Elevated CO₂ Levels Modify TiO₂ Nanoparticle Effects on Rice and Soil Microbial Communities

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Abstract

Evidence suggests that CO₂ modifies the behavior of nanomaterials. Thus, in a few decades, plants might be exposed to additional stress if atmospheric levels of CO₂ and the environmental burden of nanomaterials increase at the current pace. Here, we used a full-size free-air CO₂ enrichment (FACE) system in farm fields to investigate the effect of elevated CO₂ levels on phytotoxicity and microbial toxicity of *n*TiO₂ (0, 50, and 200 mg kg⁻¹) in a paddy soil system. Results show that *n*TiO₂ did not induce visible signs of toxicity in rice plants cultivated at the ambient CO₂ level (370 μmol mol⁻¹), but under the high CO₂ concentration (570 μmol mol⁻¹) *n*TiO₂ significantly reduced rice biomass by 17.9% and 22.1% at 50 mg kg⁻¹ and 200 mg kg⁻¹, respectively, and grain yield by 20.8% and 44.1% at 50 mg kg⁻¹ and 200 mg kg⁻¹, respectively. In addition, at the high CO₂ concentration, *n*TiO₂ at 200 mg kg⁻¹ increased accumulation of Ca, Mg, Mn, P, Zn, and Ti by 22.5%, 16.8%, 29.1%, 7.4%, 15.7% and 8.6%, respectively, but reduced fat and total sugar by 11.2% and 25.5%, respectively, in grains. Such conditions also changed the functional composition of soil microbial communities, alerting specific phyla of bacteria and the diversity and richness of protista. Overall, this study suggests that increases in CO₂ levels would modify the effects of *n*TiO₂ on the nutritional quality of crops and function of soil microbial communities, with unknown implications for future economics and human health.

Keywords: CO₂, TiO₂ nanoparticles, rice, phytotoxicity, microbial communities

1. Introduction

Driven largely by economic and population growth, anthropogenic greenhouse gas emissions have increased since the pre-industrial era. Such emissions have contributed to raise the atmospheric concentration of carbon dioxide (CO₂) from approximately 280 mmol mol⁻¹ in pre-industrial times to approximately 400 mmol mol⁻¹ in current times (IPCC, 2014). Elevated levels of atmospheric CO₂ impact natural ecosystems, especially in terrestrial environments. An increase in CO₂ stimulates photosynthesis, enhances carbon deposition in soil, and changes the rhizosphere conditions of plants, leading to increases in biomass and yield of crops (Guo et al., 2011; Niu et al., 2016; Xu et al., 2016). This can also lower the pH of rhizosphere soils, facilitating metal solubility and bioavailability, and increasing the uptake of metals by plants (Guo et al., 2011; Rajkumar et al., 2013). In addition, elevated fluxes of CO₂ may result in higher energy availability in soil for microbes. This would change soil chemistry, increasing C inputs (via litter, root growth, and especially, more root exudates) that should impact functional composition and structure of the soil microbial community (Adair et al., 2009; Ainsworth, 2008; Dijkstra et al., 2005; Rajkumar et al., 2013; William et al., 2000). An elevation in CO₂ was associated with the enhancement of expression of functional genes involved in C, N, P, and S cycling (He et al., 2014).

TiO₂ nanoparticles (*n*TiO₂), with up to 10,000 t annual production in the world (Piccinno et al., 2012), are used in sunscreens, coatings, plastics, paints, cements and photocatalysis (Minetto et al., 2014; Servin et al., 2013). The extensive applications of *n*TiO₂ could inevitably lead to their continuous

