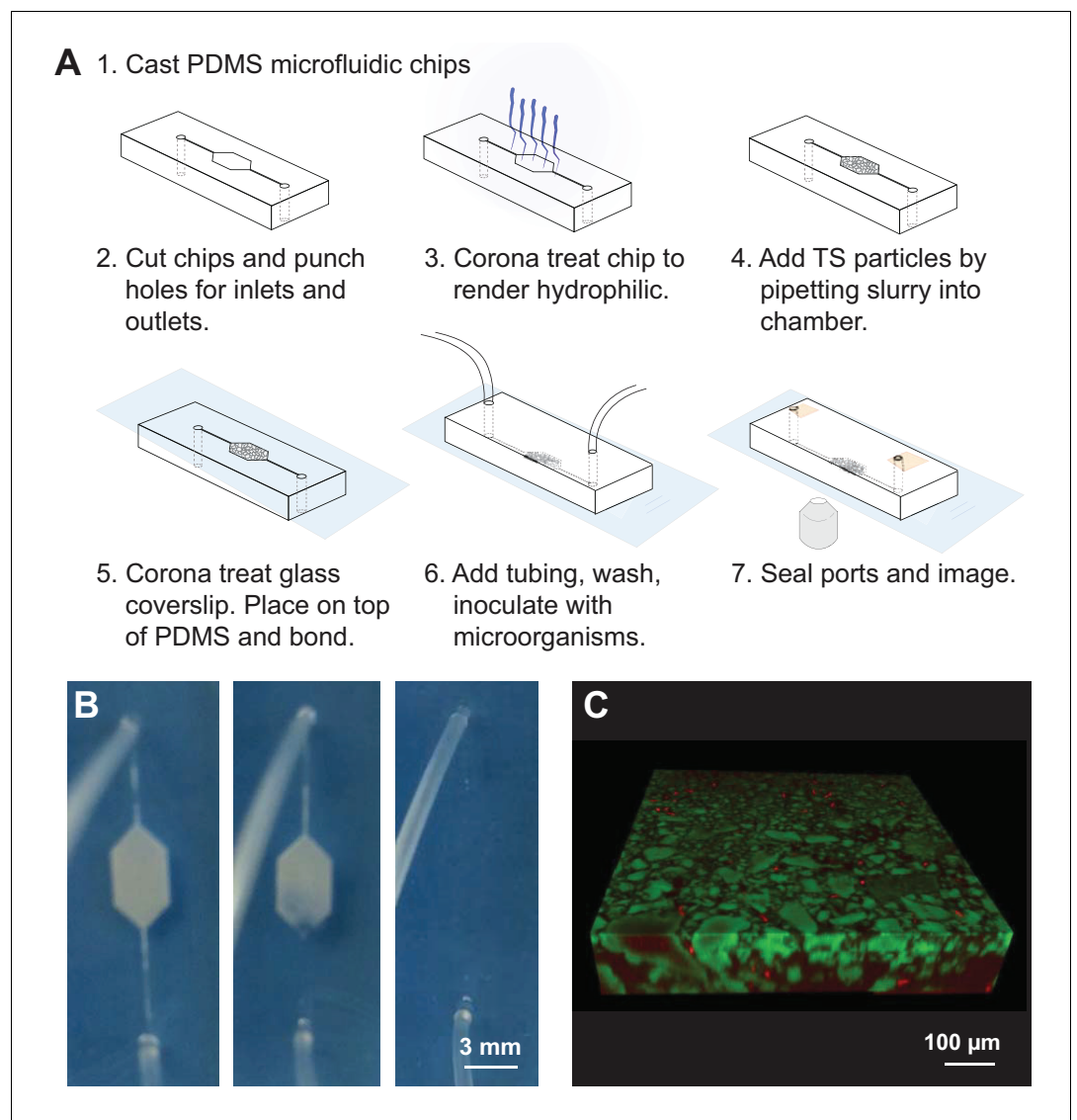


---

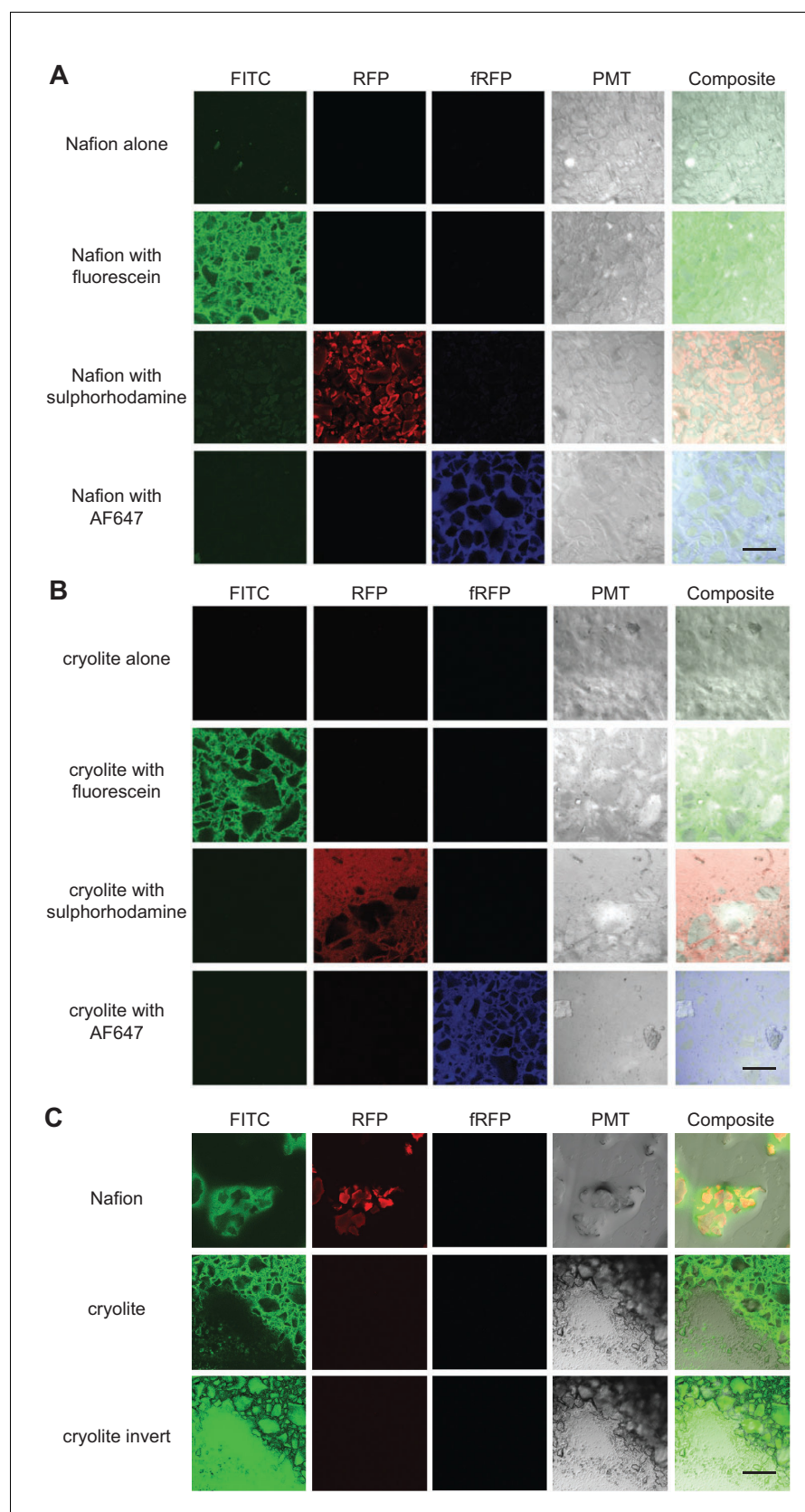
## Figures and figure supplements

Transparent soil microcosms for live-cell imaging and non-destructive stable isotope probing of soil microorganisms

**Kriti Sharma *et al***



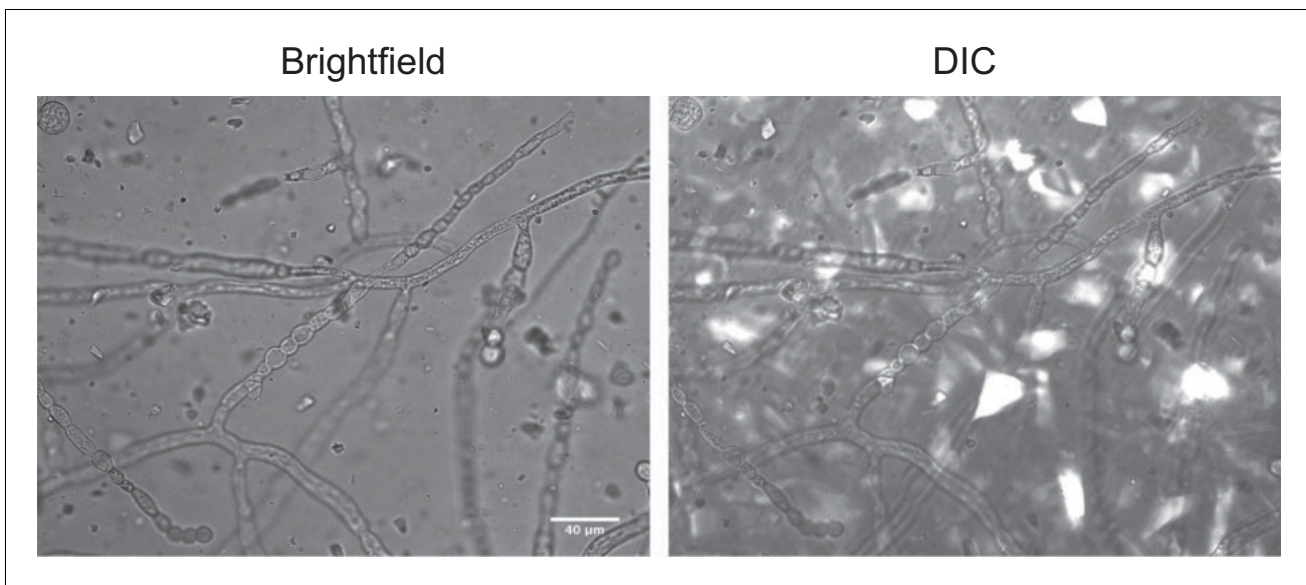
**Figure 1.** Transparent soil (TS) microcosms. (A) Manufacture process of microcosm fluidics chambers. (B) 20% ethanol added after chip manufacture hydrates dry, hydrophobic Nafion and renders it transparent. Microfluidics chamber ( $3 \times 5$  mm hexagon, with  $200 \mu\text{m}$  wide channels) filled with Nafion and attached by tubing to syringe with 20% ethanol, held in syringe pump. As ethanol is slowly flowed into the microcosm by the syringe pump, the Nafion hydrates and becomes transparent. Rehydrated Nafion can then be washed with media, washing away ethanol and rendering microcosms suitable for cell culture. (C) Three-dimensional confocal rendering of fluorescently labeled *E. coli* cells visualized to  $100 \mu\text{m}$  depth in Nafion-based TS microcosm by confocal microscopy. Sulforhodamine-stained Nafion particles (false-colored green), and *E. coli* cells constitutively expressing cyan fluorescent protein ( $P_{spacC}$ -*cfp*, false-colored red). Scale bar =  $100 \mu\text{m}$ .



