Electronic Supporting Material (ESI) for

Intergenerational responses of wheat (*Triticum aestivum* L.) to cerium oxide nanoparticles exposure

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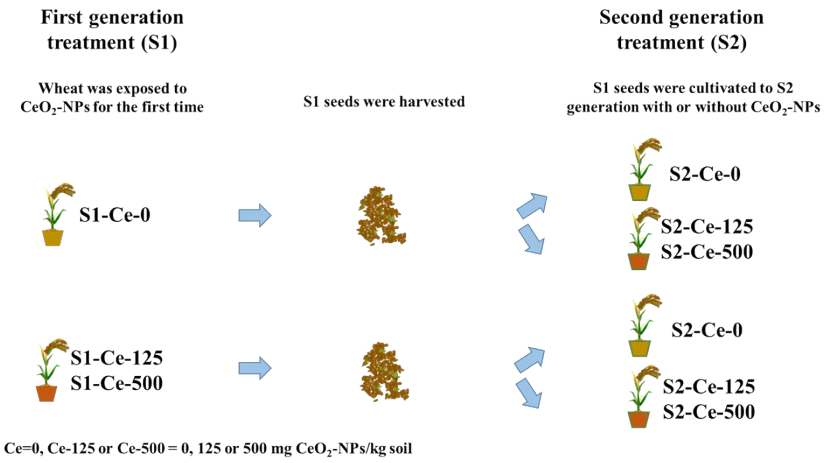
Determinations of plant growth and yield

Plant height was assessed using five measurements from randomly selected plants in each pot by measuring from soil surface to the flag leaf. The fully exposed and completely dried spikes were counted just before harvest and recorded as the number of spikes formed. For shoot biomass and water content determination, six plants were harvested from each pot and the weight was recorded before and after oven drying. In the case of yield components, the following parameters were measured using five random samples from each pot: spike length, number of spikes, number of spikelets/spike, number of grains/spike, and gain yield/spike. Grain yield from each pot was measured and recorded as grain yield/pot. The average of five measurements from each pot for agronomic and yield components, except grain yield/pot, was obtained and inputted as one replicate measurement for statistical analyses.



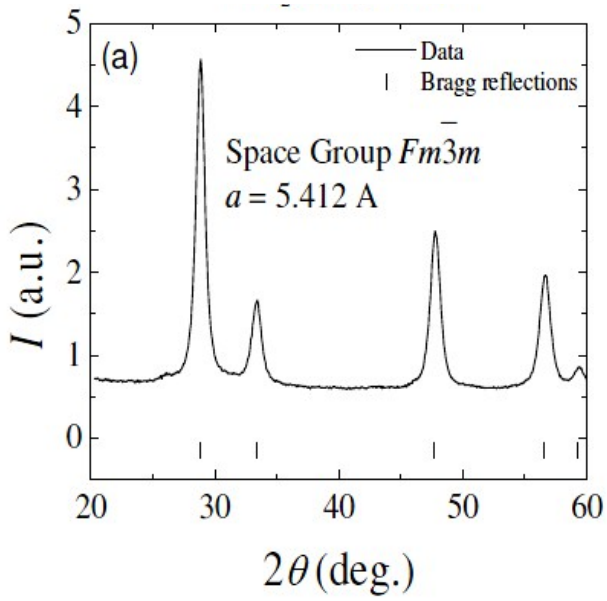
Factorial Treatment Combinations in 125 mg CeO_2-NPs/kg

First generation treatment (S1)	Second generation treatment (S2)	
	S2-Ce-0	S2-Ce-125
S1-Ce-0	S1-Ce-0/S2-Ce-0	S1-Ce-0/S2-Ce-125
S1-Ce-125	S1-Ce-125/S2-Ce-0	S1-Ce-125/S2-Ce-125

Factorial Treatment Combinations in 500 mg CeO_2-NPs/kg

First generation treatment (S1)	Second generation treatment (S2)	
	S2-Ce-0	S2-Ce-500
S1-Ce-0	S1-Ce-0/S2-Ce-0	S1-Ce-0/S2-Ce-500
S1-Ce-500	S1-Ce-500/S2-Ce-0	S1-Ce-500/S2-Ce-500

SI Figure 1. Experimental set-up for first (S1) and second (S2) generations exposure to 125 or 500 mg CeO_2-NPs/kg factorial treatments in wheat cultivated to grain production.

