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Meta-analysis of biodynamic (BD) preparations reveal the bacterial population involved in improving soil health, crop yield and quality

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ABSTRACT

Background: Bacterial community found in biodynamic preparations (BD500–BD507) can help improve soil health, plant development, yield, and quality. The current work describes a metagenomic investigation of these preparations to identify the bacterial communities along with the functional diversity present within them.
Results: Metagenome sequencing was performed using the Illumina MiSeq platform, which employs next-generation sequencing (NGS) technology, to provide an understanding of the bacterial communities and their functional diversity in BD preparations. NGS data of BD preparations revealed that maximum operational taxonomic units (OTUs) of the phylum Proteobacteria were present in BD506 (23429) followed by BD505 (22712) and BD501 (21591), respectively. Moreover, unclassified phylum (16657) and genus (16657) were also highest in BD506. Maximum alpha diversity was reported in BD501 (1095 OTU) and minimum in BD507 (257 OTU). Further, the OTUs for five major metabolic functional groups viz carbohydrate metabolism, xenobiotic degradation, membrane transport functions, energy metabolism, and enzyme activities were abundant in BD506 and BD501.
Conclusion: The bacterial communities in BD506 and BD501 are found to be unique and rare; they belong to functional categories that are involved in enzyme activity, membrane transport, xenobiotic degradation, and carbohydrate metabolism. These preparations might therefore be thought to be more effective. The investigation also found a highly varied population of bacteria, which could explain why BD preparations work well in the field. In view of this, the BD preparations may be utilized for unexploited bacterial communities for sustainable agriculture production.

1. Background

Modern farming practices require extensive use of chemical fertilizers (CF) for higher crop production, but these methods are expensive and create several enormous problems related to health and the environment[1]. Under these circumstances, concerns are raised against the residual effects of chemicals on fresh fruits and vegetables that are consumed every day. The use of chemicals in agriculture sectors both fertilizers and pesticides (PS) may damage soil health by reducing microbial diversity[2] acidification of the soil, depleting minerals, and subsequently causing soil and water pollution[3–4].

There has been a worldwide revival of attention towards eco-friendly, and sustainable agriculture practices for better crop quality and soil health. Natural/organic nutrient management strategies which include organic farming and other quite close nature-based alternate farming systems viz Permaculture, Rishi Krishi, Panchgavya, Natueco farming (Nature ecological farming), and Zero Budget Natural Farming (ZBNF), Biodynamic farming are considered as the potential options for CF and PS.

Abbreviations: BD preparations, Biodynamic preparations; OTUs, Operational Taxonomic Units; NGS, Next Generation Sequencing; PCR, Polymerase Chain Reaction; PCR-SSCP, Polymerase Chain Reaction-Single Strand Conformation Polymorphism; PLFA, Phospholipid fatty acids; DGGE, Denaturing Gradient Gel Electrophoresis; IAA, Indole acetic acid; IBA, Indole-3-butyric acid.

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Permaculture is the type of agricultural system that directly uses the patterns and features found in natural ecosystems[5]. Rishi Krishi is a natural farming method created by farmers in Madhya Pradesh and Maharashtra state of India. The preparation and procedure for this approach can be found at www.rishikrishi.co.in[5]. Panchgavya is a mixture of five cow-based components— curd, dung, ghee, milk and urine —that are acquired from cows. The aforementioned ingredients are mixed thoroughly in the 2:1:1:2:3 ratio and subsequently fermented for seven days, with frequent stirring[5–6]. Naturéco farming, also known as nature-based farming, works in synchronization with the natural world, minimizing reliance on outside inputs, and applying science to the resources found in the farm's surroundings in order to maximize benefits without endangering the ecology. The principal components of natueco are amrutmitti and amrutjal[5,7]. Zero Budget Natural Farming (ZBNF) is a farming approach that relies on crops growing naturally without the use of pesticides, fertilizers, or other external substances. The term “zero budget” describes the zero net cost of production, which includes intercrops, border crops, and multi-crops. ZBNF has 4 key elements viz Bijamrit (seed treatment), Jiwamrit (microbial culture), Achhadana (mulching), and Whapasa (soil aeration)[5,8]. Biodynamic (BD) farming is the agricultural system, centric to reduce the use of excessive chemicals in agriculture, besides the restoration of soil health[5,9].

BD farming is one of the oldest organized organic agriculture farming systems which enhances soil fertility and consequently improves plant growth and yield through the application of BD preparations[10]. Although, BD preparations do not add significant nutrients themselves, to stimulate the processes of nutrient and energy cycling, hasten decomposition, and improve soil and crop quality even by applying as few grams per ton of organic material[11]. There are eight BD preparations viz BD500, BD501, BD502, BD503, BD504, BD505, BD506, and BD507 which are prepared using a fermentation process and are reported to possess unique characteristics features[5,12–14]. BD500 is used as a spray (25 g/acre in 13 L of water) to revive soil health and improve seed germination, and root development[15]. BD501 is sprayed to improve plant immunity, seed and fruit quality as well as the photosynthesis process. BD502- BD507 are utilised for BD compost preparation. BD502 has high potassium (K) and sulphur (S) content. In order to provide plants with optimal nourishment, BD502 aids plants in absorbing trace elements in extremely dilute quantities. BD503 has a high concentration of S and Calcium (Ca). BD503 stimulate plant growth by refining soil health by improving soil life and stabilising nitrogen (N) in the compost. BD504 has a considerable amount of S, K, Ca and Iron (Fe). Due to this BD504 plays a direct or indirect role in the growth and development of plants. BD505 is abundant with Ca and aids in protecting plants from various plant diseases. BD506 facilitates the uptake of silicon (Si) and potassium (K) from the soil. BD507 aids in increasing the plant's phosphorus availability[5,14,16].