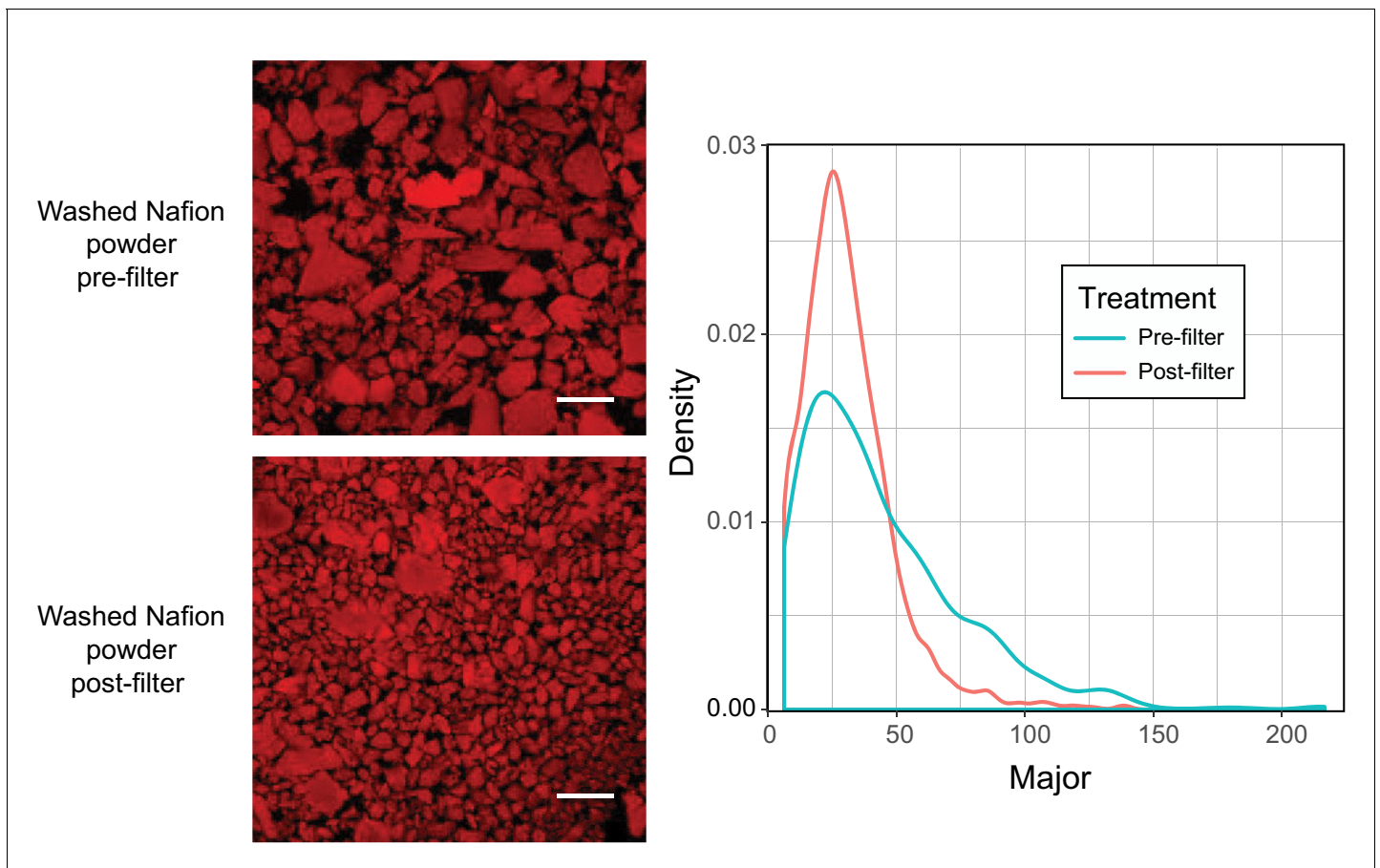
**Figure 2.** Visualization of TS matrices. Nafion (A) and cryolite (B) were packed into a microwell microscope slide, saturated with water containing the fluorophore indicated, and imaged on a confocal laser scanning microscope  
*Figure 2 continued on next page*

*Figure 2 continued*

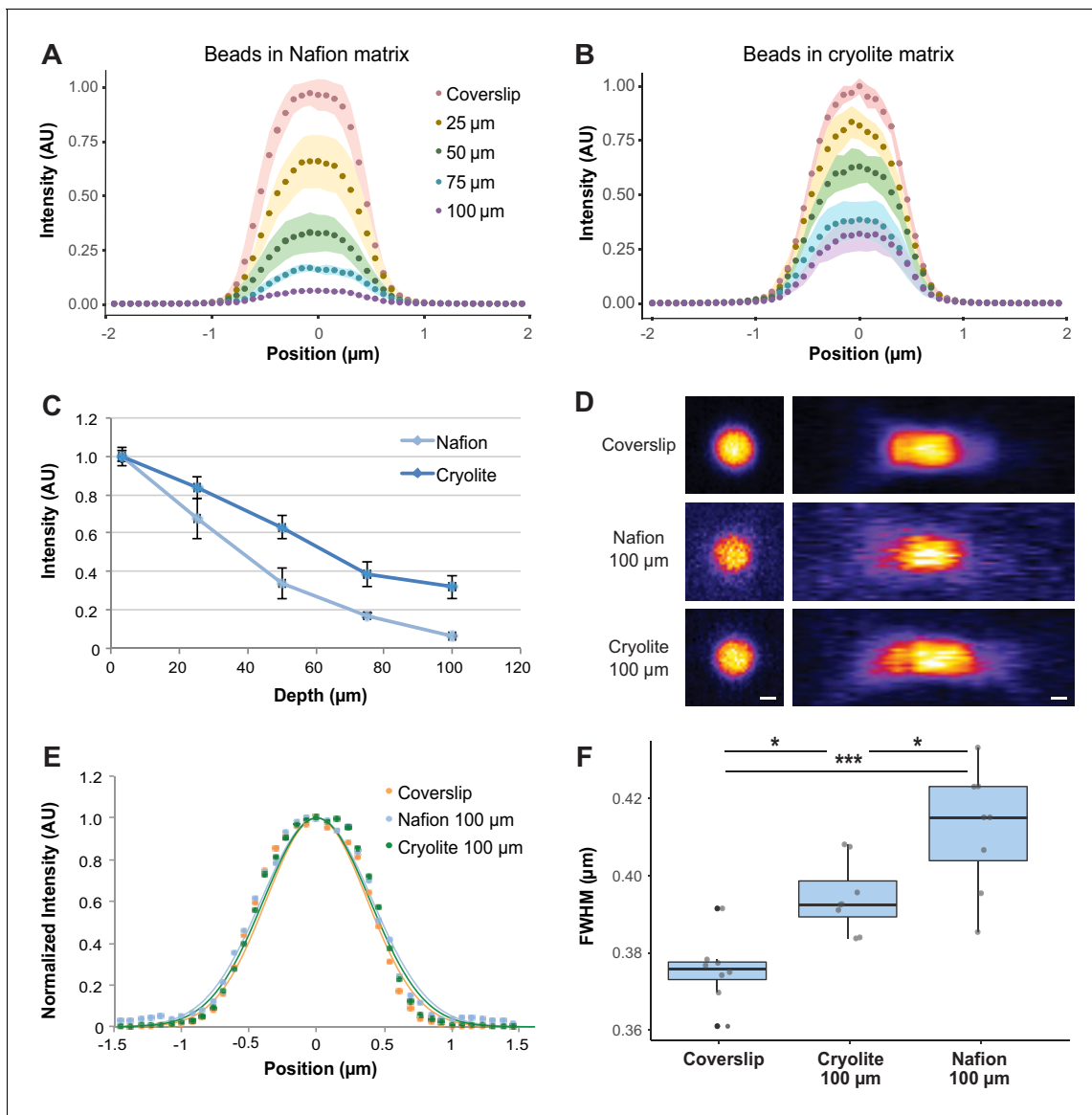
with the noted filter sets. A single Z-slice about 10  $\mu\text{m}$  deep into the TS matrix is shown here. 'Cryolite with fluorescein inverted' is the inverted image of 'cryolite with fluorescein', highlighting particles rather than the pore space. Partially hydrated microcosms (C) have air-filled pockets (black). Image size of each square is 850  $\times$  850  $\mu\text{m}$ . Scale bars are 250  $\mu\text{m}$ .



