Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting Information

Unravelling the Interactions between Nano-Hydroxyapatite and the Roots of Phosphorus Deficient Barley Plants.

Augusta E. Szameitat[‡ a], Anurag Sharma[‡ a], Francesco Minutello[‡ a], Andrea Pinna[a], Meriem Er-Rafik[b], Thomas H. Hansen[a], Daniel P. Persson[a], Birgit Andersen[a], Søren Husted* [a]

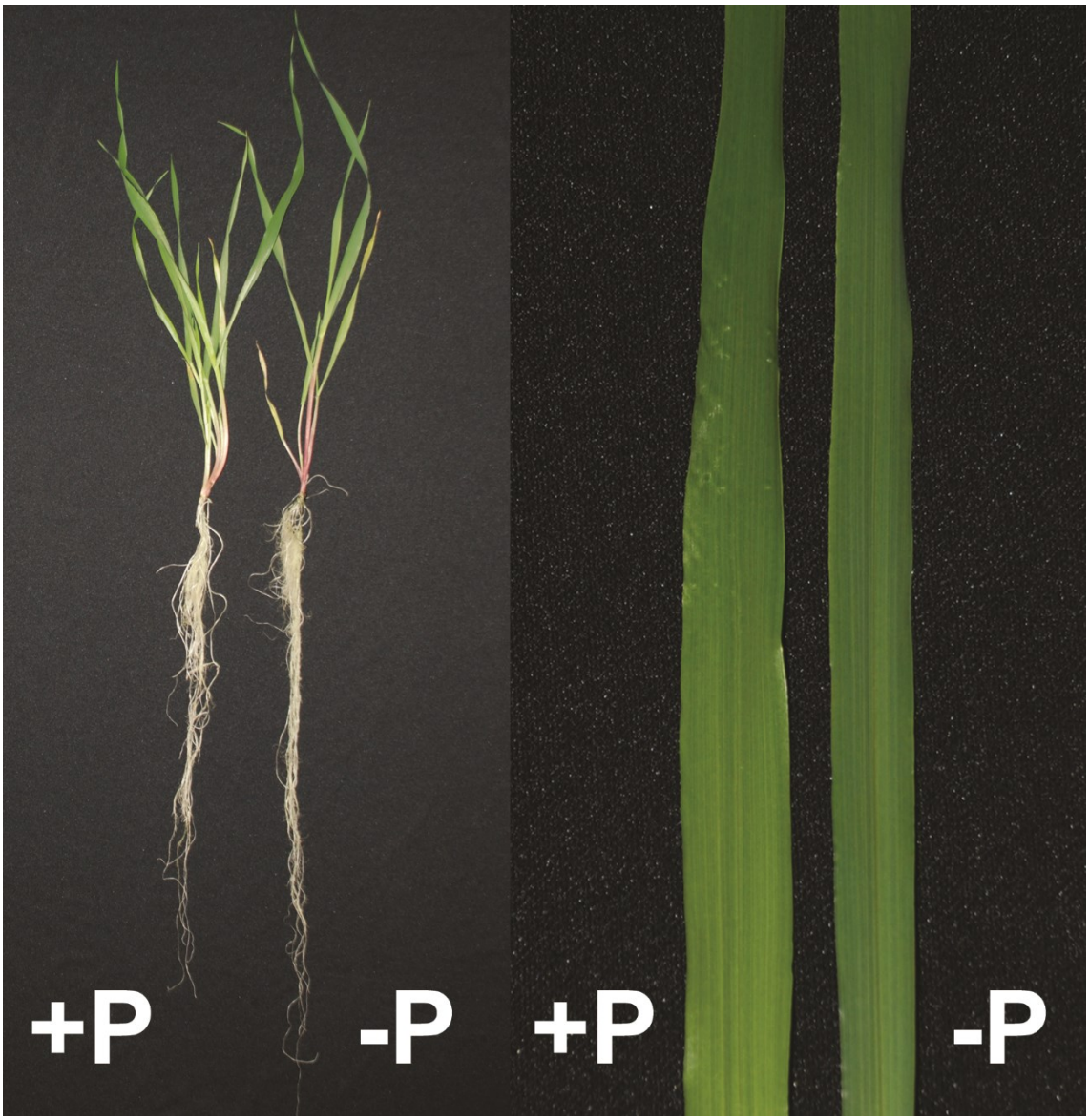

Fig. S1. Pictures of untreated, 21 days-old P sufficient (+P) and P deficient (-P) barley plants. Left: entire plants. Right: detail of youngest fully evolved leaves.