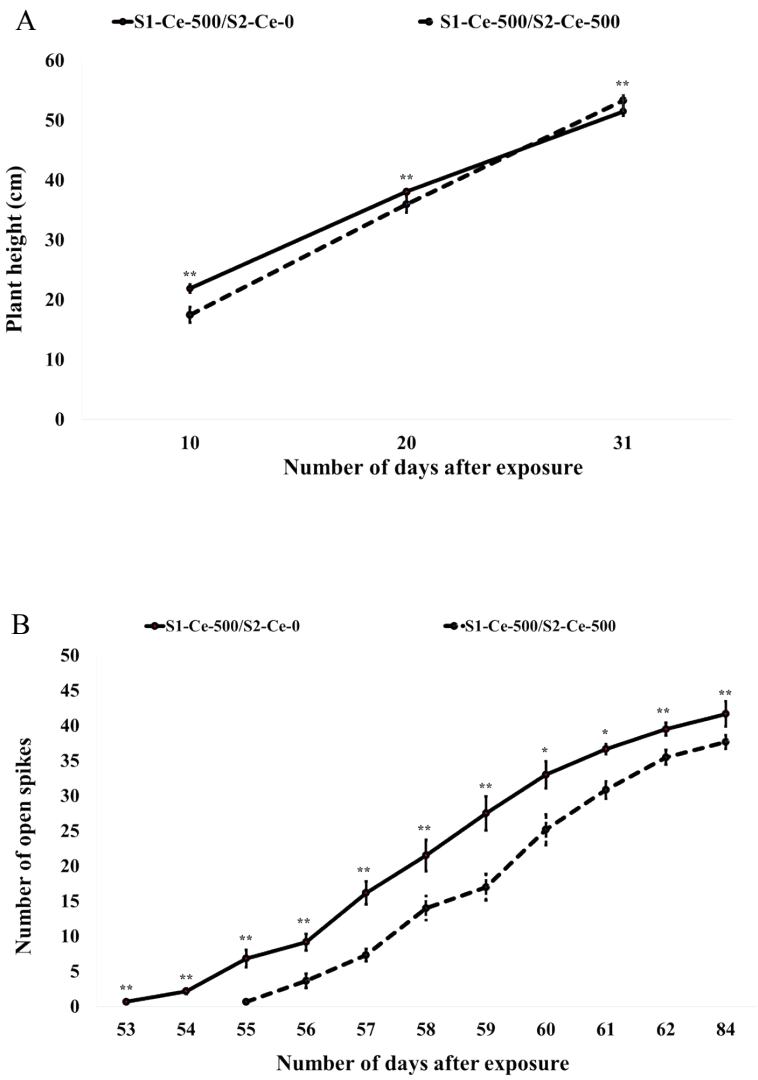


SI Figure 2. X-ray diffraction pattern of $n$$CeO_2$. Powder XRD patterns were collected in the Bragg-Brentano geometry within 20°-60° 2θ-range (λ=1.5406Å).

SI Table 1. Hydrodynamic size, zeta potential, pH and Ce ion concentration of CeO2 NPs suspension.[a]

CeO2 NPs concentration (mg L^{-1})	pH	Zeta potential (mV)	Diameter (nm)	Ce ion (mg L^{-1})
125	5.48 ± 0.21a	35.3 ± 0.7a	527.4 ± 66.5b	1.08 ± 0.37a
500	5.09 ± 0.21a	20.4 ± 0.2b	616.2 ± 8.5a	2.11 ± 0.32a

[a]Values are means ± SE (n = 3). Means with the same letter are not significantly different at $p < 0.05$.



