

## Soybean Plants Modify Metal Oxide Nanoparticle Effects on Soil Bacterial Communities

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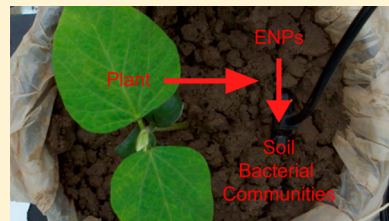
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### Supporting Information

**ABSTRACT:** Engineered nanoparticles (ENPs) are entering agricultural soils through land application of nanocontaining biosolids and agrochemicals. The potential adverse effects of ENPs have been studied on food crops and soil bacterial communities separately; however, how ENPs will affect the interacting plant–soil system remains unknown. To address this, we assessed ENP effects on soil microbial communities in soybean-planted, versus unplanted, mesocosms exposed to different doses of nano-CeO<sub>2</sub> (0–1.0 g kg<sup>-1</sup>) or nano-ZnO (0–0.5 g kg<sup>-1</sup>). Nano-CeO<sub>2</sub> did not affect soil bacterial communities in unplanted soils, but 0.1 g kg<sup>-1</sup> nano-CeO<sub>2</sub> altered soil bacterial communities in planted soils, indicating that plants interactively promote nano-CeO<sub>2</sub> effects in soil, possibly due to belowground C shifts since plant growth was impacted. Nano-ZnO at 0.5 g kg<sup>-1</sup> significantly altered soil bacterial communities, increasing some (e.g., *Rhizobium* and *Sphingomonas*) but decreasing other (e.g., *Ensifer*, *Rhodospirillaceae*, *Clostridium*, and *Azotobacter*) operational taxonomic units (OTUs). Fewer OTUs decreased from nano-ZnO exposure in planted (41) versus unplanted (85) soils, suggesting that plants ameliorate nano-ZnO effects. Taken together, plants—potentially through their effects on belowground biogeochemistry—could either promote (i.e., for the 0.1 g kg<sup>-1</sup> nano-CeO<sub>2</sub> treatment) or limit (i.e., for the 0.5 g kg<sup>-1</sup> nano-ZnO treatment) ENP effects on soil bacterial communities.



## INTRODUCTION

As production and use of engineered nanoparticles (ENPs) rise, ENPs will increasingly enter the environment.<sup>1</sup> ENPs enter agricultural soils through land application of wastewater treatment plant biosolids or nanocontaining pesticides and fertilizers, and thus may pose risks to agricultural ecosystems.<sup>2,3</sup> To assess soil exposure, experiments have used various ENPs (e.g., TiO<sub>2</sub>, ZnO, CuO, Ag, and carbon nanotubes), but mainly in the absence of plants. Such experiments have either found limited effects of ENPs to soil bacterial communities (e.g., from fullerenes<sup>4,5</sup>) or direct toxicity (e.g., TiO<sub>2</sub>).<sup>6,7</sup> ENPs can alter soil bacterial communities,<sup>8–10</sup> including the abundance of some functionally significant bacterial groups (e.g., N<sub>2</sub>-fixing bacteria).<sup>11–13</sup> Metal oxide ENPs (e.g., CeO<sub>2</sub>, ZnO, CuO, and FeOx) may also be bioavailable to soil-grown plants and may translocate from soils into plant tissues,<sup>14–16</sup> where they cause physiological and genetic damage.<sup>14,16–18</sup> However, it is still unknown how plants cultivated in soils may change ENP impacts on soil microbial communities.

Plant roots alter soil processes in ways that may either change bacterial bioavailability of ENPs or modify ENP toxicity. For example, roots release low molecular weight organic

compounds (i.e., sugars, amino acids and organic acids), polymerized sugars (i.e., mucilage), and sloughed-off root cells.<sup>19</sup> Plants allocate 30–60% of fixed carbon to their roots, and around 40–90% of this is ultimately released into soil.<sup>20</sup> Because soil organic matter can mitigate ENP toxicity by adsorbing and immobilizing some ENPs,<sup>4,21</sup> the overall greater amount of available organic material in planted soils may further attenuate ENP toxicity. For example, roots secrete chelators that sequester metallic ions,<sup>19</sup> and thus could reduce the bioavailability of toxic ions released from soluble metal oxide ENPs (e.g., ZnO).

To assess how plants alter the effects of ENPs on soil bacterial communities, we studied how nano-CeO<sub>2</sub> and nano-ZnO affect soil microbial communities and how those impacts are altered by the presence of growing soybean plants. Nano-CeO<sub>2</sub> is commonly used as a fuel additive to improve combustion efficiency, while nano-ZnO is widely used in

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sunscreens, cosmetics, and coatings.<sup>22</sup> Both ENPs are therefore increasingly being introduced into the environment.<sup>1,23</sup> We exposed mesocosms containing either only soil or soils planted with soybean seedlings to a range of ENP concentrations (0.1, 0.5, 1.0 g kg<sup>-1</sup> for nano-CeO<sub>2</sub>, and 0.05, 0.1, 0.5 g kg<sup>-1</sup> for nano-ZnO). We harvested mesocosm soils when the plants were mature and analyzed the soil bacterial community composition by two independent methods: terminal restriction fragment length polymorphism (T-RFLP) and PhyloChip analysis.

## MATERIALS AND METHODS

**Experimental Design.** As described previously,<sup>14</sup> surface soil (0–20 cm) was collected from an organic farm in Carpinteria, CA (N 34° 23' 40", W 119° 28' 40"), sieved to 2 mm and stored at 4 °C. This soil is mapped as grading between a Fluventic Haploxeroll (Goleta series) and an Aquic Xerofluvent (Camarillo series). Soil pH (6.78), texture (sandy loam, 66% sand, 22% silt, and 12% clay), soluble salts (0.58 dS m<sup>-1</sup>), cation exchange capacity (8.72 mequiv per 100 g), organic matter (1.44%), total C (0.71%), total N (0.07%), and other nutrients were characterized by the UC Davis Analytical Laboratory (Davis, CA; <http://anlab.ucdavis.edu/>) and were previously reported.<sup>14</sup>

The dry powder nano-CeO<sub>2</sub> (Meliorum, Rochester, NY, U.S.A.) used in this study was comprised of ceria with a primary rod shape (67 × 8 nm<sup>2</sup>); the dry powder specific surface area was 93.8 m<sup>2</sup> g<sup>-1</sup>, isoelectric point 7.5, and purity 95%.<sup>24</sup> The nano-ZnO (Meliorum, Rochester, U.S.A.) was composed of zincite with a spheroid shape (24 nm); the specific surface area was 42.1 m<sup>2</sup> g<sup>-1</sup>, isoelectric point 9.2, and purity 97%.<sup>24</sup> The ENP-amended soils were prepared approximately 24 h prior to use, by adding dry powder nanoparticles to sieved soils to achieve the target exposure doses (0.1, 0.5, and 1.0 g kg<sup>-1</sup> for nano-CeO<sub>2</sub>, and 0.05, 0.1, and 0.5 g kg<sup>-1</sup> for ZnO). To homogeneously distribute the ENPs into the ENP-amended soils, a 10-fold dilution method was used, as described previously.<sup>14</sup> In brief, powder nanoparticles were initially added to soil in doses of 10, 50, and 100 g kg<sup>-1</sup> for CeO<sub>2</sub>, and 5, 10, and 50 g kg<sup>-1</sup> for ZnO, and mechanically mixed for 5 min with separate hand-held kitchen mixers. The nanoparticles were then diluted 10-fold twice to achieve the target doses by adding unspiked soils into each mixture, and homogenizing as described above. Soil without ENPs was used as the control.

For each of the seven ENP treatments, eight experimental pots were prepared with four receiving transplanted soybean seedlings and the remaining four serving as unplanted controls. The full details of the experimental pot preparation, the soybean seedling preparation, and the transplantation were reported previously.<sup>14</sup> In brief, each experimental pot (4 L, polyethylene/polypropylene blend) contained 400 g of washed gravel (1.25–2.5 cm) underneath 2.4 kg of soil. To facilitate harvesting, the soil was contained within a polyethylene bag perforated with 5 mm holes ( $n = 20$ ) to form a mesh. The mesh and gravel aided in water drainage and prevented root rot. Soybean seedlings were prepared by sowing the dwarf soybean seeds (variety: Early Hakucho, product #5555, Park Seed Company, Greenwood, SC, U.S.A.) in peat-filled seed starting pellets (4 cm diameter). Once the true leaves emerged (18 days after sowing), soybean seedlings were ranked into four size classes, and transplanted into half of the experimental pots such that each ENP treatment received one seedling from each size class. The transplanting time was referred to as “day 0” of the exposure experiment.

Both unplanted and planted pots were placed in a greenhouse for 48 days until soybean plants developed fully to the later stage of pod development (except for one replicate of the low dose nano-ZnO treatment in planted soils, because the plant died after transplantation). The greenhouse was under full sunlight, with temperatures ranging from 12 to 31 °C.<sup>14</sup> During the experiment, the soil water content averaged 0.15 m<sup>3</sup> m<sup>-3</sup> by watering the experimental pots with tap water approximately every 72 h.<sup>14</sup>

As described previously,<sup>14</sup> plant and soil samples were harvested after 48 days of ENP exposure and preprocessed within 2 days, including manually removing most roots then sieving (2 mm) out any remaining root material from the planted treatments. For planted treatments, aboveground plant tissues were first cut from the root systems with a razorblade and then divided into stems, leaves, and pods and weighed separately. The belowground plant tissues were harvested by carefully breaking apart the soils, rinsing roots in deionized H<sub>2</sub>O three times, and removing nodules from the roots by using forceps. Subsamples of each tissue type were either analyzed immediately, refrigerated (4 °C), or oven-dried (70 °C for 72 h) to examine ENP effects on plant growth, nutritional value, and ENP translocation from the soil into plant tissues, as reported previously.<sup>14,15,25</sup> Soils from the unplanted treatments were also homogenized and sieved to 2 mm. The sieved soil was subsampled, with portions stored at 4 °C for measuring soil enzyme activities, and the rest stored at –80 °C for later DNA extraction to characterize soil bacterial community structure and diversity.

**Soil DNA Extraction.** Total DNA was extracted from 0.3 g soil subsampled from each of the four end point replicates for each treatment (except for the low dose nano-ZnO treatment in planted soils where  $n = 3$ , Supporting Information (SI) Table S1) using the Powersoil DNA Isolation Kit (Mo Bio, Carlsbad, CA, U.S.A.) and quantified using the Quant-iT DNA Assay Kit High Sensitivity (Invitrogen, Eugene, OR, U.S.A.), according to the manufacturer’s instructions. Besides end point soils, we also extracted DNA from subsampled soils when ENPs were just mixed with soils (0-day exposure), with one replicate per treatment (SI Table S1).

**Polymerase Chain Reaction (PCR) and T-RFLP.** Genes encoding 16S rRNA were amplified using the extracted DNA as template and using universal bacterial primers HEX-labeled 8F (AGA GTT TGA TCC TGG CTC AG) and 1389R (ACG GGC GGT GTG TAC AAG),<sup>26</sup> following the reaction chemistry and thermal cycling program described previously (see the SI for details).<sup>7</sup>

The *Hha*I digested PCR products (600 ng, see the SI for details) were shipped to Michigan State University (the Research Technology Support Facility) on dry ice for T-RFLP analysis using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, U.S.A.). T-RFLP profiles were aligned using the crosstab Excel macro “treeflap”<sup>27</sup> and only those fragments between 50 and 1000 bp in size with relative peak heights >1% were included in the analysis. The relative abundance data, defined as the peak height proportions of specific restriction fragments in a whole community, were used to explore the response patterns of the overall community. Two T-RFLP outliers, one from the low dose nano-ZnO treatment in unplanted soils and another from the low dose nano-CeO<sub>2</sub> treatment in planted soils, were not included in the statistical analyses (SI Table S1).

**PhyloChip Analysis and Data Preprocessing.** In addition to T-RFLP analysis, extracted DNA was also concentrated and shipped on dry ice to Second Genome (San Francisco, CA) for PhyloChip analysis, using protocols described previously.<sup>28</sup> The mean fluorescence intensity of each operational taxonomic unit (OTU) was  $\log_2$  transformed to generate hybridization scores for statistical analysis (see the SI for details). Hybridization scores were previously shown to correlate well with known concentrations of 16S rRNA genes in a mixture of twenty-six 16S rRNA genes (average correlation coefficient,  $r = 0.941$ ), indicating the capacity of PhyloChip for quantitative community analysis.<sup>28</sup> An OTU was defined by a group of highly similar 16S rRNA gene sequences (a probe set), and assigned to a set of higher taxa (kingdom, phylum, class, order, family, and genus) based on the probe sequences, using a Bayesian method by Second Genome. The Bayesian annotation, as opposed to adopting the annotation from full-length reference sequences, prevents overspecification of the OTUs. The resulting sample-OTU matrix was used for community analysis. One PhyloChip outlier from the low dose of nano-ZnO treatment in planted soils was not included in the statistical analyses (SI Table S1).

