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The Susceptibility of Soil Microbial Metabolism and Diazotroph Functional Groups to Silica Nanoparticles

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The agro-ecological effect of silica nanoparticles on soil metabolism has been investigated by microcalorimetry with specific enzymatic tests (urease, catalase and fluorescein diacetate hydrolase, FDA) and diazothroph account. Three SNP doses (10, 25 and 50 mg kg-1) corresponding to each dimension (9, 12 and 40 nm) were used. The thermodynamic parameters obtained from the power-time curves showed an increase, with some exception, of total heat output, microbial growth rate constant (k) and heat output peak (Pmax) by increasing SNP dose, indicating a general stimulation of microbial populations. Urease and FDA activity, except catalase, showed similar positive size-dose response thus supporting microcalorimetric analysis. However, no direct effects on diazotrophs were found. The results confirmed the capability of SNP to maintain or enhance nitrogenous nutrient availability and to promote microbial deoxygenation of the soil microenvironments. SNP did not depress total microbial biomass (no changes in FDA) or impair plant nutrition and the diazotroph taxa.

1. Introduction

Engineered nanoparticles (NP) are now becoming a significant fraction of the material in global economy. Their application in the fields of material sciences, industrial engineering and medical care has increased in last few years owing to their special and novel physical and chemical properties (Tso et al., 2010, Keller et al., 2013; Wehling et al., 2013). The reduction of particle size to nanoscale dimension not only provides benefits to diverse medical and technological areas but also poses potential risks for human health and ecosystem safety once dispersed into the environment (Dominique et al., 2013, Arne et al., 2013). Now NP pollution is an increasing environmental problem and worldwide concern (Yotova et al., 2015; Feng et al., 2015; Wu et al., 2014; Li and Zhang, 2014). Silicon is the second most abundant element on the earth's crust, but silica nanoparticles (SNP) may be not beneficial for many organisms as their bulk (Schaller et al., 2013). Previous research on phytotoxicity of SNP showed that they were non-toxic (Lee et al., 2009). By contrast, serious damage was detected in pulmonary and angiocarpy of adult mice after inhalation of SNP (Chen et al., 2008). Disperse SNP caused cell membrane damage of *Saccharomyces cerevisiae* (García et al., 2011). SNP showed size-dependent toxicity for microorganisms which increased by decreasing particle sizes (Wehling et al., 2013). SNP were also found to exhibit size-dependent toxicity toward the alga (*Chlorella kessleri, Pseudokirchneriella subcapitata*) (Fujiwara et al., 2008, Van et al., 2011).

The abuse of NP will cause accumulation over time in the form of aggregates and colloids, producing an unpredictable anthropogenic waste in the agroecosystem (Thul et al., 2013). Emissions of NP into soils represent up to about a quarter of the material flows, mostly from disposal of biosolids to land, thus highlighting the importance of understanding the impact of NP on agriculture soils (Keller et al., 2013). Therefore, more data are required about the behavior of NP in soil and their interactions with microorganisms especially under intensive cultivation. Heat transfer in conjunction with other specific bio-tests has been successfully applied to assess the impacts of environmental hazardous pollutants on soil microbial metabolism (Mahmoudi and Mejri, 2015; Hongyang,2015; Biserni and Garai, 2016). In microcalorimetry, the heat evolution is positively correlated to the amount of glucose degradation, microbial biomass and activity in glucose-

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addition calorimetric assays (Kimura and Takahashi, 1985). In addition, developing new approaches to assess soil enzyme functional diversity is necessary for our comprehending of the relations between resource availability, microbial community structure and function, and ecosystem processes (Caldwell, 2005). As such, this study focuses on the effects of various sizes of SNP on microbial metabolism in agricultural soil. The thermogenic metabolic energy flux, enzymatic activities (urease, catalase, fluorescein diacetate hydrolase) and soil diazotroph number were systematically monitored as they are markers fertility and health.

