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Response of soil enzyme activity and bacterial community to black phosphorus nanosheets

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Environmental significance

With increasing production and application of black phosphorus (BP) nanosheets, they will inevitably be released into the environment. However, the potential ecological risk of BP nanosheets is barely known. Herein, we explored the effect of BP nanosheets on enzyme activity, bacterial community structure, and bacterial function of black soil and burozem. Slightly short-term or negligible disturbance was observed in the soils following exposure to BP nanosheets. Compared to black soil, burozem was more sensitive to BP nanosheets, because the rich organic matter in black soil could interact with BP nanosheets and attenuate the bioavailability. This study provided a preliminary understanding of the impacts of BP nanosheets on soil ecological environment.

Abstract

Currently, black phosphorus (BP) is widely applied in a variety of fields. Concerning the potential toxicity of BP nanosheets towards mammalian cells and bacteria, there is a great need to evaluate their ecological effects. This study was focused on the effect of BP nanosheets on enzyme activity, bacterial community structure, and bacterial function of black soil and burozem. We found BP nanosheets had no significant impact on enzyme activities in black soil during the 60-day exposure. In contrast, the activities of urease and catalase in burozem were significantly inhibited at day 10 and day 30, respectively, which were gradually recovered at day 60. The introduction of BP nanosheets significantly decreased the community richness in burozem at day 10, but had no effect either on the diversity or the richness in black soil. The discrepancies were attributed to the stronger interaction of BP nanosheets with organic matter-rich black soil, leading to a lowered bioavailability of BP nanosheets. Nevertheless, BP nanosheets were predicted to have negligible effect on bacterial function in both soils. It is worthwhile to further investigate the in-depth mechanisms and dose-dependent effect in order to lay the foundation for safe application and discharge of BP nanosheets.

Introduction

As newly emerging two-dimensional (2D) nanomaterials in the post-graphene age, black phosphorus (BP) nanosheets have some superior features compared to graphene and other 2D nanomaterials,[1,2,3] which endows them with great performance in the applications of optoelectronic devices,[4,5] theranostics,[6] and clean energy.[7] In addition, BP nanosheets have are effective adsorbents for ionic organic pollutants,[8] rendering them applicable to environmental remediation. Very recently, the production cost of high-quality BP has been reduced to 0.235 Euro per gram, which opens up possibilities for their extensive applications in the future.[9, 10] Therefore, BP nanosheets will inevitably be released into the environment in the process of production and application. Given that BP nanosheets exhibited negative effects on mammalian cells and bacteria by generating intracellular oxidative stress and destroying membrane integrity,[11, 12] it is essential to evaluate the impact of BP nanosheets on the ecological environment before their massive production and application.

Soil environment is considered to be the ultimate sink for nanomaterials.[13] Soil microorganisms, as the basic components of soil ecosystems, play an important role in soil biological processes[14] and ecological functions, such as biogeochemical cycling, plant productivity, and climate regulation.[15] Soil enzyme activity and microbial community are sensitive to the environmental perturbations, which, hence, serve as ecological indicators of soil response to nanomaterials.[16,17] It has been reported that some graphene-family nanomaterials imposed moderate effects on the soil bacterial community.[18] Johansen *et al.* demonstrated fullerene (C_{60}) at a dose of 50 mg kg^{-1} exerted a negative effect on the fast-growing soil bacteria.[19] In addition, TiO_2 and ZnO nanoparticles were reported to reduce soil bacterial biomass and diversity.[20] Compared to these nanomaterials, BP nanosheets possess some special features in terms of electrochemical property, surface characters, and degradability, which may influence their ecological effects. Moreover, the soil properties, such as soil texture, organic matter content, and moisture content, have been shown to potentially

influence the sensitivity of soil microbial community to the nanomaterials.[21, 22] For example, the bacterial community was more susceptible to metal oxide nanoparticles in soils containing less organic matter and clay fraction.[22]

Herein, the effects of BP nanosheets on enzyme activity and bacterial community structure in black soil and burozem were evaluated. Bacterial metabolic activity was assessed based on soil enzyme activity. The response of soil bacterial abundance to the exposure of BP nanosheets was monitored by real-time quantitative polymerase chain reaction (qPCR). Illumina MiSeq sequencing provided detailed information regarding to the changes of bacterial composition upon BP exposure. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) method[23,24] was used to predict the influence of BP nanosheets on function of soil bacterial community. This study provides fundamental data for the ecological risk assessment of BP nanosheets, which will guide their safe application in the future.

Materials and methods

Materials

Bulk BP crystals (99.998%) were obtained from XFNANO Materials Tech Co. Ltd. (Nanjing, China) and stored in nitrogen atmosphere under dark condition. BP nanosheets were prepared by the previously published method (details in the supplementary information).[11] Black soil and burozem were collected in Heilongjiang and Liaoning Province, and classified as Vertisol and Entisol, respectively, according to the United States Department of Agriculture (USDA) soil taxonomy.[25] Soil physicochemical properties were listed in **Table 1**. SYBR Green I was purchased from Takara Biomedical Technology Co., Ltd. (Beijing, China). Phosphatase kit was bought from Shanghai Yunchun Bio-tech Inc. (Shanghai, China).

