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# Wheat exposure to cerium oxide nanoparticles over three generations reveals transmissible changes in nutrition, biochemical pools, and response to soil N

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ABSTRACT

This study investigated the effects of third generation exposure to cerium oxide nanoparticles ($CeO_2$-NPs) on biomass, elemental and $^{15}N$ uptake, and fatty acid contents of wheat (*Triticum aestivum*). At low or high nitrogen treatment (48 or 112 mg N), seeds exposed for two generations to 0 or 500 mg $CeO_2$-NPs per kg soil treatment were cultivated for third year in soil amended with 0 or 500 mg $CeO_2$-NPs per kg soil. The results showed that parental and current exposures to $CeO_2$-NPs increased the root biomass in daughter plants with greater magnitude of increase at low N than high N. When wheat received $CeO_2$-NPs in year 3, root elemental contents increased primarily at low N, suggesting an important role of soil N availability in altering root nutrient acquisition. The $\delta^{15}N$ ratios, previously shown to be altered by $CeO_2$-NPs, were only affected by current and not parental exposure, indicating effects on N uptake and/or metabolism are not transferred from one generation to the next. Seed fatty acid composition was also influenced both by prior and current exposure to $CeO_2$-NPs. The results suggest that risk assessments of NP exposure may need to include longer-term, transgenerational effects on growth and grain quality of agronomic crops.

## 1. Introduction

Contemporary nanophytotoxicity studies have focused on documenting immediate toxicity responses and engineered nanoparticles (NPs) uptake in plants and have neglected the long-term intergenerational implications of NPs exposure (Geisler-Lee et al., 2014; Rico et al., 2017). The majority of studies, especially recent metabolic investigations, reveal that NPs do not cause acute toxic effects in plants but induce subtle phenological or phenotypic modifications which eventually can alter the quality and composition of seeds (Zhao et al., 2016). When grown in succeeding generations, seed quality may affect physiological and biochemical processes that alter growth, survival, and productivity in progeny plants. Therefore, multigenerational exposure to engineered nanoparticles may have long-term environmental and ecological implications that need to be investigated.

Cerium oxide nanoparticles ($CeO_2$-NPs) exhibit negligible dissolution in environmental media and they are predicted to accumulate and persist in soil, and therefore interact with plants in nanoparticulate form (Rico et al., 2017; Hoppe et al., 2019). Various studies have shown that $CeO_2$-NPs do not cause plant mortality (i.e. plants go to full maturity and harvest) but significantly alter macromolecular (e.g. carbohydrates, protein, fatty acids) and nutrient (e.g. Ca, P, K, Mn, Fe) compositions of seeds even in the absence of Ce accumulation (Ma et al., 2016; Rico et al., 2017; Duncan and Owens, 2019). In addition, $CeO_2$-NPs exposure has been found to alter N uptake and/or metabolism in wheat, depending on the form of N provided (Rico et al., 2018). Therefore, it is highly possible that repeated exposures to $CeO_2$-NPs may alter seed quality and performance of plants in terrestrial environments.

Intergenerational studies in plants exposed to engineered nanoparticles have been increasingly reported in the literature. Reports have shown that first generation exposure to $TiO_2$-NPs promoted growth but adversely affected the photosynthetic ability of basil treated again with $TiO_2$-NPs in the second generation (Tan et al., 2018). Other studies have shown that CuO-NPs modified gene expressions in successive generations of exposed *Arabidopsis thaliana*, $CeO_2$-NPs induced plant

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retardation in multigenerational exposure in tomato but enhanced growth and seed maturity in wheat, and ZnO-NPs induced minimal intergenerational effects on seed composition of *Phaseolus vulgaris* (Wang et al., 2016; Rico et al., 2017; Medina-Velo et al., 2018). For multigenerational studies, Ma et al. (2016) reported reduced growth and productivity in *Brassica rapa* exposed to $CeO_2$-NPs for three generations while Geisler-Lee et al. (2014) found drastically reduced germination rates in three-generation treated *A. thaliana*. Wang et al. (2012) found that progenies of tomato (*Solanum lycopersicum* L.) previously grown in cerium oxide nanoparticles were smaller and weaker with higher reactive oxygen species content.

Repeated exposures of wheat to $CeO_2$-NPs may affect the responses of progeny to succeeding NPs exposure. Studies have shown that environmental stresses may interact in their effect on plants, and that parental exposure may impart fitness and tolerance attributes in offspring exposed to the same stress. For example, *A. thaliana* that experienced metal stress (i.e. Ni, Cd) for three generations imparted tolerance to metal exposure in the offsprings (Rahavi et al., 2011). Progeny generation of salt-stressed *A. thaliana* also exhibited improved survival rate and reproductive output when exposed to similar salt stress (Boyko et al., 2010; Suter and Widmer, 2013). Soil nutrient conditions experienced by parents also have been found to result in significant effects on size of offspring of *Senecio* sp (Aarssen and Burton, 1990) and biomass and carbon storage in progeny of *Plantago lanceolate* (Latzel et al., 2014). Likewise, nitrogen-stressed rice imparted increased tolerance to nitrogen limitation for two progeny generations (Kou et al., 2011).

The current study is the third in a series of long-term, full life-cycle studies of wheat exposed to $CeO_2$-NPs. We found that at second generation exposure, wheat exhibited greater delay in grain production and maturity, lower elemental concentrations, and altered nitrogen metabolism that were not observed during the first generation exposure (Rico et al., 2014, 2017). This study investigated the influence of multigenerational exposure to $CeO_2$-NPs on the growth, reproductive output, and seed quality of third generation wheat cultivated in low or high nitrogen amended soil. Several parameters were measured including biomass yield, seed production, nutrient accumulation, cerium uptake, $^{15}N$ discrimination, and fatty acid concentrations. The goals were 1) to identify the effects of parental exposure to $CeO_2$-NPs on growth, nutrient content, and development in 3$^{rd}$ generation progeny; 2) to identify whether parental exposure alters the response of 3$^{rd}$ generation progeny to $CeO_2$-NP exposure; and 3) to identify whether edaphic conditions such as soil N availability alters these responses. The results showed that although soil N level often affected the degree of response, very few significant interactions were present, allowing us to focus on the primary effects of parental vs current $CeO_2$-NP exposure at the two different levels of soil N.

