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Research Article

BIODYNAMIC PREPARATIONS BD500 AND BD501: A META-ANALYSIS OF BACTERIAL COMMUNITIES SUPPORTING SOIL AND CROP PRODUCTIVITY

***T. Tamilvani and Velu Rajesh Kannan**

Rhizosphere Biology Laboratory, Department of Microbiology, Bharathidasan University, Trichy, Tamil Nadu, India

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ABSTRACT

Biodynamic preparations, including BD500 and BD501, are widely used in sustainable agriculture to enhance soil fertility, microbial diversity, and crop productivity. In this study, we conducted a comprehensive physicochemical and metagenomic analysis of BD500 and BD501 to elucidate the bacterial communities associated with these preparations. Physicochemical characterization revealed that BD500, derived from cow dung, is rich in organic carbon and essential nutrients, whereas BD501, a silica-based preparation, exhibits a more alkaline environment with lower nutrient content. High-throughput sequencing of the 16S rRNA gene was performed to assess microbial diversity, community structure, and taxonomic composition. Operational Taxonomic Unit (OTU) clustering, alpha and beta diversity analyses, and rank-abundance and rarefaction curves confirmed sufficient sequencing depth and highlighted differences in microbial richness, evenness, and community organization between the two preparations. Taxonomic profiling revealed the presence of bacterial taxa involved in nutrient cycling, organic matter decomposition, and soil health maintenance, with BD500 supporting a more diverse community and BD501 harboring specialized taxa likely contributing to plant physiological stimulation. Overall, the results demonstrate that BD500 and BD501 possess complementary roles in biodynamic agriculture, combining nutrient enrichment and microbial stimulation to improve soil quality and potentially enhance crop yield and quality. These findings provide valuable insights into the functional and ecological roles of biodynamic preparations in sustainable farming systems.

Keywords: Soil microbial diversity, 16S rRNA sequencing, Operational Taxonomic Units (OTUs).

INTRODUCTION

Modern agricultural systems predominantly rely on extensive applications of chemical fertilisers (CFs) and pesticides to achieve higher crop yields and meet the food demand of a rapidly growing population. Although these inputs have significantly increased agricultural productivity, their persistent and intensive use poses serious environmental and human health challenges. Chemical fertilizers and pesticides can lead to residual accumulation in soils and crops, adversely affecting soil microbial communities, reducing soil biodiversity, and altering soil physico-chemical properties such as pH, nutrient balance, and organic matter content. These changes contribute to soil degradation, water and air pollution, and food safety concerns due to chemical residues in fresh produce consumed daily by humans (Madhulika *et al.,* 2025; Liu *et al*., 2020). The deterioration of soil biological health under conventional agricultural practices has spurred global interest in sustainable and eco-friendly farming approaches that aim to preserve soil fertility, enhance crop quality, and reduce reliance on external chemical inputs. In this context, natural and organic nutrient management strategies have gained traction as potential alternatives to conventional CF-based systems. These strategies include organic farming, permaculture, Rishi Krishi, Panchgavya-based farming, Nature Ecological Farming (Natueco), Zero Budget Natural Farming (ZBNF), biodynamic (BD) farming, and other nature-inclusive agricultural systems. Such approaches promote soil biological activity and ecosystem functioning through the use of organic amendments and minimal

*Corresponding Author: T. Tamilvani, Rhizosphere Biology Laboratory, Department of Microbiology, Bharathidasan University, Trichy, Tamil Nadu, India Email: tamilmicro89@gmail.com.

external inputs (Reganold & Wachter, 2016; Gomiero, 2021).

Permaculture, for example, mimics the structure and dynamics of natural ecosystems, integrating biodiversity, efficient resource cycling, and sustainable land use to enhance soil health and crop productivity. By reducing dependency on synthetic fertilizers and pesticides, permaculture supports resilient agricultural systems. Similarly, Panchgavya is an organic formulation derived from cow dung, cow urine, milk, curd, and ghee mixed in specific proportions and fermented. Panchgavya has been reported to improve soil microbial diversity, stimulate plant growth, and enhance nutrient uptake through its bioactive components, which include beneficial microorganisms, enzymes, and phytohormones.

Biodynamic (BD) farming is another holistic agricultural system developed to further reduce chemical inputs and restore soil vitality. Considered one of the oldest organized organic farming systems, BD farming enhances soil fertility and crop performance primarily through the application of specially prepared BD formulations rather than direct nutrient addition. These preparations are designed to stimulate nutrient and energy cycling, accelerate organic matter decomposition, and improve soil biological functions even when applied in small quantities. The BD system typically uses eight preparations (BD500–BD507), each produced via controlled fermentation of natural materials and exhibiting unique functional characteristics. BD500 and BD501, for example, are often applied as soil or foliar sprays to improve seed germination, root development, stress resilience, and photosynthesis, whereas BD502–BD507 are incorporated into compost to enhance nutrient stabilization and microbial activity.

