Appendix A. Supplementary information

Long-term outdoor lysimeter study with cerium dioxide nanomaterial

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The Supplementary information includes nine figures and four tables on 17 pages.

Content

Figures

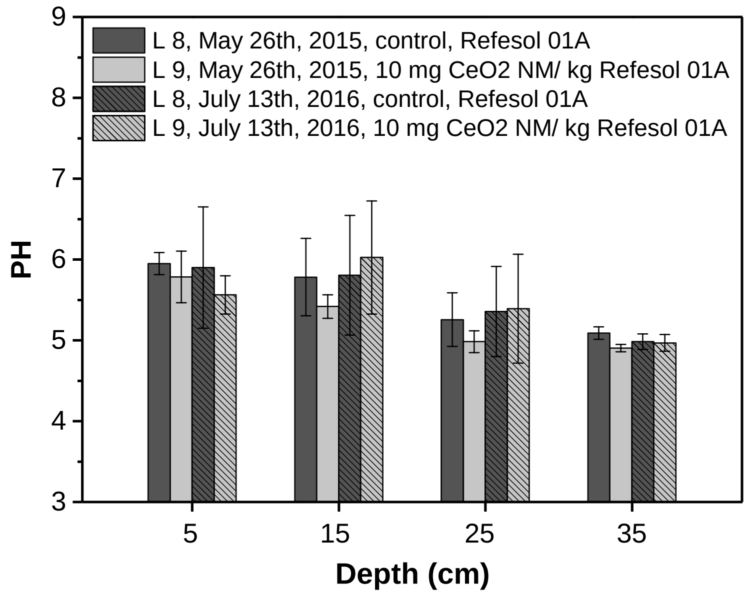

Fig. S1: Soil pH in the rainwater applied lysimeters at different points in time: The cerium dioxide nanomaterial (CeO_2 NM) was applied by artificial rainwater to lysimeter 9 (L 9). Lysimeter 8 (L 8) received artificial rainwater without CeO_2 NM (control). From each lysimeter, five borehole samples were taken and subdivided in four layers (0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm). The bars show the standard deviation of the five replicated pH measurements per layer.

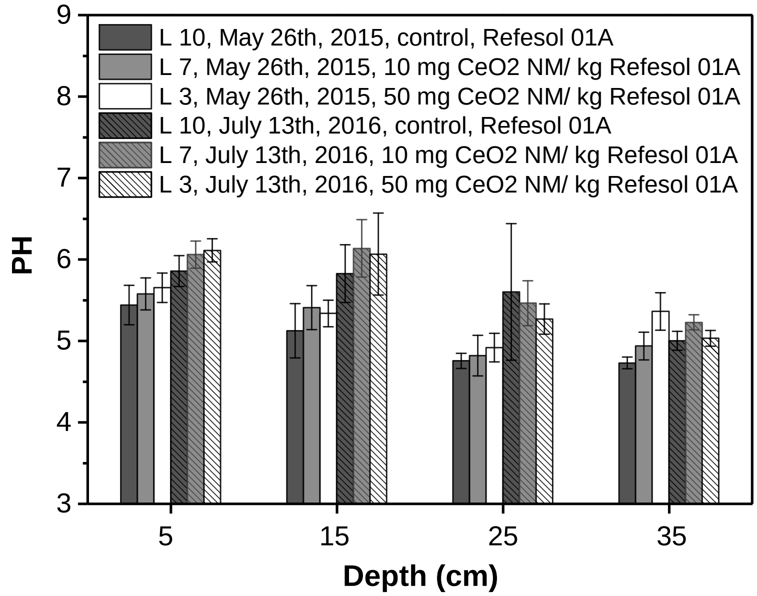

Fig. S2: Soil pH in the sewage sludge applied lysimeters at different points in time: The cerium dioxide nanomaterial (CeO_2 NM) was applied by sewage sludge to lysimeter 3 (L 3) and Lysimeter 7 (L 7). Lysimeter 10 (L 10) received sewage sludge without CeO_2 NM (control). From each lysimeter, five borehole samples were taken and subdivided in four layers (0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm). The bars show the standard deviation of the five replicated pH measurements per layer.