**Figure 2—figure supplement 1.** Cryolite crystals are invisible under brightfield illumination, but visible under DIC. *M. fragilis* fungus was grown from spores for 24 hr at 30°C in a microfluidic chamber packed with crystalline cryolite and saturated with aqueous minimal salts growth medium (MSN, minimal salts with free ammonium and 2% glucose). Images of the fungus were taken through the cryolite matrix, about 25  $\mu\text{m}$  up from the cover slip. Images taken on Zeiss Axio Observer widefield light microscope, 40x objective, water immersion lens, under brightfield or DIC illumination. Scale bar = 40  $\mu\text{m}$ .

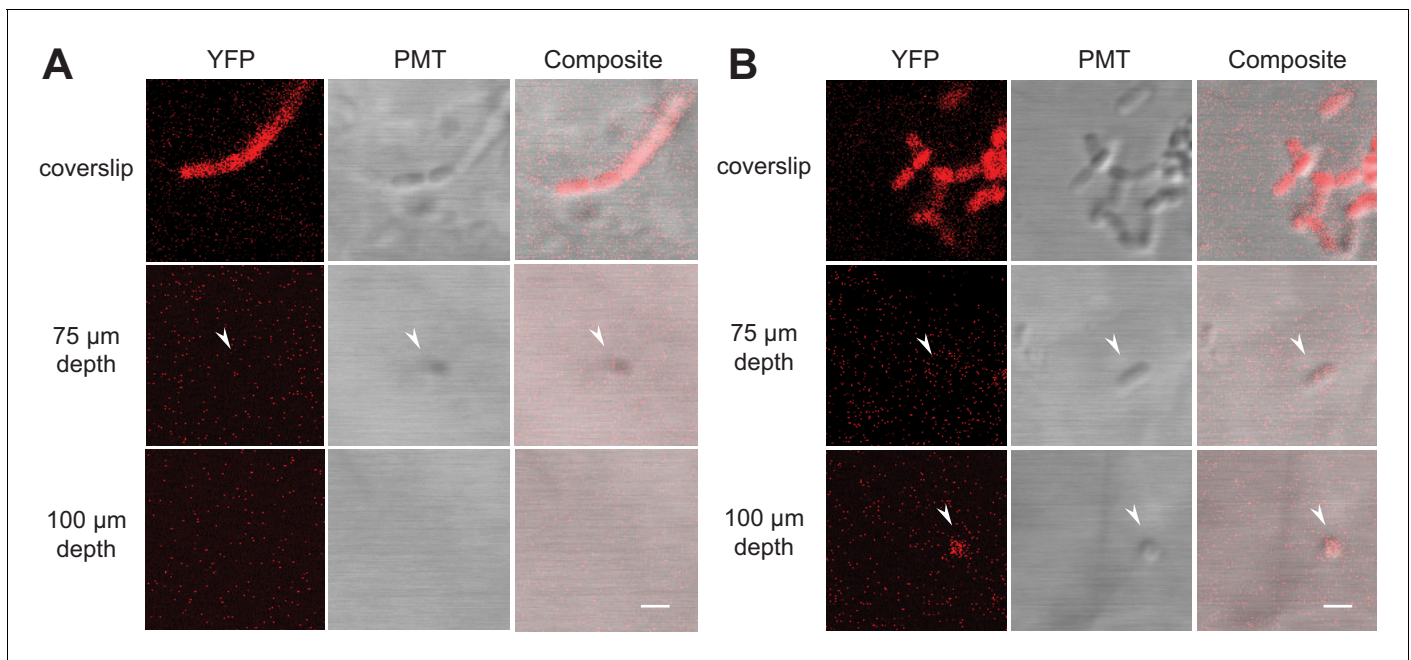


**Figure 2—figure supplement 2.** Particle size distribution of PowdION Nafion powder before and after filtering through 40  $\mu\text{m}$  cell strainer. Commercially available Nafion powder was washed (see Materials and methods) and filtered through 40  $\mu\text{m}$  cell strainer. Particles were stained with sulforhodamine to render particles fluorescent in the RFP channel (false-colored red). Particles were imaged by confocal microscopy and 15  $\mu\text{m}$  z-stacks flattened into maximum intensity projections. Images were analyzed in Fiji image analysis software by binarizing and applying watershed process, then calculating particle area size, represented in density plot. Scale bar = 100  $\mu\text{m}$ .



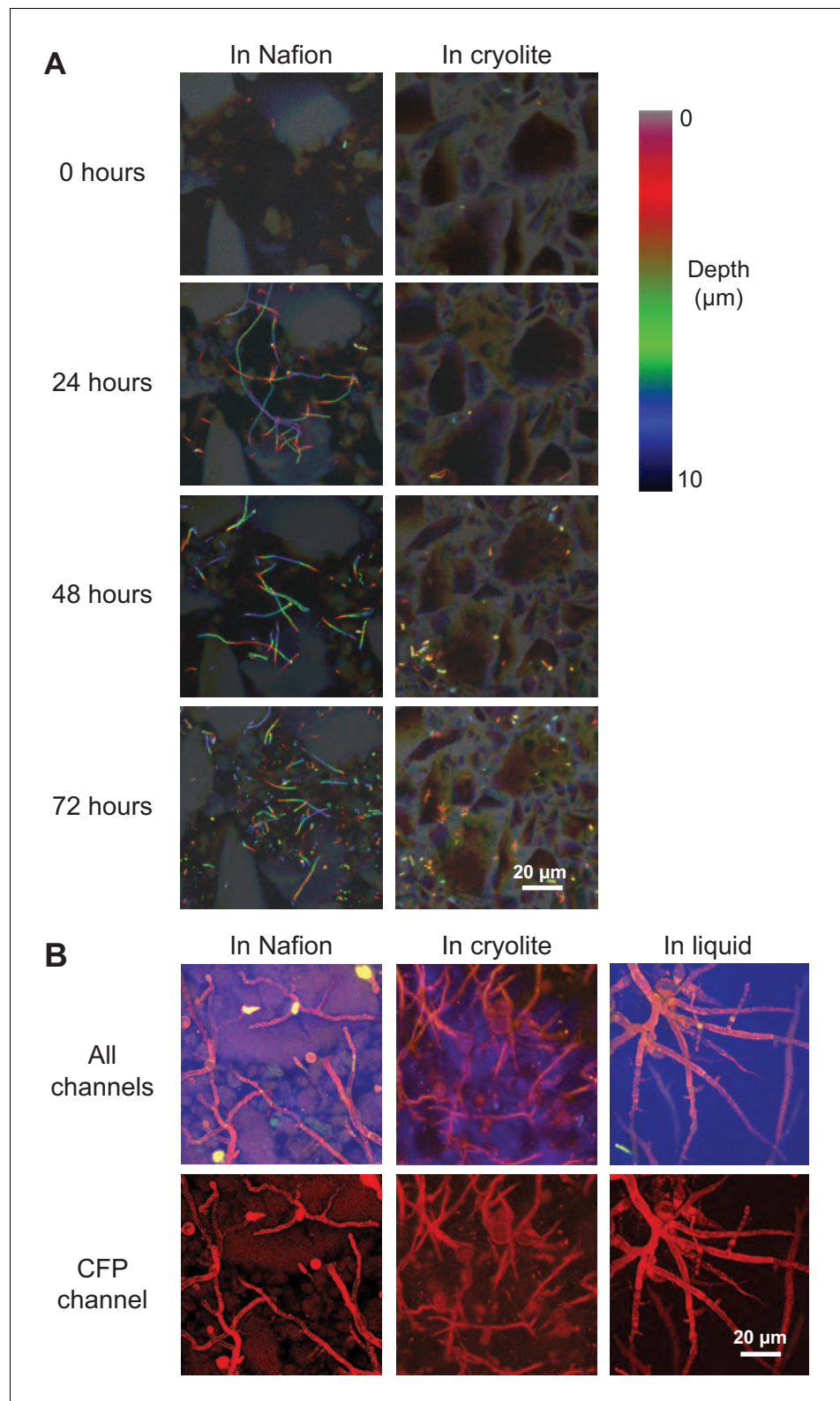
**Figure 3.** Optical properties of TS microcosms. 1  $\mu\text{m}$  FITC-fluorescent beads were mixed into TS matrices saturated with water and imaged by confocal microscopy. Average lateral intensity profiles of beads at different depths within a Nafion (A) or cryolite (B) matrix (n = 8 beads per depth) are shown. (C) Maximum intensities of beads at different depths (n = 8 beads per depth) indicate a greater decay of bead brightness with depth in Nafion than in cryolite. (D) Images of 1  $\mu\text{m}$  beads acquired at the coverslip and 100  $\mu\text{m}$  into Nafion and cryolite microcosms, lateral (xy; left panels) and axial (xz; right panels) views. Image intensity normalized for each image; intensities are not comparable between categories. Scale bar is 0.5  $\mu\text{m}$ . (E) Normalized average lateral intensity profiles of beads at coverslip and at 100  $\mu\text{m}$  depth and fitted Gaussian models. (F) Full-width half-maximum (FWHM) values derived from fitted Gaussian curves of lateral intensity profiles of individual beads are a measure of spatial resolution. FWHM of beads at the coverslip are significantly lower than FWHM of beads 100  $\mu\text{m}$  deep in cryolite (Tukey-Kramer HSD p-value=0.01014) and Nafion microcosms (Tukey-Kramer HSD p-value =  $8.6 \times 10^{-6}$ ), and FWHM for beads in cryolite is significantly lower than beads in Nafion at the same height (Tukey-Kramer HSD p-value=0.0160).





