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Absence of Nanoparticle-Induced Drought Tolerance in Nutrient Sufficient Wheat Seedlings

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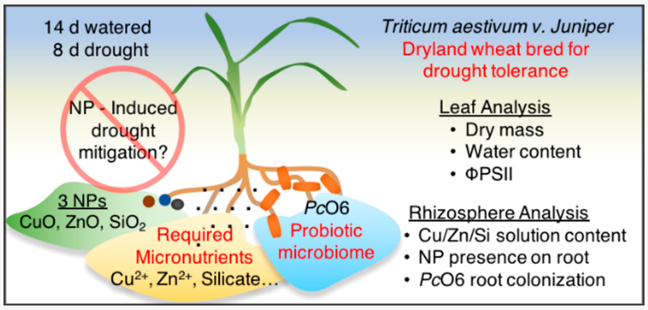
ABSTRACT: Strategies to reduce crop losses due to drought are needed as climate variability affects agricultural productivity. Wheat (*Triticum aestivum* var. Juniper) growth in a nutrient-sufficient, solid growth matrix containing varied doses of CuO, ZnO, and SiO_2 nanoparticles (NPs) was used to evaluate NP mitigation of drought stress. NP amendments were at fertilizer levels, with maxima of 30 Cu, 20 Zn, and 200 Si (mg metal/kg matrix). Seeds of this drought-tolerant cultivar were inoculated with *Pseudomonas chlororaphis* O6 (*Pc*O6) to provide a protective root microbiome. An 8 day drought imposed on 14 day-old wheat seedlings decreased shoot and root mass, shoot water content, and the quantum yield of photosystem II when compared to watered plants. *Pc*O6 root colonization was not impaired by drought or NPs. A dose-dependent increase in the Cu, Zn, and Si from the NPs was observed from analysis of the rhizosphere solution, and this process was not affected by drought. Consequently, fertilizer concentrations of the NPs did not further improve drought tolerance in wheat seedlings under the growth conditions of adequate mineral nutrition and the presence of a beneficial microbiome. These findings suggest that potential NP benefits in promoting plant drought tolerance occur only under certain environmental conditions.



KEYWORDS: *CuO, ZnO, SiO_2, drought, probiotic, microbiome, PSII, rhizosphere solution, NP dissolution, PcO6*

INTRODUCTION

Climate variability has profound effects on agriculture.[1,2] Increases in global mean temperature reduce crop yield and quality. For wheat (*Triticum aestivum*), a global staple crop, each degree Celsius increase is expected to lower yields by about 6%.[3] With its origins in the Middle East, wheat exhibits drought tolerance and is often grown in semiarid areas under dry-land farming conditions. However, dry soils may lead to nutrient stress as well as water stress. Soils in semiarid to arid regions typically have high pH associated with deficiencies of essential plant nutrients via decreased solubility and enhanced precipitation and/or sorption to mineral surfaces.[4] In addition, drought reduces uptake of mass-flow-dependent nutrients (such as Zn and Cu) and their translocation from the roots to the shoots.[5]

Dimkpa and Bindraban summarize the roles of essential and other beneficial elements in plants[6] and Cu, Zn, and Si are among those that promote plant vigor. For example, Cu and Zn are cofactors in superoxide dismutase, which reduces cell stress from reactive oxygen species. Cu is part of the ethylene-sensing system and is a cofactor for plastocyanin in photosynthesis electron transport. Zn has a crucial role in regulating gene expression via multiple zinc finger proteins and is a cofactor in carbonic anhydrase that affects the efficacy of photosynthesis. Cu and Si are involved in plant cell wall strengthening, contributing to resilience against biotic and abiotic challenges.[7−9]

A growing body of literature suggests that metal and metal oxide nanoparticles (NPs) may mitigate water stress and other abiotic and biotic pressures in crops.[9−15] Benefits are often attributed to the NPs serving as sources for essential elements. Typically, the effects of ions released from NPs vs bulk formulations are compared to growth with no amendments. This paper will address whether growth matrix supplementation with CuO, ZnO, and SiO_2 NPs, in the presence of sufficient phytoavailable nutrients from dissolved mineral salts, provides "nano-enabled" mitigation of water stress.

Several nanoformulations have been reported to improve plant vigor when water is withheld. For droughted wheat, benefits from ZnO NP are noted by Dimpka et al.,[11] Sedghi et

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al.,[16] and Taran et al.[14] Dimkpa et al.[11] report ZnO NPs to have protectant effects on droughted sorghum and earlier work with composites containing CuO NP stimulated drought stress relief in soybean.[17] The addition of elemental Cu NPs promotes growth and grain yield for maize.[18] For SiO_2 NPs and Si salts, positive effects on droughting are reported for a variety of plants including sugar cane[19] and rice.[20] Under sufficient watering, Si supply has no effects on the growth and physiological parameters of rice; however, Si applications significantly increase photosynthetic rate, transpiration rate, and chlorophyll maximum quantum yield of rice plants under drought stress.[21] Such photosynthesis measurements are commonly made for assessing drought responses in the plant.[22−25] For maize, growth and yield increase under drought when SiO_2 NPs[10] and potassium/silica NPs[26] are applied. Si and SiO_2 NPs also aid barley under drought stress.[27]

Plant vigor is additionally promoted by certain rhizosphere microbes that increase nutrient bioavailability from soil minerals and/or induce mechanisms that increase stress tolerance.[28−30] Effective strains have been identified with several microbial genera: *Bacillus subtilis* protected the herb, *Trigonella*, against drought[28] as did strains of *Azospirillum lipoferum* for wheat.[29] A pseudomonas isolate, *Pseudomonas chlororaphis* O6 (*Pc*O6), cultured from wheat roots grown in a calcareous soil under dryland farming conditions,[31] also induces drought tolerance. *Pc*O6 protective mechanisms upon wheat colonization include (1) production of volatile 2R,3R butanediol, which causes stomatal closure;[30] (2) biofilm formation on the roots,[9,32] which provides an extra hydrated layer over the roots;[33,34] and (3) priming the host plant for expression of genes conferring stress tolerance.[15] Findings with another pseudomonad suggest a role for the production of phenazines in promoting biofilms to enhance tolerance to drought under field conditions.[35,36] Thus, mitigation of drought stress in the plant may be boosted by protectant microbes in the rhizosphere. Therefore, studies of the efficacy of NPs as soil amendments should consider the status of the plant's microbiome.

