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Review

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CuO and ZnO Nanoparticles Modify Interkingdom Cell Signaling Processes Relevant to Crop Production

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ABSTRACT: As the world population increases, strategies for sustainable agriculture are needed to fulfill the global need for plants for food and other commercial products. Nanoparticle formulations are likely to be part of the developing strategies. CuO and ZnO nanoparticles (NPs) offer potential as fertilizers, as they provide bioavailable essential metals, and as pesticides, because of dose-dependent toxicity. Effects of these metal oxide NPs on rhizosphere functions are the focus of this review. These NPs at doses of ≥10 mg metal/kg change the production of key metabolites involved in plant protection in a root-associated microbe, *Pseudomonas chlororaphis* O6. Altered synthesis occurs in the microbe for phenazines, which function in plant resistance to pathogens, the pyoverdine-like siderophore that enhances Fe bioavailability in the rhizosphere and indole-3-acetic acid affecting plant growth. In wheat seedlings, reprogramming of root morphology involves increases in root hair proliferation (CuO NPs) and lateral root formation (ZnO NPs). Systemic changes in wheat shoot gene expression point to altered regulation for metal stress resilience as well as the potential for enhanced survival under stress commonly encountered in the field. These responses to the NPs cross kingdoms involving the bacteria, fungi, and plants in the rhizosphere. Our challenge is to learn how to understand the value of these potential changes and successfully formulate the NPs for optimal activity in the rhizosphere of crop plants. These formulations may be integrated into developing practices to ensure the sustainability of crop production.

KEYWORDS: *root microbiome, metabolites, acyl homoserine lactones, siderophore, phenazine, Pseudomonas chlororaphis O6, indole-3-acetic acid, drought stress*

1. INTRODUCTION

1.1. Overview. This review focuses on the impact of nanoproducts on key processes in the plant's rhizosphere affecting plant health. The effects are observed both on the plant and as a common type of bacterium that colonizes the root surface. Basic laboratory studies at the cellular, biochemical, and transcriptome levels show that CuO and ZnO nanoparticles (NPs) have an impact on metabolites acting as signals in the communication between the plant and the bacterial cells for functions in the rhizosphere. The work illustrates the need for the development of nanoproducts for agriculture to consider not only plant responses to NPs but also the essential role played by the plant's microbiome in plant productivity.

1.2. Potential Uses of Metal Oxide NPs in Agriculture. Nanosized materials will be engineered and formulated for agricultural use, although currently commercialization is in its infancy. Yield and quality of crops are being challenged by the growing human population, shrinking prime agricultural land and irrigation water, and weather instability. Additionally, there are problems associated with accumulation and runoff from applications of the conventional commercial pesticides used in agriculture, industry, and domestic settings. Many of the filed patents feature modification to nanosize particles of materials with proven efficacy as bulk products as fertilizers or pesticides. Nanomaterials also offer potential as delivery vehicles and as sensors for key processes in crop production.[1−6] Entry of NPs into agricultural soils could also occur from purposely applied sludges or by accident, such as agricultural use of polluted water.[7,8] A recent review indicated that sludge contained "vast stores of metal-containing nanoparticles".[8] Additionally, trophic transfer of NPs through natural food chains demonstrates that their applications to soils and plants extends exposure to multiple organisms from different kingdoms.[9−11] As with applications of all chemicals in agriculture, a balance must be obtained between positive and negative consequences for the nanoagricultural formulations. Aspects of potential risks of the application of nanoproducts are extensively reviewed.[12−16]

NPs containing essential metals, such as Fe, Mg, Zn, Cu, and Mn, are proposed as fertilizers at low doses and as pesticides at higher doses[17−20] because these metals are vital for cellular function yet toxic above a threshold.[21,22] Fertilization is important for soils in which the bioavailability of essential metals is limited. Restricted availability could be due to lack of metal-containing minerals or the removal of the metal resources

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through continuous cropping of the soil. Also, the metals could be present but held in the soil in non-bioavailable forms as minerals or with organic matter.[23] Worldwide there are many soils producing plants deficient in Zn, leading to low Zn nutrition when they are used as foods.[24−26] Zn deficiency is present in 30% of the world's population and impairs growth, reproduction, nervous system development, and the immune system.[25,26] Cu-deficient soils occur less frequently worldwide but are present[27] in part due to low bioavailability because of mineral complexation[23] and the strong association of Cu with organic matter.[28,29] Symptoms of Cu stress occur on plants grown on the deficient soils such as wither tip of cereals.[27]

The potential use as fertilizers for metal oxide NPs stems from increased metal accumulation in plants grown with the NPs in different matrices, including field soils[30−33] (Figure 1).

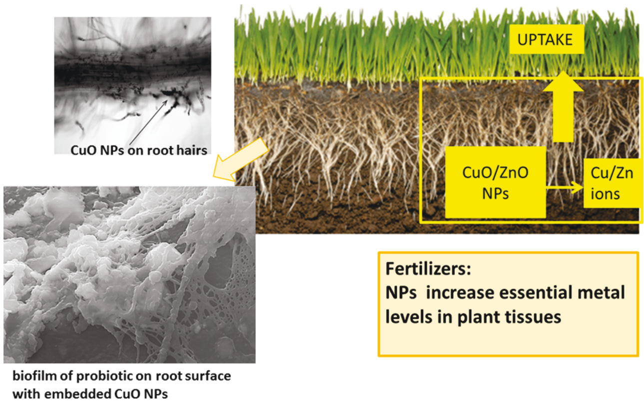

Figure 1. Fertilizer potential of CuO and ZnO NPs. The essential metals, Cu and Zn, are bioavailable to the plant from their metal oxide NPs through processes in the rhizosphere influenced by soil properties, the root, and its microbiome. Insets show the biofilm that forms over a wheat root surface by cells of a probiotic, *Pseudomonas chlororaphis* O6; NPs are embedded within the biofilm (SEM large image). In the absence of root colonization, association of CuO NPs with root hairs on the wheat seedling root is revealed (light microscopy small image).

