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Compositional and functional responses of bacterial community to titanium dioxide nanoparticles varied with soil heterogeneity and exposure duration

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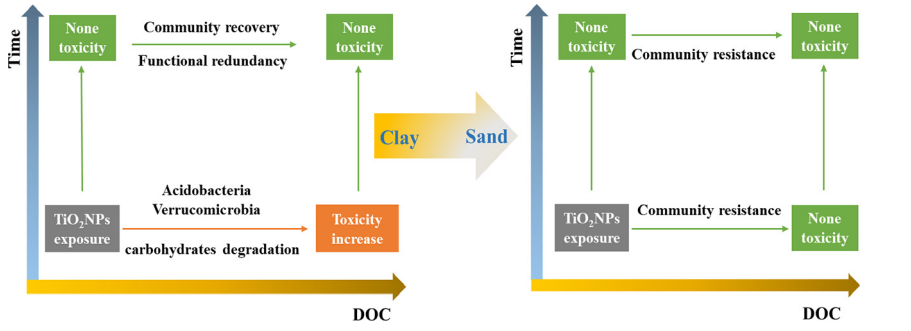
HIGHLIGHTS

- Impacts of TiO_2NPs on bacterial community varied with soil heterogeneity over time.
- No significant TiO_2NPs impact was observed in clay-LOM and sandy soils.
- TiO_2NPs significantly affected community composition and function in clay-HOM soil.
- TiO_2NPs suppressed Acidobacteria and Verrucomicrobia, and carbohydrates degradation.
- The influence of soil heterogeneity on the effects of TiO_2NPs appeared transiently.

GRAPHICAL ABSTRACT



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ABSTRACT

Titanium dioxide nanoparticles (TiO_2 NPs) are widely used as nano-agrochemicals. In this study we investigated the influence of soil heterogeneity on bacterial communities exposed to TiO_2 NPs over time. Clay and sandy soils with low- and high-organic matter contents were exposed to environmentally relevant concentration of TiO_2 NPs (1 mg/kg) and soil bacterial communities were sampled after short-term (15 days) and long-term exposure (60 days). After short-term TiO_2 NPs exposure, significant effects regarding the enzyme activity, bacterial community structure and composition, and community functioning were observed in the clay soils with high organic matter (clay-HOM) but not in other soil groups. Response alterations were observed to taxa belonging to Acidobacteria and Verrucomicrobia, and functional pathways related to carbohydrates degradation. These results indicated that soil heterogeneity play more important roles in shaping the bacterial community in soil with low clay fraction and less organic matter, while TiO_2 NPs selection was the main driver in inducing the compositional and functional impacts on the soil bacterial community in the presence of clay soil with high organic matter

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Enzyme activity
Soil bacterial community

content. As exposure time increased, the bacterial community recovered after a long-term exposure of 60 days, suggesting that the bacterial evolution and adaptation could overcome the TiO_2 NPs selection after long-term exposure. Our results highlighted the importance of soil heterogeneity including clay fraction and organic matter and exposure duration in assessing the impact of nanoparticle on soil bacterial activity, community and function. By comprehensively evaluating the risks of nanoparticles on soil ecosystem and explicitly and explicitly include spatial and temporal variations, the benefit of nano-agrochemical products has the potential to be promoted in future applications.

1. Introduction

Titanium dioxide nanoparticles (TiO_2 NPs) are widely used in food additives, agricultural plant protection products, personal care and cosmetics, photocatalysts and UV protectors etc. (Tan et al., 2018). Throughout their life-cycle that ranges from production, transportation, application to disposal, TiO_2 NPs are inevitably released into the environment (Moll et al., 2017). It is estimated that approximately 760 tons of TiO_2 NPs per year are released into soils by application of sewage sludge (Gottschalk et al., 2009; Zhang et al., 2015). A recent study also illustrated that TiO_2 NPs could also be directly applied to soils through the use of agricultural products (Abdel Latef et al., 2018). With the increasing demands from the global market and their inevitable release into the environment, more research is needed to understand the fate and ecological risk assessment for TiO_2 NPs (Li et al., 2016a, 2016b; Rana et al., 2020).

The fate of TiO_2 NPs in soil could be affected by soil properties, like e.g. texture, mineral and organic particles, pH, and ionic strength (Simonin and Richaume, 2015). Metal oxide nanoparticles have been found in small soil aggregates rich in labile organic carbon, microbial biomass and clay (Tourinho et al., 2012). This indicates that these nanoparticles could interact with the clay fraction, organic matter components and microbes present (Antisari et al., 2013; Abbas et al., 2020). Clay components have been reported to facilitate the TiO_2 NPs transport (Cai et al., 2014). Moreover, studies have shown that high dissolved organic matter and clay contents promoted TiO_2 NPs dispersion in soil suspension, while high ionic strength, zeta potential, and pH inhibited TiO_2 NPs stability (Fang et al., 2009). Therefore, organic materials such as organic waste and residues, manures, humic acid, have been added in the soil products containing metallic nanoparticles as a sustainable and remedial approach for controlling the mobility, bioavailability and toxicity of TiO_2 NPs (Zhao et al., 2020). As organic matter could potentially influence the mobility and bioavailability of TiO_2 NPs, it is critical to investigate the influence of soil heterogeneity i.e. texture and organic matter on the ecological risk of TiO_2 NPs in the soil ecosystem.

Soil bacterial community plays important roles in soil biogeochemical processes including organic carbon decomposition, nitrogen fixation, mineral recycling, plants nutrient acquisition, etc. (Falkowski et al., 2008). In terms of potential ecological risk of TiO_2 NPs in soil, previous studies have reported that TiO_2 NPs could alter the soil bacterial community composition and inhibit bacterial activity (Ge et al., 2012; Simonin et al., 2016; Zhai et al., 2019b). When released to complex soil matrices, TiO_2 NPs interact with the soil constituents like organic matter present in the clay fraction of a given soil. These interactions will influence the effect of TiO_2 NPs on soil bacterial community functioning over time (McKee and Filser, 2016). Soil bacterial respiration was for example, found to be negatively affected by short-term (7 days) TiO_2 NPs exposure in clay soil with high organic matter content, whilst it was recovered after long-term incubation of 90 days (Simonin et al., 2015). However, many studies only provided insight from a single model of soil and did not take the heterogeneity of soils into consideration. It remains unknown how the soil bacterial community diversity is changed, which taxa are sensitive, and what soil bacterial mediated processes are disrupted in TiO_2 NPs exposure over time. Soil bacterial respiration was for example, found to be negatively affected by short-term (7 days) TiO_2 NPs exposure in clay soil with high organic matter content, whilst it was recovered after long-term incubation of 90 days.