SI Figure 3. Plant growth (A) and grain formation (B) of wheat cultivated to grain production in second generation plants exposed to CeO_2-NPs during the first (S1), second (S2), or both generations. Values are means ± SE (n = 6 except for S1-Ce-0/Ce-0 wherein n = 5). *, ** indicate significance at $P \leq 0.05$, 0.01, respectively.

SI Table 2. Two-way ANOVA measuring the effects of first (S1) and second (S2) generations CeO_2-NPs treatments on growth and yield parameters in wheat.

parameters	125 mg CeO_2-NPs/kg			500 mg CeO_2-NPs/kg		
	S1	**S2**	**S1×S2**	**S1**	**S2**	**S1×S2**
Plant height at						
10 DAE	0.0010***	0.2148	0.3847	<0.0001***	0.0071***	0.1652
20 DAE	0.0014***	0.6087	0.5807	<0.0001***	0.0864*	0.8593
31 DAE	0.0022***	0.0252**	0.6037	0.0029***	0.0620*	0.5617
Biomass	0.3980	0.8279	0.8540	0.0236**	0.5718	0.2393
Number of spikes	0.2155	0.9018	0.1668	0.0071***	0.1986	0.0686*
Number of grains per spike	0.1916	0.3038	0.3854	0.0776*	0.4116	0.5049
Open spikes at						
53 DAE	0.1944	1.0000	1.0000	0.0072***	0.0072***	0.0072***
54 DAE	0.0448**	0.4008	0.4008	<0.0001***	<0.0001***	<0.0001***
55 DAE	0.4272	0.2956	0.1844	0.0007***	<0.0001***	0.0019***
56 DAE	0.4918	0.0753*	0.2038	0.0005***	0.0006***	0.1305
57 DAE	0.4386	0.1579	0.3494	0.0038***	0.0046***	0.0199**
58 DAE	0.7515	0.3692	0.4570	0.0051***	0.0123**	0.3324
59 DAE	0.8573	0.2848	0.3333	0.0084***	0.0046***	0.1324
60 DAE	0.5558	0.1383	0.1961	0.0259**	0.0368**	0.1758
61 DAE	0.9120	0.1752	0.0890*	0.0143**	0.0389**	0.0409**
62 DAE	0.7118	1.0000	0.0167**	0.0011***	0.1123	0.0861*
84 DAE	0.2155	0.9018	0.1668	0.0071***	0.1986	0.0686*
Dry spikes at						
81 DAE	1.0000	0.1944	1.0000	0.0082***	0.0928*	0.0928*
82 DAE	0.3050	0.0651*	0.3521	0.0044**	0.4930	0.3075
83 DAE	0.3827	0.0613*	0.1053	0.0133**	0.8766	0.2626
84 DAE	0.2637	0.0670*	0.0926*	0.0227**	0.6445	0.5321
85 DAE	0.6628	0.1328	0.2084	0.0458**	0.4473	0.3731
87 DAE	0.8290	0.2874	0.3758	0.0556*	0.1993	0.3864
88 DAE	0.6229	0.0963	0.1669	0.0161**	0.1328	0.2081
89 DAE	0.4690	0.1200	0.6896	0.4991	0.5934	0.3320

*, **, *** indicate significance at $P \leq 0.1, 0.05, 0.01$, respectively.