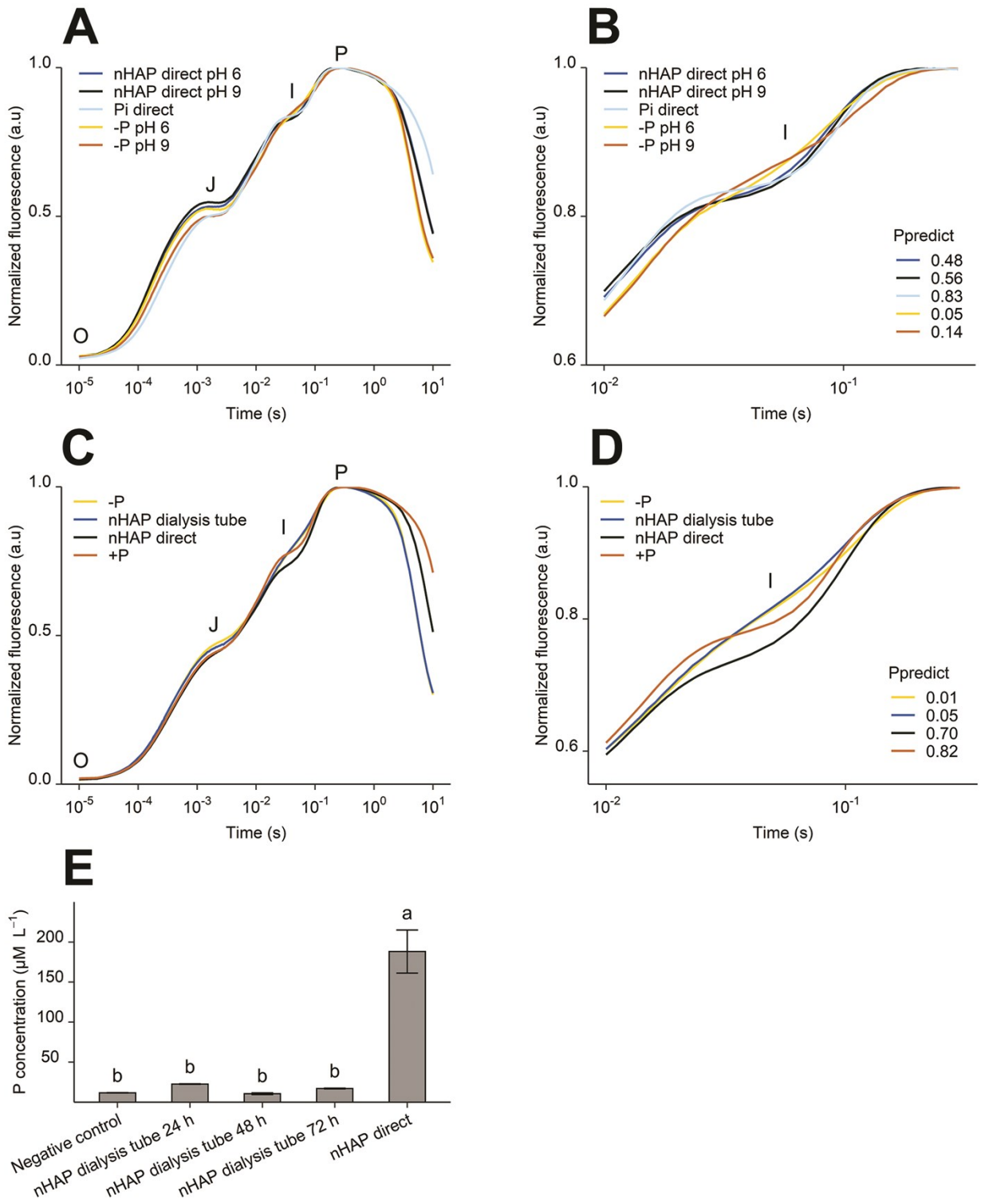

Fig. S2. (A, B) Plant response to $nHAP_{direct}$ at different hydroponic pH (6 and 9). **(C, D)** Persistence of P deficiency in $nHAP_{DT}$ after 5 days. **(A)** OJIP transients indicating the nutritional status of plants after exposure to different P treatments for 48 hours (n=3 with 4 technical replicates). **(B)** Magnification of (A) illustrating only the "I" portion of the OJIP transients. **(C)** OJIP transients indicating the nutritional status of plants after exposure to different P treatments for 5 days (n=3 with 4 technical replicates). **(D)** Magnification of (C) illustrating only the "I" portion of

the OJIP transients. The presence of an I-step and P-predict values >0.65 indicate P sufficiency; the absence of an I-step and P-predict values <0.40 indicate P deficiency; P-predict values in the range 0.40-0.65 indicate moderate P availability. **(E)** Release of P from dialysis tubes into the hydroponic solution. Dialysis tubes containing a colloidal suspension of nHAP were sealed and submerged into low-P hydroponic solutions at pH 5.5. No plants were present in this experiment. Samples from the hydroponic solution were collected 24, 48 and 72 h after nHAP application. Results are means ± standard error of the mean (n=3), and letters represent significant changes ($P<0.05$) analyzed by a one-way ANOVA and Tukey's multiple comparison test.