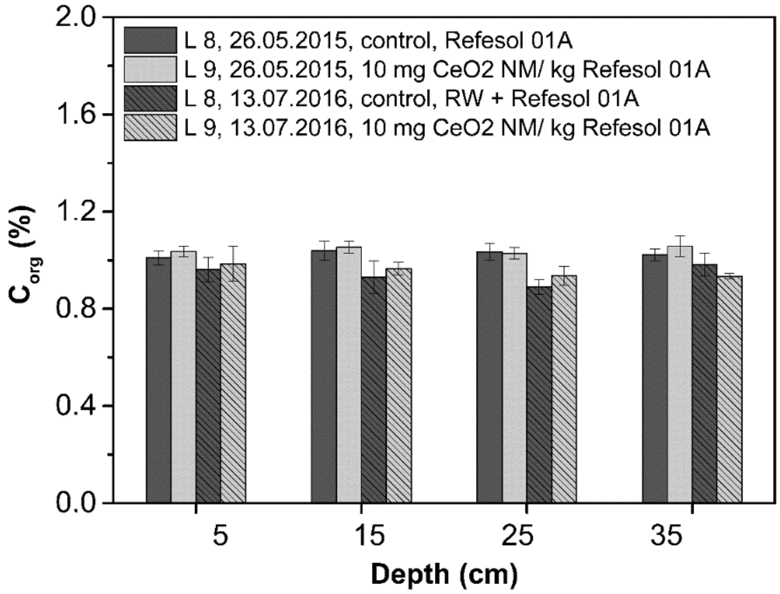

Fig. S3: Soil organic carbon content (C_{org}) in the rainwater applied lysimeters at different points in time: The cerium dioxide nanomaterial (CeO_2 NM) was applied by artificial rainwater to lysimeter 9 (L 9). Lysimeter 8 (L 8) received artificial rainwater without CeO_2 NM (control). From each lysimeter, five borehole samples were taken and subdivided in four layers (0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm). The bars show the standard deviation of the five replicated C_{org} measurements per layer.

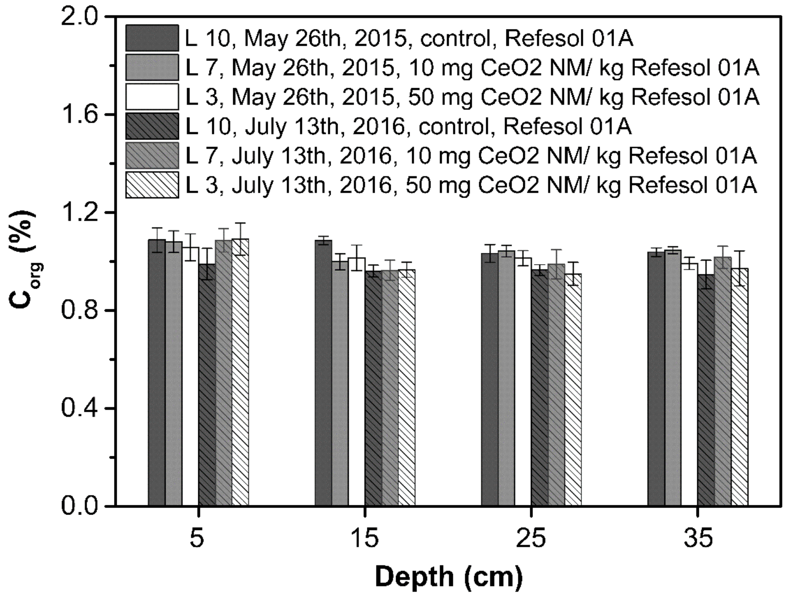

Fig. S4: Soil organic carbon content (C_{org}) in the sewage sludge applied lysimeters at different points in time: The cerium dioxide nanomaterial (CeO_2 NM) was applied by sewage sludge to lysimeter 3 (L 3) and Lysimeter 7 (L 7). Lysimeter 10 (L 10) received sewage sludge without CeO_2 NM (control). From each lysimeter, five borehole samples were taken and subdivided in four layers (0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm). The bars show the standard deviation of the five replicated C_{org} measurements per layer.