Soil enzyme activity

After being exposed to various concentrations of BP nanosheet (0, 10, and 50 mg

kg^{-1}), 50 g soil was collected from each sample at day 0, 10, 30, and 60, respectively, to study the effect of BP nanosheets on soil enzyme activities. Urease and acid phosphatase were selected, since they are involved in the biogeochemical cycles of nitrogen and phosphorus.[26] Moreover, catalase activity was also analyzed, because it is regarded as a general indicator of soil microbial activity and soil health.[27] The soil urease activity was measured by phenol-hypochlorite assay.[28,29] Briefly, soil sample (5 g) was mixed with methylbenzene (1 mL) and standing at room temperature for 15 min. Then 10 mL carbamide (10 wt%) and 20 mL citrate buffer (pH = 6.7) were added and the mixture was incubated at 37 °C for another 24 h. Afterwards, 1 mL of supernatant was mixed with ethanol solution of sodium phenol (4 mL, 1.35 mol L^{-1}), sodium hypochlorite (3 mL, 0.9 wt%), and deionized water (42 mL). After standing for 20 min at room temperature, the soil urease activity was quantified by measuring the UV absorbance at 578 nm with a double beam UV-vis Spectrophotometer TU-1901 (PERSEE, China). The urease activity was expressed as the released mass (mg) of NH_4^+-N from 1 g dry soil during 24 h. Soil catalase activity was measured by permanganate titration method.[28, 30, 31,] Soil sample (5 g) was mixed with 0.5 mL of methylbenzene and kept at 4 °C for 30 min. Then 25 mL of cold H_2O_2 solution (3 wt%) were added immediately and incubated for 1 h at 4 °C, followed by the addition of 25 mL of cold H_2SO_4 (2 mol L^{-1}). Afterwards, 1 mL supernatant, 5 mL deionized water, and 5 mL H_2SO_4 were mixed together and titrated with potassium permanganate solution (0.02 mol L^{-1}). The titration difference between the treatment group and control was measured to determine the catalase activity. The catalase activity was expressed as the consumed volume (mL) of 0.1mol/L $KMNO_4$ from 1 g dry soil during 1 h. According to the pH of different soil types, acid soil phosphatase in black soil and neutral soil phosphatase in Burozem were detected[32] by the phosphatase kit (Yuchun Bio-tech Inc., China) to evaluate the influence of BP nanosheets on the biogeochemical cycles of phosphorus. After incubating the mixture of soil (1 g) and methylbenzene (0.5 mL) overnight, the sample was prepared following the instruction and the phosphatase activity was measured by a UV-vis Spectrophotometer at a wavelength of 660 nm. The phosphatase activity was

expressed as the released volume (mL) of 1 nmol/L Phenol from 1 g dry soil during 24 h. The enzyme activities of soils without any treatment were listed in Table S2. The enzyme activities of testing groups were normalized to that of control group (day 0)[30], representing as enzyme activity (%) = enzyme activity (BP-treatment)/enzyme activity (control)×100%。

DNA extraction and quantification

DNA was extracted from the soil samples using benzyl chloride.[33] In brief, 0.5 g soil samples collected at a predetermined time point (day 0, 10, 30, 60) were mixed with 0.5 mL extraction buffer (100 mM Tris-HCl and 50 mM EDTA, pH=9.0) and 1 mL TENP buffer (50 mM Tris, pH 8.0; 20 mM EDTA, pH 8.0; 100 mM NaCl; 1% polyvinyl pyrrolidone), centrifuged at 12000 rpm for 3 min at 4 °C, followed by decanting the supernatant. The process was repeated for 3 times to thoroughly remove the humus. Afterwards, 50 μL SDS (20 wt.%), 300 μL benzyl chloride, 300 μL potassium acetate, 600 μL Tris-saturated phenol, 600 μL chloroform-isoamyl alcohol, 600 μL isopropanol, 800 μL ethanol (70 wt.%), and 30 μL TE buffer were sequentially added. The concentration of total DNA was quantified by Nanodrop 2000c (Thermo Scientific, USA) based on the characteristic absorption at 260 nm. The DNA integrity was evaluated by agarose gel electrophoresis.

Quantification of the abundance of soil total bacteria

The abundance of soil total bacteria was measured based on 16S rRNA gene copy numbers by real-time qPCR (ABI 7500, USA). The V3 region of the bacterial 16S rRNA gene was amplified using PCR amplification instrument by the following program: denaturation at 95 °C for 30 s, 35 cycles of denaturation at 95 °C for 5 s, and annealing at 59 °C for 34 s using the primers 338F (5'-CCTACGGGAGGCAGCAG-3') and 518R (5'-ATTACCGCGGCTGCTGG-3').[34] The dissolution curve was detected by the following program: 95 °C for 15 s and descending to 60 °C at 0.2 °C s^{-1}, during which

the fluorescence signal was collected, kept at 60 °C for 60 s, and then 95 °C for another 15 s. The amplification reactions were performed in 20 μL of mixtures containing: 10 μL SYBR Premix Ex Taq II, 0.4 μL ROX Reference Dye II, 1 μL template DNA, 0.8 μL each primer (2.5 μM), and 7 μL double-distilled H_2O (ddH_2O). The mixture containing all the PCR reagents except DNA templates was set as negative control to test the potential DNA contamination and primer dimer formation. The standard curves for black soil and burozem were constructed to be $y = -2.399x + 19.56$ ($R^2 = 0.988$) and $y = -4.833x + 21.39$ ($R^2 = 0.975$), respectively, by serial 10-fold dilutions according to the reported method.[35] The gene copy numbers of black soil and burozem at day 0 were 6.06 ± 0.91 and 8.72 ± 0.59 ($\times 10^{12}$ copies g^{-1} dry soil), respectively.