**Figure 4.** Fluorescently labeled bacteria in TS microcosms. *B. subtilis* 3610 cells expressing constitutive YPet were inoculated into TS microcosms in MSgg, incubated at room temperature (22°C) for 48 hr, and Z-stacks acquired by confocal microscopy. Cells were not fixed, but imaged live. Single slices (0.75 μm thick) acquired at the coverslip, 75 μm, and 100 μm deep into (A) Nafion and (B) cryolite TS microcosms are shown (frames from **Videos 1, 2, 3** and **4**, YFP and DIC channels only). White arrows indicate examples of single visible bacteria. Scale bars = 1 μm.



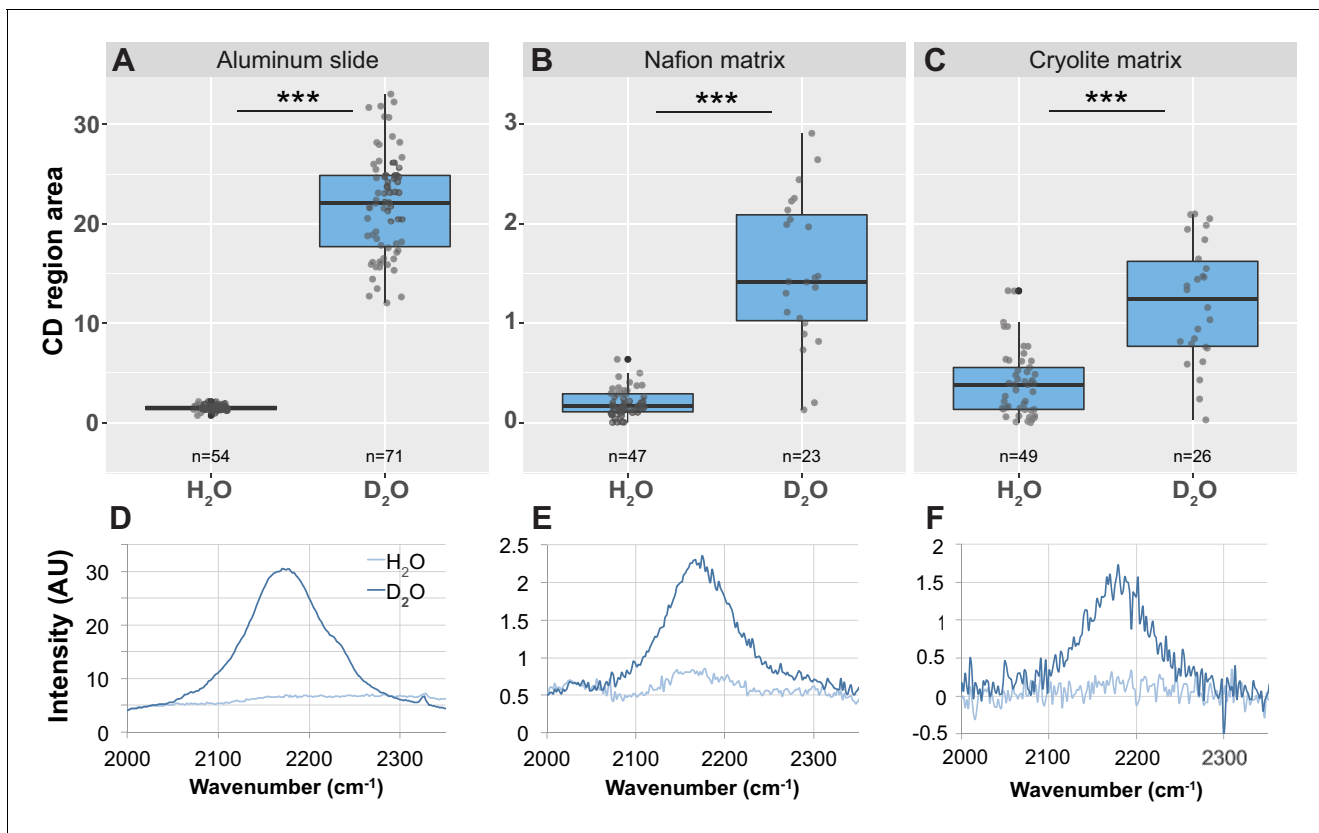


**Figure 5.** Non-destructive imaging of *B. subtilis* and *M. fragilis* in TS over time. (A) Cells of a *B. subtilis* 3610 *eps-tasA* biofilm mutant strain expressing constitutive YPet were inoculated into TS microcosms in MSgg, incubated at room temperature (22°C), and imaged over time. Images are ten-micron thick Z-stacks flattened into color-coded Z-projections. Filaments in Nafion (left) are chains of single cells, some of which have segmented, and some of

