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High-throughput sequencing approach to access the impact of nanozeolite treatment on species richness and evenness of soil metagenome

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ABSTRACT

Microbial diversity is a very crucial component for the soil health maintenance. The present study investigated the effects of nanozeolite on bacterial diversity of soil from the agricultural field which was under 4-year field trial with wheat crop. Nanozeolite was amended in the treated plot whereas, the control plot did not receive any treatment. The bacterial population was targeted through the hypervariable (V3) region, which is a part of 16SrDNA. The 16SrDNA region is a conserved region among the bacterial species, but to investigate the diversity among the same species the hypervariable region are the best suited sequences. More than 1 million reads per treatment revealed very high levels of diversity. The majority of the sequences were attribute to the Proteobacteria (about 23–25%), 15% and 30–35% fitted into Actinobacteria and unknown phylum, respectively. Significant higher abundances of bacterial species with NZ treatment encompassed the population associated with nutrient cycling, residue decomposition and xenobiosis. The alpha diversity index also indicated better diversity and evenness within the treated soil than untreated soil. Our findings support the importance of nanozeolite for better survival of soil microorganisms especially bacteria.

1. Introduction

Microbial community in soil is very diverse where the maximum percent is covered by prokaryotic populations. Just 1 g of soil houses about 10 billion microorganisms and thousands of different types of species (Knietch et al., 2003). Soil microbial activity has the capacity to reverse the deteriorating soil properties, since it participates in the major biogeochemical cycling. Therefore, soil microbial diversity is the main focus for the sustainable agricultural practices in long term (Brown et al., 2002; FAO, 2012). Global adoption of soil conservation practices in agriculture is necessary to reverse soil degradation, and to maintain soil fertility and soil biodiversity. Zeolites are naturally occurring crystalline aluminum silicates which assist in water infiltration and retention in soil due to its porous property and the suction exerted by it. It can retain nutrients and hence supposed to improve crop yield (Prasad et al., 2014). The bulk size of nanozeolite limits some extraordinary properties which are shown by their nanosized (0–100 nm) counterparts. The nanozeolite has higher cation exchange capacity, surface area, ion adsorption and complexation etc. (Mukhopadhyay, 2014).

The traditional techniques allow cultivation of about less than 1 % of total microbial population which limits the study based on it (Schloss and Handelsman, 2003). The limitation of cultivable techniques can be delineated through the application of metagenomic approaches which can be applied to study a range of soil environments (Rajendhran J, Gunasekaran, 2008; Handelsman, 1998). The present study investigated the effect of nanozeolite on bacterial population of agriculture field through 16SrDNA targeted soil metagenome sequencing. Further research can be done to understand the effect of nanozeolite on the microbial communities under different conditions, especially for different soil types.

2. Materials and methods

2.1. Details of study area

The study was performed in a field experiment on wheat system, established in the winters of 2014–2015 at Norman E. Borlogue Crop

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Research Centre of G.B. Pantnagar, Dist. Udham Singh Nagar (Uttarakhand), India. This center is situated at a 29°N latitude and 79.3°E longitudes.

2.2. Experimental design

The experimental design was strip plot design with three replications of every treatment. Treatments consist of nanozeolite treated and control (No treatment). Each plot measured 2 m width × 5 m length with the net plot area of 1.5 × 4 (6 m^2). Nanozeolite was applied by foliar spray @ 0.03 g/1.5 L (Rossi et al., 2019). UP 2526, a wheat variety was used at the rate of 125kg/ha which is suitable for late sown irrigated conditions development by GBPUA&T, Pantnagar.

2.3. Soil sampling

Sampling was performed 3 weeks after harvesting the winter crop, wheat. An area of 0.4 m^2 was covered on each plot with a metal square and the surface 0–10-cm layer was determined. Soil sample of approximately 300 g was taken from the middle of each square with the help of an auger after the removal of crop residue. The soil samples were collected in labeled bags and the procedure was repeated (3 times) in each of the three replications in the field. The discrete soil samples from replicates were mixed properly so as to form a composite soil sample of about 2.5 kg per plot, with three plots per treatment. Plant residues were removed in laboratory and the samples were homogenized and passed through a 2 mm sieve before analysis (Souza et al., 2013).

2.4. Soil physicochemical properties

The soil is classified as silty clay loam. The soil pH was measured using pH meter after mixing soil: water ration (1:2.5) comprising 10 g of air dried soil (Jackson, 1973). The main chemical and physical properties of the experimental soil are given in Table 1.