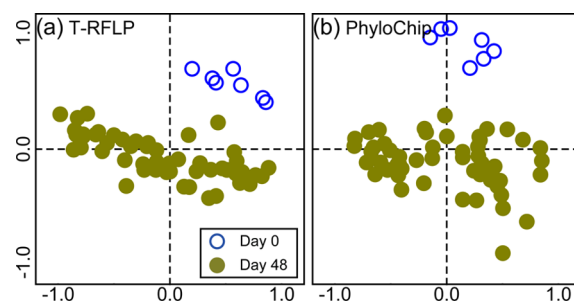
**Statistical Analyses.** Distance-based redundancy analysis (dbRDA)<sup>29</sup> was performed to examine the Bray–Curtis distances within T-RFLP profiles and within PhyloChip profiles. In this way, two data sets (T-RFLP and PhyloChip) were separately used for determining community shift patterns. As a constrained ordination approach, dbRDA uses a multivariate regression-based model to correlate bacterial community variables with environmental variables.<sup>29</sup> Furthermore, as an extension of classical redundancy analysis which processes Euclidean distances between samples, dbRDA can analyze other types of community distance, e.g., Bray–Curtis.<sup>29</sup> For dbRDA, the T-RFLP profiles or PhyloChip profiles were used as community variables, and significant ( $P < 0.05$  by the Monte Carlo permutation test with 999 permutations<sup>29</sup>) environmental variables (plant treatment, nano-ZnO, and exposure time) were used as constrained variables.

To identify the OTUs sensitive to ENP exposure, we conducted regression analysis on the PhyloChip results to examine the relationship between the hybridization score of each OTU and the ENP exposure dose, and to define the sensitive OTUs as those whose hybridization scores significantly changed according to ENP exposure. To limit the potential high false discovery rate caused by multiple testing, we corrected the original  $P$  values using the Benjamini–Hochberg (BH) procedure.<sup>30</sup> If the statistical test on the regression equation was significant after Benjamini–Hochberg correction (adjusted  $P < 0.05$ ), then the OTU was identified as a sensitive OTU with either a positive or negative correlation with ENP exposure. Regression analysis was also conducted to examine nano-CeO<sub>2</sub> and nano-ZnO effects on soil bacterial richness, defined as the number of different terminal restriction fragments (TRFs) or PhyloChip OTUs. Student's  $t$  test, coupled with Benjamini–Hochberg correction (adjusted  $P < 0.05$ ), was conducted on the PhyloChip results to identify bacterial OTUs that differed in the presence or absence of soybean plants.

Analyses were conducted using CANOCO (Microcomputer Power, Ithaca, NY, U.S.A.), R (<http://www.r-project.org/>), or SigmaPlot (Systat Software, San Jose, CA, U.S.A.).

## RESULTS AND DISCUSSION

**Soil Bacterial Community Structure and Diversity.** In the dbRDA graphs, bacterial community data from 0-day exposure samples clustered together (Figure 1 and SI Figure

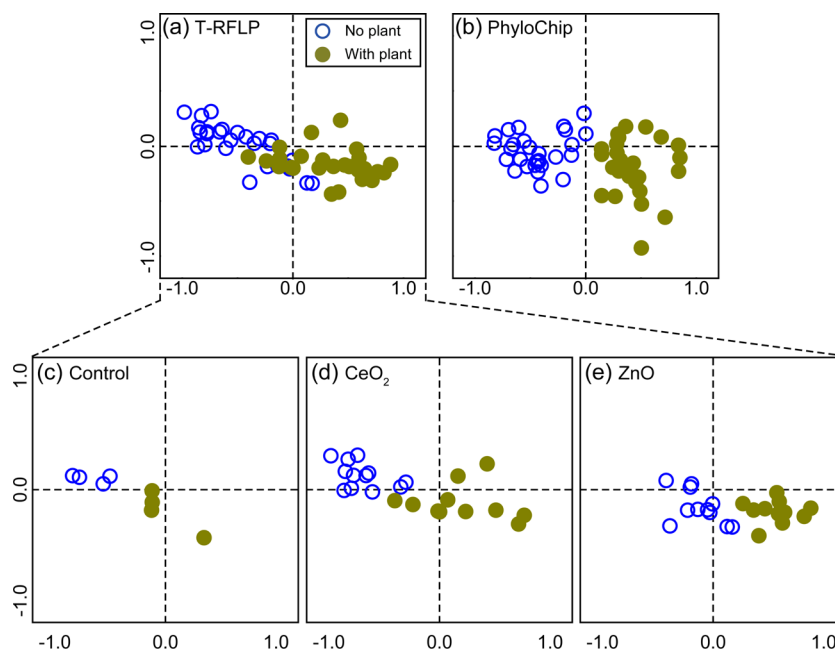


**Figure 1.** Exposure time effects on soil bacterial communities as analyzed by distance-based redundancy analysis (dbRDA), showing that soil microbial communities at day 0 were more similar to each other, irrespective of nanoparticle treatment, than to communities after 48 days of incubation. The  $x$ - and  $y$ -axis represent the first and the second ordination axis, respectively. Soil bacterial communities were independently characterized by both terminal restriction fragment length polymorphism (T-RFLP, a) and PhyloChip (b). In these graphs, the soil treatments (without or with ENPs at various doses, and with or without plants) are not distinguished. Open symbols are from day 0, i.e., immediately after soils and ENPs were mixed; closed symbols are after 48 days.

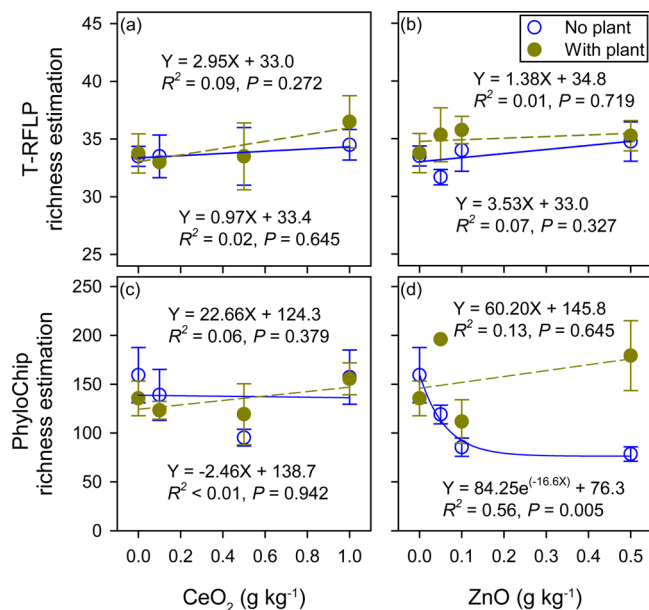
S1), indicating no ENP effects on either DNA extraction or further community analysis methods. In a prior study, exposure time was an important factor in ENP effects: with 0-day exposure, soil bacterial communities were unaffected by nano-TiO<sub>2</sub> and nano-ZnO, although soil DNA was reduced after 15 and 60 days.<sup>8</sup> In the 48-day samples here, soil bacterial community data for the unplanted treatments distributed to the left side of the first axis that mainly differentiated plant effects, while data for the planted treatments distributed to the right side (Figure 2 and SI Figure S1), indicating that soybean plants overall changed soil bacterial communities. With T-RFLP analysis, although soil bacterial community data for the planted treatments were not totally separate from the unplanted treatments, the same trend of bacterial community shifts was observed (Figure 2a). For example, when we further examined plant effects for different ENP treatments, bacterial community data for unplanted versus planted treatments were mostly different (Figure 2c–e). With PhyloChip analysis, irrespective of the ENP treatments, soil bacterial communities differed between planted and unplanted treatments (Figure 2b). Plant roots are known to alter the composition and diversity of rhizosphere microbial communities.<sup>31</sup> In this study, the soils in planted pots were mostly rhizosphere, since the rooting density was high and, thus, roots likely affected the entire soil volume.

We then examined nano-CeO<sub>2</sub> and nano-ZnO effects on the estimated richness of soil bacterial communities. T-RFLP analysis did not show measurable effects of nano-CeO<sub>2</sub> and nano-ZnO for either unplanted or planted soils ( $P > 0.05$  for all combinations, Figure 3a,b). Also, nano-CeO<sub>2</sub> did not significantly change soil bacterial richness, as estimated by either T-RFLP or PhyloChip, in either unplanted or planted soils ( $P > 0.05$  for all combinations, Figure 3a,c). However, in unplanted soils, bacterial community richness (as estimated by PhyloChip at the OTU level) exponentially decreased as a function of nano-ZnO dose ( $P < 0.05$ , Figure 3d). This pattern





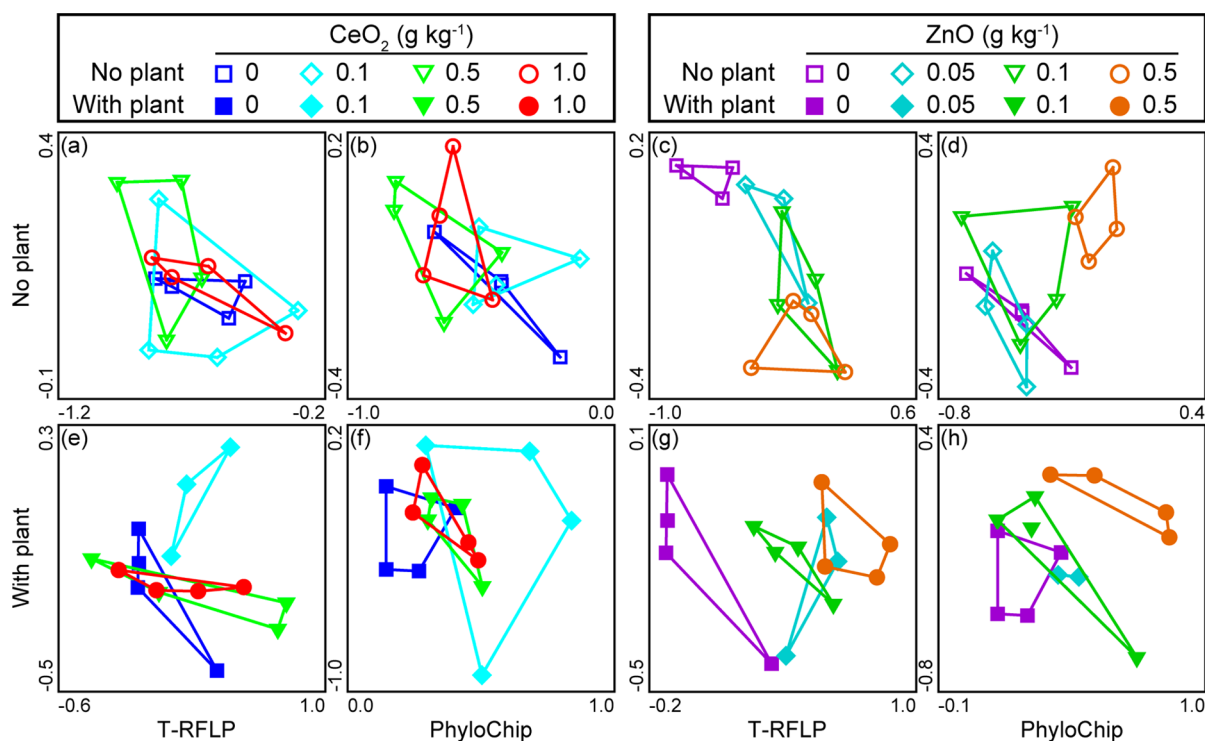
**Figure 2.** Plant effects after 48-days on soil bacterial communities analyzed by distance-based redundancy analysis (dbRDA). Time 0 data are shown in Figure 1 but not here. The  $x$ - and  $y$ -axis represent the first and the second ordination axis, respectively. Soil bacterial communities were independently characterized by both terminal restriction fragment length polymorphism (T-RFLP, a, c, d, and e) and PhyloChip (b). With T-RFLP analysis, plant effects at different ENP conditions, i.e., ENP-free control (c), nano-CeO<sub>2</sub> (d), and nano-ZnO (e), were separately plotted. Open symbols are for unplanted soils; closed symbols are for planted soils.



**Figure 3.** Effects of nano-CeO<sub>2</sub> (a and c) and nano-ZnO (b and d) on the number of terminal restriction fragments (a and b), and the number of bacterial OTUs estimated by PhyloChip analysis (c and d) in unplanted and planted soils after 48 days exposure. The  $x$ -axes display doses and not measures of actual exposure. Error bars indicate the standard error of the mean (with T-RFLP analysis,  $n = 4$  except for the low dose of nano-ZnO treatments in unplanted and planted soils, and the low dose of nano-CeO<sub>2</sub> treatment in planted soils where  $n = 3$ ; with PhyloChip analysis,  $n = 4$  except for the low dose of nano-ZnO treatments in planted soils where  $n = 2$ ). In each graph, the upper regression line equation corresponds to the “With plant” (closed symbols) soils, and the lower regression line equation corresponds to the “No plant” (open symbols) soils.

did not occur in planted soils ( $P > 0.05$ , Figure 3d), indicating that the presence of soybean plants may have reduced the nano-ZnO effects on soil bacterial diversity. The apparent contradiction between the PhyloChip and T-RFLP results could be attributed to the methodological difference between the two methods. Compared to T-RFLP that generally detects abundant bacteria (relative abundance  $>1\%$ ), PhyloChip is more sensitive and is able to detect more rare bacterial taxa in environmental samples.<sup>28</sup>