Increased metal is due to both the plant response to the NPs and the metal released by dissolution in the rhizosphere or in planta. Uptake of intact NPs into plants is observed, but with high variability between plant species. For instance, no ZnO NPs are detected in cells of soybean roots,[34] whereas aggregates of ZnO NPs are detected in maize xylem.[35] CuO NPs applications to maize roots result in transport of the NPs into the leaves and then redistribution back to the roots.[36] Translocation of NPs into shoots also occurs in rice grown with CuO NPs, although the Casparian strip acts as a physical barrier.[32] Although magnetite NPs are not transported into wheat shoots,[37] uptake and transport of iron oxide NPs occur in pumpkin.[38] Intracellular trafficking is reported. ZnO NPs are internalized by root cells of a wetland plant,[39] and TiO_2 NPs enter maize root cells.[40] The degree of bioaccumulation of metal from CuO NPs is influenced by other factors such as weathering of the NPs.[41] These variabilities illustrate the complexity of predicting the efficacy of the NPs in agricultural settings that are discussed in more detail in this review.

At higher doses than those suitable for plant fertilization, toxic effects of Zn and Cu on cellular functions raise the possibility of their use as pesticides where pathogenic fungi and bacteria could be targeted. For instance, inhibited growth of plant pathogenic fungi and oomycetes is demonstrated with dose-dependent effects (e.g., >100 mg metal/L).[42−46] Early and current work assessing toxic effects of NPs on bacteria focus on their potential as an alternative control strategy for human pathogenic bacteria, a strategy needed because of the development of resistance to traditional antibiotics.[47] Initial responses in cell toxicity may involve binding to cell walls.[48] Changes to membrane potentials[49] and disturbance of the redox balance within a cell contribute to toxicity. The ability of the Cu ion to exist in two redox states catalyzes formation of superoxide anion due to reduction of molecular oxygen. Also, both Zn and Cu interact with −SH groups, such as those found in proteins and a key intracellular antioxidant, glutathione. Inactivation of enzyme activity and depletion of glutathione contribute to cellular stress from accumulated reactive oxygen species (ROS).[50,51] Indeed, a review of the impact of metal oxide NPs on plants focuses on detoxification of ROS as an important survival mechanism.[13]

The positive value of NPs in agriculture is tempered by the need to predict negative effects, such as phytotoxicity and deleterious effects on essential soil microbe functions.[12,16] Understanding at the molecular level of the basics involved in variability between responses with different NPs and plants and soils plus their microbiomes (Figure 2) will promote confident use of nanoagricultural products. However, currently such studies at these levels are limited.[52]

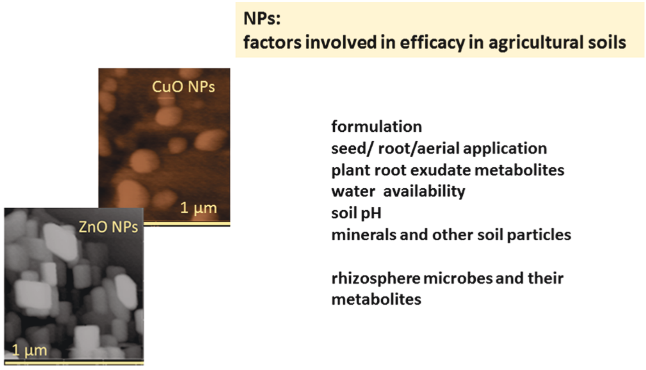

Figure 2. Metal oxide NP uses in agriculture and factors that will need to be addressed to optimize their efficacy in the rhizosphere for agricultural applications.

This review addresses, in a model system, cellular, biochemical, and transcriptome changes induced through the presence of CuO and ZnO NPs in the rhizosphere on both the plant and root-colonizing bacterial cells. The work described has been performed under defined laboratory conditions in shake and agar plate culture for the microbial studies and in growth boxes with sand or with soils for the plant studies. We do not know whether the responses observed with sublethal levels of CuO and ZnO NPs occur in agricultural field native soils. Rather, we review the findings to promote awareness of potential changes in plant−microbe signaling that are set in place with exposure of roots and their associated microbial cells to the NPs.

1.3. Root Microbiome. This review highlights that plants under field conditions grow in association with microbes. This fact contrasts to the many plant−NP studies in which the role of a microbiome was not considered. Certain microbial plant colonizers exert changes in the plant that parallel those observed with microbes termed probiotics, which are essential