2. Materials and methods

2.1 Soil analysis

The experimental loam soil was collected on the top (2–5 cm) of an arable field planted with winter wheat from Hebei Province, China. The soil was thoroughly sieved < 2mm, air dried and kept at 4 ºC in refrigerator before using. The properties of soil is pH7.2, sand/silt/clay 13.3/70.8/15.9, CEC (cmol kg^{-1}) 7.6, total N (μg g^{-1}) 0.8, total organic C (mg g^{-1})15.4, available P (μg g^{-1}) 13.2, available K (μg g^{-1}) 96.7, field capacity (v/v) 0.32.

2.2 Preparation of soil with nanoparticle

Three commercial SNP amorphous powders at *ca.* 9, 12, 40 nm with > 99.9% purity were obtained from Boyugaoke Inc. (Beijing, China). The surface area were 300 ± 30 m^2 g^{-1} (9nm), 200 ± 20 m^2 g^{-1} (12 nm) and 110 ± 25 m^2 g^{-1} (40nm), respectively. The size of SNP were determined by scanning electron microscopy and the surface area of SNP was examined using the BET method.

SNP were suspended in distilled water and sonicated to achieve a homogeneous mixture before adding to the soil samples in a test-microcosm. Each microcosm consisted of 50 g of soil in 200 mL sterile plastic bottles and NP mixtures were added to achieve a final concentration of 10, 25, 50 mg kg^{-1}, respectively. Microcosm without SNP was used as control. Three triplicates of each microcosm were incubated at 25 °C for one month and soil moisture was adjusted at 25% of water holding capacity by adding sterile deionized water.

2.3 Microcalorimetric analysis

An isothermal TAM III multi-channel microcalorimeter (TA instruments, New Castle, DE, USA) was used to record the metabolic thermogenetic flux curves. Each 4.0 ml stainless steel ampoule was loaded with 1 g soil containing SNP. Then 200 μL nutrient solution containing 5.0 mg glucose and 5.0 mg ammonium sulphate was added to the soil. The calorimetric parameters such as total heat output (Q_{total}), microbial growth constant (k), peak time and the height of peak was calculated from the power-time curves. Q_{total} was computed by integrating the power-time curves. A classical equation is used to fit the exponential growth phase, $\ln P_t = \ln P_0 + kt$, where t is the time, P_t is the output of power at time t, and the P_0 is the power at the initial stage of the exponential growth (Guo et al., 2012).

2.4 Measurement of soil enzyme activity and diazotroph counting

The activity of urease was measured using colorimetric determination of ammonium released from urea hydrolysis (Zhuang et al., 2011, Guo et al., 2012). Catalase activity was determined by back-titrating residual H_2O_2 with 0.1 mol L^{-1} $KMnO_4$ solution (Du et al., 2011). The FDA hydrolytic activity was carried out at 490 nm as absorption of the hydrolysis product fluorescein (Schnürer and Rosswall, 1982). Soil samples (5 g) were placed in Erlenmeyer flasks containing sterile water for shaking (180 rpm, 30 min) and followed by continuous dilutions for plate counting. Viable counts of soil diazotroph were performed on Ashby Mannitol Phosphate Agar incubated 28 ºC for 7 days. All bioassays conducted with materials were in accordance with national and institutional guidelines for the protection of human subjects and animal welfare.

3. Results and discussions

3.1 Dose-size effects of silica nanoparticles

Figure 1 depicts the thermal output curves of soil samples containing various concentrations and sizes of SNP. Two major peaks (P_{max1} at the time P_{t1} and P_{max2} at the time P_{t2}) were observed in the thermograms. These peaks showed a tendency to appear at shorter times with the increase in SNP concentration and even shorter than the untreated controls. However, no significant differences of P_t were observed by changing size of SNP. In general, P_{max1} is smaller than P_{max2}. Both P_{max1} and P_{max2} increases with the concentration of SNP. SNP is likely to express indirect positive effects on microbial metabolism, acting as a biochemical concentrator of substrates at the surface (Dinesh et al., 2012), thus, allowing dormant populations to benefit from the enriched surrounding habitat to grow faster than the control. Q_{total} increases with the increase in the concentration of SNP whereas k does not change much with the increase in dose and size of SNP. The average relative values of P_{max1} showed a regular increase with increasing SNP size; P_{max2}, even showing values higher than P_{max1}, also showed a flattening at 9-12 nm and a dropping in the 40 nm test. The percent increase of P_{max1} and P_{max2} had the same order of magnitude at 9-12 nm, while at 40 nm size showed an

opposite trend, *i.e.*, maximum stimulation at short time P_{t1} (8-10 h) and lower metabolic rates at longer P_{t2} (30 h) time, comparatively to the percent increase control.

