Supporting Information

Title: Silica nanomaterials and earthworms synergistically regulate maize root metabolite profiles via promoting soil Si bioavailability

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Dissolution of SiO_2 NMs in liquid culture medium

The fastest growing colony of SSB isolated from earthworm cast was selected to assess the Si release from SiO_2 NMs in an *in vitro* experiment. Erlenmeyer flasks (250 mL) with 100 mL of culture medium (1% sucrose, 0.05% yeast extract, 0.1% $(NH_4)_2SO_4$, 0.2% Na_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.01% NaCl, 0.01% $CaCO_3$, 10 mg L^{-1} SiO_2 NMs, pH 7.2) were inoculated with the SSB (final cell concentration in medium was 10^6 CFU mL^{-1}).[1] Uninoculated medium containing SiO_2 NMs served as control. Each treatment was replicated three times. The flasks were incubated on an incubator shaker at 180 r min^{-1} for seven days at 30 °C. After seven days, the culture suspension was centrifuged at 8000 r min^{-1} for 15 min and the soluble Si in the supernatant was estimated by the colorimetric molybdenum blue method.[2]

Table S1. Zeta potential and hydrodynamic diameter of SiO_2 NMs in ultrapure water.

NMs	Zeta potential (mV)	Hydrodynamic diameter (nm)
10 mg L^{-1} SiO_2 NMs	-17.28 ± 0.65	407.86 ± 28.92

Table S2. The sequences of specific primers used for the qRT-PCR analysis.

Gene	Primer	Sequence (5' to 3')	Reference
ZmLsi1	Forward	GATCCAGGTCCCGTTCTACTG	Bokor *et al*.[3]
ZmLsi1	Reverse	GACGAGCGAGTGCCAGTG	Bokor *et al*.[3]
ZmLsi2	Forward	ACGTGCCAACAGGTGCTTCTTATG	Bokor *et al*.[3]
ZmLsi2	Reverse	TACGATCGAGGCATACAATTATG	Bokor *et al*.[3]
ZmLsi6	Forward	TTCAGGTGCCCTTCTACTGG	Bokor *et al*.[3]
ZmLsi6	Reverse	ACGACGATCTCGATGAGGAG	Bokor *et al*.[3]
18S rRNA	Forward	ACCTTACCAGCCCTTGACATATG	Nelissen *et al*.[4]
18S rRNA	Reverse	GACTTGACCAAACATCTCACGAC	Nelissen *et al*.[4]

Table S3. Two-way ANOVA conducted to determine the interactive effects of Si treatments and earthworm on maize plant growth performance and soil Si bioavailability. The test results are shown with the test statistic F-value and significance levels as follows: $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ and $^{NS}p > 0.05$.

Mazie and soil traits	**Si treatments**	**Earthworm**	**Si treatments × Earthworm**
Leaf dry weight	6.16 **	14.57 ***	1.26 NS
Stem dry weight	1.23 NS	1.97 NS	2.65 NS
Root dry weight	20.74 ***	4.89 *	4.10 *
Pn	0.63 NS	3.61 *	1.83 NS
Rubisco activity	54.06 ***	57.21 ***	51.99 ***
Leaf Si	20.43 ***	7.27 *	15.90 ***
Stem Si	1.50 NS	0.07 NS	0.19 NS
Root Si	51.92 ***	9.15 **	8.67 **
ZmLsi1	190.63 ***	5.98 *	2.93 NS
ZmLsi2	262.15 ***	0.25 NS	0.57 NS
ZmLsi6	663.91 ***	5.24 *	1.55 NS
Bulk soil silicic acid	1.32 NS	0.02 NS	0.15 NS
Bulk soil SSB	0.0001 NS	0.61 NS	1.09 NS
Bulk soil DOC	0.08 NS	0.03 NS	0.10 NS
Rhizosphere silicic acid	34.11 ***	0.03 NS	0.42 NS
Rhizosphere SSB	0.35 ***	81.98 NS	1.50 NS
Rhizosphere DOC	28.89 ***	3.69 NS	3.28 NS
Phenylalanine	86.10 ***	15.33 **	3.35 NS
Histidine	80.96 ***	0.004 NS	3.04 NS
Glutamic acid	148.06 ***	43.83 ***	15.02 **
Maltose	95.33 ***	89.63 ***	49.02 ***
Fructose	46.29 ***	29.92 ***	16.75 **
4-Methoxycinnamic acid	76.43 ***	22.83 ***	8.35 *
2-Hydroxycinnamic acid	56.99 ***	13.49 **	5.39 *
Malic acid	37.16 ***	0.22 NS	2.72 NS
Caffeic acid	53.27 ***	43.31 ***	25.72 ***
Citric acid	58.80 ***	9.09 **	16.91 **
Chlorogenic acid	64.81 ***	0.43 NS	3.68 NS
Fumaric acid	15.84 **	9.98 **	6.91 *