In this study, a drought-tolerant wheat variety (*Triticum aestivum*, v. Juniper) was seed-inoculated with the rhizobacterium *Pc*O6 and provided with essential elements from the inorganic salts in a half-strength Hoagland's solution to test whether NP amendments of CuO, ZnO, and SiO_2 enhance drought tolerance. Sand was used as a solid growth matrix that would not introduce inorganic nutrients or organic materials into the system, which strongly influence NP remodeling and dissolution. This growth matrix also does not contain clays to act as water-holding particulates, which could have localized effects on root water availability. Similarly, other particulates that would sequester NPs or their dissolved ions would not be present. Thus, in this model plant growing matrix, the plant rhizosphere would be influenced only by root and microbial metabolism and the selected amendments. We have previously examined drought as a stressor in wheat seedlings using this model growth matrix.[9] Here, the impacts of the NP and drought treatments on the wheat leaf photosynthetic potential were measured *in situ* with chlorophyll fluorescence measurements for the operating quantum yield for photosystem II (Φ_{PsII}).[22−25] At harvest, shoot and root mass were measured and water content was calculated. NP presence on the rhizoplane was examined by scanning electron microscopy and energy-dispersive X-ray spectroscopy (EDS). The presence of Cu, Zn, and Si from the dissolution of the NPs in the rhizosphere solutions was evaluated to confirm that the NPs would provide bioavailable nutrients. Root colonization by *Pc*O6 and possible impairment by the NPs were assessed by screening for culturable colonies. This experimental design testedwhether the NPs: (1) provide unique benefits to water-stressed wheat beyond correcting a nutrient deficiency, (2) do not impair *Pc*O6 root-colonization, and (3) whether Φ_{PsII} assessment provides a nondestructive method to monitor water stress and NP effects on wheat seedlings before visible cues.

■ MATERIALS AND METHODS

Chemicals. The NPs, obtained as powders and stored protected from light, were CuO NPs (American Elements; Los Angeles, Ca, USA; product CU2-OX-035-NP.100N; diameter <100 nm, purity = 99.95%), ZnO NPs (SkySpring Nanomaterials; Houston, TX, USA; product 8410DL; 10−30 nm nominal size, purity = 99.8%), and SiO_2 NPs (Evonik Industries; Piscataway, NJ, USA; Aerosil 200 Fumed Silica; 12 nm primary particles, purity >99.8%). For planting, half-strength modified Hoagland's solution was used,[37] with $FeCl_3$ as the Fe source. The full chemical composition of this solution, as verified by analytical characterization using inductively coupled mass spectrometry (ICP-MS) (Agilent 7700x Agilent technologies, Santa Clara, CA) following EPA method 6020, is provided in SI Table S1.

Nanoparticle Characterization. Nanoparticle size, surface charge, and elemental purity are presented in SI Table S2. Hydrodynamic radii were measured by dynamic light scattering (DLS) using a Wyatt DynaPro Nanostar DLS. Surface charge was measured using a Brookhaven ZetaPlus Zeta Potential Analyzer. NPs were dispersed in 10 mM MOPS buffer solution at neutral pH and further diluted with the buffer (DLS) or KCl (zeta potential) to approximately 10 mg/L for analysis. Element presence, size distribution, and agglomeration profiles were determined by scanning electron microscopy (FEI Quanta FEG 650 SEM) with energy-dispersive X-ray spectroscopy (EDS, Oxford Instruments) using an X-Max Detector. NPs were diluted in dd-H_2O, probe-sonicated for 3 min, and drop-cast on an aluminum surface for imaging with SEM. NP purity was confirmed by acid digestion and analysis by inductively coupled mass spectrometry (ICP-MS) (Agilent 7700x Agilent technologies, Santa Clara, CA) following EPA method 6020.

Wheat Planting. Hard red winter wheat seeds (*Triticum aestivum* v. Juniper) were surface sterilized with 10% H_2O_2 for 10 min, rinsed thoroughly with sterile double-distilled water (dd-H_2O) (resistance >18 MΩ cm), and inoculated by 10 min immersion in a suspension of *Pc*O6 at $\sim 10^4$ CFUs/mL. The *Pc*O6 cells had been grown as biofilms on agar plates containing minimal medium for 48 h at 22 °C before suspension. Quartz sand (4075 grit; Unimin Corp; New Canaan, CT, USA) was washed with water, 0.495 mm sieved (W.S. Tyler Co., Cleveland, OH), and dried at 105 °C. Sand for the ZnO NP trials was additionally autoclaved at 121 °C for 30 min before use. The NPs were added to the dry sand in a bucket, sealed, and vigorously shaken for homogeneity. The doses were CuO NPs (0, 0.5, 5, 15, or 30 mg Cu/kg sand), ZnO NPs (0, 2.5, 5, 10, 20 mg Zn/kg sand), or SiO_2 NPs (0, 5, 50, 100, 200 mg Si/kg sand). The sand was transferred to plastic growth pots using 800 g per container (4.5″ x 4.5″ x 5.25″; United States Plastic Corp., Lima, OH, USA). Each pot was watered with 150 mL of half-strength modified Hoagland's solution to provide complete inorganic nutrition. This brought

