Interactive effects of drought, organic fertilizer, and zinc oxide nanoscale and bulk particles on wheat performance and grain nutrient accumulation.

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Abstract

Drought (40% field moisture capacity), organic fertilizer (O-F; 10%), and nano vs. bulk-ZnO particles (1.7 vs. 3.5 mg Zn/kg) were assessed in soil to determine their interactive effects on wheat performance and nutrient acquisition. Drought significantly reduced (6%) chlorophyll levels, whereas nano and bulk-ZnO alleviated some stress, thereby increasing (14-16%) chlorophyll levels, compared to the control. O-F increased (29%) chlorophyll levels and counteracted Zn's effect. Drought delayed (3-days) panicle emergence; O-F, nano and bulk-ZnO each accelerated (5-days) panicle emergence under drought, relative to the control and absence of O-F. Drought reduced (51%) grain yield, while O-F increased (130%) yield under drought. Grain yield was unaffected by Zn treatment under drought but increased (88%) under non-drought condition with bulk-ZnO, relative to the control. Drought lowered (43%) shoot Zn uptake. Compared to the control, nano and bulk-ZnO increased (39 and 23%, respectively) shoot Zn in the absence of O-F, whereas O-F amendment enhanced (94%) shoot Zn. Drought increased (48%) grain Zn concentration; nano and bulk-ZnO increased (29 and 18%, respectively) grain Zn, relative to the control, and O-F increased (85%) grain Zn. Zn recovery efficiency was in the order O-F>nano-ZnO>bulk-ZnO, regardless of the water status. Grain Fe concentration was unaffected by drought, under which O-F significantly reduced grain Fe, and nano-ZnO significantly reduced grain Fe, in the absence of O-F. Nano and bulk-ZnO also significantly reduced grain Fe, with O-F amendment under drought. Drought can have dire consequences for food and nutrition security, with implications for human health. This study demonstrated that drought-induced effects in food crops can be partially or wholly alleviated by ZnO particles and Zn-rich O-F. Understanding the interactions of drought and potential mitigation strategies such as fertilization with Zn-rich organic manure and ZnO can increase options for sustaining food production and quality under adverse conditions.

Keywords: Cow manure; Drought; Organic fertilizer; Wheat; Zinc; Zinc oxide nanoparticles

1. Introduction

Drought negatively affects soil water availability, crop growth and productivity, leading to economic losses (Lesk et al., 2016). In addition, nutrient mobility and uptake by plants can be impeded in soil with low moisture (Al-Kaisi et al., 2013; He and Dijkstra 2014; Karim and Raman 2015; Moreno-Jiménez et al., 2019), with potentially cascading effects on metabolism (e.g., hormones and chlorophyll), phenological development and acquisition of nutrients (Hajiboland and Amirazad 2010; Nikolaeva et al., 2010; Defez et al., 2017; Dimkpa et al., 2017a, 2019a, 2020). Not surprisingly, plant macronutrient (nitrogen, phosphorus and potassium; NPK) use efficiencies that are already low, <50%, under normal soil moisture conditions (Baligar et al., 2001) are exacerbated by drought, further reducing fertilizer efficacy. The combined reduction in grain yield and nutritional quality induced by drought results in double burden, leading to food and nutrition insecurity in humans (Fischer et al., 2019).

From a plant-soil perspective, the effects of drought on soil moisture and plant productivity can be mitigated by soil amendment or fertilization, of which among others, organic matter and zinc (Zn) have been described (Karim et al., 2012; Bodner et al., 2015; Bindraban et al., 2020a). As organic matter, manure can influence soil moisture holding capacity by increasing soil infiltration, minimizing soil evaporation, and capturing and storing water for plant's future use. Together, these benefits of organic manure allow for plant root proliferation and penetration to access soil nutrients under otherwise difficult conditions. However, organic manure, in and of itself, can serve as fertilizers, contributing substantial amounts of different nutrients to the soil (Haynes and Naidu, 1998; Sheppard, 2019), depending on the source - animal or plant - and its history. Accordingly, organic matter can also influence the effects of native nutrients or applied mineral fertilizer-nutrients by modulating their mobility and availability under varying soil water conditions (Tagwira et al., 1992; Moreno-Jiménez et al., 2019). Similarly, micronutrients such as Zn can regulate plant response to drought and mitigate negative effects. Up-regulation of abscisic acid to optimize stomatal closure and conserve water; increased production and activity of oxidative enzymes, and up-regulation of genes involved in water stress have been noted as potential mechanisms (Zengin 2006; Karim and Rahman 2015; Taran et al., 2017; Yang et al., 2018; Lamaoui et al., 2018).

The form of Zn that plants are exposed to could differentially influence the nutrient's role in modulating plant response to drought stress. ZnO nanoparticles (≤100 nm in at least one dimension) are more reactive than their bulk scale (≥ 1000 nm) counterparts due to their small size and enhanced surface area, as well as potentially engineered specific properties through coatings and/or functionalization to facilitate nutrient delivery (Graham et al., 2016; Medina-Velo et al., 2017). Multiple enhanced effects of nanoscale compared to bulk-scale ZnO have been observed in wheat under drought stress (Dimkpa et al., 2020). In contrast, no major differences were observed between ionic and nanoscale Zn in drought-stressed soybean, except for greater Zn accumulation due to ionic Zn treatment (Dimkpa et al., 2017a). This points to rapid dissolution into ions as a key fate of nanoscale ZnO in plant-soil systems (Wang et al., 2013). However, soil organic matter status can influence the bioavailability of nanoscale ZnO to plants (Medina-Velo et al., 2017; Moghaddasi et al., 2017; Dimkpa 2018).

Many drought-impacted soils around the world suffer from a simultaneous lack of organic matter and Zn (Moreno-Jiménez et al., 2019), potentially making crops grown on such soils more susceptible to the impacts of drought. Nevertheless, studies are unavailable that simultaneously addressed the effects of drought, organic amendment, and ZnO nanoparticles on crop performance and nutrient acquisition. We postulate that Zn and organic manure may interact with drought to modulate the plant responses. Consequently, the objective of the present study was to explore the interactive effects of drought, organic amendment and ZnO nano vs. bulk particles on wheat performance (vegetative and reproductive) and accumulation of nutrients (Zn, N, P, K, Fe, Ca, Mg and S). In particular, grain accumulation of micro (Zn and Fe) and secondary (Ca, Mg and S) nutrients were of importance, as these nutrients are essential for human health, but often are lacking in many staple diets due, in part, to excessive stripping of nutrient from soil not accompanied by replenishment via fertilization (Jones et al., 2013). The soil used in this study is Zn-deficient and very low in organic matter. To this end, cow dung was used as the source of organic manure due to its prevalent use as an organic amendment in the United States. Comparisons were made with a non-drought condition to better highlight the impact of the drought, organic manure and ZnO treatment interactions.

2. Materials and Methods

2.1. Chemicals

Commercial ZnO nanoparticles (18 nm) were purchased from US Research Nanomaterials, Inc., Houston, Texas, USA. Bulk (>1000 nm) ZnO powder was purchased from Sigma-Aldrich, St Louis, Missouri, USA. The ZnO nanoparticles were previously characterized in water suspensions using UV-vis spectrophotometry and transmission electron microscopy (Dimkpa et al., 2018, 2020), showing a spectral peak of 374 nm that is indicative of ZnO nanoparticles, and variably-shaped particles of both nano and super-nano scale dimensions, indicating propensity for aggregation. Organic manure was supplied through composted cow dung (Black KOW; Black Gold Compost Co. Oxford, Florida) that was acquired from a local Supply Store. The manure is labelled by the manufacturer as containing 0.5% each of N, P, and K; however, it was further characterized in this study using standard analytical methods: scalar segmented flow analyzer for N and P, inductively coupled plasma-optical emission spectroscopy (ICP-OES) for metals, and CNS analyzer for C and S, to obtain more detail on the chemical properties (Table 1).

2.2. Soil preparation

The soil used in the study is a sandy loam collected from Plains, Texas. Its chemical properties are presented in Table 1. The soil was divided into two portions and one portion was amended with cow manure at 10% of the soil weight. Separately, the manure-unamended and amended portions were fertilized (by mixing in a rotary shaker) with N and P at 100 and 75 mg/kg, respectively. Because the soil contained more than adequate levels of K, no additional K was provided (see also Wang et al., 2018). Subsequently, 8 kg of each soil was weighed into individual pots.

2.3. Plant growth

A greenhouse-based pot experiment involving winter wheat (*Triticum aestivum* L. var. Dyna-Gro 9522) was conducted in Muscle Shoals, Alabama (34.7448° N, 87.6675° W) between November and May (temperature, 1-33 ^{o}C; relative humidity, 25-92%). Three wheat seeds were sown in the potted soils, which upon germination was thinned down to one. Two weeks post-germination, the soils were fertilized with ZnO nano (2.17 mg/kg) and bulk (4.34 mg/kg) particles by subsurface incorporation, whereby 3 cm-deep holes were made about 3 cm away from the base of the plantlets and the dry powders were placed and the holes covered with soil. These levels of ZnO amounted to Zn rates of 1.7 mg Zn/kg for ZnO nanoparticles, and 3.5 mg Zn/kg for bulk ZnO particles. Thus, twice the rate of Zn was used in the case of the bulk product. Lower rate of nano Zn was used due to increased reactivity of nanomaterials compared to bulk materials. Moreover, using a lower rate of the nanoparticles allowed to assess one of the goals of nanotechnology, which is to reduce the input rates of chemical fertilizers in agriculture (see Kottegoda et al., 2017; Dimkpa et al., 2020). One-week post Zn treatment, a portion of the plantlets was exposed to drought stress by maintaining the soil at 40% of field moisture capacity (FMC) until harvest, approximately 210 days after sowing. The method for achieving 40% FMC is as previously described (Dimkpa et al., 2020). Ultimately, three Zn treatments were established in 3 replicates: control; nano ZnO and bulk ZnO, without and with amendment of cow manure. Each of these treatments was then duplicated for drought stress and non-drought (80% FMC) conditions. This resulted in a total of 12 treatments, which were placed into a randomized complete block design. During the growth period, chlorophyll was measured at three intervals (two, six and ten weeks after drought stress imposition) using a Soil-Plant Analyses Development (SPAD) meter (Konica Minolta). The time to panicle emergence from the primary shoot was monitored on a daily basis after drought imposition. At full maturity, plant height was measured, and plants were harvested and processed for shoot biomass and grain yield. Shoot tissues were analyzed for N, P, K, Zn, Fe, S, Ca and Mg.

