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Effects of Manganese Nanoparticle Exposure on Nutrient Acquisition in Wheat (*Triticum aestivum* L.)

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Abstract: Nanoparticles are used in a variety of products, including fertilizer-nutrients and agro-pesticides. However, due to heightened reactivity of nano-scale materials, the effects of nanoparticle nutrients on crops can be more dramatic when compared to non nano-scale nutrients. This study evaluated the effect of nano manganese-(Mn) on wheat yield and nutrient acquisition, relative to bulk and ionic-Mn. Wheat was exposed to the Mn types in soil (6 mg/kg/plant), and nano-Mn was repeated in foliar application. Plant growth, grain yield, nutrient acquisition, and residual soil nutrients were assessed. When compared to the control, all Mn types significantly ($p < 0.05$) reduced shoot N by 9–18%. However, nano-Mn in soil exhibited other subtle effects on nutrient acquisition that were different from ionic or bulk-Mn, including reductions in shoot Mn (25%), P (33%), and K (7%) contents, and increase (30%) in soil residual nitrate-N. Despite lowering shoot Mn, nano-Mn resulted in a higher grain Mn translocation efficiency (22%), as compared to salt-Mn (20%), bulk-Mn (21%), and control (16%). When compared to soil, foliar exposure to nano-Mn exhibited significant differences: greater shoot (37%) and grain (12%) Mn contents; less (40%) soil nitrate-N; and, more soil (17%) and shoot (43%) P. These findings indicate that exposure to nano-scale Mn in soil could affect plants in subtle ways, differing from bulk or ionic-Mn, suggesting caution in its use in agriculture. Applying nano Mn as a foliar treatment could enable greater control on plant responses.

Keywords: Bioaccumulation; macronutrients; manganese oxide nanoparticles; near-neutral soil; translocation factor; wheat

1. Introduction

Manganese (Mn) is an essential nutrient that is required in trace amounts by plants, where it is involved in photosynthesis, respiration, and nitrogen (N) metabolism. In addition, Mn is also involved in conferring tolerance to root and shoot pathogens [1–4]. In accordance with these cellular-level roles, the agronomic benefits of Mn application has been demonstrated in different crops. For instance, the growth and/or yield of maize, beans, soybean, wheat, and sugarcane increased several folds upon fertilization with Mn in the ionic form at relevant doses [5,6]. However, Mn can be toxic to plants at elevated exposure levels, depending on soil chemical properties, such as low pH and high redox potential, both of which can enhance Mn bioavailability and alter its oxidation state in favor of the more toxic Mn^{2+} form [2,5,7].

The advent of nanotechnology and increased inclusion of nanoscale (1–100 nanometer in size) components in various products have included the use of nanoparticles of micronutrients as fertilizers and agropesticides. Recent reviews in this area describe both the negative and positive aspects of nanoparticle nutrients on plant growth and productivity [8–12]. Notably, because nanoparticles are more reactive than their bulk-scale equivalents, these materials may cause greater toxicity or beneficial effects on agricultural crops. Thus, with better understanding and management, the beneficial aspects of nano-enabled fertilizers can become a highly valued tool for addressing the problem of global food security [12,13].

However, the literature contains very few studies that are focused on nano-Mn behavior in plant systems, particularly when compared to the number of reports on nano-ZnO, nano-CuO, or nano FeO [9–11,14]. The few published studies show that nano Mn treatment resulted in an increase in mung bean growth, 52% and 38%, for root and shoot [3]; a 22% increase in eggplant yield [15]; non-significant effects on root elongation of White Mustard [16]; no effect on lettuce germination, but a dose-dependent increase in plant root length [17]; and, suppression of disease progression over time in watermelon that was not accompanied by significant yield increase [18].

In addition to effects on plant growth and yield, nano-scale metallic elements are known to modulate plant accumulation of nutrients. For example, nano-ZnO increased N and potassium (K) accumulation in sorghum, while the effect on phosphorus (P) accumulation was mixed [19]. However, P mobilization and accumulation from native soil was increased in lettuce by nano Fe_3O_4 and TiO_2 [20]. Similarly, a nano-formulation of ZnO, CuO, and B_2O_3 altered N, P, and K accumulation in soybean [21]. Despite its reported role in N metabolism, as demonstrated in physiological observations [4], little has been done to evaluate the role of nano-Mn in the accumulation of N and other macro nutrients in crop plant species. Moreover, no prior reports could be found on the impact of nano-Mn on the productivity wheat, which is a globally important food crop. This study was initiated to evaluate the effect of nano-Mn on wheat yield and Mn dynamics, and to understand the accumulation of macro nutrients (NPK) as influenced by nanoscale Mn relative to bulk and ionic Mn. Both soil and foliar application routes were investigated. Such studies contribute to obtaining a better understanding of the possibilities and limitations of nano-enabled strategies for crop productivity and quality enhancement.

2. Materials and Methods

2.1. Chemicals and Soil

Three sources of Mn were used in this study, nanoparticle (nano) Mn-oxide (Mn_2O_3), bulk particle Mn-oxide, and manganese chloride ($MnCl_2 \cdot 4H_2O$). Mn-oxide nano was purchased from US Research Nanomaterials (Houston, TX, USA). The product was described by the manufacturer as spherical-shaped 30 nm particles, with a 99.2% purity. It was previously further characterized in an aqueous suspension by other workers [22], indicating a zero-h hydrodynamic size of 1387 nm, surface charge (zeta potential) of 0.8, and a pH of 7.2. Bulk Mn_2O_3 and Mn salt were both from Fisher Scientific (Fair Lawn, NJ, USA). As the present study was conducted in soil, the nano Mn was used "as is" without any further characterization; this is due to the complexity of soil medium, such as the presence of natural nano-size colloids that would obfuscate the outcome of in vitro characterization relative to soil. The soil that was used in the study was collected from Plains, Texas. This soil, "Brownfield", is a sandy loam with a pH of 6.87 and different levels of N, P, and K: respectively, 4.0, 2.05, and 246.0 mg/kg. The DTPA-extractable Mn level was 6.4 mg/kg soil.