The bacterial diversity and richness in soil can positively influence plant growth, yield, quality, and soil health[17–18]. Alpha diversity indices are widely used to characterize microbial communities in any ecosystem[19]. It has two components viz species richness and evenness indices. The quantity of distinct species found in a given niche is measured by species richness. The nonparametric abundance-based estimators viz Chaos1 and ACE are generally used to calculate species richness. Evenness, on the other hand, is a metric for the relative abundance of various species that make up a community[20]. Shannon-Weaver and Simpson diversity indices generally offer better insight into the composition viz diversity, rarity and evenness (commonness) of the community.

Many studies have examined the potential benefits of BD preparations for enhancing soil microbiota, soil properties, yield, and quality in a range of crop plants, including perennial fruit trees[6,21–23]. It is now widely documented that only < 1 % of bacteria are culturable, whereas the rest are unculturable[24–27]. Earlier, only the study on culturable microbiota present in BD preparations was done by many researchers[28–29], that are not reflect the actual microbial diversity and function present in BD preparations. Moreover, a preliminary investigation in our laboratory also signposted the culturable bacteria present in BD preparations[30]. Nevertheless, not enough research has been done to ascertain the microbial compositions and their function in the improvement of crop and soil health.

Earlier, many molecular techniques viz PCR-SSCP[31], PLFA[32], and DGGE[33] had been developed for the exploration of microbial communities under various habitats. Since the development of Next Generation Sequencing (NGS) technologies, metagenomics has been utilized as a suitable method to comprehend the microbial diversity and linked function in any environmental sample without requiring culture. Its benefits over substitute methods include superior precision, low cost and high throughput. This technique is most frequently used to study the variety of microbial communities in various habitats, the interactions between various microorganisms, and communities' adaptation to alteration of habitats[34].

The information about the principle underlying the efficacy of BD preparations is negligible. There is a lot of literature now available on the use of high-throughput sequencing techniques to study soil microorganisms, but none on the study of BD preparation. In light of the aforementioned, a metagenomic approach was followed to study the bacterial diversity and community structure in various BD preparations. In any ecosystem, bacteria are one of the important key biotic components (in the form of decomposers) and their number and diversity are always higher than other microflora. They decay the large and complex living/nonliving organic and inorganic compounds into small and simpler ones viz nitrate, phosphate, carbon dioxide, water, amino acids, simple sugars, mineral salts etc and easily make them available to plants[35–37]. Moreover, the different genera of bacteria also synergistically interact with plants (producers) and improve their growth directly via producing Indole (IAA, IBA), solubilizing phosphorous and other minerals, fixing environmental N in soil etc or indirectly by protecting the plants from pathogens[38–39].

2. Methods

2.1. Biodynamic preparation samples and experimental design

The BD preparation samples were procured from the leading producer of BD preparations named Supa Biotech (P) Ltd.; Nainital, Uttarakhand, India (latitude: 29.39 N, longitude: 79.46 E). The eight different types of BD preparation viz BD500, BD501, BD502, BD503, BD504, BD505, BD506, and BD507 were taken for the metagenomic study. BD500 and BD501 are prepared from fresh cow dung and silica paste incubated in cow horns, respectively. BD502, BD503, BD504, and BD506 are made from the *Achillea millefolium* L. (yarrow flower), *Matricaria chamomilla* L. (chamomile), *Urtica parviflora* (Himalayan stinging nettle), and *Taraxacum officinale* (dandelion), respectively. BD505 is made by stuffing the crushed bark of *Quercus glauca* (oak tree) into the skull cavity of any domestic animal whereas the extract of *Valeriana officinalis* L. (valerian flowers) is used for the preparation of BD507[5,14,40–41]. After procurement, the BD preparation samples were then divided into three parts. The first portion of the sample was dried by air for physicochemical analysis. The second portion of the sample was preserved at 4 °C for microbiological analysis, while the third portion was kept in a tight-pack cryotube and stored at −80 °C for metagenomic analysis.

2.2. Isolation of bacterial genomic DNA from biodynamic preparations

The genomic DNA isolation kit (Chromos Biotech Pvt. Ltd., India) was used to isolate the DNA from BD preparations by following the given instructions by the manufacturer. The integrity of the isolated

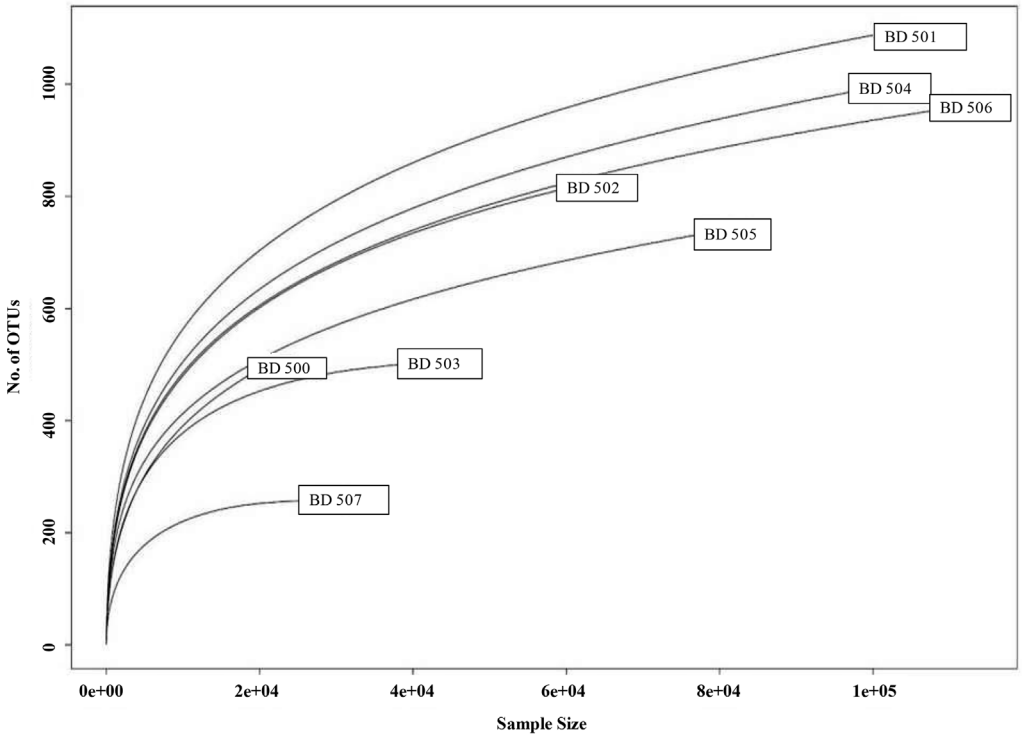

Fig. 1. Rarefaction curve: representing number of OTUs (species richness) in different BD preparations.