Table S4. Permutational multivariate analysis of variance (PERMANOVA) was conducted to determine the interactive effects of SiO_2 NMs treatment and earthworm on maize root metabolite profiles.

Treatment	F value	p value
SiO_2 NMs	35.89	$p < 0.001$
Earthworm	17.38	$p < 0.001$
SiO_2 NMs × Earthworm	9.03	$p = 0.006$

Figure S1

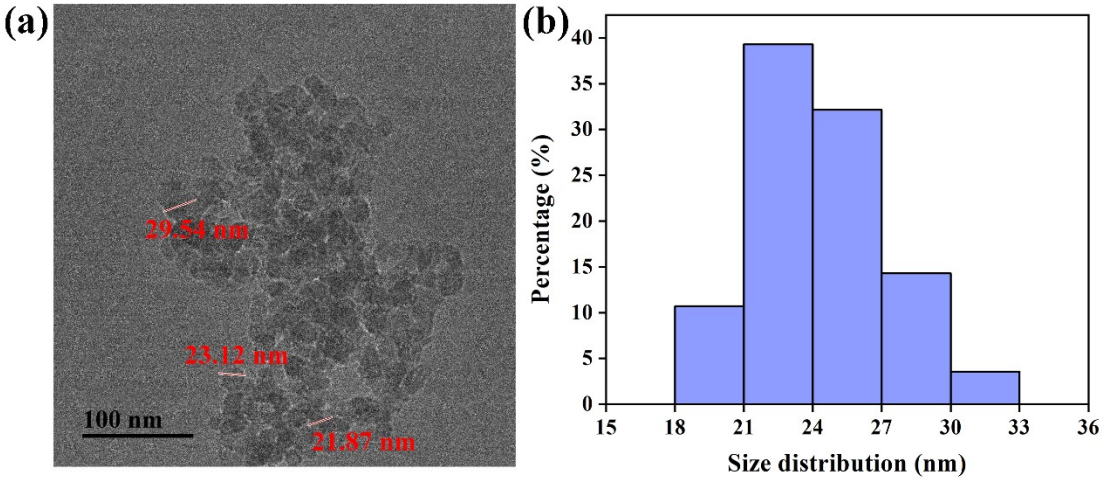

Fig. S1. TEM image (a) and size distribution (b) of SiO_2 NMs.

