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

Supplementary Material

Soil activity and microbial community response to nanometal oxides were not due exclusively to a particle size effect

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Table S1. Extracellular enzymes assayed in soil exposed to metal oxides. Abbreviation in this study, commission number (EC), function, common substrates, and corresponding substrate for fluorometric analysis are presented.

Enzyme	Abbreviation / EC	Enzyme function	Common substrates	Substrate proxy
Acid phosphatase	AP / 3.1.3.2	Involved in organic P mineralization. Hydrolyze phosphomonoesters releasing phosphate groups.	Phosphomonoesters (e.g., mononucleotide, sugar phosphates) and others (e.g., β-glycerophosphate, phenylphosphate, β-naphthyl phosphate, and *p*-nitrophenyl phosphate)[1]	4-methylumbelliferyl-phosphate
β-1,4-glucosidase	BG / 3.2.1.21	Involved in cellulose degradation. Hydrolysis of β-D-glucopyranosides to glucose.	Cellobiose and other β-1,4-glucans.	4-methylumbelliferyl-β-D-glucoside
β-1,4-*N*-acetylglucosaminidase	NAG / 3.2.1.14	Hydrolysis of terminal, non-reducing β-N-Acetylglucosamine residues from oligosaccharides into N-acetylglucosamine.	Chitooligosaccharides.	4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide

Table S2. Reactions to determine extracellular soil enzymes fluorometrically, according to Saiya-Cork *et al.*, 2002.[2]

Reagent (µl)	Blank	Negative control	Quench standard	Reference standard	Sample
200 mM substrate solution*		50			50
Acetate Buffer	50	200			
Sample suspension	200		200	200	200
Standard**			50	50	
Final Volume			250		

* 4-methylumbelliferyl-β-D-glucoside for BG; 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide for NAG; and 4-methylumbelliferyl-phosphate for AP.

**10 mM 4- methylumbelliferone

Table S3. Sequencing primers and thermocycler conditions for paired-end 16S rDNA V4/ITS1 community sequencing on the Illumina platform according to the protocols from the Earth Microbiome Project (http://earthmicrobiome.org/)

Primer	Illumina adapters	Primer sequence	Thermocycler conditions
	16S rRNA V4		
Forward	5' Illumina adapter: AATGATACGGCGACCACCGAGATCTACACG CT Golay barcode: XXXXXXXXXXXX Forward primer pad: TATGGTAATT Forward primer linker: GT	515FB forward primer GTGYCAGCMGCCGCGGTAA	94 °C for 3 min *35 cycles* 94 °C for 45 s 50 °C for 60 s 72 °C for 90 s 72 °C for 10 min 4 °C hold
Reverse	Reverse complement of 3′ Illumina adapter: CAAGCAGAAGACGGCATACGAGAT Reverse primer pad: AGTCAGCCAG Reverse primer linker: CC	806RB reverse primer GGACTACNVGGGTWTCTAAT	
	ITS		
Forward	5' Illumina adapter: AATGATACGGCGACCACCGAGATCTACAC Forward primer linker: GG	ITS1-F forward primer TTGGTCATTTAGAGGAAGTA AAAGTCGTAACAAGGTTTCC	94 °C for 1 min *35 cycles* 94 °C for 30 s 52 °C for 30 s 68 °C for 30 s 68 °C for 7 min 4 °C hold
Reverse	Reverse complement of 3' Illumina adapter: CAAGCAGAAGACGGCATACGAGAT Golay barcode: NNNNNNNNNNNN Reverse prime linker: CG	ITS2 Reverse Primer CGTTCTTCATCGATGCVAGAR CCAAGAGATC	

Table S4. Abundance of prokaryotic (16S rDNA gene V4) and fungal (ITS1) phyla in survey dataset.

Kingdom	Phylum	ASVs per phylum (%)
Bacteria	Proteobacteria	18.78
Bacteria	Bacteroidota	15.63
Bacteria	Acidobacteriota	13.19
Bacteria	Actinobacteriota	8.90
Bacteria	Chloroflexi	8.71
Bacteria	Myxococcota	7.47
Bacteria	Verrucomicrobiota	7.31
Bacteria	Planctomycetota	6.06
Bacteria	Gemmatimonadota	3.41
Bacteria	Bdellovibrionota	2.03
Bacteria	Armatimonadota	1.46
Bacteria	Firmicutes	1.43
Bacteria	Latescibacterota	0.78
Bacteria	Patescibacteria	0.78
Bacteria	Elusimicrobiota	0.75
Bacteria	Cyanobacteria	0.42
Bacteria	Desulfobacterota	0.39
Archaea	Crenarchaeota	0.34
Bacteria	Methylomirabilota	0.29
Bacteria	RCP2-54	0.29
Bacteria	Abditibacteriota	0.21
Bacteria	Dependentiae	0.21
Bacteria	Nitrospirota	0.21
Bacteria	Fibrobacterota	0.18
Bacteria	Spirochaetota	0.18
Bacteria	Sumerlaeota	0.18
Bacteria	FCPU426	0.13
Bacteria	NB1-j	0.10
Bacteria	WPS-2	0.08
Archaea	Nanoarchaeota	0.03
Bacteria	Entotheonellaeota	0.03
Bacteria	Fusobacteriota	0.03
Bacteria	SAR324_clade	0.03
Fungi	Ascomycota	48.92
Fungi	unidentified	24.22
Fungi	Basidiomycota	12.94
Fungi	Mortierellomycota	9.21
Fungi	Glomeromycota	2.25
Fungi	Chytridiomycota	0.87
Fungi	Rozellomycota	0.66
Fungi	Mucoromycota	0.36
Fungi	Kickxellomycota	0.30
Fungi	Zoopagomycota	0.12
Fungi	Calcarisporiellomycota	0.06
Fungi	Monoblepharomycota	0.06
Fungi	Basidiobolomycota	0.03