Similarity in community composition was plotted using distance-based redundancy analysis to illustrate nano-CeO<sub>2</sub> and nano-ZnO effects in more detail for with, versus without, plant treatments (Figure 4). There were no apparent effects on overall soil bacterial communities from nano-CeO<sub>2</sub> in unplanted soils since all three doses of nano-CeO<sub>2</sub> (0.1, 0.5, and 1.0 g kg<sup>-1</sup>) resulted in overlapping similarity maps including with the ENP-free control, by both T-RFLP and PhyloChip analyses (Figure 4a and b). However, the low dose of nano-CeO<sub>2</sub> (0.1 g kg<sup>-1</sup>) had a distinctively different effect in planted soils. With T-RFLP analysis, 0.1 g kg<sup>-1</sup> nano-CeO<sub>2</sub> resulted in distinctly separated similarity maps (Figure 4e); with PhyloChip analysis, there was more spread in the replicates for 0.1 g kg<sup>-1</sup> nano-CeO<sub>2</sub>, yet the treatments were distinct (Figure 4f). Because 0.1 g kg<sup>-1</sup> nano-CeO<sub>2</sub> did not affect soil bacterial communities in unplanted soils (Figure 4a,b), the observed community shifts in planted soils in response to 0.1 g kg<sup>-1</sup> nano-CeO<sub>2</sub> were probably not related to the direct effects of nano-CeO<sub>2</sub>. In a previous paper from this study, Priester et al. showed that, compared to the ENP-free control, 0.1 g kg<sup>-1</sup> nano-CeO<sub>2</sub> exerted the most pronounced effects on plant growth parameters, such as lower leaf count, lower leaf cover, and lower root biomass; in particular, plants harvested from 0.1 g kg<sup>-1</sup> nano-CeO<sub>2</sub> treatment were significantly shorter (final stem length;  $P = 0.05$ ) than controls.<sup>14</sup> Therefore, it is noteworthy that the previously



**Figure 4.** Effects of nano-CeO<sub>2</sub> (a, b, e, and f) and nano-ZnO (c, d, g, and h) on soil bacterial communities in unplanted (open symbols) or planted (solid symbols) soils, analyzed by distance-based redundancy analysis (dbRDA). The *x*- and *y*-axis represent the first and the second ordination axis, respectively. These data are also plotted in Figures 1 and 2 to display overall study effects of time or planting, respectively. Here, the data are separately displayed for each ENP, with (filled symbols) versus without (open symbols) plants, to examine detailed treatment effects. Soil bacterial communities were independently characterized by both terminal restriction fragment length polymorphism (T-RFLP, a, c, e, and g) and PhyloChip analysis (b, d, f, and h).

reported stunted plant growth at 0.1 g kg<sup>-1</sup> nano-CeO<sub>2</sub> is accompanied herein by bacterial community shifts in planted soils in response to 0.1 g kg<sup>-1</sup> nano-CeO<sub>2</sub>. Furthermore, higher concentrations of nano-CeO<sub>2</sub> had little effect on plant growth,<sup>14</sup> which also accompanies the minimal effects of medium and high nano-CeO<sub>2</sub> on soil bacterial communities herein. Although we did not measure plant root exudates, they are likely to have been reduced due to the stunted growth when plants were grown in the presence of 0.1 g kg<sup>-1</sup> nano-CeO<sub>2</sub>, since root exudates often correlate with root and shoot biomass (SI Figure S2).<sup>32,33</sup> The quantity and composition of root exudates may also have been changed in the 0.1 g kg<sup>-1</sup> nano-CeO<sub>2</sub> treatment as a result of plants mounting a defense against abiotic stress. Alternatively, damaged or stressed plants may be leakier. Perhaps relatedly, nano-Ag caused a significant release of dissolved organic carbon through affecting aquatic plants,<sup>34</sup> and nano-Ag toxicity to *Chlamydomonas reinhardtii* was mitigated by algal-produced organic compounds.<sup>35</sup> It is therefore conceivable that, although bacterial community richness was not significantly affected by nano-CeO<sub>2</sub>, 0.1 g kg<sup>-1</sup> nano-CeO<sub>2</sub> impacted soil bacterial community composition indirectly through reducing plant growth and, thus, changing the quantity and composition of plant root exudates (SI Figure S2).

Differently from the effects of nano-CeO<sub>2</sub>, nano-ZnO resulted in dose-dependent effects on soil bacterial communities. For planted or unplanted soils, the high dose of nano-ZnO (0.5 g kg<sup>-1</sup>) resulted in distinctly separated similarity maps from the ENP-free control, yet the similarity maps of low and medium nano-ZnO treatments (0.05 and 0.1 g kg<sup>-1</sup>) tended to distribute between the control and high nano-ZnO data (Figure

3c, d, g, and h). The similarity maps from T-RFLP versus PhyloChip showed some differences: with T-RFLP analysis, the similarity maps of low and medium nano-ZnO treatments were more similar to the high nano-ZnO treatment, while with PhyloChip analysis the similarity maps of low and medium nano-ZnO were more similar to the ENP-free control. Our previous study using a California grassland soil also showed that nano-ZnO significantly altered soil bacterial communities in a dose dependent manner,<sup>8,11</sup> indicating that nano-ZnO is bioavailable to soil bacteria.

**Sensitive Bacterial OTUs.** We defined sensitive bacterial OTUs as those whose hybridization scores showed significant relationships to the concentrations of either nano-CeO<sub>2</sub> or nano-ZnO (adjusted *P* < 0.05 after multiple testing corrections using BH procedure). Among the 514 bacterial OTUs that could be classified at the phylum level, no OTUs were significantly correlated with nano-CeO<sub>2</sub> (adjusted *P* > 0.05 for all), while 103 OTUs (20.0%) were significantly correlated with nano-ZnO in either unplanted or planted soils, or both (adjusted *P* < 0.05 for these 103 OTUs, SI Table S2). We also identified 261 OTUs that differed in the presence and absence of soybean plants (adjusted *P* < 0.05 for these 261 OTUs), with 68 OTUs sensitive to nano-ZnO (SI Table S3). We previously found a dose-dependent effect of nano-CeO<sub>2</sub> on nodule N<sub>2</sub> fixation potential,<sup>14</sup> while here we did not find dose-dependent nano-CeO<sub>2</sub> effects on soil rhizobia. Because nodule N<sub>2</sub> fixation could be modulated by multiple processes (e.g., nodule formation, bacteroid infection of nodules, or fixation rates),<sup>36</sup> reduced N<sub>2</sub> fixation potentials in root nodules does not require decreased rhizobia populations in soils.

Among the 103 OTUs sensitive to nano-ZnO, 15 OTUs were positively correlated with nano-ZnO, while 88 OTUs were negatively correlated with nano-ZnO (Table 1), indicating a

**Table 1. Number and Proportion of Bacterial OTUs Sensitive to nano-ZnO Exposure in Unplanted and Planted Soils<sup>a</sup>**

	positive response		negative response		sum	
	number	%	number	%	number	%
no plant	2	0.4	60	11.7	62	12.1
with plant	12	2.3	6	1.2	18	3.5
both	1	0.2	22	4.3	23	4.5
sum	15	2.9	88	17.1	103	20.0

<sup>a</sup>Bacterial OTUs were examined using PhyloChip analysis.

mainly adverse effect of nano-ZnO on soil bacteria. Our previous study using a different soil also showed that more native soil bacterial taxa within communities showed negative, versus positive, responses to nano-ZnO exposure.<sup>11</sup>

Interestingly, fewer bacterial OTUs showed significant responses to nano-ZnO in planted versus unplanted soils (41 versus 85, Table 1), indicating that the cultivation of soybean plants during ENP exposure may reduce ENP effects on some individual taxa. The mitigation effects could be partially attributed to the additional input of organic materials introduced by plant roots in the form of root exudates and sloughed root cells, although this was not directly tested. The newly introduced organic materials may attenuate ENP toxicity by immobilizing ENPs or sequestering metallic ions released by soluble metal oxide ENPs.<sup>4,19</sup> Perhaps relatedly, nano-CuO and nano-ZnO were more toxic to bacterial community growth (by the leucine incorporation method) in mineral soil (40 g kg<sup>-1</sup> organic-C) than in organic-matter-rich soil (154 g kg<sup>-1</sup> organic-C).<sup>37</sup> Furthermore, soil sorption reduced the bioavailability and antibacterial activity of fullerene (C<sub>60</sub>) and the sorption capacity increased with soil organic matter content,<sup>21</sup> indicating the role of soil organic matter in attenuating ENP toxicity. In our experimental system, there was more aboveground and belowground biomass when plants were grown in the presence of nano-ZnO, and belowground biomass was significantly higher in the high nano-ZnO treatment than in the control ( $P < 0.05$ , Student's *t* test).<sup>14</sup> The stimulated plant growth could be attributed to the increased Zn uptake in the presence of dissolvable nano-ZnO, since Zn is a micronutrient that is needed for plant photosynthesis.<sup>25</sup> It is conceivable that the stimulation of plant growth by nano-ZnO could have caused more belowground exudation.<sup>32,33</sup>

ENP translocation from the soil into the plant is an unlikely explanation for the attenuated ENP effects herein, since less than 1% of the total applied Zn was translocated into the plants.<sup>14</sup> However, given the abundant root biomass in the pots, the root surface area could have been high enough to sorb Zn ions and thus decrease their bioavailability and related effects to soil bacteria. Therefore, in addition to exudates and root cell sloughing, the sorption to root surfaces might be a mechanism by which bioavailable Zn is decreased. However, this mechanism was not studied directly, e.g., by measuring Zn associated with the roots before, versus after, root washing.<sup>14</sup>

The taxonomic affiliations (kingdom, phylum, class, order, family, and genus) of the 103 sensitive OTUs to nano-ZnO are summarized in SI Table S2, showing that the phylum *Proteobacteria* contains the highest number of sensitive OTUs

(35), followed by *Firmicutes* (17), *Cyanobacteria* (14), *Acidobacteria* (8), *Planctomycetes* (8), *Bacteroidetes* (6), *Gemmatimonadetes* (5), *Actinobacteria* (3), *Verrucomicrobia* (3), *WS3* (2), *OP11* (1), and *Synergistetes* (1). On the basis of the taxonomic affiliations, some of the sensitive OTUs are potentially significant to terrestrial N and C cycling. For example, OTU569 (the genus *Ensifer*, also called *Sinorhizobium*), known as a symbiotic N<sub>2</sub>-fixing bacterium, negatively correlated with nano-ZnO in unplanted soils (adjusted  $P < 0.05$ , SI Table S2). However, OTU112 (the genus *Rhizobium*), another symbiotic N<sub>2</sub>-fixing bacteria, positively correlated with nano-ZnO in planted soils (adjusted  $P < 0.05$ , SI Table S2). We also found that two bacterial groups, the family *Rhodospirillaceae* (OTU048 and OTU499) and the genus *Clostridium* (OTU454), which contain free-living N<sub>2</sub>-fixing bacteria,<sup>38,39</sup> negatively correlated with nano-ZnO in unplanted soils (adjusted  $P < 0.05$  for all, SI Table S2). Furthermore, OTU147 and OTU550 (the genus *Azotobacter*), another free-living N<sub>2</sub>-fixing taxa,<sup>40</sup> negatively correlated with nano-ZnO in both unplanted and planted soils (adjusted  $P < 0.05$  for all combinations, SI Table S2). Besides the OTUs involved in N<sub>2</sub> fixation, we also found some sensitive OTUs, i.e., OTU127 and OTU356 (the genus *Sphingomonas*, SI Table S2), that are known to be associated with the degradation of a variety of recalcitrant organic compounds.<sup>41</sup>

Previous bacterial population studies have reported greater toxicity of nano-ZnO to *Sinorhizobium meliloti* relative to nano-CeO<sub>2</sub>,<sup>42</sup> and the detrimental effects of nano-WO<sub>3</sub> (<10 mg L<sup>-1</sup>) and nano-Ag (around 10 mg L<sup>-1</sup>) on *Azotobacter vinelandii*,<sup>40,43</sup> which partially support our observations regarding N<sub>2</sub>-fixing bacteria. In soil exposure experiments without plant cultivation, *Bradyrhizobium canariense* was reported to be sensitive to nano-Ag;<sup>12</sup> *Flavobacteriales* and *Sphingomonadales* were suppressed by nano-Cu and nano-ZnO;<sup>44</sup> *Rhizobiales* and *Sphingobacteriaceae* were negatively affected by nano-CuO.<sup>13</sup> Our previous soil microcosm experiment also found that both nano-TiO<sub>2</sub> and nano-ZnO affected bacterial taxa associated with nitrogen fixation (the order *Rhizobiales*), methane oxidation (the family *Methylobacteriaceae*), and recalcitrant organic compound decomposition (families *Sphingomonadaceae* and *Streptomycetaceae*).<sup>11</sup> Because of the indigenous community dissimilarities between soils, some identified sensitive taxa differed across studies and soils. However, some functionally significant bacterial groups, i.e., the group associated with N<sub>2</sub> fixation and the group associated with recalcitrant organic matter decomposition, were repeatedly identified as sensitive bacteria by multiple studies, suggesting potential ecosystem consequences of ENP exposure.

In conclusion, our results demonstrate that, although nano-CeO<sub>2</sub> did not affect soil bacterial communities in unplanted soils, 0.1 g kg<sup>-1</sup> nano-CeO<sub>2</sub> had a distinctive effect in planted soils, probably due to its previously reported effects on plant growth<sup>14</sup> which may have indirectly affected soil bacterial communities through changed root exudates. In contrast, nano-ZnO, apparently, directly impacted soil bacterial communities, with 0.5 g kg<sup>-1</sup> nano-ZnO significantly altering communities in both unplanted and planted soils. However, the number of sensitive bacterial OTUs to nano-ZnO was reduced by around 50% in planted soils in comparison with unplanted soils (Table 1), indicating that the presence of soybean plants reduced nano-ZnO effects on soil bacterial communities. The mitigation effects of soybean plants on nano-ZnO toxicity could be from Zn ion sorption to plant roots, which would reduce Zn



availability to soil bacteria; it could also be from carbon that is introduced into soils by plant roots in the form of root exudates and sloughed root cells. Such plant-derived compounds could also modify the bioavailability and toxicity of nano-ZnO, e.g., by immobilizing ENPs and sequestering toxic ions released from dissolving nanoparticles. This is in contrast to the results with 0.1 g kg<sup>-1</sup> nano-CeO<sub>2</sub>, whereby the plant response to nano-CeO<sub>2</sub> could have created, not ameliorated, a soil bacterial community shift. Thus, plants—likely through their effects on belowground biogeochemistry—can either promote (i.e., for the 0.1 g kg<sup>-1</sup> nano-CeO<sub>2</sub> treatment) or limit (i.e., for the 0.5 g kg<sup>-1</sup> nano-ZnO treatment) ENP effects on soil bacterial communities. As ENPs increasingly enter the soil environment, they will alter how soils function and may therefore affect agricultural plants and possibly the food we harvest from them. But the effects of ENPs will be altered by those very plants, creating feedbacks that alter the functioning of the plant–soil–ENP system. Developing effective policies to address the new field of nanotoxicology requires understanding both the direct toxicological effects of ENPs on humans and other sensitive species of plants and animals, but also the indirect effects on environmental systems. Assessing these requires integrating focused laboratory experiments with whole system microcosms as presented here.

