Soil pH effects on the interactions between dissolved zinc, non-nano- and nano-ZnO with soil bacterial communities

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Soil pH effects on the interactions between dissolved zinc, non-nano and nano ZnO with soil bacterial communities

Daniel. S. Read[1*], Marianne Matzke[1*], Hyun S. Gweon[1], Lindsay K. Newbold[1], Laura Heggelund[1,2], Maria Diez Ortiz[1,3], Elma Lahive[1], David Spurgeon[1], and Claus Svendsen[1].

[1]NERC Centre for Ecology & Hydrology, Benson Lane, Crowmarsh Gifford, Wallingford, OX10 8BB, UK.

[2]Department of Environmental Engineering, Technical University of Denmark, Miljoevej, building 113, 2800 Kgs. Lyngby, Denmark.

[3]LEITAT Technological Center, C/ de la Innovació, 2, 08225 Terrassa (Barcelona), Spain.

*Joint first authors.

Correspondence: Dr D.S. Read, E-mail: dasr@ceh.ac.uk

Abstract

Zinc oxide nanoparticles (ZnO NPs) are used in an array of products and processes, ranging from personal care products to antifouling paints, textiles, food additives, antibacterial agents and environmental remediation processes. Soils are an environment likely to be exposed to manmade nanoparticles due to the practice of applying sewage sludge as a fertiliser or as an organic soil improver. However, understanding on the interactions between soil properties, nanoparticles and the organisms that live within soil is lacking, especially with regards to soil bacterial communities. We studied the effects of nanoparticulate, non-nanoparticulate and ionic zinc (in the form of zinc chloride) on the composition of bacterial communities in soil with a modified pH range (from pH 4.5 to pH 7.2). We observed strong pH dependent effects on the interaction between bacterial communities and all forms of zinc, with the largest changes in bacterial community composition occurring in soils with low and medium pH levels (pH 4.8 and 5.9). The high pH soil (pH 7.2) was less susceptible to the effects of zinc exposure. At the highest doses of zinc (2500 mg/kg dw soil) both nano and non-nano particulate zinc applications elicited a similar response in the soil bacterial community, and this differed significantly to the ionic zinc salt treatment. The results highlight the importance of considering soil pH in nanotoxicology studies, although further work is needed to determine the exact mechanisms controlling the toxicity and fate and interactions of nanoparticles with soil microbial communities.

Introduction

Zinc oxide nanoparticles (ZnO NPs) are amongst those with the highest production volume and therefore a high environmental release potential. ZnO NP applications range from personal care products (e.g. sun screen, toothpaste) to antifouling paints, textiles, food additives, antibacterial agents and environmental remediation processes (Lopes et al. 2014). As a result, sewage treatment works have been identified as having a high potential burden, with sludge containing up to 17.1 mg/kg dry weight (dw) followed by sewage sludge treated soils with 3.25 mg/kg dw (compared to average soil concentrations predicted to be around 0.093 mg/kg dw) (Gottschalk et al. 2009). However these values are likely to further increase with the increasing use of ZnO NPs in consumer products and processes.

Single species studies have highlighted varying sensitivity of bacteria to zinc nanoparticles (Baek &An 2011, Brayner et al. 2006, Dimkpa et al. 2011, Hernandez-Sierra et al. 2008, Jiang et al. 2009, Jones et al. 2008, Mortimer et al. 2008, Negi et al. 2012). These studies have been used to provide an insight into the mechanisms of nanoparticle toxicity, and have indicated the role of reactive oxygen species (ROS) (Choi &Hu 2008, Kumar et al. 2011b, Lu et al. 2012), DNA damage (Kumar et al. 2011b, a), oxidation of proteins, interruption of energy transduction and photo catalytic oxidation (Gou et al. 2010, Klaine et al. 2008). Although single species studies are highly informative in terms of modes of toxic action, they lack the complexity of natural systems comprising hundreds to thousands of interacting species (Roesch et al. 2007). Soil microorganisms form the base of soil ecosystems as they are the key players of many soil functions such as biogeochemical cycling, plant productivity and climate regulation. Estimated numbers are in the range of 10^9 cells/g soil with a diversity of 10^4 species (Griffiths and Philppot, 2013). Including environmentally more realistic systems into the hazard assessment of nanoparticles will consequently lead to a more complete environmental risk assessment of engineered nanomaterials.

