Chapter 10
Interaction of Engineered Nanomaterials with Soil Microbiome and Plants: Their Impact on Plant and Soil Health

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Abstract A large numbe of nanomaterials-based products are being commercially engineered and produced. Many of these engineered nanomaterials (ENMs) are disposed in soil in significant quantities. Furthermore, nanomaterials are being specially tailored for use in agriculture as nano-fertilizers, nano-pesticides, and nano-based biosensors. The behavior of ENMs in soil and their persistence depends on their chemical nature and soil characteristics. Furthermore, nanoparticles like silver and zinc oxide possess well-known antimicrobial activities. The presence and persistence of these nanomaterials in soil can alter the quality of the soil microbiome, thus influencing key microbial processes like mineralization, nitrogen fixation and plant growth promoting activities. It is, therefore, extremely important to understand how nanomaterials influence the soil microbiome and associated chemical and biochemical processes. Such investigations will provide necessary information for eventual regulation of the appropriate use of nanomaterials for sustainable agriculture and increased agricultural productivity. This chapter discusses some of these issues.

Keywords ENMs · Plant microbiome · Soil microbiome

10.1 Introduction

The industrial production of engineered nanomaterials (ENMs) is rapidly increasing with its commercial and domestic use consequently, their quantities reaching various environments including soil are also increasing (Keller et al. 2013). ENMs may be released to the environment during production, or during fabrication of ENM-containing products, during the use of such products or via disposal following use. ENMs may be added to soil directly or may experience various transformations

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before reaching soil. Therefore, it is important to understand the types of nanomaterials that are released to the environment especially to the soil and their release routes. Another key concern is to evaluate ENM fates in soil and how soil affects various properties of ENMs. For example, soil pH may modify certain physical and chemical properties of ENMs. Finally, it is both urgent and important to understand how nanomaterials affect the overall health of plants, the plant microbiome, soil and the soil microbiome. This chapter discusses the effect of ENMs on soil and plant microbiomes, as both greatly affect the health of the plant as well as soil fertility (Berendsen et al. 2012; Sergaki et al. 2018).

How ENMs influence soil and plant microbiomes depends on various factors. Some nanomaterials may be more microbicidal than others. ENM dose, size, chemical nature and various other properties influence their potential microbicidal activity. Nanomaterials that are microbicidal at high concentration may promote the growth of microorganisms at lower concentrations (Khan et al. 2018). Some nanomaterials may have selective activity against only a select group of microorganisms. Therefore, the ENMs in soil may influence important geochemicsl processes mediated by microorganisms. Similarly, toxicity of ENMs to plants also depends on a number of factors and their effect on plants can not be generalized. This chapter discusses these factors and the nanomaterials of immediate concern to soil fertility, plant health and the plant microbiome.

10.2 Plant and Soil Microbiomes

Recent studies on microbiomes from various habitats has made it clear that microorganisms are ubiquitous, abundant, and irreplaceable due to the diverse and vital roles they play in nature. One habitat that harbors the highest density of microorganisms is soil. It is established that one gram of soil may contain up to 10^8 bacterial cells, thus contributing greatly to soil biomass. The biomass of bacteria and fungi in the soil is 10^2–10^4 times higher than that of other microorganisms such as archaea, protists, and viruses. The quantity of soil microbial biomass rivals biomass occurring above-ground (˃1000 kg/hectare) (Fierer 2017). Abundance, diversity, and type of microorganisms present in soil depend on soil type, available nutrients, oxygen availability and climatic conditions. Soil represents a diverse environment which varies greatly with location; microbial communities may vary even within a distance of a few millimeters. In a review on soil microbiomes, it has was stated that most of the bacterial and archaeal species found in different soils are rare, and only a few microbial types occur abundantly in all studied samples (Fierer 2017). Based on data from 66 soil samples it was determined that, according to their abundance, the predominant fungi in soil belong to Agaricomycetes (Basidiomycota), Archaeorhizomycetes (Ascomycota), Zygomycota, Sordariomycetes (Ascomycota), Leotiomycetes (Ascomycota), Dothideomycetes (Ascomycota), Eurotiomycetes (Ascomycota), *Glomeromycota* and *Chytridiomycota*. The predominant bacteria in these samples belong to *Acidobacteria*, *Verrucomicrobia*, *Bacteroidetes*, *Proteobacteria* (*Alphaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria,* and *Betaproteobacteria*), *Planctomycetes* and *Actinobacteria*. The archaea can be arranged in the following order of abundance: *Thaumarchaeota* (Crenarchaeota), marine benthic group archaea (MBGA; *Crenarchaeota*), *Thermoplasmata* (*Euryarchaeota*), *Parvarchaeota*, and *Euryarchaeota* (unclassified groups) (Janssen 2006). These microorganisms play important roles in soil including nutrient fixation (carbon and nitrogen), nutrient solubilization (phosphate and zinc), mineralization, and loss of nutrients from soil through processes like methane production and denitrification (Jacoby et al. 2017; Prakash et al. 2015). Therefore, the role of microorganisms in nutrient cycling is irreplaceable.