Soil microorganisms' bacteria and fungi are essential components of the soil ecosystem, playing pivotal roles in organic matter degradation, nutrient cycling, and plant growth promotion. They convert complex organic and inorganic compounds into simpler forms such as nitrate, phosphate, amino acids, and mineral salts that plants can assimilate. In addition, many bacterial genera directly promote plant growth through phytohormone production (e.g., indole-3-acetic acid) and solubilization of essential minerals, or indirectly by suppressing soilborne pathogens. Consequently, microbial diversity and richness are strongly linked to soil health, crop yield, and quality. Alpha diversity indices, including species richness and evenness estimators such as Chao1, ACE, Shannon–Weaver, and Simpson indices, are commonly used to quantify microbial community diversity and structure in environmental samples. These indices provide insights into the community composition, the relative abundance of taxa, and the functional potential of microbial assemblages in response to different management practices (Hill *et al.,* 2021; Babalola *et al*., 2025).

Traditional culture-based methods have significant limitations, as only a small fraction (<1%) of soil microbes are culturable under laboratory conditions, leading to incomplete representation of microbial diversity and functions. Prior studies using culture-dependent techniques have provided limited insights into the microbial composition of biodynamic preparations or amended soils, underscoring the need for culture-independent approaches. With the advent of high-throughput sequencing and metagenomic technologies, researchers can now characterize complex microbial communities directly from environmental DNA, enabling a comprehensive understanding of both taxonomic diversity and functional gene potential without the need for culturing. Metagenomics and amplicon sequencing have become essential tools for soil and rhizosphere microbiome studies, revealing previously uncharacterized microbial taxa and functional pathways involved in nutrient cycling, disease resistance, and plant growth promotion (DeFord, 2024; Raina *et al*., 2025). Recent metagenomic studies have demonstrated significant differences in soil microbial community structures under diverse agricultural practices, highlighting how organic and regenerative systems can enrich beneficial microbiota and functional gene diversity compared to conventional management. Such insights underscore the influence of land use and management history on microbial ecology, nutrient cycling, and ecosystem services critical to sustainable agricultural productivity.

Despite the growing body of research on soil microbiomes and organic amendments, there remains a knowledge gap regarding the precise microbial composition, functional roles, and mechanisms of action of biodynamic preparations in soil ecosystems. Although some studies have begun to explore the microbiota of BD preparations themselves, comprehensive characterization and linkage to functional outcomes in soil and plant systems are limited. This gap underscores the importance of employing metagenomic approaches to unravel the complex microbial ecology of BD preparations, offering potential insights into their efficacy in improving soil health, crop growth, and long-term system resilience. In light of these considerations, the present study employed a metagenomic strategy to investigate microbial diversity, community composition, and potential functional traits within biodynamic preparations. By leveraging high-throughput sequencing technologies, this research aims to provide deeper insights into the microbial foundations of biodynamic farming and its implications for sustainable soil and crop management.

METHODS AND METHODS

Biodynamic preparation samples and experimental design

Biodynamic (BD) preparation samples were prepared using soil collected from Mahadevapattinam Village, Thiruvarur District, Tamil Nadu, India. BD500 and BD501 preparations were selected for the present study. BD500 was prepared using fresh cow dung, while BD501 was prepared using a silica paste; both preparations were incubated in cow horns following standard biodynamic practices. After preparation, the BD samples were divided

into three portions for subsequent analyses. The first portion was air-dried and used for physicochemical analysis. The second portion was stored at 4 °C for microbiological analysis. The third portion was placed in sterile, airtight cryotubes and stored at −80 °C for metagenomic analysis.

Sample Physicochemical Properties

The physicochemical and biological properties of the samples were analyzed using standard analytical procedures. Soil moisture content was determined using the gravimetric (weighing) method. Soil pH was measured using a calibrated pH meter following the microelectrode method (Zhang *et al*., 1999). Electrical conductivity (EC) was determined according to the method described by Hardie and Doyle (2012), and organic carbon content was estimated using the procedures of Walkley and Black (1934) and Piper (1966). Available nitrogen was determined using the Kjeldahl method (Subbiah and Asija, 1956). Available phosphorus was estimated spectrophotometrically following the method described by Tale and Ingole (2015), while available potassium was determined using the flame emission method (Jackson, 1973). Available sulphur was extracted using a 0.15% $CaCl_2$ solution as described by Williams and Steinbergs (1959). Exchangeable calcium and magnesium were estimated using the EDTA titration method (Raij, 1966). General soil physical characteristics, including texture, structure, color, and bulk density, were determined following Blake and Hartze (1986). Soil porosity, moisture content, and water-holding capacity were determined using standard procedures described by Sankaram (1966).