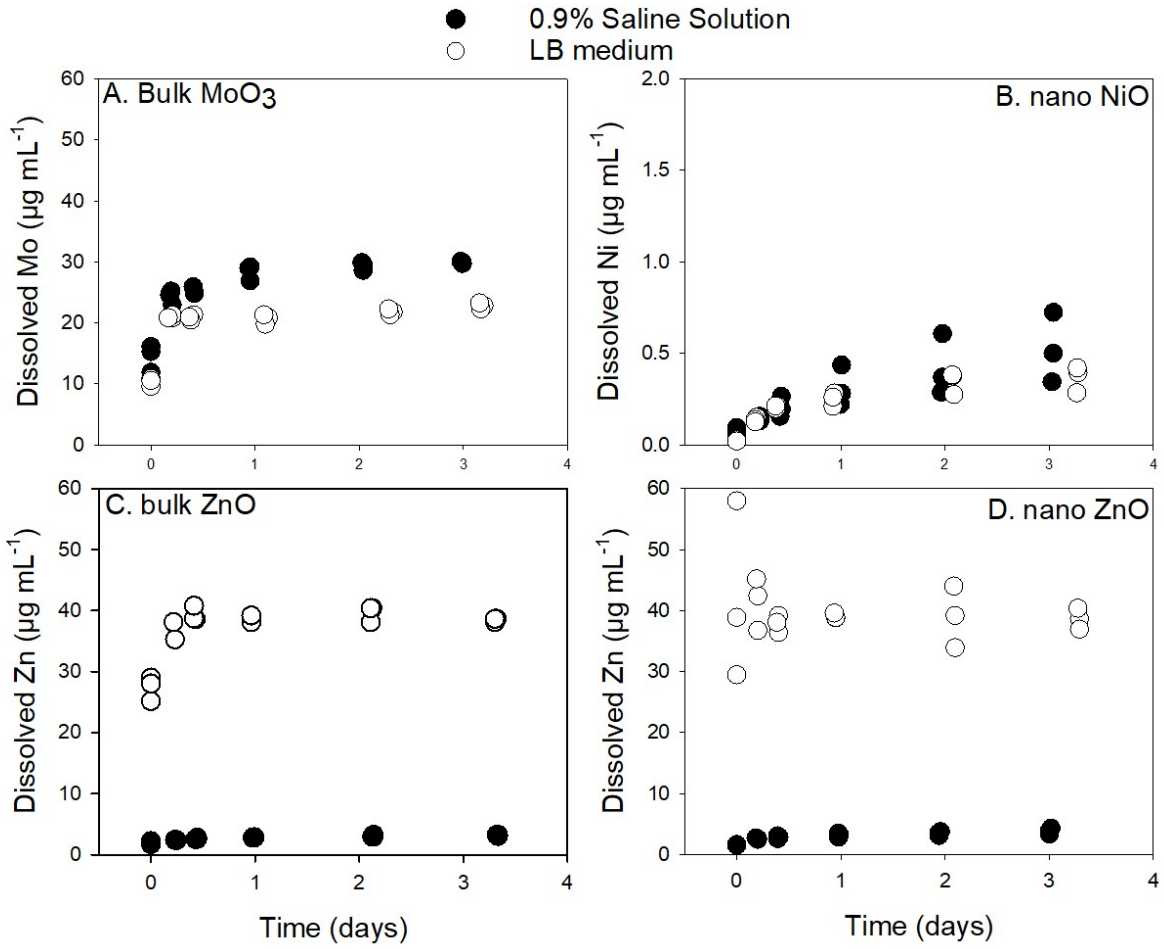

Figure S1. Dissolution curves of (A) bulkMoO_3, (B) nanoNiO, (C) bulkZnO, and (D) nanoZnO, at 50 µg mL^{-1}, in 0.9% saline solution and LB medium. Notice that scale in Y axis for nanoNiO (B) is different. Solubility of nanoMoO_3 and bulkNiO was negligible.