The plant microbiome can simply be defined as the community of microorganisms that live on plants, including the root surface, inside the root and on other surfaces such as leaves, stems, flowers, etc. These habitats are referred to as the phyllosphere, rhizoplane, rhizosphere, and endosphere. The plant microbiome is a complex system; interactions between the plant and its microbiome are affected by a number of factors including type of plant, age, health and nutrients secreted by the plant, the physical environment, the initial microbial load in soil and on the plants, and other factors. It has been demonstrated that plants are capable of selecting specific microorganisms for colonization of the rhizosphere (Lugtenberg and Kamilova 2009). Even within a plant under identical conditions, microorganisms present in the phyllosphere, rhizoplane, rhizosphere and endosphere may vary greatly. Research on the plant microbiome has focused on: (a) the extensive interplay among different microorganisms including bacteria, archaea, fungi, and protists; (b) plant-specific microbiomes to the level of cultivar; (c) the vertical transmission of core microbiomes; (d) the function of endophytes; and (e) unexpected functions and metabolic interactions (Berg et al. 2015). Some well-known examples of plant-microbe relationships are legume-rhizobia interactions and mycorrhizal associations, wherein the microbial partner provides nutrients for its host plant.

In addition to providing nutrients, microbiomes perform other support functions for plants (Hunter 2016). Microbial communities are so important that plants recruit specific microbial communities by the secretion of plant exudates (Berendsen et al. 2018). It has been demonstrated that flowering time and biomass yield of *A. thaliana* is controlled by its characteristic microbiome (Panke-Buisse et al. 2014). In another study on *A. thaliana* it was demonstrated that when plants are exposed to downy mildew disease they assemble protective microbial communities within their rhizosphere (Berendsen et al. 2018). The successful use of a microbial community for protecting *Nicotiana attenuata* against wilt disease has been demonstrated (Santhanam et al. 2015). The rhizosphere microbiome of *Phaseolus vulgaris* was shown to host a high population of the Bacteroidetes group of bacteria (Pérez-Jaramillo et al. 2017). While studying the domestication of *Phaseolus vulgaris* it was observed that domestication of plants changed the plant microbiome (Perez-Jaramillo et al. 2016): a decrease in the population of the Bacteroides group of bacteria, and an increase in populations of Actinobacteria and Proteobacteria was observed. Domestication also was found to adversely affect populations of symbiotic nitrogen fixers and mycorrhiza. Detailed studies on plant microbiomes will help in developing sustainable agriculture, minimizing dependency on fertilizers and pesticides while increasing the yield and nutrient content of crops.

Since different plants host distinct microbiomes that shift with varying conditions, it is necessary to understand the plant-specific microbiome which possibly can be customized for specific needs such as improved growth, protection against disease and better quality product (Fitzpatrick et al. 2018). Knowledge of microbiome will therefore make agriculture more extensive and less dependent on agrochemicals. In addition, it is of immense importance to understand how pollutants in soil may harm the microbiomes of plants and soil. Unparalleled urbanization in countries like China has resulted in extensive and serious soil pollution, it is estimated that 16% of Chinese soil, including 19.4% of farmland, is contaminated. An estimated 80% of these pollutants are toxic inorganic compounds (Yang et al. 2014). Nanomaterials are emerging as potential soil pollutant as they are being widely used and ultimately ends up in soil. According to some estimates, tons of nanomaterials are annually being produced and ending up in landfills and soil (Keller et al. 2013). Owing to their known antimicrobial activities, nanomaterials are expected to upset plant and soil microbiomes (Khan et al. 2016). This chapter discusses, in detail, nanomaterial pollution in soil and its consequences on plant and soil microbiomes and health.

10.3 Nanomaterials – A Brief Introduction

Nanomaterials are generally defined as materials that have at least one of their dimensions measuring less than 100 nm (Kreyling et al. 2010). Nanomaterials may be naturally occurring (e.g., volcanic ash) or engineered (TiO_2 and carbon nanotubes). Engineered nanomaterials are used in a number of commercial products. According to an estimate by the project on emerging nanotechnologies (PEN), more than 1824 products containing nanomaterials are already in the market (Berube et al. 2010). Fig. 10.1 shows some nanomaterial-based products. The extensive use of nanomaterials in industry is a consequence of their unique characteristics. Among various properties, the most important is their extremely small size and high volume to specific surface area ratio (VSSA). It has also been proposed that materials having a VSSA $\geq 60\ m^2/cm^3$ should be defined as nanomaterials (Kreyling et al. 2010). Nanomaterials represent an enormous class of compounds that are grouped based on size and other properties such as chemical nature, dimensionality, shape, and size. On the basis of chemical nature, nanomaterials are classified as organic, for example nanosized lipid micelles, and inorganic, such as silver nanoparticles. Nanomaterials can contain one repeating constituent unit or more than one type of repeating unit; the latter is termed a nanocomposite. Nanomaterials can be classified as 0D, 1D, 2D or 3D based on their dimensionality (Sun et al. 2014b). Nanomaterials having all dimensions in nanoscale like quantum dots and nanodispersions are termed 0D nanomaterials; materials having two dimensions in the nanoscale like nanotubes and nanowires, are referred to as 1D nanomaterials. 2D nanomaterials are

