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Biochar Effects on Soil Microbiota

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Abstract

Soil contamination through overuse of pesticides has become a global challenge for future food security. Therefore biochar a carboneous material produced from thermal decomposition of biomass has been recognized as an alternative approach to conserve beneficial microbiota and contaminated soil. In this study, bacterial communities associated with mixed and sandy soil types were monitored after addition of pesticides and biochar via sequencing of the V3–V4 region of the 16S rRNA gene using Illumina Miseq system. Bacterial richness was found higher in mixed soil samples compared to sand soils visualized by rarefaction curve. PCoA analyses resulted with large variation of microbial composition between sand and mixed soil types. Alpha (α) diversity indices (Observed, Shannon) increased while Inverse Simpson remain similar and increased for pesticides treated mixed soil samples and untreated sand soil samples. Alpha(α) diversity indices (Observed, Shannon and Inverse Simpson) were higher for pesticides mixed soil samples and Observed species, Shannon diversity increased with lower Inverse Simpson index in sandy soil samples after addition of biochar. Overall the biochar application seemed to improve the microbial composition of bacteria in pesticides amended samples of mixed and sandy soil. This study confirmed the importance of biochar in soil remediation practices as it changes soil physical chemical properties and changes microbial composition and supports high crop yield.

1. Introduction

Soil is a natural resources that compose of minerals, organic matter and living organisms which releases nutrients for the growth of plants (Baer & Birgé, 2018). As much as 98.8 % of human food is supplied by the soil (Kopittke et al., 2019) thus having a huge contribution to food security (Hamidov et al., 2018). Soil has been used for agricultural practices since the evolution of human civilization. Agricultural soil with its biodiversity also have a positive impact in climate change mitigation through storage of atmospheric carbon dioxide in the form of soil organic carbon (SOC) (Brar et al., 2013; Li et al., 2020). Soil microbiota consists of bacteria, archaea, eukaryotes and viruses and forms complex ecosystem. Beneficial microorganisms help in nutrients cycle, degradation of organic substances, biogeochemical cycles, reduce toxicity of harmful chemicals, suppress pathogens and promotes growth of plants (M. Liu et al., 2019; Meena et al., 2020; Yu et al., 2013). Earth composes of approx.4–6 × 10^{30} number of prokaryotes (Sleator et al., 2008). The microorganisms can give quick response to any changes in soil and thus are important biological indicators when measuring soil health (Geisseler et al., 2017). Soil health is the ability of soil to support plants and animals growth and provide various ecosystem services (Kibblewhite et al., 2008). Healthy soil's good texture and organic matter contents are the cause of its rich microbial diversity than poor soil (Kunin et al., 2008; R. Wang et al., 2017). Healthy soil reduces erosion along with leaching of nutrients and toxicity of chemicals in soil (Stirling et al., 2016). The overall physio- chemical and biological properties of soil are responsible in quantifying soil conditions (Jian et al., 2020). Microbial composition and their biomass may be affected by the vegetation type, climate and physio chemical properties of soil (W. Han et al., 2021; Kang et al., 2021).

Global issues of pesticides use

The rising population, agricultural expansion, overuse of fertilizers and pesticides, wastages of food are some major emerging causes for global soil contamination and challenges to future food security (Gupta, 2019). United nation estimates population increase of 7.7 billion in 2019 to 9.7 billion by 2050 (United nations, 2019) and to support this increasing population by 2050, food production should be increased by 70% (Food and Agricultural Organization 2009). Green revolution is the outcome of modern agriculture that started in the 20th century with the use of advanced agricultural methods and machineries, agrochemicals (fertilizers and pesticides) and

genetically modified plants (Gupta, 2019; Khush, 2001). Agricultural yield has been intensively achieved but with a huge expense on environmental health (Singh & Singh, 2017). Thereby use of pesticides is an important key factor for boosting intensive production yield (Silva et al., 2019). Pesticides are toxic chemicals or organisms used to control or kill harmful organisms like weeds and diseases that can affect plant growth (Cabrera, 2017) and provide desirable, quality and less expensive food supplies (Yavari et al. 2015). Based on target organisms, they are classified into herbicides, insecticides, fungicides, rodenticides, molluscicides, nematicides and plant growth regulators (Aktar et al., 2009; Mahmood et al., 2015). Recent agriculture rely on huge quantities of pesticides (Diez et al., 2013) as they are cost effective and easily available. The problem is that their excess use and residual deposition for longer time cause environmental pollution of air, water, soil and can affect plants, animals, microbiota including human (Aktar et al., 2009; Al-Zaidi et al., 2011). Likewise long term accumulation of its residues biomagnifies through trophic level causing impacts to living organisms (Sánchez-Bayo, 2011). Meena et al. (2020) reported in total pesticides applied, the target organisms only get 0.1 % of it and the remaining amount are viable sources of environmental pollution. Among the type of pesticides, herbicides is one that kills unwanted plants or weeds in agriculture. For. e.g. Glyphosate is a nonselective herbicides and considered as active ingredient of roundup (Hagner et al., 2019). The use of glyphosate was started from 1970s, binds in soil and easily degraded by microorganisms (Kanissery et al., 2019). It affects living organisms including beneficial microorganisms present in soil. World Health Organization's International Agency for Research on Cancer has assured glyphosate being carcinogenic to human health (Sharma & Lai, 2019).

Biochar

Biochar is produced by heating biomass residues at low or absence of oxygen at high temperature (350–700°C) through a method called pyrolysis (Rawat et al., 2019). The temperature and feedstock types determine physio chemical properties of biochar (Tomczyk et al., 2020). Increased pyrolysis temperature increases pH, surface area, pore size, amount of ash, electric conductivity, oxygen content with decrease in production of biochar, cation exchange capacity (CEC) and Hydrogen(H), Carbon (C) and Nitrogen (N) content (Hassan et al., 2020; Nardon et al., 2014).