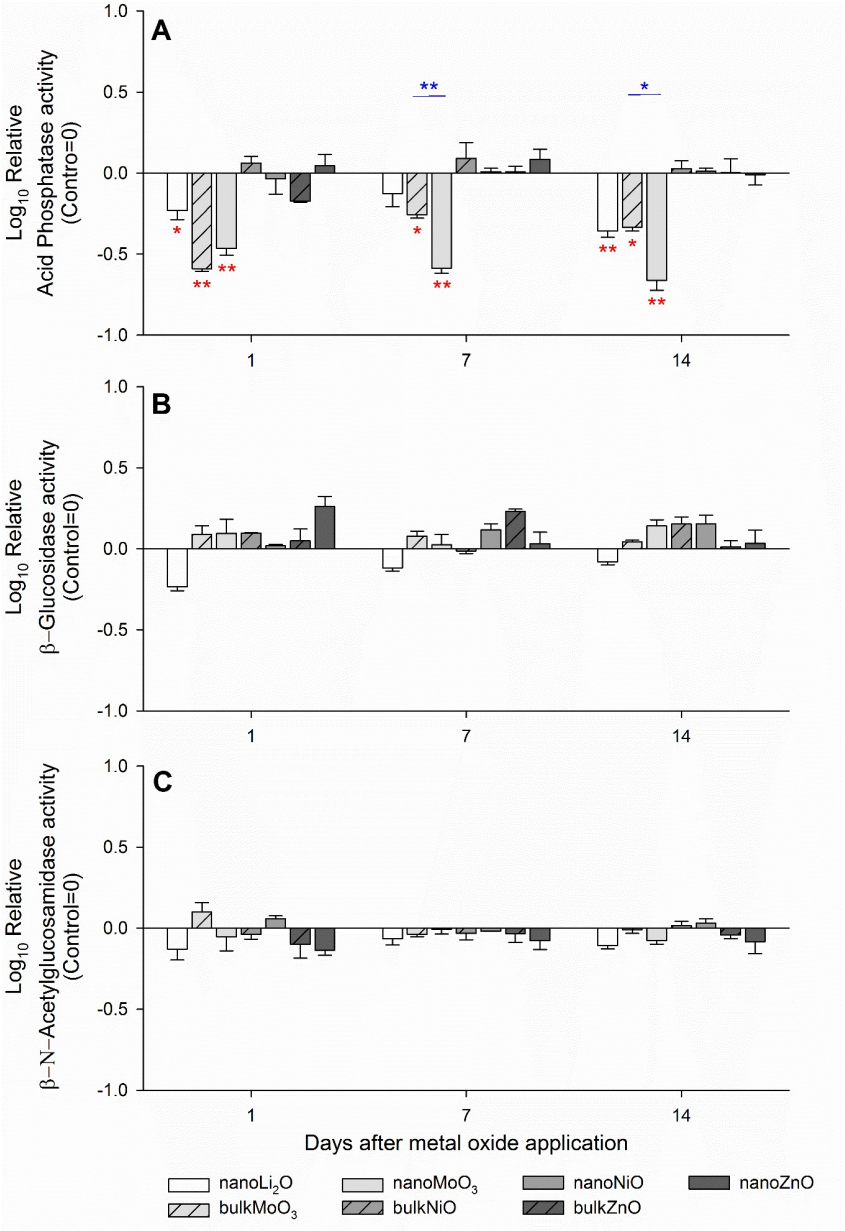

Figure S2. Soil acid phosphatase (A), β-glucosidase (B), and β-N-acetylglucosamidase (C) activity in soil under nanoLi_2O, (bulk and nano) MoO_3, (bulk and nano) NiO, and (bulk and nano) ZnO. Control was soil with no metal oxide addition. Base 10 logarithm of the relative response (i.e., soil response to metal oxide / response of soil control) are presented, where positive and negative numbers represent an increase or decrease in activity, respectively. Error bars represent ±1 standard error (n=3). Asterisk(s) and underlined asterisk(s) represent significance of treatment (metal oxide *vs* control) and size (bulk *vs* nano) effect, respectively. One or two asterisks identify significant difference (p<0.05) or highly significant difference (p<0.001), respectively.