2.4. Plant nutrient analyses

Harvested plant tissues were separated into shoot (leaves, stems and panicle head) biomass and grain, and separately oven-dried (60 °C) to constant weight. Shoot biomass and grain weights on dry basis were collected, and the dried tissues were ground into powder using a Model 4 Thomas

Wiley Laboratory Mill (Pennsylvania, USA) for shoot biomass, or using a ZM 100 Retsch grinder (Restch GmbH, Haan, Germany) for grain. A subset of ground tissue was acid-digested (in 3 mL sulfuric acid + 1 mL H_2O_2), heated for one h at 350 ºC and cooled to ambient temperature, before being equilibrated with d-H_2O and subjected to the following analytical procedures to determine elemental content: Skalar segmented flow analysis for N and P, and ICP-OES (model Spectro Arcos, SPECTRO Analytical Instruments GmbH, Kleve, Germany) for K, S, Zn, Ca, Mg, and Fe. Sulfur was extracted using a nitric and perchloric acid (5 mL; 1:1, v:v) digestion that oxidizes the plant S to sulfate (SO_4) (Blanchar et al., 1965). This digestion was done for 1 hr at 150 ºC, followed by capping with a funnel, and increasing the temperature to 200 ºC until only 1 mL of solution remained. Soil samples were recovered from the pots for each treatment to determine residual Zn levels. To this end, soils were sieved and extracted for Zn using DTPA (1:2 w/v [soil:DTPA solution]). All samples were shaken for 2 h, filtered, and then analyzed by ICP-OES.

2.5. Data Analysis

Data were analyzed using a generalized linear mixed model (GLMM) with a factorial arrangement within a randomized complete block design using Statistical Analysis System (SAS) Version 9.4. The factors, namely Zn treatment, water status (drought vs. non-drought) and organic fertilizer status (without and with amendment), were handled as fixed effects. The error estimated as the residual was handled as a random effect. Residuals from all response variables showed normal distribution and variances associated with the experimental factors were found to be homogeneous; as a consequence, the normal distribution was used to fit the GLMM, and the error variance-covariance matrix was homogeneous. P values equal or lower than 0.1 were considered significant both in the main effects of the Analysis of Variance or in

Least Square Mean (LS Mean) comparisons of significant effects. The GLIMMIX procedure from SAS was utilized for these analyses.

3. Results and Discussion

In this study, wheat was exposed or not to drought (40 vs. 80% FMC), cow manure, and ZnO nano or bulk particles in order to assess the interactive effects of these factors on crop parameters related to metabolism, development, productivity and nutrient acquisition, as a function of an adverse growth condition. At 40% FMC, the drought condition imposed in this study can be considered moderate, as opposed to severe. The measured parameters showed triple, double, or no interactive responses to the experimental factors. Based on data in Table 1 indicating the high content of nutrients in the cow manure relative to the soil, the cow organic treatment is henceforth in this study referred to as ‘‘organic fertilizer’’.

3.1. Interactive effects of drought, organic fertilizer and ZnO on chlorophyll production

Prior studies have demonstrated SPAD to be predictive of chlorophyll content (e.g., Ling et al., 2011; Liu et al., 2019). The SPAD values reported herein were averaged from three measurements taken at different times during wheat growth. There was no triple interaction effect of water status, organic fertilization and Zn treatment on the production of chlorophyll, based on SPAD measurements (Table 2). However, the separate interaction of water status and Zn treatment, and of organic fertilization and Zn treatment were significant for SPAD. Drought significantly affected SPAD values, relative to the non-drought condition (Table 2). Under drought, SPAD values were significantly higher in the Zn treatments relative to the control; however, there was no difference between the nanoscale and bulk particle treatments. In contrast, under non-drought condition, nano and bulk ZnO resulted in similar SPAD values as the control; however, nano ZnO lowered SPAD values when compared to bulk ZnO (Figure 1). Organic fertilizer amendment increased SPAD values, relative to its absence; and in the absence of organic fertilizer, ZnO nano and bulk particles significantly increased SPAD values, compared to the control. With organic fertilization, the significant effects of Zn treatments on SPAD levels were negated (Figure 1). Studies show that the individual factors of drought, organic fertilization and Zn can modulate plant chlorophyll content in their own right (e.g., Ganeshamurthy and Reddy 2000; Keyvan 2010; Nikolaeva et al., 2010; Dimkpa et al., 2017a,b, 2018; Taran et al., 2017). Mechanistically, depending on intensity, drought can modulate the production of reactive oxygen species (ROS) (Foyer and Noctor 2005; Taran et al., 2017), of which the chloroplast is a unique organelle for intensive production due to unbalanced rates of electron transport and CO_2 fixation. Whereas ROS have both positive and negative effects on plants (reviewed in Mittler 2017), under severe drought, ROS over-accumulation can result in oxidation of lipids and loss of pigments such as chlorophyll. The negation of the Zn treatment effect on SPAD levels by organic fertilizer, which was corollary to increased SPAD levels, led us to analyze the Zn content of the cow manure; the data showed that its Zn content on per kg basis (10.5 mg) was about 3-6 times higher than the Zn rates used from the ZnO nano (1.7 mg) and bulk (3.5 mg) particles. However, by applying 0.8 kg of the organic fertilizer per pot, additional 8.4 mg Zn was added to the pots with the cow manure treatment. The increased Zn exposure in the presence of organic fertilizer likely contributed in nullifying the effects of nano and bulk ZnO particles on SPAD, and indeed on several other parameters, as discussed subsequently. Zn stimulation of chlorophyll is due to the element being a cofactor of enzymes involved in chlorophyll biosynthesis. Notably, Zn in different forms (ionic, nano and bulk oxides) can stimulate chlorophyll production in crop plants, regardless of the water status (Subbaiah et al., 2016; Dimkpa et a. 2017a,b, 2018; Taran et al., 2017; Munir et al., 2018), although in this study, we observed this effect only under drought stress. Munir et al., (2010) showed under non-drought condition a dose-dependent effect of Zn (as nanoparticles) on chlorophyll production in wheat. In the present study, the higher SPAD values resulting from bulk ZnO relative to nano ZnO may reflect the dynamics of Zn bioavailability, whereby a higher Zn exposure rate during peak metabolic stage from bulk ZnO, in combination with improved soil moisture in the non-droughted soil, would have contributed to this outcome.

3.2. Interactive effects of drought, organic fertilizer and ZnO particles on panicle emergence

There was significant triple interaction effect of water status, organic fertilization status, and Zn treatment on panicle emergence by the plants (Table 2). On average, the panicles of plants exposed to drought emerged 3 days later than those of the non-droughted plants. Similarly, compared to plants without organic fertilization, organic fertilizer significantly lowered time to panicle emergence by 5 and 2 days, under drought and non-drought conditions, respectively (Figure 2). Thus, the effect of organic fertilizer on panicle emergence was stronger under drought condition. When organic fertilizer was absent under drought condition, nano ZnO, but not bulk ZnO**,** significantly lowered the time to panicle emergence by 5 days, compared to the control. In contrast, the nano ZnO treatment effect was negated by organic fertilization under drought. Contrary to the drought condition with no organic fertilization, bulk ZnO, but not nano ZnO reduced the time to panicle emergence in the non-drought condition by 5 days, when compared to the control. However, this effect was also negated by organic fertilization (Figure 2). The inter-relationship between drought and Zn regarding panicle emergence confirms findings from previous studies in sorghum and wheat (Dimkpa et al., 2019a, 2020). However, limited information is available in the literature on the interactive effect of organic fertilization and Zn on plant phenological development. In one related example the interaction of a commercially available organic potting mix (Miracle Grow®) and various types of Zn products lowered time to maturity of kidney bean, compared to the same Zn products in a normal agricultural soil (Medina-Velo et al., 2017). Separately, an amendment of cow manure reportedly reduced the number of days to flowering in maize (Amanullah and Khalid 2015). Given that the amendment of N and Zn-rich organic fertilizer facilitated panicle emergence in the current study, the observed interactive effect of organic fertilizer and Zn treatment could be related to a possible interplay of N and Zn, as reported in wheat by Sher et al. (2018). The countering of the effect of ZnO nano and bulk particle treatment on panicle emergence by the organic fertilizer is likely related to the high Zn content in the cow manure. We noted that bulk-scale ZnO (at higher rate), unlike nanoscale ZnO (at lower rate), did not affect time to panicle emergence under drought; but there was an affect under the non-drought condition. This is consistent with findings from our previous study (Dimkpa et al., 2020). In contrast, Medina-Velo et al. (2017) demonstrated that bulk ZnO and uncoated ZnO nanoparticles similarly reduced the time to maturity in kidney bean under non-drought condition, when compared to ionic Zn and coated ZnO nanoparticles. It can be imagined that the solubility dynamics and activity of the bulk ZnO were differently affected by the water status than the nano. This likely was reflected in the lower Zn recoverability of the bulk, compared to nano ZnO, in the droughted soil (see below). However, the nutritional and amendment effect of the organic fertilizer improved Zn availability from the bulk ZnO, in addition to its own Zn content.

3.3. Interactive effects of drought and organic fertilizer, and effect of ZnO particles on shoot growth.

Water and organic fertilization status showed a significant interaction effect on plant growth (Table 2). Shoot height was significantly greater under non-drought than under drought condition. Under drought, organic fertilizer significantly inhibited shoot growth. This effect was, however, counteracted by adequate water in the non-drought system (Figure 3). There was no interaction of water status, or of organic fertilization with Zn treatment. Thus, each Zn treatment data was averaged across the respective drought and organic fertilizer conditions. On that basis, the nano and bulk ZnO particles each significantly increased plant height, compared to the control (Figure 3). On its own, the effect of drought in reducing shoot elongation was not unanticipated, as drought is generally known to negatively affect plant growth (Ahmad et al., 2018). This is also the case for the Zn-induced increase in stem elongation, which agrees with previous reports (Dimkpa et al., 2018; Sher et al., 2018). In contrast, the organic fertilizer-induced inhibition of stem elongation under drought, but not under adequate water condition, was somewhat surprising. It seems likely that enhanced exposure to Zn from the organic fertilizer amendment + ZnO particles, in combination with other unknown components of the cow manure, led to a phytotoxic response in terms of shoot elongation. This effect was clearly evident only in the absence of adequate soil moisture. It is plausible that continued exposure over the plants' life cycle to high amounts of the 'chemical cocktail' (including Zn) in the cow manure and the absence of adequate moisture to dilute or dissipate the compounds in the root zone could have increased their point source concentration and inhibited stem growth.