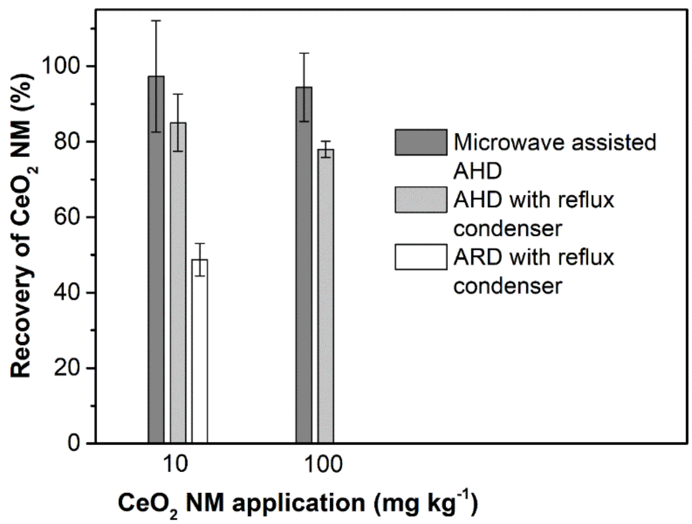

Fig. S5: Recovery rates for cerium dioxide nanomaterial (CeO_2 NM) spiked soils using different digestion methods: The CeO_2 NM was mixed to soil (Refesol 01A) as dry powder. Microwave assisted acid-hydrogen-peroxide digestion (AHD), AHD in a vessel with reflux condenser, and aqua regia digestion (ARD) in a vessel with reflux condenser were measured in triplicates. Error bars show the standard deviation.

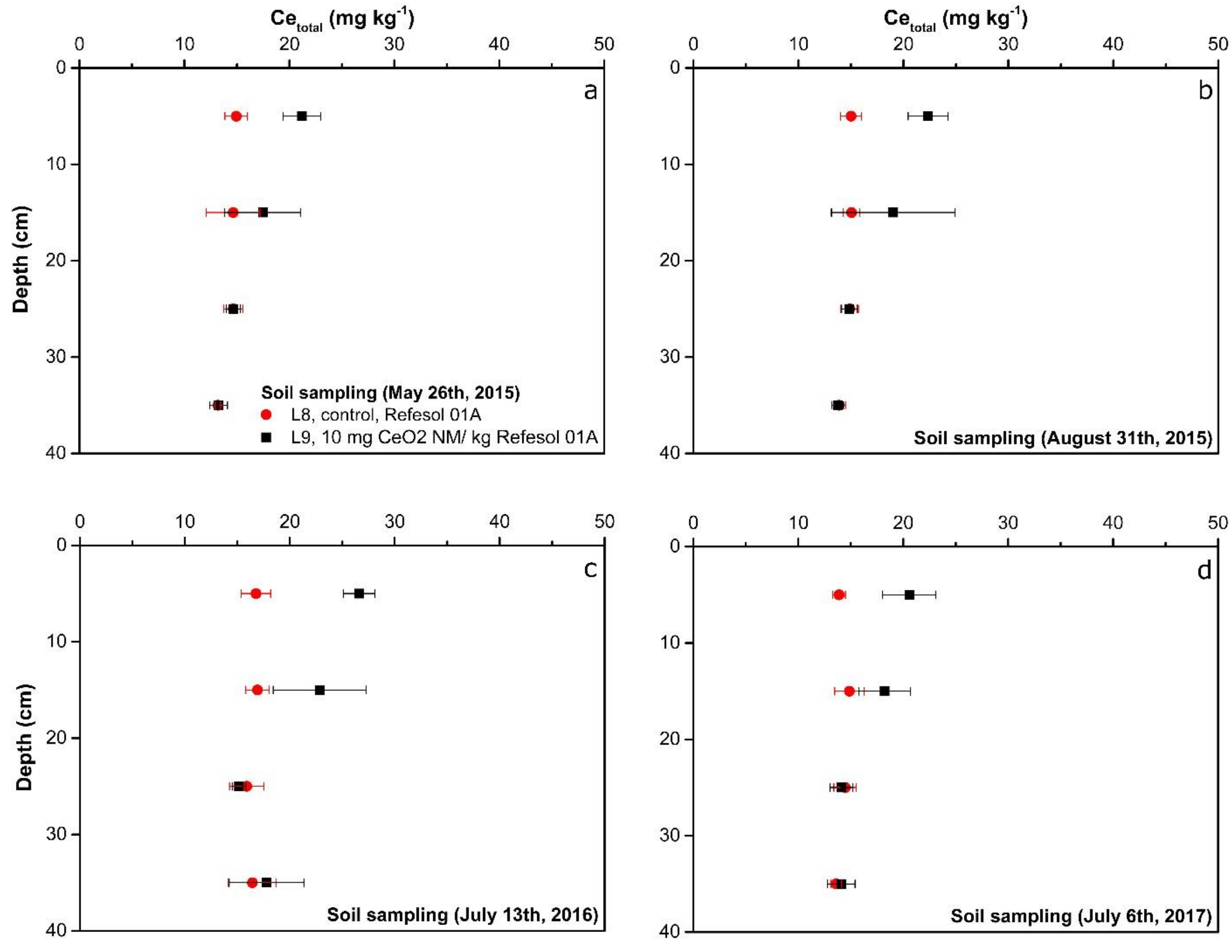

Fig. S6: Total Ce concentrations in the lysimeter soil: The cerium dioxide nanomaterial (CeO_2 NM) was applied by artificial rainwater to lysimeter 9 (L 9). Lysimeter 8 (L 8) received artificial rainwater without CeO_2 NM (control). From each lysimeter, five borehole samples were taken and subdivided in four layers (0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm). The bars show the standard deviation of the five replicated Ce measurements per layer.