Previous work has focused on wastewater systems, identified as potential accumulators of engineered nanoparticles, has highlighted effects on ecological functioning including methane production, nitrogen and phosphorous removal, the production of extracellular polymeric substances (EPS) and protease activity (Choi &Hu 2008, Mu &Chen 2011, Mu et al. 2011, Mu et al. 2012, Musee et al. 2011, Zheng et al. 2011). The toxicity of nanoparticles in natural systems has been shown not only to be species dependent, but reliant on various factors to do with the properties of the nanoparticles themselves (e.g. nanoparticle composition, size, surface area and shape), but also their interaction with the environment (pH and availability of natural organic matter) (Musee et al. 2011). Studies investigating the impact of nanomaterials on soil microbial communities are still relatively scarce due

to the complex nature of the topic. However, they do include a diverse range of nanomaterials such as silver, cerium oxide, iron oxide, copper, carbon based nanomaterials, silica, zinc and titanium (Ge et al. 2011, Kumar et al. 2011b, a) in a wide variety of measured endpoints including; diversity and modification of the community composition, substrate induced respiration, DNA quantity or impact on enzyme activities. The response of soil microbial communities to the addition of zinc oxide (ZnO) nanoparticles has been shown to be highly varied and dependent upon the properties of the systems and the form of zinc that is applied. These include reductions in both microbial biomass and diversity (Ge et al. 2011), growth inhibition (Rousk et al. 2012) and species-specific responses to zinc exposure (Collins et al. 2012, Ge et al. 2012).

While some variation in biotic responses may arise from the varying composition of microbial communities being tested, it is becoming apparent that variation also arises from differences in both the nanoparticles being used and how different exposure media affects the speciation and characteristics of NPs once in the soil environment. As well as the characteristics of NPs (e.g., size, shape, surface charge), other soil properties including organic matter (Coutris et al. 2012, Waalewijn-Kool et al. 2014), clay contents (Fang et al. 2009), soil moisture (Ge et al. 2013) and the presence of plants (Ge et al. 2014) have been shown to affect physical and chemical processes, resulting in differing rates of NP dissolution, agglomeration, and aggregation (Tourinho et al. 2012). However, soil pH appears to be one of the key factors affecting the behaviour of metal NPs in soil and recent work has shown the role this plays in NP dissolution, mobility and bioavailability to soil organisms (Heggelund et al. 2014, Waalewijn-Kool et al. 2013).

The aim of this study was to elucidate the role that soil pH plays on mediating the effect of zinc on soil bacterial communities, and; whether the form in which zinc is dosed (nanoparticulate zinc oxide, non-nanoscale zinc oxide and ionic zinc dosed as the chloride salt) influences soil bacterial community composition.

MATERIALS AND METHODS

Chemicals

Two zinc oxide particle forms were used in these experiments; uncoated 30 nm ZnO nanoparticles (ZnO NP) and 200 nm ZnO particles (ZnO P) (both Microniser Pth Ltd, Dandenong, Australia). Both the 30 nm ZnO NPs and 200 nm ZnO particles (hereafter referred to as ZnO NP and ZnO P) had no coatings or surface modification and were white odourless dry powders with close to spherical shaped particles. Primary particle sizes were verified by TEM (JEOL 2010 analytical TEM operating at 200kV). The hydrodynamic diameter of the particles in the stock suspension (nominal concentration 7.5 mg Zn/ml) and the zeta potential were determined by Dynamic Light Scattering (DLS) and Laser Doppler Electrophoresis (LDE) respectively using a Malvern Zetasizer Nano ZS. Full details and results of particle characterisation can be found in Heggelund *et al* (2014) which reports all the details of the exposures from which samples for microbial community analysis were taken (prior to the addition of earthworms). The ionic zinc reference material used in these experiments was zinc chloride ($ZnCl_2$) (BHD Chemicals, Poole, UK).

Soil and experimental setup

The experimental procedures are fully described in a previous study investigating the toxicity of ZnO NPs and particles as well as $ZnCl_2$ to the earthworm *Eisenia fetida* in a single soil at varying pH levels (Heggelund et al. 2014). In brief, soil was collected from Wareham forest (Ordnance Survey Grid Reference: SU108058, Dorset, United Kingdom) and processed by removing large roots, homogenised and sieved through a 5-mm mesh followed by air drying (initial moisture content was approximately 14% w/w). A summary of the soil properties can be found in Table S1. Soil pH was adjusted by the addition of 0.2%, 0.45% and 1% w/w calcium carbonate (CaCO3, Sigma Aldrich) to give pH values of 4.5, 5.9 and 7.2 (range ± 0.3 pH units) referred to hereafter as the low-, medium- and high- pH soils, respectively. 450 g dry weight of the respective pH soil was added to the test container (1 L glass Kilner jars). The soils were spiked with ZnO NP, ZnO P and $ZnCl_2$. The concentration range for the ZnO NP and $ZnCl_2$ treatments was 238, 381, 610, 976, 1520, and 2500 mg Zn/kg dw soil and the ZnO P treatment was 381, 976, and 2500 mg Zn/kg dw soil, with four replicates at each concentration at each of the three pH levels. An additional ten control soils with no zinc treatment were set up for each pH (total 210 containers). The ZnO particles and zinc chloride were dispersed in soil solution at a high concentration before being added to the soil to give the appropriate zinc concentration in the soil. After spiking, water was added to the soil bringing the moisture content to 45% of the water holding capacity of the soil. Soils were incubated for seven days at 20 °C. At the end of the seven day period, soils were homogenised and 2 ml tubes were filled with soil from each container and stored at -80^{o}C for molecular analysis.