Illumina MiSeq high-throughput sequencing

The collected soil samples were sent to Personalbio Co., Ltd. (Shanghai, China) for high-throughput sequencing on the Illumina MiSeq platform. The PCR amplification of soil DNA was based on 16S rRNA gene using the primers 338F (5'-barcode+ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The barcodes are seven-base-pair sequences in the primer 338F to distinguish different samples in the same library. After sequencing, the raw reads were deposited in the NCBI Sequence Read Archive (SRA, https://submit.ncbi.nlm.nih.gov/subs/sra/) database (Accession number: SRP184895).

Data analyses

In order to get high quality sequences, Qiime (version 1.9.0) was used to filter and process the original sequence.[36] After removing the PCR chimeras, the high quality sequences were clustered into operational taxonomic unit (OTU > 97% sequence similarity). The raw data was standardized in order to eliminate the difference of sequencing depth, so that all comparisons were performed at the same sequencing depth (< 9082 sequence counts). The alpha diversity and OTU taxonomic

classification were analyzed by Qiime. Principal component analysis (PCA) was performed by R programming language. The Spearman rank correlation coefficient among the dominant genera with the abundance in the top 50 was calculated. The correlation networks between related dominant genera (which $\rho > 0.8$ and $P < 0.05$) and metabolic pathways at Kyoto Encyclopedia of Genes and Genomes (KEGG) database were visualized by Cytoscape (http://www.cytoscape.org/). Bacterial function prediction was conducted by PICRUSt. All the experiments were carried out triplicates and one-way analysis of variance (ANOVA) followed by Tukey test was proceeded in order to evaluate the statistical significance. In the diversity analysis, the soil samples were abbreviated following the sequence of soil type - acquisition time - concentration of BP nanosheets.

Results and discussion

Effect of BP nanosheets on soil enzyme activity

The activities of urease, catalase, and phosphatase in black soil and burozem were monitored after exposure to BP nanosheets for different time intervals. No significant effect of BP nanosheets on the enzyme activities in black soil was observed over the entire experimental period of 60 days **(Figure 1a, c, e)**. In contrast, BP nanosheets posed a significant effect on the activities of urease and catalase, but not on neutral phosphatase, in burozem **(Figure 1b, d, f)**. The urease activity was reduced by 17.05% and 23.26% at day 10 after exposure to BP nanosheets at a dose of 10 and 50 mg kg^{-1} ($P < 0.05$), respectively, which was fully recovered at day 60 **(Figure 1b)**. On the contrary, the catalase activity was increased by 15.52% ($P < 0.05$), when burozem was treated with 50 mg kg^{-1} of BP nanosheets for 10 days **(Figure 1d)**. However, a sharp decrease of catalase activity was observed at day 30, which was partially recovered to 81.46% and 85.37% of control at BP dosages of 10 and 50 mg kg^{-1}, respectively, at day 60. According to our previous study, BP nanosheets could enhance the level of reactive oxygen species (ROS) in the bacterial cells,[37] which may exert negative effects on the soil bacteria, so that the urease activity was inhibited. Since catalase was regarded as one of the major defense units against ROS,[38] the

catalase activity was enhanced at day 10 in order to eliminate the excess ROS in soil environment. Similarly, Servin et al. reported that the catalase activity in plants was increased upon $nTiO_2$ exposure to protect chloroplast membranes from ROS attack.[39] However, catalase was overburdened to maintain the microbiological quality of burozem, so its activity was decreased to 24.14% and 20.69% at day 30 at BP dosages of 10 and 50 mg kg^{-1}, respectively **(Figure 1d)**. Interestingly, the activities of both urease and phosphatase in the testing groups were comparable to that in the control group at day 60, indicating that the effect of BP nanosheets on the enzyme activities in burozem was not permanent. This was, on the one hand, attributed to the degradation of BP nanosheets at ambient condition over time, and on the other hand, due to the accommodation of bacterial function during BP exposure.

The physiochemical properties of soil, including texture, organic matter, pH, etc., are important factors to influence enzyme activity of nanoparticle-treated soils.[40] In our study, the enzyme activities in burozem were more sensitive to BP nanosheets compared to that in black soil. Moreover, BP nanosheets showed stronger interaction with black soil than burozem (Figure S1), which can be attributed to the higher content of organic matter in black soil (**Table 1**), resulting in the decreased bioavailability of BP nanosheets in black soil.