SI Table 3. Two-way ANOVA measuring the effects of first (S1) and second (S2) generations CeO_2-NPs treatments on growth parameters in wheat.

parameters	125 mg CeO_2-NPs/kg			500 mg CeO_2-NPs/kg		
	S1	**S2**	**S1×S2**	**S1**	**S2**	**S1×S2**
Photosynthetic Rate	0.1870	0.4047	0.6872	0.4170	0.8354	0.4754
Stomatal conductance	0.7613	0.4112	0.6820	0.2785	0.8382	0.8505
Transpiration	0.8572	0.3745	0.7694	0.1644	0.8286	0.9769
Water use efficiency	0.2629	0.9989	0.5668	0.1935	0.1439	0.4429
Spike length	0.5788	0.1009	0.4336	0.5981	0.3068	0.5244
100-grain weight	0.6733	0.3200	0.4192	0.6922	0.1444	0.9632
Grain yield per pot	0.1690	0.5633	0.3916	0.3681	0.6004	0.7524

SI Table 4. Shoot biomass and yield characters of wheat cultivated to grain production in first (S1) and second (S2) generations CeO_2-NPs treatments that showed statistical differences. Values are means ± SE (n = 6 except for S1-Ce-0/Ce-0 wherein n = 5).

	S1/S2	S2-Ce-0	S2-Ce-125	**Mean**	**S1/S2**	S2-Ce-0	S2-Ce-500	**Mean**
dry shoot biomass (g)	S1-Ce-0	39.9 ± 0.6	39.9 ± 0.4	**39.9 ± 0.3**	S1-Ce-0	39.9 ± 0.6	40.2 ± 0.6	**40.1 ± 0.4**
	S1-Ce-125	40.4 ± 0.4	40.2 ± 0.5	**40.3 ± 0.3**	S1-Ce-500	41.8 ± 0.6	40.9 ± 0.2	**41.4 ± 0.3****
	Mean	**40.2 ± 0.3**	**40.0 ± 0.3**		**Mean**	**41.0 ± 0.5**	**40.6 ± 0.3**	
	S1/S2	S2-Ce-0	S2-Ce-125	**Mean**	**S1/S2**	S2-Ce-0	S2-Ce-500	**Mean**
number of spikes	S1-Ce-0	35.6 ± 0.9	37.0 ± 0.9	**36.4 ± 0.7**	S1-Ce-0	35.6 ± 0.9	36.3 ± 0.8	**36.0 ± 0.6**
	S1-Ce-125	38.5 ± 1.3	36.8 ± 1.1	**37.7 ± 0.8**	S1-Ce-500	41.7 ± 1.8	37.7 ± 1.0	**39.7 ± 1.1*****
	Mean	**37.2 ± 0.9**	**36.9 ± 0.7**		**Mean**	**38.9 ± 1.4**	**37.0 ± 0.7**	
	S1/S2	S2-Ce-0	S2-Ce-125	**Mean**	**S1/S2**	S2-Ce-0	S2-Ce-500	**Mean**
number of grains per spike	S1-Ce-0	51.6 ± 1.5	51.9 ± 2.7	**51.7 ± 1.4**	S1-Ce-0	51.6 ± 1.8	49.1 ± 1.8	**50.2 ± 1.3**
	S1-Ce-125	47.4 ± 2.4	51.0 ± 2.1	**49.2 ± 1.4**	S1-Ce-500	47.5 ± 1.7	47.2 ± 1.6	**47.4 ± 1.2***
	Mean	**49.3 ± 1.4**	**51.5 ± 1.4**		**Mean**	**49.4 ± 1.3**	**48.2 ± 1.2**	

*, **, *** indicate significance at P ≤ 0.1, 0.05, 0.01, respectively.