## ■ ASSOCIATED CONTENT

### ● Supporting Information

Additional details regarding PCR/T-RFLP and PhyloChip analyses, effects of different environmental variables (plant, ENP dose, and exposure time) on soil bacterial communities (Figure S1), effects of 0.1 g kg<sup>-1</sup> nano-CeO<sub>2</sub> on N<sub>2</sub> fixation, aboveground and belowground biomass, and potentially, on root exudates (Figure S2), the number of independent DNA extracts, T-RFLP profiles, and PhyloChip runs (Table S1), the taxonomic affiliations of 103 bacterial OTUs sensitive to nano-ZnO exposure (Table S2), and bacterial OTUs that differed in the presence and absence of soybean plants (Table S3). This material is available free of charge via the Internet at <http://pubs.acs.org/>.

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### Notes

The authors declare no competing financial interest.

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# Soybean plants modify metal oxide nanoparticle effects on soil bacterial communities

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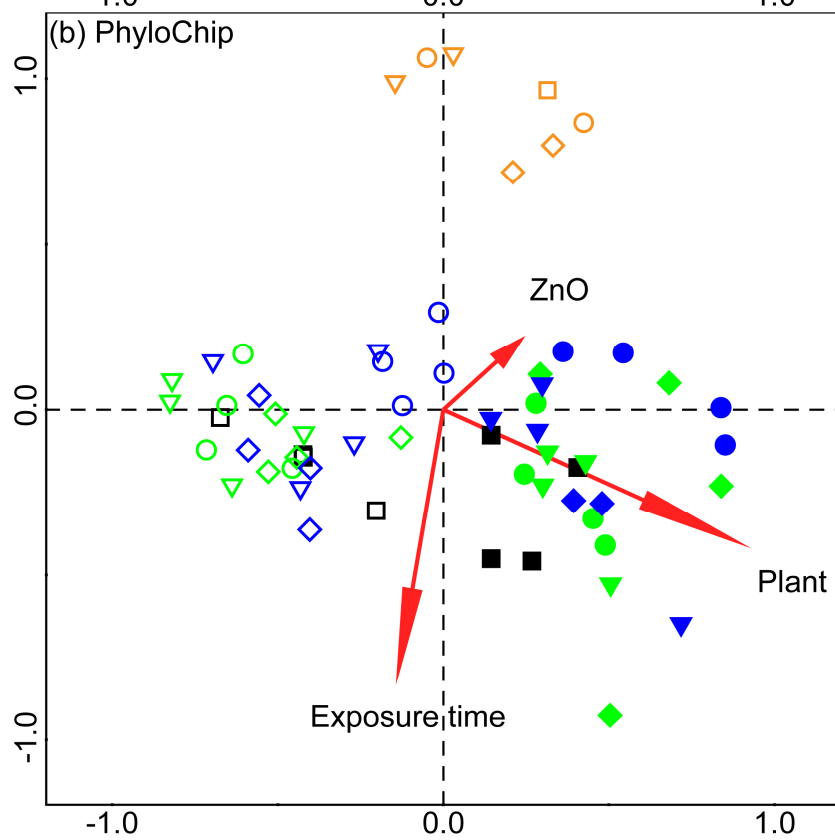
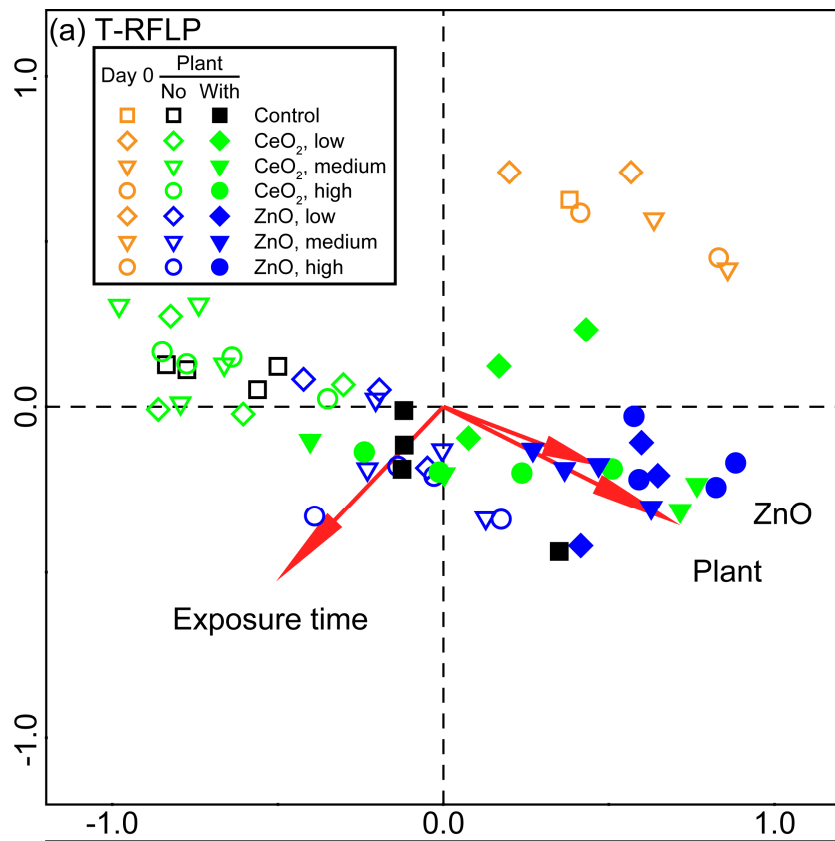
## Materials and Methods

**Polymerase Chain Reaction (PCR) and T-RFLP.** Negative controls, containing all PCR components except DNA templates, were included to test for contamination. For each sample, PCR products from duplicate reactions were pooled to reduce random PCR bias. The size and quality of PCR products were verified by 1.2% agarose-gel electrophoresis (Flashgel DNA system; Lonza, Allendale, NJ, USA). PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA), and quantified using the Quant-iT dsDNA Assay Kit, Broad Range (Invitrogen, Eugene, OR, USA), according to the manufacturer's instructions.

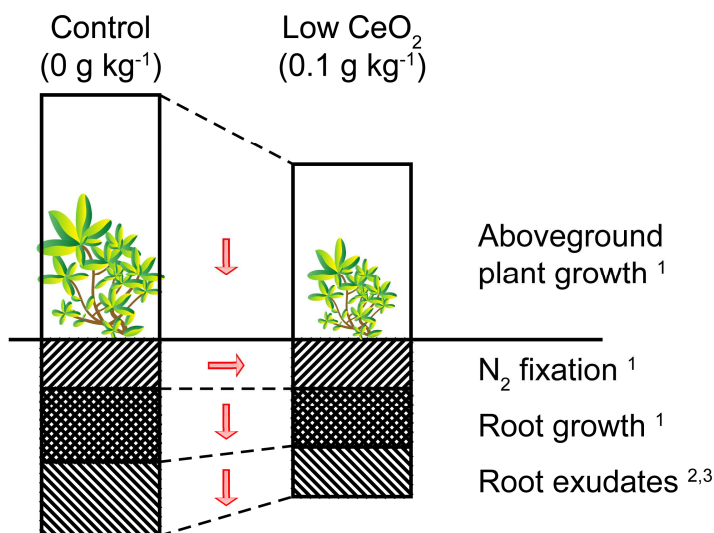
The purified PCR products (600 ng) were digested at 37°C for 16 h using the *HhaI* restriction enzyme (New England BioLabs, Ipswich, MA, USA), deactivated at 65°C for 20 min, and purified again using the QIAquick Nucleotide Removal Kit (Qiagen, Valencia, CA, USA), according to the manufacturer's instructions.

**PhyloChip Analysis and Data Preprocessing.** To calculate the overall fluorescence intensity of each probe set that represents a specific OTU, the central nine pixels of individual probes were ranked by intensity, and the 75th percentile intensity values were used. Probe intensities were background-subtracted, i.e. scaled to the quantitative standards,<sup>4</sup> and collected as integer values ranging from 0 to 65,536 ( $2^{16}$ ). Fluorescence intensities of individual probes within each probe set were averaged after discarding the highest and lowest values, and the mean was  $\log_2$  transformed and rounded to decimal numbers ranging from 0 to 16. For compatibility with the statistical analysis, the mean was multiplied by 1000 then rounded to the nearest integer, allowing a range of 0 to 16,000. The mean was referred to as the hybridization score of a specific OTU.<sup>4</sup>





**Figure S1.** Distance-based redundancy analysis (dbRDA) to illustrate the effects of different environmental variables (plant, ENP dose, and exposure time) on soil bacterial communities. The x- and y-axis represent the first and the second ordination axis, respectively. Soil bacterial communities were independently characterized by both terminal restriction fragment length polymorphism (T-RFLP, a) and PhyloChip analysis (b). Control: ENP-free control; Low: low dose of nano-CeO<sub>2</sub> (0.1 g kg<sup>-1</sup>) or nano-ZnO (0.05 g kg<sup>-1</sup>); Medium: medium dose of nano-CeO<sub>2</sub> (0.5 g kg<sup>-1</sup>) or nano-ZnO (0.1 g kg<sup>-1</sup>); High: high dose of nano-CeO<sub>2</sub> (1.0 g kg<sup>-1</sup>) or nano-ZnO (0.5 g kg<sup>-1</sup>). The red arrows point to microbial communities whose treatments were significantly related to the variable (arrow label); the lengths of the arrows indicate the variables' effects magnitudes on soil microbial community composition.



**Figure S2.** Conceptual illustration of the possible effects of 0.1 g kg<sup>-1</sup> nano-CeO<sub>2</sub> on N<sub>2</sub> fixation, aboveground and belowground soybean biomass, and root exudates. In a previous paper from this study regarding plant growth and N<sub>2</sub> fixation potential, Priester et al. showed that, compared to the ENP-free control, 0.1 g kg<sup>-1</sup> nano-CeO<sub>2</sub> did not alter N<sub>2</sub> fixation, but stunted aboveground and belowground plant growth.<sup>1</sup> Although not quantified, plant root exudates are likely to have been reduced due to stunted plant growth when plants were grown in the presence of 0.1 g kg<sup>-1</sup> nano-CeO<sub>2</sub>, since it has been reported in other studies that root exudates positively correlate with root and shoot biomass.<sup>2,3</sup> Our prior interpretation regarding N<sub>2</sub> fixation at this low nano-CeO<sub>2</sub> dose was that the plants preferentially allocated photosynthate to root nodules so that the N<sub>2</sub> fixation apparatus was functional.<sup>1</sup> Decreased root growth and aboveground plant biomass would support the interpretation, and would support that root exudates also would have been decreased. With less, or even compositionally different, bioavailable plant-derived C compounds entering planted soils, it is logical that bacterial communities in the low nano-CeO<sub>2</sub> treatment would vary from the communities in planted control soils.



**Table S1.** The number of independent DNA extracts, T-RFLP profiles, and PhyloChip runs.

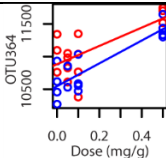
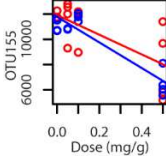
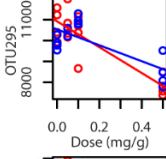
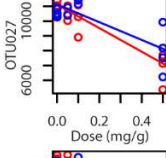
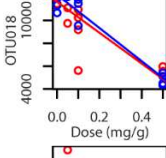
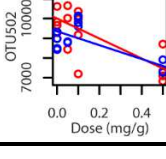
Dose (g kg <sup>-1</sup> )	Control	CeO <sub>2</sub>			ZnO		
	0	0.1	0.5	1	0.05	0.1	0.5
DNA extracts*							
Day 0	1	1	1	1	1	1	1
No plant	4	4	4	4	4	4	4
With plant	4	4	4	4	3	4	4
T-RFLP profiles **							
Day 0	1	1	1	1	1	1	1
No plant	4	4	4	4	3	4	4
With plant	4	3	4	4	3	4	4
PhyloChip runs ***							
Day 0	1	1	1	1	1	1	1
No plant	4	4	4	4	4	4	4
With plant	4	4	4	4	2	4	4

\*One replicate of the low dose nano-ZnO treatment in planted soils was not included in DNA extraction and the following T-RFLP and PhyloChip analyses, since the plant died after transplantation.

\*\*Two T-RFLP outliers, one from the low nano-ZnO treatment in unplanted soils and another from the low nano-CeO<sub>2</sub> treatment in planted soils, were not included in the statistical analyses.