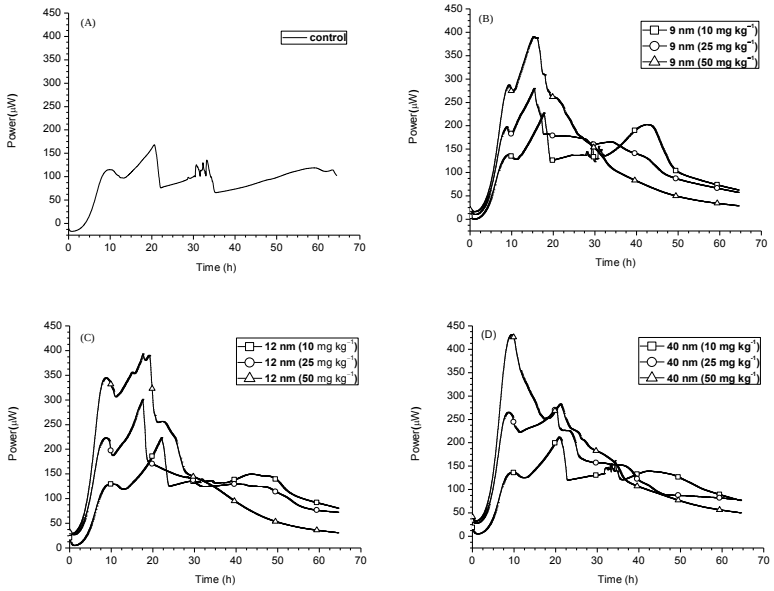

Figure 1: Thermogenesis metabolism curves of soil microorganism spiked with various sized of silica nanoparticles: (A) control, (B) 9, (C) 12, and (D) 40 nm

Kinetics of glucose degradation is proportional to kinetics of microbial growth (Castello et al., 2014). For this reason, the microbial growth rate constant *k* is considered as the apparent degradation rate of the substrate (Barja and Núñez, 1999). Therefore, *k* was regarded as a sensitive parameter expressing minor changes in the mean life adaptability of the many microbial populations and in their response to the stressing effects caused (Koga et al., 2003). It has been found that generally NP with different sizes caused different environmental effects at equivalent concentrations (Gao et al., 2008). This variability in microbial activities of the soil might have been caused by superoxide and other reactive oxygen species (Yang et al., 2010). SNP were found to exhibit size-dependent toxicity toward the alga (Fujiwara et al., 2008). It also has been demonstrated that the cytotoxicity of SNP with the same morphology was strongly related to the particle size (Napierska et al., 2009). Most of studies have proved that metal oxide NP display effect of size-dependent effectiveness and efficiency on bacteria (Ma et al., 2013).

3.2 Enzymatic activities

Soil enzyme activity is considered as direct indicators of the soil community to metabolic demand and available nutrients. It has been argued that maintaining critical functions may ultimately be more important than maintaining taxonomic diversity in soil microbial communities (Caldwell, 2005). Urease activity in soil originated from soil microbes containing urease and approximately 17-77 % soil bacteria and 78-98 % soil fungi have capacity to hydrolyze urea into ammonia. Table 1 displays the effect of SNP on the soil urease activity. It is found that the soil urease activity significantly ($p < 0.05$) increases with increase concentration of SNP but to a lesser extent with the increase in particle size. The highest urease activity was found in coincidence of the highest SNP dose and size, contrarily to the trend observed for the thermodynamic parameters P_{max2}. It seems that the soil catalase activity does not affect too much by the dose of SNP ($p > 0.05$). There is also not much difference in the soil catalase activity for difference sizes of SNP. Since catalase is not affected by the size and dose of SNP, it could be a good candidate for assessing oxidation–reduction potential of stressed soils. By the contrast, FDA is significantly stimulated by SNP concentration ($p < 0.01$) at all sizes. FDA shows higher activity with the increase in the concentration of SNP. The trends are similar for each type of SNP. At 10 mg kg^{-1} SNP, the effect is very small and is not significantly different ($p > 0.05$) from that of the control. Our results are different to that of ZnO NP which can inhibit soil protease, catalase, dehydrogenase, phosphatase and peroxidase (Du et al., 2011, Kim et al., 2011).