## 2. Materials and methods

### 2.1. Experimental design

The experiment was a $2 \times 2 \times 2$ treatment combination of seed type (i.e. seeds whose parents were exposed for 2 generations vs not exposed), $CeO_2$-NPs exposure (i.e. 0 or 500 mg $CeO_2$-NPs per kg soil), and soil N (i.e. 48 or 112 mg N added; low N or high N soil). From our second-generation study (Rico et al., 2017), seeds were harvested from plants grown for two consecutive generations in soil amended with 0 or 500 mg $CeO_2$-NPs per kg soil (C1C2 or T1T2) and were cultivated to produce third generation plants grown in soil amended with 0 or 500 mg $CeO_2$-NPs per kg soil (C3 or T3). For example, C1C2 or T1T2 seeds were cultivated in control (C3) or $CeO_2$-NPs amended soil (T3) giving four treatment combinations of C1C2C3, C1C2T3, T1T2C3, and T1T2T3 each for low N or high N soil. High N treatment was achieved by adding Yoshida nutrient solution (Yoshida et al., 1976) that contained the normal amount of ammonium nitrate ($NH_4NO_3$, 80 mg N per L) whereas low N treatment was created by adding nutrient solution that contained zero or half of the $NH_4NO_3$ concentration (0 or 40 mg N per L). At the end of the experiment, the low or high N treatment received a total of 48 or 112 mg N from the nutrient solution (SI Table 1). Only the $NH_4NO_3$ component of the nutrient solution was modified. Each treatment combination had six replicates.

### 2.2. Soil preparation and $CeO_2$-NPs addition in soil

The soil was a 3:1 (v:v) mixture of Sunshine Mix #2 potting soil (i.e. no added fertilizer, SunGro Horticulture) and sand thoroughly mixed using a cement mixer. The soil mixture contained 0.18% N or 360 mg N per pot. $CeO_2$-NPs (Meliorum Technologies, Rochester, NY) were rods with primary size of $67 \pm 8 \times 8 \pm 1$ nm (length × diameter), surface area of 93.8 $m^2/g$, 95.14% purity, but their dispersed particle size in DI water was $231 \pm 16$ nm (Keller et al., 2010). A 100 mg $CeO_2$-NPs were sonicated in 50 mL Millipore water at 25 °C for 30 min in a water bath (Branson Ultrasonics, Danbury, CT). The $CeO_2$-NPs suspension poured evenly in pot containing 200-gram dry weight equivalent of soil mix to give the necessary 500 mg $CeO_2$-NPs per kg soil treatment. The pots were prepared and aged in the growth chamber three days before seedlings were transplanted.

### 2.3. Plant cultivation and management

Wheat seedlings were prepared and grown to full maturity as described previously (Rico et al., 2017). Two nine-day-old seedlings were transplanted in each pot (one seedling/100 g dry weight soil) and grown in growth chamber (Environmental Growth Chamber, Chagrin Falls, OH) with these conditions: 16-h photoperiod, 20/10 °C, 70% humidity, 300 μmol/$m^2$-s light intensity for the first 40 days, after which the conditions were kept at 16-h photoperiod, 25/15 °C, 70% humidity, 600 μmol/$m^2$-s light intensity until harvest. Yoshida nutrient solution was prepared and added during the experiment as described in the SI (SI Table 1). Ladybugs (family Coccinellidae) were used as a biological control to prevent possible wheat green bug (*Schizaphis graminum*) infestation. At harvest, plant materials were oven-dried and weighed for total biomass. Two soil core samples were collected from each pot in soil experiment to estimate total root biomass.

### 2.4. Elemental analysis

The methods for microwave digestion of plant samples, preparation of calibration standards, instrumentations for trace element analysis, and performance of quality check and control were adopted from Avula et al. (2010). Plant materials were digested in 5 mL concentrated plasma pure nitric acid (SCP Sciences, Champlain, NY) using microwave system (CEM Mars 6, Matthews, NC), and the digestate was diluted to 50 mL using Millipore water. Blanks, duplicates, and NIST-1547 peach leaves as reference standard (NIST, Gaithersburg, MD) were used to validate the digestion and analytical methods. Trace metal analysis was performed using Agilent 7900 Inductively Coupled Plasma - Mass Spectroscopy (Agilent Technologies, Palo Alto, CA). Analyses of blank and spiked samples were repeated every 12 samples to ensure instrument stability and performance.

### 2.5. Analysis of C, N and $^{15}N$

Roots, shoots, and grains collected during harvest were analyzed for C, N and $^{15}N$ uptake. Analysis was performed using an Elementar Vario Isotope Cube (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a Isoprime 100 isotope ratio mass spectrometer (Isoprime Ltd, Stockport UK) as previously described in Rico et al. (2017).

Three laboratory isotope standards were analyzed to assess quality assurance or check calibration. The final values were expressed relative to Air as internal standard. The $\delta^{15}N(‰)$ of $NH_4NO_3$ was 4.98 ± 0.18 and that of the soil was -1.87 ± 0.28. Whole-plant $\delta^{15}N$ was calculated according to Robinson et al. (2000) and Kalcits and Guy (2013) as described in Rico et al. (2018).

### 2.6. Fatty acid analysis

The method for simultaneous extraction and methyl esterification of fatty acids was adopted from Gajewska et al. (2012) and Rico et al. (2013). The esterification mixture was prepared by mixing 200 mg of finely ground wheat grains, 1 mL methanolic sulfuric acid (5% $H_2SO_4$ in methanol), and 1 mL of the internal standard (i.e. 1 mg/mL tridecanoic acid in toluene) that gave 0.5 mg tridecanoic acid per mL in the reaction mixture. The mixture was vortexed and heated for 1.5 h in 80 °C water bath. After cooling to room temperature, 1 g $Na_2SO_4$ was added before extracting the fatty acid methyl ester twice with 1 mL hexane. The organic phase was collected in amber vial and analyzed for fatty acid methyl esters using Varian 430 GC gas chromatograph equipped with flame ionization detector. Additional information on the operating conditions were presented in SI Table 2.

