Transparent soil microcosms allow 3D spatial quantification of soil microbiological processes in vivo

Helen F. Downie, Tracy A. Valentine, Wilfred Otten,
Andrew J. Spiers, Lionel X. Dupuy

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Figures – 1, 2, S1

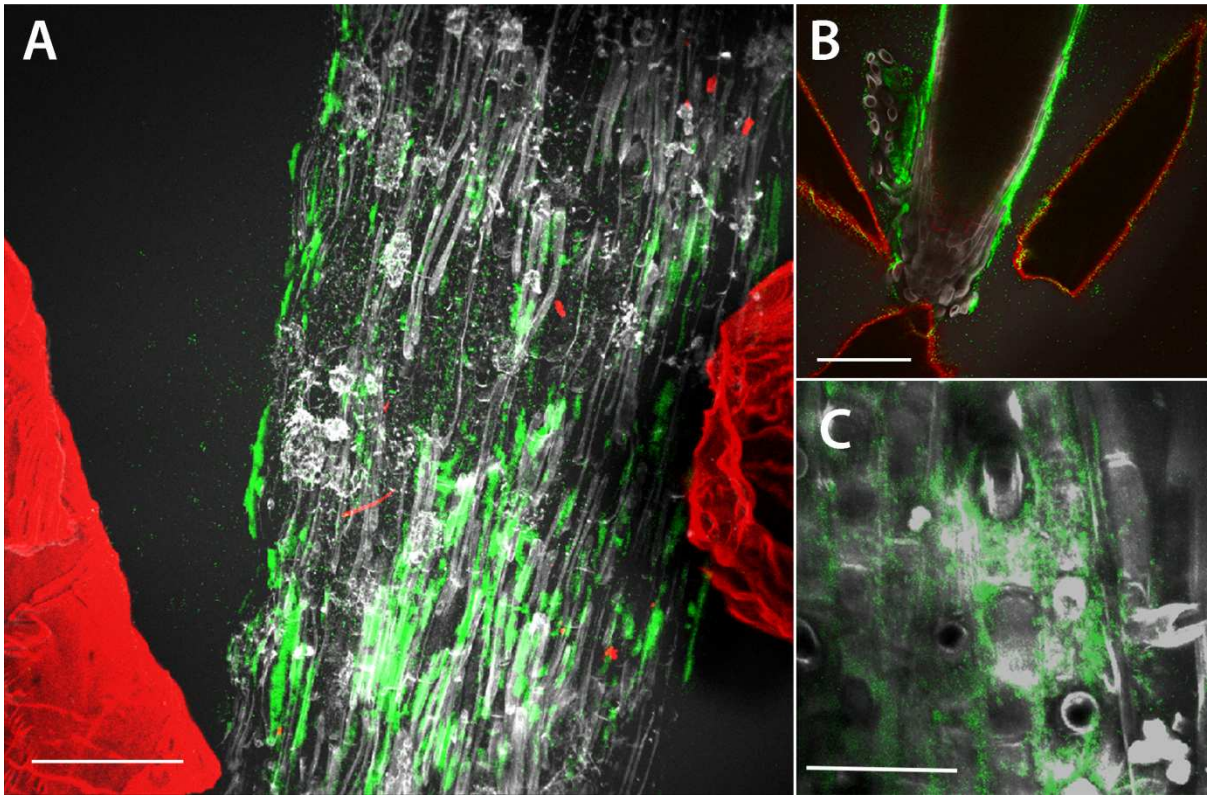

Figure 1. Maximum projection confocal images of GFP-labelled *Pseudomonas fluorescens* colonies (green) on the surface of lettuce root tissues (grey) *in situ* in transparent soil with Nafion particles from the substrate labelled with sulphorhodamine B fluorescent dye also visible (red). (A) The majority of the bacterial fluorescens is associated with the root tissue. Scale bar = 150 µm. (B) Bacteria are present on the root tip and in this case also the surfaces of Nafion particles in close proximity to the root have bacterial fluorescens associated with them. Scale bar = 150 µm. (C) At higher resolution, bacterial colonisation was predominantly observed in the intercellular junctions of root epithelial cells. Scale bar = 45 µm.