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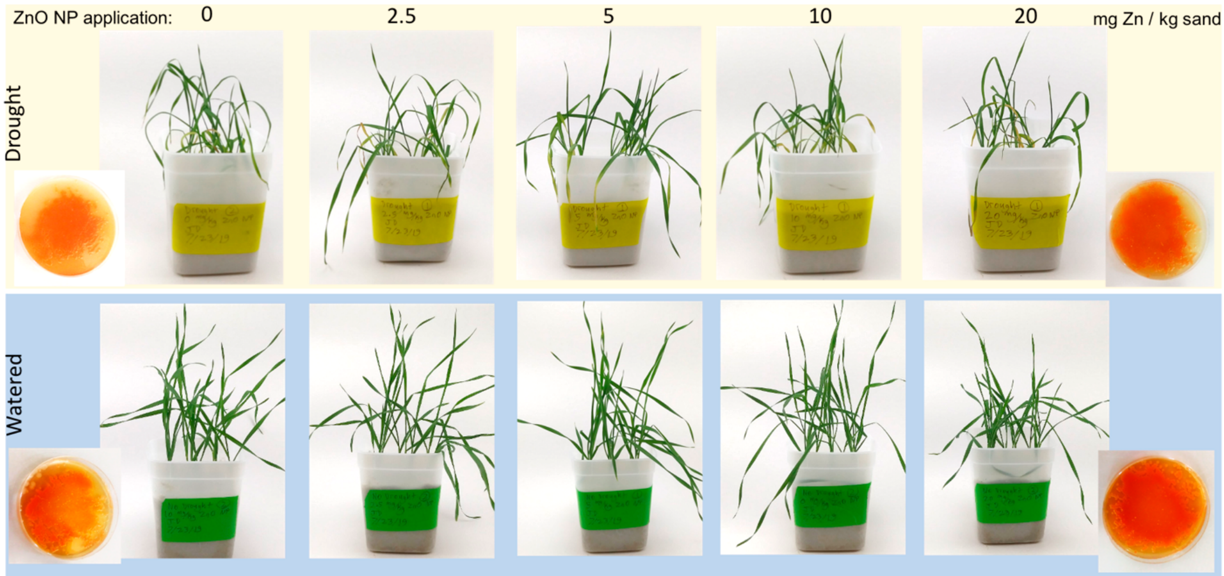

Figure 1. Wheat response at ZnO NPs dosing from 0 to 20 mg Zn/kg sand at day 22 (14 d of watered growth followed by 8 d with or without drought). *Pc*O6 isolated from roots at harvest is shown growing on LB agar in insets indicating root colonization under all conditions of watering, drought, NP-free, and highest ZnO NP challenges. Plant stand appears invariant to ZnO NPs. Photos are indicative of plants from 3 replicate experiments, each with 3 pots per treatment. Similar results were observed for CuO and SiO_2 NP trials (not shown).

the sand to 187.5% water holding capacity, as the water holding capacity of this sand was previously determined to be 0.10 g/g.[38] Nine *Pc*O6-inoculated wheat seeds were planted in each pot at depths of 1−2 cm. Three independent studies for the CuO and SiO_2 NP treatments, and four separate studies with the ZnO NPs, were performed. Each treatment within each NP study was replicated with three different pots.

Wheat Growth. Wheat was grown for 14 d with water followed by 8 d of drought under soft white LED light panels (Yescom USA Inc.; City of Industry, CA, USA) with 16/8 h photocycle periods. The light panels were fixed at approximately 30 cm above the base of the pots until the plants reached approximately 20 cm in height when the lights were raised to 45 cm. The photon flux reached ∼650 μmol/m^2/s at maximum shoot height at 22 d growth at 25 °C with air circulation provided by small fans. The pots were rotated daily. A moisture content of 0.169 g H_2O/g growth matrix (135 g H_2O/pot, 169% water holding capacity) was maintained by adding dd-H_2O daily based on the weight of each pot. After 14 d, the water content of the pots receiving the drought treatment was reduced to 0.025 g H_2O/g growth matrix (20 g H_2O/pot, 25% water holding capacity). These water contents were determined experimentally to induce conditions of no water stress and water stress (while not reaching the permanent wilting point), respectively. A moisture release curve for this sand growth matrix is provided in the Supporting Information, Figure S1. At 7 and 14 d, 25 and 40 mL, respectively, of half-strength modified Hoagland's solution was added, followed by sterile water to restore moisture content to 135 g H_2O/pot. The photosynthetic function of the leaves was measured at defined intervals after the onset of the drought period.

Assessment of Wheat Leaf Photosynthetic Function. At 14 d, the photosynthetic potential of the leaves from the control and the droughted plants was assayed using chlorophyll fluorescence (LI-6800 Portable Photosynthesis Unit, LI-COR Biosciences), which provided a nondestructive method for tracking drought stress. These measurements were repeated every 2 d during the 8 d drought period, including the day of harvest. The quantum yield (Φ_{PsII})was measured on plants that had been light-adapted for at least 1 h during the 16-h light period. The LI-6800 chamber environmental parameters were flow, 600 μmol/s; ΔP, 0.2 kPa; H_2O, RH_{air} 55%; CO_2, 500 μmol/mol; Fan, 5000 rpm; T_{air}, 25 °C; Light, 200 μmol/m^2/s, red-90%, blue-10%; fluorometer, dark mode rate of 500 Hz, light mode rate of 50 kHz, flash mode rate of 250 kHz. At least two wheat leaves were selected from each pot for each set of light-adapted measurements.