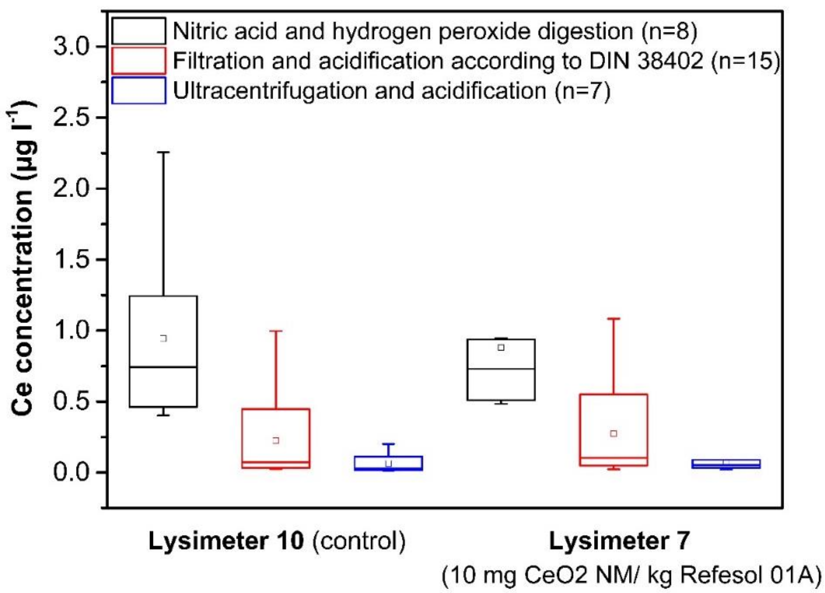

Fig. S7: The Ce concentrations in the different fractions of percolating water: The cerium dioxide nanomaterial (CeO_2 NM) was applied by sewage sludge to lysimeter 7 (L 7). Lysimeter 10 (L 10) received sewage sludge without CeO_2 NM (control). From each lysimeter, the percolating water was sampled after every rainfall event, and subsequently filtered and acidified before measurement of the Ce concentrations (n = 15, red boxplots, $Ce_{0.45µm}$). The monthly taken fresh water samples were digested (n = 8, black boxplots, Ce_{total}) or ultracentrifuged and acidified (n = 7, blue boxplots, $Ce_{dissolved}$) before the measurement of the of the Ce concentrations. According to the Mann-Whitney U test, no significant differences were found between L 10 and L 7 in the similarly treated groups (Table S3).