Biochar is prepared from various feedstocks such as animal, poultry and plant remains due to which there is difference in physical and chemical characteristics of biochar types produced (Adekanmbi et al., 2020). Safaei khorram et al. (2016) summarized biochars produced from different feedstocks vary in their adsorption, desorption, leaching of different pesticides. Nardon

et al. (2014) found increase in some macro elements like Magnesium (Mg), Sodium (Na), Calcium (Ca), Potassium (K) in some biochars feedstocks by increasing temperature. Omotade et al. (2020) found animal sources with high nutrients compared to feedstocks from plant source. Likewise biochar prepared from plant sources has high carbon (C) and Oxygen (O) contents than animal manure and sewage sludge with high Nitrogen (N) and Sulphur (S) contents (Pan et al., 2021). Askeland et al. (2019) studied higher yeild of biochar from straw (500^{o}C and 750^{o}C) than biochar from pine (sawdust) whereas biochar yield from pine was high at 350^{o}C than sawdust.

Biochar production is an environment friendly approach that utilizes agricultural wastes for adsorbing pollutants and promotes human and environmental health with economic benefits (Duwiejuah et al., 2017) but its production is only based to lab and needed to be produced at large scale for field application (Safaei khorram et al., 2016). But large scale application is possible only when prior study on estimates of nutrient use, carbon sequestration, changes in soil quality and food productivity is done (Filiberto & Gaunt, 2013). Various soil remediation approaches are developed but biochar is a promising cost effective alternative to conserve soil health of pesticides contaminated soil based on adsorption principle (Y. Liu et al., 2018). Biochar production is based on use of various feedstocks which can be the probable source of contaminants for healthy soils (Hassan et al., 2020) and biochar with high pH cause plants to have nutrient insufficiencies and only poor soil and highly acidic soil have advantages from its application (Hunt et al. 2010).

Microbial responses in pesticides and biochar amended soil

Various human caused factors such as addition of biochar and pesticides can affect diversity and microbial community structure present in soil. Pesticides in soil are degraded by microorganisms like bacteria into useful nutrients for their growth and development (Huang et al., 2018; Ljiljana et al., 2007) while some microorganisms may have suppressive effects from pesticides use (Muturi et al., 2017). The degradation of pesticides by microbes depend upon several factors such as soil texture, pH, organic substances etc (Tiryaki & Temur, 2010). Al-Ani et al. (2019) studied soil microbial activities and found their number dependent on pesticides types, their concentration and incubation period. They also observed decrease in microbial activities and microbes like bacteria, fungi, actinomycetes due to change in amount of pesticides. Likewise (Edrees, 2019) found higher number of bacterial colonies in pesticides treated soil for 5 years than soil treated for 20 years in the study done in Khatt agriculture in Dhala Governate, Yemen. The author reported that the longer time of use of pesticides, more it affects useful microorganisms and their activities, degrades useful substances causing poor quality of soil with long term accumulation of pesticides residues.

Moreover soil texture and form, weather, temperature also determines pesticides movement in the soil (Gavrilescu, 2005).

Biochar in soil promotes microbial activities, adsorption of organic and inorganic compounds as it changes soil properties (Krishnakumar et al., 2014). The biochar physio chemical properties induces physio chemical changes in soil thus changes the microbial composition of soil (Gul et al., 2015). Changes in microbial activities and their structure is due to the modification in microhabitat and metabolic processes in biochar amended soils (Zhu et al., 2017). Rich microbial composition are observed in biochar with large surface area and developed pores as they provide suitable shelter for microorganisms (Jaafar et al. 2014). The black color of biochar absorb heat thus enhances growth and activities of microorganisms in soil (Gul et al., 2015). These microorganisms use the solube organic and inorganic substances adsorbed in biochar for their growth and development (Thies & Rillig, 2009). Biochar properties like large surface area, charged surface groups adsorb and disable organic contaminants (Nartey & Zhao, 2014) hence decreases pesticides accessibility to microbes, plants and other organisms present in soil (Safaei khorram et al., 2016). The higher the adsorption of pesticides by biochar the lower its degradation (Zhelezova et al., 2017). The high adsorption behavior of biochar is found with reduced efficiency of pesticides to control harmful pests in soil (Yavari et al., 2015). The aged biochar has reduced adsorption capacity to contaminants compared to fresh biochar (Zhelezova et al., 2017).

Determination of microbial community structure in environmental samples

With advance of metagenomics at the beginning of 21st century the problems with identification of uncultivable microbes has now been solved (Garrido-Cardenas & Manzano-Agugliaro, 2017). The next generation sequencing (NGS) method is culture independent and can be used to identify genome of microorganisms (Schloss & Handelsman, 2008) followed by library preparation and bio informatics (Méndez-García et al., 2018). In addition to species composition, it studies evolution and metabolic processes of species present in an ecosystem (Hugenholtz & Tyson, 2008). NGS has reformed the studies of microbial species in the soil and in particular DNA metabarcoding has commonly been used to find out the changes in community structure and diversity of microorganisms in the soil after application of biochar (Jenkins et al., 2017). DNA metabarcoding uses the sequence differences in the area of the genome, such as 16S rRNA in bacteria and the internal transcribed spacer (ITS) in fungi, to differentiate species (Polinski et al., 2019) (B. Gao et al., 2021).This is an cost effective approach for identifying soil microorganisms

and their diversity from many samples (Peters et al., 2018). It uses short reads and produces huge number of DNA sequences in parallel from samples under study (Bush et al., 2019). This approach is based on various lab methods, bioinformatics and computational study of the sequenced data (Francioli et al., 2021). In NGS using single marker or targeted gene amplification using barcode primer pairs, purification, and DNA libraries are formed before sequencing (Bharti & Grimm, 2019). Various studies use Illumina Miseq platform to sequenceV3-V4 region of 16SrRNA gene which are amplified to study the composition of microbes communities in the environmental samples (Lo & Chong, 2020; Rozanov et al., 2020). Illumina Miseq is cost effective comparative to HiSeq (Pichler et al., 2018) platform but produces imperfect quality of sequences due to sequencing problem with low sequence diversity 16SrRNA amplicon (Fadrosh et al., 2014). Microbial composition data may be affected by the components like DNA extraction method, primers choice and sequencing approaches hence use of standards and tools are required for accuracy in the result obtained (Fouhy et al., 2016).

