Supplementary materials

Influence of cerium oxide nanoparticles on two terrestrial wild plant species

Figure S1 – Preparation of the experiment: filling the pots with the nCeO₂ amended substrate.

Figure S2 – Plantlets of H. lanatus and D. tenuifolia 10 d after sowing.
Figure S3 – Plants of *H. lanatus* and *D. tenuifolia* 30 day after sowing.

Figure S4 – Plants of *H. lanatus* (in the background) and *D. tenuifolia* before biomass harvesting.
1. Detection of nCeO2 in plant fractions

Small portions (0.03 g) of fresh roots and leaves were harvested, rinsed three times with deionized water and homogenized with 8 mL of 2 mM citrate buffer at pH 4.5, using an ultrasonic bath for 5 minutes. After the homogenization, for every sample 2 mL of the enzyme solution (0.05 g of enzyme dissolved in 2 ml of MilliQ water) were added. The final supernatants were analyzed via single particle inductively coupled plasma mass spectrometry (sp-ICP-MS) NexION 350 (Perkin Elmer, Waltham, MA, USA) to obtain the size distribution of nCeO2.

Table S1 – Most frequent particle size, mean particle size, number of peaks and content of dissolved Ce determined by sp–ICP–MS analysis after enzymatic extraction on roots and leaves of H. lanatus and D. tenuifolia treated with nCeO2 200 mg L⁻¹.

| Species   | Plant fraction | nCeO₂ size (nm) | Most frequent nCeO₂ size (nm) | Mean nCeO₂ size (nm) | Pulses (n) | Dissolved Ce (µg L⁻¹) |
|-----------|----------------|----------------|-------------------------------|---------------------|------------|------------------------|
| H. lanatus| Roots          | 25             | 30 ± 1.45                     | 36 ± 1.34           | 5785 ± 257 | 0.27 ± 0.03            |
|           | Roots          | 50             | 51 ± 1.53                     | 56 ± 1.65           | 1327 ± 49  | 7.07 ± 1.10            |
|           | Leaves         | 25             | 23 ± 1.20                     | 28 ± 1.84           | 1124 ± 64  | 0.14 ± 0.01            |
|           | Leaves         | 50             | 30 ± 0.58                     | 36 ± 1.14           | 1140 ± 73  | 0.24 ± 0.05            |
| D. tenuifolia| Roots       | 25             | 50 ± 3.46                     | 53 ± 3.35           | 11,909 ± 711 | 14.57 ± 1.13            |
|           | Roots          | 50             | 79 ± 0.88                     | 82 ± 0.87           | 2855 ± 76  | 100.30 ± 1.45          |
|           | Leaves         | 25             | 19 ± 1.20                     | 26 ± 0.51           | 818 ± 29   | 0.05 ± 0.02            |
|           | Leaves         | 50             | 25 ± 0.33                     | 32 ± 0.84           | 1208 ± 24  | 0.13 ± 0.01            |

2. Plant biomass allocation patterns

Experimental biometric dataset was used to evaluate biomass allocation patterns to roots, stems and leaves of studies species in response to nCeO2 treatments.

Table S2 – Two-way ANOVA p value determined for biometric variables of H. lanatus and D. tenuifolia. ns is not significant at p≤.05, *, ** and *** indicate significance at p≤.05, p≤.01 and p≤.001, respectively.

| Source          | Roots DW | n. Stems | Stems DW | Leaf area | Leaves DW | Total DW |
|-----------------|----------|----------|----------|-----------|-----------|----------|
| Species         | .0000 ***| .0000 ***| .0000 ***| .0000 *** | .9552 ns  | .0123 *  |
| Treatment       | .3394 ns | .0094 ** | .0574 ns | .0005 *** | .0482 *   | .2017 ns |
| Species x Treat | .0045 ** | .0157 *  | .0670 ns | .0958 ns  | .6577 ns  | .1859 ns |

Table S3 – Biomass allocation variables calculated from plant measurements (Poorter et al, 2011).

| Variable               | Abbreviation | Definition                                      | Unit    |
|------------------------|--------------|-------------------------------------------------|---------|
| Root Mass Fraction     | RMF          | Root dry mass/Total plant dry mass               | g g⁻¹   |
| Stem Mass Fraction     | SMF          | Stem dry mass/Total plant dry mass               | g g⁻¹   |
| Leaf Mass Fraction     | LMF          | Leaf dry mass/Total plant dry mass               | g g⁻¹   |
| Shoot to Root ratio    | S/R ratio    | (Leaf + Stem dry mass)/Root dry mass             | g g⁻¹   |
| Leaf Area Ratio        | LAR          | Leaf area/Total plant dry mass                   | m² kg⁻¹ |
| Specific Leaf Area     | SLA          | Leaf area/Leaf dry mass                          | m² kg⁻¹ |
Figure S5. Stems dry matter ± standard deviation of *H. lanatus* and *D. tenuifolia*. Comparison between control and plants grown in presence of 200 mg kg⁻¹ nCeO₂ having respectively 25 nm and 50 nm. For each species the statistically significant difference (p ≤ 0.05) between treatments is indicated by the letters using one-way ANOVA followed by Tukey’s test.

