Supplementary information

Response of soil enzyme activities and bacteria communities to black phosphorus nanosheets

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Materials and Methods

Preparation of BP nanosheets

BP nanosheets were prepared by the previously published method.[1] In brief, BP crystals (10 mg) were grinded into small pieces in the glove box using an agate mortar with a pestle. Then 10 mL oxygen-free Milli-pore water was added and the mixture was sonicated for 8 h with a ultrasonic cell disruption (JY 88-11N). Then the mixture was centrifuged at 800 rpm for 20 min to remove the un-exfoliated BP crystals. The concentration of BP nanosheets in the supernatant was determined to be 1036 ± 54 μg/mL by the inductively coupled plasma optical emission spectrometry (ICP-OES).

Soil collection and experimental design

Black soil (10-20 cm) was collected from a state farm (48°14′ N, 125°11′ E) in Qiqihar, Heilongjiang Province, with total carbon (TC) 85.80 ± 0.75g kg^{-1}, total nitrogen (TN) 122.50 ± 2.23 g kg^{-1}, organic matter 53.53 ± 1.24 g kg^{-1}, pH 6.37 ± 0.04. Burozem (10-20 cm) was collected from a National Field Scientific Observation and Research Station (41°31′ N, 123°24′ E) in Shenyang, Liaoning Province, with TC 76.37 ± 0.49 g kg^{-1}, TN 61.40 ± 5.88 g kg^{-1}, organic matter 16.87 ± 1.31 g kg^{-1}, and pH 7.48 ± 0.03. Detailed characterizations of soil physicochemical properties were shown in Table S1. Both soils were sieved (< 2 mm) after removing small stones and plant roots and stored at 4 °C.

The soils were pre-incubated at 37 °C for one week and the BP suspensions were homogenized by bath sonication for 30 min before use. 100 mL suspensions of BP nanosheets (200 and 1036 μg mL^{-1}) were sprayed to 2 kg soil samples to obtain the final BP concentrations of 10 and 50 mg kg^{-1}, respectively. The same volume of sterilized water was used as a negative control. Then, the mixtures were manually stirred for 10 min to make BP nanosheets uniformly distributed in the soils. The soils were incubated at 37 °C for 60 days, during which the soil moisture was maintained constantly at 21.27 ± 2.88 wt% of the dry soil by regularly replenishing sterilized water. All the treatments were performed in triplicate.

Characterizations of soil properties

Soil pH was measured with a pH meter (pH-22, HORIBA, Japan) with the soil to water ratio of 1:2.5. The TC and TN were determined by a multi N/C analyzer (N/C2100s, analytikjena, Germany). The soil organic matter was determined by potassium dichromate titration.[2] Soil texture measurement was performed by Agricultural industry standard: NY/T 1121.3-2006 [3] which black soil represents clay and burozem represent silty clay.[4]

Interaction of BP nanosheets with soils

The soil solution was prepared with the soil/water ratio of 1/50 and kept for 24 h at room temperature.[5] Then 10 mL of soil solution was mixed with 40 mL of BP suspension to obtain the final concentration of BP nanosheets at 50 mg kg^{-1}. After incubation for 24 h at room temperature, the mixture was centrifuged at 4000 rpm for 5 min and the UV absorbance of the supernatant was scanned from 185 to 800 nm. The interaction between BP nanosheets and soils was indicated by the absorbance difference between supernatants of BP suspension with or without soils.

Interference of BP nanosheets with soil enzyme assays

For the phenol-hypochlorite assay (to determine urease activity), 1 mL aqueous suspension of BP nanosheets (50 mg/mL) was mixed with 1 mL methylbenzene. After standing at room temperature for 15 min, 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. After filtration, 1 mL 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). The same volume of sterile water without BP nanosheets was set up as control. After standing for 20 min at room temperature, the UV absorbance at 578 nm was record with a double beam UV-vis Spectrophotometer TU-1901 (PERSEE, China). All the experiments were performed in triplicate.