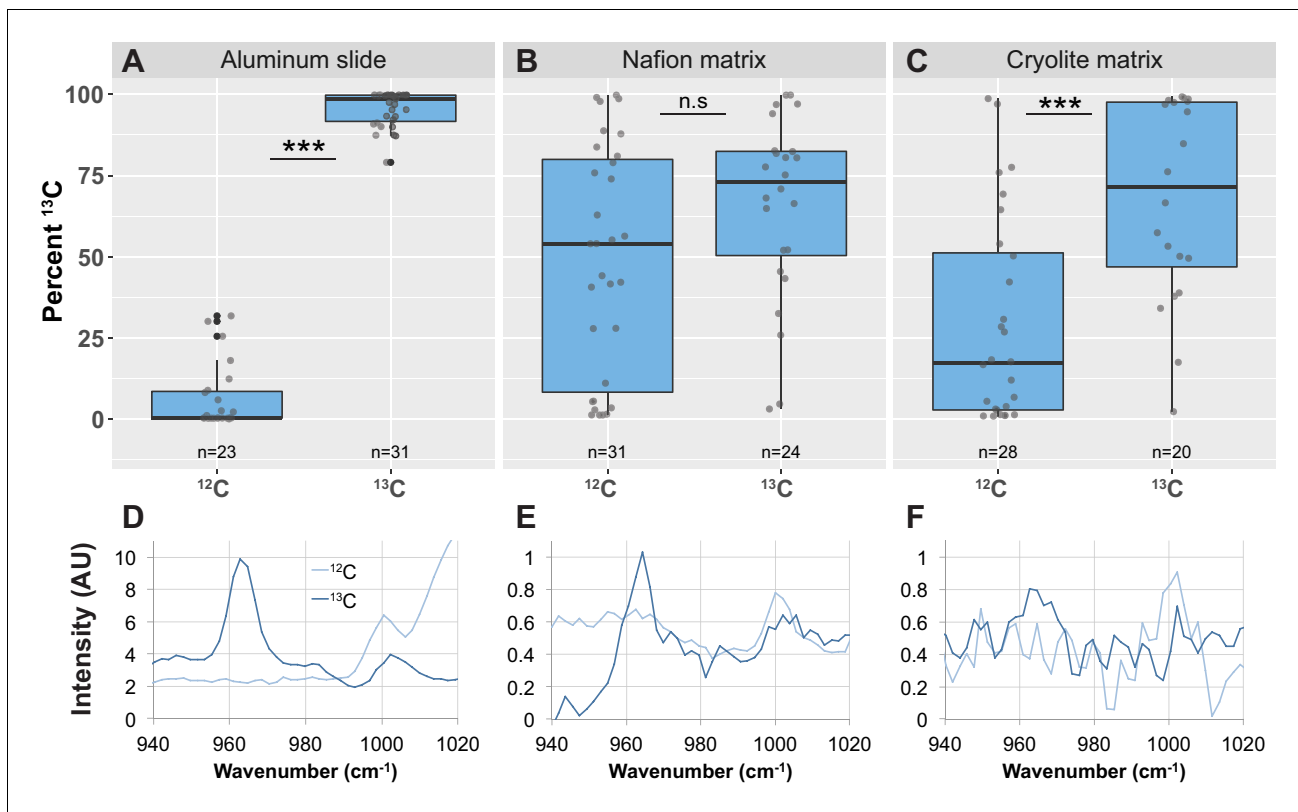
Figure 5 continued on next page

*Figure 5 continued*

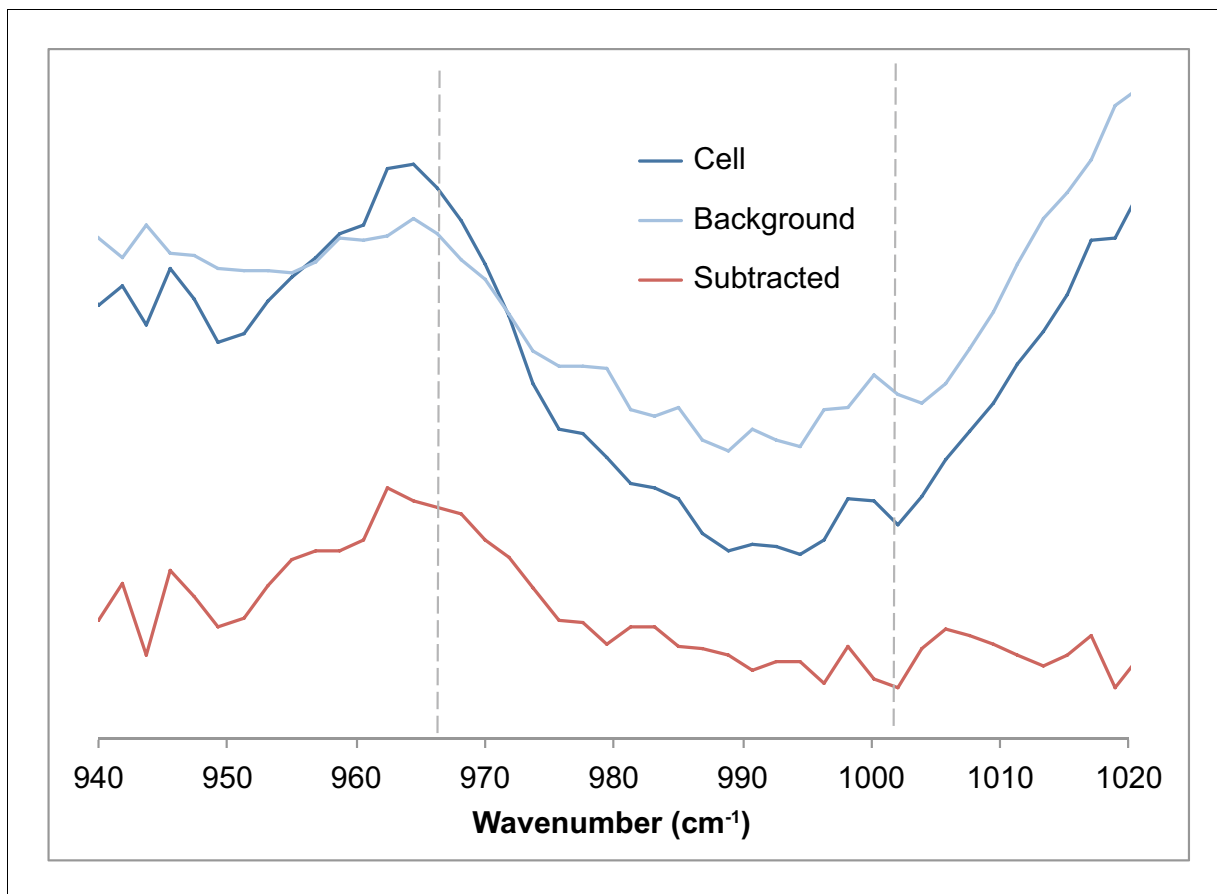
which have not yet expressed autolysins for segmentation and thus appear as filaments (**Chen et al., 2009**). Scale bar = 20  $\mu\text{m}$ . **(B)** *M. fragilis* spores were inoculated into microcosms with or without TS and incubated for 48 hr at 30°C in MSN minimal salts with 2% glucose. Confocal micrographs of 25  $\mu\text{m}$  Z-stacks flattened into maximum intensity projections; sulforhodamine-stained Nafion (false-colored green), *M. fragilis* autofluorescence in YFP channel (false-colored red), PMT channel (false-colored blue). Scale bar = 20  $\mu\text{m}$ .