2.5. Nanocompound's properties

- Nanozeolite (NS6130-09-905) was purchased from intelligent materials Pvt. Ltd. India (Table 2). The nanozeolite is the nanoform of zeolites which are abundantly present in soil and the chemical structure is alumino-silicate with good cation exchange capacity (Tosheva and Valtchev, 2005).

2.6. Soil DNA isolation, quantification and amplification

Metagenomic DNA was isolated using 1 g of each sample with HiPurA ™ Soil DNA Purification Kit MB542 (HiMedia), following the manufacturer's procedures. DNA was quantified and purity was verified in a NanoDrop spectrophotometer at 260 and 280 nm. DNA purity and quantity were also verified by electrophoresis in 1% agarose gels and samples adjusted to 50 ng/L (Nakayama et al., 2016).

Table 2
Properties of nanozeolite.

Properties	Nanozeolite
Stock No	NS6130-09-905
Size	< 80 nm
pH	7–8
Refractive index	1.47
Purity	99.9%
Elemental analysis	SiO_2 : 63–70% Al_2O_3 : 12–14% Fe_2O_3 : 0.7–1.9% Ti_2O: 0.2% K_2O: 2.5–3.8% Na_2O: 0.1–0.5% CaO: 2.4–3.7% MgO: 0.9–1.2% MnO: 0.008, P_2O_5 0.02–0.03% Cr_2O_3: 0.1%
Chemical formula	$(Ca, K_2, Na_2, Mg)_4$ $Al_8Si_{40}O_{96}$ $24H_2O$
Chemical name	Calcium Potassium Sodium Alumino-silicate
Cation exchange capacity	1.74mmol/g
Surface area	39m^2/gr
Bulk density	0.6–0.8 g/cm^3

2.7. Metagenomic sequencing

The metagenomics analysis (V3 region) was done by Agrigenome Labs Private Limited, where sequencing platform used was Illumina Miseq with Paired End (150bp × 2) Library type (Tosheva and Valtchev, 2005). The sequence data from this study were submitted to the NCBI Sequence Read Archive (SRA) with the accession number PRJNA511992 (Gloor et al., 2010; Caporaso et al., 2010).

2.8. Preprocessing of raw reads

Out of the total reads (1,189,731 for control and 1,319,553 for the nanozeolite treated), Chimeras sequences were removed with the help of USERACH, which uses the UCHIME method to remove chimera de-novo (Edgar et al., 2011).

2.9. Determination of bacterial community

The preprocessed consensus V3 sequences were analyzed further. Uclust program was used to pool and cluster the pre-processed reads on the basis of sequence similarity (similarity cutoff = 0.97) into Operational Taxonomic Units (OTUs). Quantitative Insights into Microbial Ecology (QIIME) program was used for the entire downstream analysis (Caporaso et al., 2012). Representative sequence was identified for each OTU and aligned against SILVA core set of sequences using PyNAST program (DeSantis et al., 2006 a; DeSantis et al., 2006b). Representative sequences were aligned further against reference chimeric data sets. Then, taxonomy classification was performed using RDP classifier against SILVA 16srRNA genes database. Further, a heat map was generated using QIIME program.

2.10. Statistical analysis

The alpha diversity within the samples was calculated using Shannon, Chao1 and observed species metrics. The metric calculation was performed using QIIME software (Knight et al., 2010).

Table 1
Physico-chemical properties of the experimental site.

S. No	Particulars	Value	Method employed
1	Soil texture	Silty clay loam	Deshpande et al (1971)
2	pH (1:2.5) Soil: water ratio	7.5	Beckman glass electrode pH meter (Jackson, 1973)
3	EC (m mhos/cm at 25 °C	0.205	Water and soil analyzer kit
4	Organic carbon (%)	0.74	Walkely and Black's (Brown et al., 2002)
5	Available nitrogen (kg ha^{-1})	213.14	Modified Kjeldahl method (Jackson, 1973)
6	Available P_2O_5 (kg ha^{-1})	24.14	Olsen's method (Jackson, 1958)
7	Available K_2O (kg ha^{-1})	137.08	Flame emission spectrometry Method (Jackson, 1958)