For the permanganate titration method (to determine catalase activity), 1 mL aqueous suspension of BP nanosheets (50 mg/mL) was mixed with 0.5 mL methylbenzene and the mixture was kept at 4 °C for 30 min. Then 25 mL cold H_2O_2 solution (3 wt%) was added immediately and the mixture was incubated for 1 h at 4 °C, followed by the addition of 25 mL cold H_2SO_4 (2 mol L^{-1}). After filtration, 1 mL supernatant, 5 mL deionized water, and 5 mL H_2SO_4 were mixed together and titrated with $KMNO_4$ solution (0.02 mol L^{-1}). The same volume of sterile water without BP nanosheets was set up as control. The consumed volume of $KMNO_4$ solution was record. All the experiments were performed in triplicate.

Based on the data from Table S4 and S5, there was no significant difference between BP-treated and control groups ($P > 0.05$), confirming that BP nanosheets did not interfere with the reagents of enzyme assays.

Table S1 Physiochemical properties of soil samples during culturing with BP nanosheets.

Sample	pH	TOC (mg/kg)	TN (mg/kg)	Organic matter (mg/kg)
BL - 0 d	6.37 ± 0.04	85.80 ± 0.75	122.50 ± 2.23	62.30 ± 1.87
BL-10 d - 0	6.21 ± 0.04	84.47 ± 1.63	131.87 ± 3.16	61.24 ± 0.90
BL-10 d -50	6.03 ± 0.05	84.33 ± 2.80	116.90 ± 2.72	59.78 ± 1.05
BL-60 d - 0	6.14 ± 0.07	82.53 ± 0.45	100.33 ± 1.62	61.82 ± 1.13
BL-60 d -50	5.98 ± 0.05	89.87 ± 1.14	97.33 ± 8.01	62.94 ± 5.59
BU - 0 d	7.48 ± 0.03	76.37 ± 0.49	61.40 ± 5.88	16.92 ± 1.41
BU-10 d - 0	7.39 ± 0.03	76.73 ± 1.08	61.90 ± 1.65	15.36 ± 1.01
BU-10 d -50	7.24 ± 0.04	76.43 ± 3.17	60.83 ± 1.10	15.59 ± 1.10
BU-60 d - 0	7.33 ± 0.02	84.27 ± 3.07	53.80 ± 3.20	15.07 ± 0.95
BU-60 d -50	7.16 ± 0.03	79.67 ± 2.80	51.17 ± 5.40	15.04 ± 0.81

Note: BL = black soil, BU = burozem; Means ± standard errors are presented (n = 3).

Table S2. Enzyme activity of untreated soils.

Enzyme activity	Black soil	burozem
Urease	0.025 ± 0.002	0.044 ± 0.002
Catalase	1.18 ± 0.07	1.49 ± 0.07
Acid phosphatase	15.62 ± 0.45	-
Neutral phosphatase	-	1.61 ± 0.63

Soil urease activity [mg NH_4^+-N g^{-1} dry soil.24 h^{-1}], Soil catalase activity [mL $KMnO_4$ (0.1 mol/L) g^{-1} dry soil.h^{-1}], Soil acid phosphatase activity [mL Phenol (1 nmol) g^{-1} dry soil.24 h^{-1}], Soil neutral phosphatase activity [mL Phenol (1 nmol) g^{-1} dry soil.24 h^{-1}]. Means ± standard errors are presented (n = 3).

Table S3 Number of OTUs and diversity indexes of microbial communities treated with different concentrations of BP nanosheets.

Sample	OTUs	ACE	Chao 1	Shannon
BL-0 d	4738	3799.20 ± 483.38 a	3450.94 ± 427.04 a	10.51 ± 0.15 a
BL-10 d-0	4250	3249.00 ± 189.43 a	2976.58 ± 157.19 a	10.29 ± 0.05 a
BL-10 d-50	4080	3162.44 ± 8.76 a	2917.17 ± 5.73 a	10.24 ± 0.02 a
BL-60 d-0	4157	3245.29 ± 154.71 a	2959.80 ± 235.56 a	9.78 ± 0.93 a
BL-60 d-50	4026	3203.30 ± 129.94 a	2939.78 ± 87.85 a	10.13 ± 0.17 a
BU-0 d	4684	3863.63 ± 220.12 x	3596.87 ± 182.85 x	10.89 ± 0.11 x
BU-10 d-0	4539	3319.34 ± 388.20 xy	3108.81 ± 157.43 x	10.71 ± 0.09 x
BU-10 d-50	4228	2627.29 ± 175.79 y	2591.44 ± 122.98 y	10.75 ± 0.06 x
BU-60 d-0	4003	2663.04 ± 364.29 y	2557.69 ± 270.69 y	10.61 ± 0.08 x
BU-60 d-50	4014	2557.00 ± 187.06 y	2520.66 ± 133.47 y	10.74 ± 0.003 x

Note: BL = black soil, BU = burozem. Letters (a, b) and (x, y) indicated significant difference among BL and BU, respectively, at the level of 0.05. Means ± standard errors are presented (n = 3).