Soil bacterial abundance

During the 60 days, the gene copy numbers showed no significant difference between the BP-treated groups and control group for both soil types **(Table 2)**, indicating the exposure of BP nanosheets had limited effect on soil bacterial biomass.

Based on the data of high-throughput sequencing, the effect of BP nanosheets on the bacterial community was further analyzed with respect to the relative abundance at the phyla and genus levels. The dominant phyla including *Actinobacteria*, *Proteobacteria*, *Acidobacteria*, *Chloroflexi*, *Gemmatimonadetes*, *Nitrospira*, and *Planctomycetes* accounted for > 85% of the total bacteria species in both soil types **(Figure 2a and b)**, among which most phyla were not sensitive to the exposure of BP

nanosheets. When BP nanosheets were introduced into the black soil, the relative abundance of *Acidobacteria* slightly increased from 7.61 ± 0.71% to 11.35 ± 1.34% ($P < 0.05$) at day 60 **(Figure 2a and c)**, which was due to the decreased pH during BP exposure (Table S1), since it has been reported that acidic pH is favorable for the growth of *Acidobacteria* in both laboratory culture[41] and soil environment.[42] Moreover, the relative abundance of *Actinobacteria* in the BP-treated black soil was significantly higher than control at day 10 ($P < 0.05$). Phosphatase encoding gene (*pho D*) mainly existed in *Actinobacteria*,[43] and thus the growth of *Actinobacteria* may be stimulated by the degradation of BP nanosheets. However, the growth-promoting effect vanished at day 60 **(Figure 2c)**. As for burozem, the relative abundance of *Bacteroidetes* was mildly increased from 1.62 ± 0.26% to 2.25 ± 0.29% ($P < 0.05$) after the exposure of BP nanosheets for 60 d **(Figure 2d)**. In addition, BP nanosheets inhibited the growth of *Latescibacteria* with the relative abundance decreased from 1.17 ± 0.06% to 0.95 ± 0.10% ($P < 0.05$) **(Figure 2d)**. Unlike in black soil, no significant change of *Acidobacteria* abundance was observed in burozem. The constant neutral pH in burozem during BP exposure could be the reason for the unchanged *Acidobacteria* abundance.[41]

Similarly, the relative abundance of most bacteria at the genus level was also not significantly affected by BP nanosheets. At day 60, the relative abundance of *Pseudomonas* in the BP-treated sample was higher than control in both soils **(Figure 3c and d)**. The most abundant genus in the *Acidobacteria* phylum, *RB41*, exhibited slightly increased abundance in the BP-treated black soil (3.37 ± 1.07%) compared to control (1.72 ± 0.21%) at day 60 **(Figure 3c)**, which contributed to the abundance enhancement of *Acidobacteria* **(Figure 2c)**. In contrast, the relative abundance of *RB41* showed no significant difference between BP-treated soils and control in burozem, indicating that the sensitivity of *RB41* to BP nanosheets was distinct in different soil types. Some phosphorus metabolism bacteria, such as *Gemmatimonas*[44] and *Bacillus*,[45] showed different responses to BP nanosheets in different soil types **(Figure 3c and d)**. For example, BP nanosheets promoted the growth of *Bacillus* in

burozem, but inhibited it in black soil at day 60. The in-depth mechanism will be further studied in terms of bacteria metabolism and stress tolerance.

Bacterial diversity and community structure

The Illumina sequencing data of 30 soil samples containing 1,174,366 sequences clustered into 377,188 operational taxonomic units (OTUs) at a 97% similarity level. Base on the OTU level, we calculated the richness and diversity indices (ACE, Chao1, and Shannon), as shown in **Figure 4** and Table S3. All the indicators demonstrated BP nanosheets had no effect on the bacterial richness and diversity in black soil across the entire culturing period (**Figure 4a-c,** $P > 0.05$), but the bacterial richness was significantly decreased by 16.60% (calculated based on the Chao 1 index) in burozem at day 10 (**Figure 4d-e**, $P < 0.05$). This result suggested that BP nanosheets may impose negative effects on the bacterial community in burozem in the early stage (day 10). However, after culturing for 60 days, there were no difference of bacterial richness between the BP-treated samples and control. Based on the Shannon index, a decrease of bacterial diversity were observed between 0 day and 60 day samples, which decreased by 2.60% and 1.44% for control and BP treatment, respective **(Figure 4f)**, suggesting that the bacterial community was less dynamic by BP treatments.[46]

By using the principal component analysis (PCA) and unweighted pair group method with arithmetic mean (UPGMA) methods, we investigated the response of bacterial community structure to the exposure of BP nanosheets. The results indicated that the bacterial community structure of burozem was more sensitive than that of black soil (Figure S2). Specifically, most of the BP-treated black soil samples were distributed closely to control and there was no obvious clustering trend during the 60 days (Figure S2a). In contrast, the BP-treated burozem samples were prone toclustering more closely to each other and separation from control at day 60 (Figure S2c). The UPGMA analysis showed coincident results with the PCA method (Figure S2b, d).