3.4. Interactive effects of drought, organic fertilizer and ZnO particles on shoot biomass

There was significant triple interaction effect of water status, organic fertilizer status, and Zn treatment on plant biomass (Table 2); this was evidenced in Figure 4 by the different Zn treatment patterns across the four conditions of water status (drought vs. non-drought) and organic fertilizer status (without and with). In general, drought significantly reduced plant biomass, relative to the non-drought condition, and organic fertilizer amendment significantly increased plant above-ground biomass, irrespective of plant water status. But in no case across the four combinations did the Zn treatments result in significant above-ground biomass gain, relative to the control. Under drought condition, there was no effect of both types of ZnO particles on plant biomass in the absence of organic fertilization; whereas with organic fertilizer amendment, the oxide particles, strongly so bulk ZnO, reduced plant biomass compared to the control. In the non-drought condition with no organic fertilization, bulk ZnO also reduced plant biomass, compared to the control. This effect was, however, reversed by organic fertilizer amendment (Figure 4). As with the present study, our previous study involving the same soil, ZnO nanoparticles, and wheat cultivar also did not indicate greater shoot biomass due to Zn treatment (Dimkpa et al., 2018), and both Zn types also had insignificant effect on wheat biomass in the study of García-Gómez et al. (2015). There seems to be a role of particle size in the present finding for biomass production that is distinct from organic fertilization and water status.

3.5. Interactive effects of drought, organic fertilizer and ZnO particles on grain yield

There was a triple interaction effect of water status, organic fertilization status, and Zn treatment on wheat grain yield (Table 2). The Zn treatment pattern changed across the four conditions resulting from combining water status and organic fertilizer status. Drought significantly reduced grain yield compared to the non-drought condition. However, organic fertilization significantly increased grain yield, under both drought and non-drought conditions (Figure 5). There was no statistically significant effect of treatment with nano and bulk of ZnO particles on grain yield under drought, although there was indication of potential for yield promotion with the Zn treatment. In the non-drought condition lacking organic fertilizer amendment, bulk ZnO, but not nano ZnO, significantly increased grain yield, compared to the control. This effect was, however, offset by the organic fertilizer (Figure 5). A global meta-analysis of studies of wheat production in field conditions under drought (40% water reduction) indicated an average decrease of 20.6% (Daryanto et al., 2016). Here, we show under greenhouse conditions that drought stress at 40% FMC reduced grain yield by more than 100%. However, organic fertilization interacted with water status to mitigate grain yield reduction caused by drought. The rate of improvement in grain yield due to the organic fertilizer was similar in the non-drought and droughted conditions. Notably, bulk ZnO particles strongly promoted yield in the absence of organic fertilization and drought. The lack of overall strong effect with the ZnO nanoparticles treatment in the absence of organic fertilizer was surprising, given previous findings in wheat (Dimkpa et al., 2018, 2020). However, other reports in this crop have also noted no significant grain yield increase with ZnO nanoparticles (e.g., Zhang et al., 2018).

3.6. Interactive effects of drought, organic fertilizer and ZnO particles on Zn accumulation

Zinc accumulation by the plants was assessed for above-ground (shoot + grain) uptake and grain concentration (Figure 6). There was no triple interaction effect of water status, organic fertilization status and Zn treatment on Zn accumulation in both assessments (Table 2). Thus, Zn treatment and organic fertilization values were averaged across each water status for the drought analysis, indicating significantly lowered Zn above-ground accumulation as a result of drought (Figure 6A). In contrast to drought, there was an interaction effect of organic fertilization and Zn treatment on above-ground Zn accumulation (Table 2). In the absence of organic fertilizer, both nano and bulk ZnO particles significantly increased above-ground Zn, compared to the control; the effect was similar between the ZnO particle types. Organic fertilizer also increased the levels of Zn in the treatments but counteracted the effects of the ZnO particles on shoot Zn uptake, when compared to the control (Figure 6A). These findings resulted in differential mean Zn recovery efficiencies (RE; defined as above-ground [shoot + grain] accumulation from Zn treatment/rate of applied Zn x 100) of the ZnO treatments under the different organic fertilization and watering scenarios. Notably, adequate watering in the control treatment (no Zn) resulted in a Zn RE that was 47% higher than in the droughted condition. However, under drought, nano and bulk ZnO treatment had low Zn REs of 5.0 and 1.6 %, respectively, while the Zn RE for the organic fertilizer control, organic fertilizer + nano ZnO, and organic fertilizer + bulk ZnO was 14.9, 5.6 and 3.3%, respectively. In contrast, under non-drought condition, Zn REs were higher: 7.2 and 3.6% for nano and bulk ZnO treatment, respectively; and 19.6, 6.9 and 4.2.%, respectively, for the organic fertilizer control, organic fertilizer + nano ZnO, and organic fertilizer + bulk ZnO. Collectively, These data demonstrate that (i) drought could impair Zn recovery in both unfertilized and fertilized systems; (ii) there is higher Zn recoverability from nano ZnO than bulk ZnO irrespective of soil water status; (iii) Zn in the organic fertilizer is in a more bioavailable form than Zn in the nano or bulk ZnO particles; and (iv) addition of Zn lowered RE (in the case of organic fertilizer controls) or even negated (in the case of the organic fertilizer + nano or bulk ZnO) in the drought condition, compared to the non-drought condition. Reported Zn recovery efficiency is around 13%, on the basis of Zn-sulfate (Fageria and Baligar, 2005); as ionic Zn, Zn-sulfate is the most soluble and bioavailable Zn form (Wang et al., 2013; McBeath and McLaughlin, 2014). With the particulate ZnO, higher release of Zn ions could be expected from the nano than from the bulk product (McBeath and McLaughlin, 2014), explaining the higher plant RE from the nano product, in spite of lower application rate. However, as discussed previously (Dimkpa et al., 2018), particle dissolution into ions is not a dead-end process, given that the ions could be re-complexed in the soil matrix, and hence, become non-bioavailable Zn. This likely explains the overall low REs in these treatments. In the case of the organic fertilizer, we speculate that the Zn is likely predominantly present in a chelated form with organic acids in the cow manure (Zeng et al., 2011), which increased plant mobilization and facilitated its recovery from the soil. Hence, the REs were closer to that reported by Fageria and Baligar (2005).

Specifically for grain, there were separate interactions of drought and organic fertilizer, and of organic fertilizer and Zn treatment (Table 2). Drought increased grain Zn concentration when compared to the non-drought condition; this effect was notably contrary to the observation for shoot uptake. Organic fertilizer markedly increased grain Zn content, regardless of the water status (Figure 6B). In the absence of organic fertilizer, the nano ZnO significantly increased grain Zn concentration relative to the control, while bulk ZnO particles showed a median effect. As with shoot uptake, organic fertilizer increased the overall levels of Zn in the treatments but counteracted the stimulating effect of nano ZnO on grain Zn content (Figure 6B). The strong inhibition of Zn uptake by drought is consistent with previous for wheat and other crops (Dimkpa et al., 2017a, 2019a, 2020). Also, as expected based on prior studies (Dimkpa et al., 2018, 2020), Zn treatment promoted Zn uptake which, notably, occurred to similar degrees between the nano and bulk ZnO particles, despite the difference in their exposure concentrations. The improved Zn uptake with organic fertilizer is likely due to the high Zn content of the cow manure, and is consistent with previous reports of organic fertilizer (including cow manure, in one case) improving the bioavailability of Zn in soil-plant systems in the long-term (Tagwira et al., 1992; Rupa et al., 2003; Moghaddasi et al., 2017). With respect to the grain, both decreases and increases in Zn concentration under drought stress have been observed. Magallanes-López et al. (2017) reported reduced grain Zn in 46 Durum wheat cultivars under full (>500 mm) and moderate (300 mm) irrigation. In contrast, Velu et al. (2016) reported increased grain Zn concentration in 54 Bread wheat cultivars exposed to moderate or severe (180 mm) drought stress, compared to fully irrigated plants. In general, wheat grain Zn concentration appears to be increased by drought, based on a detailed review of the subject (Etienne et al., 2018). Our finding of increased grain Zn concentration under drought is, thus, not surprising, especially given that the wheat variety used in this study is a Bread wheat (Dyna-Gro 9522). The increased grain Zn by nano ZnO further confirms a role for nanoscale Zn in food fortification for human nutrition, as demonstrated in studies involving maize, sorghum, wheat, soybean and kidney bean (Subbaiah et al., 2016; Dimkpa et al., 2017, 2019a,b; Medina-Velo et al., 2017; Zhang et al., 2018). Notably, organic fertilization enhanced overall grain Zn, even though it negated the benefit of Zn fortification by nano ZnO. This finding contrasted with the study of Medina-Velo et al., (2017) showing increased grain Zn in kidney bean by nano and bulk-scale ZnO that was not negated by organic amendment. However, it should be noted that significantly higher (>62 mg/kg) nano and bulk ZnO levels were used in that study, compared to the present one (≤4.3 mg/kg).

3.7. Interactive effects of drought, organic fertilizer and ZnO particles on grain Fe, S, Mg and Ca contents

In addition to Zn, other nutrients assessed for their grain contents as a function of water status, organic fertilization and ZnO nano and bulk particles exposure included Fe, S, Mg and Ca. For Fe, the interaction effect of water status, organic fertilization and Zn treatment was significant (Table 2). Whereas drought imposition by itself did not significantly lower grain Fe concentration, organic fertilization under drought decreased grain Fe concentration. Compared to the control, Zn treatment under drought in the absence of organic fertilizer resulted in significantly lower grain Fe in the case of the nanoparticle, while a median effect was evident for the bulk particle. With organic fertilizer addition, both Zn types strongly reduced grain Fe, compared to the control (Figure 7A). Under the non-drought condition, nanoscale ZnO, and even more so bulk ZnO particles, significantly reduced grain Fe concentration in the absence of organic fertilizer. This effect was, however, eliminated by addition of organic fertilizer (Figure 7A). The slight increase in the grain Fe content under drought vs. non-drought stress (approx. 259 vs. 251 mg/kg), albeit insignificant, is consistent with the reported slight grain Fe increases in both Bread and Durum wheat cultivars induced by moderate drought (Velu et al., 2016; Magallanes-López et al., 2017). Reduced grain Fe with organic fertilization offered an interesting scenario of Fe-Zn interaction. Both of these nutrients occured to about the same degree in the planted system with the organic fertilizer. However, Fe being a trivalent metal, will typically be more competitive than divalent metals such as Zn for binding to biological materials (Dimkpa et al., 2009), in this case root surface. Yet, it is likely not a concidence that lower grain Fe was concomitant with higher grain Zn (compare Figure 6B vs. Figure 7A, in the absence of organic fertilizer). This potential antagonistic interaction between Zn and Fe can be viewed from either one of two standpoints in which Zn appeared to have been favored: (i) inhibition of Fe uptake from root to shoot by Zn [as shown for bean (Dimkpa et al., 2015)], resulting in reduced Fe translocation to the grain, or (ii) competition between Zn and Fe in the xylem transport system [as reported in barley (Alam et al., 2001)]. This assumption of Zn out-competing Fe for grain translocation can further be corroborated by the lower grain Fe contents in the ZnO nano and bulk particle treatments without the Zn and Fe-rich organic fertilizer. Notably, ZnO particles are reserviors of Zn ions that are released slowly over time to potentially impact such interactions.