### 2.7. Data analysis

A 3-way ANOVA was performed on the data, using soil N status (i.e., high vs low), parental exposure (i.e., C1C2 vs T1T2), and current exposure (i.e., C3 vs T3) as the main treatment variables. Although levels of statistical significance varied between the high and low N treatments, there were very few significant interactions resulting from the soil N treatment. Therefore, we focused the presentation of results on the main effects of the two $CeO_2$-NP treatments (i.e., parental vs current $CeO_2$-NP exposure), separating high and low N treatments. The ANOVA was performed using the General Linear Model in SAS statistical package (SAS Institute, Cary, NC). In third generation experiment, two-way ANOVA comparison between parental exposure indicates comparing C1C2 (means of C1C2C3 and C1C2T3) with T1T2 (means of T1T2C3 and T1T2T3), and between current exposure indicates comparing C3 (means of C1C2C3 and T1T2C3) with T3 (means of C1C2T3 and T1T2T3). Tables 1 and 2 were presented to reflect this arrangement of treatments and means. For the second generation experiment (fatty acid data only), two-way ANOVA comparison between parental exposure signifies comparing C1 (means of C1C2 and C1T1) with T1 (means of T1C2 and T1T2), and comparison between current generation indicates comparing C2 (means of C1C2 and T1C2) with T2 (means of C1T2 and T1T2).

## 3. Results and discussion

### 3.1. Plant biomass

Both prior exposure and third generation exposure to $CeO_2$-NPs significantly increased root biomass at low and high N soil. The ANOVA showed much stronger statistical levels in root biomass in T1T2 and T3 at low N ($p < 0.001$ and $p < 0.01$, respectively) than high N ($p < 0.05$ and $p < 0.10$, respectively) (SI Table 3). Compared to C3, T3 enhanced root biomass production at low N soil (25% increase) higher than high N soil (11% increase) demonstrating that $CeO_2$-NPs boost plant growth even when soil nitrogen levels are low (Table 1). Similarly, T1T2 parental exposure produced more root biomass at low N (31% increase) than at high N (16% increase) relative to C1C2 controls (Table 1). Interestingly, T1T2 yielded root biomass increases (16–31%) much higher than T3 (11–25%) (Table 1).

There were no differences in shoot biomass, grain yield, and grain weight at low N despite the increases in root biomass (SI Table 3). At high N, shoot weight increased and grain weight decreased in T1T2 compared to C1C2. The increase in shoot weight was in agreement with high root biomass. The decrease in grain weight could be due to the plants using photosynthates to produce more grains (e.g. yield was not affected) than bigger and heavier grains.

This study showed that $CeO_2$-NPs were not harmful to plant (i.e. grain yield was not affected), but rather promoted root biomass production even at high $CeO_2$-NPs concentration (i.e. T3 = 500 mg per kg soil). Our previous study also showed that $CeO_2$-NPs do not affect total grain yield even with modifications in plant and grain development (e.g. delayed spike formation and maturity) (Rico et al., 2017).

**Table 1**
Effect of parental or current exposure to $CeO_2$-NPs on root, shoot and grain biomass of wheat cultivated at low or high N soil.[a]

| **Low N** | | | | **High N** | | | |
|---|---|---|---|---|---|---|---|
| **Root biomass (g)** | | | | **Root biomass (g)** | | | |
| | **C1C2** | **T1T2** | **Mean** | | **C1C2** | **T1T2** | **Mean** |
| **C3** | 6.83 ± 0.32 | 8.23 ± 0.32 | **7.53 ± 0.30** | **C3** | 8.25 ± 0.78 | 9.62 ± 0.43 | **8.93 ± 0.17** |
| **T3** | 7.80 ± 0.46 | 11.01 ± 0.88 | **9.40 ± 0.68***** | **T3** | 9.26 ± 0.60 | 10.62 ± 0.39 | **9.94 ± 0.40*** |
| **Mean** | **7.32 ± 0.31** | **9.62 ± 0.61****** | | **Mean** | **8.75 ± 0.49** | **10.12 ± 0.32**** | |
| **Shoot biomass (g)** | | | | **Shoot biomass (g)** | | | |
| | **C1C2** | **T1T2** | **Mean** | | **C1C2** | **T1T2** | **Mean** |
| **C3** | 25.12 ± 0.53 | 25.00 ± 0.47 | **25.06 ± 0.34** | **C3** | 24.26 ± 0.39 | 26.07 ± 0.79 | **25.17 ± 0.50** |
| **T3** | 24.67 ± 1.00 | 25.74 ± 0.71 | **25.20 ± 0.61** | **T3** | 23.64 ± 0.52 | 25.63 ± 0.49 | **24.64 ± 0.45** |
| **Mean** | **24.90 ± 0.55** | **25.37 ± 0.42** | | **Mean** | **23.95 ± 0.33** | **25.85 ± 0.45***** | |
| **Grain yield (g)** | | | | **Grain yield (g)** | | | |
| | **C1C2** | **T1T2** | **Mean** | | **C1C2** | **T1T2** | **Mean** |
| **C3** | 28.43 ± 1.40 | 30.44 ± 0.80 | **29.43 ± 0.83** | **C3** | 30.05 ± 0.42 | 27.05 ± 1.97 | **28.55 ± 1.06** |
| **T3** | 30.99 ± 0.75 | 29.90 ± 0.98 | **30.44 ± 0.61** | **T3** | 28.29 ± 0.86 | 27.75 ± 0.54 | **28.02 ± 0.49** |
| **Mean** | **29.70 ± 0.85** | **30.17 ± 0.61** | | **Mean** | **29.17 ± 0.53** | **27.40 ± 0.98** | |
| **Hundred grain weight (g)** | | | | **Hundred grain weight (g)** | | | |
| | **C1C2** | **T1T2** | **Mean** | | **C1C2** | **T1T2** | **Mean** |
| **C3** | 4.00 ± 0.17 | 4.05 ± 0.03 | **4.02 ± 0.08** | **C3** | 4.37 ± 0.05 | 4.09 ± 0.13 | **4.23 ± 0.08** |
| **T3** | 4.18 ± 0.07 | 3.99 ± 0.14 | **4.08 ± 0.08** | **T3** | 4.33 ± 0.09 | 4.23 ± 0.08 | **4.28 ± 0.06** |
| **Mean** | **4.09 ± 0.09** | **4.02 ± 0.07** | | **Mean** | **4.35 ± 0.05** | **4.16 ± 0.08**** | |

[a] Low or High N indicates total addition of 48 or 112 mg N as nutrient solution throughout the duration of the experiment; C1C2 or T1T2 indicates parental exposure to 0 or 500 mg $CeO_2$-NPs per kg soil at 1st and 2nd generations; C3 or T3 denotes current or 3rd generation exposure to 0 or 500 mg $CeO_2$-NPs per kg soil. Values are means ± SE; n = 6 per treatment combination. *, **, ***, **** represent significance at $p < 0.10$, 0.05, 0.01, 0.001, respectively.