Prediction for the influence of BP nanosheets on bacterial function

Since only negligible, mild, or short-term effects on enzyme activity **(Figure 1)** and bacterial community **(Figure 2-4)** were observed, a more subtle technique is desirable to explore the ecological risk of BP nanosheets. Ma et al. demonstrated that microbial functional traits are sensitive indicators for mild disturbances.[47] Although PICRUSt cannot determine the actual function, it can translate 16S rRNA sequencing data into predicted metagenomes, which have been used to predict functional changes in microbial communities.[48] In both black soil and burozem samples, the nearest sequenced taxon index (NSTI) values were less than 0.23 (Figure S3), insuring the reliability and accuracy for the functional predictions.[24] At level 2 KEGG, most pathways were related to Metabolism (approximate 52%), followed by Environmental Information Processing (16%) and Genetic Information Processing (15%) (Figure S4 and S5). Compared to control groups, abundances of major predicted metagenomes of KEGG pathways (relative abundance > 1%) were not significantly altered in both soils after treated with BP nanosheets ($P > 0.05$) **(Figure 5)**. Based on PICRUSt-predicted metagenomes, Li et al. found that Ag nanoparticles (NPs) imposed obvious inhibition on predicted abundances of amino acid, carbohydrate transport and metabolism, and signal transduction mechanisms.[49] Similarly, CuO NPs also exhibited negative effects on the pathways related to the membrane transport, translation, and metabolism, which was mainly attributed to the nanometer size effect, not the metal ions.[50] Compared to these NPs, BP nanosheets were much more eco-friendly, thus offering the possibility of large-scale production and application.

Conclusion

In our study, we investigated the ecological effect of BP nanosheets by evaluating the variations of enzyme activity, bacterial community, and bacterial function in black soil and burozem upon BP exposure. Both enzyme activity and bacterial community structure in black soil were less susceptible to the BP nanosheets than that in burozem. The discrepancies were attributed to the stronger interaction of BP nanosheets with

organic matter-rich black soil, leading to a lowered bioavailability of BP nanosheets. However, BP nanosheets had negligible effect on the functional properties of bacterial communities in both soils based on the predicted metagenomes. Overall, BP nanosheets are more eco-friendly than many other nanomaterials that have stronger and persistent effects on the soil environment.[19] This study provided a preliminary understanding of the impacts of BP nanosheets on the soil environment. In the future study, the ecotoxicology of BP nanosheets under different environmental conditions, such as soil texture (sandy, loam, clay), moisture content, artificial disturbance (fertilizers), and the intrinsic toxic mechanism needs further assessment.

Conflicts of interest

There are no conflicts to declare.

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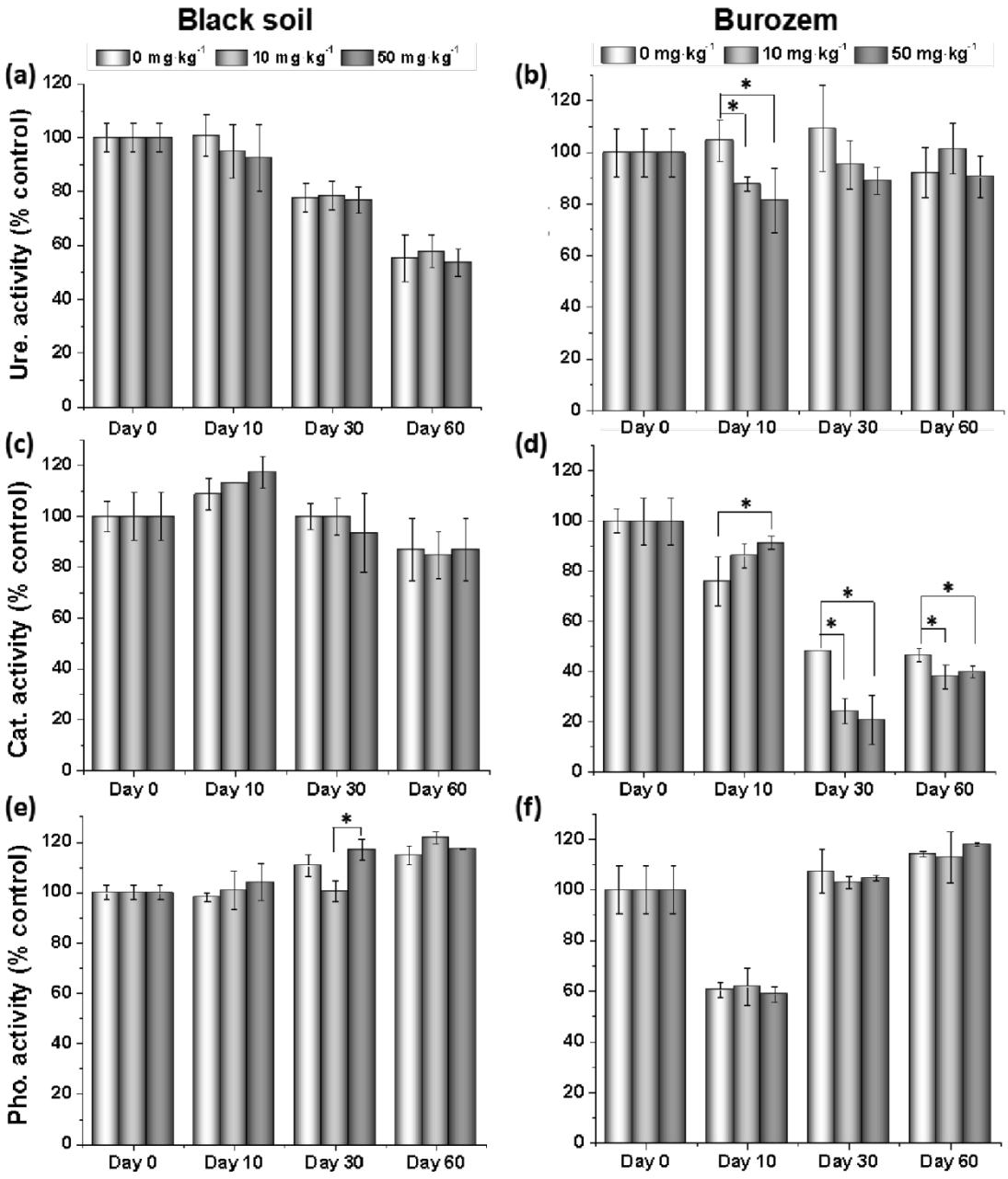