SI Table 5. Two-way ANOVA for elemental concentrations of wheat cultivated to grain production in first (S1) and second (S2) generations CeO_2-NPs treatments.

parameters	125 mg CeO_2-NPs/kg			500 mg CeO_2-NPs/kg		
	S1	**S2**	**S1×S2**	**S1**	**S2**	**S1×S2**
			Root			
Ce	<0.0001***	<0.0001***	<0.0001***	0.0406**	<0.0001***	0.0421**
Al	0.0072***	0.0614*	0.0420**	0.0097***	0.1664	0.0054***
Fe	0.0193**	0.1623	0.1221	0.0314**	0.1790	0.0202**
Mn	0.0075***	0.1670	0.2094	0.0025***	0.2417	0.2361
Ca	0.6937	0.0599*	0.1867	0.4386	0.9972	0.0047***
K	0.9543	0.1056	0.3765	0.6623	0.1283	0.5910
Mg	0.6908	0.1265	0.1139	0.1139	0.4197	0.0426**
P	0.9856	0.5543	0.9600	0.3803	0.0691	0.2160
			Shoot			
Ce	0.2256	0.0200**	0.0289**	0.0474**	<0.0001***	0.0004***
Al	0.8213	0.8972	0.7536	0.8973	0.5922	0.4584
Fe	0.5637	0.2659	0.9025	0.4148	0.2821	0.8836
Mn	0.7548	0.3740	0.9450	0.1384	0.2033	0.6202
Ca	0.6371	0.4521	0.6458	0.9314	0.3376	0.9457
K	0.3196	0.5668	0.2183	0.0622*	0.6885	0.9007
Mg	0.8734	0.3978	0.7263	0.6671	0.6175	0.7303
P	0.0695*	0.4275	0.7888	0.3149	0.9870	0.5855
			Grains			
Ce	0.6009	0.6307	0.5988	0.5609	0.3771	0.2042
Fe	0.4677	0.9932	0.8863	0.6107	0.2270	0.8982
Mn	0.4493	0.0430**	0.0016***	0.2167	0.3138	0.7866
Ca	0.1604	0.0400**	0.0684*	0.8447	0.2341	0.6931
K	0.0430**	0.1124	0.0338**	0.2249	0.2598	0.5196
Mg	0.0059***	0.1454	0.0506*	0.5992	0.4789	0.8398
P	0.0070***	0.0688*	0.0172**	0.8990	0.3529	0.7219

*, **, *** indicate significance at $P \leq 0.1, 0.05, 0.01$, respectively.

SI Table 6. Two-way ANOVA for C and N content in wheat shoots at mid-life growth stage (41 days after exposure) in first (S1) and second (S2) generations CeO_2-NPs treatments.

parameters	125 mg CeO_2-NPs/kg			500 mg CeO_2-NPs/kg		
	S1	**S2**	**S1×S2**	**S1**	**S2**	**S1×S2**
Shoot C (%)	0.1408	0.0997*	0.4564	0.0754*	0.8143	0.0969*
Shoot $\delta^{13}C$ (‰)	0.0286**	0.0108**	0.1483	0.9468	0.7137	0.6684
Shoot N (%)	0.4083	0.2906	0.4300	0.0120**	0.8685	0.9301
Shoot $\delta^{15}N$ (‰)	0.1345	0.4838	0.8777	0.4281	0.9579	0.8418
Shoot C:N	0.4874	0.2478	0.4353	0.0092***	0.9786	0.9416

*, **, *** indicate significance at $P \leq 0.1, 0.05, 0.01$, respectively.

SI Table 7. Two-way ANOVA for C and N content in wheat cultivated to seed production in first (S1) and second (S2) generations CeO_2-NPs treatments.

