mu$ m	12	9	18	5	5	5		
	5 $\mu$ m	14	13	15	8	5	5	4	
Clay	4 mm	23	15	24	8	13	6	3	5

## B *In vitro*

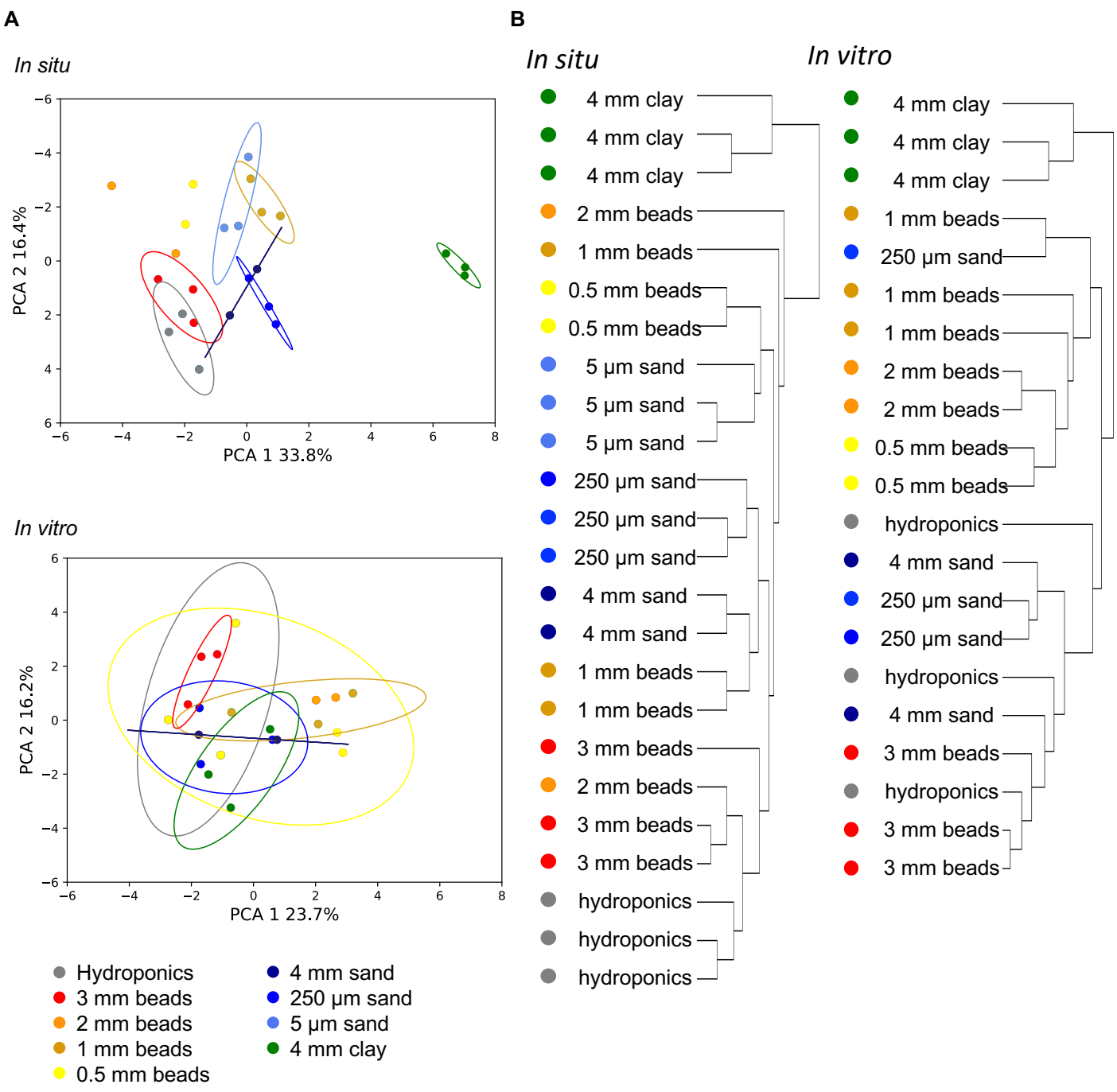
		Glass beads					Sand	
		0 mm	3 mm	2 mm	1 mm	0.5 mm	4 mm	250 $\mu$ m
Glass beads	3 mm	5						
	2 mm	4	0					
	1 mm	5	3	1				
	0.5 mm	4	5	1	0			
Sand	4 mm	3	1	1	0	2		
	250 $\mu$ m	1	3	3	2	0	0	
Clay	4 mm	9	11	8	12	7	2	1