release into the environment, raising concerns about their potential impacts in ecosystem components. However, there are only a few studies investigating the uptake and translocation of $n\text{TiO}_2$ in plants, especially on food crops (Gardea-Torresdey et al., 2014). Servin et al. (2013) established for the first time the translocation of $n\text{TiO}_2$ from the root to the fruit of cucumber, suggesting that these nanoparticles could be introduced into the food chain with unknown consequences. There are also few studies about the effects of $n\text{TiO}_2$ on soil microbial communities (Hu et al., 2016). Ge et al. (2011, 2013) found that $n\text{TiO}_2$ ($0.5\text{--}20\text{ mg g}^{-1}$) altered the bacterial community composition reducing their diversity. Ge et al. (2014) found that plants could change nanoparticle effects on soil microbial communities by affecting the belowground biogeochemistry. Moreover, it can be speculated that elevated CO_2 levels can affect plant growth, changing the soil pH and dissolved organic matter (DOM) contents in pore water (Ainsworth, 2008; Guo et al., 2011); thus, changing the behavior of nanoparticles and their phytotoxicity and microbial toxicity. More research is needed in order to investigate the response of crops grown in soils impacted with nanoparticles under elevated CO_2 levels. So far, there is only one study investigating changes in soil microbes under combined effects of nanoparticles and elevated CO_2 (Yadav et al., 2014). This study concluded that $n\text{Fe}_2\text{O}_3$ and $n\text{ZnO}$ had positive (at 100 mg kg^{-1}) or adverse (at 500 and 1000 mg kg^{-1}) effects on microbial abundance and activities; those effects were intensified by an elevated CO_2 concentration (Yadav et al., 2014).

The reason for such modifications in biological effects was possibly due to an increase in the dissolution of $n\text{Fe}_2\text{O}_3/n\text{ZnO}$, induced by elevated CO_2 levels, which increased the availability of ionic species for microbial uptake (Guo et al. 2011). For $n\text{TiO}_2$, which is considered fairly stable and insoluble in soil (Keller et al., 2013), there are no studies investigating their response to elevated CO_2 levels.

Rice (*Oryza sativa* L.) is one of the most important plants in the world, and the first staple food in Asia, providing nutrition to a large proportion of the world's population (Yang et al., 2007). Over the last decade, a considerable number of publications have reported the effects of elevated CO_2 levels on growth, yield and quality of rice crops (Ainsworth and Long, 2005; Taub et al., 2008; Wang et al., 2015). There is sufficient evidence which shows that elevated CO_2 could enhance growth and yield of rice but modify the nutritional content (Ainsworth and Long, 2005; Gifford et al., 2000; Guo et al., 2011; Kimball et al., 2001; Taub et al., 2008; Wang et al., 2015). However, to the authors' knowledge, there is no information about the response of rice crops to elevated CO_2 levels in the presence of nanoparticles. Here, we used a full-size (14 m diameter) free-air CO_2 enrichment (FACE) system in farm fields to investigate the effect of elevated CO_2 levels on phytotoxicity and microbial toxicity of $n\text{TiO}_2$ in a paddy soil system. We analyzed the changes caused by $n\text{TiO}_2$ at ambient/elevated CO_2 levels in seedling microstructure, Ti uptake, biomass and yield of harvested rice, nutritional quality of the harvest, as well

as changes in the soil microbial community.

2. Materials and methods

2.1 TiO₂ nanoparticles

The *n*TiO₂ was purchased from Sigma-Aldrich, St Louis, MO, USA. According to the vendor, these NPs have a purity > 97%, particle size <100 nm, and surface area >14.0 m² g⁻¹. These nanoparticles are not uniform in morphology and have a wide size distribution, with diameters varying from 20–100 nm, predominated by 20 ± 5 nm (93.3%) (Du et al., 2011).

2.2 FACE system

The FACE system was established in the town of Xiaoji, Jiangdu, Jiangsu Province, China (119°42'E, 32°35'N) and has been previously described in detail (Guo et al., 2011). In brief, the FACE system consists of octagonal plots, either for ambient CO₂ conditions (ambient plots, CO₂ concentration about 370 μmol mol⁻¹ reflecting the current local concentration) or elevated CO₂ levels (FACE plots, CO₂ concentration about 570 μmol mol⁻¹ reflecting predicted concentration after 50 years). Three ambient plots and three FACE plots were used in this experiment. This region has an annual mean temperature of 15 °C, annual precipitation of 980 mm, and annual no-frost period of approximately 220 days.