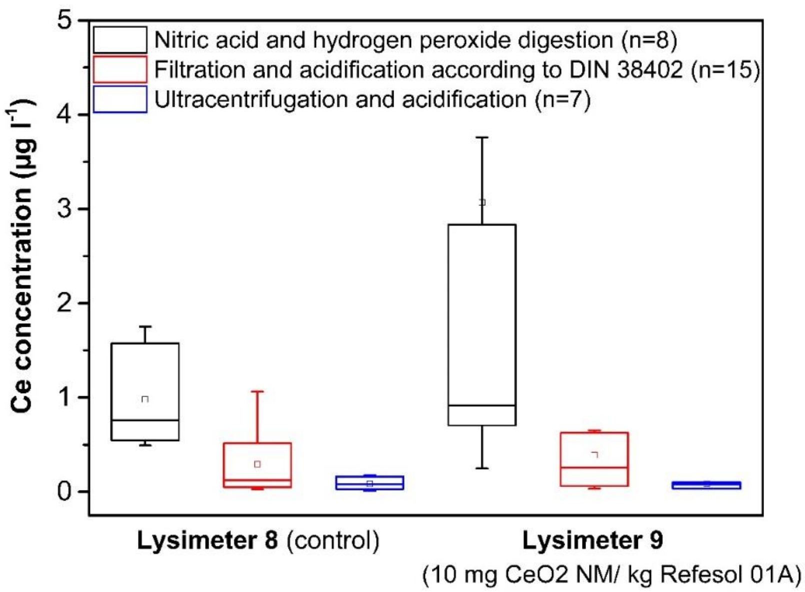

Fig. S8: The Ce concentrations in the different fractions of percolating water: The cerium dioxide nanomaterial (CeO_2 NM) was dispersed in artificial rainwater which was applied to lysimeter 9 (L 9). Lysimeter 8 (L 8) received artificial rainwater without CeO_2 NM (control). From each lysimeter, the percolating water was sampled after every rainfall event, and subsequently filtered and acidified before measurement of the Ce concentrations (n = 15, red boxplots, $Ce_{0.45µm}$). The monthly taken fresh water samples were digested (n = 8, black boxplots, Ce_{total}) or ultracentrifuged and acidified (n = 7, blue boxplots, $Ce_{dissolved}$) before measurement of the of the Ce concentrations. A single outliner was obtained directly after the CeO_2 NM application (Ce_{total} = 16 µg l^{-1}, May 21st, 2015). According to the Mann-Whitney U test, no significant differences were found between L 8 and L 9 in the similarly treated groups (Table S3).

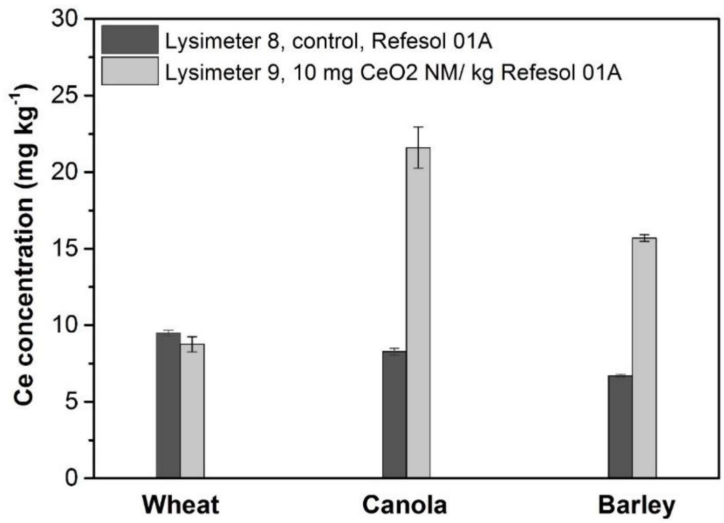

Fig. S9: Total Ce concentrations in the plant roots: The cerium dioxide nanomaterial (CeO_2 NM) was dispersed in artificial rainwater that was dosed to lysimeter 9. Lysimeter 8 received artificial rainwater without CeO_2 NM (control). The washed roots were digested with HNO_3 and H_2O_2. Afterwards, the Ce concentrations were determined in triplicates, bars show the standard deviation.

Tables

Table S1: Time line of the lysimeter experiments with cerium dioxide nanomaterial (CeO_2 NM).

Date	Sewage sludge spiked lysimeters (L 3, L 7, L 10)	Rainwater spiked lysimeters (L 8, L 9)
January, 2015	Liming	Liming
April 27th – May 21th, 2015		Application of CeO_2 NM via rainwater[a]
May 2nd, 2015	Application of CeO_2 NM via sewage sludge	
May 2nd, 2105	Ploughing	
May 22nd, 2015		Ploughing
May 26th, 2015	Soil sampling and wheat sowing	Soil sampling and wheat sowing
August 31st, 2015	Soil and wheat sampling and ploughing	Soil and wheat sampling and ploughing
September 2nd, 2015	Canola sowing	Canola sowing
July 13th, 2016	Soil and canola sampling and ploughing	Soil and canola sampling and ploughing
September 08th, 2016	Barley sowing	Barley sowing
July 6th, 2017	Soil and barley sampling	Soil and barley sampling

[a]Artificial rainwater according to Siemens et al. (2008).

Table S2: Inhibition of ammonium oxidizing bacteria (AOB) in the outdoor lysimeter experiment with cerium dioxide nanomaterial after application via artificial rainwater and sewage sludge.