This study aimed to use a metabarcoding approach to (i) observe bacterial composition of sandy and mixed soil (ii) observe if pesticide (Glyphosate) impact the bacterial composition of the two soil types (iii) to observe if biochar will alter the bacterial composition after treatment with pesticide.

2. Materials and Method

2.1. Biochar characterization

The tested biochar was derived from biomass of scots pine (*Pinus sylvestris*) woodchips produced at the biochar production facility standard Bio AS, Bø i Telemark, Norway. It was produced through combustion of biomass through pyrolysis at temperature approx. 700^{o}C. The other physiochemical properties of biochar are presented in (Table 1).

Table 1: Physiochemical properties of biochar (scots pine) used at field experiment

PH	Carbon	Nitrogen	Water	Ash	Surface Area
7.6	91.5%	0.28%	3.8%	1.9%	378m^2g^{-1}

The biochar solution was prepared by mixing 100ml volume of grinded powder with 900ml of water i.e one litre of biochar solution (10% biochar concerntration) was poured around 1/3 rd part of each pellets.

2.2. Soil sampling

The soil samples from sand and mixed soil types were collected at Standard bio of Telemark. They were categorised into total 24 pellets on the basis of treatments and control with their replicates under natural environment table (1). Horse pasture mix (SPIRE) was sown in all soil cravattes for visual observation of soil health conditions and treatment effects.

The soil (>0.25 g) with pesticides and control was collected from each pellets by using sampling tubes such as falcon tubes and resealable freezer bags. The sampling was again taken after few weeks after the addition of biochar in the same pellets with pesticides and control using the same methods. The collected soil samples were taken to lab and stored at freezing temperature at -20^{o}C for further analysis.

Table 2: Soil samples for mixed and soil types with control and treatments

Soil type	Untreated with replicates	Pesticides with replicates	Untreated +Biochar with replicates	Pesticides + Biochar with replicates	Total
Sand	3	3	3	3	12
Mixed	3	3	3	3	12
Total	6	6	6	6	24

2.3 Lab method

2.3.1 DNA extraction protocols

The soil samples of two soil types sand and mixed soil with control (untreated) and treatments (pesticides biochar) were taken to lab for further for DNA extraction. DNA extraction of these soil samples were performed by using DNeasy Powersoil Pro Kit following the manufacturer's instructions *(*www.qiagen.com*)*. Total DNA was captured through silica membrane and their quantification were carried out by using Qubit 3.0 fluorometer and Nano droplite that measures nuclei acid concentration at 260nm and purity at 260/280 ratio (Thermo Fisher Scientific Inc.). The purified DNA was taken for further sequencing analysis.

2.3.2 DNA analysis

Library preparation and next generation sequencing

The extracted DNA was sent to Norwegian sequencing centre (www.sequencing.uio.no) in Oslo for library preparation and Illumina sequencing of V3-V4 region of 16S region. The protocols for for library preparation and 16S metagenomics sequencing were extracted from (Fadrosh et al., 2014). DNA was amplified using primers designed to target V3 and V4 regions of the prokaryotic 16SrRNA gene. The primers 319F and 806 R with the following sequences used:
319F forward primer: 5' ACTCCTACGGGAGGCAGCAG 3'
806R reverse primer: 5' GGACTACNVGGGTWTCTAAT 3' (www. sequencing.uio.no)

2.4 Statistical analysis

Raw reads were demultiplexed with an inhouse software at University in Oslo (www.sequencing.uio.no). The resulting fastq sequences were combined into a Qiime artifact in Qiime2 v.2020.6 (https//qiime2.org/). DADA2 was further used to merge the paired sequences, denoising and chimera removal. The result of DADA2 is amplicon sequence variants (ASV) that replaces operational taxonomic units (OTU), representing real biological nucleotide variation. Qiime was used to assign the taxonomy to the ASVs using a Naive Bayes classifier algorithm trained against the Silva (v) database. R Studio Version 3.6 was used for statistical analysis. The samples were first rarefied, randomly subsampling all samples to the number of reads in the sample with smallest number of reads using the r package Phyloseq (joey711.github.io/phyloseq/). The rarefraction curve balances the differences in library size of largest samples by making it similar to the library size of smallest samples for better distinguision of alpha diversity (Willis, 2019) and also demonstrates how depth diversity can be studied. In order to study the effect of soil type on the alpha diversity, rarefaction curves for each sample was plotted. Further the beta diversity was calculated using Bray Curtis distance in VEGAN and PHYLOSEQ. The effect of treatment (pesticide and bio char) on the alpha diversity indices (Observed, Shannon, Invsimpson) were further compared using the PhyloSeq package.

3. Results

3.1 Bacterial community structure

A total of 2166982 usable reads were produced from 24 samples of two soil types of which 1202978 (μ = 100248) usable reads of sand samples (untreated, untreated+ biochar, pesticides, pesticides+ biochar) and 964004 (μ=80334) usable of mixed soil samples (Table A-1). The highest usable reads 97614 was obtained from untreated mixed soil sample and 123530 reads from biochar amended sandy soil sample. A total of 3876 OTU's for 24 samples of mixed and sand soil types were detected with bacterial domain. Bacterial OTU's were identified to species level. The OTU-

1186 contained the highest number of reads representing Chloroflexi phylum in sand samples whereas OTU-237 received highest reads representing Acidobacteriota phylum in mixed soil samples with control and treatments.