DNA was confirmed on 0.8 % agarose gel by electrophoresis in TAE buffer and further visualisation under UV in uvitec (Bangalore Genei, India). The quantitative yield and quality of the isolated DNA were then assessed using a Nanodrop spectrophotometer (Nanodrop ND 1000).

2.3. Amplification of 16S rRNA gene sequence

The amplification of the 16S rRNA gene from the isolated bacterial genomic DNA was performed by following the protocol described earlier[42]. The hyper-variable region (V3-V4) of the 16S rRNA gene was amplified using the modified 341F forward primer (5′-CCTACGGGN GGCWGCAG-3′) and 785R reverse primer (5′-ACTACHVGGGTATC TAATCC-3′). 25 ng of bacterial genomic DNA, 10 μM of forward and reverse primers, and KAPA HiFi HotStart Ready Mix make up the PCR reaction mixture. The PCR protocol was set up as follows: 95 ˚C for 5 min (initial denaturation); amplification which includes 25 cycles of 94 ˚C for 30 s (denaturation), 55 ˚C for 45 s (annealing), and 72 ˚C for 30 s (extension); 72 ˚C for 7 min (final extension); and 4 ˚C for an infinite period.

2.4. Amplicon purification, quality checking and library preparation

The amplicons were purified using Ampure beads to eliminate any remaining primers. Subsequently, 8 PCR cycles were run to construct the sequencing libraries with Illumina barcoded adapters. On the Illumina MiSeq platform, the library underwent further sequencing with the target of 0.5 million reads per sample using 2x 250 Paired-end (PE) chemistry. The quality of the sequence data was assessed using easily accessible online available tools viz FastQC (v0.11.7)[43] and MultiQC[44]. The data was investigated to determine the distribution of base call quality, percentage of bases over Q20, Q30, percent GC, and contamination of sequencing adapter in the sample. The QC threshold (Q20 > 95 %) was found to be passed by every sample (Table 1).

2.5. Sequencing and metagenomic analysis

The methods previously reported were used for the 16S rRNA gene sequencing of soil bacteria[45]. To get rid of the degenerate primers, the readings were trimmed by 20 bp from the 5′ end. Trim Galore was used to eliminate adaptor sequences and low-quality bases from the

Table 1
Summary of raw sequence data and quality.

Sample-ID	Number of reads	Read Length	GC%	% Bases > Q20
BD 500	125,546	301	53	99.22
BD 501	468,524	301	53.5	99.05
BD 502	334,338	301	53.5	98.48
BD 503	191,948	301	53.5	98.88
BD 504	506,268	301	53.5	99.1
BD 505	371,382	301	53.5	99.03
BD 506	504,650	301	54.5	99.36
BD 507	162,446	301	54	98.52

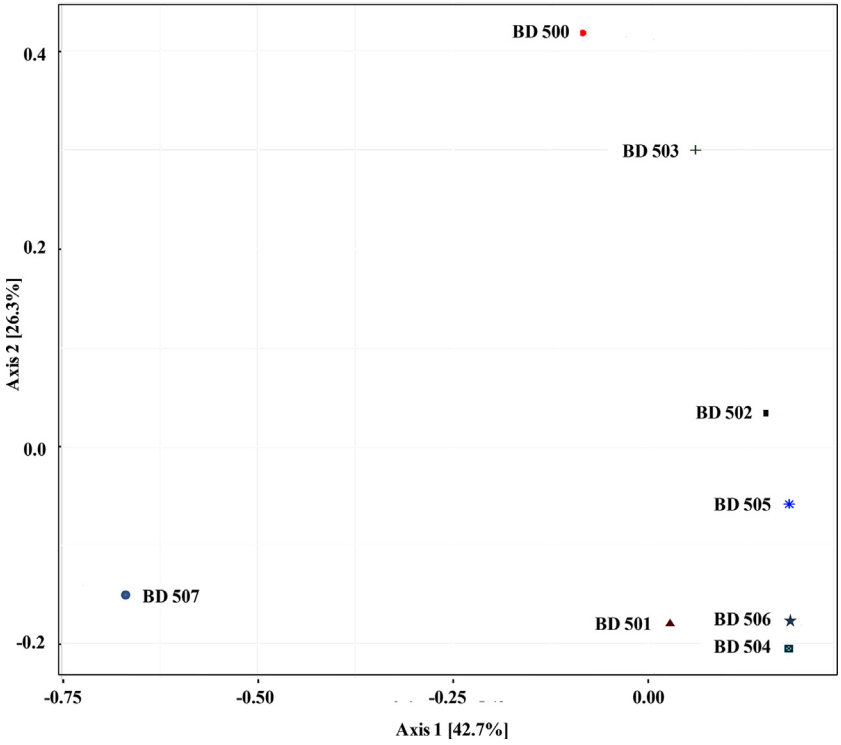

Fig. 2. Interaction between the samples (BD500 –BD 507) by Principal Component Analysis.

Table 2
Diversity indices as recorded in 8 BD preparations.