## C Defined medium

		Glass beads		Sand	
DM		3 mm	1 mm	4 mm	250 $\mu$ m
Glass beads	3 mm	15			
	1 mm	23	17		
Sand	4 mm	21	16	14	
	250 $\mu$ m	45	44	37	43
Clay	4 mm	51	53	48	51
					40

**Figure S6 | ANOVA-Tukey test results** Percent of significantly different metabolites in pairwise comparisons of *in situ* collected exudates (A), *in vitro* collected exudates (B), and substrates incubated with defined medium (C). Data are means of 3 biological replicates, ANOVA,  $p = 0.05$ .

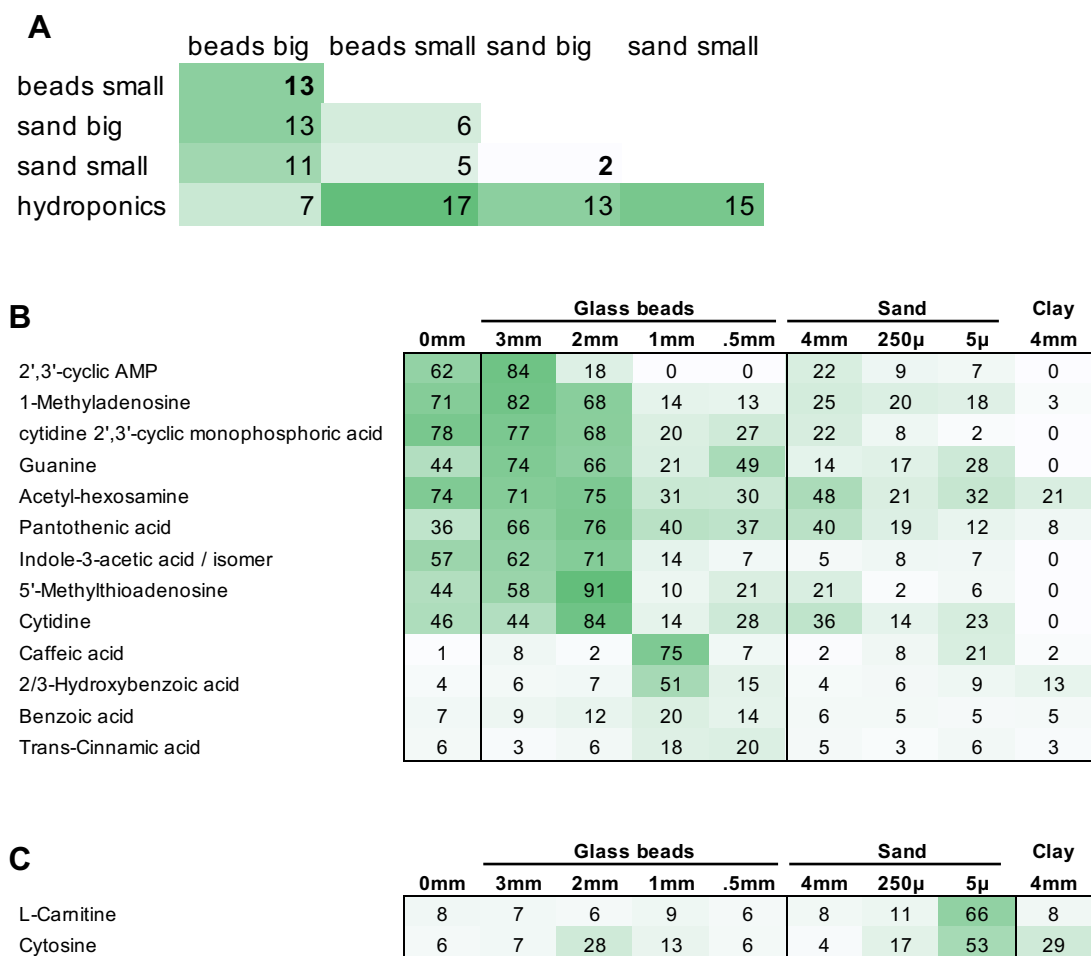
# Figure S7: Additional analysis exudate metabolic profiles



**Figure S7 | *In situ* and *in vitro* exudate metabolite profile analyses** Principal component (PC) analysis of metabolite profiles of root exudates collected *in situ* or *in vitro* (A), and hierarchical clustering of the same datasets (B). PC analyses of the data grouped by root morphology is displayed in Fig. 4.