2.3 Crop cultivation

Rice (*Oryza sativa* L. cv. Wu yun jing 14) was grown in plastic pots (5 kg soil

per pot; 20 cm in diameter, 35 cm in height) filled with soil collected (top 20 cm) from a local farm. The soil was Shajiang-Aquic Cambosols with 57.8% sand, 28.5% silt, 13.7% clay, and sandy-loamy texture. The soil had a pH of 7.2, organic matter content of 18.4 g kg⁻¹, total N content of 1.59 g kg⁻¹ and total Ti content of 7.9 g kg⁻¹. Soil samples were air-dried, crushed and then amended with *n*TiO₂ as powder; control soil was not amended. Specified amounts of *n*TiO₂ were added and thoroughly mixed into the soil to have concentrations of 0, 50, and 200 mg kg⁻¹. These concentrations were chosen based on a previous study (*n*TiO₂ at about 100 mg kg⁻¹) carried out on wheat (Du et al., 2011) and these concentrations for *n*TiO₂ were predicted to plausibly occur in soil hot spots in 2025 (Ge et al., 2013). The amended and unamended (control) soils were then watered to field water capacity and placed in plastic pots. Each treatment consisted of three replicate pots. The rice seedlings (25 days after sowing) were transplanted with three hills (three plants per hill) in each pot in June 2012 and harvested in December 2012. All pots were fertilized three times with urea (46%) and compound fertilizer (N: P₂O₅: K₂O = 15:15:15, %), 36% applied in mid-June, 24% in mid-June and 40% mid-July. The total N fertilization reached 12.5 g m⁻² over the life cycle of the plants.

2.4 Microscopy studies of rice seedlings

Fresh rice seedlings were harvested 30 days after transplant and thoroughly washed with deionized water. Root tips and leaves were cut and prepared for scanning electron microscopy (SEM, Philips SEM-505, The Netherlands) with

energy dispersive spectroscopy (EDS) analysis and transmission electron microscopy (TEM, Hitachi H-600, Japan) following standard procedures (Ni et al., 2005).

2.5 Analysis of harvested rice

Mature plants were harvested 60 days after transplant when grains reached maturity. Biomass and yield were determined in three hills from each pot. Plant samples were thoroughly washed with deionized water, divided into leaves, shoots, roots, and grains, dried at 70 °C to constant weight and prepared for digestion and further analyses. Dried samples were ground to pass through a 0.2-mm sieve, digested with HNO₃ and HClO₄ (4:1, v/v) and analyzed using inductively coupled plasma–optical emission spectrometry (ICP–OES, Optima 5300, Perkin–Elmer, USA). All assays were conducted in triplicate within the relative standard deviation (RSD) <10%. During measurement, blanks and standard solutions were measured every 20 samples with a good recovery (85–110%). Fat, protein, reducing sugar, and total sugar contents were measured according to methods (Fang et al., 2008; Sun et al., 2008) which is described in detail in the supporting information.

2.6 Analysis of soil microbial communities

After plant harvest, fresh soil samples were homogenized and sieved through a 3-mm sieve. DNA was extracted using MoBio Power Soil DNA Kit (MoBio Laboratories Inc., CA, USA) following the manufacturer's recommendation. The purity of extracted DNA was evaluated at A260/280 nm

in a Take3 microplate using a Synergy H4 Hybrid Multi-Mode Microplate reader (BioTek, VT, USA) prior to storage at -80 °C. Bacterial 16S rRNA genes were amplified by PCR from the DNA using V4 oligonucleotide primers specific for the domain of bacteria. The V4 hypervariable region for 16S rRNA genes was amplified using 563F and V4R cocktail, while the 16S rRNA V9 fragment was amplified using the primers 1380F and 1510R (Table S1) (Xie et al., 2016a and b). Sequencing adaptors were ligated to high quality pooled DNA using the Ion Xpress™ Plus Region Library Kit (Life Technologies, CA, USA). Purified products were quantified by the Qubit™ dsDNA HS Assay Kits (Invitrogen, CA, USA) and then adjusted to 10 ng/μL by using molecular grade water. All purified PCR products were pooled equally for subsequent sequencing. Sequencing adaptors were ligated to high quality pooled DNA by use of the Ion Xpress™ Plus Region Library Kit (Life Technologies, CA, USA). Before high-throughput sequencing, all amplicons were assessed for region size distribution and concentration through the use of a Bioanalyser 2100 (Agilent Technologies, CA, USA). The samples were adjusted to a final concentration of 26 pM and then attached to the surface of Ion Sphere particles (ISPs) by use of Ion OneTouch™ 200 Template Kit v2 DL (Life Technologies, CA, USA) according to the manufacturer's instructions. ISPs were enriched on the Ion OneTouch™ ES (Life Technologies, CA, USA). ISPs with template were sequenced on “318” micro-chips through the use of Ion PGM Sequencing 300 Kit under the Ion Torrent Personal Genome Machine (Life Technologies, CA,

USA) for 130 cycles (520 flows).