parameters	125 mg CeO_2-NPs/kg			500 mg CeO_2-NPs/kg		
	S1	**S2**	**S1×S2**	**S1**	**S2**	**S1×S2**
			Root			
C (%)	0.0117**	0.7483	0.0758*	0.8184	0.8296	0.5985
$\delta^{13}C$ (‰)	0.9218	0.4990	0.6433	0.9551	0.7617	0.2655
N (%)	0.8981	0.7645	0.7136	0.3934	0.5534	0.1748
$\delta^{15}N$ (‰)	0.7343	0.0684*	0.3507	0.0408**	0.2342	0.3729
C:N	0.7874	0.7753	0.5388	0.6439	0.6118	0.1498
			Shoots			
C (%)	0.9618	0.6255	0.8092	0.0098***	0.6766	0.9170
$\delta^{13}C$ (‰)	0.5795	0.6130	0.5433	0.0302**	0.7349	0.5937
N (%)	0.2395	0.7673	0.6676	0.0069***	0.5938	0.9640
$\delta^{15}N$ (‰)	0.4944	0.8390	0.6460	0.2426	0.2652	0.7521
C:N	0.3588	0.7178	0.6428	0.0067***	0.6029	0.9895
			Grains			
C (%)	0.6795	0.6345	0.1503	0.3069	0.4096	0.4859
$\delta^{13}C$ (‰)	0.7014	0.0291**	0.0081***	0.3150	0.0675*	0.0541*
N (%)	0.8482	0.2575	0.4595	0.5996	0.3664	0.4332
$\delta^{15}N$ (‰)	0.0339**	0.2536	0.8348	0.0208**	0.8946	0.7898
C:N	0.8559	0.2596	0.6108	0.8492	0.5597	0.6035

*, **, *** indicate significance at $P \leq 0.1, 0.05, 0.01$, respectively.

SI Table 8. Isotope analysis of wheat at mid-life growth stage (41 days after exposure) in first (S1) and second (S2) generations CeO_2-NPs treatments. Values are means ± SE (n = 6 except for S1-Ce-0/Ce-0 wherein n = 5).