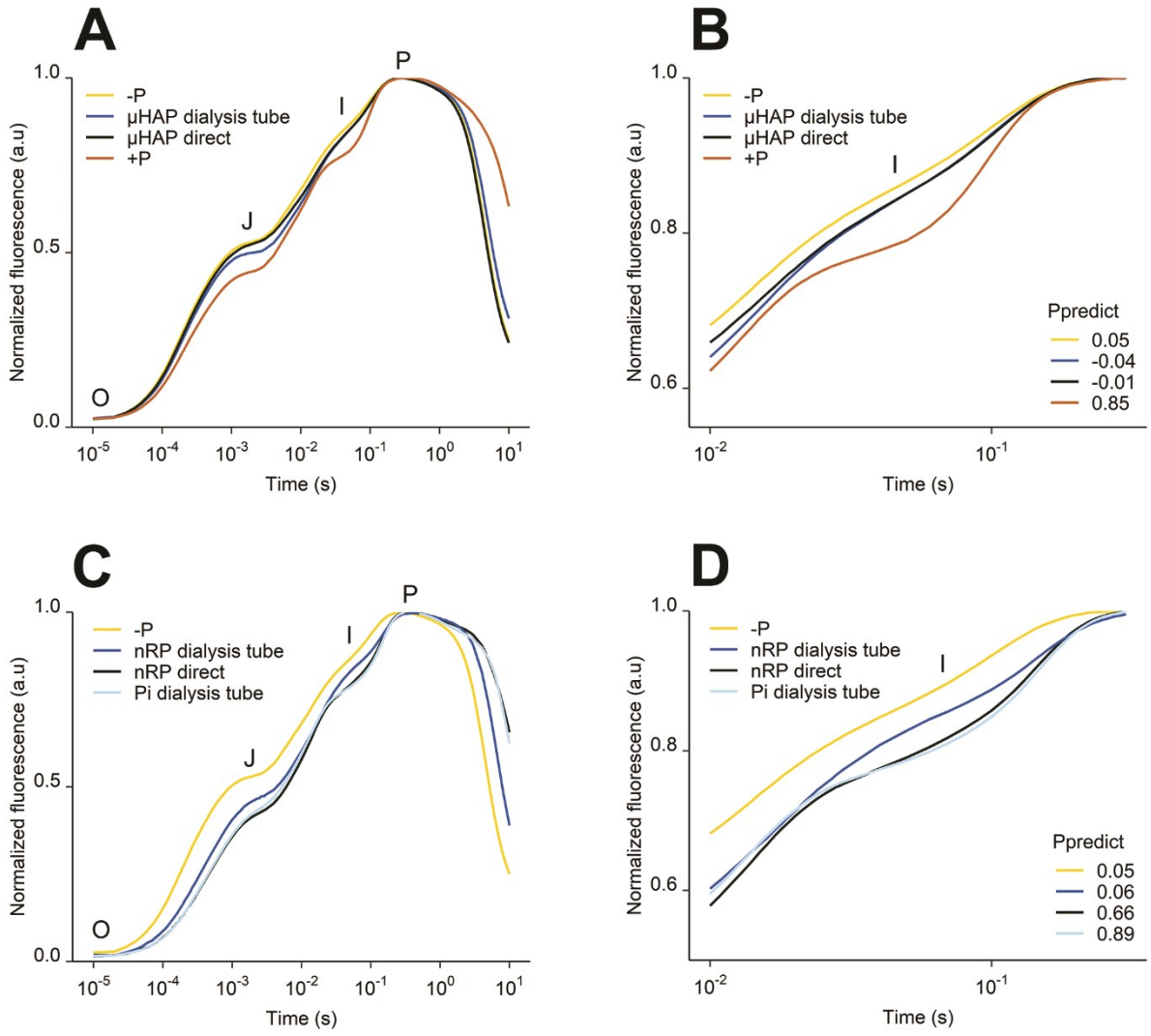

Fig. S3. Plant response to micro-HAP (µHAP) and nano-rock phosphate (nRP) treatments. **(A)** OJIP transients indicating the nutritional status of plants a week after exposure to µHAP. **(B)** Magnification of (A) illustrating only the "I" portion of the OJIP transients. **(C)** OJIP transients indicating the nutritional status of plants 72 hours after exposure to nRP. **(D)** Magnification of (C) illustrating only the "I" portion of the OJIP transients. Both µHAP and nRP were either directly applied in the hydroponic solution or placed in a sealed dialysis tubing submerged in the hydroponic solution (n=3 with 4 technical replicates). The presence an I-step and P-predict values >0.65 indicate P sufficiency; the absence of an I-step and P-predict values <0.40 indicate P deficiency.