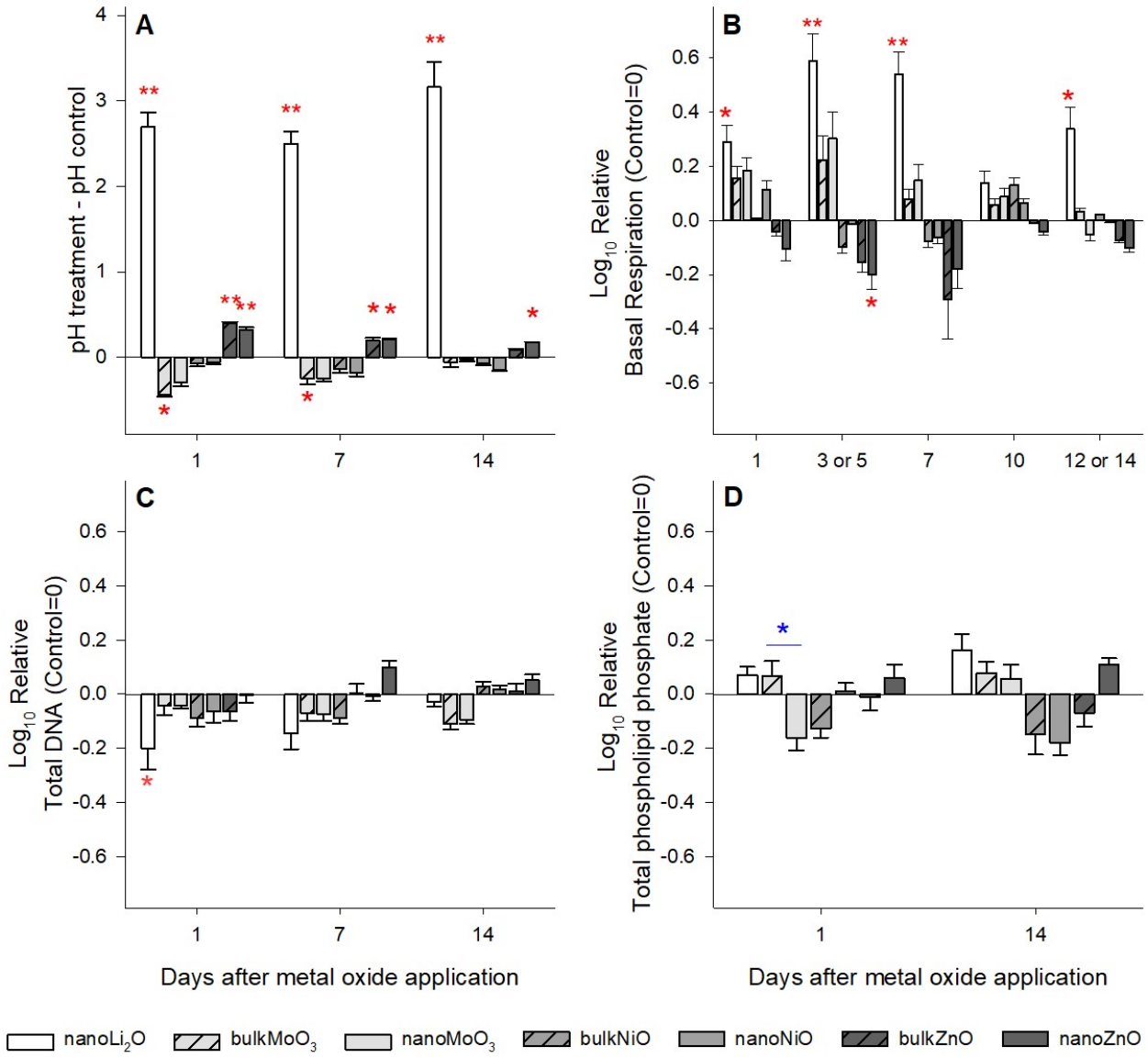

Figure S3. Soil responses to metal oxide addition on pH (A), soil basal respiration (B), total DNA (C), and biomass as total phospholipid phosphate (D) during 14 days of incubation. Control was soil with no metal oxide addition. Except for pH, base 10 logarithm of the relative response (i.e., soil response to metal oxide / response of soil control) are presented, where positive and negative numbers represent an increase or decrease in activity, respectively. Asterisk(s) and underlined asterisk(s) represent significance of treatment (metal oxide *vs* control) and size (bulk *vs* nano) effect, respectively. One or two asterisks identify significant difference (p<0.05) or highly significant difference (p<0.001), respectively.