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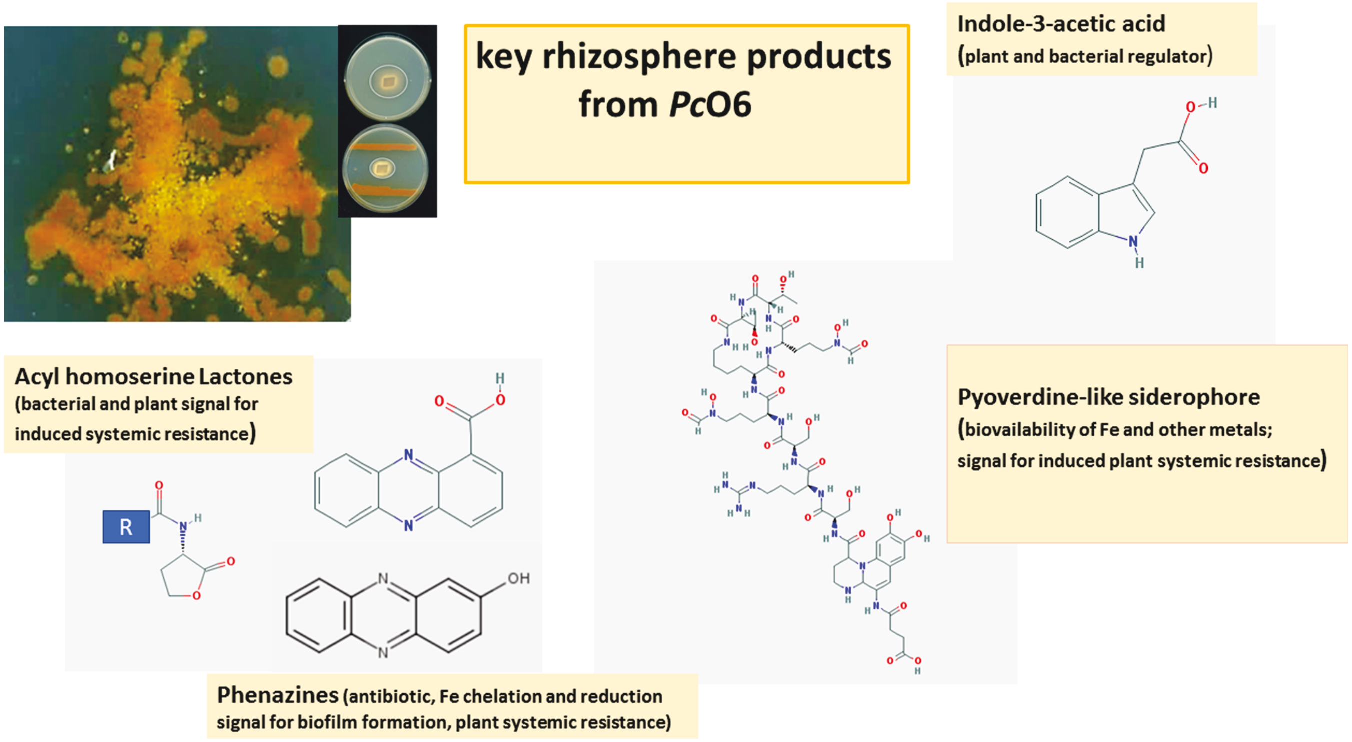

Figure 3. Key rhizosphere metabolites produced by the probiotic root colonizer, *Pseudomonas chlororaphis* O6 (*Pc*O6). The metabolites include acyl homoserine lactones (AHSLs) that are inter- and intracellular cross-kingdom signaling compounds. In *Pc*O6 they stimulate production of antifungal metabolites, a family of phenazines that are multitasked in promoting plant health. The pyoverdine-like siderophores increase rhizospheric iron bioavailability to the microbe and plant. The plant growth regulator indole-3-acetic acid is produced from tryptophan by *Pc*O6. AHSLs, phenazines, and siderophores also prime plant defense mechanisms. Growth on plate medium of orange-colored *Pc* O6 cells from the biofilms coating the roots of a colonized wheat seedling is shown (large image) and on medium where secreted products inhibit the radial growth of *Fusarium proliferatum* mycelia (small image). Sublethal doses of CuO and/or ZnO NPs modify the production of each of the shown metabolites.

for human health.[53] Outcomes for the plant from colonization with the probiotic-like microbes include enhanced growth, changes in morphology, antagonism of microbial pathogens, stimulation of defense mechanisms against environmental stress, and improved nutrition. Biofortification of soils with probiotic-like microbes will likely be an important aspect of the practices employed for sustainable agriculture.[54−57] Currently there are several commercial formulations of microbes sold as biofertilizers or biocontrol agents to combat microbial diseases.[55,56] Thus, it is interesting that there is an overlap in agricultural use of these beneficial microbes as fertilizers and pesticides, with proposed uses for NPs.[2,6,17−20]

Microbial populations are greater in the rhizosphere, the region around the root influenced by exudation of plant metabolites, than in the surrounding soil.[58] These plant exudates provide nutrients to support microbial growth.[59−62] However, the complex microbial community structure in soils is disturbed by NP amendments, with both increases and decreases for certain taxa occurring dependent on NP, plant, and soil properties.[63,64] The soil microbes involved in the nitrogen cycle are among those with documented sensitivity.[65−67] Changes in function of a population, such as a loss in function of nitrogen-fixing rhizobium isolates, would in turn affect plant performance.[30,65,68] Clearly, any formulation for agricultural use should not have deleterious effects on the organisms involved in essential element cycling.[16]

The framework for discussion of NPs use in agriculture in this review centers on studies with a pseudomonad able to colonize the roots of many plant species, a process that improves the plants' growth and stress resilience.[69] *Pseudomonas chlororaphis* isolate O6 (*Pc*O6) (Figure 3) was cultured from the roots of commercial dry land wheat at the end of the growing season in calcareous soil. Like other pseudomonads, it is adaptable to using many substrates for growth, utilizing the sugars, organic acids, and amino acids that are major metabolites in all root exudates.[58−62,70] *Pc*O6 is typical of the many microbes with biological control activity.[71−73] For example, colonization of plant roots with *Pc*O6 confers systemic protection against viral, bacterial, and fungal pathogens in the plant. Protection also arises from direct antagonism of microbial pathogen growth caused by production of antibiotics.[74−77] *Pc*O6 is lethal to root knot nematodes, with HCN production playing a major role.[78] Killing of the larval stage of certain insect pests is conditioned by production of an insecticidal peptide toxin, Fit D.[72,79] Root colonization by *Pc*O6 also stimulates in the host plant drought tolerance and resilience to heavy metals.[80,81] Thus, the consequence of contributing C and N in root exudates to support colonization of beneficial microbes is the provision of an umbrella of protection for the plant against the array of stresses experienced in a field environment. However, such generalist root colonizers would be among the rhizosphere organisms challenged by NPs when introduced into agricultural soils.