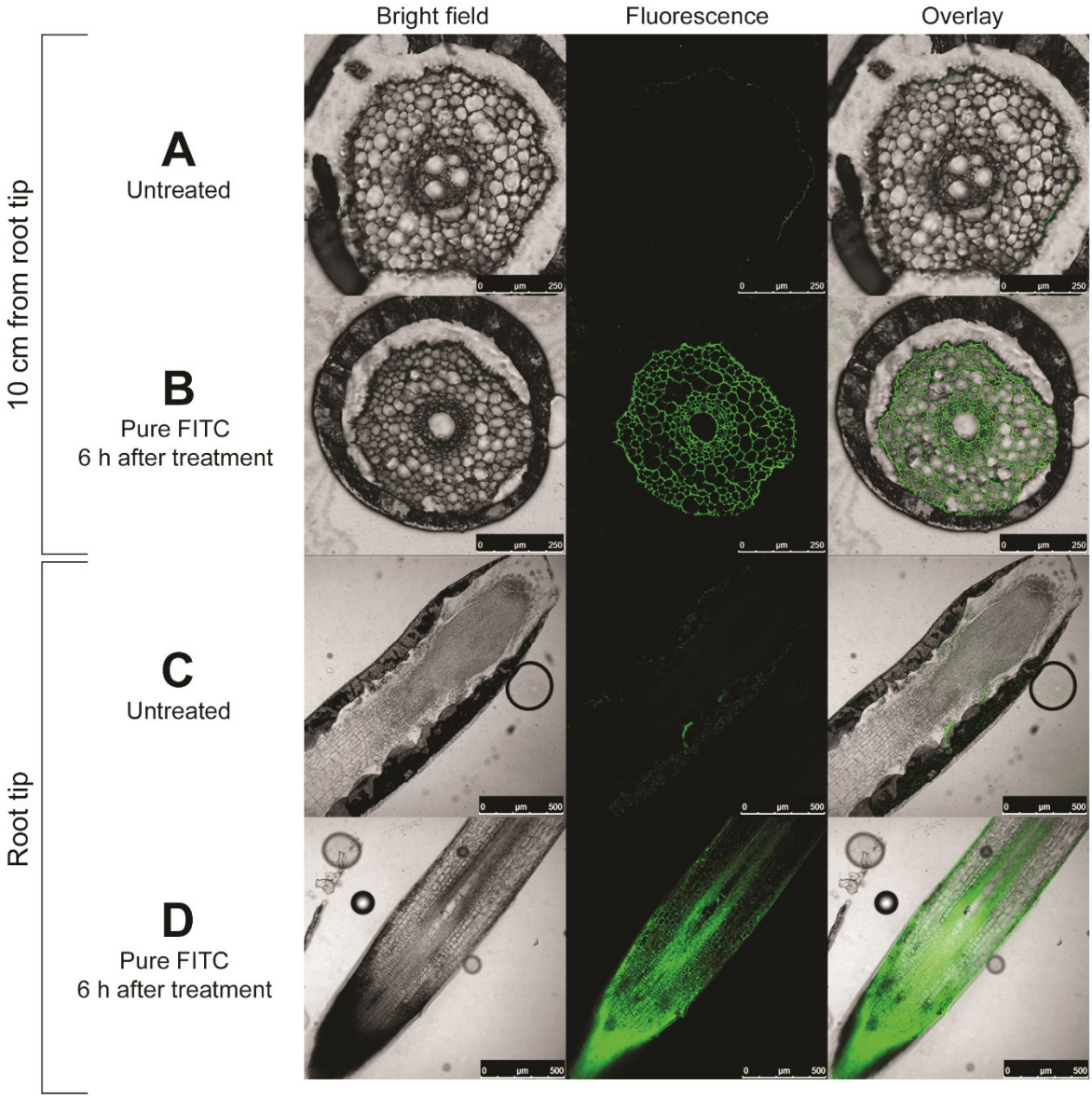

Fig. S4. Positive and negative controls used in CLSM analysis. **(A, C)** untreated plants and **(B, D)** plants treated for 6 h with pure FITC. Cross sections **(A, B)** were prepared from samples collected at 10 cm from the root tip. Longitudinal sections **(C, D)** were prepared from root tip samples. First, second and third columns show pictures from bright field, fluorescence and overlay channels, respectively. Excitation beam was set at 488 nm and emissions were detected in the range 500-530 nm. The same CLSM settings were used for all treatments. The paraffin used for sample preparation can easily be identified as a thick dark layer surrounding all root sections.