For S, there was no main effect of drought; however, drought interacted with organic fertilization to significantly increase grain S concentration (Table 2). The respective Zn treatment effects on grain S were averaged across the given water status and organic fertilization conditions. In both drought and non-drought conditions, grain S concentration increased with organic fertilizer addition (Figure 7B). The lack of drought effect on grain content is consistent with observation in a winter wheat cutivar (Gooding et al., 2003), as well as in soybean and sorghum (Dimkpa et al., 2017a, 2019a). The increase in grain S with organic fertilization can be attributed to the high S content in the cow manure, compared to the deficient level in the soil (Table 1).

For Mg, there were no interaction of plant water status, organic fertilization, and Zn treatment (Table 2). However, averaging the data from the Mg measurements show that each of these factors independently affected grain Mg significantly: drought increased grain Mg, relative to no drought (and regardless of organic fertilization and Zn treatment); organic fertilization decreased grain Mg, relative to absence of organic fertilizer (and irrespective of water status and Zn treatment); and bulk ZnO, but not nano ZnO, particles decreased grain Mg, relative to the control (and irrespective of water status and Zn treatment) (Figure 8). The increase in grain Mg concentration under drought is consistent with data for wheat and maize in a previous report (Etienne et al., 2018). In contrast to wheat, drought reduced sorghum and barley grain Mg concentrations (Dimkpa et al., 2019a; Etienne et al., 2018), which in the case of sorghum was counteracted by fertilization with ZnO nanoparticles (Dimkpa et al., 2019a). In soybean, both drought and Zn treatment had no effect on grain Mg (Dimkpa et al., 2017a). Decreased wheat grain Mg in the presence of organic product contrasted with the report of Medina-Velo et al., (2017) for kidney bean, but likely resulted in this case from competitive cationic interactions at the root zone that affected Mg uptake. Accordingly, grain Mg concentration was lowered by Zn treatment, especially by bulk ZnO with higher Zn rate.

In the case of Ca, drought had no effect on grain concentration. Amendment of organic fertilizer also did not affect grain concentration but did interact with Zn treatment (Table 2), wherein grain Ca was significantly reduced by bulk ZnO in the absence of organic fertilizer. Conversely, Zn treatment did not significantly affect grain Ca in the presence of organic fertilizer; however, nano ZnO particles significantly reduced grain Ca in the presence of organic fertilizer, compared to bulk ZnO (Figure 8). The lack of drought effect on grain Ca concentration agrees with Rose et al. (2015) and Dimkpa et al. (2017a) for wheat and soybean, respectively, under moderate drought exposure. However, it contrasted with that of sorghum showing a drought-induced increase in grain Ca (Dimkpa et al., 2019a). Contrary to data from Medina-Velo et al. (2017) for kidney bean, organic amendment did not increase grain Ca concentration in this study.

3.8. Effects of drought and organic fertilizer on above-ground (shoot and grain) N, P, and K accumulation.

There was no interactive effect of water status, organic fertilization and Zn treatment on shoot or grain NPK content. However, water status and/or organic fertilization significantly influenced plant NPK accumulation independently (Table 2). Therefore, all Zn treatments were averaged across drought vs. non-drought, and organic fertilizer amended vs non-amended conditions, as a function of above-ground (shoot + grain) NPK contents. The data show that NPK accumulation was significantly inhibited by drought; that P accumulation was significantly increased by organic fertilization, and that accumulation K was inhibited by organic fertilization (Figure 9). The reported effects of drought on NPK accumulation in crop plants in the literature vary depending on species, degree of water deprivation and ascociated fertilization regimes (Etienne et al., 2018; Soares et al., 2019). Lower N under drought correlated with lower SPAD levels (Figure 1), confirming the use of chlorophyll as an indictor of plant N levels (Bindraban, 1999). The inhibition of total above-ground wheat NPK acquisition by moderate drought reported herein is consistent with drought effects on these nutrients in previous studies (Shabbir et al., 2016; Danish and Zafar-ul-Hye 2019; Dimkpa et al., 2020). The plant P-enhancing effect of organic fertilizer also agreed with Gaind et al., (2006). In this regard, the organic fertilizer could stimulate phosphatase activity of P-solubilizing microbes in soil, with its organic acid content helping to solubilize P, thereby increasing the bioavailable P fraction for plant uptake (Gaind et al., 2006; Bindraban et al., 2020b). Moreover, adequate water availability in soil facilitates P mobilization (He and Dijkstra 2014). In contrast to P, reduced K accumulation by organic fertilizer agrees with organic (biochar) amendment-induced decrease in K uptake reported by Danish and Zafar-ul-Hye (2019), and could be related to cationic antagonistic interaction between K and other metals (Ca, Mg, Zn and Fe) in the cow manure. It would also appear that the plants elaborated mechanisms to exclude accumulation of K at toxic levels, considering the high levels of K in the admixture of soil and cow manure (Table 1).

3.9. Effects of organic fertilizer and Zn treatment on post-harvest residual soil Zn

Drought did not affect residual Zn in the post-harvest soil (data not shown). However, there was a significant interaction of organic fertilization and Zn treatment on residual Zn (Table 2), whereby the addition of organic fertilizer significantly increased soil Zn content. Under each organic fertilization status, bulk, but not nanoscale, ZnO significantly increased the residual soil Zn, compared to the control treatment (Figure 10). Higher residual Zn in the organic fertilizer-amended soil directly reflects the high Zn content of the cow manure. Higher soil Zn was also reported in a cow manure-amended soil, relative to non-amended soil, although the initial Zn content of the cow manure was not determined prior to application in soil (Mogahaddasi et al., 2017). Similarly, the effects of Zn treatment on residual Zn reflects the relative levels of Zn in the treatments (see also Mogahaddasi et al., 2017). In this regard, unlike the bulk ZnO particle treatment, application of Zn at 1.7 mg/kg from ZnO nanoparticles was not high enough to allow significant residual Zn after plant harvest, especially considering Zn amounts taken into the plant shoot and grain.

4. Conclusion

We demonstrate in this study that moderate drought can have profound effects on wheat metabolism, phenological development, productivity and nutrient accumulation, and that fertilization with organic manure (cow dung) and nano and bulk ZnO particles can interact with drought to modulate some of these outcomes. Addition of cow manure increased the final soil organic matter (from 0.92 to 2.58%). However, while the ‘‘organic matter’’ attributes of the cow manure may have modulated soil moisture in the droughted growth system, its mechanism of action seems to be based in large path on its Zn content, as indicated by its counteracting of Zn effects on chlorophyll level, shoot biomass, grain yield, shoot and grain Zn, and grain Fe, under the non-drought condition where soil moisture was not a concern. Thus, the cow manure acted both as an organic amendment and as an organic fertilizer capable of supplying nutrients. With ZnO nano and bulk particles, some of the effects were particle size-specific, relative to the control and under a given growth condition. These include the acceleration of panicle emergence by nano ZnO under drought, and by bulk ZnO under adequate water; decrease in shoot biomass by bulk ZnO with adequate water; and increase in grain yield by bulk ZnO under adequate water. Given that the preponderance of these size-specific effects was demonstrated by bulk ZnO particle, they could also be related to the higher Zn rate used in that treatment, which also allowed for greater residual soil Zn. However, despite higher Zn fertilization rate from the bulk ZnO, the nano ZnO notably increased shoot Zn uptake and grain Zn concentration similarly, if not slightly higher, as the bulk ZnO particles. This demonstrates the enhanced reactivity of nanoscale materials allowing for less Zn application rate to achieve food fortification with Zn. Ultimately, the fortification of wheat grain by Zn is critical for global human nutrition and health which, as demonstrated here, can be achieved in a Zn-deficient soil by mineral Zn fertilization or amendment with Zn-rich organic manure. However, the possibility of Zn-induced reduction in the grain content of non-target essential nutrients such as Fe, Mg and Ca must also be considered.

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References

Ahmad, Z., Waraich, E.A., Akhtar, S., Anjum, S., Ahmad, T., Mahboob, W., Hafeez, O.B., Tapera, T., Labuschagne, M., Rizwan, M., 2018. Physiological responses of wheat to drought stress and its mitigation approaches. Acta Physiol. Plantarum 40, 80.

Alam, S., Kamei, S., Kawai, S., 2001. Effect of iron deficiency on the chemical composition of the xylem sap of barley. Soil Sci. Plant Nutr. 47, 643-649.

Al-Kaisi, M.M., Elmore, R.W., Guzman, J.G., Hanna, H.M., Hart, C.E., Helmers, M.J., Hodgson, E.W., Lenssen, A.W., Mallarino, A.P., Robertson, A.E., Sawyer, J.E., 2013.

Drought impact on crop production and the soil environment, 2012 experiences from Iowa. J. Soil Water Conservation 68, 1.

Amanullah, Khalid, S., 2015. Phenology, growth and biomass yield response of maize (*Zea mays* L.) to integrated use of animal manures and phosphorus application with and without phosphate solubilizing bacteria. J. Micro. Biochem. Technol. 7, 6.

Baligar, V.C., Fageria, N.K., He, Z.L., 2001. Nutrient use efficiency in plants. Commun. Soil Sci. Plant Anal. 32, 921-950.

Bindraban, P.S., 1999. Impact of canopy nitrogen profile in wheat on growth. Field Crops Res. 63, 63-77.