Figure S2

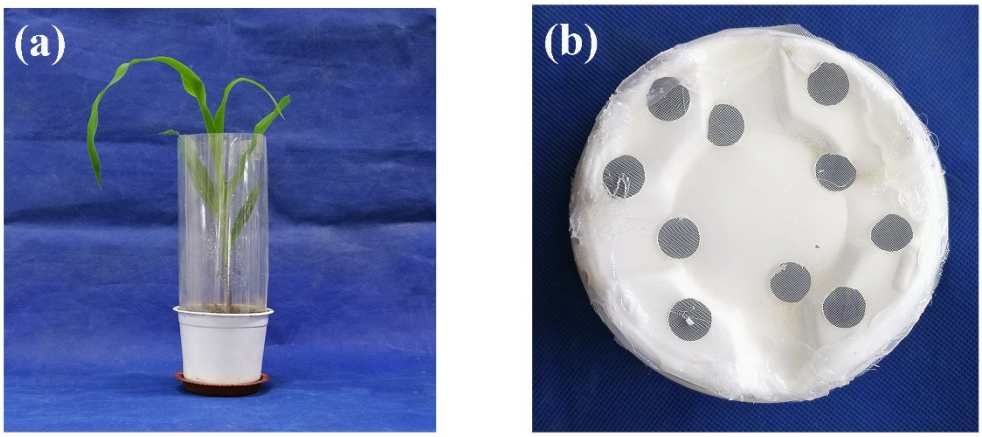

Fig. S2. Experimental culture pot design: the top (a) and bottom (b) of pot were covered with transparent plastic cylinders (height 20 cm) and nylon mesh (30 μm pore diameter) for keeping from earthworm escaping.

Figure S3

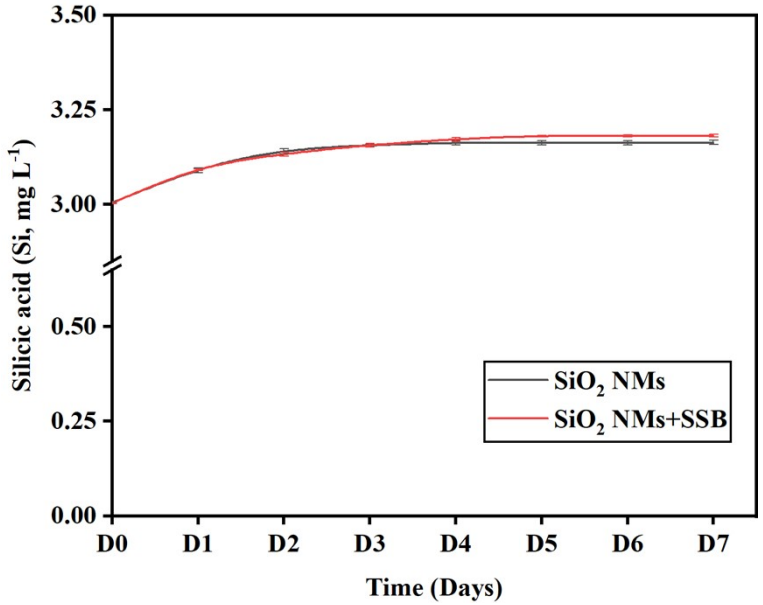

Fig. S3. The dissolution of SiO_2 NMs in the absence and presence of silicate solubilizing bacteria for seven days (n = 5). SiO_2 NMs: 10 mg L^{-1} SiO_2 NMs. SSB: silicate solubilizing bacteria.

Figure S4

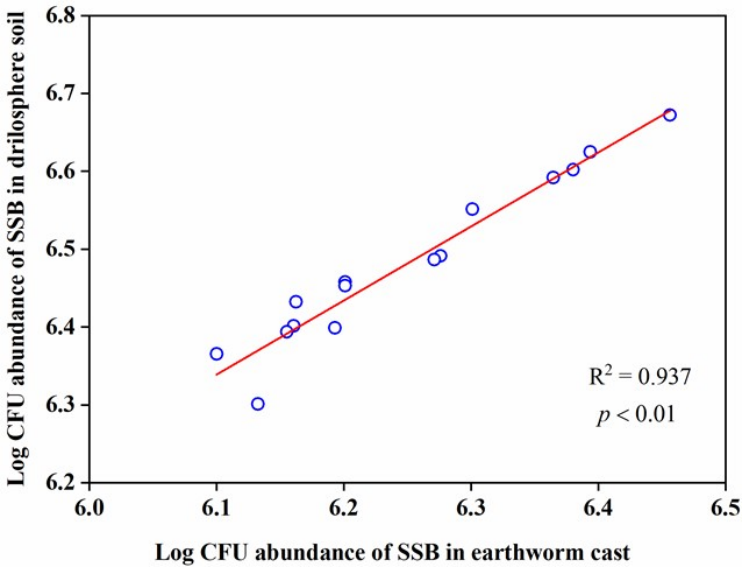

Fig. S4. Spearman correlations of the CFU abundance of silicate solubilizing bacteria (SSB) between earthworm cast and drilosphere soil.