2.7 Statistical analyses

All PGM quality filtered sequence data were exported as fastq files and the PGM's adaptor sequences were automatically trimmed through the use of default parameter under ION server (version 3.6.2). Data analysis was performed by using the Quantitative Insights Into Microbial Ecology (QIIME) toolkit (Caporaso et al., 2010). Reads containing ambiguous 'N', homopolymer and reads length shorter than 150 bps were discarded. Operational Taxonomic Units (OTUs) were selected using the UPARSE pipeline with a sequence similarity cut-off of 97% (usearch software v7) (Edgar, 2010); chimeric sequences were also removed (Edgar et al., 2011). Sequences that did not align in the appropriate zone were removed. Sequences of each OTU were assigned to representative taxa using QIIME's parallel wrappers for RDP classifier (Wang et al., 2007). Alpha rarefaction was performed using Phylogenetic Diversity, Chao1, and observed species metrics.

The data were expressed as mean $\pm$ standard deviation ($n = 3$). Statistical significance of differences among treatments were determined using one-way analysis of variance and covariance (ANOVA), followed by Tukey's pair-wise comparisons at a significance level of 0.05.

3. Results and discussion

3.1 TiO₂ nanoparticles internalization in rice seedlings

TEM images showed some nanosize dark particles in cortex cells of roots in ambient-CO₂-level plots (hereafter called ambient plots, named AC for plots without nanoparticles and AT for plots with nanoparticles) exposed to *n*TiO₂ at 200 mg kg⁻¹ (AT200) and in elevated-CO₂-level plots (hereafter called FACE plots) exposed to *n*TiO₂ at 50 and 200 mg kg⁻¹ (FT50 and FT200, Fig. 1c, e and f). In contrast, no obvious nanoparticles or aggregates were found in TEM images of rice roots exposed to *n*TiO₂ at 50 mg kg⁻¹ in ambient plots (AT50, Fig. 1b). Root cells from control plants (no nanoparticles, AC and FC) did not show particles, as expected (Fig. 1a, d). Ti distribution pattern obtained using SEM with EDS analysis was semiquantitative, but still it showed a difference in Ti distribution pattern in root tissues between ambient plots and FACE plots. In comparison with control plants, Ti was internalized in ambient exposed (AT) roots and FACE exposed (FT) roots (Fig. S1). In AT roots, Ti was mainly located in the parenchyma region. In FT roots, it was primarily detected in epidermal cells. Such difference between AT plots and FT plots in Ti distribution in root cross section was mainly attributed to nanoparticle agglomeration status during plant exposure (Larue et al., 2012), indicating more aggregation of *n*TiO₂ in FT plots. Elevated CO₂ levels decrease the soil pH (Guo et al., 2011), leading to lower surface charge and more negativity of the ζ potential, resulting in the aggregation of *n*TiO₂ (Solovitch et al., 2010). TEM images of root epidermal cells support this hypothesis (Fig. 1g-l). More nanoparticles were observed adhered out of cell wall in the root epidermis of

FT plants (Fig. 1k, l), compared with AT (Fig. 1h, i). Nanoparticles adhered to root surface might cause changes in the microenvironment of the contact area, affecting root exudation or increasing extracellular reactive oxygen species (ROS) which could damage cell membranes (Du et al., 2011). More nanoparticles adhered to root epidermis revealed that FT plants might be more vulnerable to stress damage by nanoparticles.

Since $n\text{TiO}_2$ was detected in root vascular cylinder, it was expected to translocate from roots to leaves with the transpiration stream (Laurette et al., 2012). To test this hypothesis, TEM images were taken from leaf samples. Images showed changes in organelles but no nanoparticles or aggregates (Fig. 2). In AT50 and AT200, $n\text{TiO}_2$ alone did not cause changes in organelles (Fig. 2b and c). However, leaves in ambient plots showed an accumulation of starch granules, unlike the leaves from FACE plots. Photosynthesis is closely related to the amount of starch accumulated in leaf (Nakano et al., 2000; Sawada et al., 2001). Starch accumulation may alter the normal structure and function of chloroplasts by disrupting the structure of thylakoids and grana, resulting in interrupted intracellular CO_2 transport, and downregulation of photosynthesis (Hao et al., 2013; Nafziger and Koller, 1976). Besides, in rice leaves from FT, the chloroplasts were swollen and thylakoids were bent and loosely arranged (Fig. 2e and f), compared with those from FC (Fig. 2d). These are taken as non-specific stress symptoms of chloroplasts under toxicity stress, which suggest important disturbances in metabolic functions of these organelles.