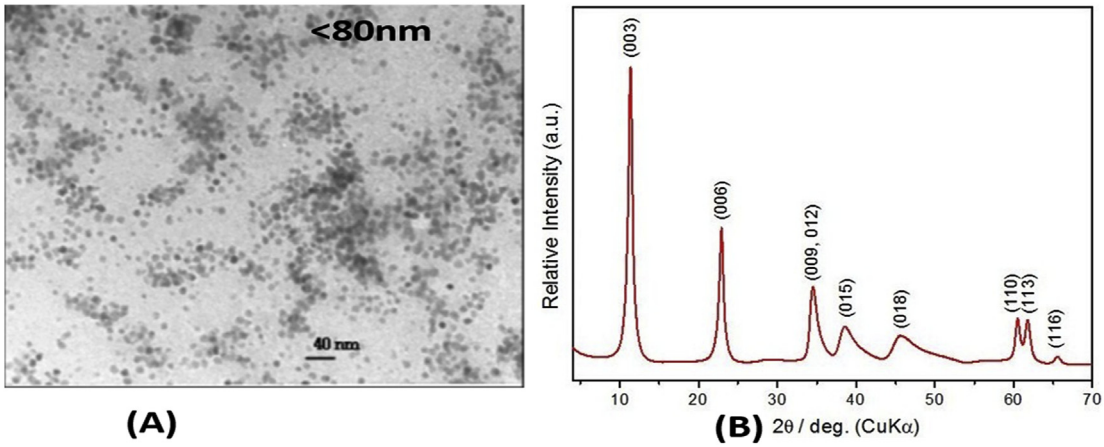

Fig. 1. Scanning Electron Microscopy Image of Nanozeolite, (B) XRD pattern of Nanozeolite (Source: Intelligent Material Pvt. Limited).

3. Results

3.1. Property of Nanozeolite

Nanozeolite (NS6130-09-905) with particle size < 80 nm are the alumino-silicates with a good cation exchange capacity (1.74mmol/g) due to which is responsible for their high affinity towards cations like Na^+, K^+, Ca^{2+} etc. (Navorotsky et al., 1995). The X-Ray Power Diffraction (XRD) patterns of nanozeolite exhibit diffraction peaks similar to that of zeolite (Fig. 1).

3.2. Soil metagenomics

Total DNA was isolated using HiPura TM soil DNA purification Kit with good quality and quantity. The nanodrop concentration for control and nanozeolite treated soil DNA was 57.7 and 36.5 respectively which was further confirmed by qubit concentration which was 85 and 69.5 respectively. The purity of DNA was also checked on the basis of 260/280 ratio which was observed to be 1.63 and 1.57 for control and treated respectively. The results authorized the quality of DNA for further amplification and sequencing process. Soil microbial community is diverse and harbor highest prokaryotic diversity in comparison to any environment.

The 16S rRNA next-generation sequencing run produced 1,189,731 for control and 1,319,553 raw sequences for nanozeolite treated soil. Out of the total reads only 754,086 in control and 934,839 in treated sample passed the filters (conserved region filter, spacer filter and read quality filter). More than 80% percent of reads had Phred score > Q30 (Ewing et al., 1998). Those sequences were used as input in the Quantitative Insights into Microbial Ecology (QIIME) analysis workflows (Knight et al., 2010). Different sequence filters were used to eliminate the unrequired sequences. After the removal of chimeric sequences nine main phyla within the root Bacteria were identified: Actinobacteria, Bacteroidetes, Firmicutes, Cyanobacteria, Acidibacteria, Proteobacteria, Chloroflexi, Gemmatimonadetes and Nitrospirae. The number of 16S OTUs assigned to each Phylum is shown in Fig. 2. Nanocompound treated soil sample showed higher population of Acidobacteria (195259 abundant reads: 23.23%), Proteobacteria (218317 abundant reads: 25.97%), Chlorofexi (79155 abundant reads: 9.42%), Nitrospira (28757: 3.42%) and Cyanobacteria (15122 abundant reads: 1.79%) than control, with a range of 116127 (16.89%), 141412 (20.57%), 67284 (9.79%), 28757 (3.42%) and 10246 abundant reads in Cyanobacteria (1.49%) respectively. Top 10 enriched class categories were analyzed, which comprise of Actinobacteria, Bacteroidetes, Proteobacteria, Chloroflexi, Firmicutes, Cyanobacteria, Acidobacteria, Gemmatimonadetes, Nitrospirae and others (Fig. 2). Phylum Proteobacteria was dominant in both the soils types with higher population in