Figure 1. Enzyme activities of urease (a, b), catalase (c, d), and phosphatase (e, f) in black soil (a, c, e) and burozem (b, d, f) under exposure to different concentrations (0, 10, 50 mg kg^{-1}) of BP nanosheets over time. Ure., Cat., and Pho. represented urease catalase, and phosphatase, respectively. Error bars indicate one standard deviation of the mean (n=3).

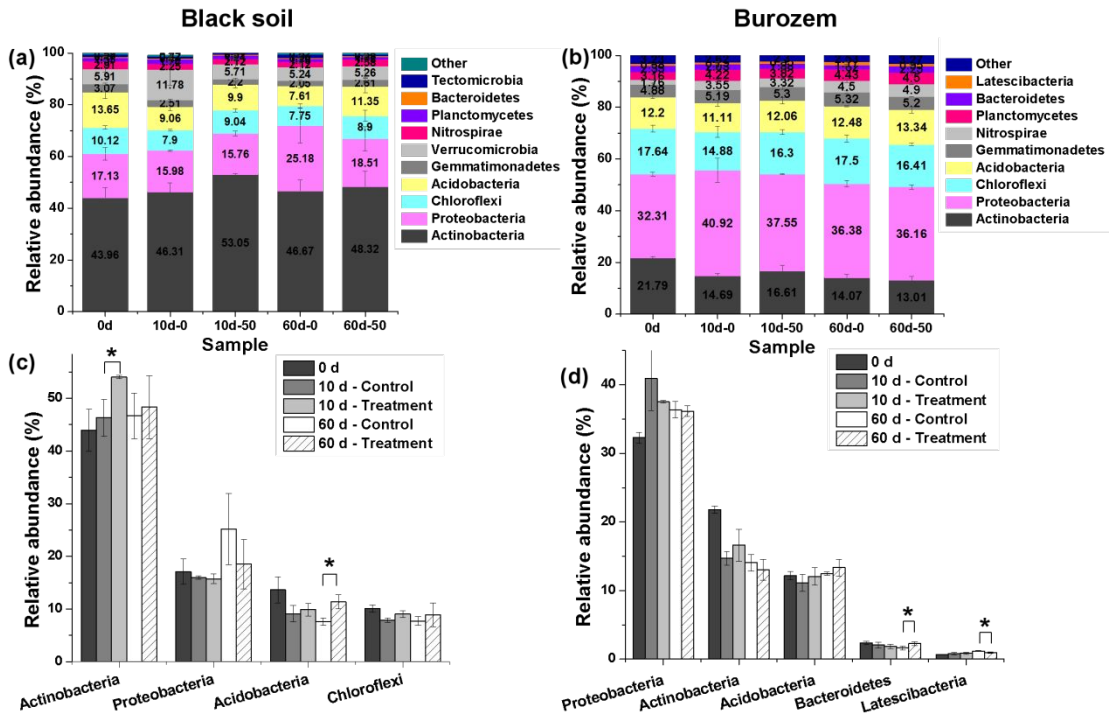


Figure 2. Relative abundance of the soil bacteria at the phylum level (a, b) and the effect of BP nanosheets (50 mg kg^{-1}) on the relative abundance of most dominant phyla (c, d) in black soil (a, b) and burozem (c, d). Error bars indicate one standard error of the mean (n = 3) and * indicates $P < 0.05$.