Table S4 Absorbance at 578 nm.

Group	Absorbance
BP-treated	0.035 ± 0.003
Control	0.038 ± 0.001

Note: Means ± standard errors are presented (n = 3).

Table S5 Consumed volume of $KMnO_4$ solution.

Group	Volume (mL)
BP-treated	7.47 ± 0.58
Control	7.47 ± 0.58

Note: Means ± standard errors are presented (n = 3).

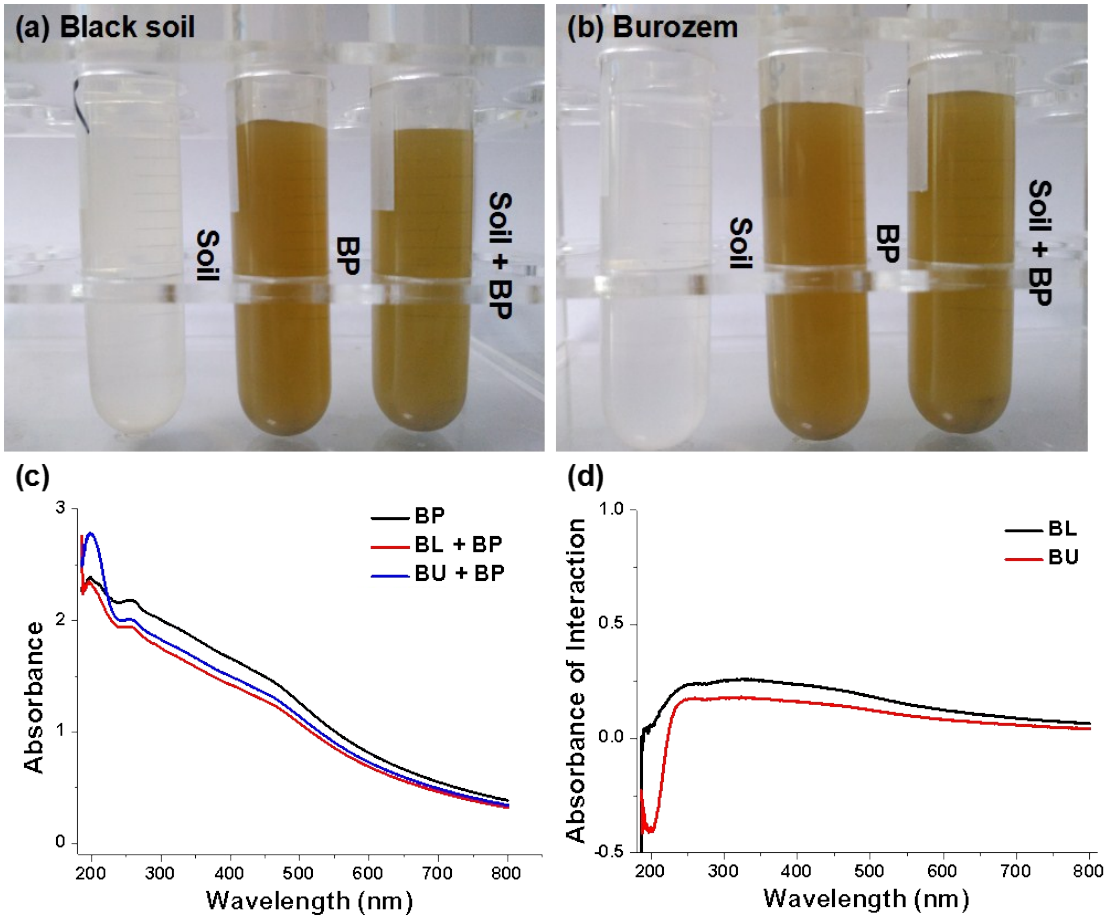

Figure S1. The photos of supernatants of soil, BP nanosheets, and their mixture for black soil (a) and burozem (b). (c) The UV absorbance of supernatants of BP nanosheets and mixture of BP and soil. (d) The absorbance of difference between supernatants of BP suspension with or without soils.