Pore water concentrations

Zinc concentrations in the soil pore water were calculated in a parallel set of 200 g dry weight of soil of each pH, spiked with each form of zinc as described in Heggelund *et al*. (2014). This was done for all ZnO treatments and for three of the six $ZnCl_2$ treatments (381, 987 and 2500 mg Zn/kg). After dosing, the soils were saturated with MilliQ water and left for 10 days to equilibrate. Subsequently the soil pore water was extracted by centrifugation at 2482 g for two hours, and the supernatant centrifuged at 18,330 g for a further hour to minimise the number of remaining soil particles. The centrifuged soil pore water extracts were acidified by addition of 1 ml/ml 1 M nitric acid and zinc concentration in the pore water analysed by Atomic Absorption Spectroscopy (AAS) (Perkin Elmer 1100B).

Molecular assessment of bacterial composition

Soil DNA extractions were carried out from 0.25 g of homogenised soil from all 210 containers. Total nucleic acids were extracted from the samples using the PowerSoil®-htp 96 Well Soil DNA Isolation Kit using standard manufacturer instructions (MO BIO Laboratories, Inc). Approximately 20–30 ng of purified template was used per PCR. For T-RFLP analysis, a ≈500 bp region of the 16S small subunit ribosomal RNA (SSU rRNA) was amplified using 6-FAM labelled forward primer 27F (3'-AGAGTTTGATCMTGGCTCAG) and reverse primer 536R (Suzuki et al. 1998). Thermal cycling conditions were as follows: initial denaturation at 94°C for 2 min followed by 30 thermal cycles of 94°C for 1 min, 52°C for 1 min and 72°C for 3 min. The PCR products were gel-purified using a QIAquick Gel Extraction Kit (QIAGEN) and 20 µl of product was digested for 4 h at 37°C in a 30 ml total reaction volume using 20 units restriction enzyme MspI (Promega). Digestion products (0.5 µl) were combined with denatured 0.5 µl LIZ600 size standard (Applied Biosystems) and 8.5 µl of Hi-Di formamide (Applied Biosystems) analysed on an Applied Biosystems 3730 DNA sequencer and the sizes of restriction fragments were calculated. Binning analysis was performed using Genemarker (Softgenetics).

A subset of samples representing two replicates of each control soil at each of the three pH levels, and one sample from each treatment (NS30, NS200, and ZnCl) at each pH level (4.8, 6.0 and 7.0) were characterised in more detail using 454 Next generation Sequencing (NGS). Briefly, pyrosequencing was carried out at Molecular Research LP, (Lubbock, Texas, USA). Microbial tag encoded FLX amplicon pyrosequencing (TEFAP) was conducted using 16S V1-V3 spanning primers Gray28F GAGTTTGATCNTGGCTCAG and Gray519r GTNTTACNGCGGCKGCTG. Generation of the sequencing library used a one-step PCR with for 30 cycles, a mixture of Hot Start and HotStart high fidelity taq

polymerases, and amplicons originating and extending from the forward primers. Pyrosequencing analyses utilized Roche 454 FLX instrument with Titanium reagents.

Data analysis

All statistical analyses were carried out in R (v.3.0.1) (R Core Team 2013) using the package 'Vegan' v2.0-10 (Oksanen et al. 2013). To visualise the relationship between TRFLP profiles from soils with different treatments we used Non-Metric Multidimensional Scaling (NMDS) using the 'metaMDS' function, based on dissimilarities calculated with the Bray–Curtis index. Proximity of the points on the NMDS plot characterises the between sample similarity of bacterial communities, with points close together representing similar communities and points further apart representing dissimilar bacterial communities. NMDS was initially used to examine the overall effect of pH modification on soil bacterial communities. Additionally, bacterial diversity, as measured using Shannon's diversity Index (H') on the TRFLP peaks was examined for each pH class using the control samples. Bray-Curtis distances were plotted against zinc dose to examine community dose response relationships. The function 'Adonis', which uses a permutational analysis of sums of squares using semi-metric and metric distance matrices, was used to test for statistically significant differences between the bacterial communities in the control soils and treated soils. The F-statistic was plotted on a scatterplot to visualise the scale of the effect against the dose of ionic, particulate and nano zinc.

For the 454 data, sequence processing, denoising, error checking and clustering was carried out as described in Read *et al.* (2015). The taxonomy of OTUs was determined by RDP Classifier with 80% bootstrapping classification confidence (Wang et al. 2007) using the Greengenes Oct 2012 database (McDonald et al. 2012).

RESULTS AND DISCUSSION

Material characterisation

Material characterisation TEM analysis indicated that the particles were spherical and relatively monodisperse in the case of ZnO NPs. The non-nanoscale material (ZnO P) contained a higher proportion of faceted rod-shaped material. Images use for characterisation of the particles in distilled water and dosing solution are presented in Heggelund *et al.* (2014). The average primary particle diameters of the ZnO material batches as measured by TEM were 29.8 nm ± 9.4 (mean ± standard deviation) for the ZnO NPs and 300 nm ± 164 (length) and 188 nm ± 102 (width) for the non-nanoscale ZnO (Waalewijn-Kool et al. 2012).