3.2 Bacterial composition of two soil types (sand and mixed)

3.2.1 Rarefaction curve

The rarefaction curve balances the differences in library size of largest samples by making it similar to the library size of smallest samples for better distinguishing of alpha diversity (Willis, 2019) and also demonstrates how depth diversity can be studied. The species richness for mixed and sandy soil samples were plotted for sample size (number reads) after rarefaction Fig (1). The solid lines represent the rarefaction curve for mixed and sand soil samples. The untreated soil samples, untreated and biochar samples, pesticides treated samples, pesticides and biochar treated samples for mixed soil type are represented by red color and for sandy soil by blue color. The species richness for mixed soil was higher than sandy soil. The bacterial richness of all treatments and control were obtained at sequencing depth of 60000 reads. The flattened curves for both mixed and sand samples indicated each samples were uniformly and fairly sampled (Fan et al., 2020) or all types of microorganisms are sampled .

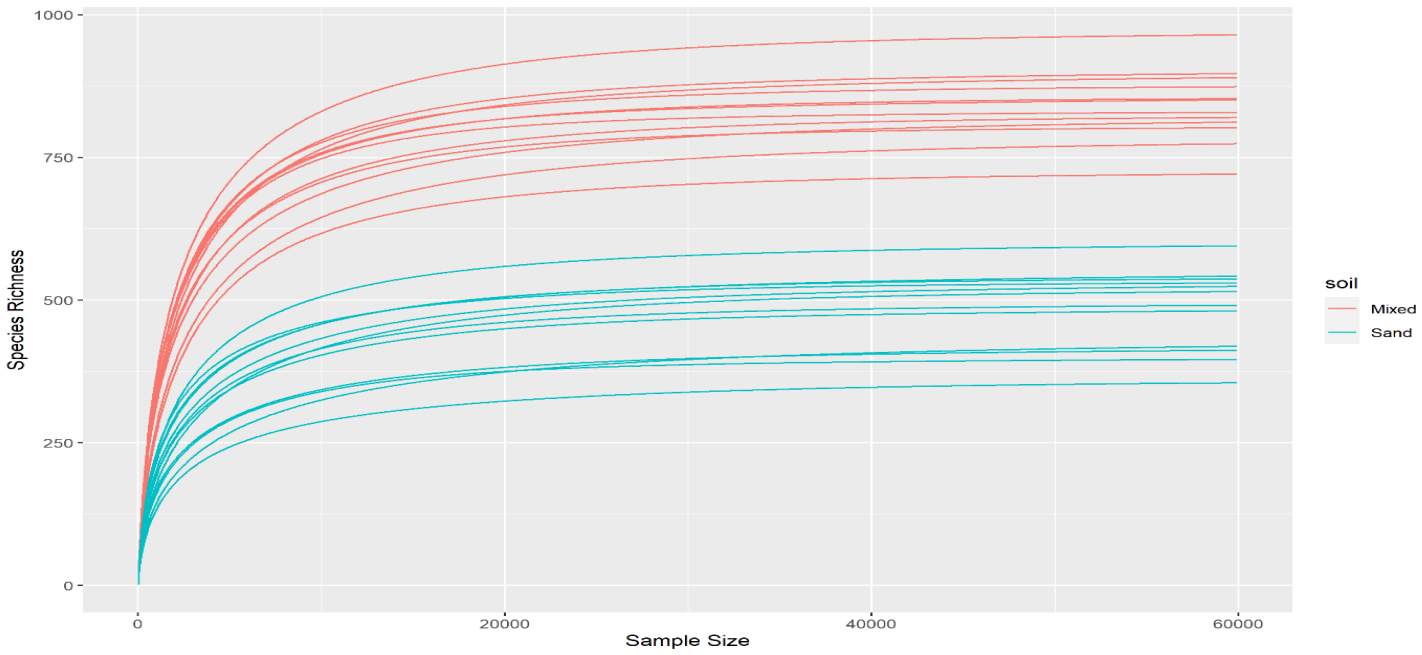

Fig. 1: Rarefaction analyses of the mixed and sandy soil types

3.2.2 Beta (β) diversity of mixed and sandy soil

The PCoA (Principal Coordinate Analyses) analysis was used to display the similarities between microbial communities. The first principal component axis separated the two soil types (fig 2). There was large variation between mixed and sandy soil. The mixed soil samples (untreated, untreated and biochar, pesticides, pesticides and biochar represented as dots were more concentrated suggesting a more similar microbial composition within the sample. The two sand soil samples (pesticides, pesticides and biochar) lying vertically at the bottom and top of axis 1 were spaced far from other sand samples indicating dissimilar bacterial composition with each other from other clustered sand samples. The other remaining sandy soil samples such as pesticides samples, biochar and pesticides and untreated samples showed similar bacterial communities.