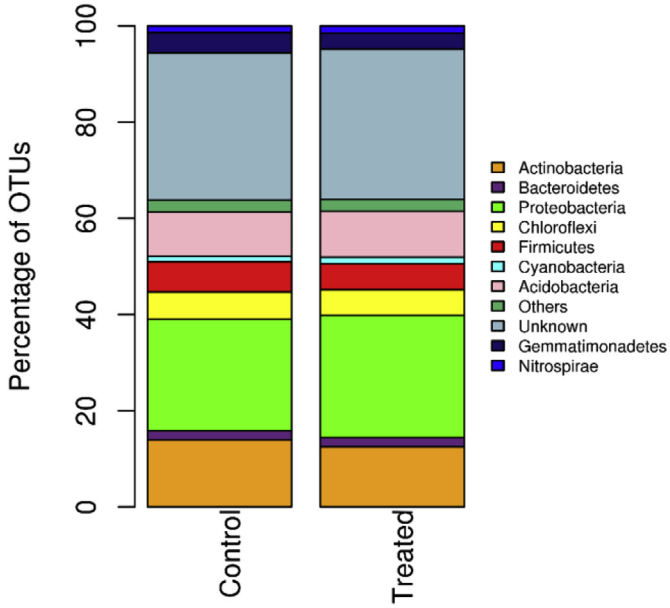

Fig. 2. Taxonomy classification of OTUs at phylum level for the sample. Only top 10 enriched class categories are shown.

nanozeolite treated soil sample.

3.3. Alpha diversity with samples and rarefaction curves

Alpha diversity is the diversity in population at local level (Gaston, 1996). The chao1 index is the measure of species richness while Shannon metric measures abundance of observed OTU, and accounts for both richness and evenness. The observed species metric identifies unique OTUs in the sample (Li, 2016). The nanozeolite treated soil was observed to have greater species richness and evenness in comparison to untreated control. The rarefaction curve exhibited a steeper slope for nanozeolite treated soil sample than control demonstrating a greater genetic richness in nanozeolite treated soil sample. The analysis of species richness (Chao 1), evenness (Shannon index) and observed species diversity indicates that nanozeolite treated soil samples had diverse bacterial population which is evenly distributed among different groups. The rarefaction curve for each of the metric is provided in Fig. (3).