3.3 Viable counting of soil diazotroph

Soil diazotroph plays a critical role in the nitrogen enrichment for plant growth as promoters of nitrogen fixation and stimulators of the nitrogen cycle in soils, which is also a good criterion for assessing soil ecosystem quality and plant health. These bacteria can contribute to 10-50 % of the total N requirement of wheat

(Kennedy and Islam, 2001). Figure 3 depicts the effect of SNP on soil diazoroph. The colony forming units of soil diazotroph are not affected by the SNP. There is no sign of inhibition or stimulation effect of SNP in terms of size and concentration on the number of diazotroph bacteria. However, other soils contaminated with ZnO and TiO_2 NP resulted in the reduction of N-fixation capacity of soils N-fixing microorganisms (Priester et al., 2012). Several studies reported other NP show inhibition effect on the N-cycling bacteria and N-fixation rate of aquatic organism (Yang et al., 2013). It is obvious that our SNP do not exhibit inhibition effect on the soil microbes which are different from other types of NP.

Table 1: Effect of silica nanoparticles on the soil enzyme activities (A) urease, (B) catalase and (C) FDA hydrolysis (Urease, mg NH^{4+}-N g^{-1}soil 24 h^{-1}; Catalase, mL $KMnO_4$ g^{-1} soil 0.5 h^{-1}; FDA Hydrolysis, mg Fluorescein g^{-1} soil 0.5 h^{-1})

	Size	control	10 mg kg^{-1}	25 mg kg^{-1}	50 mg kg^{-1}
Urease	9 nm	0.98	0.97	1.03	1.18
	12 nm		1.03	1.08	1.17
	40 nm		1.18	1.20	1.25
Catalase	9 nm	1.14	1.14	1.17	1.23
	12 nm		1.13	1.16	1.19
	40 nm		1.13	1.18	1.22
FDA Hydrolysis	9 nm	13.01	13.74	16.25	19.65
	12 nm		13.44	19.27	20.58
	40 nm		14.28	18.48	19.86

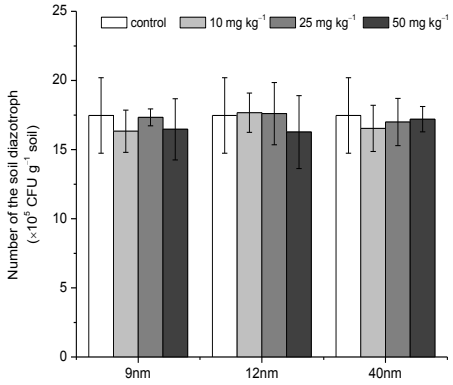

Figure 3: Effect of silica nanoparticles on the soil diazotroph

4. Conclusion

Microcalorimetry and biomarkers of metabolic processes related to soil fertility, *i.e.*, urease, catalase, FDA, and diazotroph are used to estabilsh an efficient and fast screening methodology for NP. The reason for strengthening microcalorimetry with additional biomarkers, arises from the general knowledge and the wide scientific consensus that the total thermal effect observed under microcalorimetric tests is the result of the catabolic degradation of a substrate with little anabolic reactions contribute to the final state of equilibrium. The combination of easily executable, efficient and reproducible analytical tests such as the enzymatic assays and microcalorimetric analysis that gives a continuous and longer monitoring of the metabolic processes, are very promising for revealing many and still unknown reasons of microbial biomass activity and structure. Such methodology, before being systematically applied in many agro-ecosystems, must be affined and validated on a wide range of cultivated and uncultivated soils. It has been demonstrated that the effect of SNP is strongly related to the dose and particle size but smaller SNP only showed minor effects.

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