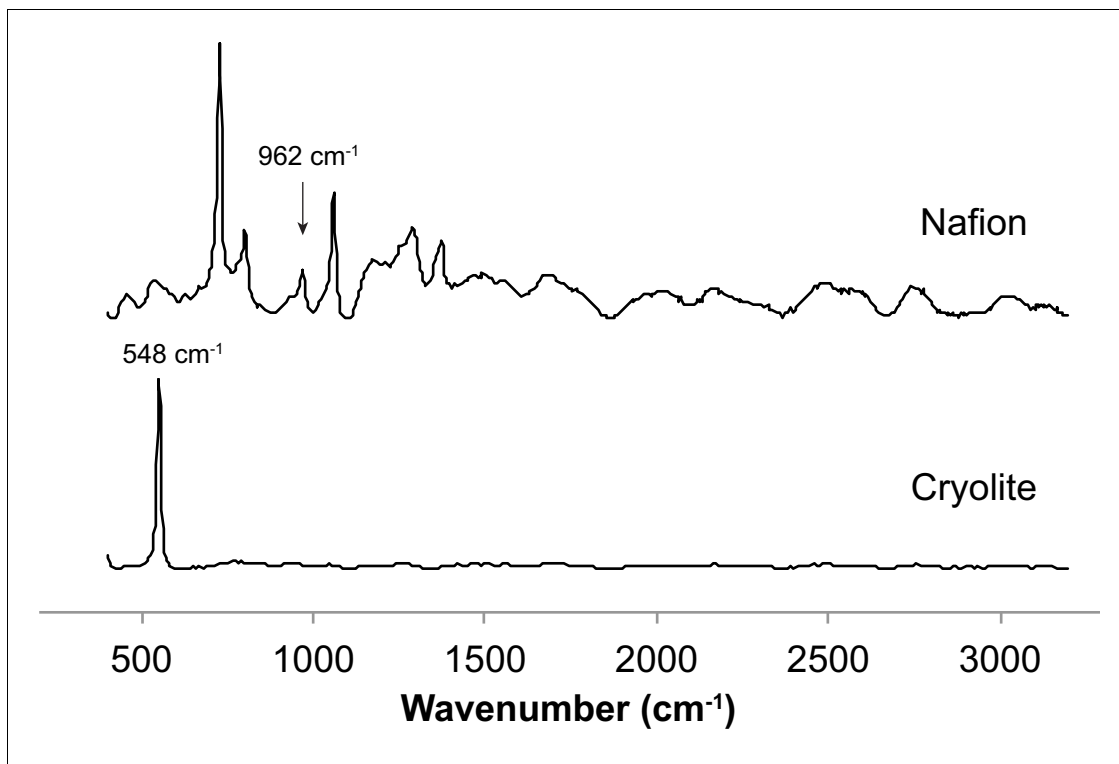
**Figure 6.** Detection of CD peak shifts and D<sub>2</sub>O labeling in *B. subtilis* cells in TS microcosms. *B. subtilis* 3610 cells were grown in minimal salts growth medium made with either regular water or 50% heavy water (deuterium oxide, D<sub>2</sub>O). Cells were then either spotted onto an aluminum slide (A, D) or inoculated into separate Nafion (B, E) or cryolite (C, F) microcosms. Raman spectra of single cells were obtained by microspectroscopy on the aluminum slide, or within the TS matrix from cells embedded anywhere from 15 to 85  $\mu$ m deep within the matrix. Average background subtracted spectra cells grown in H<sub>2</sub>O or D<sub>2</sub>O show a broad peak in the CD region of the spectrum between 2050 and 2250 in D<sub>2</sub>O-labeled cells (D, E, F). CD region for individual cell spectra was calculated as the area under the curve between 2150 and 2200 cm<sup>-1</sup>. Each dot represents CD region for an individual background-subtracted cell spectrum (A, B, C). Each boxplot represents a single separate biological replicate (e.g. all H<sub>2</sub>O-grown cells inoculated into Nafion came from a single culture, and all H<sub>2</sub>O-grown cells inoculated into cryolite came from a separate single culture). D<sub>2</sub>O-labeled cells have larger CD area than H<sub>2</sub>O-labeled cells on all three substrates (Welch's t-test p-value <  $1.3 \times 10^{-5}$  for all three substrates).