Harvesting Methods. Before the harvest at 22 d, all watered and droughted pots were restored to 135 g H_2O/pot and equilibrated for 10 min. The plants were carefully removed from the sand. Root-adhering sand was returned to the sand retained in the pots.

One root per pot was placed onto LB 2% agar plates and then removed; the liquid was spread over the surface to confirm colonization by *Pc*O6 and the absence of any other microorganisms. For SEM analysis, 0.5 cm of the root tips, 3 per treatment, were excised and immersed in anhydrous methanol for 30 min.[39] The root sections were transferred to ethanol before critical point drying using a Tousimis Autosamdri-931 Series apparatus or dehydration in HDMS (ZnO NPs trials 1−3) before SEM imaging.

Shoots were cut above the coleoptiles, and the wet weight was immediately determined. The shoot tissue was oven-dried at 60 °C for at least 48 h before the dry mass was measured. These values were used in determining the water content of the shoots. After the shoots were removed at the coleoptile, the roots were gently removed from the sand. The sand was then mixed; the solution in each pot was vacuum extracted, filtered through a 0.2 μm P-Nylon membrane (#8055-NS, Life Science Products, Frederick, CO), and analyzed for pH (Accumet 13−620−290, Accumet Engineering, Inc. Westford, MA) and EC (Accumet 13−620−100). Roots were growing throughout each pot so that the collected solution was rhizosphere influenced. Separate aliquots of this filtered rhizosphere solution (4.5 mL) were centrifuged at 20800×*g* for 15 min,

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a time and speed calculated by the Stokes−Einstein equation to pellet CuO/ZnO NPs larger than 30 nm and SiO_2 NPs larger than 50 nm, and the supernatant was collected (3 mL) and acidified. Previously, centrifugation was shown to remove CuO NPs > 30 nm.[38] After centrifugation, Cu, Zn, and Si contents were analyzed by ICP-MS.

Statistical Analysis. For chlorophyll fluorescence data, a restricted maximum likelihood (REML) method was used to analyze data with each pot treated as a statistical unit. All measurements for each pot on a given day were averaged for a total statistical sample size of n = 9 per treatment. The measurements from each pot on drought day 0 were used as model parameters to account for any variations that may have occurred before the drought period began. Pots from separate trials were considered as replicates, with the trial being treated as a blocking factor. The REML method was utilized to determine differences between the effects of drought status, days of drought, and NP concentration using SAS (p-value <0.05). The shoot water contents, shoot dry masses, pH, EC, and Cu, Zn, and Si concentration were blocked by the trial number and analyzed using one-way or two-way ANOVA (p-value <0.05).

RESULTS AND DISCUSSION

Drought Responses: Plant Stand, Root Colonization, and Shoot Water Content. Figure 1 presents representative images of droughted and watered plants for the ZnO treatments at 22 d after planting to illustrate the visual effects of the water-withholding levels on the wheat plant stand. The shoots of plants following 8 d of drought were wilted as compared to the watered controls. An improvement in the droughted plant stand was not visually evident across the investigated NP concentration ranges of 0−20 mg Zn/kg sand. A similar visual absence of drought mitigation resulted from the investigated doses of CuO (0−30 mg) or SiO_2 NPs (0−200 mg) (not shown). The selected drought conditions significantly ($p < 0.05$) reduced shoot and root water content by 36.0%, and 53.5%, respectively, across all NPs and application rates. The pooled shoot data are presented in Figure 2 with the shoot and root data for each NP type and application rate provided in the Supporting Information, Figure S2. As noted in Figure S2, the wheat shoot water content at 22 d was not statistically different ($p > 0.05$) for plants grown with NPs across all doses, including the controls without NPs; the data were thus pooled as presented in Figure 2.

The effects of the simulated drought are evident in the reduction in shoot water content (Figure 2A) and reduced shoot mass (Figure 2B). An absence of a statistical difference between NPs or application rates can be explained in part by the provision of micronutrients in Hoagland's solutions. However, as shown in Figure 2C, CuO NPs resulted in modest reductions of the dry weight for watered plants, but no concentration-dependent response for droughted plants was observed. Thus, for all NP application rates, drought effects are apparent but are not alleviated by the NPs.

At harvest, root colonization by *Pc*O6 was confirmed by the plating of root washes on rich medium (LB) agar plates, resulting in bright orange colonies (see Figure 1 for results from the ZnO NP study), typical of cells producing phenazines.[32] Previous work with *Pc*O6-inoculated wheat grown with higher concentrations of CuO and ZnO NPs also indicated the resilience of the bacterium when colonizing the roots exposed to these NPs.[9] Here, the doses of ZnO, CuO, and SiO_2 NPs were at levels similar to those of nanoformulations examined in published studies of drought amelioration. Under these conditions, root colonization by *Pc*O6 was not impaired. Impaired colonization would restrict the ability of the microbe to confer drought tolerance to plants[40] through mechanisms that include stomatal closure by microbial production of volatile metabolites,[30] induction of genes protective against plant stress,[15] and the generation of hydrated biofilms on the root surface.[9]

Plants in native agricultural soils have complex root microbiomes that include microbes, like *Pc*O6, that boost plant health, including drought tolerance.[40,41] In the present work, the NPs were premixed into the growth matrix before planting the *Pc*O6-inoculated seeds. Thus, the bacteria would be exposed to the NPs and their dissolved ions while they were colonizing the roots. In agriculture, NP accumulation through