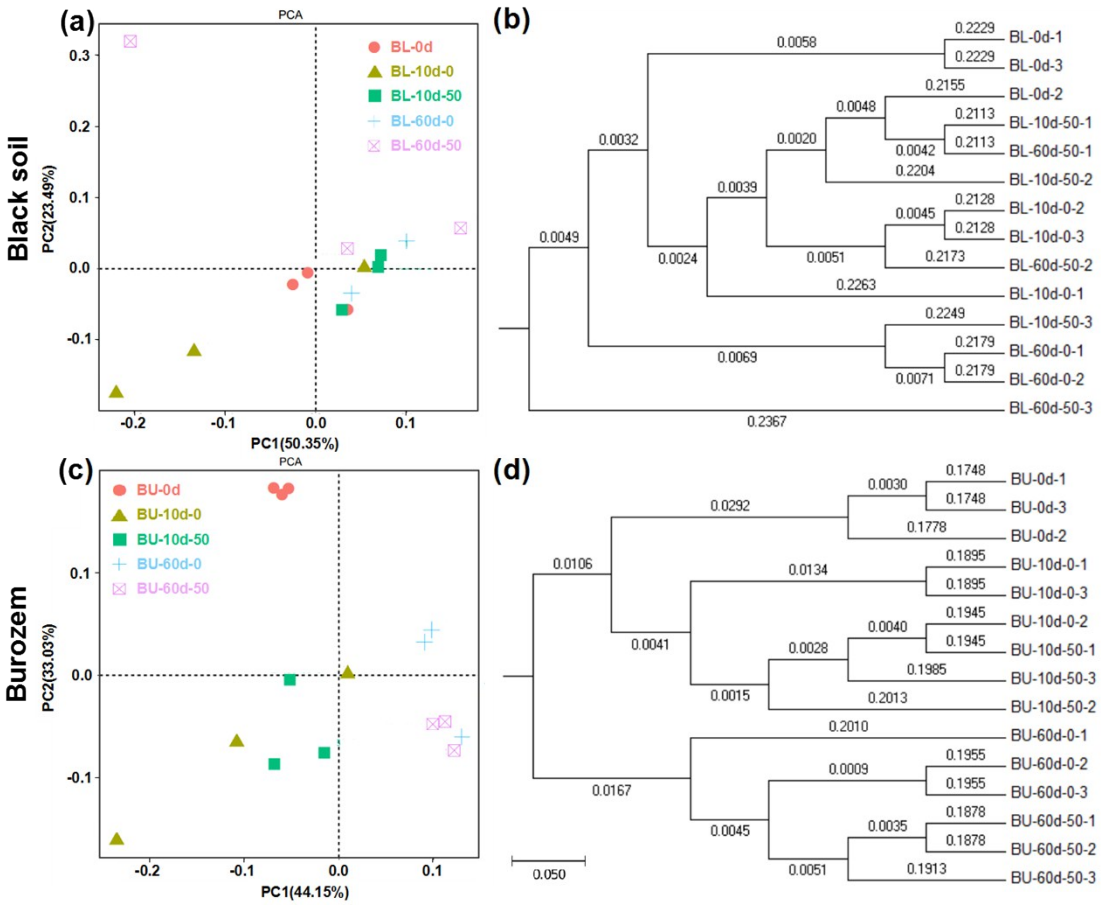

Figure S2. Results of principal component analysis (PCA) (a, c) and unweighted pair group method with arithmetic mean (UPGMA) (b, d) of bacteria communities in black soil (a, b) and burozem (c, d).