Sequence Data Analysis and Metagenomic Characterization

Metagenomic DNA was extracted from biodynamic preparation samples (BD500 and BD501) using a modified physical–chemical lysis method to ensure efficient recovery of microbial DNA. Briefly, 5 g of each BD sample was suspended in extraction buffer and subjected to mechanical disruption using glass beads, followed by sonication with a high-intensity ultrasonic processor (Vibra-Cell) to enhance microbial cell lysis. Sodium dodecyl sulphate (SDS) was subsequently added to facilitate membrane disruption, and the samples were incubated at 65 °C to promote nucleic acid release. Following incubation, the samples were centrifuged to remove particulate matter, and the supernatant containing nucleic acids was collected. The residual pellet was re-extracted with fresh extraction buffer to maximize DNA recovery, and the combined supernatants were subjected to polyethylene glycol (PEG) precipitation for partial purification of nucleic acids. The resulting pellet was resuspended in TE buffer, and proteins and polysaccharides were removed by potassium acetate precipitation.

The aqueous phase was further purified by sequential extraction with phenol–chloroform and chloroform–isoamyl alcohol. DNA was precipitated using isopropanol, pelleted by centrifugation, washed, and finally resuspended in TE buffer. The quality and quantity of the extracted DNA were assessed prior to sequencing. The purified DNA was used for high-throughput sequencing and downstream metagenomic characterization of microbial community composition and functional potential in BD500 and BD501 samples using standard bioinformatics pipelines (Yeates *et al*., 1998).

High-Throughput Sequencing, Library Preparation, and Data Quality Optimization

The concentration of extracted DNA from BD500 and BD501 samples was quantified using a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). Amplicon libraries were prepared from 40–50 ng of DNA using the MetaVx™ library preparation kit (GENEWIZ, Inc., South Plainfield, NJ, USA), following the manufacturer's instructions. The hypervariable V3–V4 regions of the bacterial 16S rRNA gene were targeted for amplicon sequencing to enable taxonomic profiling of bacterial communities (Caporaso *et al.,* 2012; Gilbert *et al*., 2014). Proprietary primers designed by GENEWIZ, targeting conserved regions flanking the V3–V4 hypervariable regions of the bacterial 16S rRNA gene, were used for PCR amplification. The quality and size distribution of the prepared libraries were assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA), and library concentrations were quantified using the Qubit 2.0 Fluorometer. Sequencing libraries were multiplexed according to the manufacturer's recommendations and sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) using a 2 × 300 bp paired-end configuration. Image analysis, base calling, and quality scoring were performed using the MiSeq Control Software (MCS) integrated within the sequencing platform (Caporaso *et al*., 2010).

Statistical analysis

Sequence analysis was performed using the Vsearch (1.9.6) (Westcott *et al*. 2015). To determine operational taxonomic units (OTUs), the 16S rRNA gene sequences were trimmed to a average length of 393 bp, sorted by using Cutadapt (v1.9.1). Operational Taxonomic Units were taxonomically annotated following a Basic Local Alignment Search Tool (BLAST) analysis against the Unite Database of each identified representative bacterial sequence done in QIIME software. Alpha diversity analysis was carried out to find the complexity of species diversity for each sample using 6 indices, which include observed species, Chao1 (Chao, 1987), Shannon (Shannon, 1948), Simpson (Simpson, 1949), abundance-based coverage estimator (ACE) (Chao and Lee, 1992) and Good's coverage (Good, 1953). Indices calculation for all the samples was done using QIIME and visualized in R software (Caporaso *et al*. 2010). Community richness was identified with Chao indices richness estimator of the total number of species in ecology (http://www.mothur.org/wiki/Ace). An index that uses Chao 1 algorithm to estimate the OTU number of samples commonly used in ecology to assess the total number of species. (http://www.mothur.org/wiki/Chao). The Shannon