**Table 2**
Effect of parental or current exposure to $CeO_2$-NPs on root cerium concentration (mg/kg) of wheat cultivated at low or high N soil.[a]

| **Low N** | | | |
|---|---|---|---|
| | **C1C2** | **T1T2** | **Mean** |
| **C3** | 2.81 ± 0.18 | 3.54 ± 0.25 | **3.17 ± 0.18** |
| **T3** | 284.11 ± 38.96 | 171.11 ± 25.93 | **227.78 ± 28.10**** |
| **Mean** | **143.62 ± 46.34** | **87.33 ± 28.12*** | |
| **High N** | | | |
| | **C1C2** | **T1T2** | **Mean** |
| **C3** | 4.62 ± 0.42 | 4.31 ± 0.56 | **4.46 ± 0.34** |
| **T3** | 298.41 ± 35.27 | 280.28 ± 57.59 | **289.35 ± 32.31**** |
| **Mean** | **151.51 ± 47.38** | **142.29 ± 49.85** | |

[a] Low or High N indicates total addition of 48 or 112 mg N as nutrient solution throughout the duration of the experiment; C1C2 or T1T2 indicates parental exposure to 0 or 500 mg $CeO_2$-NPs per kg soil at 1st and 2nd generations; C3 or T3 denotes current or 3rd generation exposure to 0 or 500 mg $CeO_2$-NPs per kg soil. Values are means ± SE; n = 6 per treatment combination. *, ** represent significance at $p < 0.05$ and 0.01, respectively.

Similar studies showed no negative effects of generational exposure in plants (Tan et al., 2018; Medina-Velo et al., 2018). In contrast, Ma et al. (2016) found reduced plant growth and biomass that resulted in decreased seed production in *Brassica napus* after three generations of exposure to $CeO_2$-NPs.

### 3.2. Cerium accumulation

Cerium was detected in the roots only. Compared to C3, $CeO_2$-NP treatment in the third generation (T3) increased root Ce concentration by 225 mg/kg (7076% increase) at low N and 285 mg/kg (6385% increase) at high N (Table 2). Surprisingly at low N, T1T2 parental exposure decreased root Ce concentration by 56 mg/kg (39% decrease) compared to C1C2. Ce content in roots (i.e. concentration × total root biomass) followed the trend recorded in T3 but not in T1T2 due to the tremendous increase in root biomass in T1T2 (Table 1). The trend in T3 (i.e. current exposure to 500 mg $CeO_2$-NPs per kg soil) increasing Ce concentration in roots has been repeatedly observed in plants, but this is the first report of parental exposure (i.e. T1T2) reducing Ce concentration in roots of daughter plants. It is not clear what caused T1T2 to decrease Ce concentration at low N only. Since $CeO_2$-NPs are adsorbed on root surface (Rico et al., 2017), it is highly possible that exudates were produced that reduced the root adsorption of $CeO_2$-NPs. There was no Ce accumulation observed in the shoots or grains, suggesting that Ce was not translocated to the aerial parts of the plants (data not shown). This is the third report in a series of long-term soil exposure studies showing the lack of Ce accumulation in wheat grains (Rico et al., 2014, 2017), in this case suggesting limited risk of $CeO_2$-NPs entry into the human food chain.

### 3.3. Elemental uptake

The root elemental concentrations were altered in T3 in low N soil (e.g. P, Mn, and Fe) and in T1T2 at high N (e.g. P and Mn) (SI Tables 4 and 5). T3 exposure increased contents of more elements at low N (i.e. Mg, P, K, Ca, Mn, Fe) compared to high N (i.e. Mg, P, Ca); however, these increases could simply be related to increased root biomass (Table 3, SI Table 6). In the case of shoot, there was no change in elemental uptake except in T1T2 at high N where Ca content increased by 6.6% compared to C1C2 (SI Tables 7, 8). This increase happened without simultaneous increase in shoot biomass, indicating that parental exposure (i.e., T1T2) promoted storage of Ca in shoot. Our previous study also showed no differences in elemental uptake in wheat shoots generationally exposed to $CeO_2$-NPs (Rico et al., 2017).

Elemental content in grains showed a different trend. At low N, T3 exposure decreased P, K, Ca, and Mn concentrations but increased Fe concentrations compared to C3 controls (SI Table 9). Following this trend, T3 decreased P and Mn but increased Fe contents by 8.8, 15.9, and 55.7% compared to C3 (Table 4). For high N, grain elemental concentrations did not change except for Fe, which decreased by 12.8% in T3 compared to C3 (SI Table 10). However, Mn and Fe contents decreased by 15.0 and 16.3% in T3 compared to C3 (Table 5). In case of parental exposure, T1T2 did not change elemental contents at low N. Surprisingly for high N, T1T2 decreased Mg, P, K, Mn, and Fe contents by 11.6, 10.6, 10.3, 7.9, 17.2% compared to C1C2 despite the lack of change in the elemental concentrations (Tables 5 and 6).

The modifications in grain elemental contents provided peculiar findings. First, the reductions in nutrient contents appeared to be due to decreases in the accumulation or movement of these elements to the grains since there were no differences in total yield. Second, parental exposure (i.e. T1T2) affected elemental content more in nitrogen-rich soil, while impacts of $CeO_2$-NPs exposure during the current year (i.e. T3) were more dominant in nitrogen-poor soil. Third, reductions of grain nutrients (i.e. Mg, P, K, Mn, Fe, and Cu) by the T1T2 treatment in high N soil were opposite to the observed lack of effects of T1 (i.e. T1 was exposed to 500 mg $CeO_2$-NPs per kg soil in first generation) on elemental uptake in grains as previously reported (Rico et al., 2017). Fourth, the modifications of Mn and Fe contents in T3 at both low and high N soil, which are in agreement with our previous findings, reveal the susceptibility of these elements to $CeO_2$-NPs exposure (Rico et al., 2017).