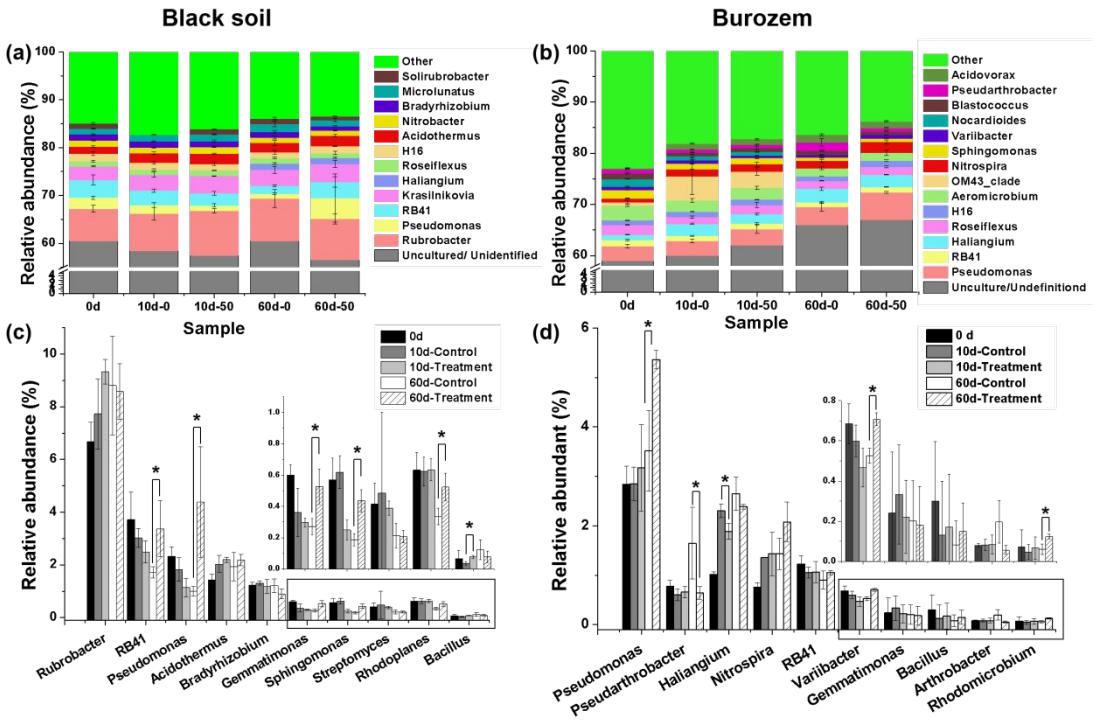


Figure 3. Relative abundance of the bacteria at the genus level (a-b) and the changes of bacterial abundance of most dominant genus (c-d) in soils after exposed to different concentrations of BP nanosheets. Error bars indicate one standard error of the mean (n = 3) and * indicate $P < 0.05$.

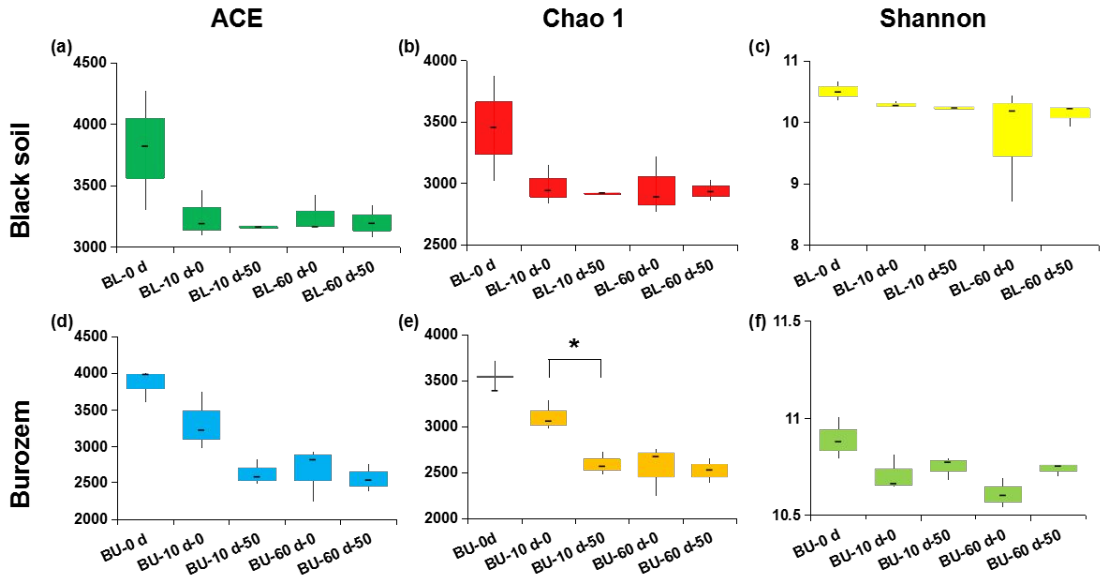


Figure 4. Comparison of alpha diversity indices of bacterial community in black soil (BL) and burozem (BU) by the boxplots. The box signifies the 75% (upper) and 25% (lower) quartiles and thus shows where 50% of the samples lie. The black line inside the box represents the median. The line outside represents the lowest datum still within 1.5 interquartile range (IQR) of the lower quartile and the highest datum still within 1.5 IQR of the upper quartile. BL and BU represent black soil and burozem, respectively.