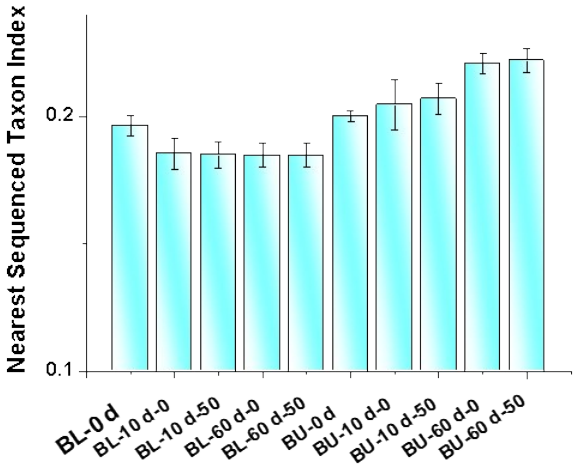

Figure S3. The nearest sequenced taxon index (NSTI) of different soil samples based on the PICRUSt method. Error bar indicates the standard deviation (n=3).

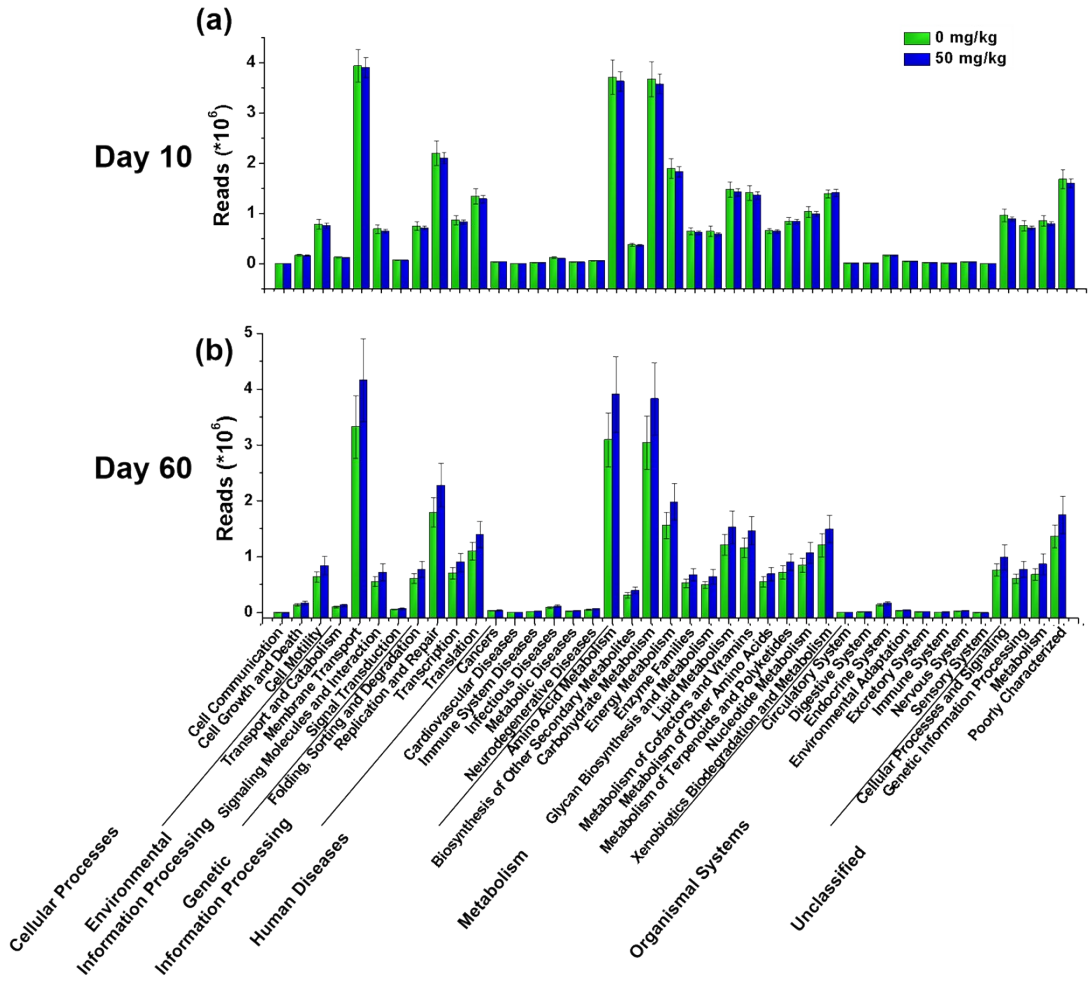

Figure S4. Predicted metagenomic base on 16S rRNA sequencing reads from black soil samples at day 10 (a) and day 60 (b) according to the pathway database of Kyoto Encyclopedia of Genes and Genomes (KEGG) at level 2. Error bar indicates the standard deviation (n=3).