The objective of this study is to investigate the influences of soil heterogeneity on the bacterial communities exposed to TiO_2 NPs over time. Soil samples were taken from clay and sandy soils with low- and high-organic matter content (LOM and HOM) exposed to TiO_2 NPs for both short-term (1 day) and long-term (60 days) incubation. Soil enzyme activity, bacterial community diversity, composition and function were investigated to answer the following key questions: 1) how does the TiO_2 NPs toxicity on bacterial community change across soil heterogeneity over time? 2) whether TiO_2 NPs exposure or soil heterogeneity determine the overall impact predominantly? 3) which featured taxa and function are sensitive to TiO_2 NPs exposure across the soil heterogeneity over time?

2. Materials and methods

2.1. TiO_2 NPs

Titanium dioxide nanoparticles (TiO_2 NPs, anatase (80%) and rutile (20%) crystal structure, 99.5% purity) were purchased from Sigma-Aldrich. The morphology of TiO_2 NP was characterized using Transmission electron microscopy (TEM) (JEOL 1010, IEOL Ltd., Japan), and the hydrodynamic size distribution was measured using dynamic light scattering (DLS) (Malvern, Instruments Ltd., UK). The physico-chemical properties of TiO_2 NPs were characterized in our previous study (Zhai et al., 2019a) and given in Fig. S1. In brief, TiO_2 NPs are spherical powders, with pristine particle size of 25 nm and specific surface area of 35–65 m^2/g. The TiO_2 NPs aggregated to larger aggregates with hydrodynamic diameter of 527 ± 124 nm in soil extracts. The TiO_2 NPs powders were dispersed in MiliQ water and then sonicated at 4 °C at 38 ± 10 KHz for 16 min to make the stock suspension (25 mg/L) following the Risk Assessment of Engineered Nanoparticles (ENPRA) protocol (Jacobsen et al., 2010).

2.2. Soils

Four different soils i.e. sandy or clay soils with low- or high-OM content were investigated in this study. Sandy soils were collected from the top 15 cm of a site dominated by deciduous trees with (high-OM content) and without fertilization (bark compost) (low-OM content) (52°11′37.9"N 4°30′16.4″E, Warmond, The Netherlands). Clay soils were collected from an agricultural field (low-OM content) and under permanent pasture (high-OM content) (52°12′28.2"N 4°30′32.1″E, Warmond, The Netherlands). The collected soils were sieved with 2 mm sieve and stored at 4 °C. The detailed characteristics of the soil samples are listed in Table S1.

2.3. Experimental design

Prior to the experiment, soils were pre-incubated for one week at 20 °C. TiO_2-NPs stock suspensions was added drop by drop using a pipet into 60 g of each soil (equivalent to 50 g dry weight) and thoroughly mixed manually for 5 min. An exposure concentrations of

1 mg/kg TiO_2 NPs was achieved, which represented a realistic environmental concentration (Sun et al., 2014). The same amount of sterilized water without TiO_2 NPs was added into the control soils. Soil microcosms were incubated at 20 °C for 15 and 60 day representing short- and long-term exposure (Zhai et al., 2019a). In total 48 microcosms with triplicate per treatment were prepared (4 soils × 2 concentrations of TiO_2 NPs (i.e. 0 and 1 mg/kg) × 2 sampling times × 3 replicates). Soil water content was maintained at 24% during the incubation using sterile water. In each microcosm, ten grams of soil were subsampled by the end of each incubation time and immediately used for bacterial community analysis.

2.4. Enzyme activity

The enzyme activity of the tested soils was measured by the dehydrogenase activity according to the 2-[4-iodophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium chloride (INT) assay (Von Mersi and Schinner, 1991). In brief, each soil sample was mixed with Tris Buffer (Tris(hydroxymethyl)aminomethane, 1 M, Sigma-Aldrich) and substrate solution (2(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (iodonitrotetrazolium chloride (INT), 10 mM, Sigma-Aldrich), and then incubated in the dark for 2 h at 40 °C. After the incubation, the soil was extracted by a solution consisting of *N*,*N*-dimethylformamide/ethanol in a 1:1 ratio, in the dark for 30 min at 20 °C to extract the developed iodonitrotetrazolium formazan (INTF). The developed INTF was determined color metrically at λ = 464 nm (UV-1800, Shimadzu, Kyoto, Japan). The dehydrogenase activity was expressed as mg INTF/g dry soil /2 h.

2.5. DNA extraction and Illumina Miseq sequencing

Soil DNA was extracted from 0.3 g of each soil sample using Qiagen DNeasy PowerSoil Kit (Hilden, Germany). DNA quality control checks were performed by downstream sequencing. PCR amplification was performed using a universal bacterial primer set (515F: 5′-GTGCCAGC MGCCGCGGTAA-3′ and 909R: 5′-CCCGTCAATTCMTTTRAGT-3′) targeting the variable V4–V5 regions of bacterial 16S rRNA genes (Liu et al., 2017). Paired-end sequencing was performed by BaseClear (Leiden, the Netherlands) using the 2 × 300 bp Illumina Miseq platform (Illumina, Inc., San Diego, CA, USA). The sequences have been deposited into the NCBI database with project number: PRJNA660920, and the sample information is provided in Table S2.

Data obtained through Illumina sequencing were processed using the Quantitative Insights Into Microbial Ecology (QIIME2) pipeline (Bolyen et al., 2019). In brief, the QIIME 2 pipeline includes sequence quality control, feature table construction, phylogenetic tree generation, diversity analysis and taxonomic analysis. Sequences quality control was performed using the software package DADA2 for modeling and correcting for Illumina-sequenced amplicon errors. Qualified sequences were processed to construct the FeatureTable which contained frequencies of each unique sequence in each sample and FeatureData which maps feature identifiers in the FeatureTable to the sequences they represent. The FeatureTable was collapsed at the genus level (i.e. level 6 of the Greengenes taxonomy) The phylogenetic tree was built using the q2-phylogeny plugin. Community diversity was analyzed through the q2-diversity plugin, which supports computing alpha and beta diversity metrics. The sampling depth was rarefied at 5500 to remove the heterogeneity. The rarefaction curve is shown in Fig. S2. The taxonomic assignment to the sequences in the FeatureData was conducted using the q2-feature-classifier plugin. Phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) was used to further translate the 16S rRNA gene amplicon data into predicted metagenomes to predict the soil bacterial community functional profile (Langille et al., 2013). MetaCyc pathway was used to annotate the predicted metagenomes. PICRUSt was processed using PICRUSt2 pipeline in QIIME2 to generate FeatureTable which contained frequencies of MetaCyc pathway abundance in each sample.