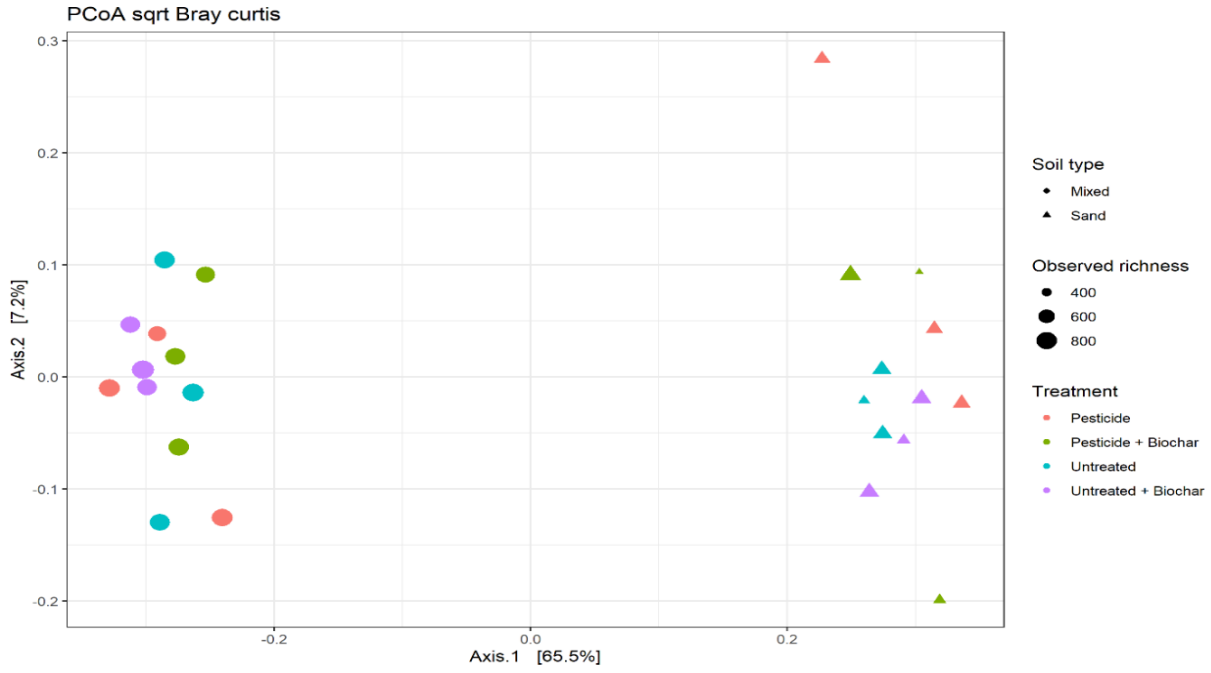

Fig: 2 PCoA analyses of bacterial community from two soil types (mixed and sand)

(with treatments (pesticides, pesticides and biochar) and control. PCoA plot was extracted from a Bray–Curtis similarity matrix of data which was changed into square root. The values expressed in parentheses represents the percentage of the total variation explained by each axis)

3.2.3 Alpha diversity indices of two soil types after treatment with pesticides

The bacterial alpha diversity for pesticides amended and untreated samples of two soil types is illustrated in boxplot fig (4. a, b). The Observed species richness and Shannon diversity index were higher for pesticides amended soil when compared with untreated mixed soil samples (Fig a). In

sandy soil, the Observed species richness, Shannon and Inverse simpson were lower for pesticides treated sand samples compared untreated samples (Fig b). There were no significant effect of pesticides treatment on the alpha diversity of any of the soil types i.e mixed soil samples (p=0.71)) and sandy soil (p=0.42). However the tendency that the Observed species richness and Shannon index was slightly higher for pesticides treated mixed soil and all three indices were slightly lower for pesticides treated sand samples.

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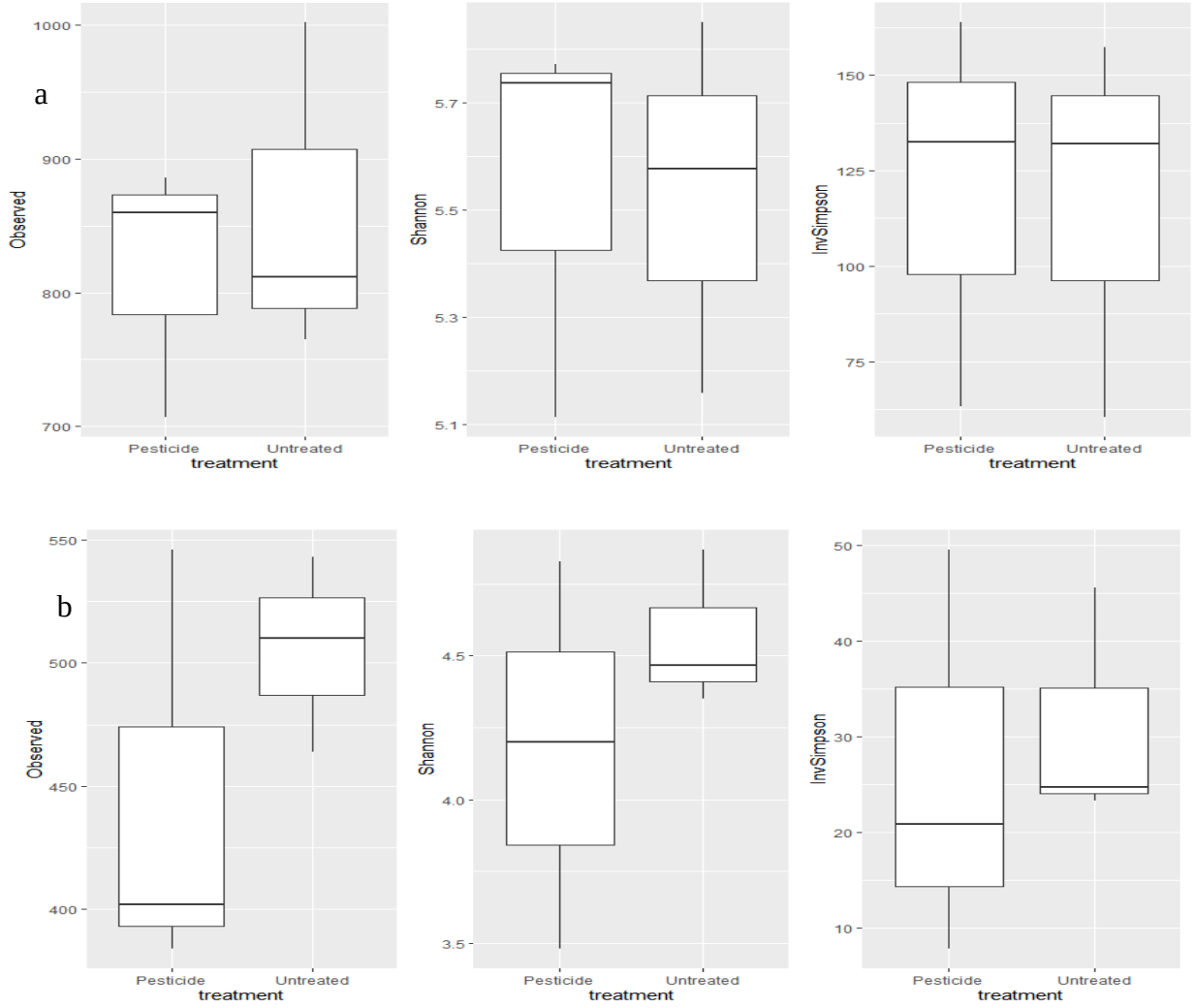