2.2. Exposure Assay

A greenhouse-based pot experiment involving winter wheat was conducted in Muscle Shoals, Alabama (34.7448° N, 87.6675° W) during November–May (temperature, 1–33 °C; relative humidity, 25–92%). Quadruplicate pots were filled with 8 kg of Brownfield soil and amended with 200:75:200 mg/kg NPK as a basal treatment. Three winter wheat seeds (*Triticum aestivum* var. Dyna Gro 9522) were sown per pot and were thinned down to one seedling after germination. Four weeks post germination, the plants were exposed to Mn in treatments consisting of control (no Mn), Mn_2O_3 nano (in soil or foliar), Mn salt (in soil), and Mn_2O_3 bulk (in soil). Each Mn treatment was at a rate of 6 mg/kg soil/plant; accordingly, while considering a native Mn level of 6.4 mg/kg, the treated plants would have been exposed to a total Mn concentration of 12.4 mg/kg. For soil treatment, exposure was achieved by placing a total of 138 mg of the dry nano or bulk powders, or 173 mg of the Mn salt (each corresponding to 6 mg/kg/plant) into the soil at a depth of 2–3 cm in the vicinity of the root. These Mn values were obtained based on the molecular weight of each Mn compound. The foliar treatment was done by separating pre-determined plants and covering the surface of each pot with a plastic sheet to prevent contamination from foliar drips. Subsequently, the leaves were sprayed with a water-based nano suspension of the equivalent amount of nano Mn as in soil (138 mg suspended in 100 mL/plant) while using a handheld sprayer. A drop (0.2–0.5 mL) of commercial detergent (Ecolab, Great Plains, TX, USA) was added to the nano suspension to act as a surfactant to facilitate wettability and stickiness of the liquid on the leaves [19]. Following spraying and drying, the plants were returned to the experimental area and grown under natural daylength up to the crop's physiological maturity, seven months later. During the process, watering of the plants was achieved by automated drip irrigation as needed, based on predetermined water holding capacity of the soil.

During plant growth, chlorophyll content, as influenced by Mn application over time, was determined using a SPAD (Soil-Plant Analyses Development) meter (Konica Minolta; Aurora, IL, USA). SPAD readings were taken at three different times corresponding to one (Time 1), three (Time 2) and six (Time 3) weeks after Mn treatment. Early (four weeks post treatment) and late (pre-anthesis) tiller numbers were recorded. Upon maturity at approximately 210 days after sowing, the final plant height for the parent shoot was measured, and plants were harvested and separated into root, shoot and grain. At that time, the root, shoot, and grain weights for each treatment were determined. The tissues were subsequently oven-dried (60 °C) until constant weight, ground into powder using a Model 4 Thomas Wiley Laboratory Mill (Philadelphia, PA, USA) for shoot and root, or a Zn 100 Retsch grinder (Restch GmbH, Haan, Germany), for grain. Ground tissues were acid-digested (3 mL sulfuric acid + 1 mL H_2O_2), heated for one h at 350 °C, and cooled to room temperature, before being equilibrated with dd-H_2O and then subjected to the following analytical procedures to determine elemental contents: Skalar segmented flow analysis for N and P, and inductively coupled plasma-optical electron spectroscopy (ICP-OES) for K and Mn. Soil samples were also collected post-harvest for each treatment to determine pH and available residual nutrient levels for N, P, K, and Mn For pH, each soil sample in replicates was directly mixed with dd-H_2O (1:1, w/v), followed by slow agitation for 15 min, incubating for a further 15 min, and measurement while using a soil pH probe. For elemental analysis, soils were ground and sieved followed by extraction; for N, ammonium-N and nitrate-N were separately extracted while using KCl; for P, extraction was by the Pi method [23]; for K, extraction was by NH_4Cl; and for Mn by DTPA (1:2 w/v [soil: DTPA solution]). All of the samples were shaken for 2 h, filtered, and subjected to analytical procedures while using the respective above instrumentations.

2.3. Data Analysis

A two-way analysis of variance (ANOVA; OriginPro 2018) was used to determine significant differences in crop responses to the treatments and in block effects for each parameter, including SPAD, plant vegetative and reproductive development, and nutrient contents in plant and soil samples. A Fisher LSD mean comparison was performed to further explore the differences with significant ($p < 0.05$) ANOVA.

3. Results and Discussion

3.1. Early Chlorophyll Formation in Wheat Is Altered by Nano Mn Application

Chlorophyll content over time was determined by SPAD measurements (counts) as a function of Mn. In the control, SPAD counts over time increased significantly ($p < 0.05$), indicating increasing chlorophyll production as the plants grew (Figure 1). However, SPAD values did not significantly differ from control as a function of time with Mn treatments, except for the decrease in T1 in the presence of nano and bulk Mn (Figure 1).

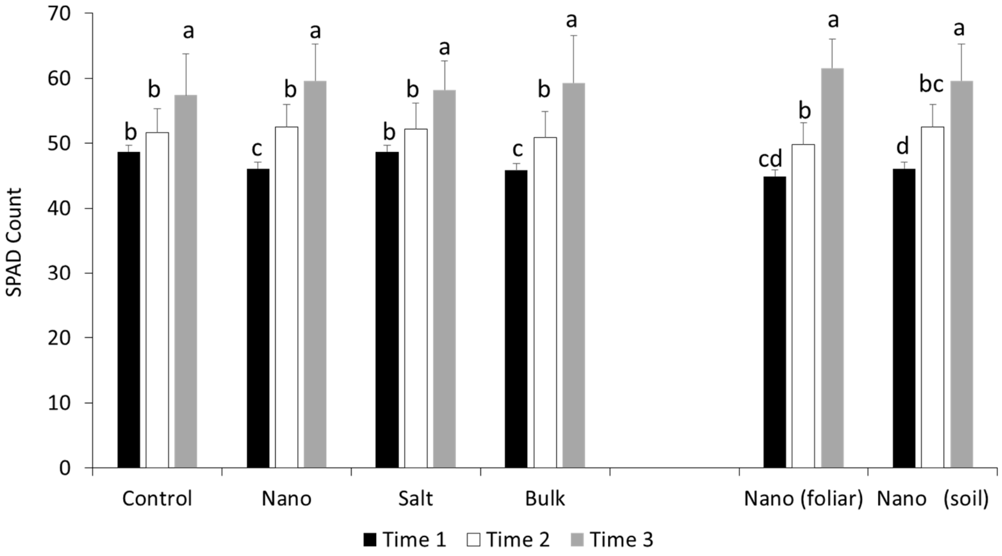

Figure 1. Effects of manganese (Mn) type (nano, salt, and bulk) and exposure route (soil v. foliar) of nano Mn on temporal (Time 1, one week post Mn treatment; Time 2, three weeks post Mn treatment; Time 3, six weeks post Mn treatment) chlorophyll levels in winter wheat leaves. Values are means and standard deviations, and different letters on bars indicate statistically significant differences among the treatments, separately for Mn type and soil versus foliar route ($p < 0.05$; $n = 4$).