2.6. Statistical analysis

All bacterial responses i.e. enzyme activity, community diversity, structure, composition and functional profile were analyzed among the different treatments (clay-LOM, clay-HOM, sand-LOM and sand-HOM) at the selected time points (short- and long-term). One-way analysis of variance (ANOVA) and post hoc Tukey HSD were performed to test the influence of soil properties and exposure time on the effect of TiO_2 NPs on soil enzyme activity (Fig. S3). Significance testing on the community alpha diversity comparisons across the different treatments was performed using the QIIME2 diversity alpha-group-significance plugin. To test whether bacterial communities differed among treatments, community dissimilarities based on both taxonomic and functional composition were illustrated using principal coordinates analysis (PCoA) based on the weighted UniFrac distance matrices. The significance of community dissimilarity was further tested using Permutational multivariate analysis of variance (PERMANOVA). To

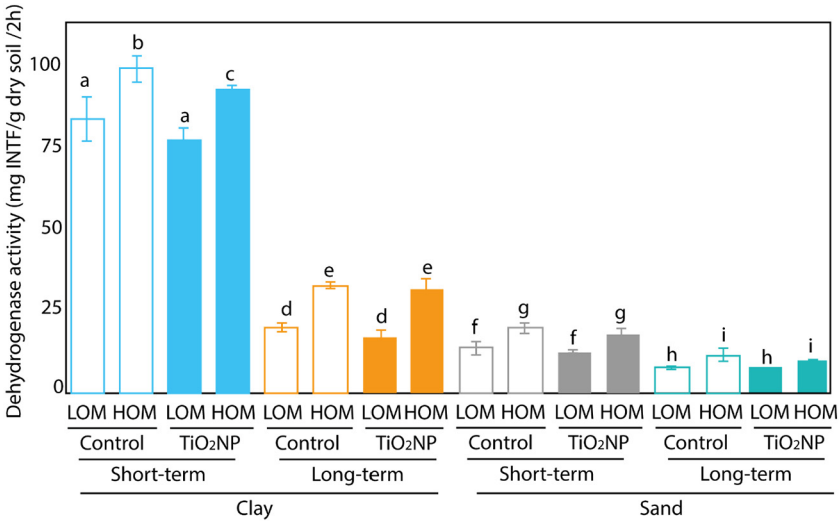

Fig. 1. Enzyme activity of the clay and sandy soil with low- and high-OM content with and without TiO_2 NP exposure for short- and long-term incubation. Enzyme activity was determined by measuring the dehydrogenase activity expressed as mg iodonitrotetrazolium formazan (INTF)/g dry soil /2 h). Different letters indicate significant changes in enzyme activity ($n = 3$).

investigate the changes in taxonomic as well as functional composition of the soil bacterial community, analysis of composition of microbiomes (ANCOM) was applied to identify features (i.e. feature taxa and feature pathways) that are differentially abundant between the low- and high-OM content clay and sandy soils with and without TiO_2 NPs exposure at each time point. Correlation between feature taxa and functional pathways were calculated using pairwise Spearman's rank processed in R v3.6.1. (psych package). Networks with coefficient at 0.7 and statistically significant ($p < 0.05$) were visualized using Gephy v0.9.1.

3. Results

3.1. Influence of soil heterogeneity on soil enzyme activity in response to TiO_2 NPs exposure over time

The impact of TiO_2 NPs on the bacterial dehydrogenase activity of the tested soils is shown in Fig. 1. In short-term incubation, the dehydrogenase activity in the clay-HOM and sand-HOM soils increased from 83.2 ± 7.8 to 98.2 ± 3.7 and from 13.4 ± 0.3 to 20.2 ± 1.6 mg INTF/kg soil/2 h, respectively. The addition of TiO_2 NPs induced no significant difference in either clay-LOM, sand-LOM or sand-HOM soils. However, the dehydrogenase activity in the clay-HOM soil significantly decreased ($p < 0.05$) from 98.2 ± 3.7 to 91.3 ± 1.4 mg INTF/kg soil/2 h, indicating that the bacterial enzyme activity was significantly affected by TiO_2 NPs exposure in clay soil with high organic matter. Moreover, TiO_2 NPs exposure reduced the enzyme activity by 7.9 ± 5.3% in the clay-LOM and 11.8 ± 6.5% in sand-LOM soils, suggesting that the impact of TiO_2 NPs exposure in sand induced more serious impact on the bacterial enzyme activity compared with clay. After long-term exposure, the dehydrogenase activity in the clay-HOM and sand-HOM soils increased from 19.2 ± 1.3 to 31.5 ± 1.2 and from 7.8 ± 0.2 to 10.6 ± 2.1 mg INTF/kg soil/2 h, respectively. The addition of TiO_2 NPs induced no significant difference in the clay and sandy soils, no matter whether the soil contained a low- or long-OM content. These results indicated that the