Figure S6. Total plant dry matter ± standard deviation of *H. lanatus* and *D. tenuifolia*. Comparison between control and plants grown in presence of 200 mg kg⁻¹ nCeO₂ having respectively 25 nm and 50 nm. For each species the statistically significant difference (p ≤ 0.05) between treatments is indicated by the letters using one-way ANOVA followed by Tukey’s test.

Table S4 – Two-way ANOVA p value determined for biometric ratios calculated for *H. lanatus* and *D. tenuifolia*. ns is not significant at p≤.05, *, ** and *** indicate significance at p≤.05, p≤.01 and p≤.001, respectively.

| Source           | Root:Shoot | RMF  | SMF  | LMF  | LAR  | SLA  |
|------------------|------------|------|------|------|------|------|
| Species          | .0000 ***  | .0000 *** | .0000 *** | .0000 *** | .0000 *** | .0000 *** |
| Treatment        | .0038 **   | .0070 ** | .1022 ns | .0618 ns | .0021 ** | .0017 ** |
| Species x Treatment | .0026 **   | .0035 ** | .0174 * | .0549 ns | .1134 ns | .0583 ns |
3. Cerium concentration in plant fractions

Table S5 – Two-way ANOVA p value determined for Ce concentration in plant fractions of *H. lanatus* and *D. tenuifolia*. ns is not significant at p≤.05, * *, ** and *** indicate significance at p≤.05, p≤.01 and p≤.001, respectively.

| Source            | Ce root | Ce stems | Ce leaves |
|-------------------|---------|----------|-----------|
| Species           | .0289 * | 0.2395 ns| .9910 ns  |
| Treatment         | .0000 ***| 0.0131 * | .0003 *** |
| Species x Treatment| .1651 ns| .0998 ns | .0020 **  |
4. Macronutrient and micronutrient concentration in plant fractions

**Table S6** – Two-way ANOVA p value for concentration of macronutrients and micronutrients in roots of *H. lanatus* and *D. tenuifolia*. ns is not significant at p≤.05, *, ** and *** indicate significance at p≤.05, p≤.01 and p≤.001, respectively.

| Source             | K      | Mg     | Na     | P     | Cu     | Fe     | Mn     | Zn     |
|--------------------|--------|--------|--------|-------|--------|--------|--------|--------|
| Species            | .0000 *** | .0000 *** | .0076 ** | .0000 ** | .0000 *** | .0000 *** | .0000 *** | .0000 *** |
| Treatment          | .4124 ns | .3942 ns | .0044 ** | .2220 ns | .8510 ns | .0013 ** | .0058 ** | .0650 ns |
| Species x Treatment| .1045 ns | .0671 ns | .5601 ns | .1701 ns | .8797 ns | .1353 ns | .0917 ns | .0000 *** |

**Table S7** – Two-way ANOVA p value for concentration of macronutrients and micronutrients in stems of *H. lanatus* and *D. tenuifolia*. ns is not significant at p≤.05, *, ** and *** indicate significance at p≤.05, p≤.01 and p≤.001, respectively.

| Source             | K      | Mg     | Na     | P     | Cu     | Fe     | Mn     | Zn     |
|--------------------|--------|--------|--------|-------|--------|--------|--------|--------|
| Species            | .0004 *** | .1435 ns | .0009 *** | .0198 | .0008 *** | .0289 * | .0000 *** | .0108 * |
| Treatment          | .2437 ns | .9615 ns | .1697 ns | .2452 ns | .8216 ns | .0075 ** | .0495 * | .4795 ns |
| Species x Treatment| .4800 ns | .6225 ns | .2653 ns | .7548 ns | .3758 ns | .4410 ns | .0612 ns | .8050 ns |

**Table S8** – Two-way ANOVA p value for concentration of macronutrients and micronutrients in leaves of *H. lanatus* and *D. tenuifolia*. ns is not significant at p≤.05, *, ** and *** indicate significance at p≤.05, p≤.01 and p≤.001, respectively.

| Source             | K      | Mg     | Na     | P     | Cu     | Fe     | Mn     | Zn     |
|--------------------|--------|--------|--------|-------|--------|--------|--------|--------|
| Species            | .0115 * | .0000 *** | .2653 ns | .3579 ns | .1970 ns | .6790 ns | .0000 *** | .0000 *** |
| Treatment          | .1777 ns | .8807 ns | .0876 ns | .2470 ns | .0132 * | .1282 ns | .1798 ns | .2486 ns |
| Species x Treatment| .0442 * | .3137 ns | .2396 ns | .0864 ns | .0947 ns | .0466 * | .1510 ns | .3278 ns |
References

Evans, G.C. The quantitative analysis of plant growth. Oxford, UK: Blackwell Scientific Publications. 1972. [https://doi.org/10.2307/2259048].

Gunn, S.; Farrar, J.F.; Collis, B.E.; Nason, M. Specific leaf area in barley: individual leaves versus whole plants. *New Phytol.*, 1999, 143, 45–51. [https://doi.org/10.1046/j.1469-8137.1999.00434.x].

Poorter, H.; Niklas, K.J.; Reich, P.B.; Oleksyn, J.; Poot, P.; Mommer, L.; Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytol.*, 2012, 193: 30–50. [http://doi.org/10.1111/j.1469-8137.2011.03952.x].