Fig. 10.1 Examples of some nanomaterial-containing products that may eventually release ENMs to soil: (**a**) paint; (**b**) teeth whitener; (**c**) automotive metal polish (**d**) Sun cream, (**e**) fabrics and (**f**) electronics

defined as nanomaterials with only one dimension in the nanometric size range, like nanofilms, while 3D nanomaterials are mesoporous structures and nanoclusters. Nanomaterials occur in various shapes such as nanotubes, nanocubes, nanosheets, nanospheres, and nanoflowers.

The small size of nanomaterials and their high surface area to volume ratio comprise the most unique properties of ENMs. Due to their small size ENMs are capable of penetration into living tissue including the blood-brain barrier in mammals and in various plant tissues (Khodakovskaya et al. 2011). The high volume to surface area ratio makes ENMs lighter and provides a greater surface for interaction and functionalization. Furthermore, ENMs can be crafted to possess the necessary properties to suit a specific purpose. Owing to their unique properties nanomaterials are used in a number of industries ranging from aeronautics to pharmaceuticals (Novikov and Voronina 2017; Aitken et al. 2006). Following consumption tons of nanomaterials are disposed in various environmental compartments such as soil, sediment and water (Keller et al. 2013).

10.4 Nanomaterials in Soil, Their Release Routes, and Fate

Soil contains a number of naturally-occurring nanomaterials such as clay, iron oxides and organic matter (Klaine et al. 2008). Several engineered nanomaterials, as discussed above, are also released to soil and other environments via the use and disposal of nanomaterial-based products. It is estimated that about 3000, 550, 5500, 55, 55, 55, 300, 0.6, 55 and 0.6 tons of TiO_2, ZnO, SiO_2, FeO_x, AlO_x, CeO_x, CNT Fullerenes, Ag and quantum dot nanomaterials, respectively, are produced worldwide annually (Piccinno et al. 2011). ENMs are released intentionally or unintentionally (a) during manufacturing; (b) during use; and (c) via disposal after use. Products that contribute the most ENMs to the environment include coatings, paints, pigments, electronics, optics, and cosmetics. It is estimated that from 0.1 to 2% of all ENMs produced are released into the environment during production (Keller et al. 2013). Release during use varies from product to product; for example, most of the nanomaterials used for academic, research and cosmetics are released into wastewater ultimately reaching wastewater treatment plants (Fig. 10.2). Most nanomaterials used in electronics, packaging, paper, plastics, and board are placed into landfills and soil (Keller et al. 2013). Approximately 63–91% of ENMs are disposed in landfills, while the second largest volume (8–28%) is disposed in soil. These data reveal that tons of nanomaterials reach soil every year. The highest volumes of tita-

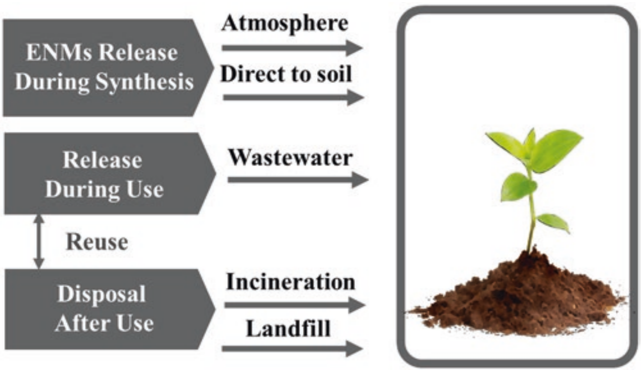

Fig. 10.2 Release routes of ENMs in soil. Few details are currently available on release routes of ENMs in various environments