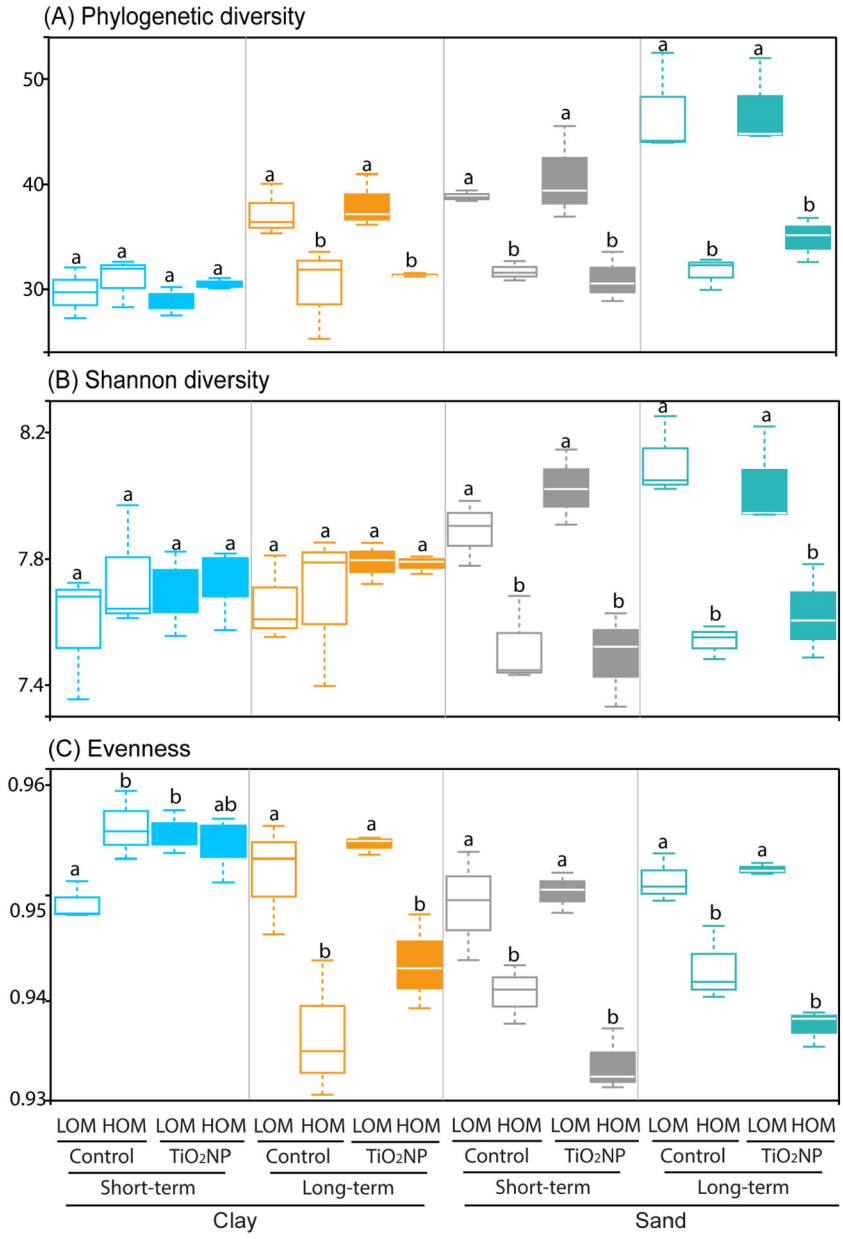

Fig. 2. Boxplots showing the alpha diversity of the bacterial communities in clay and sandy soil with low- and high-OM content with and without TiO_2 NP exposure for short- and long-term incubation. (A) Phylogenetic diversity. (B) Shannon diversity. (C) Evenness. Different letters indicate significant changes in alpha diversity indexes (n = 3).

high-OM content promoted the soil enzyme activity, while TiO_2 NPs exposure transiently inhibited this promotion in the clay-HOM treatment transiently.

3.2. Influence of soil heterogeneity on the bacterial community in response to TiO_2 NPs exposure over time

3.2.1. Responses of bacterial community diversity

In total, 386,568 sequences obtained from 48 samples, were assigned as 11,787 OTUs. The rarefaction curve becomes flat after reaching 2000 sequences (Fig. S2), indicating sufficient sequence depth of this study. The impact of TiO_2 NPs on the community alpha diversity (i.e. phylogenetic diversity, Shannon diversity and evenness) of the clay and sandy soils with different OM content is given in Fig. 2. For clay soil in short-term incubation, a high-OM content promoted the community alpha diversity indexes, and a significant increase in community evenness was observed in high-OM treatment ($p < 0.05$). However, the increase in community alpha diversity which was induced by a high-OM content was inhibited after TiO_2 NPs addition, where no significant difference in alpha diversity between the low- and high-OM content was observed in the presence of TiO_2 NPs. After long-term incubation, community phylogenetic diversity and evenness were significantly decreased in high-OM treatment ($p < 0.05$), regardless of TiO_2 NPs exposure. The sand community phylogenetic diversity, Shannon diversity and evenness significantly decreased as OM-content increased in both short- and long-term incubation, and these indexes were not affected by the TiO_2 NPs exposure. These results suggested that short-term exposure of TiO_2 NPs reduced community alpha diversity especially in the clay-HOM soil.

The impact of TiO_2 NPs on the community beta diversity (based on weighted UniFrac distance matrices) of the clay and sandy soils with different OM content is given in Fig. 3. The results of significance testing of community dissimilarity are summarized in Table S3. The environmental factors of exposure time (short- and long-term), soil texture (clay and sand) and OM content (low and high) significantly explain the variation in community dissimilarity. Although the TiO_2NP treatment did not significantly alter the bacterial communities in all the tested soils, a clear separation between the control and TiO_2NP treatment was observed in the clay-HOM soil in short-term TiO_2NP exposure. As exposure time increased, the bacterial communities of the control and TiO_2NP treatments were separated in the clay soil with both low- and high-OM content. This indicated that TiO_2NP exposure affected the community beta diversity. However, the bacterial communities of the control and TiO_2NP treatments grouped together in all the sandy soils, regardless of OM-content and incubation time. This indicated that the community beta diversity was not affected by the addition of TiO_2 NPs in all the sandy soils.

3.2.2. Responses of bacterial community taxonomic composition

The above reported community similarity results showed that the impact of TiO_2 NP on the clay and sandy soil bacterial communities changed along with the OM-content in both short- and long-term exposure. We further investigated the community composition in clay and sandy soil with low- and high-OM content in the presence and absence of TiO_2 NP exposure in short- and long-term incubation. As shown in Fig. S4, the soil bacterial community was predominated by Proteobacteria (28.6%–51.2%), Actinobacteria (2.7%–35.3%) and Acidobacteria (5.6%–16.8%). To further investigate the community compositional changes among different treatments, a differential abundance test was performed at genus level to identify features that are differentially abundant across soil sample groups (Fig. 4). The parameters of significance testing for each featured taxa are given in Table S4–11. For the control clay soil in short-term exposure, the most abundant featured taxa in low-OM content were genera belonging to Proteobacteria, Acidobacteria and Actinobacteria. The increased OM content promoted the diversity of featured taxa, with genera belonging to Chloroflexi, Verrucomicrobia and Gemmatimonadetes appearing in the bacterial community with high-OM content. After TiO_2NP addition, the featured taxa in the clay soil with low-OM content still belonged to Proteobacteria, Acidobacteria and Actinobacteria, and Verrucomicrobia. However, the TiO_2 NPs addition reduced the diversity of featured taxa in the high-OM content soil, with most featured taxa belonging to Proteobacteria. As incubation time increased, the decreased diversity of featured taxa induced by TiO_2 NPs treatment disappeared. A high OM content promoted diversity of the differentially abundant featured taxa, whatever TiO_2 NPs treatment. This indicated that as incubation time increased, the impact of TiO_2 NPs decreased. For the sandy soil in short-term incubation, increasing OM-content promoted the diversity of featured taxa, regardless of TiO_2 NPs treatment. As incubation time increased, the promoted diversity of featured taxa by OM-content disappeared in both control and TiO_2 NPs treatment. This indicated that OM-content and exposure time were the main drivers for the featured taxa and TiO_2 NP had little effect on the soil bacterial community in sandy soil.