2. MODIFICATION OF *Pc*O6 METABOLISM BY NPs

2.1. Metal Oxide NPs.

In the studies discussed in the following sections, the CuO and ZnO NPs were used "as-made" from the manufacturer.[31,45,46,70,82] Nanodimensions of the particles were confirmed by microscopy, and their chemistry was confirmed by elemental analysis with ICP-MS, XRD, and ZANES analysis.[82] Aggregation of the particles, shape modification, and dissolution under different conditions are reported.[46,82]

2.2. ZnO NPs: Phenazine and AHSL Production. *Pc*O6 is an example of the biological control agents that produce the phenazine class of antibiotics (Figure 3). The phenazines from *Pc*O6 are visualized as orange pigments secreted from the bacterial cell[74] (Figure 3). Their synthesis occurs from the shikimic acid pathway through chorismate.[84] Nutrients, such as the sugars that feed into the shikimic acid pathway in the bacterial cell, are part of the exudates from germinating seeds and plant roots.[69] Thus, the C and N in the plant exudates is repurposed by bacterial metabolism to generate the phenazines, which in turn are beneficial to plant and rhizosphere health.

Phenazines originally were examined in vitro for their direct antifungal activities, becoming associated with disease suppression.[85] Mutation, so that the bacteria lack phenazine production, reduces the extent of growth inhibition for plant pathogenic fungi by the pseudomonad.[85] Phenazines are isolated from native field soils in which wheat is grown at levels sufficiently high to cause antagonism of pathogen growth.[86] This finding indicates that phenazine production is not just a laboratory phenomenon.

Phenazines are involved in additional processes that affect plant function.[87,88] Phenazines are elicitors that prime systemic induced resistance in plants. "Priming" means that the plant responds more rapidly and to a greater extent when subsequently challenged by a stress such as drought or pathogen attack.[76,89−91] Primed defense is observed systemically at sites distant from the inducing microbe. For instance, shoot tissues are protected, whereas the inducing microbes are located only on the root surface.[76] This systemic induction of resistance augments direct antagonism of pathogen growth. These responses illustrate that the phenazines are interkingdom signaling chemicals, affecting the functioning of bacteria and the plant as well as fungi in the environment.

Effective biocontrol agents display aggressive and sustainable colonization of plant roots. One of the traits needed for sustained colonization is attachment to the root surface followed by growth of the cells embedded within a gel-like matrix as a biofilm[92,93] (Figure 1). The pseudomonad biofilms formed on plant root surfaces protect against challenges of the CuO NPs and ROS that are lethal to planktonic cells.[81,94] Resilience of biofilm cells to antibiotics generated by other rhizosphere microbes is likely.[95] Aspects of biofilm formation are covered in a another paper in this issue by Britt et al. (submitted for publication). Thus, biofilm matrix, with its intertwining polymers of polysaccharides, nucleic acids, and proteins, provides a protective environment for microbial cell survival.

Phenazines play additional roles in biofilm function. For instance, phenazine production in *P. chlororaphis* 30-84 enhances the extrusion of extracellular DNA levels, which become part of the biofilm matrix.[96] Another role of phenazines in biofilms is to accept electrons and, consequently, aid redox balancing in bacterial cells under anaerobic conditions, which are likely to occur within layered biofilms in the rhizosphere.[97] Biofilm formation in pseudomonads is promoted by an adequate iron supply.[98,99] Phenazines may, through acting as iron chelates, promote the release of metals from minerals[100,101] and, thus, enhance microbial biofilm formation. Additionally, their redox activity will reduce ferric to ferrous ions, which can be transported into cells by mechanisms that differ between these charged Fe ions.[98]

Phenazine synthesis in pseudomonads is regulated by the Gac/Rsm signal transduction pathway. In *Pc*O6, synthesis is dependent on acyl homoserine lactones (AHSLs) (Figure 3) produced by activation of the Gac/Rsm pathway.[88] Synthesis of an array of AHSLs with different fatty acid chain lengths and of families with the basic phenazine structure applies to *Pc*O6[83] and other beneficial pseudomonads.[102] When the bacterial cells reach a sufficiently high density, the AHSLs reprogram the bacterial cell population to act in unison to initiate phenazine synthesis, an event termed quorum sensing.[103] ZnO NPs affect this process. A decrease in phenazine production occurs as ZnO NPs are added to broth medium (Figure 4).[83,104] These effects were seen at 2 mg soluble Zn/L from ZnO NPs.[83] AHSLs also were reduced in levels in broths with the ZnO NP treatments (Figure 4).[83] The lack of AHSLs correlated to lowered expression from the AHSL synthase gene, *phzI*.[83] Inhibition of phenazine and AHSLs production by the ZnO NPs is duplicated in part by Zn ions but not with the same efficacy as from the NPs.[83] We speculate that the NPs are highly effective as a source of Zn for the bacterial cell through metal oxide dissolution. Dissolution of the ZnO NPs could be increased by metabolites produced by the bacterium that are metal chelators, such as siderophores (see section 2.3) or gluconic acid.[81]