The effect of nano Mn on chlorophyll content was similar for the soil versus foliar exposure. An increase in chlorophyll production was reported in prior studies upon treatment of wheat plants with Mn salt [24]; whereas, chlorophyll content was reduced by high Mn exposure (378 mg/L) [25]. Here, we found no effect of a lower Mn exposure (6 mg/kg) on wheat chlorophyll levels, except for an initial reduction by the particulate Mn types. We speculate that this initial lower SPAD values in the nano and bulk Mn is likely related to a slower dynamics of shoot Mn accumulation from these Mn types, which is reflected in the final lower accumulation in the shoot (see below). With regards to soil versus foliar exposure results, it is known that Mn is required in the shoot (leaves) for chlorophyll and photosynthesis functions. It is, thus, not surprising that the nutrient is preferentially distributed to that portion of the plant. Our data suggest that the Mn application route does not appear to be critical for its remobilization to the target tissue.

3.2. Effect of Mn Type on the Vegetative and Reproductive Growth of Wheat

Tiller number of the wheat plants averaged seven across all treatments during early development. However, tiller numbers increased with time up to anthesis (Table 1). The presence of Mn tended to increase tiller number at anthesis, although the effect was not significant (Table 1). Similarly, end-point plant height (cm) was not significantly affected by Mn. Root and shoot dry matter yields were also not significantly different among the treatments. Grain dry yield also was not significantly affected by Mn treatments (Table 1).

Table 1. Effects of Mn type (nano, salt, and bulk) and exposure route (soil v. foliar) of nano Mn on vegetative and reproductive growth of wheat. Values are means and standard deviations (n = 4; NS = not significant).

Treatment	Tiller Number (Anthesis)	Plant Height	Root Dry Weight (g/Plant)	Shoot Dry Weight (g/Plant)	Grain Yield (g/Plant)
Control	36 ± 5	98 ± 5	11 ± 6	84 ± 7	32 ± 7
Nano	41 ± 6	95 ± 4	11 ± 4	81 ± 4	37 ± 8
Salt	43 ± 8	96 ± 6	10 ± 3	82 ± 7	35 ±13
Bulk	38 ± 5	98 ± 6	10 ± 3	83 ± 4	36 ± 9
Nano (foliar)	39 ± 5	97 ± 7	8 ± 4	84 ± 5	39 ± 7
ANOVA	NS	NS	NS	NS	NS

Prior reports on the effects of Mn fertilization on wheat involving mostly the salt form have shown mixed outcomes, dependent on cultivar. For instance, Nayyar et al. [26] reported a marked grain yield increase upon exposure of wheat cultivar WL to 10–40 kg/ha (5–20 mg/kg) Mn via soil (pH 8.8); the rate of yield increase ranged between 48% and 78%, but was not dose-dependent. However, in a concurrent study on soil with pH 8.4, these authors reported significant and dose-dependent grain yield increases (31–112%) upon exposure of wheat to Mn (10–50 kg/ha). Other studies have also demonstrated an increased wheat grain yield with Mn amendment [27,28]. Prior comparative evaluation of soil versus foliar Mn application while using a salt form (5–50 kg/ha, for soil; and and 1%, for foliar in 3 applications) show increased grain yield over the control, but demonstrated marked differences between soil and foliar treatment strategies. Notably, 1% Mn applied three times as a foliar spray, amounting roughly to 3 kg/ha Mn, was superior to a 20 kg/ha soil application. Only when the soil Mn dose was increased to 40 kg/ha was the grain yield response comparable to foliar application [26]. However, in other studies, a 0.5% Mn salt foliar fertilization of wheat did not significantly increase grain yield of winter wheat variety Boomer or Harmoon [29,30]. Conversely, a 500 mg/L Mn foliar amendment increased the grain yield of wheat cultivar Giza 168 by 11% [31]. Also, late (pre anthesis) foliar fertilization with Mn under drought stress promoted grain yield of wheat cultivar Kenong 9204 by 10% under greenhouse conditions, but it did not affect yield under field conditions [32]. Thus, it would appear that cultivar effects are involved in wheat response to Mn treatment.

Reports on wheat growth or grain yield modulation by nanoscale Mn are scarce. One study described the use of a proprietary nanoscale formulation composed of Mn, Fe, Cu, and Zn that increased the yield of several winter wheat cultivars by between 12% and 29% [33]. Notably, the specific role of nano Mn in the process was not distinguished in the study, due to the product being a composite formulation. However, in other crops, the effects of nano Mn on plant vegetative or reproductive development have been reported. For instance, the root and shoot growth of mung bean was improved by treatment with both nano Mn (0.05–1.0 mg/L) and Mn salt (0.05–0.1 mg/L); interestingly, higher doses of the Mn salt (0.5–1.0 mg/L) inhibited root and shoot growth [3]. Liu et al. [17] showed a dose and Mn type-dependent effect on root growth regulation in lettuce. No effect or marginal growth promotion was observed with nano Mn at 1 to 50 mg/L range. However, bulk Mn at 50 mg/L inhibited root growth, and a strong reduction in root growth was caused by Mn salt at 20 and 50 mg/L. Similarly, root growth of mustard was unaffected by nanowire Mn, bulk Mn and Mn salt at 10–100 mg/L. However, while nanowire Mn had no effect at a higher dose of 1000 mg/L, bulk and ionic Mn significantly reduced root growth by 68% and 13% at that treatment level, respectively [16]. Notably, these vegetative studies were conducted in non-soil systems and at short exposure durations; outcomes could certainly vary for longer duration exposures in soil [11]. In a soil-based study, foliar exposure to nano Mn (0.1–1 mg/L) under *Fusarium* disease stress in soil increased tomato fruit yield by 6.2%, when compared to bulk Mn, which increased fruit yield by 31% and Mn salt, which had no effect. In contrast, eggplant fruit yield was increased 22% by nano Mn, reduced 6.6% by bulk Mn and unaffected by