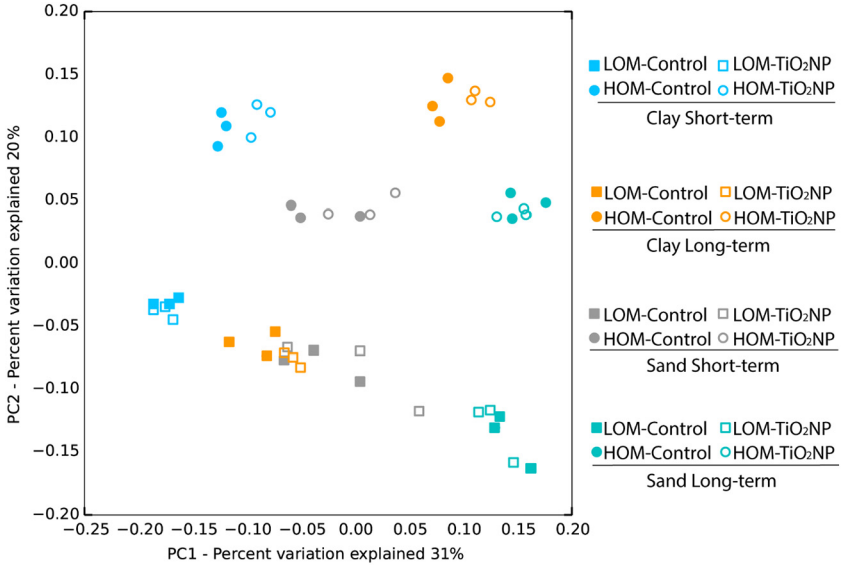

Fig. 3. Principal coordinate analysis (PCoA) of the bacterial communities in clay and sandy soil with low- and high-OM content with and without TiO_2 NP exposure for short- and long-term incubation based on taxonomic composition.

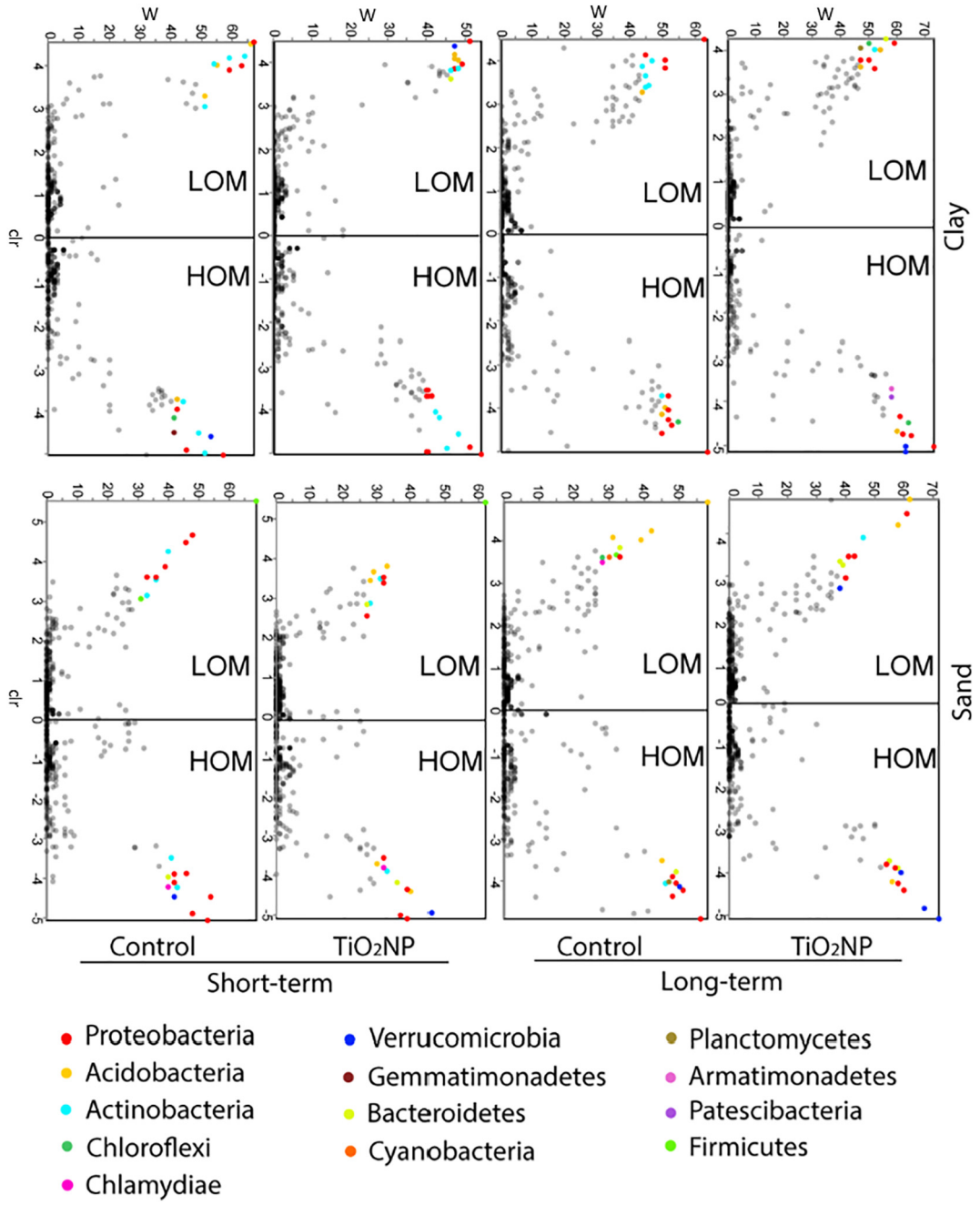

Fig. 4. Distribution of the featured taxa between the clay and sandy soil with low- and high-OM content with and without TiO_2 NP exposure for short- and long-term incubation. The volcano plot relates the ANCOM W statistic to the clr (center log transform) for the groups. The colored dots are the top ten most abundant featured taxa (at genus level) in each treatment that belong to the phyla in the legend.

3.3. Influence of soil heterogeneity on bacterial community function in response to TiO_2 NPs exposure over time

3.3.1. Responses of the bacterial community functional profile

The impact of TiO_2 NPs on the microbial community functional profile of the clay and sandy soils with different OM content is given in Fig. S5. The results of significance testing of community dissimilarity are summarized in Table S12. The exposure time (short- and long-term), soil texture (clay and sand) and OM content (low and high) significantly explain the variation in community dissimilarity. There was no significant effect of TiO_2 NPs on the microbial community functional profile of the tested soils. However, separation between the control and the TiO_2 NPs treatment was observed in clay soil in short-term exposure. After long-term exposure, the separation between the control and the TiO_2 NPs treatment had disappeared in the clay soil. For the sandy soil, there was no separation in community functional profile between the control and the TiO_2 NPs treatments regardless of OM content and exposure time.