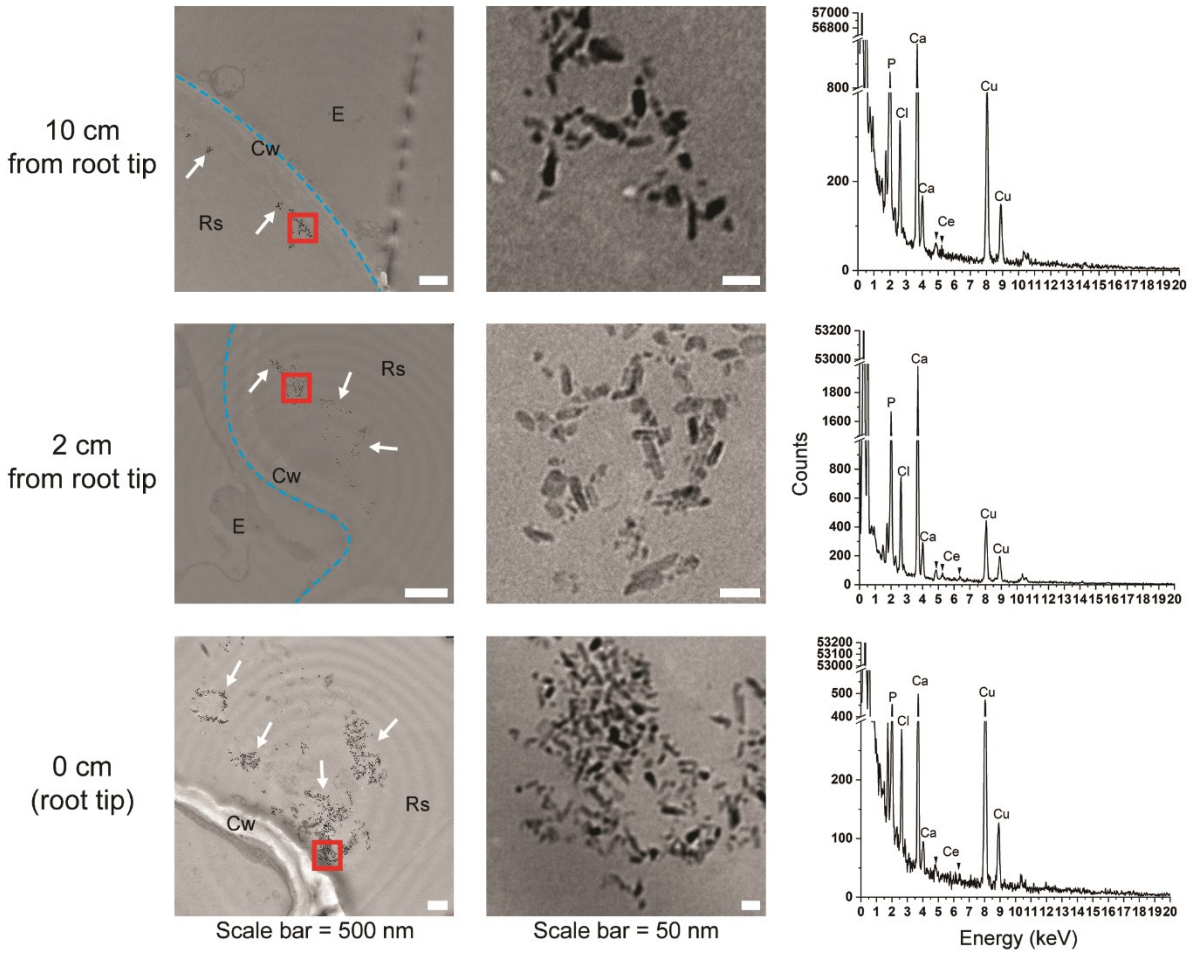

Fig. S5. TEM-EDS analysis of plant roots directly exposed to nHAP for 8 hours. Pictures illustrate cross sections prepared from samples collected at 10 cm, 2 cm and 0 cm from the root tip. Column 1 contains TEM images of root cross sections from treated plants; the areas enclosed in red squares are illustrated as magnifications in column 2; column 3 contains EDS spectra that show the elemental composition of the respective TEM images from column 2. White arrows highlight nHAP aggregates outside the red squares. Blue dashed lines highlight the boundary between epidermal cells and rhizosphere where they are not clearly visible. Abbreviations used in the Fig.: cell wall (Cw); rhizosphere (Rs); epidermis cell (E). The circular interferences in the background of micrographs are defaults from the camera confirmed by the provider.