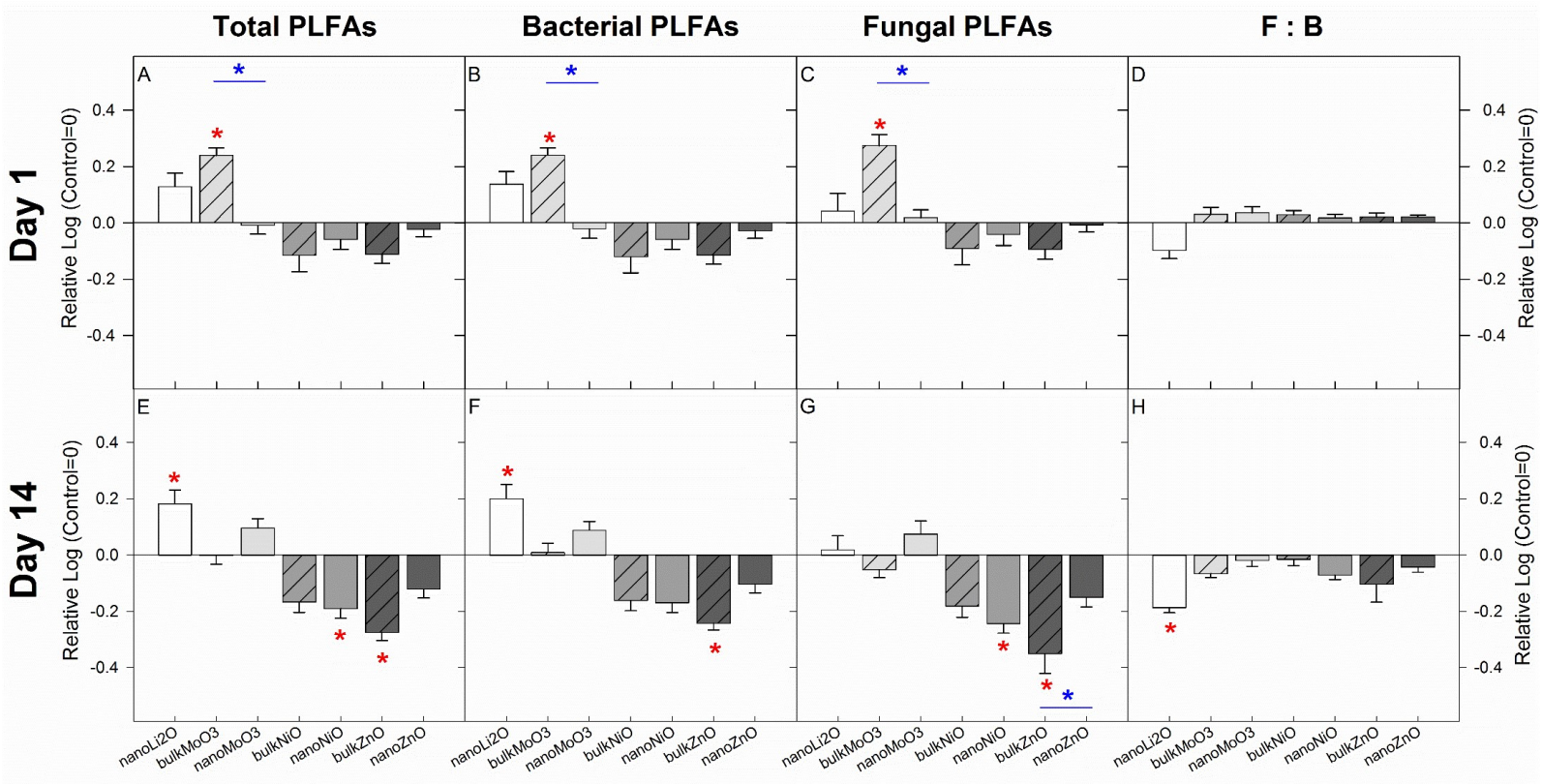

Figure S4. Soil microbial biomass of total (A and E), bacterial (B and F), fungal (C and G) and fungal:bacterial ratio (F:B in D and H) of PLFAs in soils 1 and 14 days after metal oxide application. Control was soil with no metal oxide addition. Logarithm (base 10) of the relative response (i.e., soil response to metal oxide / response of soil control) are presented, where positive and negative numbers represent an increase or decrease in activity, respectively. Error bars represent ±1 standard error (n=3). Asterisk(s) and underlined asterisk(s) represent significance of treatment (metal oxide *vs.* control) and size (bulk *vs.* nano) effect, respectively. One asterisk identifies significant differences (p<0.05).