3.3.2. Responses of bacterial community functional composition

To understand the impact of TiO_2NP on the functional composition of the soil bacterial community among the different treatments, featured pathways were further identified across soil sample groups (Fig. S6). For the control clay soil in short-term exposure, diverse feature pathways were identified in both low- and high-OM content soils (i.e. amino acid biosynthesis, fermentation, carbohydrate biosynthesis and carbohydrate degradation), indicating that the functional composition

of clay soil was altered by the OM content. However, the discrepancy disappeared after TiO_2NP treatment, with only one featured pathway identified in the low-OM-content clay (carbohydrate degradation). This suggested that the addition of TiO_2NP reduced the divergence induced by OM. After long-term exposure, there were no featured pathways identified in either control or TiO_2NP treated clay soil, indicating a decreased effect of TiO_2NP along with time. For the sandy soil, no featured pathway was identified in either the low- and high-OM content treatment in either short- or long-term exposure, and regardless of TiO_2 NPs addition. This indicated that the functional composition of the sand bacterial community was not affected by TiO_2NP treatment.

3.3.3. Co-occurrence between featured taxa and function

MetaCyc pathway was further used to annotate the predicted metagenomes, and the co-occurrence patterns of the identified feature taxa and pathways in all samples were visualized by co-network analysis (only data are shown with correlation coefficient $r > 0.9$ and significant $p < 0.01$, Fig. 5). The annotations for the functional pathways are listed in Table S13. In total 71/139 positive correlations were established among featured taxa (22) and pathways (84). Notably, the observed declined diversity of feature taxa in clay soil after short-term TiO_2 NPs treatment, i.e. *Acidobacteriales* (Acidobacteria) and *Opitutus* (Verrucomicrobia) are positively correlated with pathways related to carbohydrates degradation and biosynthesis e.g. P269 (Starch biosynthesis), P102 (Purine nucleobases degradation). This indicated that the short-term TiO_2 NPs treatment affected the functional pathways related with carbohydrates degradation and biosynthesis in clay soil bacterial community.

4. Discussion

4.1. Toxicity of TiO_2 NPs on soil bacterial communities varied as the soil heterogeneity over time

The toxicity of TiO_2 NPs on soil bacterial communities was found to varied as the soil heterogeneity. In short-term exposure of TiO_2 NPs, we observed that the enzyme activity in sand was lower than the

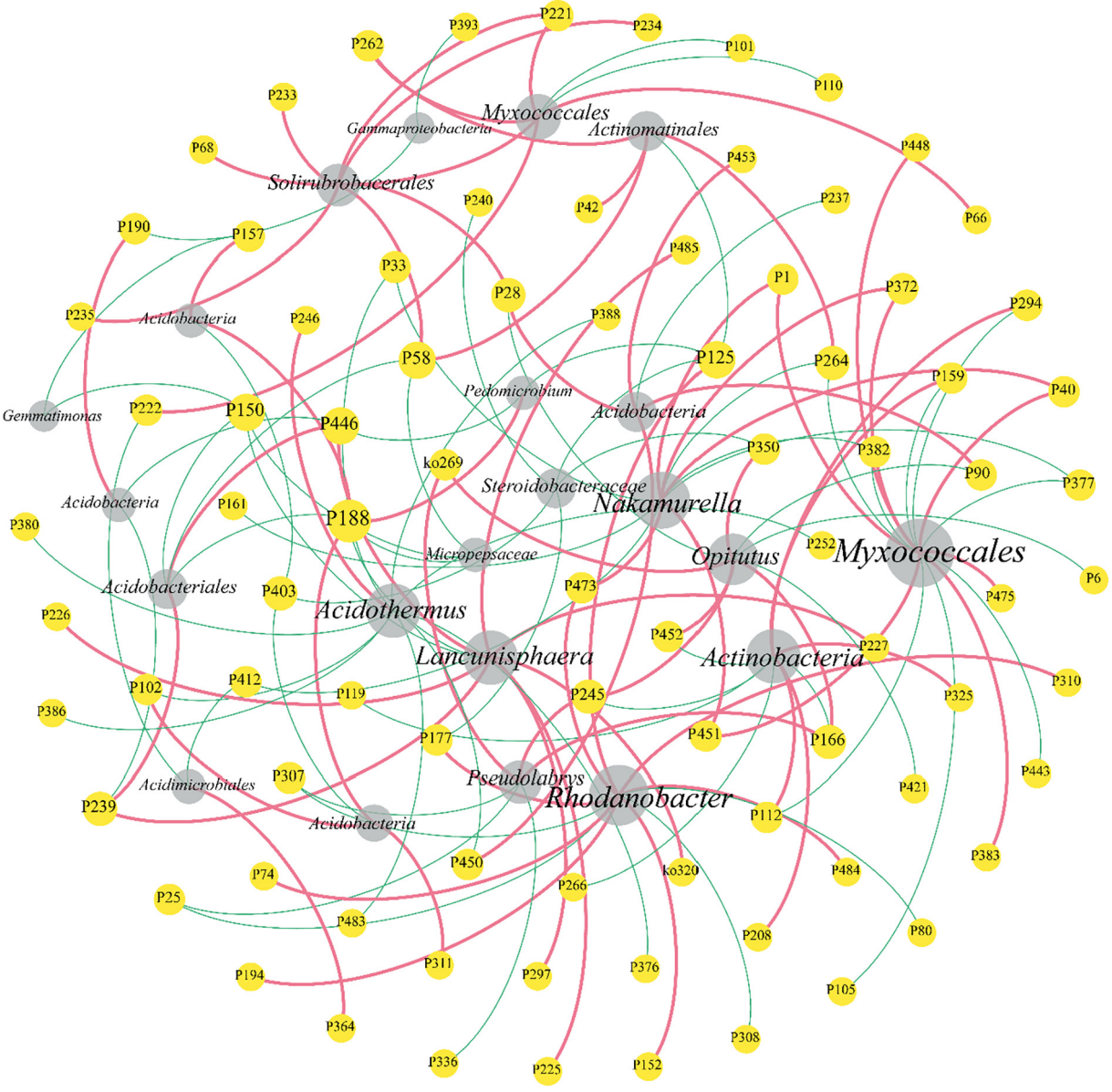

Fig. 5. Co-network analysis revealing the co-occurrence patterns of the identified significantly different featured taxa and the predicted pathways. A connection represents a strong (Spearman's correlation coefficient $r > 0.9$) and significant ($p < 0.01$) correlation. Grey nodes are taxa, and yellow nodes are pathways with annotation listed in Table S13. Red lines represent positive correlations and green lines represent negative correlations. The size of each node is proportional to the number of connections. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

