Soil enzyme activities as an integral part of the environmental risk assessment of nanopesticides

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Nanotechnology has extraordinary potential to improve agriculture, essential in the current scenario of increasing population and projected shortage of natural resources[1]. For example, nanopesticide formulations (NPFs) are showing economic and environmental advantages over the corresponding conventional formulations by better delivering and controlling the application of pesticides. This can result in lower application doses, increased crop yields and reduced contamination to soils[1,2]. Nonetheless, there is a paucity of information on the ecological and health implications of engineered nanoparticles (NPs), particularly NPFs, on terrestrial ecosystems. Before widely spreading NPFs in agricultural fields, it will be necessary to robustly evaluate their risks, including for regulatory purposes. One of the major challenges will be to determine whether the tools that are currently available for the risk assessment of more conventional pesticide formulations, including soil biochemical and microbial responses and determinations of total pesticide concentrations, would differ from those used for NPFs[2,3].

NPFs are generally composed of an active ingredient (AI) that is in contact with a NP, such as a polymeric or inorganic material[1,2]. In some cases, the NP itself can act as the AI with its own antimicrobial activity[1,4], such as Ag and chitosan-based NPs. Following their application to crops, NPFs may eventually reach soils and waters, where the AI can be released[2] and chemically transformed, leading to toxic effects to soil microbes. The mobility of the NPs will be affected by the properties of the soil (e.g., organic matter content, pH), as well as by interactions with the soil components (e.g., agglomeration, dispersion)[1]. Based upon the design of the NP and its response to stimuli, the AI will have different efficacy, release rates and risks[2], putting into question whether existing methods of risk assessment need to be refined. Global responses of soil health, such as the measurement of microbial composition and enzyme activities, are showing great potential to indicate environmental risks induced by these novel NPs[3,4], through their links to contaminant degradation and maintenance of the rhizobial microbial community.

Alterations of the soil enzyme activities, including those of the extracellular hydrolases, may affect agricultural systems through modifications in nutrient cycling and soil fertility[4]. Numerous important pathways implicating soil enzymes have been shown to be good indicators of the quality of the soil ecosystem and are likely to be affected by NPs (Fig. 1). For example, nanoscale $Cu(OH)_2$[3] and Ag[4] have been shown to negatively affect non-target soil microorganisms responsible for N, P, C cycling in soils. On a macroscopic level, the AI (particularly metals) can be assimilated by plants and biomagnified throughout the food chain. On a molecular scale, the enzymes can facilitate the breakdown of NPs, pesticides, detrital inputs, organic compounds and nutrients, which can be recovered and used by plants and other organisms. Therefore, within an integrated assessment of risk, soil enzyme activity measurements are likely to provide key information of the direct and indirect effects of the NPs on soil function[3].

In addition to enzyme activity measurements, major shifts in the diversity of the soil microbial community or the regulation of functional genes related to major microbial functions can serve as useful indicators of soil health[3,4]. Indeed, the composition of the microbial community and its diversity indices can be used as a complementary means to assess soil health. Nonetheless, interpretation of microbial community compositions acquired through 16S and 18S rRNA sequencing can be costly and challenging towards directly interpreting soil health due to the large number of phylogenic groups involved in specific soil functions. Functional genes could potentially be linked to soil functions and enzyme activities, but this would involve quantifying the expression of very large sets of genes for specific functions. In contrast, the determination of enzyme activities is generally sufficiently sensitive and analytically reliable to measure the stress induced by NPs in soils. Furthermore, temporal variations in enzyme activities distinct from no-treatment controls or conventional formulations can indicate early changes caused by the NPFs[3,4]. The low costs and simple instrumentation required to perform enzyme assays (i.e. spectrophotometer) make these analyzes highly accessible for all users of nanotechnology, allowing them to monitor soil quality and adjust agricultural practices, if required. Studies have also indicated good agreement between complex genomic analysis derived conclusions data and simple soil enzyme assay results in interpreting soil health[5,6].

In spite of the obvious benefits of using enzyme activities to evaluate soil quality, a number of caveats should be noted. The majority of available research has measured activities following short-term exposures to inorganic NPs[4], under laboratory conditions. Long-term field experiments involving chronic exposures to NPs will be necessary, especially for compounds with higher mobility or potential to accumulate in soils over time. Other factors that will require additional research include: the role of the soil type, soil management practices, application regime, dose and delivery on a field scale, target enzymes, presence of secondary products, climate impacts, climate change and the role of animals and livestock[2,3,7]. Activities are typically measured using absorbance or fluorescence spectroscopy, following hydrolysis of the respective substrates. Additional efforts are required to optimize the enzyme activity measurements in order to ensure the fewest possible analytical and spectral interferences due to the presence of the NPs; to rigorously measure NPs fate under representative conditions, including NPF concentrations; to clearly distinguish the effects of natural colloids from engineered NPs and to evaluate the mechanism of action of NPs on the metabolism of soil bacteria.

Although the measurement of enzyme activities has undoubtedly demonstrated its potential as a useful environmental indicator for these emerging contaminants[3,4], further research is needed to validate their use as an internationally accepted environmental indicator and to involve producers and users in scientific-industrial partnerships[7]. This knowledge will help contribute to the development of novel NPFs and robust regulatory approaches for ensuring that they safeguard soil microbiota, which will ultimately lead to positive environmental and economic impacts. Maintaining and protecting biodiversity, including microbiodiversity, has become an increasingly necessary field of research, which is essential for ensuring the functional resilience of soils and the proper management of natural resources. Nanotechnology clearly has the potential to improve agricultural sustainability[7]; however, without considering soil microbiota and enzymes as critical components in the assessment of ecological risk, the cost/benefit calculation for the use of NPFs will not be complete.