Mn salt when compared to control plants [15]. In other studies, nano Mn (500 mg/L) increased watermelon fruit yield by about 24 and 17.5%, dependent on whether the soil was infested or not with soil-borne disease pathogens. However, the nano form was about 6% less effective in promoting biomass yield than the bulk form under disease infestation [18]. In the current study, the obtained yield increases, though statistically insignificant (Table 1), show that Mn treatment in soil as nano, salt, or bulk tended to increase grain yield, by 16%, 9%, or 13%, respectively, whereas foliar application of nano Mn increased grain yield by 22%. Furthermore, nano Mn resulted in 5% greater grain yield when applied as a foliar treatment compared to soil application. These findings indicate that nano Mn may be more effective than other Mn forms in enhancing wheat grain yield. Also, when compared to soil application, the foliar application of nano Mn may be more effective for increasing grain yield. This outcome is not necessarily surprising, given that for Mn the target tissue physiologically is the shoot system. We speculate that the lack of a stronger effect on yield than was observed might have been due to the relatively high initial level of native bioavailable Mn in the soil, which the slight change in pH (see below) during plant growth did little to influence.

3.3. Shoot Uptake of Manganese Is Inhibited by Soil-Applied Nano Mn

Mn acquired by the plants was strongly partitioned into the shoot system relative to that in the root and grain (Figure 2). There was no effect of Mn application on root Mn concentration, regardless of Mn type. In contrast, shoot Mn concentration was significantly decreased by nano Mn; reduced level was also suggested with the bulk Mn, although the effect was statistically insignificant. Grain Mn concentration was unaffected by nano and bulk Mn, but it was significantly increased by salt Mn (Figure 2). Foliar application of nano Mn strongly influenced Mn levels in shoot and grain, relative to soil application (Figure 2).

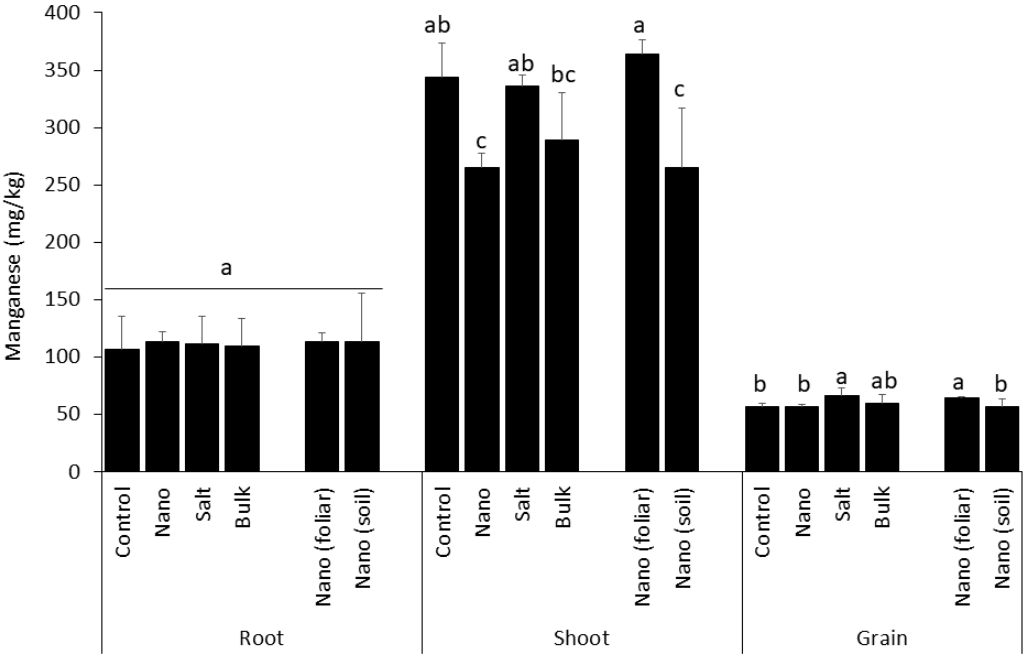

Figure 2. Effects of Mn type (nano, salt, and bulk) and exposure route (soil v. foliar) of nano Mn on manganese concentration in root, shoot, and grain of winter wheat. Values are means and standard deviations, and different letters on bars indicate statistically significant differences among the treatments, separately for root, shoot, and grain ($p < 0.05$; $n = 4$).

Across all plant tissues, the Mn products exhibited mixed effects on Mn acquisition by wheat: when compared to the control, slightly more Mn, 6.7% or 1.2%, was bioaccumulated in the plant due to Mn salt or foliar nano treatment, whereas 14% or 9.5% less Mn was bioaccumulated upon exposure to nano or bulk Mn. It should be noted that for the foliar treatment with the largest positive effect, some of the shoot Mn may be adsorbed on the leaf surface from application rather than internalized in the tissues, much the same way that some Mn in the soil treatments might be root-surface adsorbed. The nature or speciation of Mn in the wheat tissues from nano Mn exposure is not known at this time. However, Pradhan et al. [3] previously demonstrated the uptake of intact nano Mn (20 nm particle size) in mung bean root and shoot. We also reported the shoot uptake of intact nano CuO (<50 nm particle size) from a solid matrix by wheat [34,35]. In the case of foliar exposure, observations with other metallic nanoparticles suggest that nano Mn could also be taken up by plants through direct penetration and transport via stomatal openings [36,37]. Moreover, phloem-based translocation of metallic nanoparticles from leaves to other plant parts has also been previously demonstrated [38]. It is, therefore, not inconceivable that some of the 30 nm nano Mn that were used in the current study could have been taken up by wheat from the soil or leaves and redistributed to other plant parts. These aspects are being further investigated. That being said, the fact that the majority of Mn that was acquired by the plant was preferentially partitioned in the shoot is clearly related to its role in photosynthesis [3]. Thus, it was not surprising that reduced chlorophyll content by nano and bulk Mn treatments correlated with reduced shoot Mn content, as observed upon exposure to these products. However, the poor root-to-shoot transfer of nano Mn that was observed in this study does not align with the strong shoot-to-root transfer of foliar nano Mn observed in our previous study with eggplant infested with root fungal pathogen [15]. Taken together, these findings suggest a role for Mn in roots exposed to biotic stress, or differences based on whether the crop was a monocot or dicot.