Bindraban, P.S., Dimkpa, C.O., White, J.C., Franklin, F.A., Melse-Boonstra, A., Koele, N., Pandey, R., Rodenburg, J., Senthilkumar, K. Demokritou, P., Schmidt, S., 2020a. Safeguarding human and planetary health demands a fertilizer sector transformation. Plants, People Planet DOI: 10.1002/ppp3.10098.

Bindraban, P.S., Dimkpa, C.O., Pandey, R. 2020b. Exploring phosphorus fertilizers and fertilization strategies for improved human and environmental health. Biol. Fert. Soils https://doi.org/10.1007/s00374-019-01430-2.

Blanchar, R.W., Rehm, G., Caldwell, A.C. 1965. Sulfur in plant materials by digestion with nitric and perchloric acid. Soil Sci. Soc Amer Proc 29, 71-72.

Bodner, G., Nakhforoosh, A., Kaul, H-P., 2015. Management of crop water under drought, a review. Agron. Sustain. Dev. 35, DOI, 10.1007/s13593-015-0283-4.

Danish, S., Zafar-ul-Hye, M., 2019. Co-application of ACC-deaminase producing PGPR and timber-waste biochar improves pigments formation, growth and yield of wheat under drought stress. Sci. Rep. 9, 5999.

Daryanto, S., Wang, L., Jacinthe, P-A., 2016. Global synthesis of drought effects on maize and wheat production. PLoS ONE 11(5), e0156362.

Defez, R., Andreozzi, A., Dickinson, M., Charlton, A., Tadini, L., Pesaresi, P., Bianco, C. 2017. Improved drought stress response in alfalfa plants nodulated by an IAA over-producing *Rhizobium* strain. Front. Microbiol. 8, 2466.

Dimkpa, C.O., 2018. Soil properties influence the response of terrestrial plants to metallic nanoparticles exposure. Curr. Opin. Environ. Sci. Health 6, 1-8.

Dimkpa, C.O., Andrews, J., Fugice, J., Singh, U., Bindraban, P.S., Elmer, W. H., Gardea-Torresdey, J.L., White, J.C., 2020. Facile coating of urea with low-dose ZnO nanoparticles promotes wheat performance and enhances Zn uptake under drought stress. Front. Plant Sci. 11:168.

Dimkpa, C.O., Singh, U., Bindraban, P.S., Elmer, W.H., Gardea-Torresdey, J.L., White, J.C., 2019a. Zinc oxide nanoparticles alleviate drought-induced alterations in sorghum performance, nutrient acquisition, and grain fortification. Sci. Total Environ. 688, 926-934.

Dimkpa, C.O., Singh, U., Bindraban, P.S., Adisa, I.O., Elmer, W.H., Gardea-Torresdey, J.L., White, J.C. 2019b. Addition-omission of zinc, copper, and boron nano and bulk particles demonstrate element and size -specific response of soybean to micronutrients exposure. Sci. Total Environ. 665, 606-616.

Dimkpa, C.O., Singh, U., Bindraban, P.S., Elmer, W.H., Gardea-Torresdey, J.L., White, J.C., 2018. Exposure to weathered and fresh nanoparticle and ionic Zn in soil promotes grain yield and modulates nutrient acquisition in wheat (*Triticum aestivum* L.) J. Agric. Food Chem. *66,* 9645–9656.

Dimkpa, C., Bindraban, P., Fugice, J., Agyin-Birikorang, S., Singh, U., Hellums, D., 2017a. Composite micronutrient nanoparticles and salts decrease drought stress in soybean. Agron. Sustain. Dev. 37, 5.

Dimkpa, C.O., White, J.C. Elmer, W.H., Gardea-Torresdey J., 2017b., Nanoparticle and ionic Zn promote nutrient loading of sorghum grain under low NPK fertilization. J. Agric. Food Chem. 65, 8552-8559.

Dimkpa, C.O., Hansen, T., Stewart, J., McLean, J.E., Britt, D.W., Anderson, A.J., 2015. ZnO nanoparticles and root colonization by a beneficial pseudomonad influence essential metal responses in bean (*Phaseolus vulgaris*). Nanotoxicol. 9, 271–278.

Dimkpa, C. O., Merten, D., Svatoš, A., Büchel, G., Kothe, E. 2009. Metal-induced oxidative stress impacting plant growth in contaminated soil is alleviated by microbial siderophores. Soil Biol. Biochem. 41, 154-162.

Etienne, P., Diquelou, S., Prudent, M., Salon, C., Maillard, A., Ourry, A., 2018 Macro and micronutrient storage in plants and their remobilization when facing scarcity: the case of drought. Agric-Basel 8, 14.

Fageria, N.K., Baligar, V.C., 2005. Growth components and zinc recovery efficiency of upland rice genotypes. Pesq agropec bras Brasília 40, 1211-1215.

Fischer, S., Hilger, T., Piepho, H-P., Jordan, I., Cadisch, G., 2019. Do we need more drought for better nutrition? The effect of precipitation on nutrient concentration in East African food crops. Sci. Total Environ. 658, 405-415.

Foyer, C.H., Noctor, G., 2005. Redox homeostasis and antioxidant signaling, a metabolic interface between stress perception and physiological responses. Plant Cell 17, 1866–1875.

Gaind, S., Pandey, A.K., Lata, N., 2006. Microbial biomass, P-nutrition, and enzymatic activities of wheat soil in response to phosphorus enriched organic and inorganic manures. J. Environ. Sci. Health, Part B 41, 177-187.

Ganeshamurthy, A.N., Reddy, K.S., 2000. Effect of integrated use of farmyard manure and sulphur in a soybean and wheat cropping system on nodulation, dry matter production and chlorophyll content of soybean on swell-shrink soils in central India. J. Agron. Crop Sci. 185, 91-97.

García-Gómez, C., Babin, M., Obrador, A., Álvarez, J.M., Fernández, M.D., 2015. Integrating ecotoxicity and chemical approaches to compare the effects of ZnO nanoparticles, ZnO bulk, and $ZnCl_2$ on plants and microorganisms in a natural soil. Environ. Sci. Pollut. Res. 22, 16803–16813.

Gooding, M.J., Ellis, R.H., Shewry, P.R., Schofield, J.D., 2003. Effects of restricted water availability and increased temperature on the grain filling, drying and quality of winter wheat. J. Cereal Sci. 37, 295-309.

Graham, J.H., Johnson, E.G., Myers, M.E., Young, M., Rajasekaran, P., Das, S., Santra, S., 2016. Potential of Nano-formulated zinc oxide for control of citrus canker on grapefruit trees. Plant Dis. 100, 2442-2447.

Hajiboland, R., Amirazad, H., 2010. Drought tolerance in Zn-deficient red cabbage (*Brassica oleracea* L. var. capitata f. rubra) plants. Hort Sci. 37, 88-98. Haynes, R.J. Naidu, R., 1998. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. Nutr. Cycl. Agroecosys. 51, 123–137.

He, M., Dijkstra, F.A. 2014. Drought effect on plant nitrogen and phosphorus: a meta-analysis. New Phytol. 204, 924-931.

Jones, D.L., Cross, P., Withers, P.J.A., DeLuca, T.H., Robinson, D.A., Quilliam, R.S., Harris, I.M., Chadwick, D.R., Edwards-Jones, G., 2013. Review: Nutrient stripping: the global disparity between food security and soil nutrient stocks. J. Appl. Ecol. 50, 851-862.

Karim, M.R., Rahman, M.A. 2015. Drought risk management for increased cereal production in Asian Least Developed Countries. Weath. Clim. Extr. 7, 24-35.

Karim, M.R., Zhang, Y.-Q., Zhao, R.-R., Chen, X.-P., Zhang, F.-S., Zou, C.-Q. 2012. Alleviation of drought stress in winter wheat by late foliar application of zinc, boron, and manganese. J. Plant Nutri. Soil Sci. 175, 142-151.

Keyvan, S., 2010. The effects of drought stress on yield, relative water content, proline, soluble carbohydrates and chlorophyll of bread wheat cultivars. J. Animal Plant Sci. 8,1051-1060.

Kottegoda, N., Sandaruwan, C., Priyadarshana,G., Siriwardhana, A., Rathnayake, U.A., Arachchige, D.M.B., Kumarasinghe, A.R., Dahanayake, D., Karunaratne, V., Amaratunga, G.A.J., 2017. Urea-hydroxyapatite nanohybrids for slow release of nitrogen. ACS NANO 11, 1214-1221.

Lamaoui, M., Jemo, M., Datla, R., Bekkaoui, F. 2018. Heat and drought stresses in crops and approaches for their mitigation. Front. Chem. 6, 26.

Lesk, C., Rowhani, P., Ramankutty N. 2016. Influence of extreme weather disasters on global crop production. Nature 529, 84-87.

Ling, Q.H., Huang, W.H., Jarvis, P., 2011. Use of a SPAD-502 meter to measure leaf chlorophyll concentration in *Arabidopsis thaliana*. Photosynthesis Res. 107, 209-214.

Liu, S., Li, S., Fan, X.Y., Yuan, G.D., Hu, T., Shi, X.M., Huang, J.B., Pu, X.Y., Wu, C.S., 2019. Comparison of two noninvasive methods for measuring the pigment content in foliose macrolichens. Photosynth Res. 141, 245-257.

Magallanes-López, A.M., Hernandez-Espinosa, N., Velu, G., Posadas-Romano, G., Ordoñez-Villegas, V.M.G., Crossa, J., Ammar K., Guzmán, C., 2017. Variability in iron, zinc and phytic acid content in a worldwide collection of commercial durum wheat cultivars and the effect of reduced irrigation on these traits. Food Chem. 237, 499-505

McBeath, T.M., McLaughlin, M.J., 2014. Efficacy of zinc oxides as fertilisers. Plant Soil 374, 843-85.

Medina-Velo, I.A., Dominguez, O.E., Ochoa, L., Barrios, A.C., Hernández-Viezcas, J.A., White, J.C., Peralta-Videa, A.R., Gardea-Torresdey, J.L., 2017. Nutritional quality of bean seeds harvested from plants grown in different soils amended with coated and uncoated zinc oxide nanomaterials. Environ. Sci., Nano 4, 2336-2347.

Mittler, R., 2017. ROS are good. Trends Plant Sci. 22, 1.

Moghaddasi, S., Fotovat, A., Khoshgoftarmanesh, A.H., Karimzadeh, F., Khazaei, H.R., Khorassani, R., 2017. Bioavailability of coated and uncoated ZnO nanoparticles to cucumber in soil with or without organic matter. Ecotoxicol. Environ. Safety 144, 543-551.