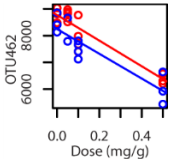
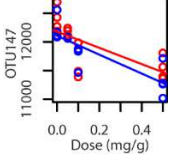
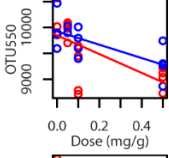
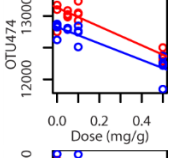
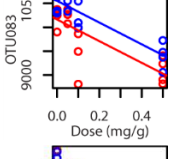
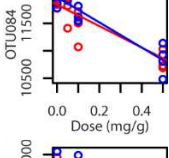
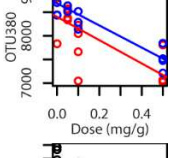
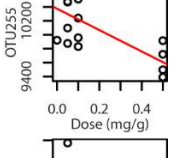
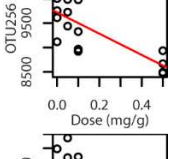
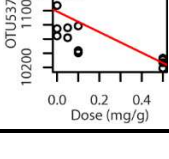
\*\*\*One PhyloChip outlier from the low nano-ZnO treatment in planted soils was not included in the statistical analyses.

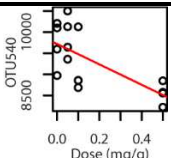
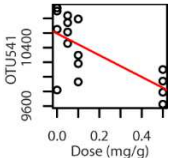
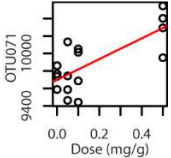
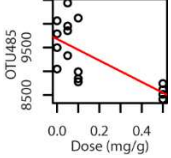
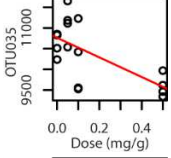
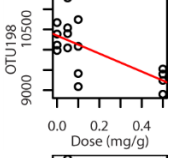
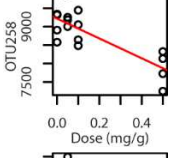
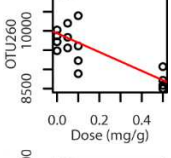
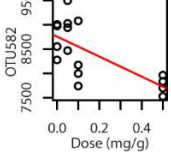
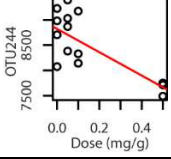
**Table S2.** The bacterial taxa (103 of 514 classified OTUs) identified as sensitive to nano-ZnO exposure through linear regression ( $Y = aX + b$ ). The hybridization score of bacterial OTU determined by PhyloChip was used as the dependent variable, and the exposure dose of nano-ZnO was the independent variable. The red lines represent unplanted treatments, and the blue lines represent planted treatments. Because the OTU is a lower level than the genus, and can be further grouped to higher taxonomic levels (kingdom; phylum; class; order; family; genus), the same genus may contain different OTUs, with some increasing and others decreasing, in the presence of nano-ZnO.

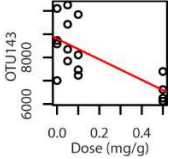
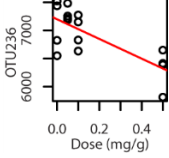
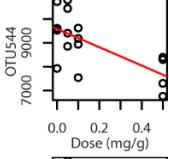
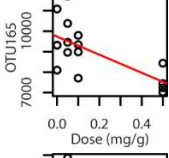
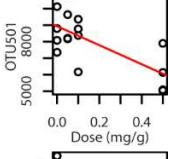
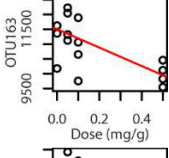
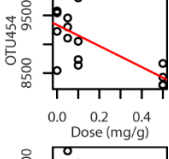
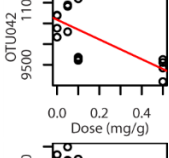
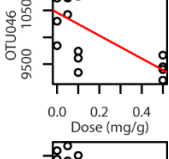
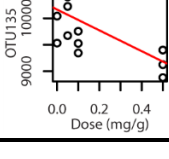
OTU	Plant	Linear regression: $Y = aX + b$				Graph	Taxonomic affiliation (kingdom; phylum; class; order; family; genus)
		a	b	$R^2$	Adjusted $P$		
otu364	No	1402	10883	0.53	0.024		<i>Bacteria;Acidobacteria;iii1-8;DS-18;unclassified;unclassified</i>
	With	1749	10546	0.76	0.003		
otu155	No	-7799	11923	0.48	0.035		<i>Bacteria;Cyanobacteria;Oscillatorio phycideae;Chroococcales;Synechococaceae;Synechococcus</i>
	With	-10147	11782	0.84	0.001		
otu295	No	-6180	10916	0.74	0.001		<i>Bacteria;Cyanobacteria;Oscillatorio phycideae;Chroococcales;Xenococcaeae;Chroococciopsis</i>
	With	-3816	10535	0.56	0.028		
otu027	No	-7976	11121	0.79	0.001		<i>Bacteria;Cyanobacteria;Oscillatorio phycideae;Chroococcales;Xenococcaeae;Xenococcus</i>
	With	-5990	11124	0.64	0.018		
otu018	No	-13516	11635	0.77	0.001		<i>Bacteria;Cyanobacteria;Oscillatorio phycideae;Oscillatoriales;Phormidia ceae;Phormidium</i>
	With	-14543	12256	0.92	0.000		
otu502	No	-5067	10002	0.50	0.029		<i>Bacteria;Cyanobacteria;Oscillatorio phycideae;Oscillatoriales;Phormidia ceae;Phormidium</i>
	With	-3519	9354	0.56	0.029		

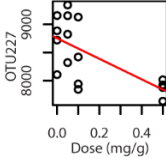
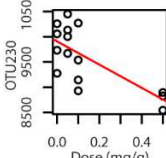
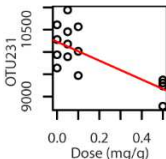
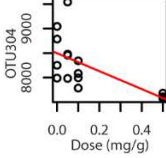
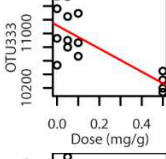
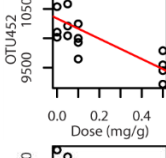
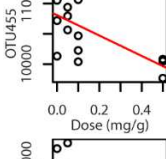
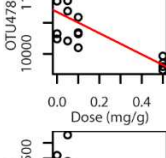
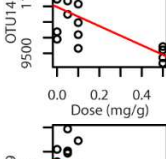
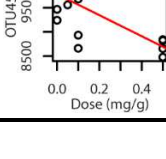
otu542	No	-5784	10656	0.75	0.001		<i>Bacteria;Cyanobacteria;Oscillatorio phycideae;Oscillatoriales;Phormidia ceae;unclassified</i>
	With	-4552	10538	0.57	0.027		
otu012	No	-1078	11799	0.55	0.021		<i>Bacteria;Gemmatimonadetes;Gemma timonadetes;Gemmatimonadales;Ge mmatimonadaceae;Gemmatimonas</i>
	With	-1814	11435	0.81	0.001		
otu433	No	-1009	11659	0.56	0.019		<i>Bacteria;Gemmatimonadetes;Gemma timonadetes;Gemmatimonadales;Ge mmatimonadaceae;unclassified</i>
	With	-1501	11472	0.73	0.005		
otu099	No	-3060	10847	0.69	0.004		<i>Bacteria;OP11;OP11- 1;GN01;unclassified;unclassified</i>
	With	-3245	11192	0.64	0.017		
otu403	No	-2017	11818	0.62	0.010		<i>Bacteria;Planctomycetes;agg27;CL5 00-15;unclassified;unclassified</i>
	With	-1927	11828	0.53	0.035		
otu475	No	-1556	10158	0.46	0.037		<i>Bacteria;Planctomycetes;Kueneniae; Kueneniales;Scalinduaceae;Candidat us_Scalindua</i>
	With	-2955	10400	0.89	0.000		
otu397	No	-1467	9316	0.53	0.024		<i>Bacteria;Planctomycetes;Planctomyc ea;Planctomycetales;Planctomycetac eae;A17</i>
	With	-1971	9209	0.73	0.005		
otu358	No	-1849	8947	0.51	0.028		<i>Bacteria;Planctomycetes;Planctomyc ea;Planctomycetales;Planctomycetac eae;unclassified</i>
	With	-1563	9328	0.58	0.025		
otu151	No	-3016	10666	0.57	0.018		<i>Bacteria;Planctomycetes;Planctomyc ea;unclassified;unclassified;unclassif ied</i>
	With	-3025	10675	0.72	0.006		
otu472	No	-1624	10534	0.55	0.019		<i>Bacteria;Proteobacteria;Alphaproteo bacteria;Rhodospirillales;Acetobacte raceae;unclassified</i>
	With	-2575	10594	0.82	0.001		

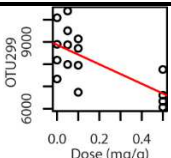
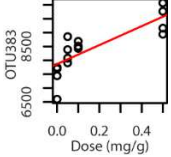
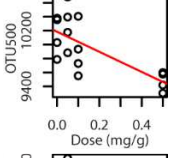
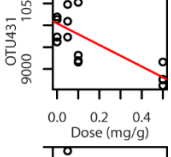
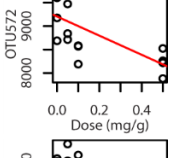
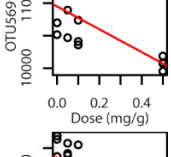
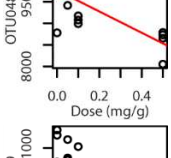
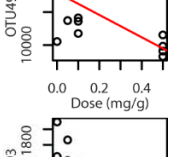
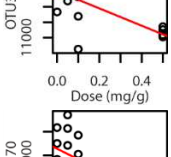
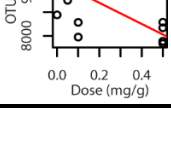


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	With	-4624	8246	0.85	0.000		
otu147	No	-1413	12179	0.50	0.029		<i>Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Azotobacter</i>
	With	-1643	12106	0.69	0.008		
otu550	No	-1816	9848	0.43	0.048		<i>Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Azotobacter</i>
	With	-1289	9924	0.48	0.049		
otu474	No	-1483	13130	0.82	0.000		<i>Bacteria;Synergistetes;Synergistia;Synergistales;Synergistaceae;vadinCA02</i>
	With	-1298	12825	0.79	0.002		
otu083	No	-2264	10162	0.58	0.018		<i>Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;unclassified</i>
	With	-2272	10552	0.78	0.002		
otu084	No	-1953	11840	0.73	0.002		<i>Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;unclassified</i>
	With	-2290	11975	0.88	0.000		
otu380	No	-2462	8401	0.56	0.019		<i>Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;unclassified</i>
	With	-2341	8688	0.79	0.002		
otu255	No	-1537	10374	0.53	0.023		<i>Bacteria;Acidobacteria;Acidobacteriia;Acidobacteriales;Acidobacteriaceae;unclassified</i>
	With	-2173	9718	0.56	0.019		
otu256	No	-2173	9718	0.56	0.019		<i>Bacteria;Acidobacteria;Acidobacteriia;Acidobacteriales;Acidobacteriaceae;unclassified</i>
	With	-1452	10989	0.46	0.037		
otu537	No	-1452	10989	0.46	0.037		<i>Bacteria;Acidobacteria;Acidobacteriia;Acidobacteriales;Acidobacteriaceae;unclassified</i>
	With	-1452	10989	0.46	0.037		