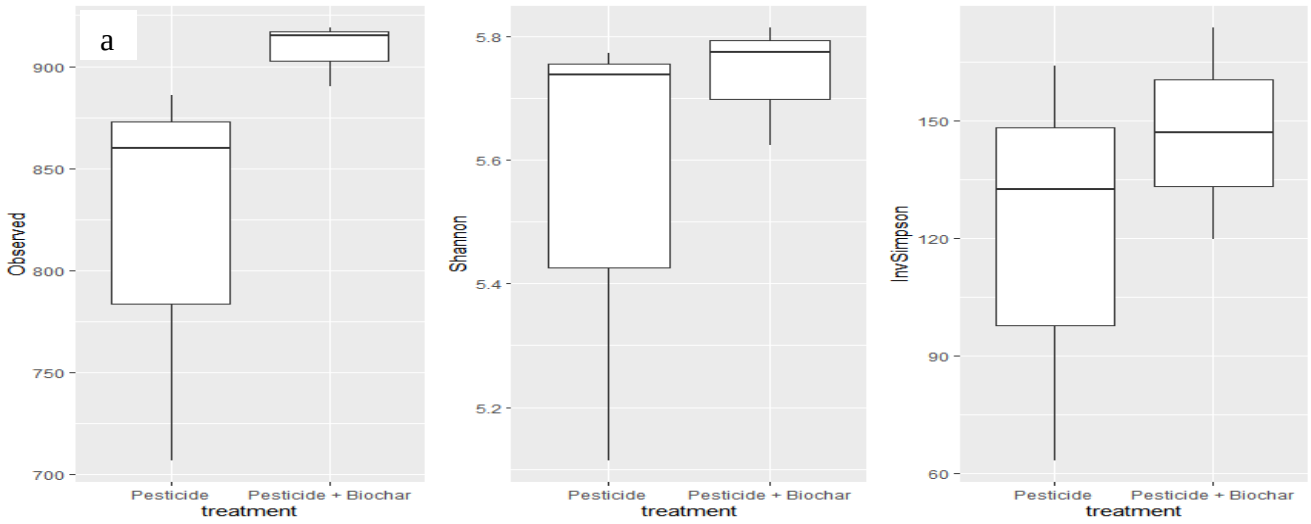
Fig.3: Alpha diversity analysis: :Observed richness,Shannon and Inverse Simpson of pesticides treated samples

((a) Mixed soil: pesticides and untreated (b)Sand soil: pesticides and untreated. The line inside the box represents the median, while the whiskersdisplay the lowest and highest values within the 1.5 interquartile range (IQR).)

3.3.2 Alpha diversity indices of two soil types after application of biochar in pesticides treated mixed and soil samples

The biochar application and its effects on alpha diversity of pesticides treated samples of both soil types are demonstrated in boxplots (fig.4.a,b). In mixed soil with pesticides, the alpha diversity indices like Observed species richness, Shannon diversity and Inverse Simpson were found higher after addition of biochar (Fig.4.a). This indicated that biochar application enhanced number of rare species and evenness of species of bacteria. Statistically there was no significant effect of biochar in on alpha diversity in pesticides treated mixed soil samples (p=0.25) but increase in diversity could be seen in the pesticides treated mixed soil after addition of biochar.

In sandy pesticides treated samples, the Observed species richness and Shannon diversity index were increased after addition of biochar while the Inverse simpson index decreased slightly (Fig.4.b). This may suggests that biochar addition in pesticides treated sandy soil was found with higher number of rare species but decrease in evenness of the common species. Statistically there was not any significant effect of biochar on alpha diversity of pesticides treated sand samples (p=0.66). However the slight increase in Observed species richness and Shannon index with slight decrease in Simpson Inverse index were observed.