Moreno-Jiménez, E., Plaza, C., Saiz, H., Manzano, R., Flagmeier M., Maestre, F.T. 2019. Aridity and reduced soil micronutrient availability in global drylands. Nature Sust. 2, 371–377.

Munir, T., Rizwan, M., Kashif, M., Shahzad, A., Ali, S., Amin, N., Zahid, R., Alam, M.F.E., Imran, M., 2018. Effect of zinc oxide nanoparticles on the growth and Zn uptake in wheat *(Triticum aestivum* L.) by seed priming method. Dig. J. Nanomat. Biostruct. 13, 315-323.

Nikolaeva, M.K., Maevskaya, S.N., Shugaev, A.G., Bukhov, N.G., 2010. Effect of drought on chlorophyll content and antioxidant enzyme activities in leaves of three wheat cultivars varying in productivity. Russian J. Plant Physiol. 57, 87-95.

Rose T.J., Raymond, C.A., Bloomfield, C., King, G.J., 2015. Perturbation of nutrient source–sink relationships by post-anthesis stresses results in differential accumulation of nutrients in wheat grain. J. Plant Nutr. Soil Sci. 178, 89–98.

Rupa, T.R., Rao, C.S., Rao, A.S., Singh, M., 2003. Effects of farmyard manure and phosphorus on zinc transformations and phyto-availability in two alfisols of India. Biores. Technol. 87, 279-288.

Shabbir, R.N., Waraich, E.A., Ali, H., Nawaz, F., Ashraf, M.Y., Ahmad, R., Awan, M.I., Ahmad, S., Irfan, M., Hussain, S., Ahmad, Z., 2016. Supplemental exogenous NPK application alters biochemical processes to improve yield and drought tolerance in wheat (*Triticum aestivum* L.) Environ. Sci. Pollut. Res. 23, 2651–2662.

Sheppard, S.C. 2019. Elemental composition of swine manure from 1997 to 2017: changes relevant to environmental consequences. J. Environ. Qual. 48, 160-170.

Sher, A.K., Naveed, G., Ahmad, K.A, Saeed, M., Masaud, S. 2018. Phenology and biomass production of wheat in response to micronutrients and nitrogen application. Sarhad J. Agric. 34, 712-723.

Soares, J.C., Santos, C.S., Carvalho, S.M.P., Pintado, M.M., Vasconcelos, M.W., 2019. Preserving the nutritional quality of crop plants under a changing climate, importance and strategies. Plant Soil 443, 1- 26.

Subbaiah, L.V., Prasad, T.N.V.K.V., Krishna, T.G., Sudhakar, P., Reddy, B.R., Pradeep, T. 2016. Novel effects of nanoparticulate delivery of zinc on growth, productivity, and zinc biofortification in maize (*Zea mays* L.). J. Agric. Food Chem. 64, 3778−3788.

Tagwira, F., Piha, M., Mugwira, L., 1992. Effect of pH, and phosphorus and organic matter contents on zinc availability and distribution in two Zimbabwean soils. Comm. Soil Sci. Plant Anal. 23, 1485-1500.

Taran, N., Storozhenko, V., Svietlova, N., Batsmanova, L., Shvartau, V., Kovalenko, M., 2017. Effect of zinc and copper nanoparticles on drought resistance of wheat seedlings. Nanoscale Res. Lett. 12, 60.

Velu, G., Guzman, C., Mondal, S., Autrique, J.E., Huerta, J., Singh, R.P., 2016. Effect of drought and elevated temperature on grain zinc and iron concentrations in CIMMYT spring wheat. J. Cereal Sci. 69, 182-186.

Wang, S., Wang, Z-H., Li, S-S., Diao, C-P., Liu, L., Hui, X-L., Huang, M., Luo, L-C., He, G., Cao, H-B., Yu, R., Malhi, S.S., 2018. Identification of high-yield and high-Zn wheat cultivars for overcoming "yield dilution" in dryland cultivation. Euro. J. Agron. 101, 57-62.

Wang, P., Menzies, N.W., Lombi, E., McKenna, B.A., Johannessen, B., Glover, C.J., Kappen, P., Kopittke, P.M. 2013. Fate of ZnO nanoparticles in soils and cowpea (*Vigna unguiculata*). Environ Sci. Technol. 47, 13822-13830.

Yang, K.-Y., Doxey, S., McLean, J.E., Britt, D., Watson, A., Al Qassy, D., Jacobson, A., Anderson, A.J. 2018. Remodeling of root morphology by CuO and ZnO nanoparticles, Effects on drought tolerance for plants colonized by a beneficial pseudomonad. Botany 96, 175-186.

Zeng, F., Ali, S., Zhang, H., Ouyang, Y., Qiu, B., Wu, F., Zhang, G., 2011. The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. Environ. Pollut 159, 84–91.

Zengin, F.K. 2006. The effects of Co^{2+} and Zn^{2+} on the contents of protein, abscisic acid, proline and chlorophyll in bean (*Phaseolus vulgaris* cv. Strike) seedlings. J. Environ. Biol. 27, 441-448.

Zhang, T., Sun, H., Lv, Z., Cui, L., Mao, H., Kopittke, P.M. 2018. Using synchrotron-based approaches to examine the foliar application of $ZnSO_4$ and ZnO nanoparticles for field-grown winter wheat. J. Agric. Food Chem. 66, 2572–2579.

Table and Figure Captions

Table 1. pH, organic matter and nutrient contents (mg/kg) of the soil and cow manure used in the study.

Table 2. P values (Pr > F) from the analysis of variance performed for the measured parameters.

Figure 1. Interactive effect of water status (drought v. non drought) and Zn treatment, and of organic fertilization status and Zn treatment, on the production of chlorophyll in wheat as determined by SPAD. Values are means and standard errors (SEs). Different uppercase letters above horizontal lines represent significant interactions of water status or organic fertilizer with Zn treatment. Different lowercase letters on bars indicate significant differences among the Zn as a function of water status or organic fertilizer amendment (n=3).

Figure 2. Triple interaction effect of water status (drought v. non drought), organic fertilizer and Zn treatment on time to panicle emergence in wheat. Values are means and SEs. Different uppercase letters above horizontal lines represent significant effect of water status (upper lines) and organic matter amendment (lower lines). Different lowercase letters on bars indicate significant difference among the Zn treatments, separately for organic fertilizer amendment within each water status (n=3).

Figure 3. Interactive effect of water status (drought v. non drought) and organic fertilizer, and effect of Zn treatment on wheat shoot growth. Values are means and SEs. Different uppercase letters above horizontal lines represent significant effect of water status. Different lowercase letters on bars indicate significant difference, separately for organic fertilization and water status interaction and Zn treatment (n=3).

Figure 4. Interactive effect of water status (drought v. non drought), organic fertilizer and Zn treatment on wheat biomass (dry weight basis). Values are means and SEs. Different uppercase letters above horizontal lines represent significant effect of water status (upper lines) and organic fertilization (lower lines). Different lowercase letters on bars indicate significant difference among the Zn treatments, separately for organic fertilization status within each water status (n=3).

Figure 5. Interactive effect of water status (drought v. non drought), organic fertilizer and Zn treatment on wheat grain yield (dry weight basis). Values are means and SEs. Different uppercase letters above horizontal lines represent significant effect of water status (upper lines) and organic fertilization (lower lines). Different lowercase letters on bars indicate significant difference among the Zn treatments, separately for organic fertilization status within each water status (n=3).

Figure 6. Effect of water status (drought v. non drought), and interaction of organic fertilizer and Zn treatment on shoot Zn uptake (left panel). Interaction of drought and organic fertilizer and of organic fertilizer and Zn treatment on grain Zn concentration (right panel). Values are means and SEs. Different uppercase letters above horizontal lines represent significant interaction of organic fertilization and Zn treatment (for left panel), and for significant interaction between water status and organic fertilization or organic fertilization and Zn treatment (for right panel). Different

lowercase letters on bars indicate significant difference among the Zn treatments, separately for water status and organic fertilization (n=3).

Figure 7. Interactive effects of water status (drought v. non drought), organic fertilizer and Zn treatment on grain Fe (left panel) and S (right panel) contents. Values are means and SEs. For Fe, different uppercase letters above horizontal lines represent significant effect of water status (upper lines) and organic fertilizer amendment (lower lines). Different lowercase letters on bars indicate significant difference among the Zn treatments, separately for organic fertilization status within each water regime. For S, uppercase letters above horizontal lines represent significant effect of water status while lowercase letter on bars indicate significant difference between the organic matter statuses (n=3).

Figure 8. Effects of water status, organic fertilizer and Zn treatment on grain Mg and Ca concentrations. Values are means and SEs. For Mg, different lowercase letters on bars indicate significant differences, separately for water status, organic fertilizer amendment and Zn treatment. For Ca, different uppercase letters above horizontal lines represent significant interaction of organic fertilizer and Zn treatment while different lowercase letters on bars indicate significant difference among the treatments as a function of organic fertilization (n=3).

Figure 9. Effects of drought and organic fertilization on N, P, and K accumulation in above-ground (shoot and grain) plant tissues. Bars are means and SEs and different letters on bars indicate significant differences, for drought and non-drought conditions, and for organic fertilization (n=3).

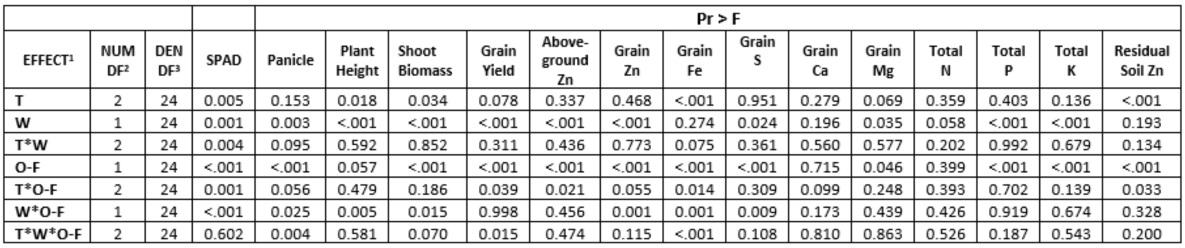
Figure 10. Interactive effect of Zn treatment and organic fertilizer amendment on residual Zn in soil after plant harvest. Values are means and SEs. Different letters on bars indicate significant

difference among the treatments, for a given organic fertilization status (n=3).