nia, iron and zinc oxides are released into soil, water and air. While, SiO_2, titania (TiO_2), iron, and zinc oxides (ZnO), and alumina oxides (Al_2O_3) are the most abundant ENMs released to the soil and landfills. Most reach soil directly due to the nature of their use; for example, pigments containing TiO_2 used for pigments directly reaches into landfills (Sun et al. 2014a). These ENMs are released into the air from manufacturing, which subsequently reaches the soil. Additionally, upon incineration burning of ENM-s containing products in waste incineration plants (WIPs) also the ENMs will eventually reach the soil. ENMs such as TiO_2 contact soil when sewage treatment effluents containing ENMs are used as irrigation water. It is estimated that irrigation with nanomaterial-containing wastewater may result in an increase of 89 μg TiO_2 per kg of soil annually (Gottschalk et al. 2009). It was found that highest concentrations of ENMs measured in soils are those treated with municipal sludge. The same study calculates that 0.28–1.28 μg kg^{-1} y^{-1} of TiO_2 is released into soil in Europe. Similarly, 0.093 and 0.050 μg kg^{-1} y^{-1} of ZnO are released into soil in Europe and the US, respectively. While, other studies have, reported that highest amounts of TiO_2 are reaching all the compartments of the environment including soil, followed by ZnO (Gottschalk et al. 2009). The fate of ENMs upon reaching soil varies with soil type and properties of the nanomaterial. ENMs may dissolve in soil water, interact with charged particles in soil, or can be taken up by organisms resulting in their bioaccumulation. The latter depends on bioavailability of ENMs. ENMs may also reach water bodies underneath and sediments.

The fate of nanomaterials in soil and other environments varies based on their inherent properties as well as the properties of the environment (Klaine et al. 2008). Size, shape, chemical nature, and surface properties are key characteristics that influence ENM behavior and fate in soil. All ENMs undergo aging (weathering); with aging, particles may undergo chemical transformation, aggregation, and disaggregation (Bundschuh et al. 2018). Chemical transformations of ENMs in soil may include dissolution, sulfidation, adsorption and desorption.

10.5 Effect of Nanomaterials on Plant Health and Plant Microbiome

Although the use of nanomaterials in agriculture is still in its infancy, some commercial nano-based products are already available in the market (Sekhon 2014; Servin et al. 2015a). Studies showing the beneficial effects of ENMs have already been published (Khan et al. 2018; Faizan et al. 2018). This shows that the ENMs are added to soil intentionally in addition to the ENMs that are released in the soil through the routes discussed above. The presence of nanomaterials in the plant rhizosphere is a unique scenario which embraces an interplay of the plant microbiome, the plant, and the ENM. For example, as discussed above, plants secrete exudates that craft the microbial community within their respective rhizosphere. The rhizospheric microbial communities are also influenced, however, by ENMs present in the soil. Furthermore, the chemical nature of the ENMs may also change in the presence of root exudates and the extracellular substances from microbes. The presence of ENMs in soil may affect plant health either directly or by influencing the plant microbiome.

10.5.1 Direct Effects of ENMs on Plants

The uptake of ENMs by higher organisms and their interaction with various biomolecules is well established. It is known that many ENMs are taken up by plants and can be transported to leaves and other aerial parts, influenceing plant growth directly (Lin et al. 2009). Both growth-promoting and inhibitory activities of ENMs have been reported. These effects depends on a number of factors including the chemical nature of ENMs and their size, surface charge and dose (Husen and Siddiqi 2014; Faizan et al. 2018). Furthermore, the ability of ENMs to migrate from soil to the plant and their propensity for transport to various tissues also play an important role in plant health. Several reports are available on migration of nanomaterials to leaves and other aboveground tissues following absorption by roots. Movement of nanomaterials in plants can either be apoplastic (i.e., through extracellular spaces and xylem vessels) or symplastic (through plasmodesmata) (Pérez-de-Luque 2017). TiO_2 NPs and multi-walled carbon nanotubes (MWNCTs) exhibited limited mobility from soil to wheat and red clover leachates (Gogos et al. 2016).

Various molecular mechanisms have been proposed of nanomaterial toxicity (Khodakovskaya et al. 2011; Servin et al. 2015b); one of the most commonly reported is induction of stress which is detected by increased activity of superoxide dismutase and peroxide dismutase. When different carbon nanomaterials including fullerenes, reduced graphene oxide, and MWCNTs were added to the rhizosphere of rice at a dose of 50–500 mg/kg of soil for 30 days, the MWCNMs triggered the induction of four phytohormones including auxins, brassinosteroids, indoleacetic acid, and gibberellins (Hao et al. 2018). In addition, increased activities of superoxide dismutase and peroxide dismutase were observed. The study concluded that the CNMs resulted in toxicity to both rice plants and microbial communities. A 60 and 75% growth inhibition of zucchini (*Cucurbita pepo*) was reported in the presence of Ag NPs and MWCNTs, respectively (Stampoulis et al. 2009). Similarly, Ag NPs inhibited the germination of ryegrass and flax (*Linum usitatissimum*). At a concentration of 1.5 g L^{-1}, Ag NPs reduced the germination of barley (*Hordeum vulgare* L.) by 13% (Yehia and Joner 2012). Accumulation of CeO_2 in soybean roots and their translocation and accumulation in edible tissue is also reported (Hernandez-Viezcas et al. 2013). It has been demonstrated that exposure to ZnO NPs (200–300 mg/L^{-1}) results in a decrease in chlorophyll content of plants leading to deterioration of plant health (Wang et al. 2016). Poly(acrylic acid) nano ceria increased carbon assimilation rates by 67% in *Arabidopsis thaliana* plants (Wu et al. 2017). Nano ceria protected the plant from abiotic stress by scavenging free radicals. This was in contrast to the findings of other works, where nanomaterials induced stress in plants through ROS generation. CuO nanoparticles inhibited denitrification, nitrification, and soil respiration. This inhibition was observed, however, at a high concentration of 100 mg/kg dry soil.