With respect to the grain, although efficient accumulation of Mn is critical for seed germination and plant establishment [39], poor remobilization of the nutrient to the grain from the shoot has been described [40]. Data from the control treatment supports inefficient remobilization of Mn to the wheat grain; the translocation factor (TF; ratio of grain Mn to shoot Mn) was only 0.16. In this regard, the ability of a specific Mn type or exposure route to influence Mn translocation to the grain might contribute to improving the grain stock towards enhancing seed quality and germination. Here, among the Mn treatments, nano Mn via soil application increased the TF to 0.22, followed closely by bulk Mn at 0.21, and salt Mn at 0.2. Thus, even though salt Mn had the highest absolute grain Mn content, its TF was equivalent to that of nano Mn. In contrast to soil nano Mn, foliar nano Mn application had a TF of 0.18.

Barman et al. [41] investigated Mn tolerance in 47 genetically diverse wheat cultivars that are based on the use of salt Mn. They reported a mean total Mn accumulation in shoot under Mn sufficient (3.3 mg/L) and deficient (no added Mn) conditions to be 44 and 26 mg/kg, respectively. Notably, the investigation was conducted in a hydroponic nutrient solution, typically with more predictable Mn behavior than in soil. In contrast, in the present study, the total above-ground Mn accumulation is about 400 mg/kg, based on the control treatment. It would, thus, appear that the potentially available Mn in this soil was high, in spite of the initial soil DTPA analysis indicating an amount that was close to the critical threshold. The manifestation of Mn toxicity in different crops may occur when shoot Mn content ranges from 176 to 3500 mg/kg [2]. For wheat, this value is reported at 720 mg/kg [5]. Since the particulate Mn types, especially nano Mn, lowered the shoot Mn content by 16–23%, it can be argued that particulate Mn types may reduce the potentially toxic accumulation of Mn under high soil Mn levels. This property is likely due to their tendency to transform into other less bioavailable forms, such as homo aggregation or hetero aggregation/precipitation into insoluble complexes with other soil constituents, such as phosphate [42]. Indeed, a role for P in inhibiting Mn accumulation in wheat has been noted [43], which may be applicable in the present study, given the relatively high level of added P in the soil.

3.4. Accumulation of Nitrogen in Shoot and Grain Is Differently Affected by Mn Type

Not surprisingly, more N was present in wheat grain than in the shoot and root tissues (Figure 3). This indicates a high root/shoot to grain translocation of N and preferential partitioning in the grain. Exposure to different Mn types influenced N accumulation by wheat, but did depend on the plant tissue. For the roots, there were no significant differences in N concentration across the Mn treatments. For the shoots, N content was significantly reduced by all Mn treatments as compared to the control. Between the Mn types, there were no statistical differences between nano and salt Mn, and between nano and bulk Mn. However, the bulk Mn treated plants, which had the lowest level of N, differed significantly when compared to plants treated with salt Mn.

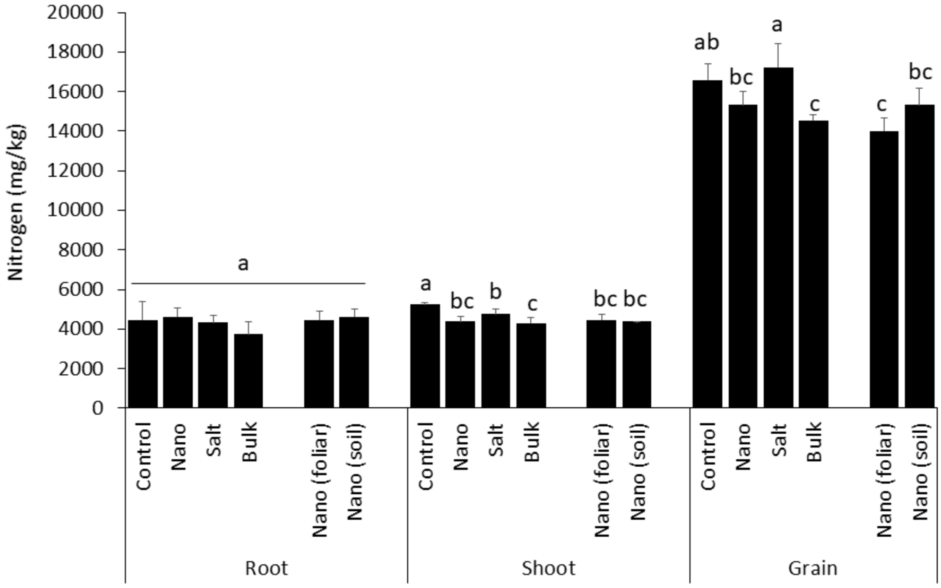

Figure 3. Effects of Mn type (nano, salt, and bulk) and exposure route (soil v. foliar) of nano Mn on nitrogen concentration in root, shoot, and grain of winter wheat. Values are means and standard deviations, and different letters on bars indicate statistically significant differences among the treatments, separately for root, shoot, and grain ($p < 0.05$; $n = 4$).

Translocation of N from shoot to grain was also differently influenced by Mn. There was an insignificant reduction in grain N concentration due to nano Mn application; salt Mn did not affect grain N concentration relative to the control. However, bulk Mn caused a significant reduction in N concentration relative to the control and salt Mn treatments (Figure 3). Regarding the different exposure routes, no significant difference was observed in N concentration in the root, shoot, or grain between soil and foliar applied Mn, although soil application resulted in 9% greater translocation of N in the grain than did foliar application (Figure 3). Collectively, Mn fertilization lowered the above-ground (shoot and grain) N accumulation by 10%, 14%, and 15% when presented to the plant as soil applied nanoparticles, bulk particles, and foliar applied nanoparticles, respectively. In contrast, the above-ground effect of salt Mn on N accumulation was a negligible 0.8%. Contrary to our finding, significant increases in the accumulation of N in wheat shoot upon soil or foliar exposure to salt Mn fertilizer have been reported previously [31,44,45]. These increases correlated with increases in shoot Mn levels. However, nano Mn has been shown to reduce shoot concentration of nitrate-N in mung bean [4]. Studies with other metallic nanoparticles suggest contrasting effects on N-related processes at the biochemical level. Rawat et al. [46] described the potential induction of N assimilation in Brassica, based on the activation of glutamate synthase gene by FeS nanoparticles. In contrast, $Cu(OH)_2$ nanoparticles negatively affected several molecular pathways that are related to N metabolism, including glutamate [47].