activity in the clay soils (Fig. 1). A significant effect of TiO_2 NPs bacterial dehydrogenase activity has previously been reported (Li et al., 2014). In this study, the limited effects of TiO_2 NPs on the clay soil could be explained by the role of clay particles on TiO_2 NPs stability and mobility. Electrostatic interactions were found to mainly drive the affinity of TiO_2 NPs to clay particles (Labille et al., 2015), and the interaction of clay particles and TiO_2 NPs could affect the TiO_2 NPs distribution between the solid soil matrix and the porewater and decreasing the mobility of the particles in the soil (Fang et al., 2009). Moreover, we also observed that the ionic strength in clay soils was higher than in the case of sandy soils (Table S1). A high ionic strength favors the attachment of nanoparticles to the clay matrix due to the electrical double layer compression (Chen et al., 2011). The formed clay-TiO_2 NPs hetero-aggregates could affect the transport of TiO_2 NPs, and may lower the impacts of TiO_2 NPs on the soil bacterial activity (Labille et al., 2015; Simonin et al., 2015).

In addition, significantly inhibition of enzyme activity only occurred in clay-HOM soil (Fig. 1). Decrease in community alpha diversity was also observed in short-term exposure of TiO_2 NPs in clay-HOM soil (Fig. 2). Moreover, although not significant, clear bacterial community separation between the control and TiO_2 NPs treatment was observed in the clay-HOM soil (Fig. 3). The absence of effects in the clay soil with low OM soil suggested that soil clay fraction was not the only factor influencing TiO_2 NPs toxicity, and the OM content also influenced the effect of TiO_2 NPs. The enhanced effect of TiO_2 NPs by OM content was also observed in soil bacteria (Simonin et al., 2015), aquatic protozoan (Gupta et al., 2017), and water flea (Fan et al., 2016). Natural organic matter could promote TiO_2 NPs dispersion due to the combined effect of increased electrostatic and steric repulsions between TiO_2 NPs particles (Thio et al., 2011). After interacting with OM, large TiO_2 NPs aggregates could be broken into small particles which may facilitate TiO_2 NPs exposure and bioavailability (Wang et al., 2016).

Moreover, the TiO_2 NPs effects on bacterial community crossed the tested soil samples were observed to change along with exposure time. In short-term exposure we observed clear community separation (based on taxonomic composition) between the control and TiO_2 NPs treatment only in the clay-HOM samples. After long-term exposure, separation between the control and TiO_2 NPs treatment also appeared in the clay soil with low-OM content. This indicated that the disruption by TiO_2 NPs of the bacterial community structure was enhanced by exposure time. Previous studies have also found that after 60–90 days exposure of TiO_2 NPs, the soil bacterial community structure modified and community diversity decreased, suggesting that aged TiO_2 NPs can affect soil bacterial community even at low concentrations and after a long exposure (Ge et al., 2011; Simonin et al., 2016). However, the inhibited enzyme activity and the community functional profile dissimilarity between TiO_2 NPs treatment and control observed in the clay-HOM samples, appeared transitory and were no longer observed after long-term exposure. This indicated that after long-term exposure compositional alterations occur but they do not yet necessarily reflect a biologically significant impact on the functioning of the soil bacterial community (Zhai et al., 2019a). A similar functional redundancy was also observed in a periphytic community in long-term exposure to TiO_2 NPs (Liu et al., 2019). These results indicate that the duration of exposure is another key factor for assessing TiO_2 NPs ecotoxicity. The organic matter-NPs aggregates and the aging of NPs potentially reduce their bioavailability and mobility in soil, and the microbial adaptation or evolutionary process may take place in long-term to cope with the external stress (Wang et al., 2016).

4.2. Compositional and functional responses of soil bacterial community to TiO_2 NPs in soils varying with clay and OM components

We further identified the sensitive taxa and pathways in response to TiO_2 NPs exposure in the tested soils based on the taxonomic and functional composition. The increased OM content was found to promote the diversity of featured taxa, with genera belonging to Chloroflexi, Verrucomicrobia and Gemmatimonadetes appearing in the bacterial community with high-OM content. Studies have reported that Chloroflexi were related with aromatic compound degradation (Colatriano et al., 2018), Verrucomicrobia were candidates for polysaccharide-degrading bacterioplankton (Cardman et al., 2014), and Gemmatimonadetes were replete of genes of carbohydrate hydrolysis (Li et al., 2016a). However, in the presence of TiO_2 NPs the promotion of community diversity in the clay soil was inhibited (Fig. 4). This indicated that TiO_2 NPs altered the community composition related to the clay fraction. TiO_2 NPs have been found to decrease bacterial diversity in silty-clay soil (Simonin et al., 2016). Moreover, we found that in absence of TiO_2 NPs, bacterial genera belonging to Acidobacteria and Verrucomicrobia were the featured taxa in the clay-HOM soil, while these featured taxa disappeared in the same soil upon TiO_2 NPs addition. The exposure to TiO_2 NPs promoted genera belonging to Proteobacteria as the most featured taxa. Similar shifts in bacterial community composition were reported in soils amended with silver nanoparticles, with significant decreases in the relative abundance of Acidobacteria and Verrucomicrobia and an increase in Proteobacteria (McGee et al., 2017). Similar to the antibacterial properties of silver nanoparticles, studies have shown that TiO_2 NPs exposure to bacteria could induce cell membrane damage, causing oxidative stress and producing reactive oxygen species (ROS) (Simon-Deckers et al., 2009). On the other hand, after being incorporated into the cell, nanoparticles could induce the generation of ROS, which increased the membrane permeability and facilitated the horizontal transfer of the antibacterial resistant genes (Su et al., 2019). The OM in the clay soil might facilitate the stability and toxicity to the soil bacterial community, inhibiting the sensitive taxa and altering the community composition. However, the decrease in the diversity of the featured taxa by TiO_2 NPs exposure appeared transiently. After long-term exposure, high-OM content no longer promoted the diversity of featured taxa, and the reduced diversity of featured taxa induced by TiO_2 NPs addition disappeared. In our previous study, we also observed that the impact of TiO_2 NPs at the environmentally relevant concentration (1 mg/kg) on soil bacterial composition appeared transiently (Zhai et al., 2019a). As exposure time increased, the physicochemical properties of TiO_2 NPs in soil could change along with time due to e.g. aging, aggregation, interaction with clay minerals and organic matter, which reduced the bioavailability of TiO_2 NPs (Tourinho et al., 2012). The adaption of the soil bacterial community could also contribute to the community recovery after long-term TiO_2 NPs exposure (Wu et al., 2018).