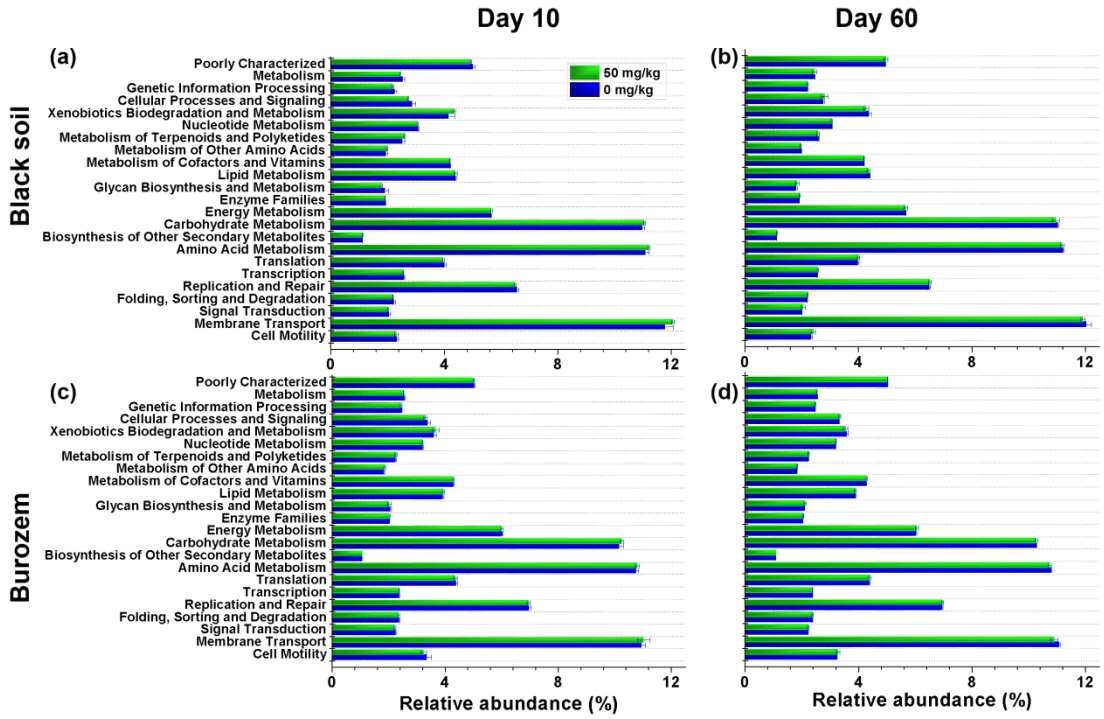


Figure 5. Relative abundance of different metagenomes of KEGG pathways in black soil (a-b) and burozem (c-d) at day 10 (a, c) and 60 (b, d) according to the pathway database of Kyoto Encyclopedia of Genes and Genomes (KEGG) at level 2. Error bars indicate one standard error of the mean (n = 3).

Table 1. Physicochemical properties of soils used in the experiment.

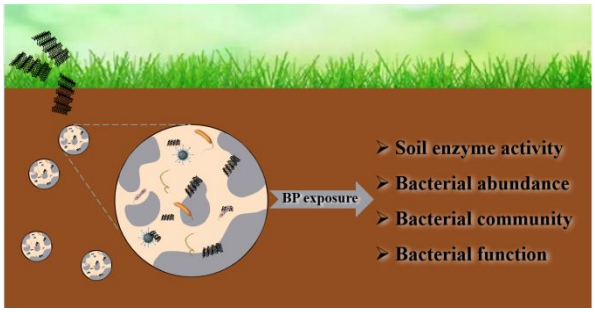
	Black soil	burozem
Sand	6.33 ± 1.53	11.33 ± 2.31
Silt	44.00 ± 1.00	62.33± 2.52
Clay	49.67± 2.52	26.33± 1.53
pH	6.37 ± 0.04	7.48 ± 0.03
TOC	85.80 ± 0.75	76.37 ± 0.49
TN	122.50 ± 2.23	61.40 ± 5.88
OM	62.30 ± 1.87	16.92 ± 1.41

Sand, clay, silt (%); TOC: total organic carbon content (g/kg); TN: total nitrogen content (g/kg); OM: organic matter (g/kg).

Table 2. 16S rRNA gene copy numbers ($\times 10^{12}$ copies g^{-1} dry soil) under the treatment of BP nanosheets (0, 10, 50 mg kg^{-1}) for 10, 30, and 60 days. Means ± standard errors are presented (n = 3).

Date	Soil type	Gene copy numbers ($\times 10^{12}$ copies g^{-1} dry soil) at various BP concentrations (0, 10, 50 mg kg^{-1})		
		0	10	50
Day 10	Black soil	5.33 ± 0.42	5.15 ± 0.29	5.66 ± 1.64
	Burozem	7.60 ± 1.80	8.12 ± 1.32	7.98 ± 0.86
Day 30	Black soil	6.08 ± 1.32	5.72 ± 0.41	5.85 ± 2.14
	Burozem	7.42 ± 0.99	7.92 ± 2.06	7.48 ± 2.27
Day 60	Black soil	5.92 ± 0.32	5.63 ± 0.85	5.06 ± 1.36
	Burozem	7.12 ± 0.53	6.60 ± 2.09	6.07 ± 1.50

TOC



Evaluation of BP nanosheets' impact on the soil ecological environment is important for their safe application and discharge.