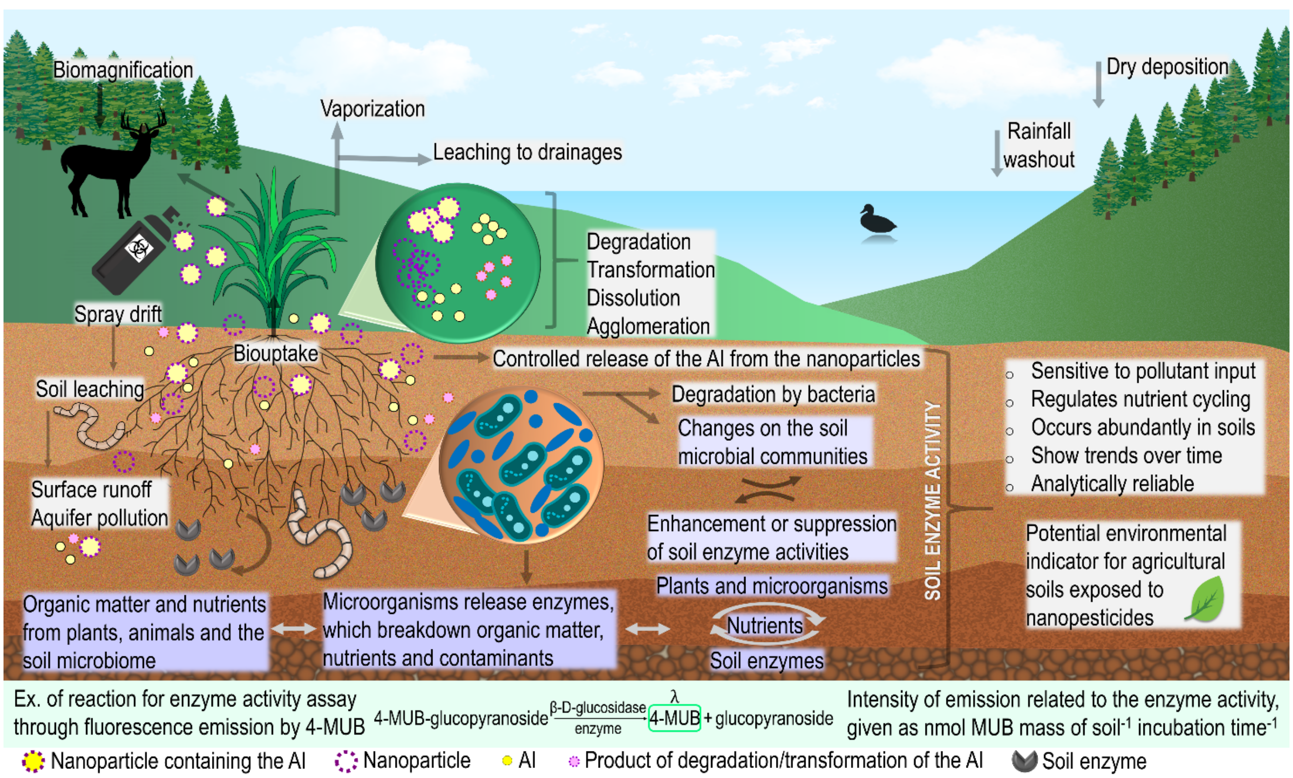

Fig. 1 – Schematic representation of some of the important pathways of an active ingredient (AI) encapsulated in nanoparticles in the environment and its interactions with soil enzymes.

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References

1. Kumar S, Nehra M, Dilbaghi N, Marrazza G, Hassan AA, Kim KH. Nano-based smart pesticide formulations: Emerging opportunities for agriculture. J Control Release. 2019;294(December 2018):131-153. doi:10.1016/j.jconrel.2018.12.012
2. Camara MC, Campos EVR, Monteiro RA, Do Espirito Santo Pereira A, De Freitas Proença PL, Fraceto LF. Development of stimuli-responsive nano-based pesticides: Emerging opportunities for agriculture. J Nanobiotechnology. 2019;17(1):1-19. doi:10.1186/s12951-019-0533-8
3. Zhang X, Xu Z, Qian X, et al. Assessing the Impacts of Cu(OH)2 Nanopesticide and Ionic Copper on Soil Enzyme Activity and Bacterial Community. J Agric Food Chem. 2020. doi:10.1021/acs.jafc.9b06325
4. Peyrot C, Wilkinson KJ, Desrosiers M, Sauvé S. Effects of silver nanoparticles on soil enzyme activities with and without added organic matter. Environ Toxicol Chem. 2014;33(1):115-125. doi:10.1002/etc.2398
5. Asadishad B, Chahal S, Akbari A, Cianciarelli V, Azodi M, Ghoshal S, Tufenkji N. Amendment of Agricultural Soil with Metal Nanoparticles: Effects on Soil Enzyme Activity and Microbial Community Composition. Environmental Science and Technology. 2018;52(4): 1908–1918.
6. Zhang H, Wang R, Chen S, Qi G, He Z, Zhao X. Microbial taxa and functional genes shift in degraded soil with bacterial wilt. Sci Rep. 2017;7:39911. doi:/10.1038/srep39911
7. Hofmann T, Lowry GV, Ghoshal S, Tufenkji N, Brambilla D, Dutcher JR, Gilbertson L, Giraldo JP, Kinsella JM, Landry MP, Lovell W, Naccache R, Paret ML, Pedersen JA, Unrine JM, White JC, Wilkinson KJ. Advancing Nanotechnology-Enabled Plant Agriculture Responsibly. *Nature Food*. 2020 (In press).