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

Supporting Information

Effect of Gold Nanoparticles on Extracellular Nutrient-Cycling Enzyme Activity and Bacterial Community in Soil Slurries: Role of Nanoparticle Size and Surface Coating

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Materials and Methods

Extracellular Enzyme Assay. The activities of five extracellular enzymes, cellobiohydrolase (CBH), β-1,4-xylosidase (XYL), β-1,4-glucosidase (GLU), β-1,4-N-acetylglucosaminidase (AGA), and acid phosphatase (AP), were determined using 4-methylumbelliferyl-linked (MUB) substrates and MUB as a standard (Table S1). Substrates and standard were obtained from Sigma-Aldrich and used as received.

5 mM and 30 mM stock solutions of MUB and MUB-linked substrates were prepared using dimethylsulfoxide (DMSO) and stored in the dark at 4°C for up to two months. Stock solutions of 80 mM sodium acetate buffer were prepared in DI water and adjusted to pH 5.1. Since enzyme activity is pH sensitive; the pH of the buffer was adjusted to the mean soil pH of the study site in $CaCl_2$ (i.e., pH 5.6) within 0.5 units.[1] Working solutions of MUB substrates (200 μM) and MUB standard (10 μM) were prepared in buffer solution. Working solutions and buffer solutions were stored in the dark at 4℃ for up to two weeks.

Extracellular enzyme activities were determined by fluorogenic substrate methods.[1-2] Briefly, 4 mL of soil slurries (containing 2 g of soil) were added to 125 mL of sodium acetate buffer. These slurries were then homogenized by magnetic stirring for 2 h and added to a black polystyrene 96-well microplate. Substrates and standard were added and enzyme activities were determined after 2 h incubation in the dark using a multi-well plate reader (Tecan Infinite m200) with 365 nm excitation and 450 nm emission settings. The main advantage of using fluorometrically-labeled substrates is that product formation can be measured directly in the microplate without extraction and purification of the product.[3] Each well was supplemented with 10 μL NaOH (1 mol L^{-1}) within 1 min before

measurement to amplify the fluorescence.[2] These assays were performed at 2 h and 30 d after treatment with nAu suspensions. The enzyme activities are expressed as nmol MUB $g\,soil^{-1}\,h^{-1}$.

Table S1. Soil enzymes assayed in this study.

Enzyme	Abbreviation	EC	Substrate
Acid phosphatase	AP	EC 3.3.3.1	4-MUB-phosphate
β-1,4-glucosidase	GLU	EC 3.2.1.21	4-MUB-β-D-glucoside
Cellobiohydrolase	CBH	EC 3.2.1.91	4-MUB-β-D-cellobioside
β-1,4-N-acetylglucosaminidase	AGA	EC 3.2.1.14	4-MUB-N-acetyl-β-D-glucosaminide
β-1,4-xylosidase	XYL	EC 3.2.1.37	4-Methylumbelliferyl-β-D-xylopyranoside