This might result in a lower photosynthesis rate and reduction of biomass (Baszynski et al., 1980; Rufner and Barker, 1984; Panou-Filothéou et al., 2001; Senser et al., 1975). Hence, in comparison with ambient plots, the photosynthetic rate of rice chloroplasts in FACE plots might increase, leading to a final increase in biomass. But, elevated CO₂ levels could also modify the effects of *n*TiO₂ by changing its aggregation status, resulting in unpredictable changes in the final biomass and physiology of FT plants.

3.2 Effects on rice biomass and yield

The biomass and yield were measured in mature rice plants (Table 1). Comparing AC with FC, it can be seen that CO₂ levels alone did increase the grain yield of rice, mainly due to an increase in number of grains. AT caused no significant effect on biomass or grain yield when compared to AC. However, FT led to significantly lower biomass and grain yield (decreased biomass by 17.9% (FT50) and 22.1% (FT200) and grain yield by 20.8% (FT50) and 44.1% (FT200)), compared to FC. Exposure to *n*TiO₂ and CO₂ led to lower grain yield, associated with lower individual grain mass. In addition, there was a significant decrease in individual grain mass in FT200 plots. However, there was no significant difference in biomass and grain yield between FT and AT plants, revealing that the increase in biomass by elevated CO₂ levels was likely offset by the increased toxicity of *n*TiO₂, as previously hypothesized. Results suggest that elevated CO₂ levels potentiate the adverse effect of *n*TiO₂ on rice growth and yield.

3.3 Effects on rice nutritional quality

No significant difference in Ti contents was found between AC and AT50, FC and FT50 (Fig. 3). A comparison of rice from ambient plots with that from FACE plots showed that elevated CO₂ levels did not affect Ti content in different rice tissues. On the other hand, AT200 had significantly more Ti in leaves and roots, compared with AC. FT200 had significantly more Ti in seeds, stems, and roots, compared with FC. The accumulation of chemical elements (e.g., Ti uptake from *n*TiO₂ treatment) in plants, especially in edible tissues or reproductive organs, is an important aspect of nanoparticle fate and may have implications for human health and agricultural productivity (Gardea-Torresdey et al., 2014). Very few studies have described the presence of *n*TiO₂ in fruits or seeds (Jacob et al., 2013; Song et al., 2013). Song et al. (2013) reported high Ti in tomato fruit, while Jacob et al. (2013) recorded minimal Ti in grains of bean and wheat plants exposed to *n*TiO₂. In this study, a higher Ti concentration was found in rice grains from FT200 plots, which might be due to decreased individual grain mass (Table 1).

Effects of *n*TiO₂ on nutrient contents in rice grain at ambient/elevated CO₂ levels are shown in Table 2. Compared with AC, FC significantly increased protein content and reducing sugar, while AT200 decreased protein and total sugar, but increased the content of reducing sugar in grains. On the other hand, grains from both FT plots had lower fat, protein, and total sugar, but higher reducing sugar contents. The data suggest that elevated CO₂ levels and *n*TiO₂

alone did interfere with protein, sugar, or fat synthesis in rice. This interference is potentiated by a combination of both factors.

Previous reports have described the effects of CO₂ on grain quality of food crops, especially reduction in the concentration of proteins (Kimball et al., 2001; Taub et al., 2008). This might be due to a decrease in Rubisco concentration (Ainsworth and Long, 2005) or dilution of protein by an increase in nonstructural carbohydrate content (Gifford et al., 2000). On the other hand, nanoparticles could be implicated in modifications of proteins, sugar, or lipid levels in crop plants, when the mechanisms of toxicity are not clear yet (Dijkstra et al., 2005; Rico et al., 2013, 2014). It is possible that nTiO₂ affected the gene expression of protein synthesis and carbohydrate metabolism involved in biosynthesis and conversion of sugar during grain development (Rico et al., 2013). In addition to nutritional contents, most of essential elements in rice grains were not affected by CO₂ or nTiO₂ alone. However, there were significant increases in P, Mg, Ca, Mn and Zn contents in grains from FT200 (Table 3). An Increase in grain essential elements could be due to increases in availability of soil metals at elevated CO₂ levels (Guo et al., 2011), as well as decreased individual grain mass of FT200 (Table 1). Taken together, these findings strongly suggest that the combination of elevated CO₂ and nTiO₂ would cause significant modifications in the nutritional quality of rice, with unknown implications to the food chain and human health.