Table 1

Property	pH	Organic Matter (%)	NH_4-N	NO_3-N	Avail. P	K	Ca	Mg	S	DTPA-Zn	DTPA-Fe
Soil	6.87 ± 0.01	0.92 ± 0.06	5.8± 0.3	0.3± 0.05	2.5± 0.1	1903 ±55	1174 ±28	150 ± 3.7	2.7 ± 0.5	0.1± 0.01	2.04± 0.6
Cow Manure	8.08 ± 0.03	17.5± 0.4	0.8± 0.2	57± 0.7	214± 4.5	3668 ± 90	3516 ± 64	745 ± 21	929 ± 64	10.5± 0.3	14.2± 0.9

Table 2



				Pr > F													
EFFECT[1]	NUM DF[2]	DEN DF[3]	SPAD	Panicle	Plant Height	Shoot Biomass	Grain Yield	Above-ground Zn	Grain Zn	Grain Fe	Grain S	Grain Ca	Grain Mg	Total N	Total P	Total K	Residual Soil Zn
T	2	24	0.005	0.153	0.018	0.034	0.078	0.337	0.468	<.001	0.951	0.279	0.069	0.359	0.403	0.136	<.001
W	1	24	0.001	0.003	<.001	<.001	<.001	<.001	<.001	0.274	0.024	0.196	0.035	0.058	<.001	<.001	0.193
T*W	2	24	0.004	0.095	0.592	0.852	0.311	0.436	0.773	0.075	0.361	0.560	0.577	0.202	0.992	0.679	0.134
O-F	1	24	<.001	<.001	0.057	<.001	<.001	<.001	<.001	<.001	<.001	0.715	0.046	0.399	<.001	<.001	<.001
T*O-F	2	24	0.001	0.056	0.479	0.186	0.039	0.021	0.055	0.014	0.309	0.099	0.248	0.393	0.702	0.139	0.033
W*O-F	1	24	<.001	0.025	0.005	0.015	0.998	0.456	0.001	0.001	0.009	0.173	0.439	0.426	0.919	0.674	0.328
T*W*O-F	2	24	0.602	0.004	0.581	0.070	0.015	0.474	0.115	<.001	0.108	0.810	0.863	0.526	0.187	0.543	0.200

[1]T: Zn Treatment; W: Water status; O-F: Organic fertilizer amendment. [2] Numerator Degrees of Freedom. [3] Denominator Degrees of Freedom

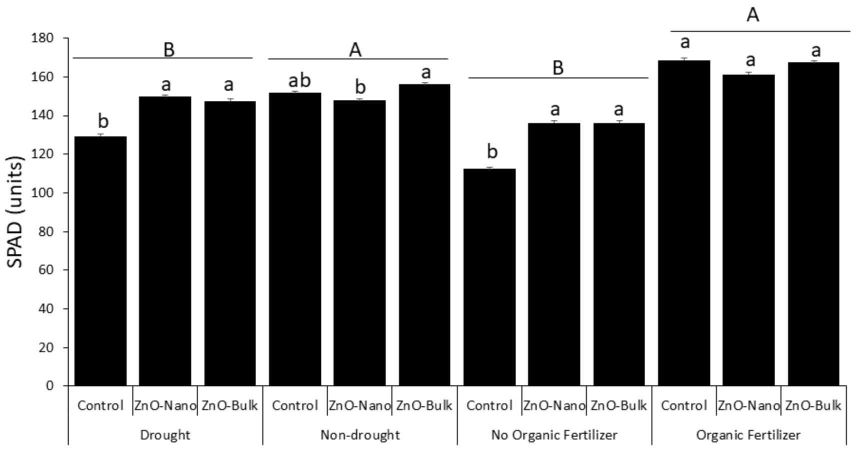


Figure 1

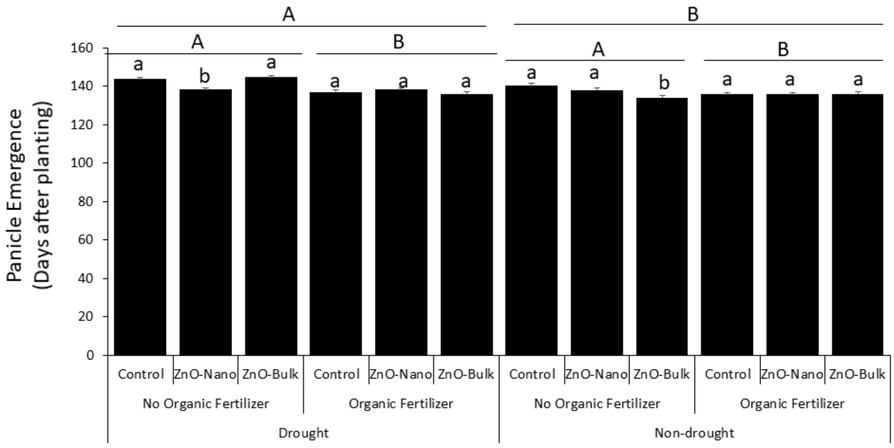


Figure 2

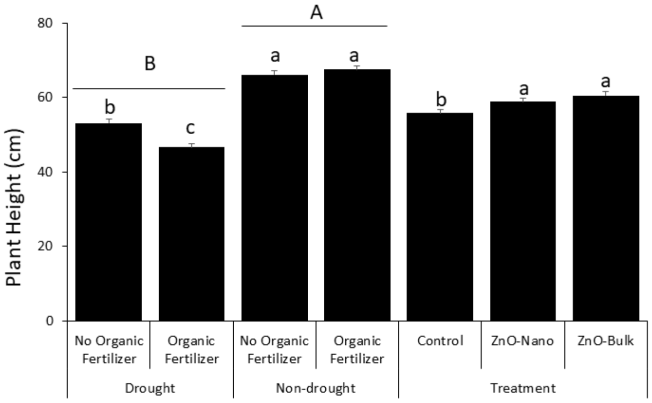
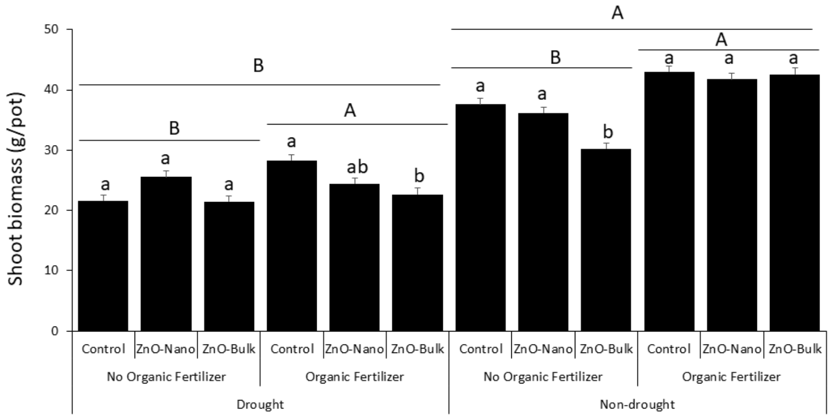


Figure 3



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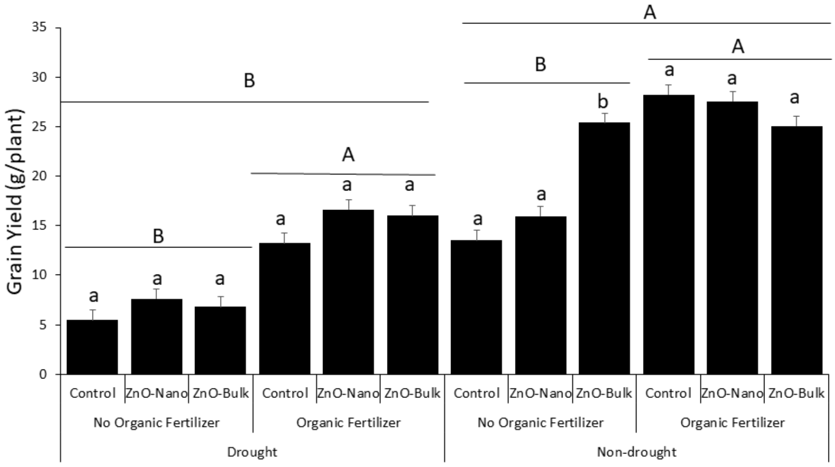