Sampling	Application	Nominal concentration [mg/kg dm soil]	Mean activity of AOB [ng NO_2-N/g dm*h]	Standard deviation [ng NO_2-N/g dm*h]	Coefficient of variation [%]	Inhibition to control [%]
September 2015	Artificial rainwater	0	89.9	5.1	5.6	
		10	87.5	2.7	3.0	2.7
	Sewage sludge	0	76.7	7.9	10.2	
		10	83.4	4.1	4.9	-8.7
		50	65.8	10.3	15.6	14.3
November 2015	Artificial rainwater	0	100.7	5.9	5.8	
		10	112.5	5.6	5.0	-11.7
	Sewage sludge	0	64.2	2.8	4.4	
		10	70.4	3.0	4.3	-9.7
		50	58.0	3.7	6.3	9.6
July 2016	Artificial rainwater	0	109.0	5.6	5.1	
		10	116.9	10.1	8.7	-7.2
	Sewage sludge	0	55.1	4.8	8.7	
		10	55.7	1.6	2.9	-1.1
		50	54.9	3.4	6.2	0.3
February 2017	Artificial rainwater	0	50.0	7.9	15.8	
		10	39.4	4.3	10.9	21.2
	Sewage sludge	0	76.6	4.0	5.2	
		10	75.0	2.6	3.5	2.0
		50	78.2	2.1	2.6	-2.2
June 2017	Artificial rainwater	0	72.1	3.4	4.7	
		10	63.1	1.3	2.0	12.5
	Sewage sludge	0	88.0	6.1	6.9	
		10	75.3	3.7	4.9	14.4
		50	93.3	7.1	7.6	-6.1

Table S3: The Mann-Whitney U test was used to determine whether the samples from the different populations of the percolating water show the same distribution (null hypothesis (H_0)). No significant differences were found for the median Ce concentrations between the control lysimeters and the CeO_2 NM applied lysimeters.

CeO_2 NM application	Fraction	Control lysimeter	CeO_2 NM lysimeter	Median Ce concentration in the control (µg l^{-1})	Median Ce concentration in the CeO_2 NM lysimeter (µg l^{-1})	Value of U	Value of P	Number of samples per group ($n_1 = n_2$)
RW[a]	$Ce_{dissolved}$	L 8	L 9	0.081	0.089	27	0.805	7
RW	$Ce_{0.45µm}$	L 8	L 9	0.122	0.257	132	0.436	15
RW	Ce_{total}	L 8	L 9	0.758	0.916	51	0.387	9
SeS[b]	$Ce_{dissolved}$	L 10	L 7	0.031	0.054	16	0.318	7
SeS	$Ce_{0.45µm}$	L 10	L 7	0.073	0.103	99	0.595	15
SeS	Ce_{total}	L 10	L 7	0.741	0.731	30	0.878	8
SeS	$Ce_{dissolved}$	L 10	L 3	0.031	0.020	27	0.805	7
SeS	$Ce_{0.45µm}$	L 10	L 3	0.073	0.160	77	0.148	15
SeS	Ce_{total}	L 10	L 3	0.741	0.991	26	0.574	8

[a]Artificial rainwater according to Siemens et al. (2008), [b]sewage sludge.

Table S4: The results, uncertainties, and fit quality parameters of the linear combination fits (LCF) between $-20 < E_0 < 50$ eV.

	L 3, Refesol 01A, July 6th, 2017		(L 3, barley root, July 6th, 2017		L 3, Refesol 01A, May 25th, 2015	
	Fraction	Uncertainty	Fraction	Uncertainty	Fraction	Uncertainty
CeO_2 bulk	0.208	0.481	0.430	0.176	0.747	0.102
CeO_2 nano	0.284	0.462	0.360	0.169	0.118	0.098
Ce-Allanite	0.000	0.175	0.153	0.065	0.088	0.038
$CePO_4$	0.522	0.200	0.000	0.073	0.000	0.042
Fit Quality parameter						
R-factor	0.02716		0.00437		0.00154	
Chi-square	1.25543		0.16870		0.05648	
Red. Chi-square	0.00923		0.00124		0.00042	

Details about ICP-MS measurements

Single-Element Rotistar standard (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) was used to calibrate the ICP-MS. Between the sample measurement, the ICP-MS device was recalibrated. The recalibration was confirmed by the measurements of an internal standard (Bernd Kraft GmbH, Duisburg, Germany).

The ISE 989 from Wageningen was used as reference material to confirm the Ce concentrations after aqua regia digestion (Ce = 43.4 mg kg^{-1} (International Soil-Analytical Exchange, 2015) *vs.* Ce = 45.5 mg kg^{-1} (standard deviation = 2.1 mg kg^{-1}, n = 3, BGR measurement 2015)).

International Soil-Analytical Exchange. 2015. Wageningen evaluating programs for analytical laboratories - Quarterly report 2015.1, January - March. *Wageningen University*, Wageningen.