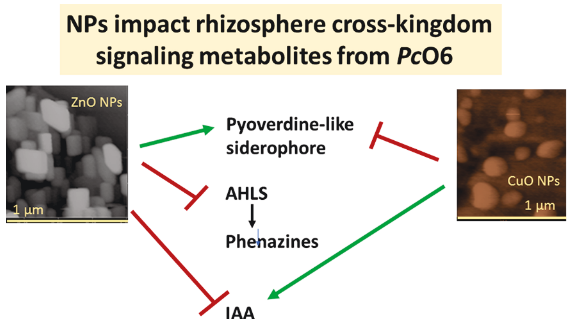

Figure 4. Summary of changes in *Pc*O6 metabolism elicited by CuO and ZnO NPs. The chemistry of these NPs is important because they have opposing effects on some of the metabolic pathways.

Reduction of both phenazines and AHSLs by exposure to ZnO NPs could change the effects of potential pathogens in the rhizosphere. The AHSLs, like the phenazines, trigger systemic defense mechanisms in the plant.[105,106] Thus, two different metabolites used in signal communication between plant and the rhizosphere microbe are modified in synthesis by the ZnO NPs. Whether other antibiotics produced by *Pc*O6, which are regulated independently of AHSLs within the Gac/RSm network, are modified by the ZnO NPs awaits study. This work with *Pc*O6 also raises the question of whether NPs have a similar impact on the metabolism of other rhizosphere bacteria that produce antimicrobial agents.

2.3. ZnO NPs and Siderophore Production. Coincident with the loss of phenazine production when *Pc*O6 is exposed to ZnO NPs is an increase in a secreted, fluorescent pyoverdine-like siderophore.[107] (Figures 3 and 4). The major function of siderophores is binding Fe^{3+} ions that are then transported and unloaded into the bacterial cell to provide this essential metal.[108,109] Expression from the large biosynthetic cluster of

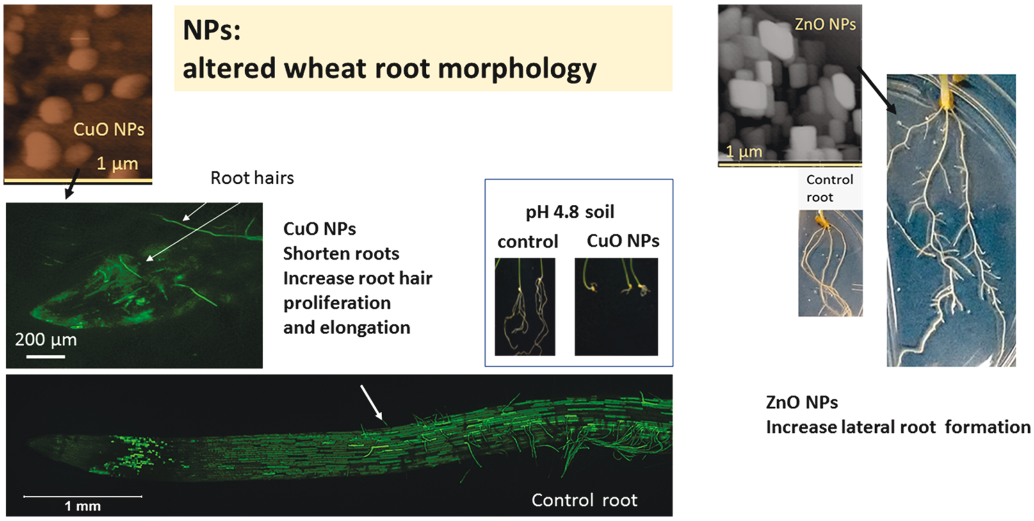

Figure 5. Changes in root morphology triggered by growth with CuO or ZnO NPs. The bright green fluorescence in cells revealed in the confocal images of the wheat seedling root tips is due to accumulated nitric oxide (NO). NO is part of the cell signaling system required for root hair formation and is detected by interaction with 4-amino-5-methylamino-2,7-difluorofluorescein diacetate (DAF-FM DA). The white arrows denote root hairs. The distance between root cap and root hair initiation is very short for the seedlings grown with CuO NPs (green arrow). The inset image depicts the intense shortening of wheat seedling roots with growth with CuO NPs in pH 4.8 soil. Soluble Cu in the rhizosphere solution is heightened because dissolution is enhanced by the proton concentration. Wheat seedling growth with ZnO NPs results in increased lateral root formation.

genes involved in pyoverdine synthesis in pseudomonads is highly regulated, with suppression occurring when Fe is adequate.[96] Although these biochemical changes were demonstrated in broth cultures, ZnO NPs also had an impact on siderophore production in *Pc*O6 cells colonizing the roots of beans growing in a solid matrix.[110] Increased pyoverdine levels were detected in bean rhizosphere solutions with the presence of ZnO NPs in the plant growth matrix.[110]