In contrast, growth-promoting activities of ENMs have also been reported (Faizan et al. 2018; Zhu et al. 2019). For example, addition of nano TiO_2 alleviated Cd stress in soybean plants by promoting plant growth; this was achieved through increased chlorophyll or carotene content following TiO_2 treatment, consequently increasing the rate of photosynthesis (Singh and Lee 2016). Nanomaterials of graphene oxide have been shown to promote seed germination through increased water retention (He et al. 2018). Priming of aged rice with 5 and 10 ppm of Ag NPs improved seed germination and seedling vigor (Mahakham et al. 2017). Similarly, addition of ZnO promoted the growth of tomato plants by providing Zn as a micronutrient (Faizan et al. 2018). Addition of a water-soluble wood-based pyrolysis waste product termed nano-onions (wsCNOs) was found to enhance the overall growth rate of gram (*Cicer arietinum*) plants (Sonkar et al. 2012). Some ENMs serve as micronutrients for plants, but also as carriers for various nutrients (Jampílek and Kráľová 2017). ENMs act as an effective carrier for nutrients due to their small size and excellent penetration capabilities (Pérez-de-Luque 2017). In experiments on *Arabidopsis thaliana* it was observed that exposure to nano-cerium resulted in increased plant biomass and numbers of rosette leaves (Tumburu et al. 2017). These findings were supported by the microarray data presented in the same study. Change in gene expression of tobacco cells upon exposure to carbon nanotubes was studied; genes involved in cell division and water transport were upregulated at low concentration of CNT. Low exposure concentrations promoted cell growth (Khodakovskaya et al. 2012). Nano Fe_3O_4 was found to be beneficial for growth of *Triticum aestivum* L., and an increase in antioxidant enzyme activity was also observed (Iannone et al. 2016). Although many reports on this aspect are available, further systematic studies are required. A careful evaluation of environmentally realistic doses should be considered before reaching any conclusion regarding toxicity of ENMs to plants.

10.5.1.1 Influence of ENMs on Soil and Plant Microbiome

In addition to directly affecting growth of plants, ENMs also influence the microbial community in the soil and plant microbiomes consequently affecting the plant health (Fig. 10.3). For example, it has been reported that nano TiO_2 and ZnO influence soil microbial communities and a comparison of the two ENMs suggests that ZnO NPs induce more pronounced toxicity than does TiO_2 (Ge et al. 2011). Bacteria that carry out nitrogen fixation and methane oxidation were among the populations that decreased significantly with treatment of ENMs (Ge et al. 2011). While the population of members of *Sphingomonadaceae* increased, members of this family are well-known for decomposition of recalcitrant organic pollutants increased.

TiO_2 was assessed for its effect on the microbiome of wheat. It was observed that populations of certain prokaryotes changed, but growth of the plant and arbuscular mycorrhizal root colonization remained largely unaffected (Moll et al. 2017). It was

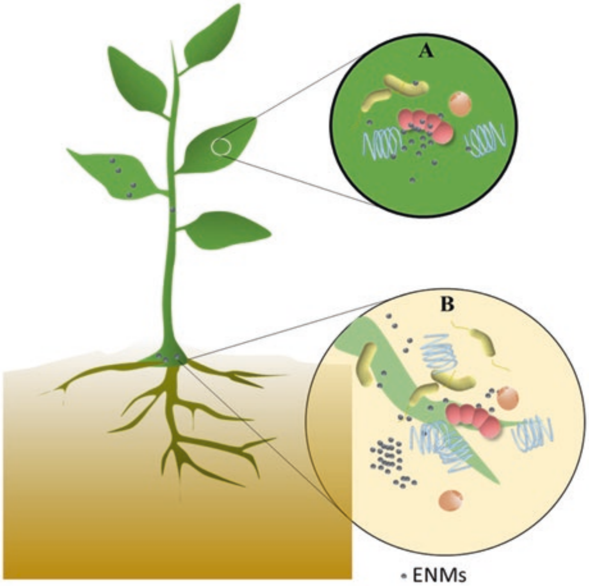

Fig. 10.3 ENMs present in soil may affect plant-microbe interactions, especially in rhizospheric microflora (**b**). These ENMs can also reach aerial parts of the plant and can affect the phyllospheric microflora (**a**)