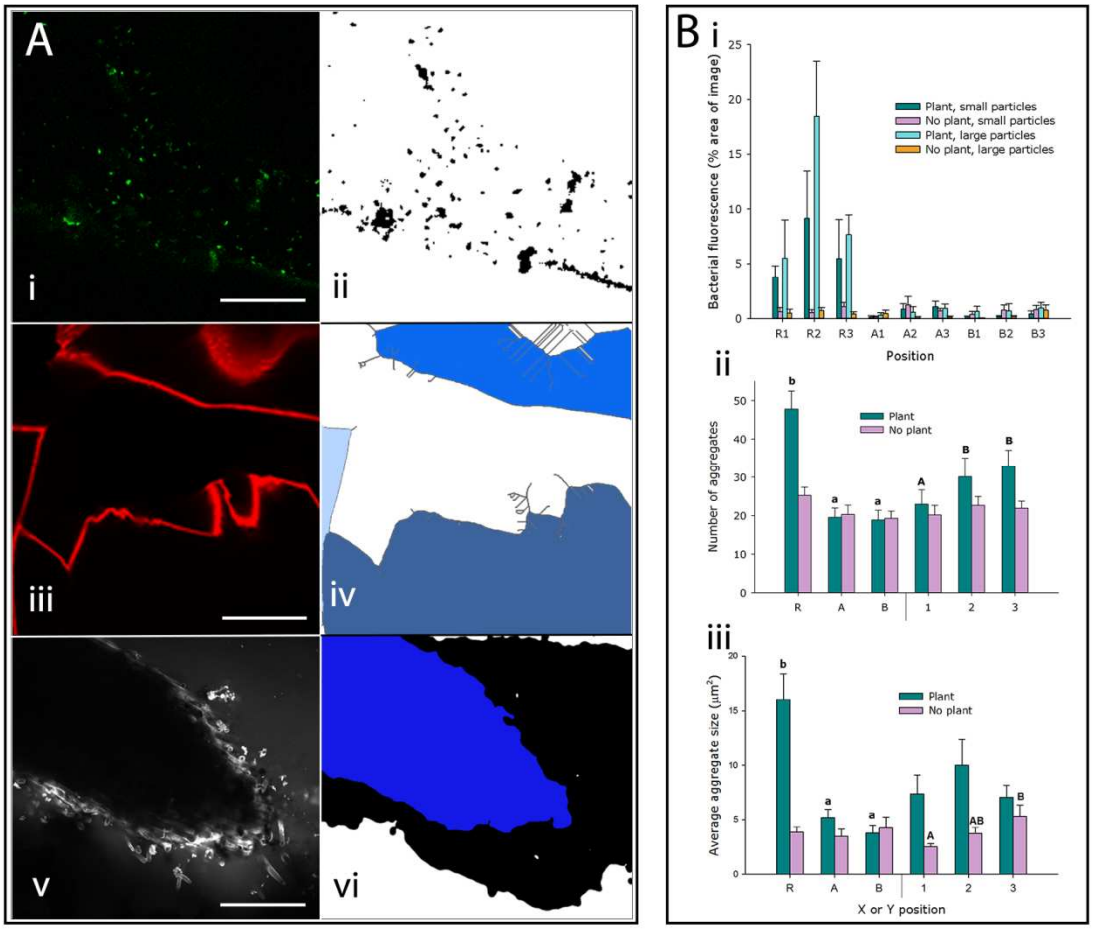

Figure 2. Quantification of *Pseudomonas fluorescens* in the rhizosphere. A) Bacteria, Nafion particles and roots were processed sequentially to allow quantification. (i-ii) Bacterial fluorescence before and after processing with a median filter and thresholding facilitated measuring the bacterial abundance. Scale bar = 40 µm. (iii-iv) Original images of particle surfaces were processed and skeletonised. Grey lines in (iv) represent skeleton of particle surfaces in (iii). It was then possible to select the volumes inside particles (shown here in blue) to measure them to correct for available area (pore space). Scale bar = 200 µm. (v-vi) Example image of a section of lettuce root before and after the application of a median filter and subsequent thresholding were applied. This allowed the selection of the internal volume of the root for measurement (shown in blue). Scale bar = 200 µm. B) Quantification of bacterial distribution in transparent soil with small (500 – 850 µm) and large particles (850 – 1200 µm). The positions R1 to B3 represent a 3 × 3 grid of points on and around the roots, where R is on the root and A and B are at intervals perpendicular to the root. 1 is the root tip and 2 and 3 are closer to the shoot. See Figure S1 for schematic. (i) There was higher bacterial abundance in images that include a section of plant root. At all other positions,