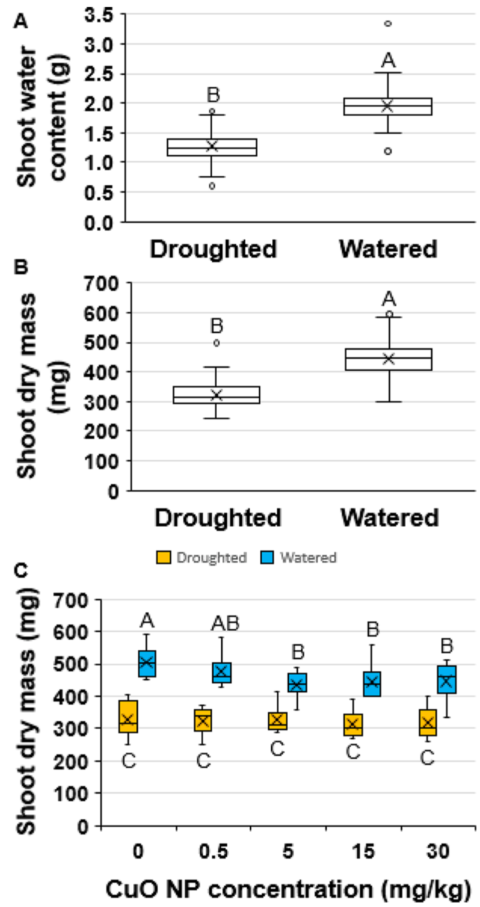

Figure 2. Wheat shoot mass and water content at harvest. Pooled NP treatments are presented in (A, B) and CuO NP treatments in (C). The 8 d drought decreased shoot water content (A) and dry mass (B), but the type and concentration of the NPs did not influence these parameters, thus all data are pooled within the watering regime. (C) Shoot dry mass as affected by NP dose was only significant for watered wheat treated with CuO NPs. NP concentrations are in units of mg core element per kg sand. Different letters indicate a statistical difference based on one-way ANOVA for the pooled data (A,B) and two-way ANOVA for the CuO treatment (C) with posthoc Tukey test, p-value <0.05. For the box and whisker plots, the line within the box is the median, the × symbol is the mean, the bottom edge and top edge of the box are the first and the third quartiles, respectively. The whiskers are the minimum and maximum value. Values outside of the whiskers are outliers, defined as points >1.5× the interquartile range.

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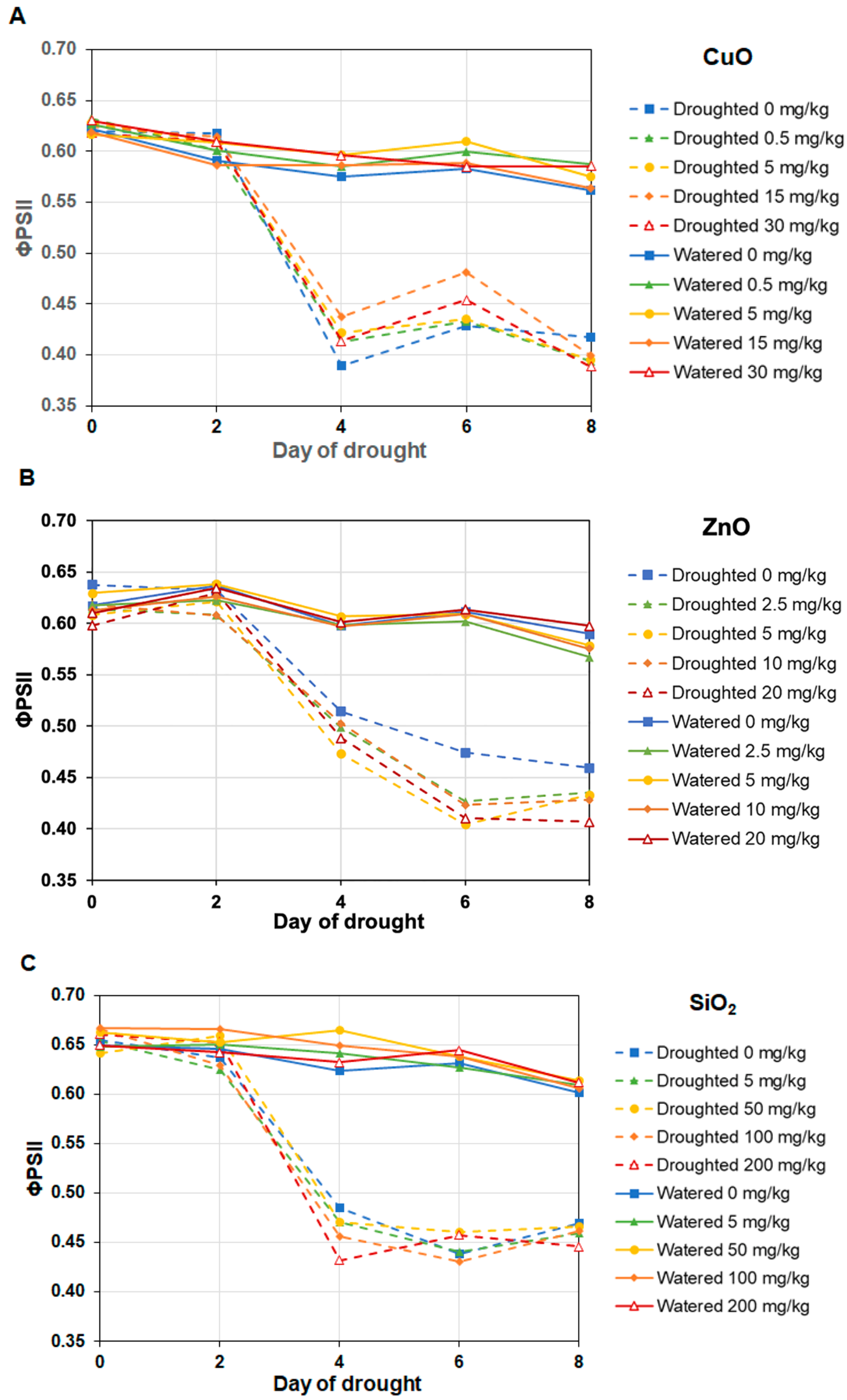