### 3.4. Changes in C, N and $^{15}N$ uptake

The C and N concentrations in roots and shoots were not affected but contents were altered in some treatments. At low soil N levels, both parental treatment T1T2 and current year treatment T3 increased the total C (30.9 and 24.6%, respectively) and N (28.0 and 32.6%,

**Table 3**
Effect of parental or current exposure to $CeO_2$-NPs on root elemental contents of wheat cultivated at low N soil.[a]

| **Changes due to generational exposure** | | | **Changes due to 3rd generation exposure** | | |
|---|---|---|---|---|---|
| | **C1C2** | **T1T2** | | **C3** | **T3** |
| **P (mg)** | 4.9 ± 0.2 | 6.1 ± 0.4*** | **P (mg)** | 5.1 ± 0.2 | 5.8 ± 0.5 |
| **Mg (mg)** | 14.1 ± 1.0 | 20.4 ± 1.8*** | **Mg (mg)** | 14.6 ± 1.0 | 20.0 ± 1.9*** |
| **K (mg)** | 30.4 ± 2.0 | 38.5 ± 3.5** | **K (mg)** | 29.2 ± 1.5 | 40.0 ± 3.5*** |
| **Ca (mg)** | 76.3 ± 4.0 | 102.8 ± 7.2**** | **Ca (mg)** | 77.7 ± 3.4 | 101.5 ± 7.9*** |
| **Mn (μg)** | 63 ± 5 | 95 ± 9**** | **Mn (μg)** | 63 ± 4 | 96 ± 9**** |
| **Fe (μg)** | 567 ± 54 | 855 ± 74**** | **Fe (μg)** | 578 ± 39 | 843 ± 86*** |

[a] Low N indicates total addition of 48 mg N as nutrient solution throughout the duration of the experiment; C1C2 or T1T2 indicates parental exposure to 0 or 500 mg $CeO_2$-NPs per kg soil at 1st and 2nd generations; C3 or T3 denotes current or 3rd generation exposure to 0 or 500 mg $CeO_2$-NPs per kg soil. Values are means ± SE; n = 12. **, ***, **** represent significance at $p < 0.05$, 0.01, 0.001, respectively.

**Table 4**
Effect of parental or current exposure to $CeO_2$-NPs on grain elemental contents of wheat cultivated at low and high N soil.[a]

| Low N | | | High N | | |
|---|---|---|---|---|---|
| | C3 | T3 | | C1C2 | T1T2 |
| P (mg) | 81.5 ± 2.1 | 74.3 ± 2.1** | P (mg) | 69.2 ± 2.8 | 61.8 ± 2.3** |
| Mn (μg) | 2354 ± 73 | 2122 ± 43*** | Mg (mg) | 34.8 ± 0.9 | 30.8 ± 0.9*** |
| Fe (μg) | 1012 ± 56 | 1238 ± 68*** | K (mg) | 128.2 ± 2.7 | 115.0 ± 4.8** |

[a] Low or High N indicates total addition of 48 or 112 mg N as nutrient solution throughout the duration of the experiment; C1C2 or T1T2 indicates parental exposure to 0 or 500 mg $CeO_2$-NPs per kg soil at 1st and 2nd generations; C3 or T3 denotes current or 3rd generation exposure to 0 or 500 mg $CeO_2$-NPs per kg soil. Values are means ± SE; n = 12. **, *** represent significance at $p < 0.05$, 0.01, respectively.

**Table 5**
Effect of parental or current exposure to $CeO_2$-NPs on grain elemental contents of wheat cultivated at high N soil.[a].

| Changes due to generational exposure | | | Changes due to 3rd generation exposure | | |
|---|---|---|---|---|---|
| | C1C2 | T1T2 | | C3 | T3 |
| Mn (μg) | 1957 ± 44 | 1770 ± 67** | Mn (μg) | 1932 ± 62 | 1795 ± 58* |
| Fe (μg) | 1162 ± 54 | 962 ± 57** | Fe (μg) | 1143 ± 55 | 981 ± 61** |

[a] High N indicates total addition of 112 mg N as nutrient solution throughout the duration of the experiment; C1C2 or T1T2 indicates parental exposure to 0 or 500 mg $CeO_2$-NPs per kg soil at 1st and 2nd generations; C3 or T3 denotes current or 3rd generation exposure to 0 or 500 mg $CeO_2$-NPs per kg soil. Values are means ± SE; n = 12. *, ** represent significance at $p < 0.10$, 0.05, respectively.

respectively) in the roots compared to their respective controls (i.e. C1C2 and C3) (Table 6). At high N, only T1T2 increased total C in shoot and root by 9.4 and 6.2%, respectively, compared to C1C2 (Table 6). Since there were no changes on C and N concentrations, the increases in C and N contents were due to an increase in root and shoot biomass. In the case of grains, T1T2 at low N enhanced C and N concentrations by 1.2 and 8.2%, respectively, compared to C1C2. The plants may have used the extra C and N to produce an increased number of grains since there was no increase in total yield or grain weight at low N (Table 1). The results also followed the trend in root biomass wherein parental exposure (T1T2) and current exposure (T3) enhanced C and N concentrations at low soil N much greater than at high soil N. These findings indicate that $CeO_2$-NPs or generationally-exposed seeds improve growth performance of daughter plants.