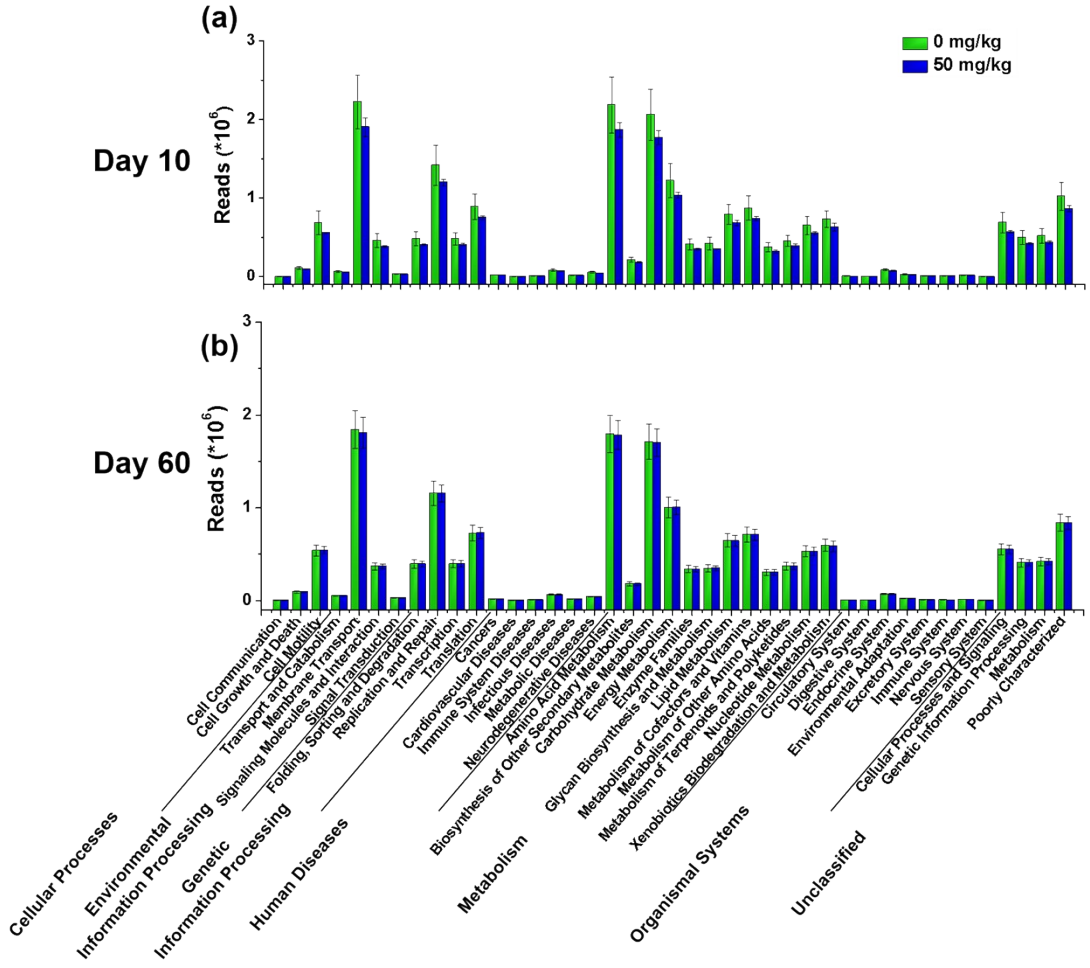

Figure S5. Predicted metagenomic base on 16S rRNA sequencing reads from burozem samples at day 10 (a) and day 60 (b) according to the pathway database of Kyoto Encyclopedia of Genes and Genomes (KEGG) at level 2. Error bar indicates the standard deviation (n=3).

References

1 X. J. Zhang, Z. M. Zhang, S. Y. Zhang, D. Y. Li, W. Ma, C. X. Ma, F. C. Wu, Q. Zhao, Q. F. Yan and B. S. Xing, Size effect on the cytotoxicity of layered black phosphorus and underlying mechanisms, *Small*, 2017, **13**, 1701210.

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4 United States Department of Agriculture (USDA), https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey/?cid=nrcs142p2_054167.

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