In addition to the bacterial community taxonomic composition, we also analyzed the functional composition in response to TiO_2 NPs exposure in the tested soils based on the MetaCyc pathways abundance (Figs. 5 and S6). Alterations in functional composition were only observed in clay soils in short-term exposure, with more featured pathways identified in the clay-HOM soil-related to carbohydrates degradation and biosynthesis. However, the presence of TiO_2 NPs disrupted the promotion by OM, with no featured pathway identified in the high-OM content. Combining the results of taxonomic composition, the short-term exposure of TiO_2 NPs could inhibit the positive effect of OM on soil bacterial community by reducing the diversity of featured taxa and pathways related to carbohydrates degradation and biosynthesis. Nevertheless, the effect of TiO_2 NPs on the functional composition disappeared after long-term exposure. Similar bacterial community functioning recovery was also found in soil, stream water, and sediments exposed to titanium dioxide, silver, and copper nanoparticles (Colman et al., 2012; Sheng et al., 2015; Moore et al., 2016; Zhai et al., 2019a). When the soil bacterial community was exposed to TiO_2 NPs, the sensitive taxa were not able to survive, which induced bacterial community compositional alterations. However, the resistant taxa may have an overproduction of extracellular polymeric substances that could form aggregates with TiO_2 NPs to reduce the toxicity (Joshi et al., 2012). Moreover, the loss of the susceptible taxa functioning

could be compensated for by the promotion of survival taxa sharing the similar functioning (Zhai et al., 2019a). The soil bacterial community may experience evolutionary adaptation after long-term exposure to TiO_2 NPs and remains sustainable functioning (Liu et al., 2019). Considering that in this study we did not observe the effect of long-term TiO_2 NPs exposure on the diversity of the featured taxa, the highly diverse species could compensate for the partial disruption of community functioning regulated by certain taxa (Rosenfeld, 2002; Strickland et al., 2009). This resulted in a functionally redundant soil bacterial community that the community functioning could recover after long-term incubation (Allison and Martiny, 2008).

Combining the responses of bacterial enzyme activity, community taxonomic composition and community functional profile, significant impacts of TiO_2 NPs exposure was observed in the clay-HOM soil. These results further indicated that TiO_2 NPs selection was the main driver in inducing the compositional and functional impacts on the soil bacterial community in the presence of clay with high organic matter content. Our previous study identified various patterns of bacterial community dynamics due to TiO_2 NPs selection, depending on the bacterial tolerance (Zhai et al., 2019b). In this study, TiO_2 NPs exposure was found to suppress bacterial genera belonging to Acidobacteria and Verrucomicrobia while promote taxa belonging to Proteobacteria. The resistant taxa could establish persistence by means of detoxification enzymes, acclimation, and horizontal transfer of resistant genes (Ernst et al., 2016), while the less tolerant taxa could cope with the stress which caused damage (Griffiths and Philippot, 2013). In addition, the impact of TiO_2 NPs was not significant in sand and clay-LOM soil, suggesting that the soil heterogeneity play more important roles in shaping the bacterial community soil with low clay fraction and less organic matter. Nevertheless, the alterations in bacterial community induced by TiO_2 NPs was found to be transient. After long-term exposure, the natural evolution and adaptation of soil bacterial community overcome the TiO_2 NPs selection, with no significance observed before and after TiO_2 NPs addition. Although we did not find a significant effect of TiO_2 NPs on the soil bacterial community functioning in long-term exposure, the disruption of the community taxonomic composition indicated that the bacterial stability decreased, which potentially makes the community more vulnerable to the next external stress. In our previous work we reported that exposure to multiple doses of TiO_2 NPs induces more severe effects on soil bacterial communities compared to exposure to a dose. Given that TiO_2 NPs are released via waste discharge or repetitively applied as agrochemicals into soil, the fate and behavior of the nanoparticles in soil matrix as well as the accumulated impacts of multiple exposures on the soil microbial communities need to be assessed. In view of the increasing application of nanoparticles-containing products and the inevitable higher release into soil environment, future investigations into the spatial and temporal impacts are urgently needed for safe development of nano-products.

5. Conclusion

In conclusion, the impact of TiO_2 NPs on soil bacterial communities depended on the soil heterogeneity and exposure time. Clay fraction and organic matter content were found to influence the effect of TiO_2 NPs on soil bacterial community. Bacterial enzyme activity inhibition, community compositional and functional disruption were observed when TiO_2 NPs exposed in clay soil with high organic matter content, where featured taxa belonging to Acidobacteria and Verrucomicrobia and functional pathways related to carbohydrates degradation were suppressed. No significant impacts of TiO_2 NPs was observed in the clay soil with low organic matter content and all the sandy soils. These results suggested that TiO_2 NPs selection was the main driver in inducing the compositional and functional impacts on the soil bacterial community in the presence of clay soil with high organic matter content, and soil heterogeneity play more important roles in shaping the bacterial community in soil with low clay fraction and less organic matter. In addition, the influence of soil heterogeneity on the effect of TiO_2 NPs appeared transiently. The bacterial evolution and adaptation overcome the TiO_2 NPs selection after long-term exposure, and the bacterial community varied with the soil heterogeneity. These findings suggested that soil heterogeneity such as soil texture, organic matter content, and exposure duration play important roles in determining TiO_2 NPs impacts on soil bacterial community. Given the large numbers of nano-fertilizers, additives, and organic amendments being added to agricultural systems, future research regarding with the time-dependent interactions between soil heterogeneity and nanotoxicity is needed to better understand the fate and ecotoxicological impacts of nanoparticles-containing products on the soil ecosystem.

CRediT authorship contribution statement

Yujia Zhai: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Lihua Chen:** Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Gang Liu:** Supervision, Validation, Visualization, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing. **Lan Song:** Supervision, Validation, Visualization, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing. **Daniel Arenas-Lago:** Data curation, Methodology, Writing - review & editing. **Lingchao Kong:** Visualization, Formal analysis. **Willie Peijnenburg:** Supervision, Validation, Funding acquisition, Project administration, Writing – review & editing. **Martina G. Vijver:** Supervision, Validation, Funding acquisition, Project administration, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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