Figure 3. Photosystem II efficiency, Φ_{PSII}, of 22 day-old wheat in response to drought and indicated NPs. NP concentrations are in units of mg core element per kg sand. The Φ_{PSII} values were statistically different ($p < 0.05$) between watered and droughted plants, but not for NP concentration effects, except when CuO NPs were applied, as discussed in the text. Data are averages of measurements from $n = 3$ pots per each of the 3 trials for a total of $n = 9$ pots. Error bars were removed for clarity.

repeated application may become a potential environmental hazard. These findings with *Pc*O6, as well as studies with *Pseudomonas putida* showing that biofilm maturity decreases susceptibility to ZnO NP toxicity, suggest that, at low doses, ZnO NPs exhibit little toxicity to even immature biofilms.[42] Postharvest assessments of wheat response to NPs may miss subtle aspects of NP-plant interactions that may improve plant vigor under drought stress. *In-situ* measurements of photosystem efficiency were thus performed on watered and droughted plants.

Photosynthetic function (ΦPSII). The effect of drought on leaf photosynthetic function was determined using nondestructive measurements of Φ_{PSII} (Figure 3). These measurements were initiated at day 14 of growth and were continued through the 8 days of drought for both the droughted and watered plants. The Φ_{PSII} values measured for

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watered plants were statistically insignificant for NP type and concentrations. Plants subjected to water withholding did not exhibit any Φ_{PSII} stress within the first 2 days, after which photosynthetic function decreased for all NPs and application concentrations. Treatments with CuO NPs showed a significant concentration-drought interaction for Φ_{PSII}, where responses to 0.5 and 15 mg Cu/kg sand differed with the droughted plants but did not differ from the measurements of the control (0 mg Cu/kg sand) (posthoc Tukey p-value >0.05). The effects of drought on Φ_{PSII} were significant relative to the watered controls, but there was no improvement in this assessment of photosynthesis for amendments with the three NPs.

The sensitivity of Φ_{PSII} to abiotic stressors, including drought, has been established.[22−25] A study by Lu and Zhang, for wheat grown for 28 d with water followed by two levels of water withholding for up to an additional 10 d, reported Φ_{PSII} values of 0.60, 0.55, and 0.50 for watered, moderately stressed, and severely stressed plants, respectively.[22−25] As seen in Figure 3, the Φ_{PSII} values by day 8 of drought were below 0.50 for all doses of the three NPs, including NP-free droughted controls. Values as low as 0.40 were recorded, indicative of severe stress; however, permanent wilt was not reached as plants watered following the 8d drought period continued to grow as discussed above. The absence of a NP effect for the three NPs across four dosing ranges is likely due to the selection of a wheat variety bred by the Idaho Agriculture Experiment Station for drought tolerance in dryland farming, combined with a nutrient sufficient growth medium, and root colonization by the probiotic, *Pc*O6. These results do not exclude the possibility of NP benefit at longer growth stages, or when delivered at higher doses, when their effects could be in part due to combating challenges of pathogenic microbes when tested in a soil matrix.

Accumulation of Metals in Rhizosolution. Amendments with NPs increased the mass of the core element of the NP in the rhizosphere solution (Figure 4). Increases in elements in the rhizosphere solutions were noted at doses above 5 mg/kg for each NP compared to that with no amendment, with the dose-dependent increase continuing. Dissolution was independent of plant drought status. The increases in Si with increasing SiO_2 NP concentrations indicated that the silica sand used as the growth medium did not saturate the rhizosphere solution. Rather, the SiO_2 NPs supplemented the soluble silica under these plant growth conditions.

All treatments had circumneutral pH values (Table S3). pH is critical in controlling the solubilities of CuO and ZnO NPs but does not affect the dissolution of SiO_2 between pH 2 and 9.[43] The predicted dissolution equilibrium concentrations of CuO, ZnO, and SiO_2 NPs were calculated in MINTEQ v3.1 in a low ionic strength solution (4 mM as $Ca(NO_3)_2$, estimated from the measured ECs, Table S3) representing the rhizosphere solution and at the measured average pH for each NP (Table S3) with precipitation disallowed. The highest dose of each NP was entered as finite crystalline tenorite (CuO), zincite (ZnO), and amorphous SiO_2 precipitate (SiO_2). This generates predicted dissolution masses of the three NPs with only the pH influencing solubility. The measured solubility of CuO (Figure 4) was enhanced over the predicted value (9.5 μg/pot) at doses above 0.5 mg/kg; the increased dissolution of the NP is due to the chelation of Cu by the organic acids,