S.N.	OTU's	Chao1	se.chao1	ACE	se.ACE	Shannon	Simpson	InvSimpson	Fisher
BD500	503	733.00	49.80	675.36	13.37	3.94	0.94	17.95	92.28
BD501	1095	1392.31	49.13	1353.52	18.67	4.89	0.98	59.19	171.08
BD502	821	1039.68	41.23	1007.31	16.06	4.38	0.96	27.63	133.67
BD503	503	518.00	6.51	520.86	11.10	3.88	0.94	17.28	80.57
BD504	993	1347.78	60.68	1257.00	18.04	4.53	0.97	32.84	153.25
BD505	739	961.51	43.59	941.71	15.69	4.29	0.97	29.40	112.44
BD506	966	1240.32	48.63	1197.11	17.56	4.30	0.96	23.23	144.79
BD507	257	257.00	0.00	257.00	7.79	3.41	0.94	15.85	39.04

trimmed reads[46]. The QC-passed reads were aligned with each other to construct contigs using Mothur software v1.39.5.0. Only contigs ranging in size from 300 bp to 532 bp were kept after being inspected for errors. Any contig that had incorrect base calls was omitted. The high-quality contigs were inspected for sequence homology and during this process, the duplicates sequences were merged in order to get the unique sequences. Despite being intended for the 16S bacterial rRNA gene, the primers used in this experiment had a good possibility to amplify the other areas non-specifically. In order to account for this, the contigs were aligned with the 16S rRNA gene of a known database. Most of the contigs were aligned to the corresponding variable region of the 16S RNA gene in the database. Contigs that aligned erratically with other database areas were removed. The UCHIME algorithm was used to eliminate the chimeric sequences[47]. Thereafter, the filtered contigs were processed for OTU construction. The OTU abundance in the aforementioned metagenomic sample was mined using PICRUSt software[48]. The inbuilt QIIME (v.1.9.0) software run on the py script was used for this purpose. The filtered sequence data were taken as input by QIIME and matched with the Greengenes reference database at a 97 % sequence similarity threshold. The UCLUST algorithm was used for cluster and OTUs assignment of similar sequences[49]. The various metabolic potentials among the BD preparations were investigated using PICRUSt.

2.6. Statistical analysis

The α-diversity and OTUs were examined using the online tool Microbiome Analyst (https://www.microbiomeanalyst.ca/faces/home.xhtml) that do a thorough statistical *meta*-analysis of output QIIME data. To assess the richness shared among sample groups. The QIIME diversity command was employed with the maximum depth parameter to compute diversity measures. The statistical analysis was done by the 'ggpubr' R package.

3. Results

In the new era of technology, the abundance of bacterial diversity in soil (both culturable and nonculturable) is analyzed by the metagenomics approach. In this method, the 16S rRNA genes fragment from extracted DNA of soil microbes were amplified and further analysed by various tools. This technique is cost-effective, consistently reliable, and accurate for bacterial classification. The V3-V4 region of 16S rRNA genes is frequently exploited in phylogenetic classifications such as genus or species in diverse bacterial populations. The consensus nature of the V3-V4 regions is recognised as appropriate for identifying the bacteria up to the species level.

Table 3
Profile of top 10 most abundant OTUs observed in 8 BD preparations at phylum, genus and species level.

Phylum level	BD 500	BD 501	BD 502	BD 503	BD 504	BD 505	BD 506	BD 507
p_Proteobacteria	5452	21,591	13,548	9754	20,771	22,712	23,429	11,985
p_Planctomycetes	2210	13,611	9442	3826	13,242	15,446	14,166	3467
p_Bacteroidetes	2260	21,609	6040	3968	9918	8739	14,821	3879
p_OD1	4244	4984	7170	11,361	11,589	7790	8808	81
k_Bacteria_unclassified	1900	6244	7398	2486	9702	6938	16,657	659
p_Actinobacteria	956	7116	3058	1662	9823	2951	10,549	217
p_TM7	1649	2877	2976	2544	3056	2749	8651	62
p_Firmicutes	410	9055	713	545	9295	531	2489	422
p_Chloroflexi	225	2185	2679	892	4869	3855	4856	21
p_Verrucomicrobia	496	5550	1776	1794	1031	1560	1730	1544
Genus level								
k_Bacteria_unclassified	1900	6244	7398	2486	9702	6938	16,657	659
c_ZB2_unclassified	3927	4860	6171	7350	10,003	6677	7629	51
p_Bacteroidetes_unclassified	652	2048	2549	1174	2870	4704	8925	74
f_Pirellulaceae_unclassified	483	3365	2687	1006	4953	5978	4038	27
c_TM7-1_unclassifiified	1119	621	2680	2306	2682	2553	7652	25
g_Planctomyces	96	3282	937	279	3233	1647	1862	15
f_Chitinophagaceae_unclassified	1111	1074	1741	2353	368	1776	2531	20
c_Gammaproteobacteria_unclassified	562	600	1294	1225	2332	1919	2446	63
o_Phycisphaerales_unclassified	18	82	1288	28	1362	1195	4189	13
o_WD2101_unclassified	829	1134	2216	1535	254	1205	881	6
Species level								
s_sulfuriphila	4	3639	0	3	8	0	31	3
s_MS3071	3	2669	10	1	21	0	15	0
s_flava	7	6	285	839	211	331	562	3
s_lwoffii	0	18	0	1	2213	0	0	0
s_copri	120	234	190	56	640	372	30	148
s_flexibilis	5	1642	4	0	6	0	89	3
s_olearia	0	847	11	0	265	0	254	3
s_maritima	1	13	76	9	742	326	47	0
s_soli	1	765	3	0	114	5	12	0
s_exiguus	0	0	0	0	0	0	1	820