3.4 Effects on soil microbial communities

Previous studies have shown that, depending on exposure concentration and mode of application, nanoparticles may have no effect or adverse effects on soil microbial communities (Ge et al., 2014). Beneficial or adverse effects of nanoparticles depend on the concentration, living organisms, and environmental conditions including CO₂ concentration (Yadav et al., 2014). In this study, the structure of bacterial communities was diverse among samples, and a total of 3,500 different OTUs were identified (Fig. 4a and c). The effects of either elevated CO₂ levels or nTiO₂ were not statistically significant with regards to the PD and Shannon indices, suggesting that elevated CO₂ levels or nTiO₂ alone had little or no effect on the diversity and richness of bacterial communities. *Proteobacteria*, *Actinobacteria*, *Acidobacteria* and *Chloroflexi* (Fig. 5a) dominated across all samples. While elevated CO₂ levels showed significant effects on *Actinobacteria* and *TM7*, nTiO₂ (at 200 mg kg⁻¹) significantly affected *Acidobacteria* and *Gemmatimonadetes* in both ambient and FACE plots. Besides, *TM7*, *Nitrospirae* and *Chlorobi*, which were not affected by nTiO₂ in AT soils, showed changes in soils of FT200 (Fig. 5a). Although the richness or diversity of the soil bacterial community (as shown in Fig. 4) were not affected by elevated CO₂ or nTiO₂, changes in the composition of the soil bacterial community might be due to the effects of elevated CO₂ or nTiO₂ on plant growth. This could change the quality and composition of plant root exudates (Ge et al., 2014).

The structure of the protistan communities was diverse among samples and

a total of 2,395 different OTUs were identified (Fig. 4b and d). Either elevated CO₂ levels or nTiO₂ alone did not cause significant change in PD values, suggesting little or no changes in its diversity. Compared with FC, soil protist from FT200 plots showed significant decrease in PD values, indicating lower diversity. On the other hand, either elevated CO₂ levels or nTiO₂ (at 50 and 200 mg kg⁻¹) alone caused significant changes in Shannon indices, suggesting modification in richness of protista. Protistan communities were dominated across all samples by *Chloroplastida*, *Fungi*, *Rhizaria* and *Alveolata* (Fig. 5b). Elevated CO₂ levels showed significant effects on *Chloroplastida*, *Fungi*, *Alveolata* and *Discoba*, while nTiO₂ (at 50 mg kg⁻¹) showed significant effects on *Chloroplastida* and *Fungi* in both ambient and FACE soils. *Rhizaria*, *Discoba* and *Discosea*, which were not affected by nTiO₂ in AT soils, showed changes in FT soils (Fig. 5b). Impacts of nTiO₂ alone on soil bacteria communities might be due to direct toxicity (Piccinno et al., 2012). However, the combined effect of nTiO₂ with elevated CO₂ levels was difficult to predict because previous reports have shown responses ranging from significant changes to no effect on soil microbes to elevated CO₂ levels (Hayden et al., 2012). In this study, elevated CO₂ levels modified the effects of nTiO₂ on microbial communities, mainly by affecting specific phyla of bacteria and the diversity and richness of protista. The data also showed that elevated CO₂ levels changed the agglomeration status of nanoparticles as well as organic matter (root exudates) in soil, most likely affecting microbial enzyme synthesis. This may lead to modifications in the dynamics of C and N cycles, resulting in changes in plant growth and yield (Guenet et al., 2012), as shown in Table 1.

4. Conclusions

In summary, this study has shown that elevated CO₂ levels increased the aggregation of *n*TiO₂ on the rice root surface, resulting in different responses of the plants and soil microbial communities. Exposure of rice to *n*TiO₂ in CO₂ enriched paddy soil resulted in altered growth, yield, and grain nutritional contents. In addition, the combination of both factors altered the structural and functional composition of soil microbial communities. This suggests that the inconsequential effect of nanoparticles on natural systems at current CO₂ level may become markedly different given the effects on the nutritional quality of crops and function of soil microbial communities under future CO₂ level, with unknown implications for future economics and human health.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online

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Figure captions

Fig. 1. TEM images of rice root tips grown under ambient (370 $\mu\text{mol mol}^{-1}$) or elevated (570 $\mu\text{mol mol}^{-1}$) CO₂ levels with or without *n*TiO₂. (a, g), Ambient control. (b, h), Ambient with 50 mg kg⁻¹ *n*TiO₂. (c, i), Ambient with 200 mg kg⁻¹ *n*TiO₂. (d, j), FACE control. (e, k), FACE with 50 mg kg⁻¹ *n*TiO₂. (f, l), FACE with 200 mg kg⁻¹ *n*TiO₂. (a, b, c, d, e, f) cortex cells. (g, h, i, j, k, l) epidermal cells. O, outside; cw, cell wall; cy, cytoplasm; arrows point to particles.