index commonly used to reflect the diversity index a for (http://www.mothur.org/wiki/Shannon) the estimation and of microbial Simpson diversity index (http://www.mothur.org/wiki/Simpson) indices were used for the identification of community diversity in all the samples. To characterize the sequencing depth and coverage, the Good's coverage (http://www.mothur.org/wiki/Coverage) was used. Rank abundance curve performed by using R packages and OTU clustered by Vsearch (1.9.6) (Rand, 1971; Hubert, and Arabie, 1985). RDP classifier (Cole *et al*. 2014) Bayesian algorithm was used to classify the OTU representative sequences of 97% similarity level, and the community composition of each sample was analyzed and summarized at all levels. Taxon assignment was performed using the QIIME (v1.9.1). For each OTU cluster, a representative sequence was screened to perform taxonomic annotation. Rank Abundance Curve (RAC) is used to analyze diversity (MacArthur, 1957; Whittaker, 1965) by R packages based on the results of OTU analysis. Rank-abundance curve reflects both species abundance and species uniformity. Species uniformity is reflected by the shape of the curve. The rarefaction curve (Heck *et al*. 1975) is a useful tool to characterize the species composition of a sample and predicting the abundance of species in a sample. It efficiently deals with the increase of detected species due to the increase in sample size. The observed numbers of OTUs were plotted against the number of extracted sequences by QIIME (1.9.1).

RESULTS AND DISCUSSION

The physicochemical characteristics of the biodynamic preparations BD500 and BD501 were analyzed to understand their compositional differences (Table 1). Variations were observed in key soil parameters, including moisture content, pH, electrical conductivity, and organic carbon content between the two preparations. BD500 exhibited comparatively higher organic carbon content, whereas BD501 showed a relatively alkaline pH. Electrical conductivity values indicated moderate soluble salt concentrations in both preparations, suggesting suitability for agricultural application. The availability of macronutrients such as nitrogen, phosphorus, potassium, sulphur, calcium, and magnesium differed between BD500 and BD501, reflecting differences in raw material composition and preparation processes.

Table 1. Physiochemical Properties of Biodynamic Preparations.

Biodynamics	pH	EC (dSm^{-1})	Total organic carbon ($g\ kg^{-1}$)	Total nitrogen (%)	Total phosphorus (%)	Total potassium (%)	Total calcium (%)	Total magnesium (%)
BD500	7.6	2.64	27.87	1.96	1.17	2.5	1.600	1.005
BD501	7.8	2.00	7.6	0.3	0.4	1.2	1.500	0.8

High-throughput sequencing of the BD500 and BD501 samples generated a substantial number of raw 16S rRNA gene reads. After quality filtering, trimming, and removal of low-quality sequences, high-quality reads were retained for downstream analysis. The average read length after trimming was approximately 393 bp, consistent with the targeted V3–V4 regions of the bacterial 16S rRNA gene. Operational Taxonomic Unit (OTU) clustering at 97% sequence similarity yielded a comprehensive representation of bacterial taxa present in the biodynamic preparations. Quality control metrics indicated that the sequencing data were robust and suitable for reliable microbial community analysis. The coverage values approached completeness, confirming adequate sequencing depth for both BD500 and BD501 samples. Rarefaction analysis demonstrated a tendency toward saturation, indicating sufficient sequencing effort to capture the majority of bacterial diversity present in the samples. These results confirm that the generated sequencing dataset was of high quality and provided a reliable foundation for subsequent taxonomic and diversity analyses of the biodynamic preparations. Operational Taxonomic Unit (OTU) clustering of the quality-filtered 16S rRNA gene sequences was performed at a 97% sequence similarity threshold, resulting in the identification of distinct OTUs across the biodynamic preparations BD500 and BD501. The OTU clustering revealed a diverse bacterial community, indicating the presence of multiple taxonomic groups within both preparations.

Representative sequences from each OTU were subjected to taxonomic annotation using the Ribosomal Database Project (RDP) classifier and further validated through BLAST analysis using the QIIME pipeline. Taxonomic assignments were successfully achieved at multiple hierarchical levels, including phylum, class, order, family, genus, and species. The annotated OTUs demonstrated variation in relative abundance and taxonomic composition between BD500 and BD501, reflecting differences in microbial community structure associated with the distinct preparation processes. Several OTUs were assigned to bacterial taxa commonly associated with organic matter decomposition, nutrient cycling, and soil health. A proportion of OTUs could not be classified at the species level, likely due to limitations in reference database

resolution and the presence of novel or poorly characterized microbial taxa.