Effect of soil pH on bacterial community composition

Soil pH has previously been shown to strongly correlate with bacterial community composition and diversity, with a positive relationship between pH and diversity observed in both natural soils (Fierer & Jackson 2006, Griffiths et al. 2011) and those with artificially modified pH ranges (Bartram et al. 2014, Rousk et al. 2010). Although the exact mechanism behind this is not known, it has been speculated to be due to bacterial taxa exhibiting relatively narrow growth tolerances to pH (Rousk et al. 2010). Despite this appearing to be a fundamental rule determining microbial composition in soils worldwide, little research has been done comparing resistance and resilience of bacterial communities across this range. In the current study, modification of soil pH resulted in a predictable shift in community composition, with an increase in Proteobacteria and reduction in Acidobacteria with increasing pH (Figure 1). An NMDS ordination (Figure 2A) of the control (no zinc addition) communities showed that increasing soil pH through the addition of calcium carbonate (from a starting pH of ≈ 4.0) to values of 4.5, 5.9 and 7.2, caused shifts in bacterial community composition along Axis 1. This was accompanied by changes in the diversity of TRFLP peaks (Shannon H' Index), where increasing the soil pH resulted in increased measured diversity (Figure 2B), in agreement with previous studies (Fierer & Jackson 2006, Griffiths et al. 2011).

Having established three pH modified bacteria communities, we wished to examine two aspects of the response of soil bacterial communities to zinc exposure; 1) the interaction between soil pH and zinc exposure on soil bacterial communities, and; 2) whether zinc form (nanoparticulate non-nanoscale and ionic) influences the response of soil bacterial communities to exposure.

The relationship between bacterial communities in each treatment (ZnO NP, ZnO P and $ZnCl_2$) in relation to treatment dose and soil pH was examined using a matrix of NMDS ordination plots (Figure 3). Exposure to zinc in all forms caused changes in the bacterial community when compared to the control samples, illustrated by the increased distance between the treatment and control points. Generally, there was a higher change in zinc exposed communities in low and medium pH soils (pH 4.5 and 5.9) versus the high pH soil (pH 7.2). This change is quantified in Figure 4, which shows the between group (control and treatment dose) F-values from an Adonis analysis. Higher F-values at higher dose concentrations indicate a larger effect of zinc on the bacterial community composition. A dose-response can be observed in the low and medium pH soils, where higher doses generally result in higher changes in community composition. However, in the high pH soil this relationship breaks down, and the community is relatively insensitive to zinc addition. These results are also reflected in an examination of community dissimilarity using Bray-Curtis distances, where increasing dose increases dissimilarity between samples (Supplementary figure S1). Possible explanations for this

pattern are twofold. Firstly there is a known interaction between pH and nanoparticle dissolution (Zhang et al. 2010). Our measurements of soil pore water zinc concentration indicated that pH controls zinc dissolution and availability, with zinc pore water concentrations being higher in lower pH soils (Figure 5). This relationship has been observed to drive similar differences in effects in previous nanotoxicity studies with invertebrates (Waalewijn-Kool et al. 2013) and is due to more acidic conditions generally favouring dissolution of ZnO and release of Zn ions (Miao et al. 2010). However, the availability of zinc (as measured by pore water concentration) does not completely explain the patterns in bacterial community composition. For example, zinc pore water concentrations in the ZnO NP treatment did not vary between the pH 5.9 and pH 7.2 treatments (Figure 5A), but there was a much larger change in community composition with increasing zinc dosage in the pH 5.9 soil compared to the pH 7.2 soil (Figure 4A). This means there may be a specific response to nanoparticles, and that this is linked to pH in a manner that does not just involve particle dissolution. One explanation is that pH has previously been observed to influence nanoparticle zeta potential and this has been related to nanotoxicity due to interactions with cell membranes (Berg et al. 2009, Cho et al. 2012, Schwegmann et al. 2010).

The second explanation is that pH-specific bacterial communities may vary in their resistance to zinc, with low pH communities being inherently more susceptible to Zn exposure than high pH communities. Our results indicated that the lower pH communities had a lower diversity, as measured using Shannon's H index on the TRFLP peaks. Although there is some debate as the validity of diversity indices as an absolute measure of diversity when used on TRFP data (Blackwood et al. 2007), these pH driven patterns are in agreement with large scale sequencing based assessments of soil bacteria diversity (Fierer & Jackson 2006). If the lower pH soils do have a lower diversity, it may explain reduced resilience to disturbance, based on lower levels of functional and taxonomic redundancy. We are unaware of any studies that address this question directly, although bacterial communities across soil pH ranges have previously been shown to vary in their functionality, including growth and rates of biogeochemical cycles (Fernandez-Calvino et al. 2011, Kemmitt et al. 2006). The design of this study, and the nature of zinc solubility in soil means that it is not possible to unravel the relative contribution of the role of pH on zinc dissolution and bacterial community structure, but it would make a valuable topic for future research.