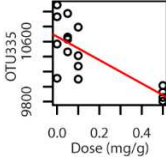
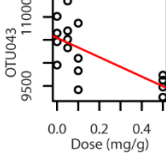
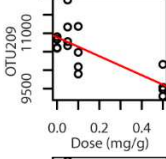
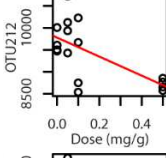
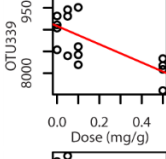
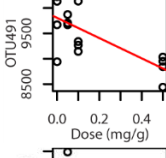
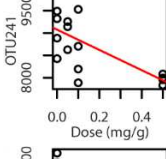
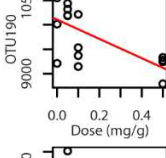
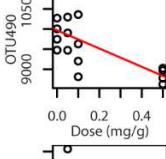
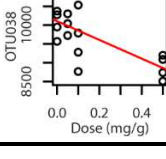
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otu541	No	-1488	10594	0.47	0.037		<i>Bacteria;Acidobacteria;Acidobacteri a;Acidobacteriales;Acidobacteriace e;unclassified</i>
otu071	No	1186	9699	0.54	0.021		<i>Bacteria;Acidobacteria;Solibacteres; Solibacterales;Solibacteraceae;Cand idatus_Solibacter</i>
otu485	No	-2258	9679	0.50	0.029		<i>Bacteria;Actinobacteria;Actinobacte ria;Actinomycetales;Micrococcaceae ;Arthrobacter</i>
otu035	No	-2367	10763	0.44	0.047		<i>Bacteria;Bacteroidetes;Bacteroidia; Bacteroidales;RikenellaceaeII;unclas sified</i>
otu198	No	-2217	10348	0.45	0.039		<i>Bacteria;Bacteroidetes;Bacteroidia; Bacteroidales;RikenellaceaeII;unclas sified</i>
otu258	No	-2688	9218	0.65	0.007		<i>Bacteria;Bacteroidetes;Bacteroidia; Bacteroidales;RikenellaceaeII;unclas sified</i>
otu260	No	-2444	9939	0.52	0.025		<i>Bacteria;Bacteroidetes;Bacteroidia; Bacteroidales;RikenellaceaeII;unclas sified</i>
otu582	No	-2060	8758	0.48	0.034		<i>Bacteria;Bacteroidetes;Bacteroidia; Bacteroidales;RikenellaceaeII;unclas sified</i>
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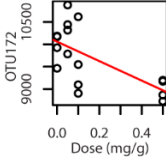
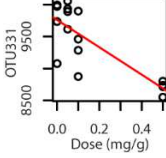
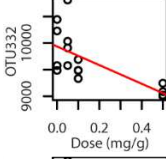
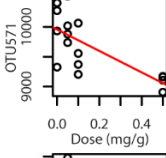
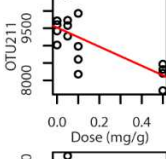
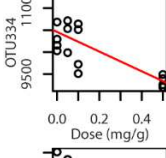
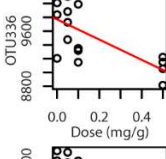
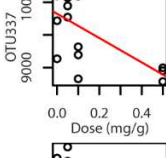
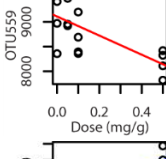
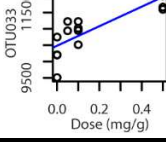
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otu544	No	-3850	9588	0.43	0.049		<i>Bacteria;Cyanobacteria;Chloroplast;Streptophyta;unclassified;unclassified</i>
otu165	No	-4414	9737	0.45	0.039		<i>Bacteria;Cyanobacteria;Oscillatorio phycideae;Chroococcales;Xenococcaeae;Chroococciopsis</i>
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otu163	No	-3113	11505	0.43	0.049		<i>Bacteria;Cyanobacteria;Synechococ phycideae;Pseudanabaenales;Pseudanabaenaceae;Leptolyngbya</i>
otu454	No	-1824	9335	0.48	0.035		<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;Clostridium</i>
otu042	No	-2386	10589	0.48	0.034		<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu046	No	-2138	10460	0.47	0.036		<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
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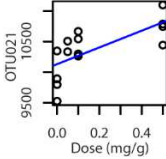
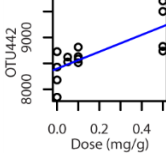
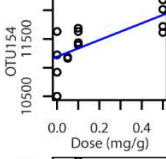
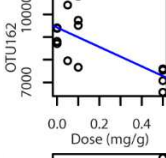
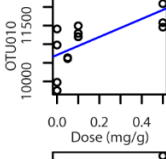
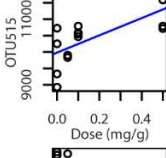
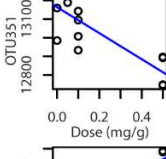
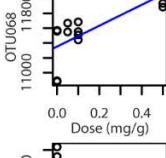
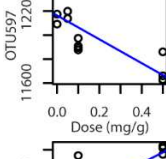
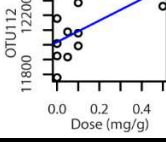
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otu231	No	-2112	10227	0.53	0.025		<i>Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; unclassified</i>
otu304	No	-1829	8497	0.44	0.043		<i>Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; unclassified</i>
otu333	No	-1693	11115	0.57	0.018		<i>Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; unclassified</i>
otu452	No	-1723	10337	0.57	0.018		<i>Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; unclassified</i>
otu455	No	-1682	10808	0.47	0.036		<i>Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; unclassified</i>
otu478	No	-1701	10673	0.53	0.023		<i>Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; unclassified</i>
otu141	No	-3044	10976	0.48	0.035		<i>Bacteria; Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; unclassified</i>
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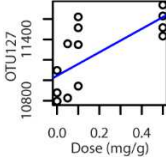
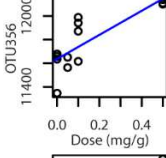
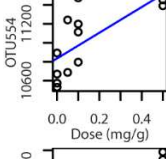
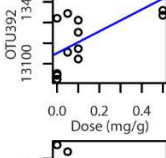
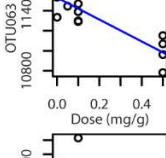
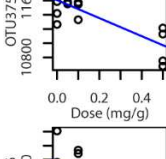
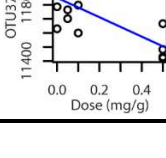
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otu500	No	-1440	10185	0.51	0.028		<i>Bacteria; Gemmatimonadetes; Gemmatimonadetes; Gemmatimonadales; Gemmatimonadaceae; unclassified</i>
otu431	No	-2465	10043	0.54	0.021		<i>Bacteria; Planctomycetes; Planctomycea; Planctomycetales; Planctomycetaceae; unclassified</i>
otu572	No	-2038	9204	0.44	0.045		<i>Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Rhizobiaceae; Agrobacterium</i>
otu569	No	-1756	10948	0.56	0.019		<i>Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Rhizobiaceae; Ensifer</i>
otu048	No	-2466	9767	0.55	0.019		<i>Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae; unclassified</i>
otu499	No	-1687	10794	0.50	0.029		<i>Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae; unclassified</i>
otu393	No	-1117	11602	0.46	0.037		<i>Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; Blastomonas</i>
otu570	No	-2226	9143	0.47	0.037		<i>Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; unclassified; unclassified</i>



otu335	No	-1557	10666	0.59	0.017		<i>Bacteria;Proteobacteria;Deltaproteo bacteria;Syntrophobacterales;Desulf obacteraceae;unclassified</i>
otu043	No	-2099	10545	0.48	0.035		<i>Bacteria;Proteobacteria;Deltaproteo bacteria;Syntrophobacterales;Syntro phobacteraceae;unclassified</i>
otu209	No	-2570	10892	0.56	0.019		<i>Bacteria;Proteobacteria;Deltaproteo bacteria;Syntrophobacterales;Syntro phobacteraceae;unclassified</i>
otu212	No	-2164	9779	0.43	0.049		<i>Bacteria;Proteobacteria;Deltaproteo bacteria;Syntrophobacterales;unclas sified;unclassified</i>
otu339	No	-2090	9083	0.47	0.037		<i>Bacteria;Proteobacteria;Deltaproteo bacteria;unclassified;unclassified;un classified</i>
otu491	No	-1997	9807	0.51	0.027		<i>Bacteria;Proteobacteria;Gammaprot eobacteria;Alteromonadales;unclassi fied;unclassified</i>
otu241	No	-2266	9075	0.46	0.038		<i>Bacteria;Proteobacteria;Gammaprot eobacteria;Chromatiales;Chromatiac eae;unclassified</i>
otu190	No	-1970	10106	0.44	0.043		<i>Bacteria;Proteobacteria;Gammaprot eobacteria;Enterobacterales;Entero bacteriaceae;unclassified</i>
otu490	No	-2269	9968	0.46	0.037		<i>Bacteria;Proteobacteria;Gammaprot eobacteria;Oceanospirillales;Halom onadaceae;unclassified</i>
otu038	No	-2487	10105	0.49	0.032		<i>Bacteria;Proteobacteria;Gammaprot eobacteria;Pseudomonadales;Pseudo monadaceae;Pseudomonas</i>

otu172	No	-2180	10060	0.43	0.049		<i>Bacteria;Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified</i>
otu331	No	-2142	9761	0.65	0.007		<i>Bacteria;Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified</i>
otu332	No	-1753	9936	0.48	0.035		<i>Bacteria;Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified</i>
otu571	No	-1797	9956	0.52	0.025		<i>Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;unclassified</i>
otu211	No	-2751	9521	0.54	0.022		<i>Bacteria;Proteobacteria;unclassified;unclassified;unclassified;unclassified</i>
otu334	No	-2238	10459	0.53	0.025		<i>Bacteria;Proteobacteria;unclassified;unclassified;unclassified;unclassified</i>
otu336	No	-1466	9769	0.48	0.034		<i>Bacteria;Proteobacteria;unclassified;unclassified;unclassified;unclassified</i>
otu337	No	-1862	9824	0.48	0.035		<i>Bacteria;Proteobacteria;unclassified;unclassified;unclassified;unclassified</i>
otu559	No	-1930	9109	0.48	0.035		<i>Bacteria;Proteobacteria;unclassified;unclassified;unclassified;unclassified</i>
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otu021	With	1367	10130	0.49	0.045		<i>Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Microbacteriaceae;Microbacterium</i>
otu442	With	1631	8397	0.56	0.029		<i>Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Nocardiaceae;Rhodococcus</i>
otu154	With	1458	11190	0.52	0.037		<i>Bacteria;Cyanobacteria;Oscillatoriothycidae;Chroococcales;unclassified;unclassified</i>
otu162	With	-4286	9422	0.51	0.038		<i>Bacteria;Cyanobacteria;Synechococophycidae;Pseudanabaenales;Pseudanabaenaceae;Leptolyngbya</i>
otu010	With	2416	10712	0.53	0.036		<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu515	With	2629	9981	0.52	0.037		<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu351	With	-697	13163	0.70	0.007		<i>Bacteria;Gemmatimonadetes;Gemmatimonadetes;Gemmatimonadales;Gemmatimonadaceae;unclassified</i>
otu068	With	1406	11352	0.62	0.018		<i>Bacteria;Planctomycetes;Planctomycea;Planctomycetales;Planctomycetaceae;Planctomyces</i>
otu597	With	-994	12163	0.70	0.007		<i>Bacteria;Planctomycetes;Planctomycea;Planctomycetales;Planctomycetaceae;unclassified</i>
otu112	With	726	12019	0.56	0.029		<i>Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Rhizobium</i>

otu127	With	1146	11045	0.50	0.040		<i>Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Sphingomonas</i>
otu356	With	1018	11637	0.72	0.006		<i>Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Sphingomonas</i>
otu554	With	1354	10835	0.60	0.021		<i>Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;unclassified</i>
otu392	With	529	13151	0.56	0.029		<i>Bacteria;Proteobacteria;Betaproteobacteria;Methylophilales;Methylophilaceae;Methylobacillus</i>
otu063	With	-1094	11531	0.76	0.003		<i>Bacteria;Proteobacteria;Betaproteobacteria;unclassified;unclassified;unclassified</i>
otu375	With	-1267	11607	0.57	0.027		<i>Bacteria;WS3;PRR-12;Sediment-1;PRR-10;unclassified</i>
otu376	With	-690	11851	0.52	0.037		<i>Bacteria;WS3;PRR-12;Sediment-1;PRR-10;unclassified</i>

**Table S3.** The bacterial OTUs that differed in the presence and absence of soybean plants. The hybridization scores of bacterial OTUs determined by PhyloChip were compared using Student's *t* test, coupled with Benjamini-Hochberg correction (adjusted  $P < 0.05$ ).

OTU	No plant		With plant		Adjusted <i>P</i>	Sensitive to ZnO	Taxonomic affiliation (kingdom; phylum; class; order; family; genus)
	Mean	S.D.	Mean	S.D.			
otu012	11739	291	11195	386	0.000	Yes	<i>Bacteria;Gemmatimonadetes;Gemmatimonadetes;Gemmatimonadales;Gemmatimonadaceae;Gemmatimonas</i>
otu474	13001	294	12664	258	0.000	Yes	<i>Bacteria;Synergistetes;Synergistia;Synergistales;Synergistaceae;vadinCA02</i>
otu364	11142	328	10729	369	0.000	Yes	<i>Bacteria;Acidobacteria;iii1-8;DS-18;unclassified;unclassified</i>
otu433	11598	262	11369	346	0.019	Yes	<i>Bacteria;Gemmatimonadetes;Gemmatimonadetes;Gemmatimonadales;Gemmatimonadaceae;unclassified</i>
otu462	8347	905	7730	868	0.030	Yes	<i>Bacteria;Proteobacteria;Deltaproteobacteria;Myxococcales;unclassified;unclassified</i>
otu358	8877	553	9208	432	0.039	Yes	<i>Bacteria;Planctomycetes;Planctomycea;Planctomycetales;Planctomycetaceae;unclassified</i>
otu331	9554	470	8741	276	0.000	Yes	<i>Bacteria;Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified</i>
otu500	10086	378	9455	245	0.000	Yes	<i>Bacteria;Gemmatimonadetes;Gemmatimonadetes;Gemmatimonadales;Gemmatimonadaceae;unclassified</i>
otu491	9707	523	8857	323	0.000	Yes	<i>Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;unclassified;unclassified</i>
otu244	8671	563	7804	313	0.000	Yes	<i>Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;unclassified;unclassified</i>
otu230	9729	545	8882	329	0.000	Yes	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu333	11052	433	10398	247	0.000	Yes	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu143	8160	1104	11098	2024	0.000	Yes	<i>Bacteria;Cyanobacteria;Chloroplast;Str</i>