To the best of our knowledge, the present study is the first demonstration of a reduction in N accumulation in wheat shoot by nano Mn, wherein the reduction in shoot N correlated with reduced shoot Mn. Taken together, these findings indicate that an impediment or reduction in shoot Mn may inhibit N acquisition by wheat plants. The consistent reduction of shoot N by all Mn types in the present study suggests a similarity in the mechanism of action of the Mn types on N uptake into shoot, likely through the accumulation of dissolved ions. However, the fact that the salt Mn treatment showed higher shoot and grain Mn levels, but the lowest inhibition of shoot N accumulation than nano and bulk Mn indicates a particle effect that appears to be related to slow particle dissolution rate, relative to the salt Mn. We posit that the concomitant reduction of Mn and N content in the plant shoot may have implications for both N and Mn roles in photosynthesis, as well as Mn role in N metabolism by crops that were exposed to nanoparticulate Mn in the environment [25]. The lowering of shoot N by particulate Mn was reflected in grain N translocation. However, the slight increase in grain N over the control with Mn salt agrees with grain N improvement in wheat by ionic Mn, as previously reported [31,44]. Taken together, there clearly is a size-independent, particle-specific effect of Mn for reducing N accumulation in the shoots and grains of crops that should be further investigated. However, soil or foliar application route, as shown with nano Mn, played little, if any role, in these effects.

3.5. Shoot Uptake of Phosphorus Is Inhibited by Soil Application of Mn

As with N, P was preferentially partitioned into the grain as compared to the shoots and roots of the wheat plants, but shoots contained more P (140%) than the roots, based on the control plants (Figure 4).

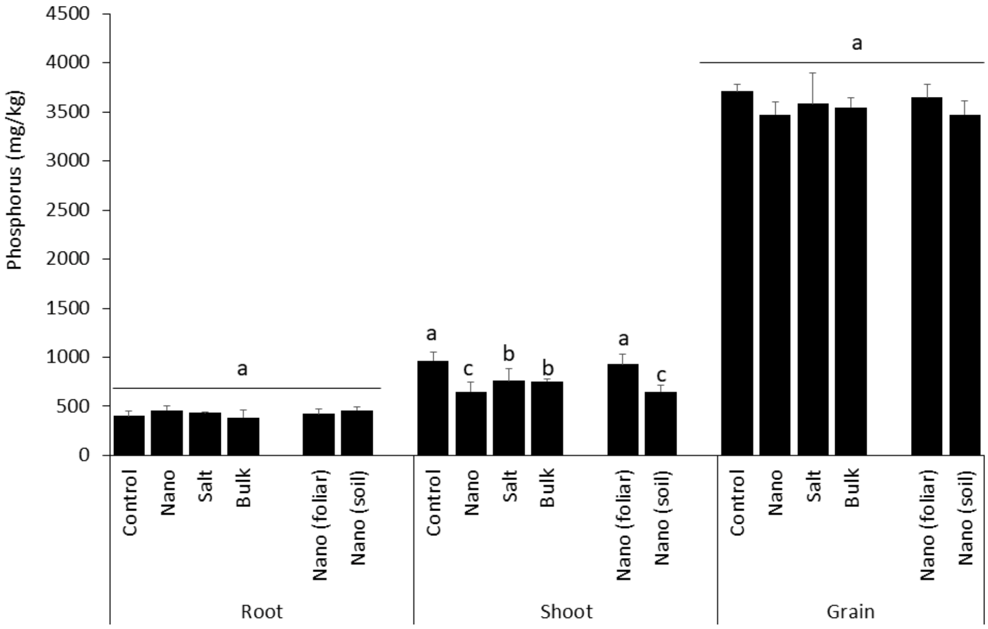

Figure 4. Effects of Mn type (nano, salt, and bulk) and exposure route (soil v. foliar) of nano Mn on phosphorus concentrations in root, shoot, and grain of winter wheat. Values are means and standard deviations, and different letters on bars indicate statistically significant differences among the treatments, separately for root, shoot, and grain ($p < 0.05$; $n = 4$).

Mn exposure did not significantly affect root and grain P concentrations, despite showing a trend for reducing P levels in the grain. In the case of nano Mn, this lack of effect occurred for both soil or foliar application routes; a 5% increase in P translocation was evident for the foliar application compared to soil-applied Mn. Conversely, shoot P concentrations were significantly reduced by all soil-applied Mn, relative to the control; the greatest reduction was with nano Mn (32%), followed by salt (22%), and then bulk Mn (21%). However, foliar treatment with nano Mn did not affect shoot P relative to control, but did significantly increase P uptake in the shoot relative to the soil route (Figure 4). When considering total above-ground P content, uptake from the root was reduced 12%, 7%, 8%, and 2% by soil applied nano Mn (soil applied), salt Mn, bulk Mn, and foliar-applied nano Mn, respectively. The relationship between Mn and P regarding plant productivity and nutrient acquisition can be both positive and antagonistic, dependent on the amount of P in the soil [48,49]. A previous report indicated significant shoot and grain P increase in wheat that was exposed to Mn salt through foliar application [31]. In the current study, although grain P translocation was efficient and unaffected by Mn, P uptake into the shoot was strongly affected by Mn in the soil. In contrast, P uptake was unaffected by Mn as a foliar treatment Also, Mn treatment reduced P accumulation in bean shoots, but not in corn, suggesting species-specificity as a factor [50]. Inhibition of P uptake by metallic nutrients under high P exposure has been reported for Zn in other crops, and it appears to be related to the formation of insoluble Zn phosphate [19,21]. The present data also highlights a strong inhibition of P uptake in wheat by different Mn types; however, in the case of nano Mn, this effect can be avoided by using a foliar route as an appropriate delivery strategy.