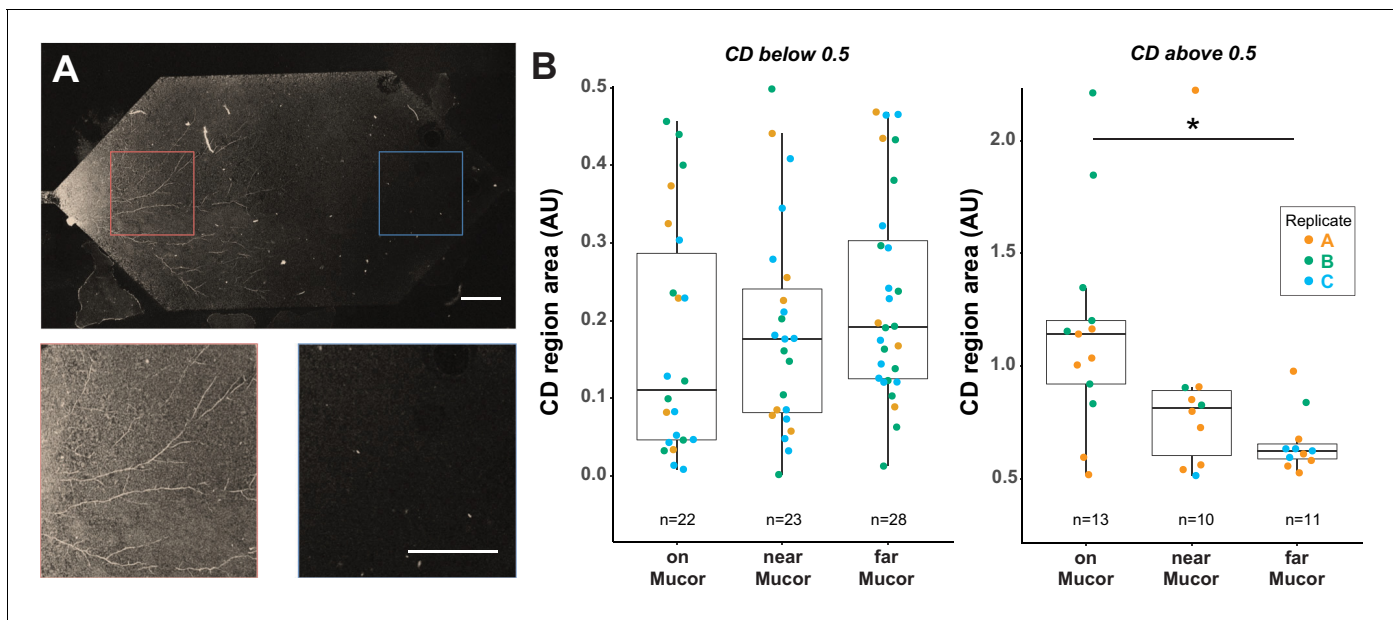
**Figure 7.** Detection of phenylalanine peak shifts and  $^{13}\text{C}$  enrichment in *B. subtilis* cells in TS microcosms. *B. subtilis* 3610 cells were grown in minimal salts growth medium made with either regular ( $^{12}\text{C}$ ) glucose or  $^{13}\text{C}$  glucose. Cells were then either spotted onto an aluminum slide (A, D) or inoculated into separate Nafion (B, E) or cryolite (C, F) microcosms. Raman spectra of single cells were obtained by microspectroscopy on the aluminum slide, or within the TS matrix from cells embedded anywhere from 15 to 85  $\mu\text{m}$  deep within the matrix. Percent  $^{13}\text{C}$  is calculated individually for each background subtracted cell spectrum (see Materials and methods). Each dot represents Percent  $^{13}\text{C}$  for an individual background-subtracted cell spectrum (A, B, C). Each boxplot represents a single separate biological replicate (e.g. all  $^{12}\text{C}$ -grown cells inoculated into Nafion came from a single culture, and all  $^{12}\text{C}$ -grown cells inoculated into cryolite came from a separate single culture). Average background subtracted spectra cells grown in  $^{12}\text{C}$  or  $^{13}\text{C}$  glucose are shown (D, E, F). On aluminum slides,  $^{13}\text{C}$ -labeled cells show a significantly higher Percent  $^{13}\text{C}$  than  $^{12}\text{C}$ -labeled cells (A; Welch's t-test p-value  $< 2.2 \times 10^{-16}$ ). In Nafion TS, the Percent  $^{13}\text{C}$  of  $^{13}\text{C}$ -labeled cells are not significantly different than  $^{12}\text{C}$ -labeled cells (B; Welch's t-test p-value = 0.2151). In cryolite TS,  $^{13}\text{C}$ -labeled cells show a significantly higher Percent  $^{13}\text{C}$  than  $^{12}\text{C}$ -labeled cells (C; Welch's t-test p-value =  $1.348 \times 10^{-4}$ ).



**Figure 7—figure supplement 1.** High Nafion background around 965 cm<sup>-1</sup> interferes with <sup>13</sup>C phenylalanine peak at 966 nm, resulting in unlabeled <sup>12</sup>C-rich cells being misclassified as <sup>13</sup>C labeled. Representative single-cell spectrum showing <sup>12</sup>C-labeled cell spectrum, a background spectrum taken 20 μm away from the cell and capturing the Raman spectrum of the Nafion matrix itself, and the subtracted spectrum (cell spectrum minus background).



**Figure 7—figure supplement 2.** Raman spectra of Nafion and cryolite. Nafion and cryolite powders were spotted onto an aluminum slide and Raman spectra acquired at 532 nm excitation wavelength. Resultant spectra are shown, with peaks of interest highlighted: 962 cm<sup>-1</sup> peak in Nafion interferes with reading phenylalanine peak shift at 966 cm<sup>-1</sup> in embedded cells; 548 cm<sup>-1</sup> peak in cryolite is the only strong peak in the spectrum.



**Figure 8.** *B. subtilis* cells on *M. fragilis* are more metabolically active than cells far from *M. fragilis* after dry-wet cycle in Nafion-based transparent soil microcosms. (A) *B. subtilis* cells grown with dead *M. fragilis* in Nafion TS microcosm undergo a dry-wet cycle, mimicking the dry-down and wet-up of soils. Cells were exposed to D<sub>2</sub>O for 16 hr during the wet-up phase. Because *M. fragilis* spores were trapped within the Nafion matrix on the side where they were inoculated, after they germinated, one side of the microcosm filled with hyphae, while the other side remains empty. Confocal microscopy in GFP channel shows autofluorescent *M. fragilis* hyphae on one side of microcosm (above, and left inset), and no growth on the other side (right inset). Scale bar is 400  $\mu$ m in both top micrograph and insets. *B. subtilis* cells measured were classified as either 'on' (cells attached to *M. fragilis* hyphae), 'near' (cells attached to Nafion on *M. fragilis*-inoculated side of microcosm, within 20  $\mu$ m radius of nearest hypha), or 'far' (cells attached to Nafion on side of microcosm without *M. fragilis*). 'Far' cells are 3 mm or more away from nearest *M. fragilis* hyphae. (B) Most cells (~60–70 percent) have no detectable activity after a dry-wet cycle, regardless of distance from *M. fragilis* (left panel, all cells with CD area less than 0.5, indicating no activity detectable by D<sub>2</sub>O uptake). However, cells that did take up D<sub>2</sub>O took up more of the label when on *M. fragilis* than cells far from *M. fragilis* (right panel, one-way ANOVA of all three categories F-statistic 4.7194, p-value=0.0160; Tukey-Kramer HSD p-value<0.0131 for cells on *M. fragilis* vs far from *M. fragilis*, Welch's t-test p-value=0.002398). Results pooled from three separate biological replicate experiments in three separate TS microcosms, indicated by color.