suggested that the change in the prokaryotic community can be used as a marker for nano TiO_2 contamination in soil. In another study (Grün et al. 2019) it was observed that silver NPs affect the soil microbial community significantly (Grün et al. 2019). The same authors demonstrated that populations of β-proteobacteria and ammonia oxidizers decreased significantly upon exposure to silver nanoparticles; in contrast, populations of *Acidobacteria*, Actinobacteria, and Bacteroidetes increased significantly (Grun et al. 2018). Exposure to Ag NPs decreased soil microbial biomass, leucine aminopeptidase activity, and abundance of nitrogen-fixing microorganisms (Grun et al. 2018). When soil was treated with C60 fullerenes of 50 nm size, growth of fast-growing bacteria was suppressed three to four-fold (Johansen et al. 2008). When TiO_2 and polystyrene nanomaterials were added to the rhizosphere of lettuce seedlings numbers of rhizospheric soil bacteria decreased, which consequently inhibited root and shoot growth (Kibbey and Strevett 2019). Soils irrigated with waste effluent containing ENMs experienced an increased population of cyanobacteria and an unknown group of Archaea. The life cycle of *A. thaliana* was significantly shortened (Liu et al. 2018).

Carbon nanomaterials have been shown to affect the microbial community of the rice rhizosphere and incur toxicity (Hao et al. 2018). In a study on tomato plants, treatment of soil with carbon nanotubes did not significantly alter the soil microbial community (Khodakovskaya et al. 2013). Among the various carbon nanomaterial, reduced graphene oxide resulted in the most significant changes to the microbial community. The antimicrobial activity of ENMs is well-known and the mechanism of their antimicrobial activity has also been extensively studied and reported. Mechanisms include: (i) bacterial cell membrane disruption; (ii) perturbation of metabolic functions such as purine metabolism; (iii) protein denaturation; (iv) DNA damage; (v) inhibition of respiration through disruption of the respiratory chain; (vi) free radical formation and induction of oxidative stress; (vi) mutagenesis; and (vii) inhibition of DNA replication through DNA binding (Khan et al. 2016). ENMs are not always microbicidal in their action but simply be inhibitory to specific microbial enzymes and processes. The toxicity of nanomaterials also depends on their inherent properties including shape, size, chemical nature, surface charge, and hydrophobicity. Furthermore, some microorganisms may be more sensitive to a nanomaterial than others. For example, some nanomaterials are more effective against Gram-positive bacteria than Gram-negative bacteria. The growth rate of bacteria and the ability to produce extracellular polysaccharides (EPS) also influence sensitivity of bacteria to engineered nanomaterials.

10.6 Effect of ENMs on Soil Microbial Processes

Many nanomaterials are known to possess microbicidal properties and hence can inhibit the proliferation of microorganisms involved in crucial biogeochemical processes such as nitrogen fixation, ammonification, denitrification, phosphate solubilization, and other plant growth promoting (PGPR) activities, thus inhibiting geochemical processes (Fig. 10.4). Study of the literature shows that ENM toxicity is reported usually at high concentrations. To the contrary, it has been demonstrated that certain nanomaterials occurring at low concentrations promote some biogeo-

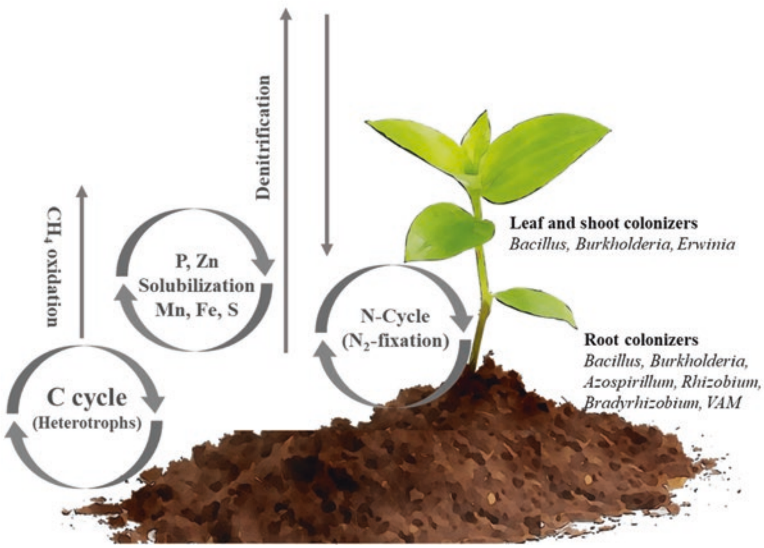

Fig. 10.4 Role of microorganisms in geochemical processes. Many of these microorganisms are free-living in soil while some live in close association with plants, playing an important role in nutrient cycling and consequently influencing plant growth and soil health

chemical processes, consequently promoting plant growth (Khan et al. 2018; Yuan et al. 2017).