Figure 5

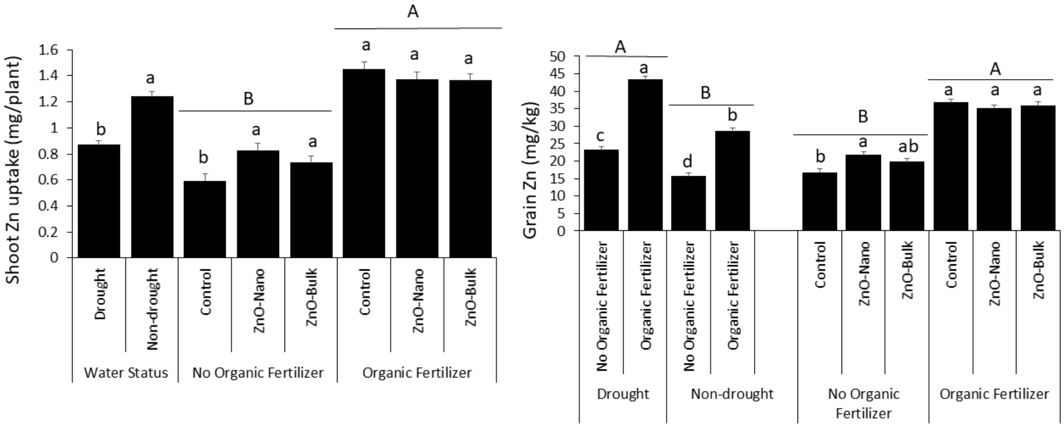

Figure 6

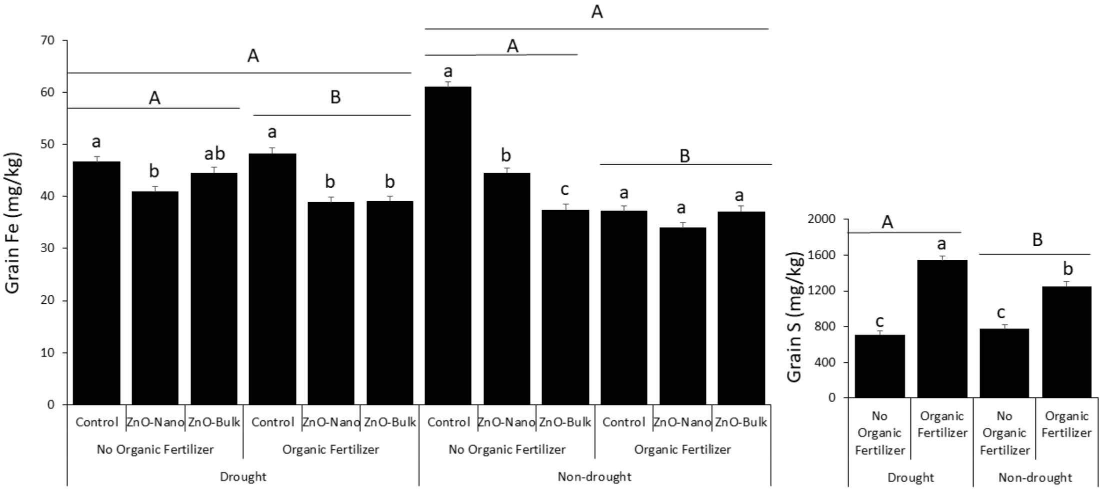

Figure 7

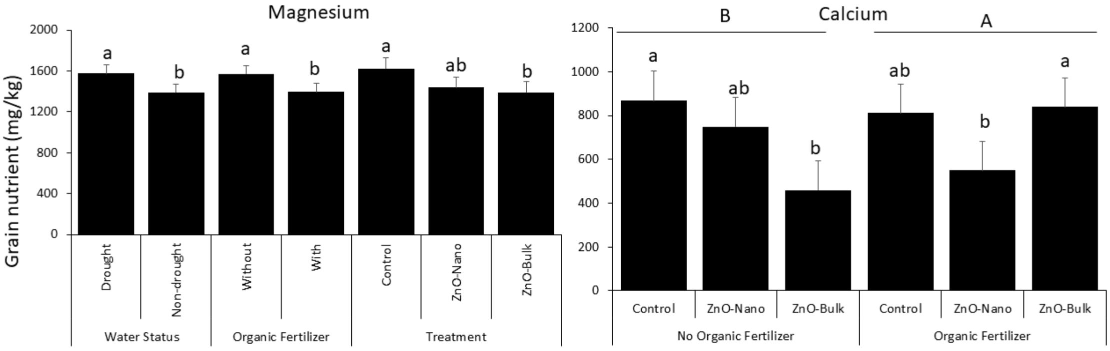

Figure 8

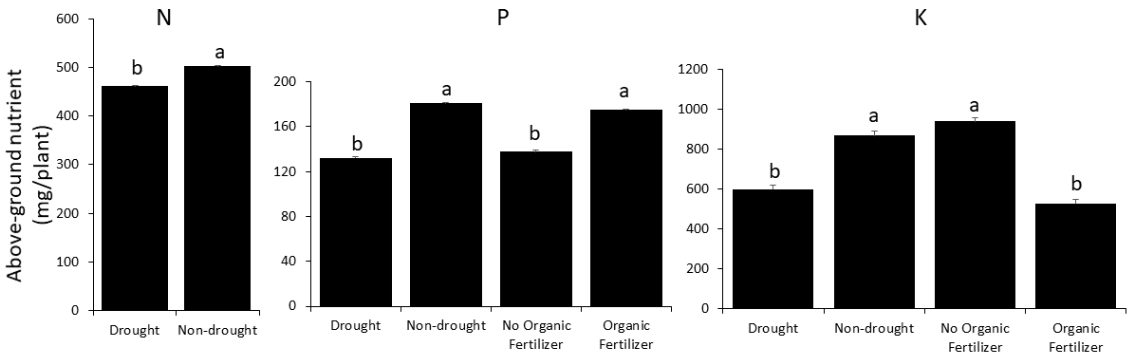

Figure 9

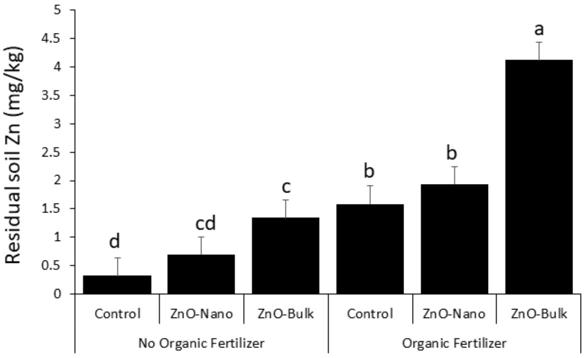
Residual soil Zn (mg/kg)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
d
cd
c
b
b
a
Control
ZnO-Nano
ZnO-Bulk
Control
ZnO-Nano
ZnO-Bulk
No Organic Fertilizer
Organic Fertilizer

Figure 10

Table 2. P values (Pr > F) from the analysis of variance performed for each response variable

				Pr > F													
EFFECT[1]	**NUM DF[2]**	**DEN DF[3]**	**SPAD**	**Panicle**	**Plant Height**	**Shoot Biomass**	**Grain Yield**	**Above-ground Zn**	**Grain Zn**	**Grain Fe**	**Grain S**	**Grain Ca**	**Grain Mg**	**Total N**	**Total P**	**Total K**	**Residual Soil Zn**
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T*W	2	24	0.004	0.095	0.592	0.852	0.311	0.436	0.773	0.075	0.361	0.560	0.577	0.202	0.992	0.679	0.134
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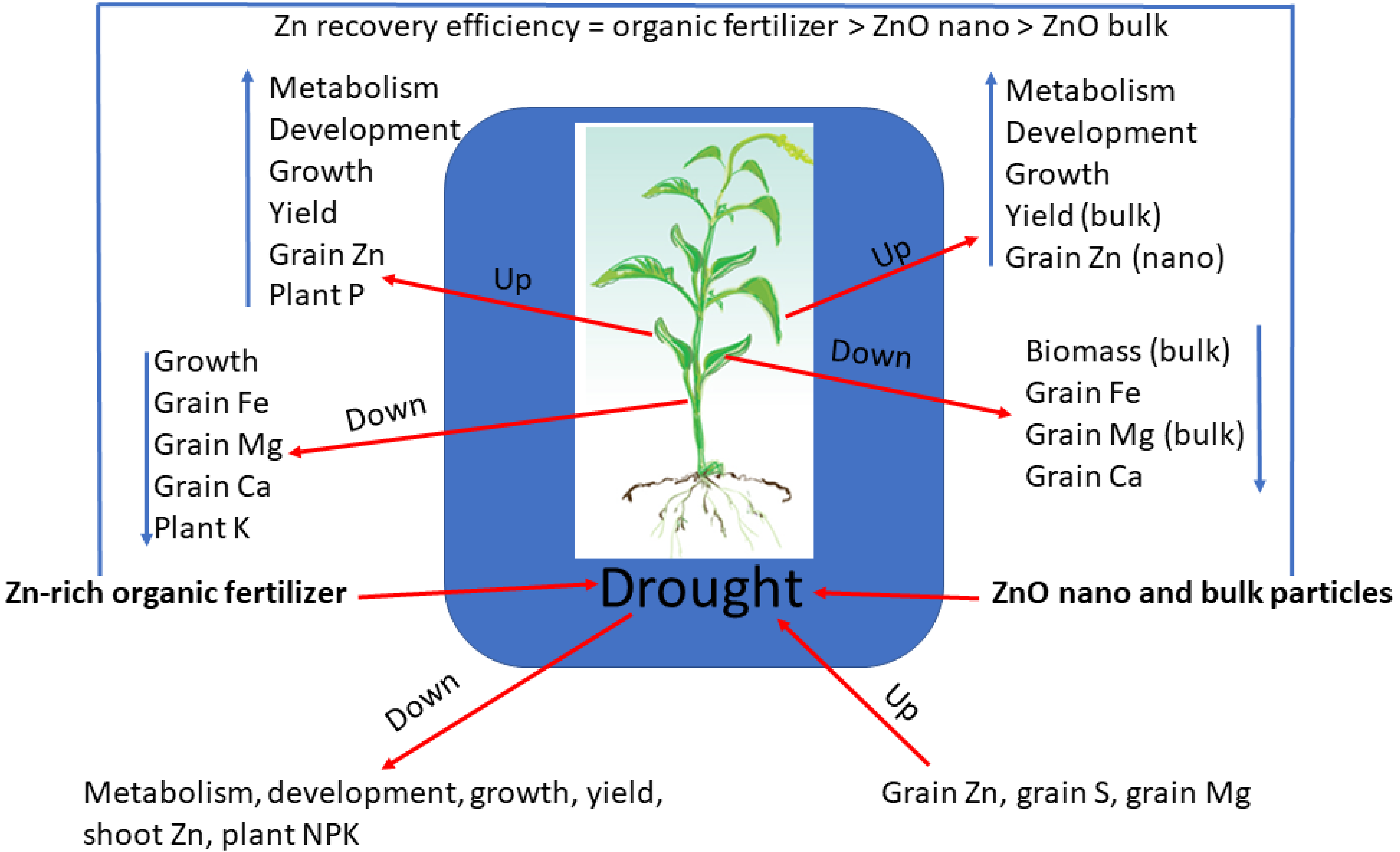
W*O-F	1	24	<.001	0.025	0.005	0.015	0.998	0.456	0.001	0.001	0.009	0.173	0.439	0.426	0.919	0.674	0.328
T*W*O-F	2	24	0.602	0.004	0.581	0.070	0.015	0.474	0.115	<.001	0.108	0.810	0.863	0.526	0.187	0.543	0.200

[1] T: Zn Treatment; W: Water status; O-F: Organic Fertilizer amendment. [2] Numerator Degrees of Freedom. [3] Denominator Degrees of Freedom


Zn recovery efficiency = organic fertilizer > ZnO nano > ZnO bulk
Metabolism
Development
Growth
Yield
Grain Zn
Plant P
Up
Growth
Grain Fe
Grain Mg
Grain Ca
Plant K
Down
Metabolism
Development
Growth
Yield (bulk)
Grain Zn (nano)
Up
Down
Biomass (bulk)
Grain Fe
Grain Mg (bulk)
Grain Ca
Zn-rich organic fertilizer
Drought
ZnO nano and bulk particles
Down
Up
Metabolism, development, growth, yield, shoot Zn, plant NPK
Grain Zn, grain S, grain Mg