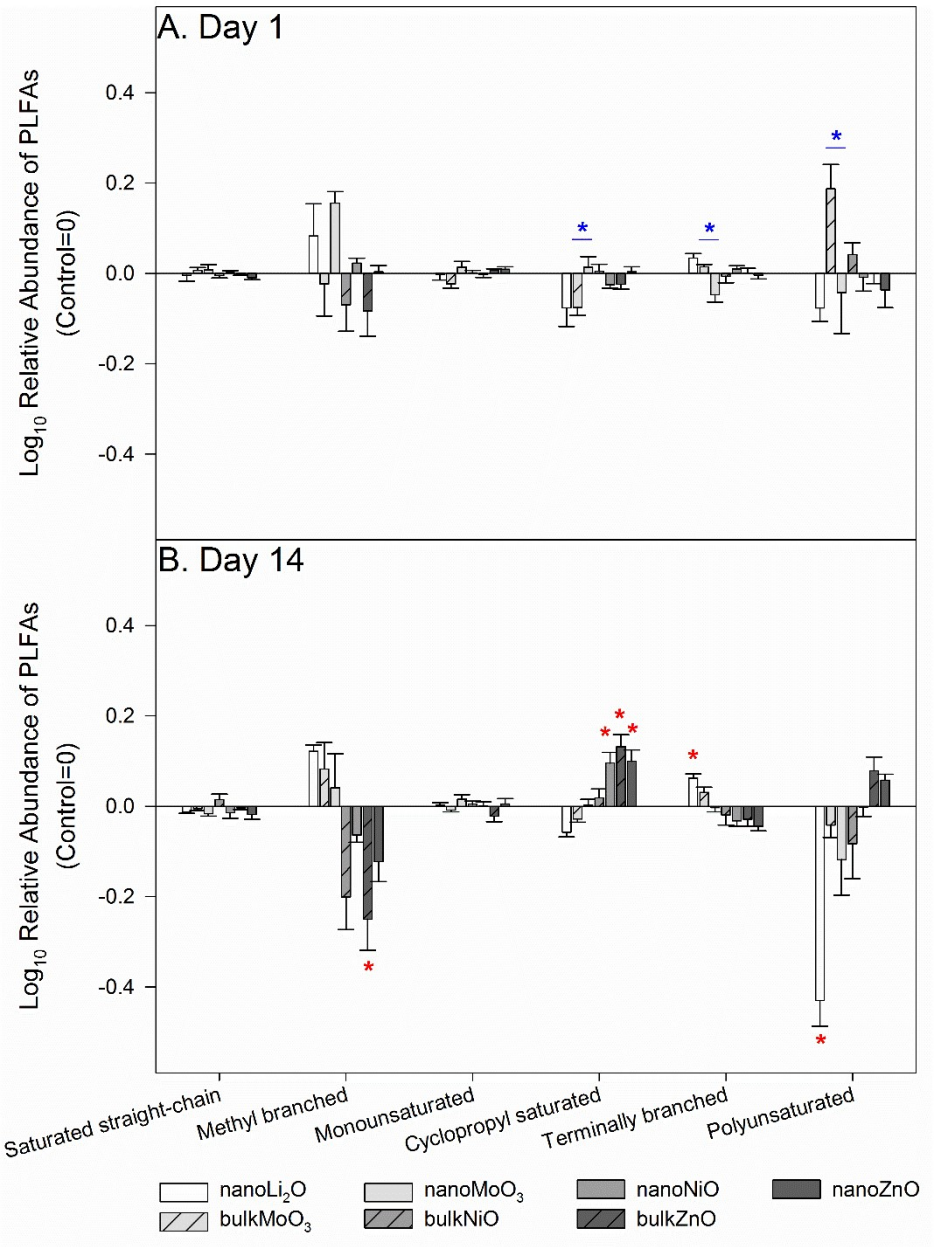

Figure S5. PLFAs groups in soils 1 (A) and 14 (B) days after metal oxide application. Control was soil with no metal oxide addition. Base 10 logarithm of the relative response (i.e., soil response to metal oxide / response of soil control) are presented, where positive and negative numbers represent an increase or decrease in activity, respectively. Error bars represent ±1 standard error (n=3). Asterisk(s) and underlined asterisk(s) represent significance of treatment (metal oxide vs control) and size (bulk *vs* nano) effect, respectively. One asterisk identifies significant differences (p<0.05).