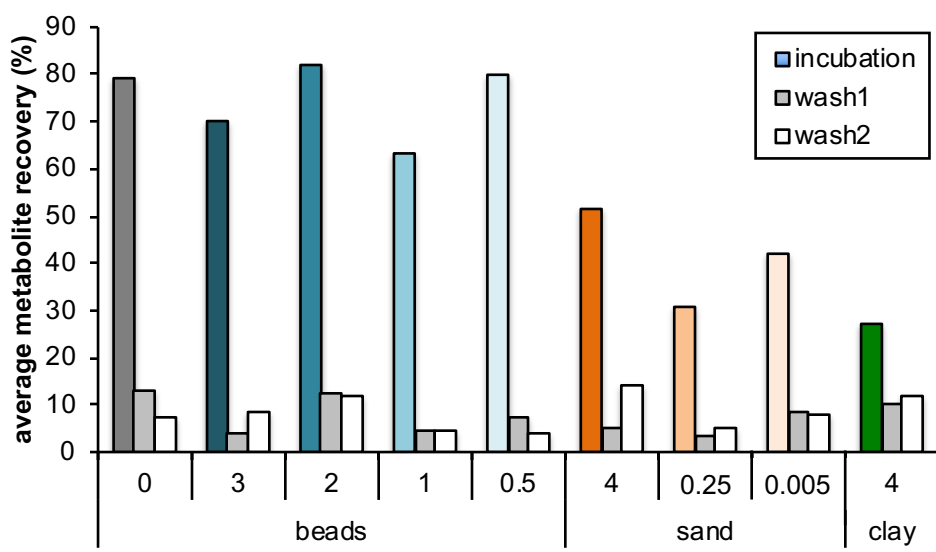


# Figure S8: Distinct metabolites in grouped in situ substrates



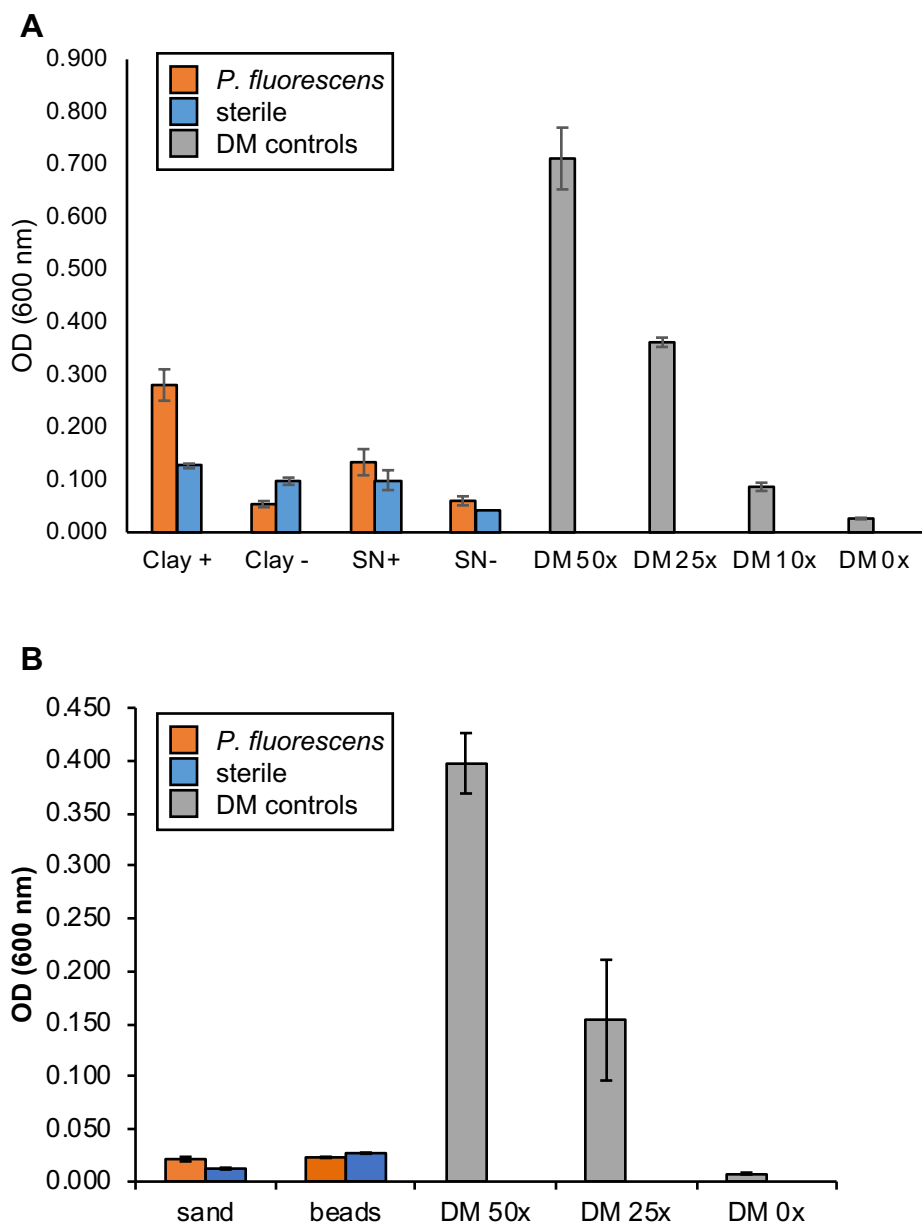
**Figure S8 | Distinct metabolites in grouped in situ substrates (A)** Number of significantly different metabolites in pairwise comparisons of metabolite profiles grouped by root morphology (ANOVA,  $p = 0.05$ ). Substrates are grouped as follows: beads big: 3 mm, 2 mm glass beads; beads small: 1 mm, 0.5 mm glass beads; sand big: 4 mm, 250  $\mu$ m sand; sand small: 5  $\mu$ m sand. Heatmap of metabolite abundances significantly different between in situ collected exudates in big vs small beads (B) and big vs small sand (C), ANOVA,  $p = 0.05$ . Values indicate average peak heights, scaled to the maximum abundance for each compound. All substrates are displayed for comparison. Full dataset is provided in Table S2.

# Figure S9: Metabolite recovery from substrates



**Figure S9 | Metabolite recovery from substrates** Relative abundance of defined medium metabolites in percent in various substrates after incubation (colored bars), after a first wash (grey bars), and a second wash (white bars). Data is normalized by metabolite abundance in defined medium incubated without substrate; n=1.

# Figure S10: Rhizobacterium utilization of clay sorbed metabolites



## Figure S10 | Rhizobacterium utilization of clay sorbed metabolites (A)

Optical density (OD at 600 nm) of *P. fluorescens* (orange bars) grown in clay pre-incubated with 50x defined medium (DM, Clay +) or 0x DM (Clay -), or in supernatant of clay pre-incubated with 50x DM (SN +) or 0x DM (SN -). Sterile controls (blue bars) indicate substrate background (no bacteria control). Data normalized by substrate background is displayed in Fig. 6. **(B)** OD of *P. fluorescens* grown in 4 mm sand and 3 mm glass beads pre-incubated with 50x DM (orange) or 0x DM (blue). *P. fluorescens* growth in different concentrations of DM without substrate are given as comparison. All ODs are means  $\pm$  SEM, n = 3.

## Table S1: Diffusion Parameters for Glass Bead Substrates of Varying Size

<b>Bead size (mm)</b>	<b>Diffusion rate (cm h<sup>-1</sup>)</b>
3	21.6
2	4.48
1	1.56
0.5	1.02

Diffusion parameters calculated based on Congo Red diffusion through a column filled with glass beads of varying sizes.

## Table S2: Metabolomics data

See excel file