Effect of Zinc form on microbial communities

Finally, we wished to address the question as to whether there was a specific microbial response related to the form of zinc (ionic, nanoparticulate or particulate). Previous studies have highlighted that the physical characteristics of nanoparticles have a major influence on toxicity, mainly focussing

on NP size (Coradeghini et al. 2013, Gliga et al. 2014), but also shape (Hua et al. 2014) and surface chemistry (Albanese et al. 2012). Size-dependent toxicity is thought to occur due to direct interactions between nanoparticles and cells, with smaller particles allowing for a higher contact rate with cell membranes (Nair et al. 2009), but also higher rates of dissolution in the environment and release of ionic metal (Ma et al. 2012). Additionally, there has been debate over whether nanoparticles are inherently toxic or that toxic mechanisms are solely linked to the release of the ionic metal.

In the present study, we observed ionic and particulate specific responses in soil microbes across all doses (Supplementary Figure S2), although this was the most pronounced at the highest dose of 2500 mg Zn/kg dw soil (Figure 6). As before, it can be seen that all forms of Zn caused the communities to change in structure compared to the control soils, regardless of soil pH. However, the bacterial communities after exposure to particulate zinc (either nano or micro-scale) clustered together, and differed to those in the dissolved zinc salt treatments. One explanation is that the addition of ionic zinc in the form of zinc chloride resulted in far higher soil pore water concentrations (ranging from 90.4 – 809 mg/L) compared to either of the particulate zinc treatments (6.93 – 14.8 mg/L) (Figure 5). The results also showed that there was little difference in the nanoparticulate (NS30) and micro-scale (NS200) zinc treatments at the highest doses, indicating that there is no clear evidence of toxicity directly related to the presence of nanoparticles rather than non-nanoparticulate zinc.

Conclusions

Determining nanotoxicity in soils is complex, largely because of the numerous ways in which nanoparticles can interact with their environment, including through dissolution, agglomeration and the addition of environmentally obtained surface coatings, all of which have been shown to modify toxicity in model systems (Albanese et al. 2012). This is further complicated by the fact that interactions with organisms can further modify the properties, and hence toxicity of nanoparticles in the environment. For example, many bacteria produces extracellular polymeric substances (EPS) and exudates that have been shown to modify nanoparticle behaviour (Duster & Fein 2014, Kroll et al. 2014). Our study was designed to investigate the interactions with pristine nanoparticles added direct to soil, a situation that is unlikely unless due to industrial contamination or spillage. A far more likely route of nanoparticle transmission is through sewage works, and the addition of sewage sludge to soils. Nanoparticle have been shown to exhibit a wide range of chemical transformations in this scenario (Lombi et al. 2013, Ma et al. 2014) changing many of the properties, such as size, shape and surface coatings, that determine toxicity. Because of this, further work should focus on environmentally relevant routes of exposure and determine the impacts on microbial communities over a range of timescales.

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8

Figure legends

Figure 1. Phylum-level bar chart showing the representative bacterial composition of the control (no zinc) and treated soils exposed 2500 mg Zn/kg dw soil of 30 nm nanoparticulate zinc (ZnO NP), non-nanoscale particulate zinc (ZnO P) and Zinc chloride ($ZnCl_2$) at each pH (4.5, 5.9 and 7.2) from 454 16S rRNA gene sequencing.

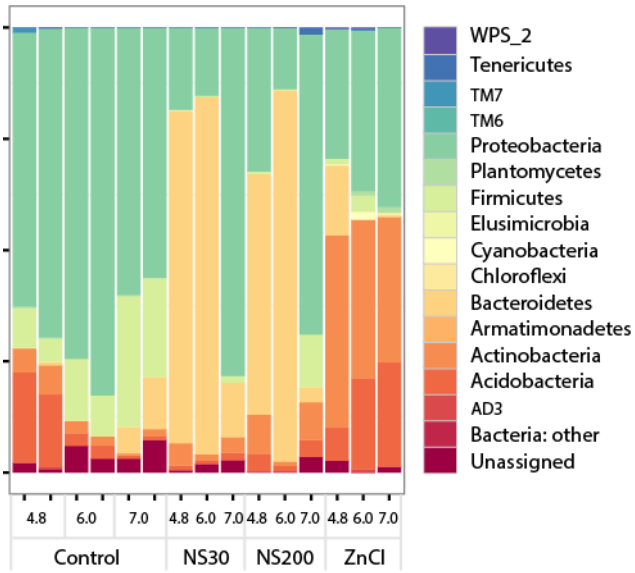
Figure 2. A: The relationship between TRFLP profiles from control soils at three pH levels in a Non metric Multidimensional Scaling (NMDS) plot (A). Colours show the three different soil pH groups (red pH 4.5, orange pH 5.9, green pH 7.2). The Shannon diversity (H) of the TRFLP profiles from control soils across each of the three pH treatments (B).

Figure 3. Non Metric Multidimensional Scaling (NMDS) plots showing the relationship between the microbial community composition in the ZnO NP (NS30), ZnO P (NS200) and Zinc chloride ($ZnCl_2$) treatments in soils of pH 4.5, 5.9, and 7.2.

Figure 4. Line charts showing the relationship between the Adonis F-test values of each dose group compared to the control for ZnO NP (NS30) (A), ZnO P (NS200) (B) and zinc chloride ($ZnCl_2$) (C). A higher F-test value indicates a higher level of significance in the difference between the groups.