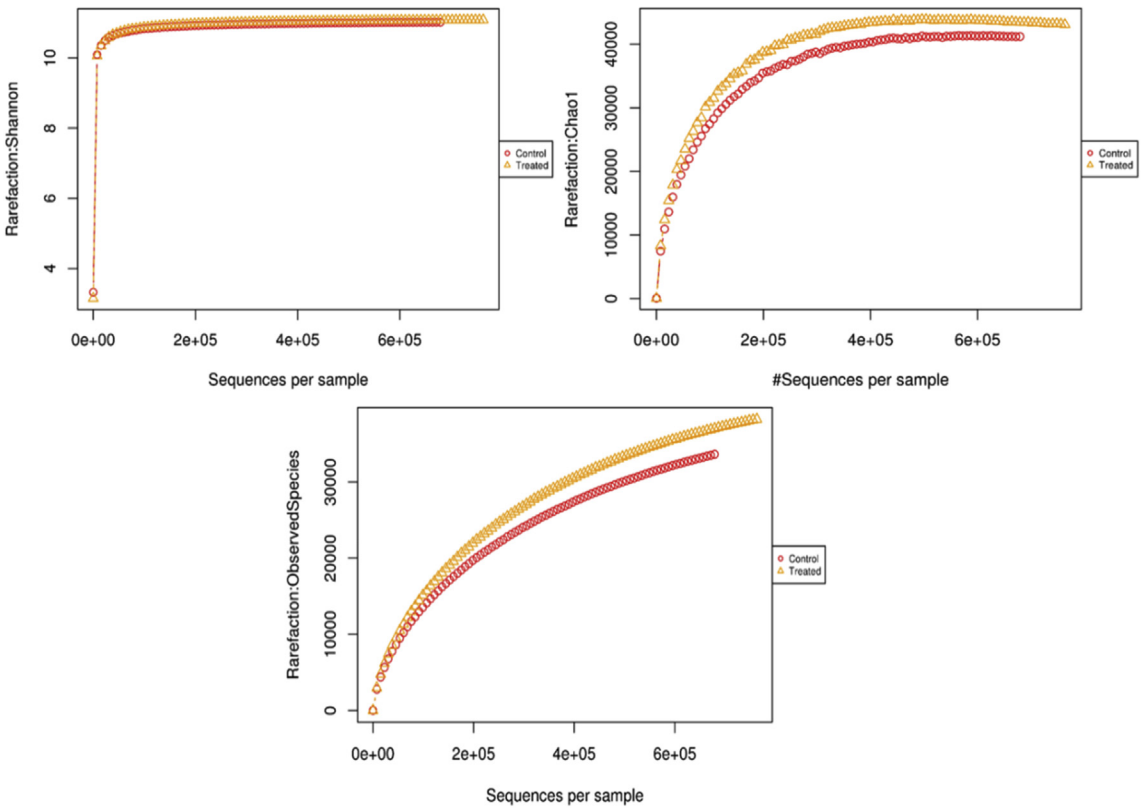

Fig. 3. (a)Shannon curve, (b) Chao1 curve and (c) Observed species curve.

4. Discussion

Microbial population in soil is an important indicator of soil health but cultivation dependent approaches are biased towards the protocol used and the dominating populations. 16SrDNA is a highly conserved region to target the bacterial population, but to assess the variability within the bacterial species V3 Hypervariable region is one of the best tools. The metagenome sequencing revealed the impact of nanozeolite treatment on prokaryotic diversity of soil, which was improved in number and diversity. The prokaryotic population among the all other microbial population was targeted through the hypervariable region of 16SrDNA to assess the major group of microbial population. The prokaryotes are the dominant and most diverse group among the microbial communities of any environment and metagenomic approaches targeting prokaryotic population can improve our access to these communities (Delmont et al., 2011). There are various tools available for the analysis of metagenome sequences these days. These tools treated the sequences and annotated to specific profile and functions. D'Argenio et al. (2014) compared two bioinformatics tool for metagenomic analysis i.e. QIIME and MetaGenome Rapid Annotation using Subsystem Technology (MG-RAST) and suggested QIIME to be better pipeline as it generate better BIOM file, which improves the diversity analysis output. Removal of unnecessary sequence is equally necessary to avoid false identification. Chimeras are the hybrids of different parent sequences that can be interpreted falsely as novel organisms so, their removal is necessary (Haas et al., 2011). According to Edgard et al. (2011) UCHIME is better than the previously used tools for chimera detection as it detects chimera de novo using the abundant data.

Among the prokaryotes, proteobacteria was observed to be positively influence with the treatment of nanozeolite. Proteobacteria is the most varied group known for versatile metabolism, due to which they can survive in different environments (Hugenholtz et al., 1998). The effect of nanozeolite treatment was mainly evident in Proteobacteria, which is most diverse group of prokaryote. The proteobacteria are mainly known for their capabilities to adjust to varied environmental conditions including versatile nutrient requirements. The higher diversity and abundance of proteobacteria could be the due to increased nutrient use efficiency and management in soil with the help of nanozeolite. The results are in correlation with our previous study where nanozeolite was reported to support the bacterial population (through culturable methods) (Khati et al., 2019). Similarly, Chavan and Nadanathangam (2019) also studied the effect of two nanoparticles (Ag NP and ZnO NP) on soil microbial community and observed that silver nanoparticles increased the abundance of proteobacteria from 43.7% in control to 62.2% in treated (about 30% increase) however the ZnO NP did not show any noticeable effect on soil bacteria or plant health. In contrary to the positive effects, some nanocompounds may have toxic response towards microbial population of soil. In an another study with three nanomaterial (TiO_2, positive polysterene and sulfate modified polystyrene) only one (sulfate modified polystyrene) nanomaterial was found to increase the rhizosphere bacterial population (Kibbey and Strevett, 2019). Nanozeolite, being a natural and native compound of soil does not pose any harmful effect on soil micro and/or macro flora. Nanozeolite due to their complex structure, small size and high cation exchange capacity may chelate nutrients and trap moisture, which may help improve the availability of resources in soil. The slow release of resources in soil would help the living micro and/or macroorganism to survive and function better (Khati et al., 2017b, 2018). The steeper rarefaction curve indicates more diverse and even distribution of bacterial population within the nanozeolite treated soil in comparison to control. The nanozeolite application can provide a suitable solution to water scarcity and nutrients deficiency in soil, and thus may help increasing the productivity while maintaining the soil health. The present study has laid the foundation for further investigation regarding application of nanocompounds for improvement in soil health so as to improve agriculture production.

Conflicts of interest

Authors declare no conflict of interest.

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