All $\delta^{15}N$ values recorded in the plant tissues were significantly lower in $CeO_2$-NP treatments (T3) than C3 controls, indicating that $CeO_2$-NPs increased discrimination against $^{15}N$ in wheat (Table 6). $CeO_2$-NP treated roots, shoots and whole-plant tissues had $\delta^{15}N$ levels ranging from 0.42 to 3.57‰, all below the $\delta^{15}N$ signature of the $NH_4NO_3$ fertilizer provided ($\delta^{15}N = 4.98$‰). At low N, T3 significantly reduced root and shoot $\delta^{15}N$ by 0.48‰ and 1.39‰, respectively, compared to C3. Whole-plant $\delta^{15}N$ also decreased significantly by 0.50‰ at T3 compared to C3. At high N, a change in isotopic signature was observed in root only, and $CeO_2$-NP exposure only decreased $\delta^{15}N$ by 0.23‰ compared to C3 controls. The results clearly show that $CeO_2$-NPs decreases $^{15}N$ uptake or retention in roots and shoots since there were decreases in $\delta^{15}N$ despite the notable increases in N content in T3 relative to C3, and there was no change in N concentration between C3 and T3 treatments. Findings from our hydroponic study revealed that wheat discriminates against $^{15}N$ when the source is $NH_4NO_3$, which suggests physiological changes occurred in plants when exposed to $CeO_2$-NPs (Rico et al., 2018).

The effects of $CeO_2$-NP on $\delta^{15}N$ were most pronounced in low N soils, where root, shoot, and whole-plant $\delta^{15}N$ signatures were affected. Other studies also have reported changes in $\delta^{15}N$ in plants exposed to TiO2-NPs and As, Cd, Pb, and Zn (Gao et al., 2013; Sutter et al., 2002; Schmidt et al., 2004). Surprisingly, the current results did not show changes in $^{15}N$ discrimination due to parental or generational exposures as we found previously (Rico et al., 2017).

### 3.5. Changes in fatty acid concentrations

Fatty acid analysis was performed in 2nd and 3rd generation grains to better assess the generational effects of exposure to $CeO_2$-NPs. Results showed that parental exposure to $CeO_2$-NPs modulated fatty acid synthesis in wheat grains, but changes in 3rd generation grains were recorded at high N soil only (Table 7, SI Tables 11, 12). Prior exposure to $CeO_2$-NPs for one generation (i.e. T1, means of T1C2 and T1T2) increased palmitic (C16:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), and total fatty acids concentrations by 14.7%, 8.8% 17.7%, 8.9%, and 12.5% compared to C1 (i.e. control in the first generation; means of C1C2 and C1T2) (Table 7, SI Table 13). Exposure to $CeO_2$-NPs during the 2nd second generation study (T2) decreased myristic (C14:0) and linolenic (C18:3) acids by 18.4% and 5.1%, respectively, compared to C2. These seeds (i.e. C1C2 and T1T2) were used in the 3rd generation study, and the high palmitic, oleic, linoleic, linolenic, and total fatty acids concentrations of T1T2 seeds possibly explain

**Table 6**
Effect of parental or current exposure to $CeO_2$-NPs on C, N and $^{15}N$ uptake of cultivated at low and high N soil.[a]

| Low N | | | | | |
|---|---|---|---|---|---|
| Changes due to generational exposure | | | Changes due to 3rd generation exposure | | |
| | C1C2 | T1T2 | | C3 | T3 |
| Total Root C (g) | 3.20 ± 0.14 | 4.19 ± 0.26**** | Total Root C (g) | 3.29 ± 0.13 | 4.10 ± 0.29*** |
| Total Root N (mg) | 50 ± 2 | 64 ± 5*** | Total Root N (mg) | 49 ± 2 | 65 ± 4**** |
| Grain C (%) | 37.83 ± 0.11 | 38.28 ± 0.05**** | Root $\delta^{15}N$ (‰) | 1.04 ± 0.09 | 0.56 ± 0.07*** |
| Grain N (%) | 0.913 ± 0.021 | 0.988 ± 0.033* | Shoot $\delta^{15}N$ (‰) | 4.96 ± 0.60 | 3.57 ± 0.37* |
| Total Grain N (mg) | 271 ± 10 | 297 ± 9* | Whole-plant $\delta^{15}N$ (‰) | 3.83 ± 0.20 | 3.33 ± 0.14* |
| **High N** | | | | | |
| Changes due to generational exposure | | | Changes due to 3rd generation exposure | | |
| | C1C2 | T1T2 | | C3 | T3 |
| Total Root C (g) | 3.83 ± 0.22 | 4.19 ± 0.26** | Root $\delta^{15}N$ (‰) | 0.65 ± 0.05 | 0.42 ± 0.07** |
| Total Shoot C (g) | 9.72 ± 0.13 | 10.32 ± 0.17*** | | | |

[a] Low or High N indicates total addition of 48 or 112 mg N as nutrient solution throughout the duration of the experiment; C1C2 or T1T2 indicates parental exposure to 0 or 500 mg $CeO_2$-NPs per kg soil at 1st and 2nd generations; C3 or T3 denotes current or 3rd generation exposure to 0 or 500 mg $CeO_2$-NPs per kg soil. Values are means ± SE; n = 12. *, **, ***, *** represent significance at $p < 0.10$, 0.05, 0.01, 0.001, respectively.

**Table 7**
Effect of parental or current exposure to $CeO_2$-NPs on fatty acid concentrations (μg/g) in wheat.[a]

| **2nd generation study** | | | | | |
|---|---|---|---|---|---|
| **Changes due to generational exposure** | | | **Changes due to 2nd generation exposure** | | |
| | **C1** | **T1** | | **C2** | **T2** |
| **Myristic acid (C14:0)** | 862 ± 79 | 704 ± 37** | **Myristic acid (C14:0)** | 863 ± 78 | 704 ± 39** |
| **Palmitic acid (C16:0)** | 3804 ± 77 | 4365 ± 80**** | **Linolenic acid (C18:3)** | 954 ± 20 | 905 ± 27* |
| **Oleic acid (C18:1)** | 2219 ± 79 | 2415 ± 50** | | | |
| **Linoleic acid (C18:2)** | 10768 ± 252 | 12672 ± 313**** | | | |
| **Linolenic acid (C18:3)** | 887 ± 27 | 966 ± 17*** | | | |
| **Total Fatty Acid** | 19167 ± 509 | 21566 ± 473**** | | | |
| **3rd generation study at high N soil** | | | | | |
| **Changes due to generational exposure** | | | **Changes due to 3rd generation exposure** | | |
| | **C1C2** | **T1T2** | | **C3** | **T3** |
| **Lauric acid (C12:0)** | 175 ± 3 | 165 ± 3** | **Myristic acid (C14:0)** | 1050 ± 36 | 933 ± 34** |
| **Linoleic acid (C18:2)** | 8562 ± 127 | 8856 ± 65* | | | |
| **Total Fatty Acid** | 14697 ± 199 | 15131 ± 123* | | | |