3.6. Above-Ground Accumulation of Potassium Is Inhibited by Soil Applied Nano Mn

Contrary to observations with N and P, overall tissue K partitioning was greater in the shoot than in the root or grain; however, grains contained more K (193%) than roots (Figure 5). Exposure of the plants to different Mn sources did not strongly affect root K concentration. However, shoot uptake of K was significantly reduced by nano Mn when compared to control plants, but not by salt or bulk Mn. Nano Mn reduced shoot K concentration by 11% as compared to other Mn sources. When compared to the control, shoot to grain K translocation was inhibited by all Mn sources, and in the case of nano Mn, by each application route. The TF in the control treatment was 0.32, implying moderate translocation efficiency; Mn exposure slightly reduced TF values, with an average of 0.29. In all tissues, exposure route of nano Mn did not significantly affect K content of the plants (Figure 5).

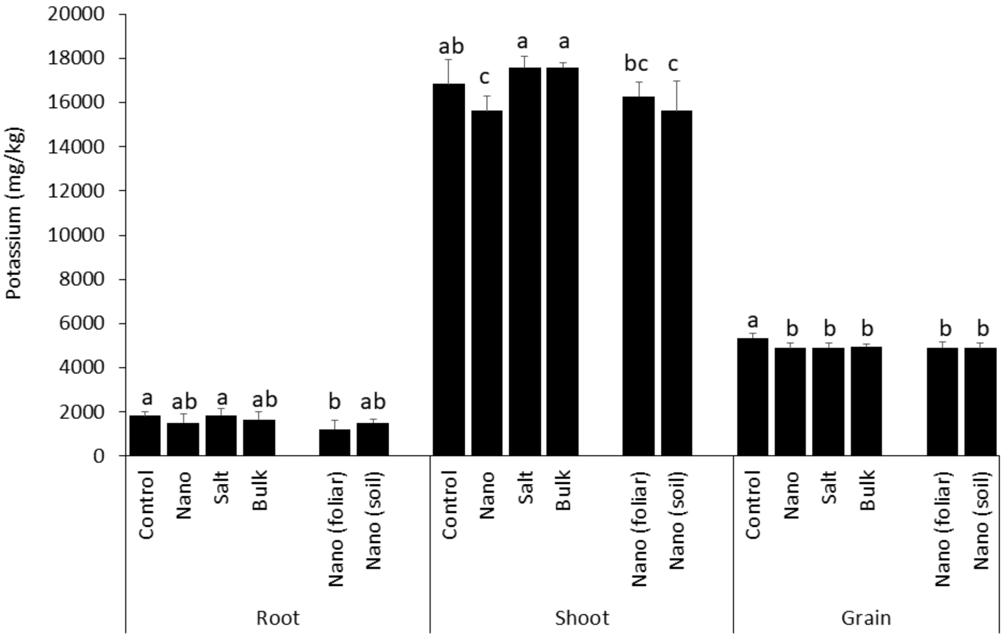

Figure 5. Effects of Mn type (nano, salt, and bulk) and exposure route (soil v. foliar) of nano Mn on potassium concentrations in root, shoot, and grain of winter wheat. Values are means and standard deviations, and different letters on bars indicate statistically significant differences among the treatments, separately for root, shoot, and grain ($p < 0.05$; $n = 4$).

Taken together, total above-ground K accumulation changed only marginally: 1.4% and 1.6% increases with exposure to salt and bulk particle Mn, respectively, and 7.6% or 4.5% decreases with soil or foliar-exposure to nano Mn. Reported Mn and K interactions show mixed outcomes for nutrient acquisition in plants [49]. In rice, Mn salt treatment caused dose-dependent antagonism against K accumulation in the shoots [51]. However, in wheat, Mn salt foliar application had no effect on shoot K levels, although the grain K content was increased [31]. Also, a significant increase in K accumulation in bean shoots from Mn salt-treated soil was reported [50]. In the current study, only nano Mn inhibited K shoot uptake, suggesting a Mn nano-specific effect on root-to-shoot transport of K. Once in the root, Mn will be a divalent cation and it will outcompete K for uptake, somewhat regardless of their respective levels in soil [52]. In the current study ratio of applied K to Mn = 33:1). Moreover, the slower dissolution of nanoscale elements compared to ionic forms implies continuous availability of soluble Mn [6], further skewing the competition between K and Mn in favor of the later. In terms of seed quality, as with most grain crops, wheat grain is generally low in K. This is attested to by the limited grain translocation efficiency of K in the absence of Mn in this study. Thus, the overall Mn-induced reduction of grain K content that was observed in the study could further complicate the nutritional quality of wheat grains.

3.7. Soil Residual Levels of N, P, and Mn Are Differently Affected by Mn Treatment

The initial Mn level of 6.4 mg/kg in the soil (pH 6.87) suggests that Mn bioavailability to plants would be intermediate, relative to a more acidic or alkaline soil. Moreover, that soil Mn level is just above the critical level of 1–5 mg Mn/kg indicated in the literature for wheat [5,26]. This suggests that wheat growing in this soil could become susceptible to Mn response, dependent on the extent of soil pH changes that might occur during plant growth. After plant harvest, the soil pH and residual levels of the added nutrients (i.e., N, P, K, and Mn) were assessed to determine whether Mn exposure altered amounts in soil during plant growth. Table 2 indicates that the post-harvest soil was acidified to 6.11 from the original level of 6.87 at pre-planting. However, Mn treatment had little impact on soil pH. The lower, but similar, pHs from the soil with the control and the different Mn treatments indicated plant factors, most likely organic acids from root exudates or associated microbial activity, rather than Mn exposure, contributed to soil acidification. However, since the N-fertilizer source that was used in the study was ammonium nitrate, soil acidification could also have been influenced by ammonium metabolism [53]. Indeed, the chemistry of Mn in alkaline soils with poor bioavailability is complex. Whereas, Mn solubility should increase strongly for every unit of pH decrease, the presence of various inorganic and organic compounds capable of complexing Mn and changing solubility may influence final Mn availability, independent of any pH change [7,54].