Fig. 2. TEM images of chloroplast in rice leaves grown under ambient (370 $\mu\text{mol mol}^{-1}$) or elevated (570 $\mu\text{mol mol}^{-1}$) CO₂ levels with or without *n*TiO₂. (a), Ambient control. (b), Ambient with 50 mg kg⁻¹ *n*TiO₂. (c), Ambient with 200 mg kg⁻¹ *n*TiO₂. (d), FACE control. (e), FACE with 50 mg kg⁻¹ *n*TiO₂. (f), FACE with

200 mg kg⁻¹ nTiO₂. Ch, chloroplast; s, starch grain; white arrow indicates thylakoids; black arrow indicates plastoglobuli.

Fig. 3. Ti concentration in harvested rice tissues from rice grown under ambient (370 µmol mol⁻¹) or elevated (570 µmol mol⁻¹) CO₂ levels with or without nTiO₂. A, ambient; F, FACE. Data are means of three replicates ± standard deviation. Different letters among columns indicate statistically significant differences in Ti content at $p \leq 0.05$.

Fig. 4. Phylogenetic diversity and richness (Shannon indice) of soil microbial communities. a, phylogenetic diversity of bacterial community. b, phylogenetic diversity of protistan community. c, richness of bacterial species. d, richness of protistan species. AC, ambient (370 µmol mol⁻¹) control; FC, FACE (570 µmol mol⁻¹) control; ATi50, ambient with 50 mg kg⁻¹ nTiO₂; FTi50, FACE with 50 mg kg⁻¹ nTiO₂; ATi200, ambient with 200 mg kg⁻¹ nTiO₂; FTi200, FACE with 200 mg kg⁻¹ nTiO₂.

Fig. 5. Composition at phyla levels of soil microbial communities. a, composition of bacteria at phyla levels. b, composition of protistans at phyla levels. AC, ambient (370 µmol mol⁻¹) control; FC, FACE (570 µmol mol⁻¹) control; ATi50, ambient with 50 mg kg⁻¹ nTiO₂; FTi50, FACE with 50 mg kg⁻¹ nTiO₂; ATi200, ambient with 200 mg kg⁻¹ nTiO₂; FTi200, FACE with 200 mg kg⁻¹ nTiO₂.

Table 1. Biomass and yield of rice crops harvested in control soil and soil amended with $n\text{TiO}_2$ at 50 mg kg^{-1} and 200 mg kg^{-1} at ambient ($370 \mu\text{mol mol}^{-1}$) or elevated ($570 \mu\text{mol mol}^{-1}$) CO_2 levels.

	Ambient			FACE		
	0	50	200	0	50	200
Tiller no.	18.3±2.9 a	18.2±1.5 a	17.5±2.2 a	19.7±2.3 a	18.0±1.8 a	17.3±2.3 a
AGB (g)	80.7±8.5 a	80.6±7.5 a	79.1±8.0 a	103.1±2.9 b	85.0±10.0 a	83.3±13.5 a
BGB (g)	10.7±1.2 a	11.4±0.7 a	11.1±1.8 a	14.5±1.6 b	11.6±1.6 a	11.8±1.4 a
TB (g)	91.4±9.2 a	92.0±7.8 a	90.3±9.6 a	117.6±3.3 b	96.6±11.5 a	91.6±15.3 a
Grain no.	76.4±10.0 a	87.4±19.7 ac	84.0±14.9 ac	96.0±12.8 bc	97.1±8.1 bc	79.6±18.3 ac
IGM (mg)	26.7±2.0 a	25.3±3.3 a	26.3±3.0 a	25.1±1.0 a	25.3±0.9 a	17.7±2.4 b
HI	0.44±0.03 abc	0.40±0.08 abc	0.44±0.02 abc	0.47±0.04 b	0.45±0.02 abc	0.35±0.12 c
Yield (g)	40.4±5.2 a	36.8±9.5 a	39.2±4.1 a	55.3±4.8 b	43.8±6.3 a	30.9±14.8a

Grain biomass and yield were determined in all plants from each pot. HI harvest index, TB total biomass, AGB aboveground biomass, BGB belowground biomass, IGM individual grain mass, FACE free-air CO_2 enrichment. Data are means of three replicates $\pm$ standard deviation. Different letters among columns indicate statistically significant differences at $p \leq 0.05$.