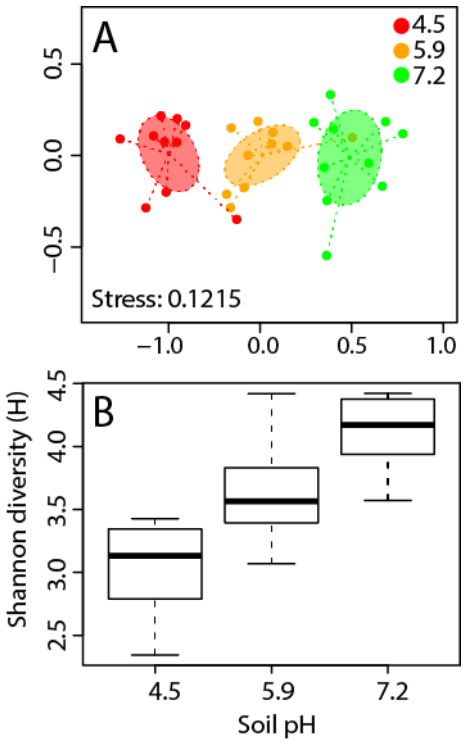
Figure 5. Dosed zinc concentration versus measured zinc concentration in pore water at each soil pH for zinc chloride ($ZnCl_2$) (A), ZnO NP (NS30) (B) and ZnO P (NS200) (C).

Figure 6. Non Metric Multidimensional Scaling (NMDS) plots showing the relationship between the microbial communities from TRFLP profiles for each treatment (ZnO NP (NS30), ZnO P (NS200) and Zinc chloride ($ZnCl_2$)) at the highest dose (2500 mg Zn/kg dw soil) in soils of pH 4.5 (A), 5.9 (B), and 7.2 (C).


WPS_2
Tenericutes
TM7
TM6
Proteobacteria
Plantomycetes
Firmicutes
Elusimicrobia
Cyanobacteria
Chloroflexi
Bacteroidetes
Armatimonadetes
Actinobacteria
Acidobacteria
AD3
Bacteria: other
Unassigned
4.8 6.0 7.0 4.8 6.0 7.0 4.8 6.0 7.0 4.8 6.0 7.0
Control
NS30
NS200
ZnCl

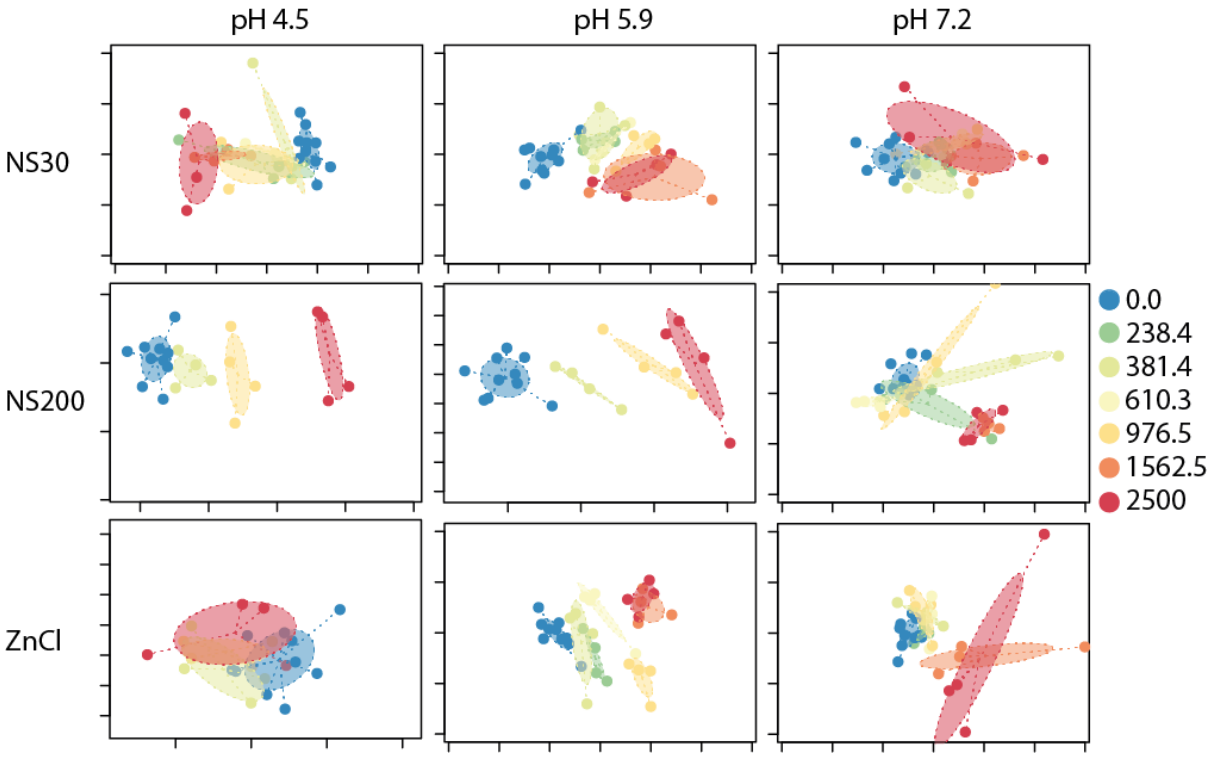
1

2


A
4.5
5.9
7.2
Stress: 0.1215
0.5
0.0
−0.5
−1.0
0.0
0.5
1.0
B
Shannon diversity (H)
2.5
3.0
3.5
4.0
4.5
4.5
5.9
7.2
Soil pH