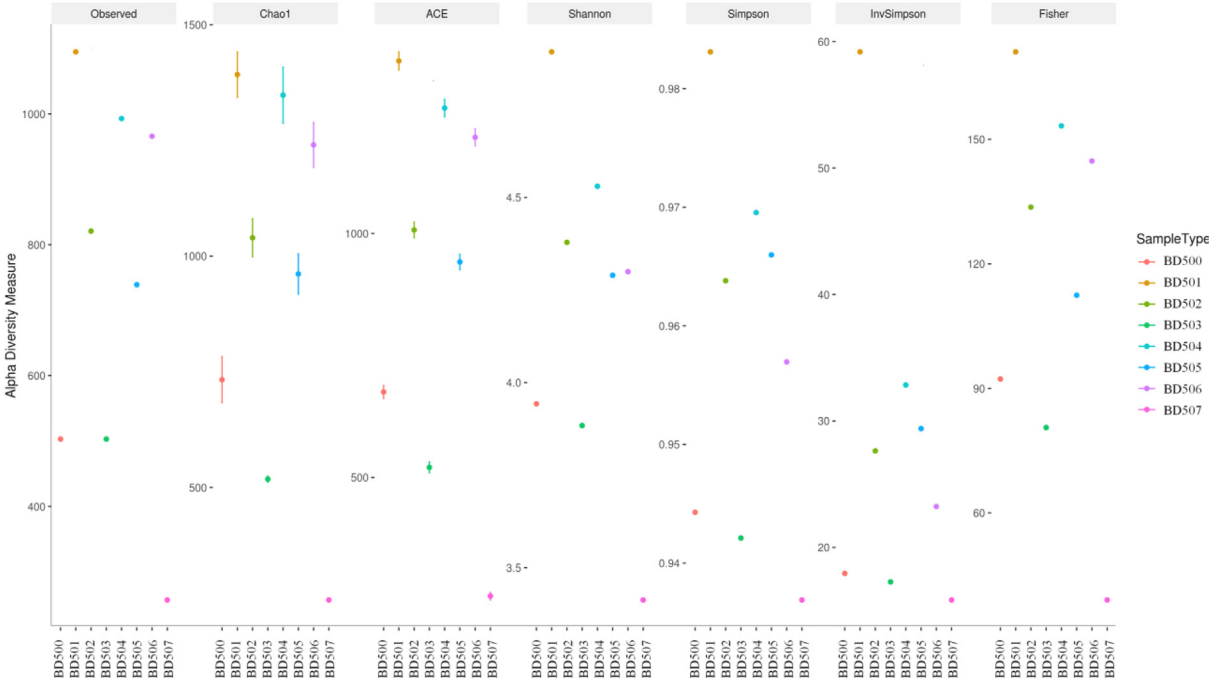

Fig. 3. Alpha diversity indices of different BD preparation.

In the present study, the hypervariable region (V3-V4) of the 16S rRNA gene of bacteria was amplified for metagenomic analysis. The basic summary statistics viz reads for samples (BD500- BD507), average read lengths for amplicon data of all the eight samples is depicted in Table 1. BD500, BD501, BD502, BD503, BD504, BD505, BD506, and BD507 yielded a total of 125546, 468524, 334338, 191948, 506268, 371382, 504650, and 162,446 reads, respectively with the average read length of 301 bp. The highest number of reads was accounted for in BD504 and the lowest in BD500.

A rarefaction curve was used to link the relationship between the number of OTUs and the number of sequences in BD preparations. It is possible to depict species richness using sample results. The plateau rarefaction curve reflects the number of newly discovered OTUs is limited with further sequences and vice-versa (Fig. 1). Among the 8 BD preparations, the maximum diversity (OTUs) was recorded in BD501 (1095 OTUs) and the minimum in BD507 (257 OTUs) (Fig. 1; Table 2). The bacterial community dynamics among BD preparations were analyzed by the Principal Component Analysis (PCoA). This plot was used to check the taxonomical distribution-based similarities among BD preparations. The PCoA plot showed that all 8 BD preparations (BD500 –BD507) were not similar to each other and had different values on axes 1 and 2. However, BD501 and BD503 were similar on axis 1 but dissimilar on axis 2; likewise, BD502, BD504, BD505, and BD506 were also relatively more similar on axis 1 but somewhat dissimilar on axis 2. The BD507 showed comparative similarities with BD501, BD504, and BD506 at axis 2, while no similarities were seen at axis 1. Among all, BD507 represented no similarities with other BD preparations (Fig. 2).

Alpha diversity is a metric used to determine the relative abundance and richness of organisms in a sample. The different Alpha diversity indices viz Shannon, Simpson, InvSimpson, Chao1, ACE, and Fisher in different BD preparations are displayed in Fig. 3. Sample richness is shown by Chao1 and ACE, while both relative abundance and richness are specified by Shannon, Simpson, InvSimpson, and Fisher. The higher value of the aforementioned indices of BD preparation indicated higher dominance and higher richness in terms of bacterial diversity. Based on this, the BD501 and BD507 were found to be the highest and lowest diversity as well as richness, respectively (Fig. 3, Table 2).

The bacterial population present in the BD preparations was classified at the phylum, genus, and species levels by means of OTUs identified by Quantitative Insights into Microbial Ecology (QIIME) analysis of the sequencing data. The bacterial population in BD preparations was accounted for in the following order: BD506 > BD501 > BD504 > BD505 > BD502 > BD503 > BD507 > BD500 (Table 3 and Fig. 4). Proteobacteria was found to be the most dominant phylum in all BD preparations. Among BD preparations, the abundance of Proteobacteria was observed to be highest in BD506 (23429) and lowest in BD500 (5452). Moreover, the abundance of phylum viz Actinobacteria, TM7, and other unclassified bacteria was found to be maximum in BD506 (10549, 8651, and 16657, respectively) and minimum in BD507 (217, 62, and 659, respectively). Similarly, phylum Bacteroidetes and Verrucomicrobia were found to be most abundant in BD501 (21609 and 5550, respectively) and minimum abundance was recorded in BD500 (2260 and 496, respectively). Likewise, OD1 and Chloroflexi were found to be maximum in BD504 (11549 and 4869, respectively) and minimum in BD507 (81 and 21, respectively). The maximum richness of phylum Firmicutes and Planctomycetes was recorded in BD504 and BD505 (9295 and 15446, respectively) while the minimum was in BD500 (410 and 2210, respectively).