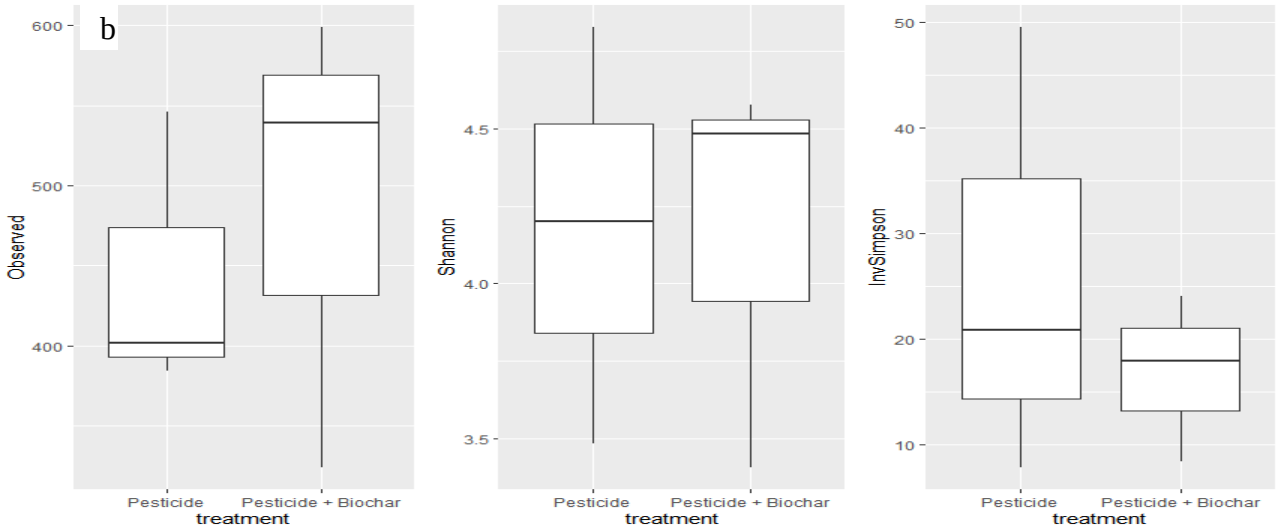


Fig. 4: Alpha diversity indices: Observed, Shannon and Inverse Simpson of biochar added pesticides samples

((a) pesticides, biochar added pesticides mixed soil samples (b) pesticides, biochar added pesticides sand samples. The line inside the box represents the median, while the whiskers display the lowest and highest values within the 1.5 interquartile range (IQR).)

4. Discussion

The aim of this research is to study the effects of biochar on microbiota of pesticides contaminated soil types (mixed and sand) through metabarcoding of 16SrRNA gene. Bacteria composition was found dominant in both types of soil (mixed and sand) environment. Lo and Chong (2020) also reported higher percentage of bacteria (97.4%) with archaea 0.2% in soil samples collected from disease free, high and low basal rot stem incidence plots. Such a difference may be due to affinity of archaea to extreme environments like high temperatures, high salts, high acidic etc (Jarrell et al., 1999). The highest usable reads found in mixed soil without receiving any treatments (pesticides or biochar) indicated the richness in bacteria before any treatments and sand samples treated with biochar received the highest reads indicating high number of micro organisms in sandy soil after the improvement of soil environment by biochar. The texture,soil type,organic matter contents ,pH may be the cause of differences in bacterial reads.

Mixed samples were found with wider and higher bacterial spectrum than sand samples. The flattened curves for both soil types was the indication of all microorganisms sampled. (Zhang et al., 2020) reported same trend of curves in the study done in soil samples to study influences of vegetation and depth in bacterial diversity of soil. The PcoA analysis showed large variation between sand and mixed soil indicating the different microbial composition and structure in the

soil types. This may be due to the variation in soil types that share different physio chemical properties. The mixed soil used in our study contained higher organic matter compared to sandy soil and varied in their texture. Mixed soil samples such as pesticides, pesticides and biochar ,untreated ,untreated and biochar showing similar microbial composition indicated that they share similar type of soil environment. The microbial composition and structure between these soil types largely varied as due to the difference in texture and soil organic matter contents. More studies reported that the differences in microbial composition in soil types due to the changes in the nutrients, pH, moisture contents of soil (Xue et al., 2018). (Seaton et al., 2020) reported the more the heterogeneity in texture of soil the higher bacterial diversity it supports. The sand soils are poor in organic substances with less moisture and may have lower capacity to withhold nutrients (Muhammad et al., 2014) which may decrease microbial activities in soil. Gavrilescu (2005) reported if the soil has high content of sand then it transports more water compared to soil with more clay and organic substances causing less activities of micro organisms due to less moisture contents in soil.

Overall the pesticides addition in unamended mixed soil samples increased the alpha diversity of microorganisms in mixed soil samples. This could be due to the higher adsorption of pesticides to the soil and lesser bioavailabity to micro organisms or could be used by some micro organisms as a source of energy through its degradation for their growth and reproduction. In sandy soil samples, the reverse effect was noticed by decreased alpha diversity of bacteria in pesticides amended soil than untreated. Several studies reported pesticides use in soil are beneficial to some microbial population as they utilise pesticides and derive energy and nutrients from their degradation (Staley et al., 2015) whereas harmful for some microorganisms and reduces their population size (Johnsen et al., 2001). The pesticides degradation and modification in soil are driven by the factors like pesticides dosages, soil type and texture, moisture content , pH, organic matter and temperature. C.-y. Wang et al. (2019) found in their result that soil pH is positively related with alpha diversity of bacteria. Haney et al. (2000) reported Glyphosate was highly degraded by microbes at higher concerntration(47, 94, 140, and 234 μg ai g−1) without causing any impact to microbial activities. The two soil types studied were different in their texture and organic matter contents. The mixed soil contained higher organic substances compared to sandy soil with good texture. Abdel Ghani et al. (2018) reported sandy soil have coarse grains and the adsorption capacity is very less compared to other. Gavrilescu (2005) reported highly sandy soil allows water to flow easily than other soil types due to which it is less added to pesticides and contain less microorganisms. Rainfall and low quality of soil have high risk of pesticides erosion especially glyphosate (Laitinen et al.,

2009) a water soluble herbicides (Sharma & Lai, 2019). This may cause desorption of glyphosate and soluble to water and toxic to micro organisms increased by its greater bioavailabity. Abdel Ghani et al. (2018) reported pesticides like fenamiphos is less adsorbed to the sandy soils as a result desorbed to the water present in the soil.