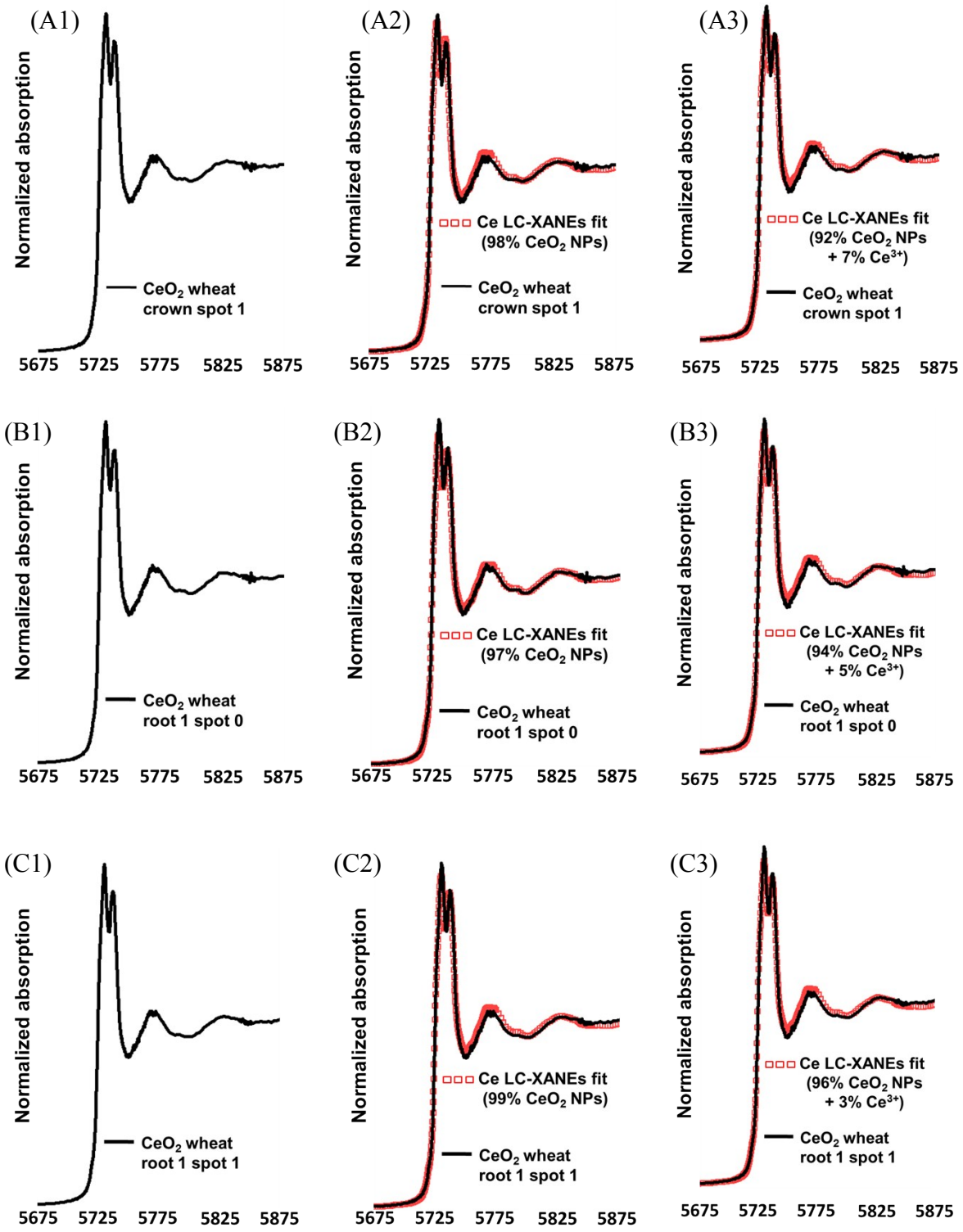
	Shoots				Shoots			
	S1/S2	S2-Ce-0	S2-Ce-125	**Mean**	**S1/S2**	S2-Ce-0	S2-Ce-500	**Mean**
C (%)	S1-Ce-0	43.85 ± 0.51	44.67 ± 0.39	**44.25 ± 0.33**	S1-Ce-0	43.85 ± 0.51	44.28 ± 0.17	**44.06 ± 0.26**
	S1-Ce-125	44.61 ± 0.13	44.93 ± 0.08	**44.77 ± 0.09**	S1-Ce-500	44.90 ± 0.17	44.32 ± 019	**44.61 ± 0.15***
	Mean	**44.23 ± 0.28**	**44.78 ± 0.19***		**Mean**	**44.37 ± 0.30**	**44.30 ± 0.10**	
	S1/S2	S2-Ce-0	S2-Ce-125	**Mean**	**S1/S2**	S2-Ce-0	S2-Ce-500	**Mean**
$\delta^{13}C$ (‰)	S1-Ce-0	-25.61 ± 0.16	-25.32 ± 0.26	**-25.47 ± 0.15**	S1-Ce-0	-25.61 ± 0.16	-25.59 ± 0.16	**-25.60 ± 0.11**
	S1-Ce-125	-26.45 ± 0.19	-25.51 ± 0.25	**-25.98 ± 0.21****	S1-Ce-500	-25.53 ± 0.30	-25.70 ± 0.22	**-25.62 ± 0.18**
	Mean	**-26.03 ± 0.17**	**-25.42 ± 0.17****		**Mean**	**-25.57 ± 0.16**	**-25.65 ± 0.13**	
	S1/S2	S2-Ce-0	S2-Ce-125	**Mean**	**S1/S2**	S2-Ce-0	S2-Ce-500	**Mean**
N (%)	S1-Ce-0	4.25 ± 0.24	3.94 ± 0.11	**4.09 ± 0.14**	S1-Ce-0	4.25 ± 0.24	4.23 ± 0.16	**4.24 ± 0.14**
	S1-Ce-125	4.26 ± 0.14	4.21 ± 0.12	**4.23 ± 0.09**	S1-Ce-500	3.78 ± 0.16	3.73 ± 0.12	**3.75 ± 0.10****
	Mean	**4.25 ± 0.14**	**4.07 ± 0.09**		**Mean**	**4.01 ± 0.16**	**3.98 ± 0.12**	
	S1/S2	S2-Ce-0	S2-Ce-125	**Mean**	**S1/S2**	S2-Ce-0	S2-Ce-500	**Mean**
$\delta^{15}N$ (‰)	S1-Ce-0	1.81 ± 0.54	2.02 ± 0.30	**1.92 ± 0.30**	S1-Ce-0	1.81 ± 0.54	1.75 ± 0.50	**1.78 ± 0.35**
	S1-Ce-125	1.18 ± 0.26	1.50 ± 0.31	**1.34 ± 0.20**	S1-Ce-500	1.42 ± 0.18	1.52 ± 0.20	**1.47 ± 0.13**
	Mean	**1.50 ± 0.30**	**1.76 ± 0.22**		**Mean**	**1.61 ± 0.28**	**1.64 ± 0.26**	
	S1/S2	S2-Ce-0	S2-Ce-125	**Mean**	**S1/S2**	S2-Ce-0	S2-Ce-500	**Mean**
C:N	S1-Ce-0	10.51 ± 0.66	11.39 ± 0.35	**10.95 ± 0.38**	S1-Ce-0	10.51 ± 0.66	10.53 ± 0.39	**10.52 ± 0.36**
	S1-Ce-125	10.55 ± 0.37	10.72 ± 0.32	**10.63 ± 0.23**	S1-Ce-500	12.00 ± 0.51	11.95 ± 0.42	**11.97 ± 0.31*****
	Mean	**10.53 ± 0.36**	**11.05 ± 0.25**		**Mean**	**11.25 ± 0.45**	**11.24 ± 0.35**	