[a] High N soil indicates total addition of 112 mg N as nutrient solution throughout the duration of the experiment; C1 or T1 denotes parental exposure to 0 or 500 mg $CeO_2$-NPs per kg soil at 1st generation, C2 or T2 represents current or 2nd generation exposure to 0 or 500 mg $CeO_2$-NPs per kg soil, C1C2 or T1T2 indicates parental exposure to 0 or 500 mg $CeO_2$-NPs per kg soil at 1st and 2nd generations; C3 or T3 denotes current or 3rd generation exposure to 0 or 500 mg $CeO_2$-NPs per kg soil. Values are means ± SE; n = 12. *, **, ***, **** represent significance at $p < 0.10$, 0.05, 0.01, 0.001 respectively.

the larger biomass of 3rd generation plants produced from T1T2. In case of 3rd generation exposure to $CeO_2$-NPs (T3), myristic acid (C14:0) decreased by 11.1% in T3 compared to C3. Parental exposure for two generations (T1T2) decreased lauric acid (C12:0) by 5.8% but increased linoleic (C18:2) and total fatty acids by 3.4% and 3.0% compared to C1C2.

Wheat grains contain around 1–3% fatty acid that small modifications in concentrations may cause significant impacts on chemical and physical properties of grains and possibly the growth and physiology of the daughter plants (Banas et al., 2007). It is not clear why fatty acid concentrations changed in high N soil and not in low N soil especially that T1T2 increased grain C and N concentrations at low N. Clearly, parental exposure and environmental factor (i.e. soil N) affected fatty acid synthesis in grains (Allen and Young, 2013; Andrianasolo et al., 2016). The findings also showed that the highly significant changes in fatty acid concentrations in parent seeds did not result in similar or even stronger effects in daughter grains (i.e. T1 induced 12.5% increase in fatty acid concentrations while T1T2 induced 3.0% increase only).

## 4. Conclusion

This work provides evidence that previous generation exposure to $CeO_2$-NPs affects the performance and nutrient profile in progeny plants. However, environmental variables such as soil N availability also modulates the influence of parental exposure. Findings revealed that parental exposure (i.e. T1T2) promoted root biomass production in daughter plants that were grown in either low or high N nutrient. Surprisingly, T1T2 parental exposure decreased the accumulation of most elements in grains in high nitrogen soil but current exposure to $CeO_2$-NPs during growth of the third generation (i.e. T3) reduced nutrient accumulation in low N soils. The mechanisms underlying the observed responses are unknown, but they may be related to changes in gene expression (Tumburu et al., 2017; Reichman et al., 2018) or epigenetic shifts that may be carried from one generation to the next (Vandegehuchte and Janssen, 2014). Overall, the results demonstrate the potential for transgenerational changes in wheat growth and grain quality in response to $CeO_2$-NP exposure and suggest that these longer-term effects should be included in any risk assessment addressing the release of ENMs to the environment.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2019.121364.

## References

Aarssen, L.W., Burton, S.M., 1990. Maternal effects at four levels in Senecio vulgaris (asteraceae) grown on a soil nutrient gradient. Am. J. Bot. 77, 1231–1240.
Allen, D.K., Young, J.D., 2013. Carbon and nitrogen provisions alter the metabolic flux in developing soybean embryos. Plant. Physiol. 161, 1458–1475.
Andrianasolo, F.N., Debaeke, P., Champolivier, L., Maury, P., 2016. Analysis and modelling of the factors controlling seed oil concentration in sunflower: a review. OCL 23, D206.
Avula, B., Wang, Y.H., Smillie, T.J., Duzgoren-Aydin, N.S., Khan, I.A., 2010. Quantitative determination of multiple elements in botanicals and dietary supplements using ICP-MS. J. Agric. Food Chem. 58, 8887–8894.
Banas, A., Debski, H., Banas, W., Heneen, W.K., Dahlqvist, A., Bafor, M., Gummeson, P.O., Marttila, S., Ekman, A., Carlsson, A.S., Stymne, S., 2007. Lipids in grain tissues of oat (Avena sativa): differences in content, time of deposition, and fatty acid composition. J. Exp. Bot. 58, 2463–2470.
Boyko, A., Golubov, A., Bilichak, A., Kovalchuk, I., 2010. Chlorine ions but not sodium ions alter genome stability of Arabidopsis thaliana. Plant Cell Physiol. 51, 1066–1078.
Duncan, E., Owens, G., 2019. Metal oxide nanomaterials used to remediate heavy metal contaminated soils have strong effects on nutrient and trace element phytoavailability. Sci. Total Environ. 678, 430–437.
Gajewska, E., Bernat, P., Dlugonski, J., Sklodowska, M., 2012. Effect of nickel on membrane integrity, lipid peroxidation and fatty acid composition in wheat seedlings. J. Agron. Crop Sci. 198, 256–294.
Gao, J., Xu, G., Qian, H., Liu, P., Zhao, P., Hu, Y., 2013. Effects of nano-$TiO_2$ on photosynthetic characteristics of Ulmus elongata seedlings. Environ. Pollut. 176, 63–70.

Geisler-Lee, J., Brooks, M., Gerfen, J.R., Wang, Q., Fotis, C., Sparer, A., Ma, X., Berg, R.H., Geisler, M., 2014. Reproductive toxicity and life history study of silver nanoparticle effect, uptake and transport in Arabidopsis thaliana. Nanomaterials 4, 301–318.

Hoppe, M., Schlich, K., Wielinski, J., Koser, J., Ruckamp, D., Kaegi, R., Hund-Rinke, K., 2019. Long-term outdoor lysimeter study with cerium dioxide nanomaterial. NanoImpact 14, 100170.

Kalcits, L.A., Guy, R.D., 2013. Whole-plant and organ-level nitrogen isotope discrimination indicates modification of partitioning of assimilation, fluxes, and allocation of nitrogen in knockout lines of Arabidopsis thaliana. Physiol. Plant. 149, 249–259.