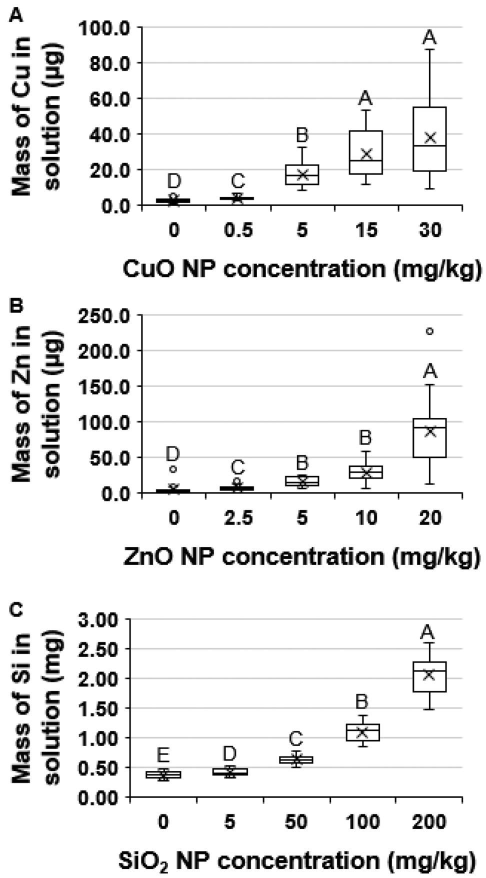

Figure 4. Element composition of wheat rhizosphere solutions. Plants were grown with and without drought in the presence of (A) CuO, (B) ZnO, and (C) SiO_2 NPs, expressed as mass of element per pot. There was no significant difference between watered and droughted pots. The contribution from the micronutrient-sufficient Hoagland solution was minimal (1.4−14 μg Cu, 5.4 μg Zn, and 0 μg Si per pot). Different letters indicate a statistical difference based on one-way ANOVA with posthoc Tukey test, p-value <0.05. For the box and whisker plots, the line within the box is the median, the × symbol is the mean, the bottom edge and top edge of the box are the first and the third quartiles, respectively. The whiskers are the minimum and maximum value. Values outside of the whiskers are outliers, defined as points outside 1.5x the interquartile range.

amino acids, and the bacterial and plant siderophores in the rhizosphere solution.[43,44] The average mass of DOC in the pore water for the CuO NP study was 2500 (±2000) μg C that would have been comprised of root exudates that enhanced solubility (Table S3).

The measured masses of Zn and Si in solution were, however, lower than thermodynamic predicted amounts (8200 μg/pot for Zn and 6900 μg/pot for Si), potentially due to dissolution and plant uptake or redistributing of Zn and Si onto the media solids. Further studies are required to describe the actual mechanisms for the observed lower amounts of Zn and Si in solution.

At harvest, less than 2% of the added Si and 1% of the added Cu/Zn were soluble. For a similar study with 100 mg Cu/kg added as CuO NPs, Hortin et al.[44] reported that a maximum of 1% and 2% of the added Cu was in solution and taken up by the plants regardless of treatment. The observations of NP dissolution indicated that doses of nanofertilizers must be adjusted to prevent undesired environmental accumulation. The findings that not all of the NPs dissolved under these

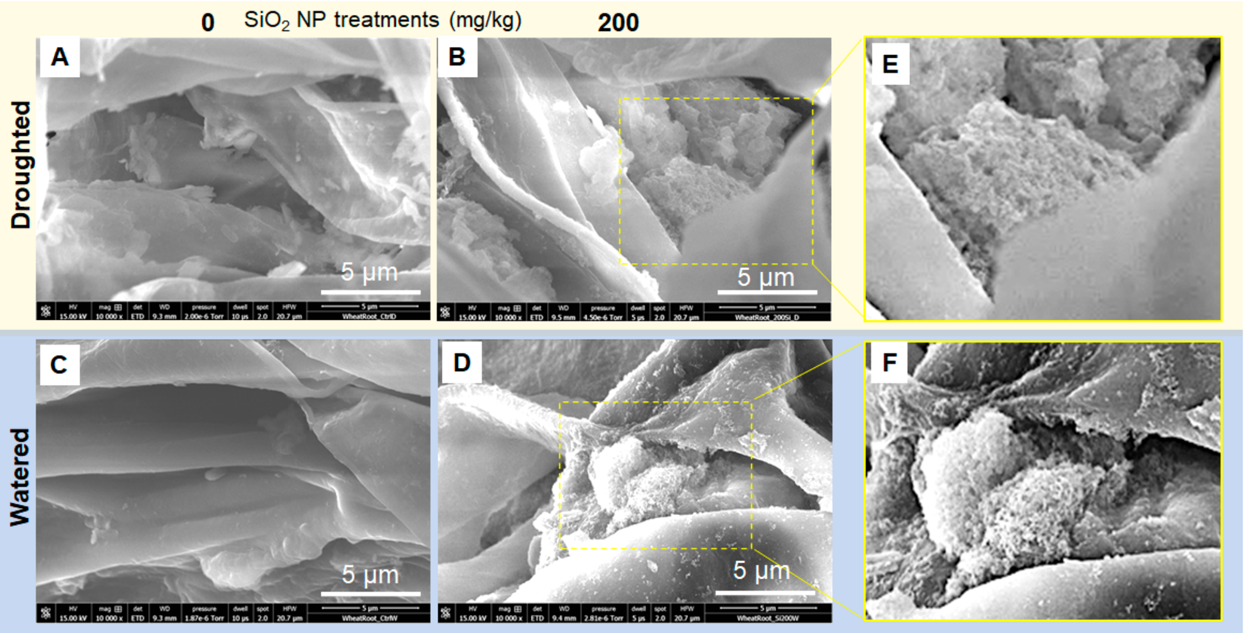

Figure 5. SEM analysis of wheat root hair morphology and SiO_2 NP distribution. The root hairs from the droughted plants (images A, B) and watered plants (images C, D) exhibit similar morphologies. The presence of SiO_2 NPs at 200 mg Si/kg of sand growth medium demonstrates NP adsorption to the root hair surfaces of both droughted (B) and watered (D) plants. The images in E and F are expanded and sharpened images of the area indicated by the dashed boxes to reveal assemblies of the spherical SiO_2 NPs. Clusters of spherical NPs could be observed only in the NP-treated samples for both droughted and watered plants.

growth conditions suggest that the core elements from the NP would remain in this growth matrix, acting as sources of the elements for a prolonged time.