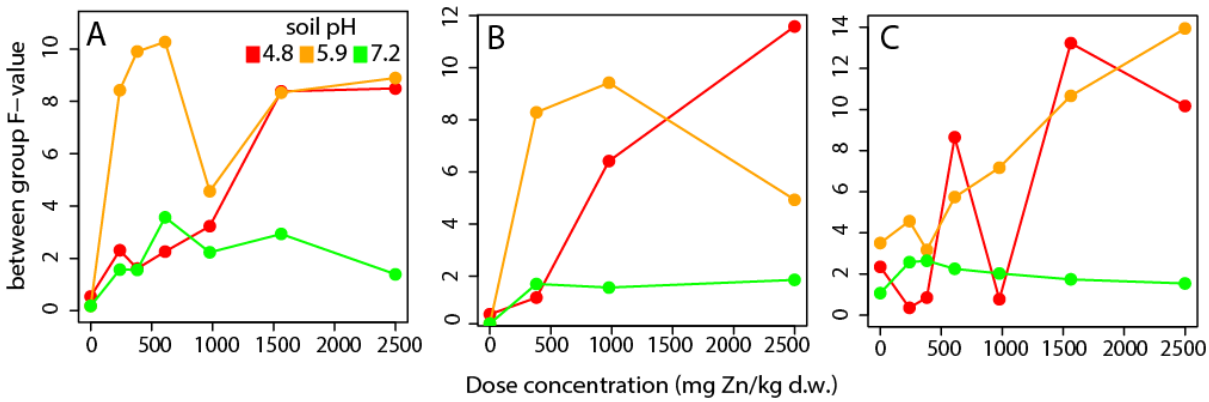
1

2


pH 4.5
pH 5.9
pH 7.2
NS30
NS200
ZnCl
0.0
238.4
381.4
610.3
976.5
1562.5
2500

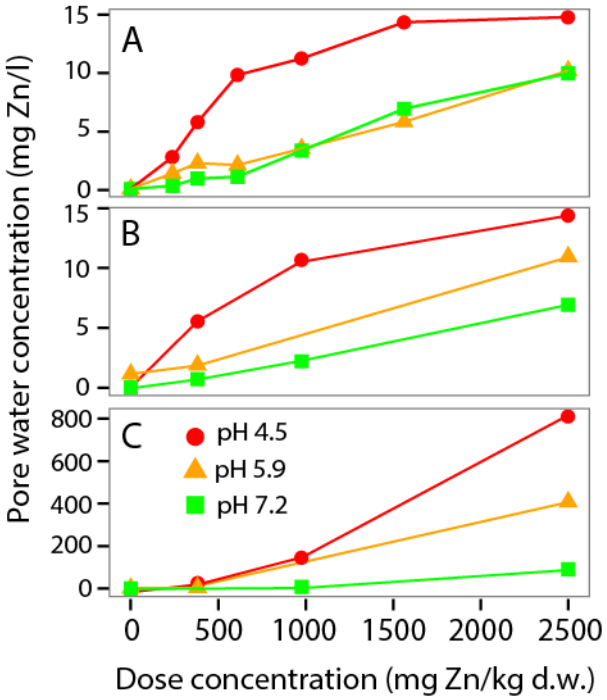
1

2


A
B
C
soil pH
4.8 5.9 7.2
between group F−value
Dose concentration (mg Zn/kg d.w.)
0 500 1000 1500 2000 2500

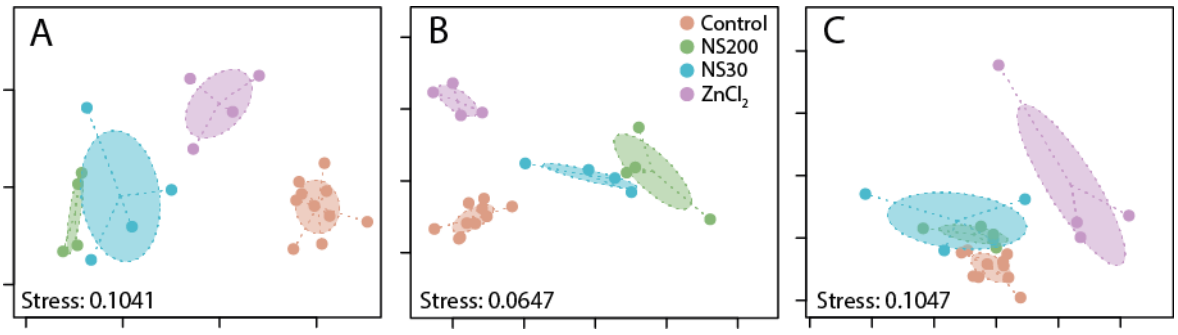
1

2


A
B
C
pH 4.5
pH 5.9
pH 7.2
Pore water concentration (mg Zn/l)
Dose concentration (mg Zn/kg d.w.)
0
5
10
15
200
400
600
800
500
1000
1500
2000
2500