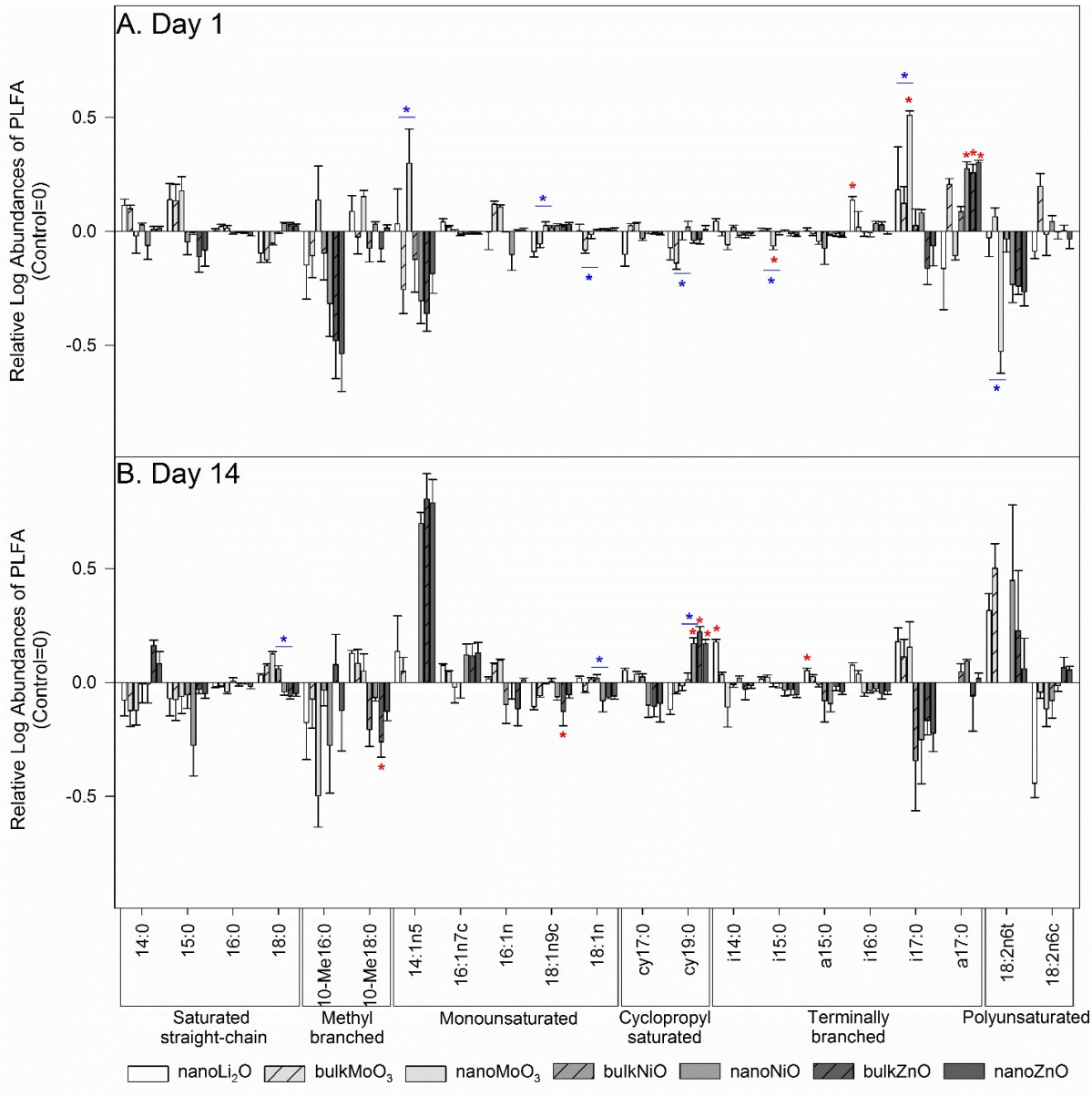

Figure S6. Individual PLFAs in soils 1 (A) and 14 (B) days after metal oxide application. Control was soil with no metal oxide addition. Base 10 logarithm of the relative response (i.e., soil response to metal oxide / response of soil control) are presented, where positive and negative numbers represent an increase or decrease in activity, respectively. Error bars represent ±1 standard error (n=3). Asterisk(s) and underlined asterisk(s) represent significance of treatment (metal oxide *vs.* control) and size (bulk *vs* nano) effect, respectively. One asterisk identifies significant differences (p<0.05).

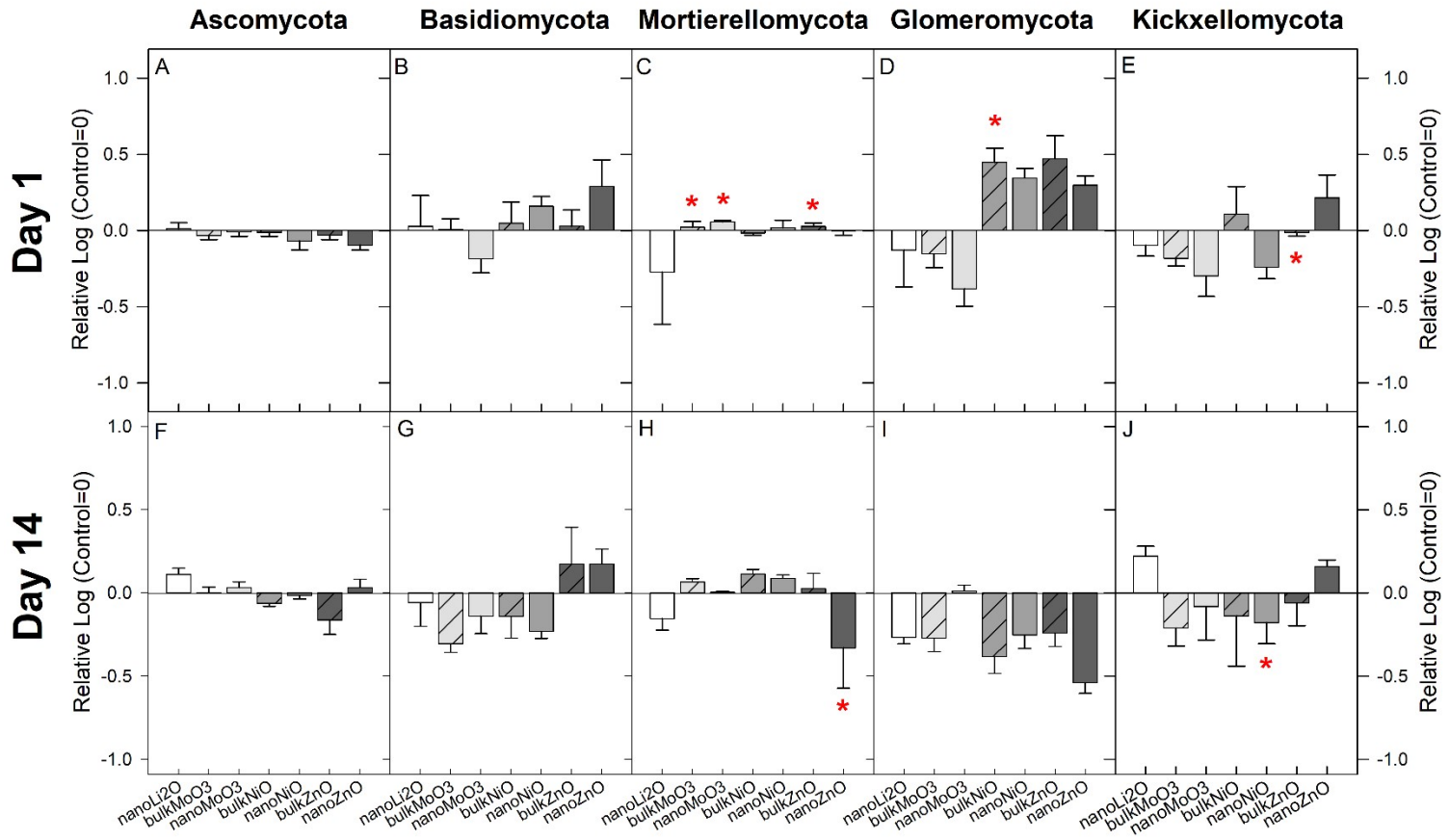

Figure S7. Differential abundance analysis of fungal (ITS1) phyla in soils, 1 and 14 days after metal oxide application. Control was soil with no metal oxide addition. Logarithm (base 10) of the relative response (i.e., soil response to metal oxide / response of soil control) are presented, where positive and negative numbers represent an increase or decrease in activity, respectively. Error bars represent ±1 standard error (n=3). Asterisk(s) and underlined asterisk(s) represent significance of treatment (metal oxide *vs.* control) and size (bulk *vs.* nano) effect, respectively. One asterisk identifies significant differences (p<0.05).