Table 2. Nutritional contents (mg kg^{-1}) in rice grains harvested in control soil and soil amended with $n\text{TiO}_2$ at 50 mg kg^{-1} and 200 mg kg^{-1} at ambient ($370 \mu\text{mol mol}^{-1}$) or elevated ($570 \mu\text{mol mol}^{-1}$) CO_2 levels.

	$n\text{TiO}_2$	0	50	200
Fat	Ambient	$22.9 \pm 1.3 \text{ abc}$	$22.4 \pm 0.7 \text{ ab}$	$21.2 \pm 0.3 \text{ abc}$
	FACE	$21.5 \pm 1.0 \text{ ac}$	$21.3 \pm 0.4 \text{ c}$	$19.1 \pm 0.6 \text{ d}$
Protein	Ambient	$104.4 \pm 1.0 \text{ a}$	$104.5 \pm 0.8 \text{ a}$	$101.3 \pm 1.5 \text{ b}$
	FACE	$111.4 \pm 0.4 \text{ c}$	$100.4 \pm 0.6 \text{ d}$	$110.7 \pm 0.6 \text{ c}$
Total sugar	Ambient	$816.0 \pm 16.5 \text{ a}$	$780.8 \pm 48.8 \text{ ab}$	$760.5 \pm 13.0 \text{ b}$
	FACE	$797.0 \pm 14.6 \text{ a}$	$752.9 \pm 15.3 \text{ b}$	$593.1 \pm 72.4 \text{ c}$
Reducing sugar	Ambient	$8.9 \pm 0.8 \text{ a}$	$8.9 \pm 0.4 \text{ a}$	$11.8 \pm 1.1 \text{ b}$
	FACE	$11.5 \pm 0.8 \text{ b}$	$12.9 \pm 0.9 \text{ b}$	$12.6 \pm 0.2 \text{ b}$

Different letters among columns indicate statistically significant differences at $p \leq 0.05$.

Table 3. Nutritional element contents (mg kg⁻¹) in rice grains harvested in control soil and soil amended with *n*TiO₂ at 50 mg kg⁻¹ and 200 mg kg⁻¹ at ambient (370 µmol mol⁻¹) or elevated (570 µmol mol⁻¹) CO₂ levels.

	<i>n</i> TiO ₂	0	50	200
K	Ambient	3464.5±279.1 a	3517.5±382.7 a	3721.7±235.8 a
	FACE	3502.9±218.6 a	3749.4±364.9 a	3751.1±145.6 a
P	Ambient	3064.9±283.4 a	3351.7±177.2 a	3164.9±115.8 a
	FACE	3250.1±194.7 a	3366.1±306.5 ab	3490.9±213.4 b
Mg	Ambient	1093.0±89.0 a	1211.8±67.9 a	1166.8±55.5 a
	FACE	1180.1±96.8 a	1246.7±129.8ab	1379.3±103.0 b
Ca	Ambient	255.0±21.2 a	272.4±22.8 a	266.1±18.5 a
	FACE	273.0±14.8 a	275.3±19.3 a	334.3±39.7 b
Mn	Ambient	39.8±3.8 a	39.2±4.6 a	39.8±3.2 a
	FACE	44.3±8.3 a	43.6±8.3 a	57.2±9.0 b
Fe	Ambient	32.9±8.1 a	27.1±4.1 a	31.9±10.8 a
	FACE	37.4±8.2 a	30.8±4.8 a	33.0±5.8 a
Zn	Ambient	23.8±3.0 a	24.9±1.1 a	24.2±1.4 a
	FACE	26.6±1.6 a	26.6±2.0 a	30.5±2.2 b
Cu	Ambient	4.8±0.4 a	4.4±0.4ab	4.8±0.4 a
	FACE	4.8±0.4ab	4.3±0.3 b	4.9±0.5 a

Different letters among columns indicate statistically significant differences at $p \leq 0.05$.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.