1

2


A
Stress: 0.1041
B
Control
NS200
NS30
ZnCl2
Stress: 0.0647
C
Stress: 0.1047

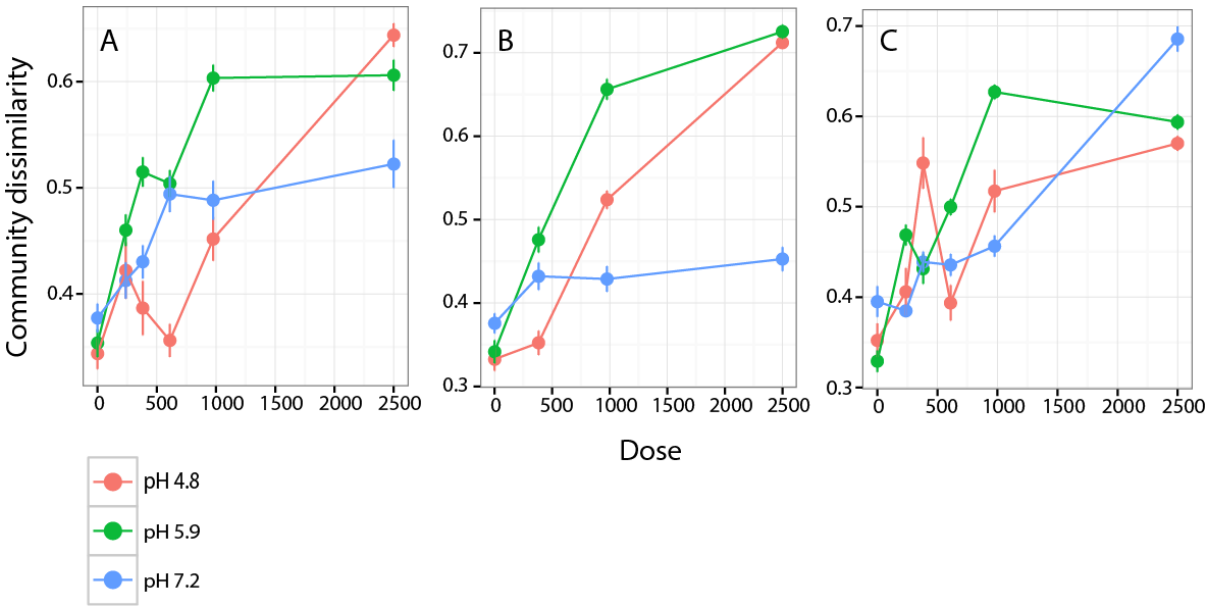
1

2

Table S1. Summary of the soil properties of the unchanged Dorset soil prior to pH amendment. Dorset soil texture, pH (in water and 0.01M CaCl2), conductivity, total carbon (% Total C) and nitrogen (% Total N), cation exchange capacity (CEC), percent base saturation (% BS), major cations concentrations (Ca2+, Mg2+, K+ and Na+) along with oxalate (% Feox, % Alox) and citrate-bicarbonate-dithionite (% FeCBD and % AlCBD) extractable iron and aluminium are shown (Heggelund et al 2014)

	% Coarse sand	% Fine sand	% Clay	% Silt	% Total C	% Total N
Texture, total carbon & nitrogen	51.5	40.2	3.5	4.7	4	0.1
	pH_{H2O}	**pH_{CaCl2}**	**Conductivity (µS)**			
pH and conductivity	4.2	3.1	422			
	Ca	**Mg**	**K**	**Na**	**CEC**	**% BS**
Exchangeable cations (cmol(+)/kg)	1.4	0.6	0.1	0.1	5.4	41
	$\%\ Fe_{Ox}$	**$\%\ Al_{Ox}$**	**$\%\ Fe_{CBD}$**	**$\%\ Al_{CBD}$**		
Extractable Fe & Al	0.04	0.03	0.11	0.03		

Supplementary Figure S1. Community dissimilarity (Bay Curtis distance) between samples for zinc treatments containing nanoparticulate zinc (A), non-nanoscale zinc particles (B) and zinc chloride (C). Points and lines represent treatments in soils of different pH levels.



Supplementary figure S2. Non Metric Multidimensional Scaling (NMDS) plots showing the relationship between the microbial communities from TRFLP profiles for nanoparticulate zinc (ZnO NP), non-nanoscale zinc particles (ZnO P) and zinc chloride ($ZnCl_2$) at zinc doses of 381.47 (A-C) and 976.56 (D-F) mg Zn / kg DW soil in soils of pH 4.5 (A, D), 5.9 (B, E), and 7.2 (C, F).