The mechanism underpinning altered expression from the siderophore genes is not clear. Increased pyoverdine production in *Pc*O6 is observed in a mutant in which the GacS environmental signaling protein of the Gac/Rsm controlling network is eliminated.[111] This is one of the few traits identified as being negatively regulated by the Gac/Rsm network in isolate *Pc*O6; currently most of the traits studied for this network, such as production of phenazine and AHSLs, are positively regulated.[88] Thus, the ZnO NPs appear to induce a *gacS*-like phenotype to the cells. In a *gacS* mutant, the translational repression, caused by the binding of the protein RsmA for genes in the *gac* regulon, cannot be relieved.[112] Studies with *P. aeruginosa* (*Pa*) show that the translation inhibitory protein, RsmA, is a strong repressor of pyoverdine synthesis; that is, deletion of RsmA in *Pa* increases pyoverdine production.[113] This response is attributed to indirect transcriptional control by RsmA of the alternative sigma factor, PvdS, that is required for expression of the pyoverdine biosynthetic genes. Transcriptional regulation of *pvdS* is correlated with levels of the intracellular cell signaling compound, c-di-GMP, that are governed by RsmA; a *Pa rsmA* mutant has higher levels of c-di-GMP.[113] This control network would explain the responses of *Pc*O6 but is puzzling in light of other studies in *Escherichia coli* in which Zn ions are inhibitory to the protein diguanylate cyclase that produces c-di-GMP,[114] meaning that with Zn inhibition, levels of c-di GMP would be lowered. Whether Zn modifies c-di-GMP levels in pseudomonad cells is not known.

The increase in pyoverdine-like siderophore production from rhizosphere bacteria triggered by ZnO NPs (Figure 4) may be advantageous to the plant. Several potential outcomes exist. First, the chelation efficiency for Fe of the siderophore could limit growth of pathogenic rhizosphere microbes. This possibility is likely because pyoverdine has high binding capacity for Fe. Indeed, siderophore production from fluorescent pseudomonads was among the first traits to be associated with suppression of disease caused by fungal pathogens in the field.[115,116] Second, plants may access the Fe from the loaded pyoverdine-like siderophores[117−119] for nutritional benefit. Third, the pyoverdine-like siderophores also are among the microbial metabolites that stimulate systemic plant resistance mechanisms.[120−122] Thus, under laboratory conditions, although the ZnO NPs reduce production of the interkingdom cell signaling metabolites, phenazines, and AHSLs from *Pc*O6, there is enhanced production of another interkingdom signaling compound, a pyoverdine-like siderophore. If these phenomena occur in native soils, there is potential for changes in plant defense against pathogen attack.

2.4. CuO NPs and *Pc*O6 Metabolism. In contrast to the increase by ZnO NPs in siderophore production by *Pc*O6, CuO NPs decrease the pyoverdine levels (Figure 4).[123] Analysis of gene expression in the *Pc*O6 cells finds lower transcript abundance from genes encoding the cytoplasmic transporter and proteins involved in periplasmic maturation of the pyoverdine precursor with CuO NPs.[123] Thus, the chemistries of these two metal oxide NPs elicit different cellular responses in the microbe.

Opposing regulation in *Pc*O6 in response to the two NPs also is observed with the production of a plant growth regulator IAA (Figure 4). In *Pc*O6, IAA is synthesized from the amino acid tryptophan, which is among the array of amino acids detected in root exudates at levels varying between plant types.[59] IAA production in *Pc*O6 was enhanced by CuO NPs but repressed by ZnO NPs.[124]The mechanisms causing these effects await elucidation. However, further complexity with the

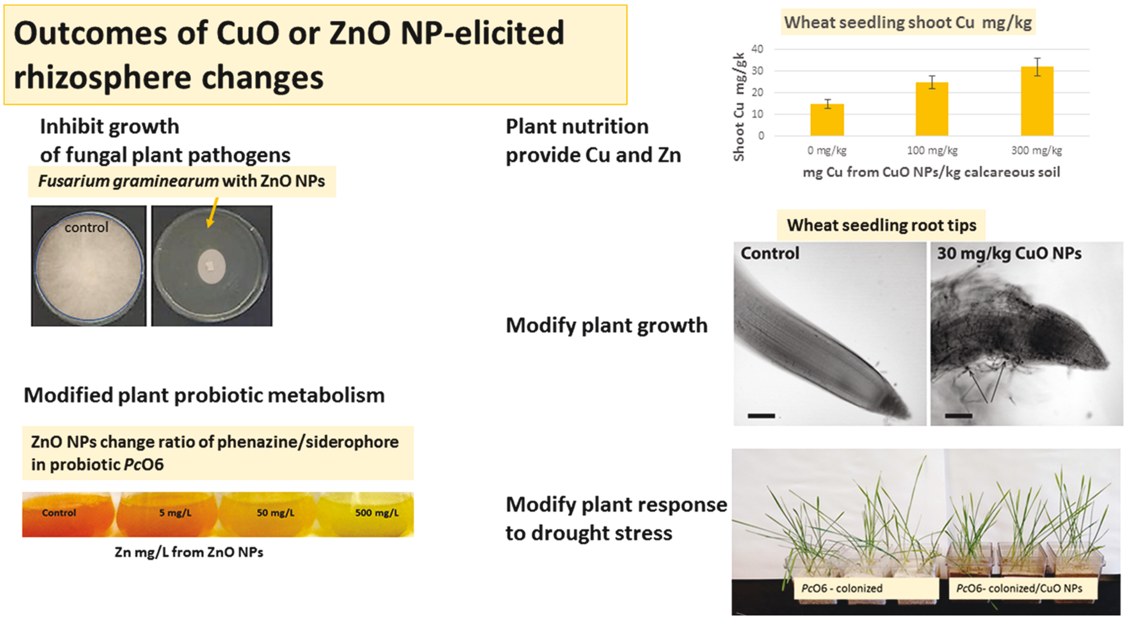

Figure 6. Exposure of rhizosphere microbes and roots to CuO or ZnO NPs has outcomes in both the plant and root-associated microbe (*Pc*O6). Nanoparticle-induced changes in the microbe would affect antagonism of fungal growth, plant root morphology, metal uptake into the shoots, and plant response to drought stress. Each of these responses has implications for plant health.