Few reports are available on the influence of ENMs on reactions of one of the most important nutrients, i.e., carbon. Reduced graphene oxide (RGO) inhibit photosynthesis in pea plants, consequently affecting carbon fixation and biomass production (Chen et al. 2019). The RGO damaged the oxygen-evolving-complex on the donor site, consequently inhibiting the activity of photosystem II (PS II). This inhibition was attributed to oxidative stress induced by RGO. Various reports reveal increased biomass following treatment with nanomaterials at low concentration. For example, upon treatment with MWNCT (100 μg/kg soil), an increase in nitrogen fixation activity was observed, leading to an increase of biomass (Yuan et al. 2017). Microorganisms play an indispensable role in the nitrogen cycle from ammonification to nitrogen fixation to denitrification. Nitrogen fixation is one of the most important processes mediated by microorganisms, both free-living and symbiotic. The influence of ENMs on nitrogen fixation by both free-living and symbiotic microorganisms has been studied. In the presence of nano TiO_2 the growth rate of *Anabaena variabilis*, its nitrogen fixation rate, and rate of nitrogen storage were inhibited (Cherchi and Gu 2010). Time of exposure was found to be a more important factor than concentration of nanomaterial on microbial processes. The negative effects of copper nanoparticles on microbial carbon and nitrogen cycles has been reported (Simonin et al. 2018). In this study, however, the low ENM concentrations (0.1–1 mg/kg of soil) were not inhibitory to the process. Furthermore, denitrification was most sensitive to CuO-NPs. The presence of plants did not mitigate the effects of the nanomaterial. In another study, a high concentration of nano-CeO_2 inhibited nitrogen fixation in soybean (Priester et al. 2012). The nodulation frequency of *Medicago truncatula* by *Sinorhizobium meliloti* decreased in the presence of Zn, Ag, and Ti nanomaterials in soil (Judy et al. 2015).

Silver is one of the most widely studied nanomaterials due to its well-known microbicidal activity. Silver NPs inhibited the growth of *Azotobacter vinelandii*, a free-living nitrogen fixer (Zhang et al. 2018). The Ag NPs resulted in cell damage, inhibition of nitrogenase activity, oxidative stress and death by apoptosis. Toxicity of the Ag nanomaterials was found to be size-dependent. The influence of single-walled carbon nanotubes (SWCNTs), multi-walled carbon nanotubes (MWCNTs) and graphene oxide (GO) on legume-*Rhizobium* symbiosis (*Lotus japonicus* and *Mesorhizobium loti* MAFF303099) has also been reported (Yuan et al. 2017). It was observed that a low concentration of MWCNT (100 μg/ml) actually promoted nitrogen fixation activity of nodules and consequently increased plant biomass by 14–25%.

The effects of ENMs on various other PGPR microbial process including nitrification, denitrification, phosphate and potassium solubilization, and microbial protection of plants against diseases are also reported. Nitrification activity of *Nitrosomonas europaea* was inhibited by silver ions released from silver NPs (Radniecki et al. 2011). Exposure to 20 nm silver NPs resulted in compromised outer membranes and inhibition of ammonium oxidase activity. ZnO, SiO_2, TiO_2, and CeO_2 nanoparticles were tested for their activity against PGPR bacteria including *Azotobacter*, phosphate-, and potassium-solubilizing bacteria and their enzymatic activities (Chai et al. 2015). A rate of 1 mg/g ZnO and CeO_2 individually hindered thermogenic metabolism, reducing *Azotobacter* colony numbers and P- and K-solubilizing bacteria. These ENMs also inhibited activities of urease and catalase and decreased fluorescein diacetate hydrolysis activities. The activity of silica (SiO_2) and alumina (Al_2O_3) nanoparticles against plant growth promoting rhizobacteria, and other PGPR bacteria like *Pseudomonas fluorescens*, *Bacillus megaterium,* and *Bacillus brevis* have been studied. Nano Al_2O_3 particles were highly toxic to these organisms at 1000 mg/L (Karunakaran et al. 2014).

The effects of a metabolite of DDT, dichloro diphenyl dichloroethylene; *p*,*p*'-DDE, on plants was influenced by the presence of C60 fullerene nanomaterials (De La Torre-Roche et al. 2012). The level of the contaminant in soybean shoots decreased by 48% while in another case it increased. This study demonstrates that nanomaterials in soil may influence the uptake of various environmental pollutants.