The pesticides treated samples of two soil types that received the biochar showed increase in bacterial diversity. The biochar addition had increased the number of rare species of bacteria without any change in the evenness of the species of untreated mixed sample. Likeewise in the sandy soil, the biochar addition increased the number of rare species while evenness of species of bacteria decreased. The fresh biochar used in our study was derived from pine wood at high temperature with wider surface area and porosity. (Nartey & Zhao, 2014; Safaei khorram et al., 2016) studied larger the surface area, porosity and higher organic contents of biochar increases its adsorption capacity to pesticides and higher the presence of useful organisms (Zhu et al., 2017). Biochar's physio chemical properties depend on feedstocks types and pyrolysis temperature maintained during biochar production (Tomczyk et al., 2020). Sopeña et al. (2012) found biochar increasing application rate increased the adsortion, desorption and less degradation by micro organisms in a herdicides isoproturon (IPU). Hall et al. (2018) found higher adsorption of Glyphosate (1 mg L^{-1}) by biochars at pyrolysis temperature(900 °C) and the adsorption varied among feedstocks types. The biochar produced especially wood biochar at high temperatures are very good for improving soil pH and volatile organic compounds (Zhu et al., 2017). (Yuru et al., 2021) reported pine wood biochar at different application rate (by weight) in nutrient poor sandy soil enhanced moisture content, nutrients (p,k,mg,ca), organic substances in soil and CEC of soil.This may support the increase of diversity of bacteria in sandy pesticides samples. Biochar prepared from woodchip and straw feedstocks at temperature (750^{o}C) increased carbon contents after the addition of biochar in the sandy and loamy soil containing herbicides(herbicide 4-chloro-2-methylphenoxyacetic acid). They also found sandy soil with lower organic carbon and nitrogen compared to loamy soil sand soil before addition of biochar. According to (G. Han et al., 2017); Siedt et al. (2021) bacterial communities are affected by use of biochar as it changes carbon supply and nutrients, changes water holding capacity, pH of soil and adsorb pesticides thus reduces its bioavailability to micro organisms. Besides other factors such as vegetation cover, temperature and relation with plants rhizospheres and temperature may also influences bacterial richness in the biochar amended soil (Jenkins et al., 2017). The increased observed richness of bacteria in the pesticides samples of two soil types in our study may be a result of positive alteration of soil environment of both soil types (Ren et al., 2020) due to biochar.

The abundances of some bacteria like proteobacteria ,acidobacteria by increased biochar amount in tobacco planting soils (L. Gao et al., 2017). They also noted decreased soil dissolved organic carbon and available nitrogen in rice straw derived biochar amended soil than non amended and nitrogen decreased in soil by addition as a result of biochar adsorption and fixation processesG. Han et al. (2017) studied increase in number of dominant Sphingomonas and Pseudomonas in the soils after utilising carbon sources from added biochar in the cotton soil with different cropping years after addition of biochar.

According to (Latini et al., 2019) nutrients rich biochar balances microbial composition by increasing or make their number stable. They also reported enhancement or reduction of microbial abundances and their activities may sometimes caused by various organic molecules present in fresh biochar. More studies highlighted the causes for increase in microbial abundances are due to high nutrients, unstable organic substances on biochar surface, suitable ecological niche formed by biochar (Gul et al., 2015), microbial competition (Lehmann et al., 2011), priming effect (Chen et al., 2018). (Gul et al., 2015) also reported the soil texture of soil types cause for changes in abundances of microorganism in soil. Zhelezova et al. (2017) found decreased adsorption of herbicides (glyphosate) in sand soils after the addition of biochar considering pH increase as the main cause. (Abdel Ghani et al., 2018) found biochar added soil had higher adsorption potential than without biochar in their study done in pesticides compound (fenamiphos and cadusafos) in sandy soil.

Statistically, no significant effects of pesticides on alpha diversity of microorganisms in unreated mixed and sandy soil and biochar in pesticides treated sand and mixed soilcould be due to less number of replicates used for study. Moreover other factors such as rainfall, short sampling period may introduce bias into the system that can make the effect less clear. However the increased tendency that the increase in observed species richness of of mixed and sand soil types. The biochar application on the surface of soil has high chance of being swept away by the rain leaving behind its less impact on soil (Palviainen et al., 2018). During our field sampling, there was continous rainfall after biochar addition in soil and could be one of the possible cause of less effect of biochar in soil health as expected. Therefore bio char could be an alternative approach to preserve soil fertility and its biodiversity, control land degradation, promotes crop production to reduce food insecurity in future (Agegnehu et al., 2017).

Conclusions

This current study highlights the positive effects of biochar on microbiota of pesticides amended soil using NGS approach following DNA metabarcoding studies of two soil types. The alpha diversity of species richness was higher in mixed soil. Large variation in microbial composition was found between two soil types. Biochar adsorption and desorption are influenced by factors such as soil type and texture, climate, soil pH,organic matter contents,moisture.The pesticides use in untreated mixed soil increased number of rare species of bacteria while no change in evenness of bacteria .In sandy soil, pesticides decreased the alpha diversity of bacteria in pesticides amended soil while decreased the alpha diversity in pesticides amended soil. Likewise biochar application increased alpha bacterial diversity in mixed soil and in sand treated with pesticides, Observed species richness, Shannon diversity increased with decrease in Inverse simpson index. Statististcally there was no significant effects of pesticides and biochar in pesticides amended soil though slight increase or decrease in the alpha diversity indices by their use were observed.The insignificant effects may be due to less number of replicates .Hence biochar has positive play positive role in supporting soil microbial diversity and remediation of contaminated soil from pesticides.

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Appendix: Supplementary tables

Table A-1: Samples of soil types (mixed and sand) with their depth of reads

Mixed samples	Reads	Sand samples	Reads
Untreated	89302	Untreated	90994
Untreated	90820	Untreated	92764
Untreated	97614	Untreated	87299
Pesticides	76004	Pesticides	123180
Pesticides	60937	Pesticides	113552
Pesticides	70840	Pesticides	119665
Untreated and biochar	71476	Untreated and biochar	66135
Untreated and biochar	83485	Untreated and biochar	123530
Untreated and biochar	73740	Untreated and biochar	99648
Pesticides and biochar	91894	Pesticides and biochar	97407
Pesticides and biochar	63877	Pesticides and biochar	90246
Pesticides and biochar	94015	Pesticides and biochar	98558

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