Gac/Rsm regulon is evident because IAA production from tryptophan also is boosted by deletion of GacS in *Pc*O6.[125] Thus, stimulation of IAA production by ZnO NPs would be expected if these NPs produced a *gacS*-like phenotype. We speculate that there must be a strong inhibitory effect of Zn at a site currently unidentified in the tryptophan-dependent synthesis of IAA.

IAA production by *Pc*O6 cells colonizing wheat roots affects root morphology when the growth matrix is supplemented with tryptophan.[126] The wheat roots shorten and prolific and elongated root hairs are observed, as reported for roots exposed to authentic IAA or other IAA-producing microbes.[127] IAA production by root-associated microbes has field significance because this trait is prevalent in isolates displaying plant growth promotion and the ability to enhance metal phytoremediation.[128,129]

These studies illustrate that the metabolism of the rhizosphere-colonist *Pc*O6 can be tuned by Cu or Zn supplied through NPs (Figure 4). The metabolites that change in level affect functions of plants and fungi that are involved in plant health.

3. PLANT RESPONSES TO NPs

3.1. NPs and Their Impact on Plant Roots. Plants closely control Cu and Zn homeostasis. The physical mechanisms to limit toxicity differ between the metals: excess Cu is held largely within plant cell walls, whereas Zn is contained within vacuoles.[130,131] Studies with CuO NPs indicate that the capture of Cu by root cell walls is important to limit Cu flux into the shoots of the rice plant.[32] Both CuO and ZnO NPs increase lignification in roots of several plant species through processes that involve altered peroxidase activities.[132,133] These changes may be a generalized response to increased ROS in the plant tissue due to enhanced metal levels.[13]

The NPs also manipulate the plasticity of root morphology with outcomes that differ between the ZnO and CuO NPs (Figure 5). Both outcomes, however, would increase root surface area, potentially providing more support for colonization by beneficial microbes. When grown with CuO NPs, the roots of wheat seedlings are shortened in length (e.g., from 12–14 cm for controls to <4 cm with CuO NPs depending on dose), and there is prolific elongated root hair formation close to the root tip[114] (Figure 5). With sand as the growth matrix for 7-day wheat seedlings, these morphological changes occur at a dose of 10 mg Cu/kg sand.[126] Shortening of root length in wheat is extreme in acidic soils (Figure 5) and correlates with dissolution of CuO NPs, whereas in calcareous soil there is mitigation of toxicity.[26] The changes in root hair patterning may not be universal for plants; for instance, Cu ion treatment of cowpea also causes root stunting, but the root hairs become shortened.[134]

The gross morphological changes observed in wheat tips grown with CuO NPs resemble those from high IAA levels.[26] We speculate that the dissolution of CuO NPs in the root tip zone allows Cu to accumulate to a level that affects the normal inverted fountain flow of IAA as it unloads from the stele. Root exposure to Cu ions is reported to inhibit a specific efflux pump in the plant cells at the base of the stele, causing IAA flux in the root tip cells to be modified.[135]

The CuO NPs were more effective in induction of root hair proliferation than Cu ions, but this could be attributed to dilution of the ion concentration in the rhizosphere space below that of the gradient of ions being released from NP aggregated on the root surface.[113] The phenomenon of CuO NP-enhanced root hair formation could benefit plants in the field because root hairs are involved in nutrient and water uptake.[136,137] It is possible that the entrapment of the CuO NPs onto the root hairs (Figure 1), as revealed by microscopic imaging,[114] is important to this phenomenon.

3.2. Lateral Root Growth Proliferation. Growth with ZnO NPs has no effect on the differentiation of epidermal cells into root hairs. However, lateral roots are induced when wheat seedlings are grown in sand and calcareous soils (Figure 5).[31] Lateral root formation is dependent on Zn in other plants,[138] and consequently the changes in wheat caused by the ZnO NPs may depend on their dissolution in the rhizosphere or root tissues. The molecular basis of stimulation of lateral root primordia is not fully understood, although oscillations in IAA in the root tip are proposed to be involved and IAA signaling is required in the transforming cells.[139−141] Clearly, any changes in IAA triggered by ZnO NPs must differ from those caused by CuO NPs because the resulting root morphologies are distinct (Figure 5). Stimulated lateral root formation by formulations with ZnO NPs could also be valuable under field conditions because they will increase nutrient and water uptake. It is notable that lateral root hairs are especially involved in uptake of Si that increases plant strength and resilience to pathogens.[142]

3.3. CuO and ZnO NPs and Their Impact on Plants' Response to Stress. At doses of ZnO or CuO NPs that cause changes in root morphology (300 mg M/kg), there are effects on transcript accumulations in the roots, as well as systemically in the shoots (Anderson et al., unpublished results). Transcript analysis in the roots show increased expression of genes associated with resistance to metal stress incited by ion exposure.[81] These findings suggest that the plant perceives metal ions released from the NPs. Colonization of the plant root with *Pc*O6 reduces the extent of transcript abundance, about 2−3-fold.[81] We speculate that the lowered transcript levels could be due to a protective effect of the *Pc*O6 biofilm (Figure 1) on the root in which NPs become embedded, likely altering the mobility of released metal and, thus, reducing the exposure of root epidermal cells to the released metal or NPs.