The abundance of the top 10 OTU at the genus level and their percent distribution among BD preparations are depicted in Table 3 and Fig. 5, respectively. In Fig. 5, the area of each fragment represented the abundance of one OTU at the genus level. The highest number of bacteria at the genus level was found in the k_Bacteria_unclassified. The abundance of *Plancomyces* accounted for the maximum in BD501, which justifies our result reported at the phylum level. At the species level, some of the OTUs were extremely dominant in particular BD preparation (Table 3 and Fig. 6). The abundant population of species *sulfuriphila* (*Petrimonas sulfuriphila*), *MS3071* (*Planctomycete MS3071*), *flexibilis* (*Serpens flexibilis*), *olearia* (*Muricauda olearia*) and *soli* (*Solimonas soli*) were recorded in BD501 preparation. Similarly, BD504 was found to be the largest population of *lwoffii* (*Acinetobacter lwoffii*), *copri* (*Prevotella copri*) and *maritime* (*Woodsholea maritime*) species. Moreover, *flava* (*Phaselicystis flava*) and *exiguous* (*Corallococcus exiguous*) were found to be the richest in BD503 and BD507 preparation, respectively.

To examine the functional characteristics of the bacteria in BD preparations, functional analysis of metagenomic data was used. Table 4 lists the metabolic roles that various BD preparations play in terms of energy metabolism, xenobiotic degradation, membrane transport, enzyme families, and carbohydrate metabolism. In BD506, the greatest abundance of OTUs was found for the metabolism of carbohydrates (14994476), energy (8733277), enzyme families (2777826), and membrane transport (15485898). For membrane transport, BD501 (14505442) had the second-highest number of OTUs, following BD506. The largest relative abundances of OTUs associated with

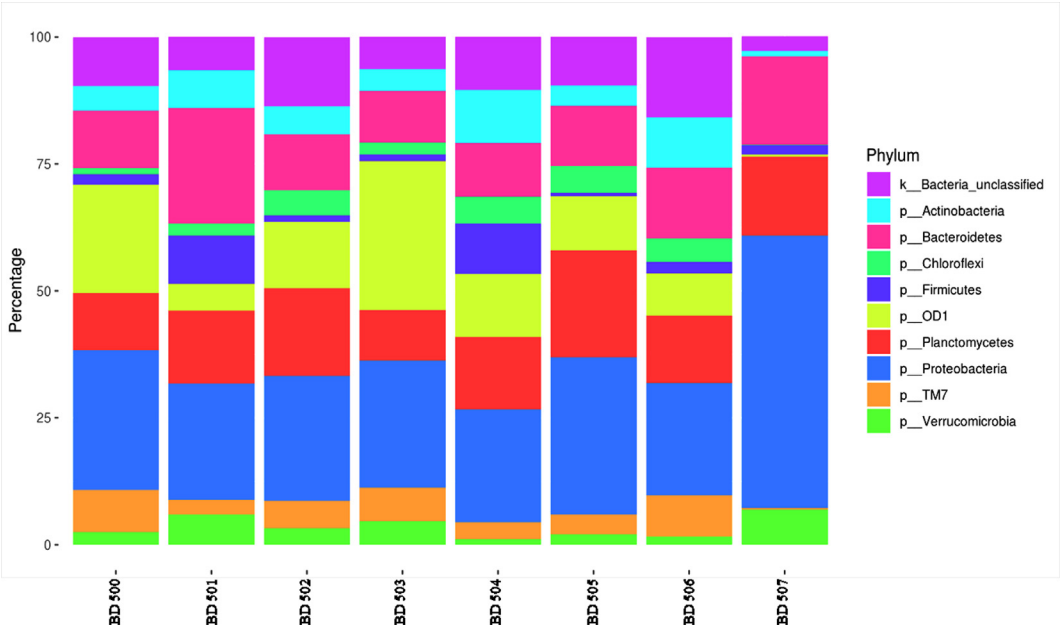

Fig. 4. Relative abundance of top 10 bacterial OTUs in soil at phylum level. One color represents one OTU and length of the color block presents the relative richness proportion of the phylum.

energy metabolism, carbohydrate metabolism, and enzyme activity were found in BD506 and BD501. BD506 (4484910) had the highest OTU for the biodegradation of xenobiotics, while BD500 (770577) had the lowest.

4. Discussion

The organic carriers are generally rich in beneficial microbes. However, the potential of different origins of organic carriers to retain microbial richness and diversity is different. It supports to maintain the population of microbes beyond the threshold limit for a longer duration probably by providing a favourable nutritional environment[50–51]. Moreover, the effective organic carrier can promote better plant growth, yields, and soil health via add of microbial richness and diversity in soil[51]. Similarly like other organic carriers BD preparation were also found to improve the plant growth via improving soil microbial diversity as well as soil quality[52]. Recently, Jayachandran and coworkers studied on physico-chemical characters of biodynamic herbal preparations (BD 502 to 507) and accounted to rich of N, K, organic C, and different other micro and macro nutrients apart from heavy microbial load[53]. Earlier, application of BD preparation in wheat was reported to enhanced spikelet production, seeds/ spikelet and grain yield under stress conditions[54]. Their study also reported that in bio-dynamically grown wheat and maize crops have better root growth, health and higher yield under stress environment apart from increased organic carbon and improved soil health. Moreover, Biody-