10.7 Role of ENMs in Protecting Plants Against Pathogens

ENMs can influence plant-pathogen interactions. Nano-formulations of some pesticides, such as DMM [sodium dodecyl sulfate-modified photocatalytic TiO_2/Ag nanomaterial conjugated with dimethomorph] is already in use (Khot et al. 2012). Many studies have reported the inhibitory effect of ENMs to plant pathogens. The inhibitory effect can be due to the direct activity of ENMs or release of metal ions from ENMs, or to augmenting the activities of microorganisms that inhibit phytopathogens (Khan et al. 2018; Elmer and White 2018; Servin et al. 2015b). Silver is known for its antimicrobial activity; thus, its effect on various phytopathogens has been reported. The inhibitory effect of Ag NPs on phytopathogenic fungi that cause disease in ryegrass was evaluated (Jo et al. 2009). Ag NPs were found to reduce the growth of phytopathogenic fungi and were also found to reduce the disease in the plants. In another study, the ability of ENMs (Fe_2O_3, TiO_2, MWCNTs and C60) to inhibit tobacco mosaic virus and turnip mosaic virus was studied (Hao et al. 2018). It was observed that ENMs decreased the pathogenicity of these viruses by decreasing the coat proteins of the viruses by 15–60%. However, it is interesting to note that quite high doses were used for the study (50-200 mg/L) . A low dose (500 ng/mL) of ENMs (Ag, SiO_2, TiO_2, and ZnO) promoted the antifungal activity of *Pseudomonas protegens* CHA0 against *C. albicans* (Khan et al. 2018). Inhibition of *F. graminearum*, a plant pathogen, by ZnO nanoparticles in vitro and in vivo has been reported (Dimkpa et al. 2013). Treatment of wheat plants with ZnO NPs reduced *F. graminearum* infection, wherein a significant reduction in *F. graminearum* CFU was observed compared to control (Savi et al. 2015). Treatment with Zn nanoparticles did not harm the plant and the levels of zinc in wheat grains were within permissible limits. In another study the effect of six different carbon nanomaterials (SWCNT, MWCNTs, GO, RGO, C60, and activated carbon) against two phytopathogenic fungi (*Fusarium graminearum* and *Fusarium poae*) was evaluated

(Wang et al. 2014). Except for C_{60} and AC all nanomaterials inhibited the growth of the two fungi. Spores of these fungi were inactivated primarily through (i) deposition on the surface of the spores; (ii) inhibition of water uptake; and (iii) plasmolysis. In field trials the inhibition of *Colletotrichum* spp. (a phytopathogen which causes anthracnose) by Ag nanoparticles was demonstrated (Lamsal et al. 2011). Application of nanomaterials before the spread of disease resulted in significantly reduced infection of pepper plants by *Colletotrichum* spp. From the review of the literature discussed above, it is concluded that ENMs may help protect plants against phytopathogens. However, toxicity concerns remain to be evaluated carefully.

10.8 Conclusion

ENMs are released to the soil both intentionally and unintentionally without evaluating the risks involved. Among different ENMs that are released into the soil, the major ENMs are SiO_2,Titania (TiO_2) iron and zinc oxides (ZnO), and alumina (Al_2O_3). The unregulated and continuous use of ENMs has resulted in the release of tons of these ENMs to soil. Studies have estimated the annual changes in their concentrations in soil; concentrations depend on persistence combined with the cumulative additions of ENMs to soil. Little evaluation has been accomplished to date regarding the risks involved from such accumulation.

Upon reaching soil ENMs experience variable fates. Among these are interactions with soil and plants, and soil and plant microbiomes. Interaction with ENMs influences the soil microbiome, plant microbiome, and overall plant health. Microbiomes of bulk soil, and the plants vary from soil to soil and plant to plant and therefore cannot be generalized. Many studies have evaluated the potential toxicity of these ENMs on soil microorganisms and plants. Microorganisms play an important role in the biogeochemical cycling of nutrients and in affecting the overall health of plants as well. Unfortunately, many studies have evaluated only very high doses of ENMs such as mg/kg levels, while realistic concentrations are far lower. In many studies, almost no effect was observed at lower doses; some studies have shown growth promoting and other positive effects of ENMs on plants and the plant microbiome, resulting in improved plant health and productivity. Hence, the use of realistic doses should be considered as a criterion for conducting and publishing such studies.

It is becoming increasingly necessary to regulate the release of ENMs during production, use, and disposal following use, as continuous and increasing quantities of ENMs released may pollute soil to the point of severe consequences. Studies show the beneficial and plant growth promoting effect of ENMs, thus arguing for their use in agriculture. Few studies have investigated the simultaneous accumulation of ENMs in plants and the greater environmental consequences of their use. For example, if Ag promotes plant growth, it is also taken up by the plant. Continuously consuming plants having elevated levels of Ag or other ENMs may become a health concern. Therefore, the wise use of nanotechnology, keeping in view all applications and consequences, will ensure the sustainable use of nanotechnology in agriculture. The targeted use of those nanomaterials having a short life in soil is desirable. Biocompatible ENMs having a short lifespan in soil that can be easily recycled or removed from soil by natural processes, should be designed.

To conclude, ENMs are a boon for the soil ecosystem if concentrations are low and application is regulated. If uncontrolled release in the soil remains unabated, their presence in soil at high concentrations may result in serious environmental and public health hazards risking crop productivity, food security and the status of soil as a sustainable resource.

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