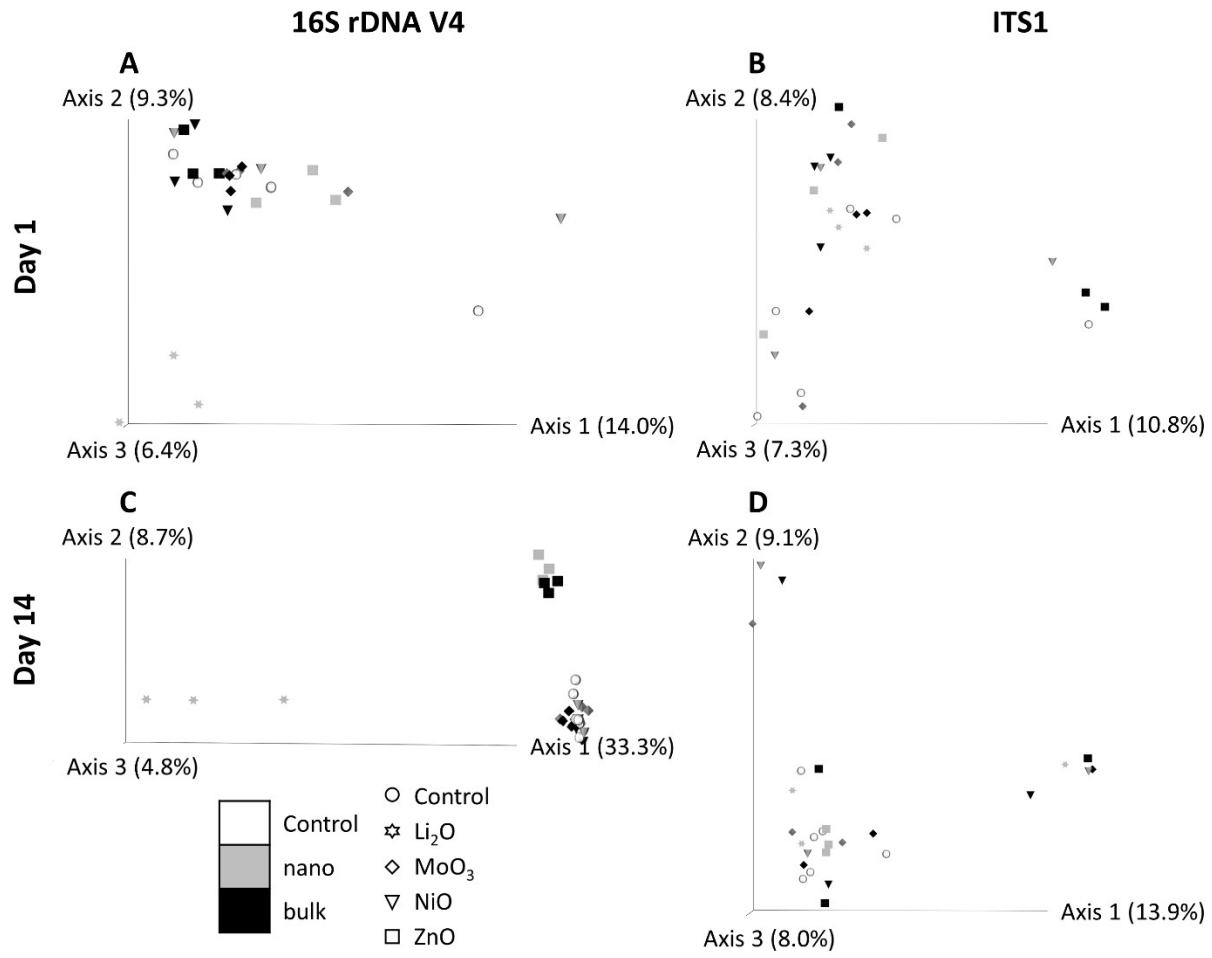

Figure S8. Principal coordinate analysis depicting the quantitative Bray-Curtis distances of the 16S rDNA gene V4 (A and C) and ITS1 (B and D) sequences in soils, 1 (A and B) and 14 (C and D) days after exposure to metal oxides. Percentages in axes represent variation explained by the components. No statistical differences (p<0.05) in β-diversity metrics between control and metal oxide were observed (**Table 5**).

References

1. M. A. Tabatabai, in *Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties*, eds. R. W. Weaver, S. Angle and P. Bottomley, Soil Science Society of America, Madison, WI 53711, 1994, DOI: 10.2136/sssabookser5.2.c37, ch. 37, pp. 775-833.

2. K. R. Saiya-Cork, R. L. Sinsabaugh and D. R. Zak, *Soil Biology and Biochemistry*, 2002, **34**, 1309-1315.