Keller, A.A., Wang, H., Zhou, D., Lenihan, H.S., Cherr, G., Cardinale, B.J., Miller, R., Ji, Z., 2010. Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices. Environ. Sci. Technol. 44, 1962–1967.

Kou, H.P., Li, Y., Song, X.X., Ou, X.F., Xing, S.C., Ma, J., Liu, B., Von-Wettstein, D., 2011. Heritable alteration in DNA methylation induced by nitrogen-deficiency stress accompanies enhanced tolerance by progenies to the stress in rice (Oryza sativa L.). J. Plant Physiol. 168, 1685–1693.

Latzel, V., Janecek, S., Dolezal, J., Klimesova, J., Bossdorf, O., 2014. Adaptive transgenerational plasticity in the perennial Plantago lanceolata. Oikos 123, 41–46.

Ma, X., Wang, Q., Rossi, L., Ebbs, S.D., White, J.C., 2016. Multigenerational exposure to cerium oxide nanoparticles: physiological and biochemical analysis reveals transmissible changes in rapid cycling Brassica rapa. NanoImpact 1, 46–54.

Medina-Velo, I.A., Zuverza-Mena, N., Tamez, C., Ye, Y., Hernandez-Viezcas, J.A., White, J.C., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2018. Minimal transgenerational effect of ZnO nanomaterials on the physiology and nutrient profile of Phaseolus vulgaris. ACS Sustainable Chem. Eng. 6, 7924–7930.

Rahavi, M., Migicovsky, Z., Titov, V., Kovalchuk, I., 2011. Transgenerational adaptation to heavy metal salts in Arabidopsis. Front. Plant Sci. 2, 1–10.

Reichman, J.R., Rygiewicz, P.T., Johnson, M.G., Bollman, M.A., Smith, B.M., Krantz, Q.T., King, C.J., Andersen, C.P., 2018. Douglas-fir (Pseudotsuga menziesii) transcriptome profile changes induced by diesel emissions generated with $CeO_2$ nanoparticle fuel borne catalyst. Environ. Sci. Technol. 52, 10067–10077.

Rico, C.M., Morales, M.I., McCreary, R., Castillo-Michel, H., Barrios, A.C., Hong, J., Tafoya, A., Lee, W.Y., Varela-Ramirez, A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2013. Cerium oxide nanoparticles modify the antioxidative stress enzyme activities and macromolecular composition in rice seedlings. Environ. Sci. Technol. 47, 14110–14118.

Rico, C.M., Lee, S.C., Rubenecia, R., Mukherjee, A., Hong, J., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2014. Cerium oxide nanoparticles impact yield and modify nutritional parameters in wheat (Triticum aestivum L.). J. Agric. Food Chem. 62, 9669–9675.

Rico, C.M., Johnson, M.G., Marcus, M.A., Andersen, C.P., 2017. Intergenerational responses of wheat (Triticum aestivum) to cerium oxide nanoparticles exposure. Environ. Sci: Nano 4, 700–711.

Rico, C.M., Johnson, M.G., Marcus, M.A., Andersen, C.P, 2018. Shifts in N and $\delta^{15}N$ in wheat and barley exposed to cerium oxide nanoparticles. NanoImpact 11, 156–163.

Robinson, D., Handley, L.L., Scrimgeour, C.M., Gordon, D.C., Forster, B.P., Ellis, R.P., 2000. Using stable isotope natural abundances ($\delta^{15}N$ and $\delta^{13}C$) to integrate the stress responses of wild barley (Hordeum spontaneum C. Koch.) genotypes. J. Exp. Bot. 51, 41–50.

Schmidt, A.C., Reisser, W., Mattusch, J., Wennrich, R., Jung, K., 2004. Analysis of arsenic species accumulation by plants and the influence on their nitrogen uptake. J. Anal. At. Spectrom. 19, 172–177.

Suter, L., Widmer, A., 2013. Environmental heat and salt stress induce transgenerational phenotypic changes in Arabidopsis thaliana. PloS One 8, e60364.

Sutter, K., Jung, K., Krauss, G.J, 2002. Effects of heavy metals on the nitrogen metabolism of the aquatic moss Fontnalis antipyretica L. Ex Hedw. – a $^{15}N$ tracer study. Environ. Sci. Pollut. Res. 9, 417–421.

Tan, W., Du, W., Darrouzet-Nardi, A.J., Hernandez-Viezcas, J.A., Ye, Y., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2018. Effects of the exposure of $TiO_2$ nanoparticles on basil (Ocimum basilicum) for two generations. Sci. Total Environ. 636, 240–248.

Tumburu, L., Andersen, C.P., Rygiewicz, P.T., Reichman, J., 2017. Molecular and physiological responses to titanium dioxide and cerium oxide nanoparticles in Arabidopsis. Environ. Toxicol. Chem. 36, 71–82.

Vandegehuchte, M.B., Janssen, C.R., 2014. Epigenetics in an ecotoxicological context. Mutat. Res-Gen. Tox. En. 764-765, 36–45.

Wang, Q., Ma, X., Zhang, W., Pei, H., Chen, Y., 2012. The impact of cerium oxide nanoparticles on tomato (Solanum lycopersicum L.) and its implications for food safety. Metallomics 4, 1105–1112.

Wang, Z., Xu, L., Zhao, J., Wang, X., White, J.C., Xing, B., 2016. CuO nanoparticle interaction with Arabidopsis thaliana: toxicity, parent-progeny transfer, and gene expression. Environ. Sci. Technol. 50, 6008–6016.

Yoshida, S., Forno, D.A., Cock, J.H., Gomez, K.A., 1976. Laboratory Manual for Physiological Studies of Rice. International Rice Research Institute, Los Baños, Laguna, Philippines.

Zhao, L., Huang, Y., Zhou, H., Adeleye, A.S., Wang, H., Ortiz, C., Mazer, S.J., Keller, A.A., 2016. GC-TOF-MS based metabolomics and ICP-MS based metallomics of cucumber (Cucumis sativus) fruits reveal alteration of metabolites profile and biological pathway disruption induced by nano copper. Environ. Sci.: Nano 3, 1114–1123.