Increased transcript levels for metal stress are observed in the shoot tissues (Anderson, unpublished results). The shoots are not in immediate contact with the bacterium and lack NPs on their surfaces.[81] Also, transcripts, anticipated to have a role in drought and pathogen stress, are elevated in the shoot tissues (Anderson et al., unpublished results), illustrating cross-talk between stress responses for metals, pathogen attack, and water deficiency. Annotation of the function of the transcripts indicates that many are associated with improved tolerance to water stress regulated by abscisic acid. This finding indicates the NPs target another plant cell signaling process in addition to modification of IAA. Preliminary studies with drought stress on plants reveals that *Pc*O6-colonized wheat seedlings growing with CuO NPs have a shoot stand after a drought period superior to that of control plants grown without these treatments (Figure 6). The underlying mechanisms are being investigated and may relate to NP-induced cell wall modifications as well as the altered transcriptome associated with drought tolerance. The findings highlight another consequence for the plant to exposure to NPs: the induction of a higher level of plant tolerance to drought could be valuable under field conditions.

4. SUMMARY OF RHIZOSPHERE EFFECTS OF CuO AND ZnO NPs

CuO and ZnO NPs alter processes important in the rhizosphere that are involved in plant health in addition to the nutritional effect of increased tissue levels of Cu and Zn (Figure 6). These changes are correlated with specific effects on cell signaling compounds that communicate between the plant and the root-colonizing microbe. The consequences of NP challenge of the probiotic *Pc*O6 involve altered levels of interkingdom signaling metabolites including antifungal products (e.g., phenazines and siderophores), quorum sensing coordinators (e.g., AHSLs), Fe-chelating siderophores (e.g., pyoverdine), and the plant growth regulator IAA Figure 6). Thus, there is potential to affect antifungal protection and Fe supply in the rhizosphere, as well as induced systemic resistance in the plant.

Growth morphology in the plant may be altered by effects on the microbial levels of IAA as well as by direct disturbance of the flux of plant-produced IAA at the root tip. Increased lateral root formation (ZnO NPs) and proliferation of elongated root hairs (CuO NPs) would have a beneficial impact on plant nutrition and water uptake. Also, there would be greater surface area for colonization by microbes. Systemic effects induced by the NPs include cell signaling to promote altered gene expression increasing the plants' resilience to drought stress. Differences in the chemistry of the metals in the metal oxide NPs govern the plant and microbial responses. Collectively, the findings illustrate the power of localized concentrations of the essential metals, Cu and Zn, to affect rhizosphere processes that are key to plant health under environmental stress.

5. FACTORS GOVERNING THE EFFICACY OF FORMULATIONS OF NPs FOR AGRICULTURE

The basic scientific findings discussed for the CuO and ZnO NPs are from studies with particles that were "as-made" from the manufacturer. Thus, any impact of engineered coatings of the NPs that could alter their bioactivity is not explored in these studies. The plants were grown without gross pore water movement, such as would result from a rain event. The growth matrix, sand, was selected to reduce factors that could chelate released metal ions. It lacked minerals, organic materials, and other microbes characteristic of native soil. The studies also are of a model system with only a single microbe being addressed, whereas crop plants associate with diverse microbes, both as endophytes and as surface colonizers. Microbial soil populations are diverse[143−,145] and respond to many inputs including current farming practices that change plant diversity and ecotypes.[146−148] The rhizosphere in agricultural soils has many microbial and eukaryotic interactive partners that will influence responses to NPs (Figure 1).

Consistent with the findings reviewed here and elsewhere is the importance of the cell's response to a gradient of metal release from the metal oxide NPs. Dissolution of the metal oxide NPs could be intense at the site of the nanoparticle at an intracellular or extracellular location with the cell.[149] Soil factors, especially pH, would influence NP dissolution. For example, acid soils would cause greater metal dissolution than a basic soil.[150] Microsites of different pH of the root surface exist; root hairs secrete protons along with the plant metabolites. NP dissolution is promoted by metal chelators, including siderophores, as well as the organic and amino acids that are part of root and microbial exudates.[46,151] These acids would have the highest concentrations as they are being released into the rhizosphere space, but metabolite levels will drop with distance from the root surface in part through consumption by rhizosphere microbes.[70] The extent and composition of organic soil matter, such as the fulvic and humic acids, also would affect metal bioavailability.[151] The chemistry and sorption properties of the soil would modify NP effects.[152] Salts with Na, K, and Ca as cations, at levels similar to those in saline soils, relieve NP phytotoxic effects.[153]

A major research thrust with nano-agrochemicals is to boost the bioavailability of the native soil. It is an innovative research field. Two recent papers show NPs increase P supply to plants.

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In one paper, where ZnO NPs increase P uptake in mung bean, the proposed mechanism involves a chemical "hand-off chain". Enhanced Zn bioavailability to the plant from the NPs allowed the roots to increase phosphatase activity, which liberated Zn from zinc phosphate and generated the metal release from NP dissolution.[154] The second paper, showing increased phosphate in lettuce upon growth with TiO_2 and Fe_3O_4 NPs, implicates several mechanisms, including NP- induced changes in root exudation that acidified the rhizosphere to enhance P release.[155] A third paper reports that Fe could be increased for rice grown in calcareous soils by the presence of coated Fe_3O_4 NPs. The proposed mechanism involved Fe release from the NPs that counteracted the effect on rice of excess Ca ions.[156] Other nanosized products are cited for improving plant resistance to pathogens. Such products contain silica[157] and elicitors of plant resistance, nanosized glucans, and chitosan.[158,159]

Engineering the formulations of the NPs by coating and mixing of chemical composition will be required to optimize field performance. The formulations will differ whether the products are applied to seeds, to the soil, or as an aerial application. The products may be time-released and targeted to provide the stimulus only at the right location and at the right time for the plant.[18] Our abilities to predict the function of NP formulations in different soils and with varied crops yet await the acquisition of information bases to coordinate multiple physical, chemical, and biological factors.

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