Table 2. Post-harvest pH and residual levels (mg/kg) of the added nutrients in soil exposed to nano, salt and bulk Mn. Values are means, and different letters associated with values indicate statistically significant differences among the treatments, separately for pH and each nutrient ($p < 0.05$; $n = 4$).

Treatment	pH	Mn	Ammonium-N	Nitrate-N	P	K
Pre-growth	6.87	12.4				
Post-harvest						
Control	6.11 a	4.65 b	4.99 a	1.33 ab	8.40 d	177 a
Nano	6.12 a	5.62 b	5.45 a	1.73 a	10.40 c	184 a
Salt	6.13 a	6.16 a	5.03 a	1.01 b	12.20 b	174 a
Bulk	6.19 a	4.61 b	4.31 a	1.06 b	16.70 a	173 a
Nano (foliar)	6.26 a	4.63 b	4.70 a	1.03 b	12.20 b	166 a

Fertilization with Mn did not strongly influence soil residual Mn levels, except for the salt Mn, which showed a significantly higher (10–34%) residual Mn, when compared to other treatments. Under the acidified condition of the soil, the overall Mn release from insoluble Mn fractions would be modified; and, additional Mn should theoretically be mobilized from such nonbioavailable fractions. This is the likely explanation for why the above-ground tissue Mn level in the control treatment was high, relative to reported averages under agriculturally relevant Mn application regimes [5,41], and the plants appear to minimize their uptake of additional Mn from the added products, especially nano and bulk Mn. Mn_2O_3 nanoparticles have been shown to be relatively soluble in aqueous suspensions, with up to 35% solubilization recorded, on average [22]. In the current study, it is difficult to accurately estimate the solubility of the added nano Mn in the soil due to the presence of native Mn at a high level. However, based on Table 2, at least 45% of the added nano Mn would have been recovered as dissolved Mn. Nevertheless, in soil, Mn dissolving from Mn-oxides or provided in soluble salts could re-associate with aggregates in soil, thereby reducing the pool of Mn ions available for plant uptake from those sources. In general, bulk oxide particles would dissolve slower than nanoparticles, as also suggested in Table 2 for nano versus bulk Mn; thus, fewer ions would be available from bulk particles for DTPA chelation. Moreover, DTPA is an ion-specific detection method; hence, more soil residual Mn was detected from the salt Mn treatment. DTPA could have obfuscated the total Mn levels from nano and bulk Mn due to it not detecting undissolved Mn fractions at the time of measurement. Future studies on this subject will involve the fractionation of the soil to determine the distribution of Mn ions from Mn nanoparticles in solution, or in various other exchangeable, organically complexed, and oxide bound forms, under differing soil pH conditions.

The residual N level was assessed as a function of the applied N-fertilizer type, namely ammonium-N and nitrate-N. Nano Mn tended to increase the levels of both forms, albeit insignificantly when compared with the control treatment. The effect of soil-applied nano Mn on nitrate-N was significant relative to other Mn forms (Table 2). The involvement of Mn oxides in influencing N transformation (nitrification-denitrification) in cropping systems has been reported. The findings indicated that, depending on soil/cropping conditions, Mn oxides may reduce both nitrate formation and nitrous oxide emission [55]. Accordingly, given the somewhat higher residual nitrate-N in the soil with nano Mn, it seems that nano Mn interfered with the transformation rate of nitrate-N, likely through denitrification inhibition in the wheat rhizosphere.

Post-harvest residual P levels were significantly increased by Mn treatment, with bulk Mn exerting the greatest effect (Table 2). The interaction of P and Mn in the present study can be characterized as antagonistic with respect to uptake upon exposure in soil. The wheat plants were fertilized with 75 mg/kg P, an amount that is at least 12 times greater than the amount of Mn that was used in the study. Therefore, the probability is high for the formation of manganese phosphate, a highly insoluble complex that could retard either P or Mn uptake by the plant [43]. Accordingly, more (24 to 99%) residual P was contained in the soil with Mn, dependent both on Mn type and exposure route. However, the fact that foliar applied Mn also yielded greater residual P in soil indicates that negative Mn and P interaction may also occur at the physiological level, unrelated to soil processes. Indeed, Pedas et al. [48] showed P inhibition of Mn uptake that was independent of P-Mn co-precipitation in soil, which they attributed to a direct effect of P on Mn accumulation, due, perhaps, to a co-mediated mechanism of P-Mn transport. In contrast to N and P, the residual K level in soil was generally unaffected by Mn treatment. The overall high level of K in the soil may be involved in limiting any influence of Mn on residual K level.

4. Conclusions

In this study, we have demonstrated the effects of nano Mn on wheat in a near-neutral soil at an exposure level that is realistic in cropping systems. At this exposure level, no indication of apparent toxicity was observed in the crop. Rather, a modest promotion of grain yield was noted that was largely similar to that caused by bulk or ionic Mn. The lack of stronger effect in this regard might be due to the original soil Mn level being above the critical level for wheat. However, it is also plausible that the limited change in soil pH, which otherwise would cause dramatic effects on Mn solubility, might have contributed to moderating the effects on vegetative and reproductive yields. Importantly, whereas all

Mn types reduced N accumulation in wheat shoots, nano Mn exhibited other subtle effects on nutrient acquisition that were not observed for the ionic or bulk Mn: reduced shoot Mn accumulation, reduced shoot P and K uptake, and increased residual nitrate-N in soil. However, despite lowering Mn shoot accumulation, nano Mn increased the efficiency of grain Mn translocation to a greater degree than other Mn types. Viewed broadly, despite no inhibition of the vegetative and reproductive development of wheat by nano Mn, which is in agreement with our previous findings with tomato, watermelon, and eggplant, the subtle outcomes on nutrient acquisition suggest caution in the use of nano-scale Mn as nanofertilizers or nanopesticides. These effects are likely related to (i) higher reactivity of nano Mn versus bulk Mn; and, (ii) slower and continuous availability of soluble Mn from nano Mn, compared to salt Mn. Alternatively, intact Mn nanoparticles could have accumulated in the plant tissues from the root, where they evoke these nano-specific effects. Notably, foliar application of nano Mn showed clear differences with soil application: higher shoot and grain Mn contents, lower soil nitrate-N, and higher soil and shoot P. Thus, applying nano Mn via foliar may be a strategy to modulate its subtle effects in soil.

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