EC: enzyme commission classification; MUB: methylumbelliferyl

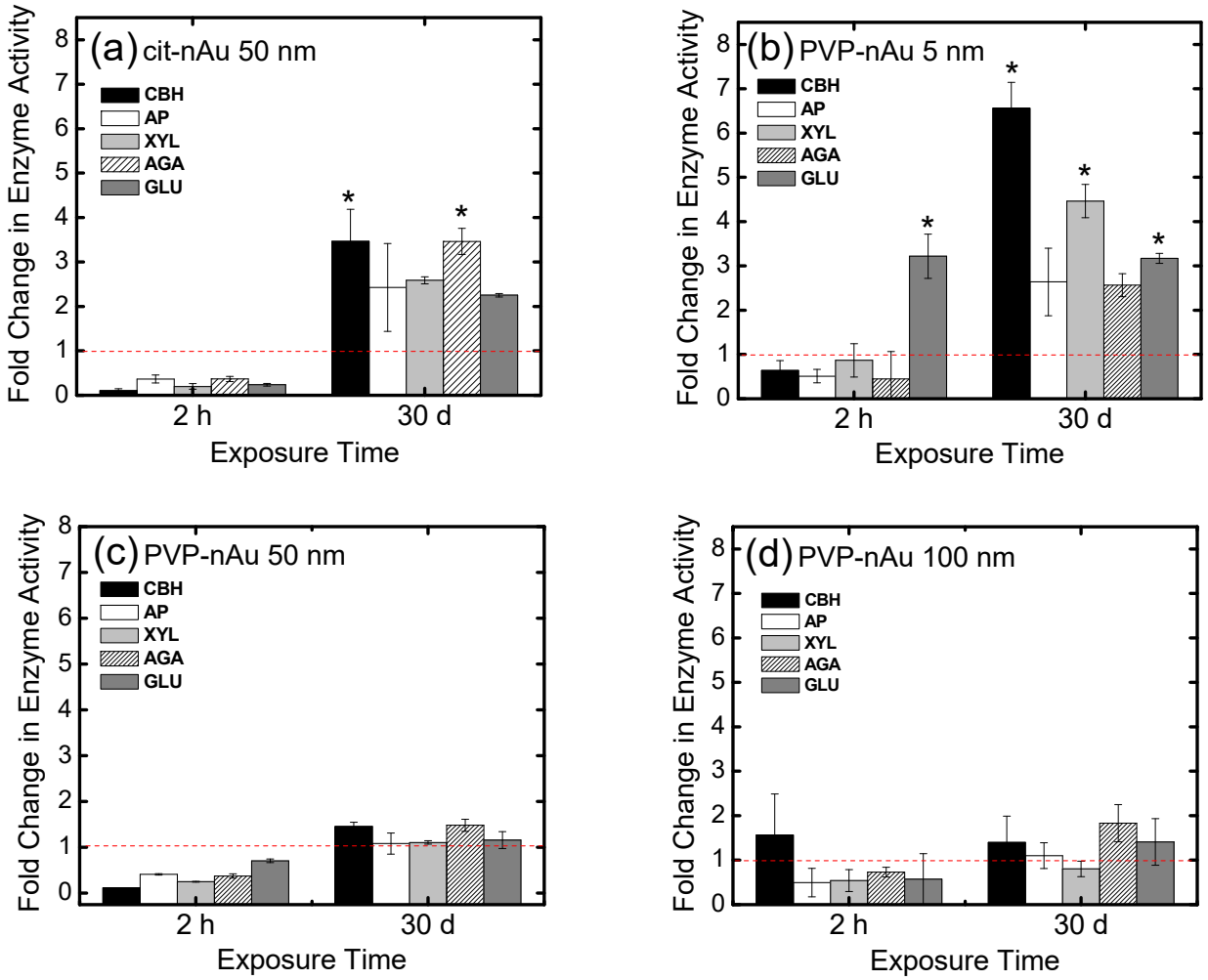

Figure S1. Extracellular enzyme activities in soils treated with 0.1 mg kg^{-1} soil of (a) 50 nm cit-nAu; (b) 5 nm PVP-nAu; (c) 50 nm PVP-nAu; and (d) 100 nm PVP-nAu determined at 2 h and 30 d of exposure. The enzyme activities of treated samples were normalized to those of the control at each time point and reported as a fold change in enzyme activity. Error bars indicate one standard deviation of means (n=4). Symbols above a bar indicate a statistically significant difference in measured values when compared to the control (determined using two-way ANOVA, *: $p<0.05$).

References

1. Saiya-Cork, K. R.; Sinsabaugh, R. L.; Zak, D. R., The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. *Soil Biology and Biochemistry* **2002,** *34* (9), 1309-1315.

2. DeForest, J. L., The influence of time, storage temperature, and substrate age on potential soil enzyme activity in acidic forest soils using MUB-linked substrates and l-DOPA. *Soil Biology and Biochemistry* **2009,** *41* (6), 1180-1186.

3. Marx, M. C.; Wood, M.; Jarvis, S. C., A microplate fluorimetric assay for the study of enzyme diversity in soils. *Soil Biology and Biochemistry* **2001,** *33* (12–13), 1633-1640.