there was a consistent area of bacterial fluorescence as a proportion of the area of backgound in images without plant roots. These values were corrected for available area. (ii) Number and (iii) average size of bacterial aggregates at the 3 horizontal (X) positions (R, A & B) and at the 3 vertical (Y) positions (1, 2 & 3) in samples with or without plants. Letters above the bars indicate the results of Fisher's protected LSD tests.

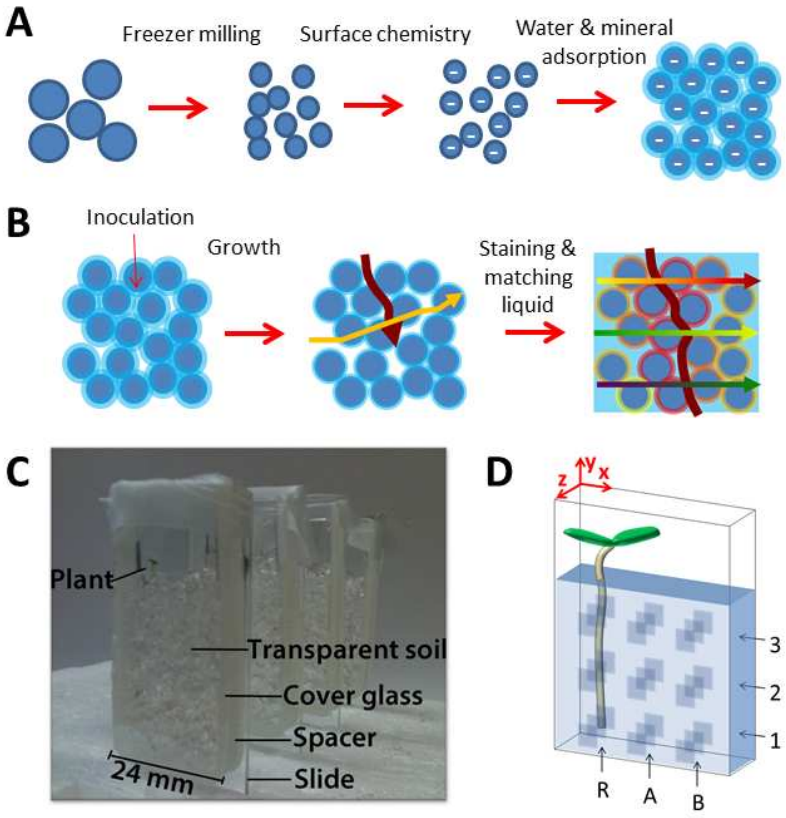

Figure S1. Protocol for the development and imaging of transparent soil microcosms. A) The preparation of the transparent soil material follows three main steps. First, Nafion precursor particles are milled in liquid nitrogen to obtain suitable particle size distribution. In a second stage, particles are treated chemically to give them an anionic charge [11, S1]. Finally, the exchange sites are saturated with the cations from Murashige & Skoog basal plant nutrient medium and the transparent soil medium water content is adjusted for optimal growth conditions. B) The culture and imaging of root and bacteria in transparent soil requires several steps. Seedlings and bacteria are inoculated in the transparent soil substrate at the start of each experiment and grow for 5 days. Samples are then saturated with half-strength M&S medium containing 1 mg ml^{-1} fluorescent brightener to stain the root tissue. Immediately before imaging, this solution was removed and replaced with pure Percoll (Sigma-Aldrich Co.) containing 1 µg ml^{-1} sulphorhodamine B (Sigma-Aldrich Co.). C) Purpose-built transparent containers were constructed for the experiment. Containers were made of a microscope slide and long cover glass with a 4 mm spacer between them on 3 sides and an opening at the top. D) 27 positions were imaged in each sample following a 3 by 3 by 3

regular grid. When plants were present, the origin of the sampling grid is the root tip and the other positions are obtained along and perpendicular to the roots. In the control samples, the reference point R1 was chosen arbitrarily.