								<i>eptophyta;unclassified;unclassified</i>
otu478	10586	438	9919	310	0.000	Yes		<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu332	9873	486	9169	305	0.000	Yes		<i>Bacteria;Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified</i>
otu256	9534	525	8797	319	0.000	Yes		<i>Bacteria;Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified</i>
otu452	10218	423	9586	319	0.000	Yes		<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu258	8952	562	8219	252	0.000	Yes		<i>Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;RikenellaceaeII;unclassified</i>
otu236	7026	420	9262	1899	0.000	Yes		<i>Bacteria;Cyanobacteria;Chloroplast;Streptophyta;unclassified;unclassified</i>
otu569	10858	436	10299	228	0.000	Yes		<i>Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Ensifer</i>
otu336	9710	406	9189	289	0.000	Yes		<i>Bacteria;Proteobacteria;unclassified;unclassified;unclassified;unclassified</i>
otu570	8973	567	8285	340	0.000	Yes		<i>Bacteria;Proteobacteria;Deltaproteobacteria;Myxococcales;unclassified;unclassified</i>
otu048	9610	614	8859	383	0.000	Yes		<i>Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;unclassified</i>
otu541	10536	412	10025	272	0.000	Yes		<i>Bacteria;Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified</i>
otu454	9228	490	8615	398	0.000	Yes		<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;Clostridium</i>
otu135	10054	523	9423	396	0.000	Yes		<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu571	9839	447	9338	311	0.000	Yes		<i>Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;unclassified</i>
otu260	9707	583	9029	455	0.000	Yes		<i>Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;RikenellaceaeII;unclassified</i>
otu339	8892	537	8277	416	0.000	Yes		<i>Bacteria;Proteobacteria;Deltaproteobacteria;unclassified;unclassified;unclassified</i>



otu537	10925	406	10509	210	0.000	Yes	<i>Bacteria;Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified</i>
otu335	10585	384	10176	253	0.000	Yes	<i>Bacteria;Proteobacteria;Deltaproteobacteria;Syntrophobacteriales;Desulfobacteraceae;unclassified</i>
otu190	9971	527	9421	359	0.000	Yes	<i>Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;unclassified</i>
otu540	9563	633	8904	452	0.000	Yes	<i>Bacteria;Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified</i>
otu231	10086	531	9569	299	0.000	Yes	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu299	8271	1101	7148	767	0.000	Yes	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;unclassified;unclassified</i>
otu046	10273	553	9732	377	0.000	Yes	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu255	10343	429	9948	251	0.001	Yes	<i>Bacteria;Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified</i>
otu485	9532	587	8995	369	0.001	Yes	<i>Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Micrococcaceae;Arthroobacter</i>
otu582	8593	537	7883	768	0.001	Yes	<i>Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;RikenellaceaeII;unclassified</i>
otu304	8423	527	7694	818	0.001	Yes	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu165	9180	1123	8247	708	0.002	Yes	<i>Bacteria;Cyanobacteria;Oscillatoriophyceae;Chroococcales;Xenococcaceae;Chroococcidiopsis</i>
otu544	9111	983	8320	702	0.004	Yes	<i>Bacteria;Cyanobacteria;Chloroplast;Streptophyta;unclassified;unclassified</i>
otu559	8923	492	8181	1043	0.004	Yes	<i>Bacteria;Proteobacteria;unclassified;unclassified;unclassified;unclassified</i>
otu241	8867	595	8402	391	0.004	Yes	<i>Bacteria;Proteobacteria;Gammaproteobacteria;Chromatiales;Chromatiaceae;unclassified</i>
otu337	9654	478	9293	281	0.004	Yes	<i>Bacteria;Proteobacteria;unclassified;unclassified;unclassified;unclassified</i>
otu334	10224	530	9850	272	0.006	Yes	<i>Bacteria;Proteobacteria;unclassified;unclassified</i>

								<i>classified;unclassified;unclassified</i>
otu393	11474	318	11717	230	0.006	Yes		<i>Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Blastomonas</i>
otu499	10741	467	10408	281	0.008	Yes		<i>Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;unclassified</i>
otu431	9810	575	9393	384	0.008	Yes		<i>Bacteria;Planctomycetes;Planctomycea;Planctomycetales;Planctomycetaceae;unclassified</i>
otu038	9853	613	9135	1065	0.009	Yes		<i>Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas</i>
otu172	9849	586	9422	450	0.011	Yes		<i>Bacteria;Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified</i>
otu227	8628	502	8060	916	0.016	Yes		<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu211	9177	662	8760	408	0.019	Yes		<i>Bacteria;Proteobacteria;unclassified;unclassified;unclassified</i>
otu572	9062	558	8710	345	0.019	Yes		<i>Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Agrobacterium</i>
otu459	9557	541	9226	343	0.024	Yes		<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Ruminococcaceae;unclassified</i>
otu035	10507	616	10125	447	0.028	Yes		<i>Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;RikenellaceaeII;unclassified</i>
otu209	10598	585	10237	442	0.031	Yes		<i>Bacteria;Proteobacteria;Deltaproteobacteria;Syntrophobacterales;Syntrophobacteraceae;unclassified</i>
otu212	9539	570	9198	412	0.034	Yes		<i>Bacteria;Proteobacteria;Deltaproteobacteria;Syntrophobacterales;unclassified;unclassified</i>
otu043	10339	523	10043	361	0.042	Yes		<i>Bacteria;Proteobacteria;Deltaproteobacteria;Syntrophobacterales;Syntrophobacteraceae;unclassified</i>
otu112	11569	240	12109	257	0.000	Yes		<i>Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Rhizobium</i>
otu597	12501	227	12077	234	0.000	Yes		<i>Bacteria;Planctomycetes;Planctomycea;Planctomycetales;Planctomycetaceae;unclassified</i>

otu392	12912	203	13249	173	0.000	Yes	<i>Bacteria;Proteobacteria;Betaproteobact eria;Methylophilales;Methylophilaceae; Methylobacillus</i>
otu356	11266	271	11721	255	0.000	Yes	<i>Bacteria;Proteobacteria;Alphaproteoba cteria;Sphingomonadales;Sphingomona daceae;Sphingomonas</i>
otu351	13293	118	13063	162	0.000	Yes	<i>Bacteria;Gemmatimonadetes;Gemmatim onadetes;Gemmatimonadales;Gemmati monadaceae;unclassified</i>
otu376	11991	233	11780	222	0.004	Yes	<i>Bacteria;WS3;PRR-12;Sediment-1;PRR- 10;unclassified</i>
otu375	11654	232	11396	325	0.004	Yes	<i>Bacteria;WS3;PRR-12;Sediment-1;PRR- 10;unclassified</i>
otu021	9851	438	10223	385	0.005	Yes	<i>Bacteria;Actinobacteria;Actinobacteria; Actinomycetales;Microbacteriaceae;Mic robacterium</i>
otu033	10472	394	10847	646	0.028	Yes	<i>Bacteria;Acidobacteria;Acidobacteria;A cidobacteriales;Acidobacteriaceae;uncl assified</i>
otu149	10843	283	12839	557	0.000	No	<i>Bacteria;Proteobacteria;Betaproteobact eria;Burkholderiales;Burkholderiaceae; Burkholderia</i>
otu102	3996	443	9067	1735	0.000	No	<i>Bacteria;Bacteroidetes;Sphingobacteria ;Sphingobacteriales;Flexibacteraceae;S pirosoma</i>
otu017	4189	451	9372	1808	0.000	No	<i>Bacteria;Bacteroidetes;Sphingobacteria ;Sphingobacteriales;Flexibacteraceae;E mticicia</i>
otu504	9762	350	11394	464	0.000	No	<i>Bacteria;TM7;TM7- 3;EW055;unclassified;unclassified</i>
otu496	10261	259	11379	335	0.000	No	<i>Bacteria;Proteobacteria;Alphaproteoba cteria;unclassified;unclassified;unclassif ied</i>
otu074	7020	690	11098	1406	0.000	No	<i>Bacteria;TM7;TM7- 3;EW055;unclassified;unclassified</i>
otu116	10756	324	12341	529	0.000	No	<i>Bacteria;Actinobacteria;Actinobacteria; Actinomycetales;Micrococcaceae;unclas sified</i>
otu156	10296	429	11922	476	0.000	No	<i>Bacteria;TM7;TM7- 3;EW055;unclassified;unclassified</i>
otu245	7740	394	10174	930	0.000	No	<i>Bacteria;TM7;TM7- 3;EW055;unclassified;unclassified</i>

otu052	12485	207	11780	208	0.000	No	<i>Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;RikenellaceaeII;unclassified</i>
otu013	4990	457	10134	2191	0.000	No	<i>Bacteria;Cyanobacteria;Chloroplast;Streptophyta;unclassified;unclassified</i>
otu005	7873	453	10559	1104	0.000	No	<i>Bacteria;TM7;TM7-3;I025;A29;unclassified</i>
otu169	8188	593	10339	818	0.000	No	<i>Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;unclassified</i>
otu108	3939	919	8114	1771	0.000	No	<i>Bacteria;Proteobacteria;Betaproteobacteria;Methylophilales;Methylophilaceae;Methylophilus</i>
otu443	5199	572	8503	1509	0.000	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu058	8485	320	10138	806	0.000	No	<i>Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bacteriovoracaceae;unclassified</i>
otu024	8320	405	9725	627	0.000	No	<i>Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Prostheco bacter</i>
otu081	12254	315	11574	215	0.000	No	<i>Bacteria;Gemmatimonadetes;Gemmatimonadetes;unclassified;unclassified;unclassified</i>
otu552	9616	326	10419	322	0.000	No	<i>Bacteria;Proteobacteria;Betaproteobacteria;Methylophilales;Methylophilaceae;Methylovorus</i>
otu434	10087	286	10780	284	0.000	No	<i>Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;unclassified</i>
otu107	8842	293	10155	722	0.000	No	<i>Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bacteriovoracaceae;unclassified</i>
otu360	6745	586	8477	839	0.000	No	<i>Bacteria;Planctomycetes;Planctomycea;Planctomycetales;Planctomycetaceae;Planctomyces</i>
otu503	10042	467	11187	541	0.000	No	<i>Bacteria;TM7;TM7-3;EW055;unclassified;unclassified</i>
otu374	10251	302	9518	344	0.000	No	<i>Bacteria;Gemmatimonadetes;Gemmatimonadetes;unclassified;unclassified;unclassified</i>
otu080	12515	199	12075	199	0.000	No	<i>Bacteria;Gemmatimonadetes;Gemmatimonadetes;unclassified;unclassified;unclassified</i>

								<i>ssified</i>
otu109	8356	895	10246	808	0.000	No		<i>Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Flexibacteraceae;Dyadobacter</i>
otu378	10754	234	11387	343	0.000	No		<i>Bacteria;TM7;TM7-1;unclassified;unclassified;unclassified</i>
otu087	11953	265	11316	323	0.000	No		<i>Bacteria;Gemmatimonadetes;Gemmatimonadetes;Gemmatimonadales;Gemmatimonadaceae;Gemmatimonas</i>
otu070	11004	244	10485	244	0.000	No		<i>Bacteria;Acidobacteria;Solibacteres;Solibacterales;Solibacteraceae;Candidatus_Solibacter</i>
otu114	8088	711	9614	743	0.000	No		<i>Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Shinella</i>
otu440	10151	296	10805	326	0.000	No		<i>Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Microbacteriaceae;Microbacterium</i>
otu562	10854	226	10379	240	0.000	No		<i>Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;unclassified</i>
otu399	11454	173	11820	186	0.000	No		<i>Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Microbacteriaceae;Agrococcus</i>
otu437	9946	313	10551	309	0.000	No		<i>Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter</i>
otu069	8457	384	9498	672	0.000	No		<i>Bacteria;Planctomycetes;Planctomycea;Planctomycetales;Planctomycetaceae;Planctomyces</i>
otu119	10649	322	11415	469	0.000	No		<i>Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Methylubiaceae;Methylubium</i>
otu014	7761	493	10430	1944	0.000	No		<i>Bacteria;Cyanobacteria;Chloroplast;Streptophyta;unclassified;unclassified</i>
otu050	9724	452	9002	273	0.000	No		<i>Bacteria;Proteobacteria;Deltaproteobacteria;unclassified;unclassified;unclassified</i>
otu427	8236	360	9139	591	0.000	No		<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;Clostridium</i>
otu097	9777	375	10727	628	0.000	No		<i>Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Sphingobacteriaceae</i>

								<i>e;Pedobacter</i>
otu061	8973	406	10149	822	0.000	No	<i>Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Flexibacteraceae;unclassified</i>	
otu589	7858	540	9467	1137	0.000	No	<i>Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Flexibacteraceae;unclassified</i>	
otu253	10184	215	10516	139	0.000	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>	
otu020	11420	520	12256	395	0.000	No	<i>Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Pseudoxanthomonas</i>	
otu309	12391	178	12080	184	0.000	No	<i>Bacteria;Proteobacteria;Alphaproteobacteria;unclassified;unclassified;unclassified</i>	
otu098	6430	1032	8584	1460	0.000	No	<i>Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Sphingobium</i>	
otu264	10351	223	10712	200	0.000	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>	
otu416	11876	224	11527	185	0.000	No	<i>Bacteria;Chloroflexi;Dehalococcoidetes;TK17;S085;unclassified</i>	
otu471	10526	283	11099	402	0.000	No	<i>Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales;Acetobacteraceae;Roseomonas</i>	
otu049	9590	522	8910	285	0.000	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>	
otu419	8602	507	9525	654	0.000	No	<i>Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas</i>	
otu022	9250	753	10478	805	0.000	No	<i>Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Microbacteriaceae;Microbacterium</i>	
otu398	11401	344	12044	472	0.000	No	<i>Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Microbacteriaceae;Agrococcus</i>	
otu174	8824	381	9515	504	0.000	No	<i>Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;unclassified;unclassified</i>	
otu152	8996	578	10065	794	0.000	No	<i>Bacteria;Proteobacteria;Alphaproteobacteria;Kiloniellales;Kiloniellaceae;unclassified</i>	



otu051	10276	514	9619	302	0.000	No	<i>Bacteria;Proteobacteria;unclassified;unclassified;unclassified;unclassified</i>
otu484	9887	490	9273	278	0.000	No	<i>Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;unclassified;unclassified</i>
otu234	9752	252	10109	217	0.000	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu153	10349	387	11017	496	0.000	No	<i>Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Kaistia</i>
otu411	8253	400	8829	361	0.000	No	<i>Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Sinobacteraceae;Nevskia</i>
otu453	9338	584	8608	344	0.000	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu120	11117	595	10244	563	0.000	No	<i>Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Lysobacter</i>
otu381	11596	260	11206	263	0.000	No	<i>Bacteria;Acidobacteria;PAUC37f;unclassified;unclassified;unclassified</i>
otu175	10070	522	9429	308	0.000	No	<i>Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;RikenellaceaeII;unclassified</i>
otu267	13518	159	13741	148	0.000	No	<i>Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas</i>
otu408	8046	537	8813	537	0.000	No	<i>Bacteria;Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Terriglobus</i>
otu268	10681	421	11216	321	0.000	No	<i>Bacteria;Proteobacteria;unclassified;unclassified;unclassified;unclassified</i>
otu466	8093	449	8843	601	0.000	No	<i>Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;RikenellaceaeII;unclassified</i>
otu497	10761	321	11232	346	0.000	No	<i>Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Paracoccus</i>
otu519	10675	351	10246	249	0.000	No	<i>Bacteria;Firmicutes;unclassified;unclassified;unclassified;unclassified</i>
otu145	9454	583	10306	646	0.000	No	<i>Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas</i>
otu235	8219	418	8894	556	0.000	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>