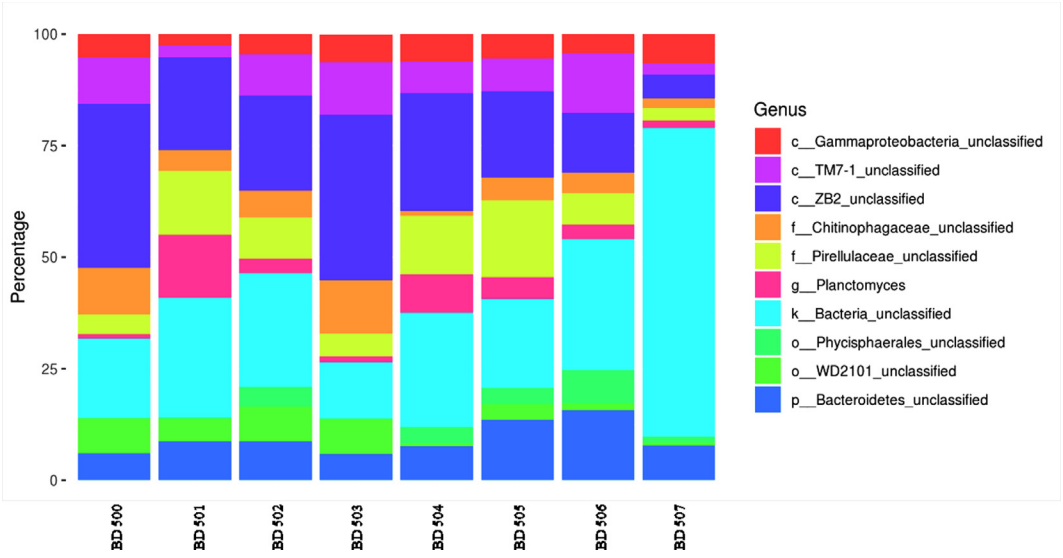

Fig. 5. Relative abundance of top 10 bacterial OTUs in soil at genus level. One color represents one OTU and length of the color block presents the relative richness proportion of the genus.

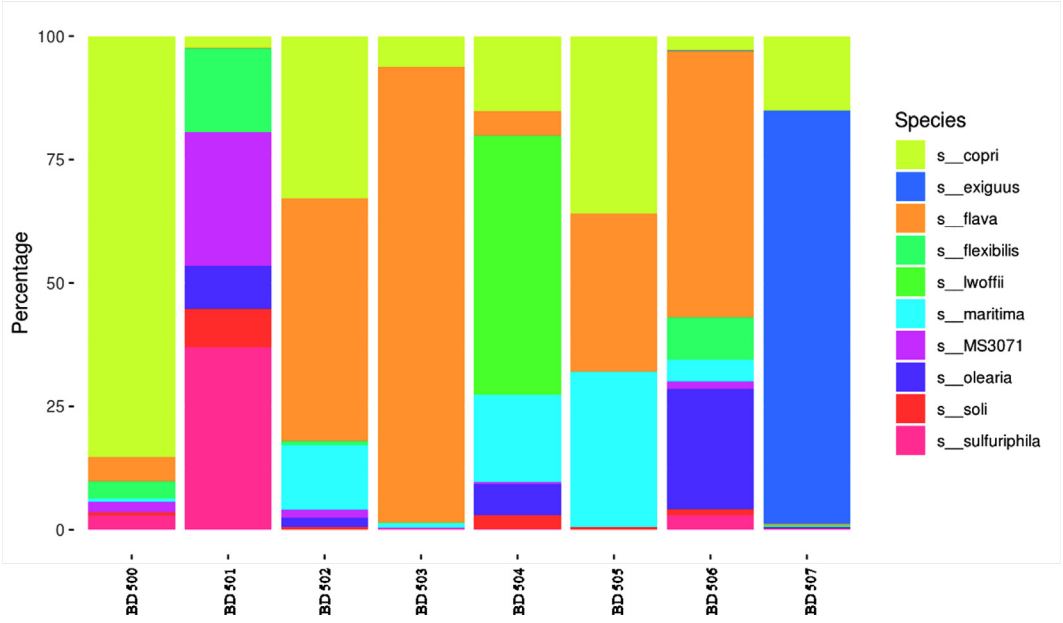

Fig. 6. Relative abundance of top 10 bacterial OTUs in soil at species level. One color represents one OTU and length of the color block presents the relative richness proportion of the species.

Table 4
Functional attributes of bacterial OTUs in BD preparations.

Functional attributes of bacteria	No. of OTUs (In Millions)							
	BD 500	BD 501	BD 502	BD 503	BD 504	BD 505	BD 506	BD 507
CM	2.81	13.69	8.39	5.54	12.95	11.30	14.99	3.96
EM	1.70	7.69	5.08	3.37	7.32	7.04	8.73	2.42
EF	0.52	2.59	1.60	1.04	2.39	2.23	2.78	0.77
MT	2.87	14.51	8.56	5.53	14.48	11.06	15.49	3.27
XBM	0.77	3.93	2.34	1.50	3.73	3.23	4.48	1.43

CM, carbohydrate metabolism; EM, energy metabolism; EF, enzyme families; MT, membrane transport; XBM, xenobiotics biodegradation

namic preparations treated with compost was found to have enriched the nitrate content of the compost and also enhanced the microbial parameters[55]. In another organic farming experiments conducted by Rodas-Gaitan and coworkers at University of Bonn in Hennef, Germany accounted that the BD compost applications improved the soil properties and nitrogen status of the microbial community[56]. In light of aforementioned, the present experiment was conducted to explore the diversity and richness of bacteria in different BD preparations in order to their precise utilization.

Proteobacteria present in agricultural samples are involved in N-fixation and consequently improve plant growth[57–59]. In our study, we found that the relative abundance of Proteobacteria was higher than other phylum. Our study is in line with the findings of Joel et al.[60]. They accounted that the relative abundance of Proteobacteria was higher in agricultural samples than in non-agricultural samples. In another study, Mhete et al. found that the abundance and diversity of Proteobacteria were maximum among different soil samples including garden soil, saline soil, and sludge-impacted soil[61]. They also described the existence of other groups of phylum viz Bacteroidetes, Actinobacteria, Firmicutes, and Chloroflexi in the aforementioned soil samples; however, their abundance and diversity were comparatively less and varied from sample to sample. Huhe et al. reported that the bacteria belonging to different classes of phylum proteobacteria were found in abundance in various stages of the composting process[62]. In our investigation, a comparable result was observed in BD preparations. George et al. determined, via amplifying 16S ribosomal DNA (rDNA), the distribution of ammonia-oxidizing members of the beta subdivision of the family Proteobacteria in a range of composting materials[63]. This finding supported that the abundance of Proteobacteria in BD preparation makes it stronger towards plant growth applicator.