Figure S5

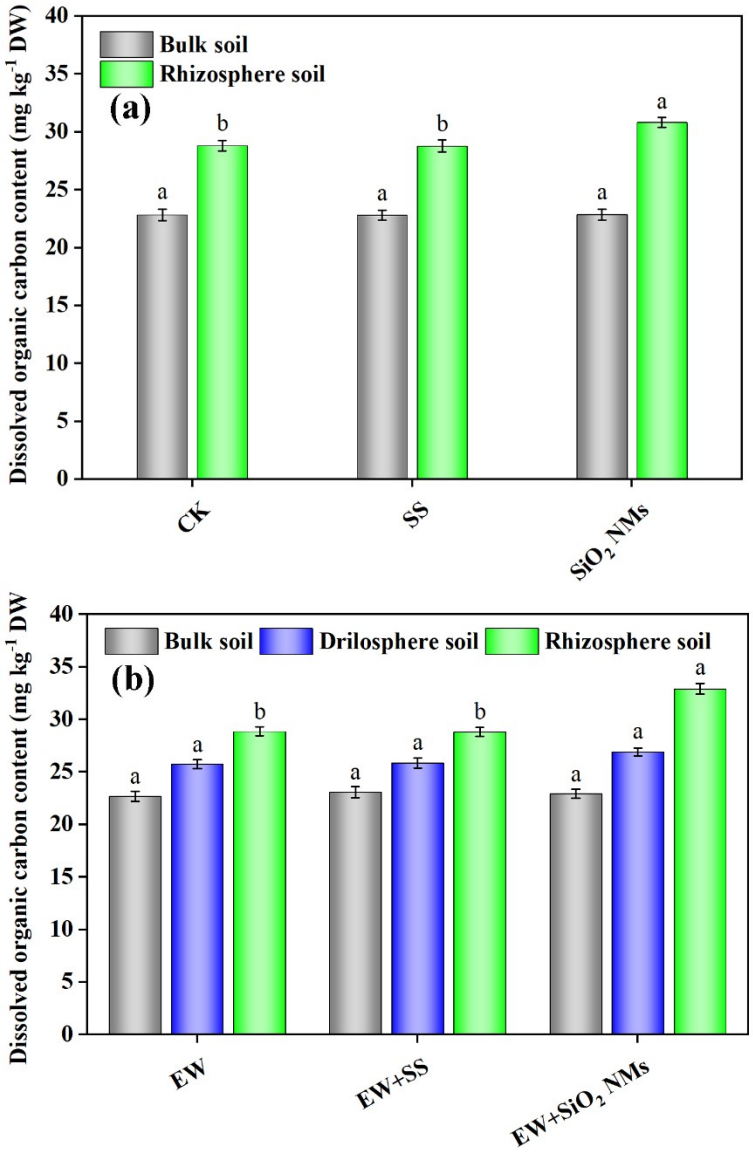

Fig. S5. (a) Dissolved organic carbon content in the bulk soil and rhizosphere soil upon exposure to sodium silicate and SiO_2 NMs in the absence of earthworms for 20 days (n = 5). (b) Dissolved organic carbon content in the bulk soil, drilosphere soil, and rhizosphere soil upon exposure to sodium silicate and SiO_2 NMs in the presence of earthworms for 20 days (n = 5). Different letters represent significant difference of each part of soils among treatments, respectively ($p < 0.05$).

References

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2. N. Bityutskii, P. Kaidun and K. Yakkonen, Earthworms can increase mobility and bioavailability of silicon in soil, *Soil Biol. Biochem.*, 2016, **99**, 47-53.
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4. H. Nelissen, B. Rymen, Y. Jikumaru, K. Demuynck, M. Van Lijsebettens, Y. Kamiya, D. Inzé and G. T. Beemster, A local maximum in gibberellin levels regulates maize leaf growth by spatial control of cell division, *Curr. Biol.*, 2012, **22**, 1183-1187.