otu379	7208	820	8493	1042	0.000	No	<i>Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;unclassified</i>
otu412	9958	291	9559	306	0.000	No	<i>Bacteria;CD12;MB-B2-116;unclassified;unclassified;unclassified</i>
otu365	8570	452	9451	829	0.000	No	<i>Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;unclassified;unclassified</i>
otu082	9090	438	9764	588	0.000	No	<i>Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;unclassified</i>
otu413	9221	303	9752	504	0.000	No	<i>Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Micrococcaceae;unclassified</i>
otu568	9587	497	9060	286	0.000	No	<i>Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;unclassified;unclassified</i>
otu355	11412	270	10959	424	0.000	No	<i>Bacteria;Proteobacteria;Deltaproteobacteria;Myxococcales;Polyangiaceae;unclassified</i>
otu096	12262	243	11901	318	0.000	No	<i>Bacteria;SM2F11;unclassified;unclassified;unclassified;unclassified;unclassified</i>
otu377	11644	221	11367	212	0.000	No	<i>Bacteria;Planctomycetes;Planctomycea;Gemmatales;Isosphaeraceae;unclassified</i>
otu409	10410	350	10819	286	0.000	No	<i>Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Rhizobium</i>
otu498	11457	276	11149	199	0.000	No	<i>Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;unclassified;unclassified</i>
otu160	10246	493	9686	376	0.000	No	<i>Bacteria;Firmicutes;Bacilli;Bacillales;unclassified;unclassified</i>
otu085	10881	327	10491	291	0.000	No	<i>Bacteria;Acidobacteria;Chloracidobacteria;unclassified;unclassified;unclassified</i>
otu451	9647	619	9014	338	0.000	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Ruminococcaceae;unclassified</i>
otu495	12670	152	12426	232	0.000	No	<i>Bacteria;Proteobacteria;Betaproteobacteria;unclassified;unclassified;unclassified</i>

otu348	10384	307	10812	375	0.000	No	<i>Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Flexibacteraceae;Cytophaga</i>
otu341	11320	418	10886	259	0.000	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu436	9919	522	9377	340	0.000	No	<i>Bacteria;Chloroflexi;Anaerolineae;Anaerolineales;Anaerolinaceae;Anaerolinea</i>
otu263	8239	445	8879	597	0.000	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu362	11034	214	11335	277	0.000	No	<i>Bacteria;Planctomycetes;Planctomycea;Planctomycetales;Planctomycetaceae;Rhodopirellula</i>
otu420	11923	329	11587	210	0.000	No	<i>Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Streptomycetaceae;Streptomyces</i>
otu025	8946	450	9561	575	0.000	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu370	12438	414	12026	248	0.000	No	<i>Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;unclassified;unclassified</i>
otu414	10738	297	11125	351	0.000	No	<i>Bacteria;Proteobacteria;Gammaproteobacteria;Methylococcales;Methylococcaceae;unclassified</i>
otu599	9098	539	8535	393	0.000	No	<i>Bacteria;Tenericutes;Mollicutes;Mycoplasmatales;Mycoplasmataceae;Mycoplasma</i>
otu259	9068	552	8488	409	0.000	No	<i>Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;RikenellaceaeII;unclassified</i>
otu588	9500	352	9956	418	0.000	No	<i>Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Saprospiraceae;unclassified</i>
otu567	10637	503	10142	318	0.000	No	<i>Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;unclassified;unclassified</i>
otu430	10628	664	9944	508	0.000	No	<i>Bacteria;Cyanobacteria;Synechococcophycideae;Pseudanabaenales;Pseudanabaenaceae;unclassified</i>
otu330	11199	366	10853	207	0.000	No	<i>Bacteria;Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified</i>
otu553	10314	312	10660	290	0.000	No	<i>Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonad</i>

								<i>aceae;Pseudomonas</i>
otu359	10729	215	10466	246	0.000	No		<i>Bacteria;Planctomycetes;Planctomycea;Planctomycetales;Planctomycetaceae;unclassified</i>
otu385	9317	391	9767	399	0.000	No		<i>Bacteria;Chloroflexi;Anaerolineae;Anaerolineales;Anaerolinaceae;A4b</i>
otu122	10126	336	10561	428	0.000	No		<i>Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas</i>
otu523	10596	509	10033	489	0.000	No		<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu016	7482	973	8886	1504	0.001	No		<i>Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Cellulomonadaceae;Actinotalea</i>
otu089	9806	447	9251	547	0.001	No		<i>Bacteria;Planctomycetes;vadinHA49;unclassified;unclassified;unclassified</i>
otu357	7618	495	8277	678	0.001	No		<i>Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Oxalobacteraceae;unclassified</i>
otu396	11211	279	10925	238	0.001	No		<i>Bacteria;Firmicutes;Bacilli;Bacillales;Paenibacillaceae;Paenibacillus</i>
otu546	7812	1236	6652	816	0.001	No		<i>Bacteria;Cyanobacteria;Synechococcophycideae;Pseudanabaenales;Pseudanabaenaceae;unclassified</i>
otu232	9526	492	9067	323	0.001	No		<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu514	10185	195	9946	243	0.001	No		<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu581	8763	434	9320	587	0.001	No		<i>Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas</i>
otu126	7078	711	7764	538	0.001	No		<i>Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Flavobacterium</i>
otu039	11600	476	11163	314	0.001	No		<i>Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;unclassified</i>
otu524	9050	640	8485	370	0.001	No		<i>Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus</i>
otu418	9699	347	10130	475	0.001	No		<i>Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonad</i>

								<i>aceae;Pseudomonas</i>
otu585	9882	565	9392	340	0.001	No		<i>Bacteria;Bacteroidetes;unclassified;unclassified;unclassified;unclassified;unclassified</i>
otu065	10786	701	11386	402	0.001	No		<i>Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Shinella</i>
otu011	9303	431	9822	573	0.001	No		<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu548	9135	884	9814	265	0.001	No		<i>Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Rhizobium</i>
otu405	11857	383	12201	296	0.002	No		<i>Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Sphingomonas</i>
otu387	5725	740	6693	1187	0.002	No		<i>Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Stenotrophomonas</i>
otu242	9936	494	9523	336	0.002	No		<i>Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas</i>
otu207	11461	363	11160	247	0.003	No		<i>Bacteria;Proteobacteria;unclassified;unclassified;unclassified;unclassified</i>
otu073	10390	238	10126	313	0.003	No		<i>Bacteria;Acidobacteria;Solibacteres;Solibacterales;Solibacteraceae;Candidatus_Solibacter</i>
otu525	9316	518	8901	338	0.003	No		<i>Bacteria;Firmicutes;unclassified;unclassified;unclassified;unclassified</i>
otu208	9741	417	9394	316	0.004	No		<i>Bacteria;Proteobacteria;Deltaproteobacteria;Myxococcales;unclassified;unclassified</i>
otu343	9978	555	9529	394	0.004	No		<i>Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;Acinetobacter</i>
otu369	10143	512	10622	525	0.004	No		<i>Bacteria;Planctomycetes;Phycisphaerae;Phycisphaerales;unclassified;unclassified</i>
otu095	10266	464	10655	377	0.004	No		<i>Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas</i>
otu395	11424	217	11154	361	0.004	No		<i>Bacteria;SM2F11;unclassified;unclassified;unclassified;unclassified</i>

otu101	10383	405	10010	414	0.004	No	<i>Bacteria;Thermi;Deinococci;Deinococcales;Deinococcaceae;Deinococcus</i>
otu469	9130	597	8654	434	0.005	No	<i>Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;unclassified;unclassified</i>
otu223	9524	534	9093	416	0.005	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu064	10060	254	9750	427	0.005	No	<i>Bacteria;Proteobacteria;Deltaproteobacteria;Myxococcales;Polyangiaceae;unclassified</i>
otu367	11493	335	11227	261	0.006	No	<i>Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Chitinophagaceae;unclassified</i>
otu302	9002	510	8483	674	0.006	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu328	11027	480	10655	373	0.007	No	<i>Bacteria;Proteobacteria;unclassified;unclassified;unclassified;unclassified</i>
otu564	7969	570	7454	657	0.009	No	<i>Bacteria;Proteobacteria;unclassified;unclassified;unclassified;unclassified</i>
otu129	9606	861	10240	632	0.009	No	<i>Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Stenotrophomonas</i>
otu092	9541	612	10017	540	0.010	No	<i>Bacteria;Proteobacteria;Gammaproteobacteria;Legionellales;Legionellaceae;Legionella</i>
otu347	10138	531	10514	360	0.010	No	<i>Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Flexibacteraceae;Cytophaga</i>
otu566	11129	433	10842	243	0.012	No	<i>Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;unclassified</i>
otu535	10588	433	10287	303	0.013	No	<i>Bacteria;Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified</i>
otu482	9392	403	8837	917	0.014	No	<i>Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Cellulomonadaceae;unclassified</i>
otu040	9657	601	9220	495	0.014	No	<i>Bacteria;Proteobacteria;Alphaproteobacteria;unclassified;unclassified;unclassified</i>
otu400	10399	541	10786	434	0.014	No	<i>Bacteria;Proteobacteria;Alphaproteobacteria;Caulobacterales;Caulobacteraceae</i>

								<i>ae;Mycoplana</i>
otu305	8822	492	8289	831	0.014	No	<i>Bacteria;Proteobacteria;Deltaproteobacteria;Syntrophobacterales;unclassified;unclassified</i>	
otu340	10715	378	10461	258	0.016	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>	
otu517	8640	478	8069	934	0.016	No	<i>Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus</i>	
otu316	10014	412	9716	362	0.017	No	<i>Bacteria;Proteobacteria;Deltaproteobacteria;unclassified;unclassified;unclassified</i>	
otu584	9128	655	8695	452	0.017	No	<i>Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Flexibacteraceae;unclassified</i>	
otu479	8944	573	8553	446	0.018	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>	
otu539	10100	661	9670	444	0.018	No	<i>Bacteria;Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified</i>	
otu363	9433	261	9199	355	0.019	No	<i>Bacteria;Acidobacteria;Solibacteres;Solibacterales;Solibacteraceae;Candidatus_Solibacter</i>	
otu090	8985	489	8599	551	0.021	No	<i>Bacteria;Planctomycetes;vadinHA49;unclassified;unclassified;unclassified</i>	
otu300	10903	191	10457	843	0.021	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>	
otu134	9562	567	9022	888	0.023	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>	
otu461	11062	262	11231	193	0.024	No	<i>Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Nocardioidaceae;Nocardioides</i>	
otu173	9615	635	9224	408	0.024	No	<i>Bacteria;Proteobacteria;unclassified;unclassified;unclassified</i>	
otu161	9874	604	9492	426	0.024	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>	
otu481	11444	333	11234	234	0.024	No	<i>Bacteria;Firmicutes;Bacilli;Bacillales;unclassified;unclassified</i>	
otu505	9340	898	9825	260	0.024	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>	
otu486	9955	555	9604	397	0.024	No	<i>Bacteria;Proteobacteria;Deltaproteobacteria;Syntrophobacterales;Desulfobacte</i>	



								<i>raceae;unclassified</i>
otu094	6958	1063	7905	1550	0.025	No		<i>Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Stenotrophomonas</i>
otu344	10740	280	10979	383	0.025	No		<i>Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Flexibacteraceae;unclassified</i>
otu137	9994	415	10345	569	0.027	No		<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu176	10506	262	10031	926	0.027	No		<i>Bacteria;Planctomycetes;Planctomycea;unclassified;unclassified;unclassified</i>
otu406	8277	1035	9080	1252	0.029	No		<i>Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Cryomorphaceae;Fluvii cola</i>
otu424	11203	261	10987	355	0.029	No		<i>Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Flavobacterium</i>
otu438	10409	543	10083	377	0.031	No		<i>Bacteria;Tenericutes;Erysipelotrichi;Erysipelotrichales;Erysipelotrichaceae;All obaculum</i>
otu317	9287	573	8957	365	0.034	No		<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu534	10151	621	9779	462	0.035	No		<i>Bacteria;Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified</i>
otu596	9692	508	9385	391	0.036	No		<i>Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;unclassified</i>
otu031	7031	867	6398	1028	0.038	No		<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu148	10933	923	10356	802	0.038	No		<i>Bacteria;Cyanobacteria;Synechococcophycideae;Pseudanabaenales;Pseudanabaenaceae;Leptolyngbya</i>
otu530	8699	392	8226	943	0.038	No		<i>Bacteria;Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified</i>
otu125	9630	360	9930	540	0.040	No		<i>Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Ulvi bacter</i>
otu261	10704	609	10356	429	0.041	No		<i>Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;RikenellaceaeII;unclassified</i>

otu426	8601	572	8282	374	0.041	No	<i>Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Cupriavidus</i>
otu140	10708	347	10414	541	0.042	No	<i>Bacteria;Actinobacteria;Actinobacteria;Acidimicrobiales;AKIW874;unclassified</i>
otu117	8713	833	9250	814	0.042	No	<i>Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Stenotrophomonas</i>
otu123	8981	789	9482	747	0.042	No	<i>Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;Pantoea</i>
otu342	10424	467	10166	304	0.042	No	<i>Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Pseudonocardia;Pseudonocardia</i>
otu407	13006	208	12853	264	0.042	No	<i>Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Streptosporangiaceae;Acrocarpospora</i>
otu476	13101	300	13305	329	0.043	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;unclassified;unclassified</i>
otu115	9774	762	10275	790	0.043	No	<i>Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Xanthomonas</i>
otu457	10660	532	10371	337	0.044	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>
otu015	9818	758	9239	1054	0.048	No	<i>Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Sphingomonas</i>
otu279	6978	771	6385	1086	0.048	No	<i>Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;unclassified</i>

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