*, **, *** indicate significance at P ≤ 0.1, 0.05, 0.01, respectively.

SI Table 9. Speciation of cerium in tissues of plants exposed to CeO_2-NPs.

Plant/plant parts	Exposure route	Findings	References
Soybean	Farmland soil	Accumulation of CeO_2-NPs with very limited transformation to Ce(III) species (12%)*	Priester et al., *P. Natl. Acad. Sci. USA* **2012**, *109*, E2451–E2456 Hernandez-Viezcas et al., *ACS Nano* **2013**, *7*, 1415-1423
Kidney beans	Low organic matter soil, and organic matter enriched soil	Cerium was found largely as CeO_2-NPs with small fraction in Ce(III) species (12%)*	Majumdar et al., *J. Hazard. Mater.* **2014**, *278*, 279-287
Wheat	Farmland soil	CeO_2-NPs were detected in the root surface*	Du et al., Environ. Sci. Technol. 2015, 49, 11884-11893
Corn roots	Natural soil	CeO_2-NPs transferred into the root tissues without any changes to chemical speciation*	Zhao et al., *J. Hazard. Mater.* **2012,** *225*, 131-138
Pumpkin shoots	Hydroponic	CeO2 NPs translocated into the shoots	Schwabe et al., *Chemosphere* **2013**, *91*, 512-520
Cucumber	Hydroponic	Presence of CeO_2-NPs with limited transformation to cerium phosphates and carboxylates (13-34% depending on plant parts)	Zhang et al., *Metallomics* **2011**, *3*, 816-822 Zhang et al., *ACS Nano* **2012**, *6*, 9943-9950
Lettuce	Sand	Cerium was mostly CeO_2-NPs with small amounts of Ce(III) species (8-23%)	Zhang et al., *Nanotoxicology* **2013**, DOI: 10.3109/17435390.2013.855829 Gui et al., *PLOS One* **2015**, 10, e0134261 Zhang et al., *Environ. Pollut.* **2016**, http://dx.doi.org/10.1016/j.envpol.2016.10.094

*Experiments that used the same CeO_2-NPs employed in this study.



SI Figure 4. Ce μXANES spectra from (A) crown spot 1, (B) root 1 spot 0, and (C) root 1 spot 1 from μXRF maps in Figures 5. Spectra in red line represents linear combination (LC) fits, and white solid line spectra represents μXANES from the sample.