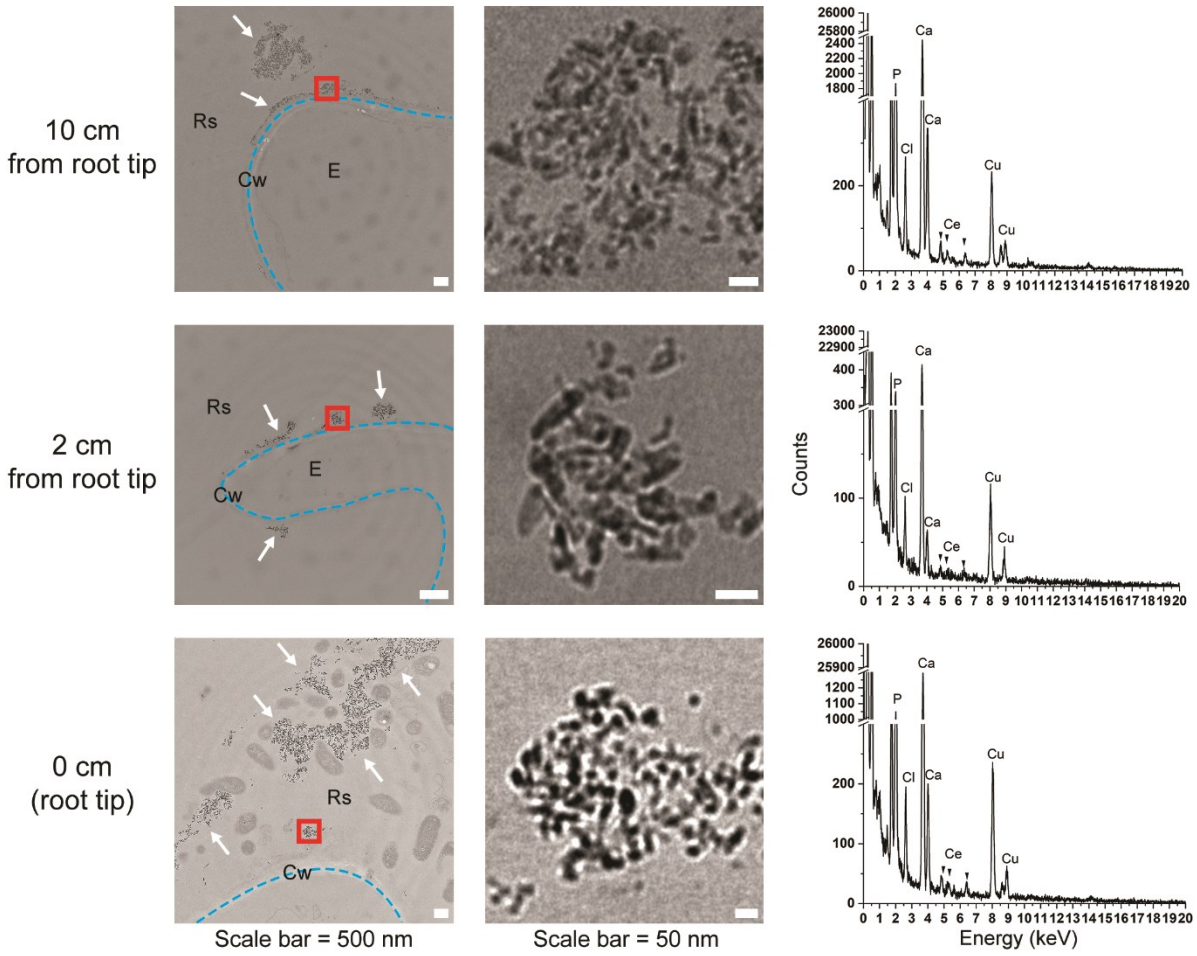

Fig. S6. TEM-EDS analysis of plant roots directly exposed to nHAP for 48 hours. Pictures illustrate cross sections prepared from samples collected at 10 cm, 2 cm and 0 cm from the root tip. Column 1 contains TEM images of root cross sections from treated plants; the areas enclosed in red squares are illustrated as magnifications in column 2; column 3 contains EDS spectra that show the elemental composition of the respective TEM images from column 2. White arrows highlight nHAP aggregates outside the red squares. Blue dashed lines highlight the boundary between surface cells and rhizosphere where they are not clearly visible. Abbreviations used in the Fig.: cell wall (Cw); rhizosphere (Rs); epidermis cell (E). The circular interferences in the background of micrographs are defaults from the camera confirmed by the provider.