The abundance of Bacteroidetes phylum in diverse BD preparations is not surprising because the abundance of Bacteroides in plant microbiome including phyllosphere, endosphere, and rhizosphere reported much higher than in the surrounding soils[64–65]. The Bacteroidetes is the most dominant phylum accounted 5–65 % of microorganisms linked to crops and causes a great impact on plant health[66–67]. Moreover, Bacteroidetes are also commonly occurring in the microflora of the gut of animals. Girija et al. reported the maximum abundance of bacteria belonging to phylum Bacteroidetes (38.3 %) followed by Firmicutes (29.8 %), Proteobacteria (21.3 %), and verrucomicrobia (2 %) in cow dung[68]. The abundance of the aforementioned phylum has been reported in several different ecosystems as degraders of polymeric organic matter [69–70]. Its abundance in agricultural systems is comparatively higher than in the same soil under non-disturbed conditions[71].

In all the BD preparations, most of the culturable bacteria belong to the genus *Bacillus* which confirmed the existence of Firmicutes. *Bacillus* is a universal PGPR in soil that plays a key role in conferring tolerance against abiotic and biotic stress to plants via inducing systemic resistance (ISR), lipopeptide production, and biofilm formation[72]. Besides this, these are reported to improve plant growth and soil health and also play other beneficial roles such as remediation of metals, improving the carbon sequestration process, facilitating phosphorous uptake, acting as a potent denitrifying agent in agroecosystems, etc.[73–75]. The greatest number of bacteria that belong to the unclassified genus indicates that these bacteria are novel or that there isn't any sequence data available for them in databases that are available to the general public.

Metagenomic data functional analysis is a useful method for revealing the functional nature of any microbial consortia by identifying the OTUs according to their metabolic roles. Montella and coworkers accounted for the abundance of genes coding for carbohydrate-active enzymes, which are involved in the degradation, modification, or formation of glycosidic bonds during the *meta*-analysis of lignocellulosic biomasses[76]. BD preparations 502—507 are used for making BD compost which stabilizes macro and micronutrient availability to plants[10]. BD preparations don't offer a lot of nutrients, they do work to speed up decomposition, enhance soil quality, and increase crops even when only a few grams per tonne of compost are used[11]. The cellulose, lignins, and hemicelluloses that are present in carbohydrates are converted into humus with the aid of BD preparations. These degrading activities are assumed to be caused by the OTUs associated with metabolic functions linked to energy metabolism, carbohydrate metabolism, and enzyme families. The genes involved in membrane transport are crucial in the processes involved in nutrient uptake and exchange, as well as in preserving the mineral balance in soil[77]. The assertion that BD preparations aid in preserving the notable concentrations of Ca, N, K, S, Fe, magnesium (Mg), silica, phosphorus (P), and other trace elements in the soil is supported by the availability of OTUs for membrane transfer[78]. The soil ecosystem is severely harmed by the persistence of xenobiotic compounds, which are man-made substances with medium-to-long-term stability in the soil. The most significant process for the breakdown of xenobiotic chemicals in soil is widely believed to be microbial metabolism[79]. It has been possible to identify certain xenobiotic-degrading microorganisms that could aid in the breakdown of xenobiotic substances. BD preparations contain bacteria with the potential to be useful for bioremediation, which is explained by the presence of OTUs with xenobiotic degradation function. Moreover, in our earlier study, we observed the culturable bacteria present in BD preparation had plant growth attributes viz indole acetic acid (IAA), ammonia, and HCN production[79]. Veeresh et al also accounted for the bacterial species that had phosphate solubilization potential[80]. Stearn observed increased root and shoot growth in maize and soybean seedlings due to higher levels of cytokinins present in horn-based BD preparations[81]. Other studies conducted by Fritz and Koepke and Giannattasio et al. accounted gibberellin and auxin-like impact of BD preparations[28,82]. The influence of BD preparations on crop growth, crop yields, and soil organic matter through the promotion of root production and root health is a crucial connecting factor that may explain several outcomes. Improved root growth in response to the application of BD preparations was connected with higher soil organic matter and biological activity as well as increased particle organic matter[52].

5. Conclusion

To the best of my knowledge, this is the first metagenomic study of BD preparations to unlock bacterial and functional diversity. This finding elucidates the mechanism by which BD preparations aid in enhanc-

ing soil health apart from improving soil fertility. This study investigation also reveals how BD preparations in nanoscale amounts enhance plant growth and yields. The dearth of genomic data on all the unculturable bacterial species in public-domain sources makes classification difficult even while basic evidence of bacterial diversity is present. This suggests that the functional metabolic qualities and uncommon character of these bacteria may add to the preparations' effectiveness in boosting crop production and quality as well as soil health. So, this study may be helpful for researchers/farmers to exploit the functionally diversified BD preparations for the reclamation of soils as well as sustainable agricultural production through an eco-friendly approach.

Statement and declarations

Authors contribution: NG and IZ: conceived the research, and designed experiments; SV: performed the experiments and BS also help them; SV, SKS and MM analyzed the data; SV, SKS, MM and AKT prepared the MS draft; NG and SKS finally edited and prepared the final version of MS.

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Consent for publication

All authors have seen the latest version of the manuscript and agree to its publication.

CRediT authorship contribution statement

Supriya Vaish: Formal analysis, Investigation, Methodology, Writing – original draft. **Sumit K. Soni:** Data curation, Formal analysis, Software, Validation, Writing – original draft, Writing – review & editing. **Balvindra Singh:** Investigation, Methodology. **Neelima Garg:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision. **Iffat Zareen Ahmad:** Conceptualization. **Muthukumar Manoharan:** Formal analysis, Writing – original draft. **Ajaya Kumar Trivedi:** Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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