Rhizoplane Characterization: Root Surface Imaging. To understand the fate of the NPs and their association with the root surface, SEM imaging was performed on root segments. Identification of root-associated SiO_2 NPs demonstrated successful delivery to the rhizoplane where NPs could interact with the roots and microbiome. No particles with a Zn or a Cu EDS signature were observed for either watered or droughted plants at the maximum application rates of ZnO or CuO NPs (SI Figures S3 and S4). However, in similar growth studies with 8 d watered plants, without a drought period, and with a higher dose (300 mg Cu/kg sand from CuO NPs), agglomerated particles with a Cu signature were identified on the abundant wheat root hairs.[45] Gao et al.[46] also demonstrated the binding of CuO NPs to wheat root surfaces as well as the release of Cu from the NPs in the rhizosphere solutions. In the present work, the inability to detect CuO and ZnO NPs at concentrations relevant for agricultural applications may be due to the low dosing of these NPs relative to the SiO_2 NPs, the duration of the study, and NP dissolution in the rooting zone, as well as to the restricted root zone that can be assessed by SEM. At the highest dose for SiO_2 NPs, 200 mg Si/kg sand, aggregates that gave a Si signature upon EDS analysis were detected on the root surfaces of plants grown with and without drought stress (Figure 5). Thus, such particles could serve as point sources for silicate release at the rhizoplane, supporting the value of NPs as sustained-release fertilizers. As the least toxic of the three NPs, SiO_2 was considered over a larger concentration range.

The findings of this study suggest that the potential benefits of NPs in promoting drought tolerance in plants occur only under certain environmental conditions. Wheat was grown exposed to CuO, ZnO, or SiO_2 NPs in a model system that mimicked optimal dryland crop production: selection of a drought-resistant cultivar (var. Juniper), complete fertilization from inorganic salts, and root colonization by a bacterium, *Pc*O6, that induces drought tolerance in wheat. Sand, used as the defined growth matrix, permitted strong root development without undefined organic materials to chelate metal ions and resulted in a circumneutral rhizosolution pH. Under these conditions, the observed dose-dependent CuO NP dissolution could be attributed to organic acids, amino acids, and bacterial and plant products, including siderophores and enzymes. Though no drought protection was observed from amendments of these NPs in these conditions, the results support that the NPs would provide essential elements supporting plant nutrition under conditions of nutrient limitation. Such provisions could also alleviate drought stress.

In the context of reported metal and metal oxide NP-mitigation of drought stress, the most reasonable explanation is that the NPs provided the essential elements otherwise lacking in the plant to promote drought tolerance through improved metabolic responses. Enzymes involved in response to oxidative stress or factors that promote photosynthesis are likely to be involved. Drought sensitivity differs between plants and with cultivar, as well as developmental age of the plant. Additionally, soil parameters, purposely not examined in the defined model growth system employed here, affect drought tolerance in the field and the agricultural and environmental impacts of added NPs. These soil properties include mineral composition, acidity or alkalinity, and varied particulate matter as well as differing and complex microbiomes. Real-time, nondestructive assessments of photosynthetic efficiency provide a sensitive assessment of plant health status as demonstrated here with the droughted wheat seedlings.

Environmental Significance and Outlook. NPs may still play a unique role as point sources for targeted and sustained nutrient delivery, as supported by the presence of aggregated

SiO_2 NPs on the root surface for the 200 mg Si/kg sand dose. In contrast, CuO and ZnO NPs at their highest doses of 30 mg Cu and 20 mg Zn/kg sand, respectively, were not detected on roots using SEM with EDS. However, this does not preclude the possibility of NP-root associations for CuO and ZnO NPs, as limitations of SEM scan regions and increased dissolution of these NPs reduce the likelihood of their detection on the 22 d plants. for the given doses. The greater reactivity of CuO and ZnO NPs in the rhizosolution, where root exudates, acids, and chelators are concentrated, has implications for the fate and transport of these metals in agricultural and environmental systems. None of the NP doses affected wheat root colonization by the bacterium, *Pc*O6, which persisted on the 22 d wheat seedling roots after colonization from a seed inoculum. These results suggest that at concentrations relevant to the use of CuO, ZnO, and SiO_2 NPs as fertilizers, it is unlikely that the microbiomes formed from beneficial pseudomonads will be impaired. Potential NP benefits to wheat, and other crops, may manifest at later growth stages and under different levels/duration of drought than those tested here.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c00453.

> Concentration of elements used in plant growth nutrient solution; CuO, ZnO, and SiO_2 nanoparticle size and surface charge characterization; drought effects on pH, electroconductivity (EC), and dissolved organic carbon (DOC) for each of the three NPs; moisture release curve for the sand growth matrix used in the study; plant tissue masses depicting root fresh mass, root dry mass, shoot fresh mass, and shoot dry masses for all 3 NP types by drought condition and for each NP type by concentration; SEM imaging of wheat roots grown for 22d in the presence of one NP type (CuO); EDS analysis of wheat roots grown for 22d in the presence of one NP type (CuO) (PDF)

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Notes

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