Taxonomic analysis revealed distinct microbial community compositions in BD500 and BD501. At the phylum level, bacterial communities were predominantly composed of major taxonomic groups commonly associated with organic and mineral-based environments. Differences in relative abundance of dominant and rare taxa were observed between the preparations, reflecting variation in microbial community structure influenced by preparation-specific factors. Statistical analyses supported the observed variations in microbial diversity and composition between BD500 and BD501. Rank-abundance curves illustrated differences in species evenness and dominance patterns, while rarefaction analysis demonstrated sufficient sequencing effort for both samples. The combined diversity and compositional analyses confirm that biodynamic preparations harbor complex and distinct microbial communities.

GC content analysis was performed to evaluate the nucleotide composition of the 16S rRNA gene sequences obtained from BD500 and BD501 samples. The GC content provides insight into the genomic characteristics of the microbial communities and can influence DNA stability and amplification efficiency during sequencing. The distribution of GC content across all reads was assessed, revealing the proportion of guanine (G) and cytosine (C) nucleotides relative to the total sequence length. The GC content profiles of BD500 and BD501 were visualized as histograms (Figure 1), indicating the variability of GC composition among the microbial populations. Analysis of GC content confirmed that the sequences were within the expected range for bacterial 16S rRNA genes, supporting the quality and reliability of the sequencing dataset. No significant anomalies or biases were observed in either sample, ensuring that the downstream diversity and taxonomic analyses were based on high-quality, representative sequences.

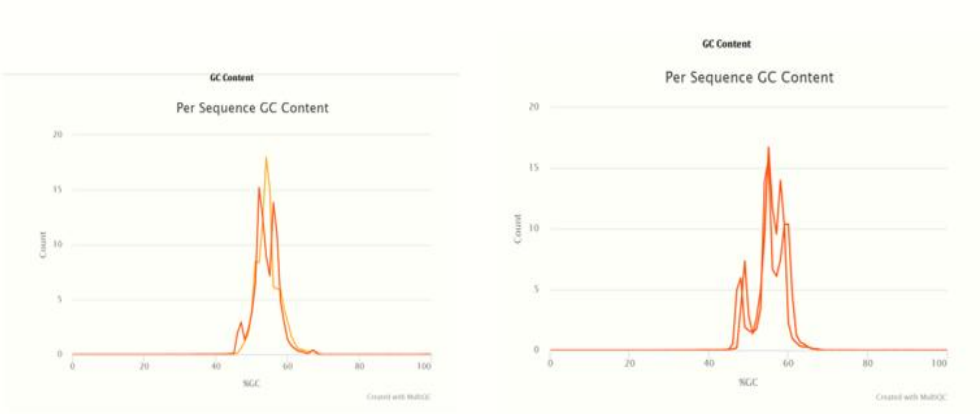


Figure 1. GC Content of BD500 and BD501 Samples.

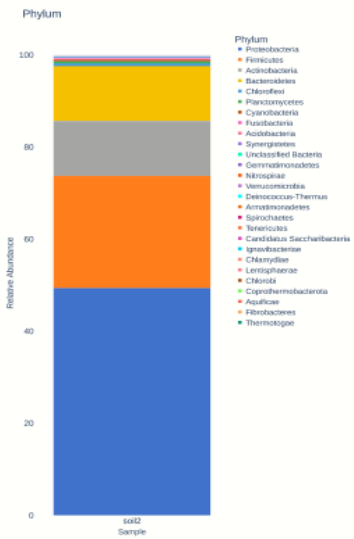
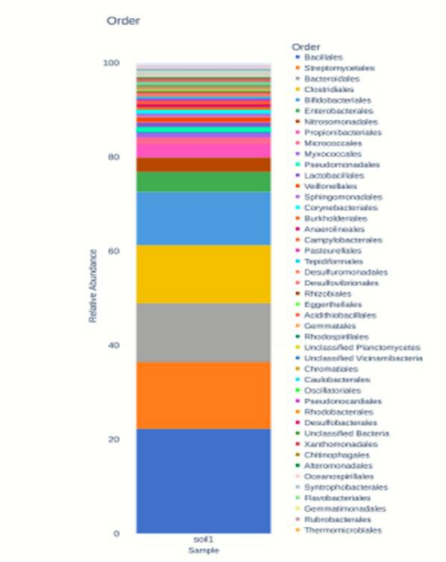


Figure 2. Taxonomic Diversity of BD500 and BD501.


Order
Relative Abundance
Sample
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Order
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Streptomycetales
Bacteroidales
Clostridiales
Bifidobacteriales
Enterobacteriales
Nitrosomonadales
Propionibacteriales
Micrococcales
Myxococcales
Pseudomonadales
Lactobacillales
Veillonellales
Sphingomonadales
Corynebacteriales
Burkholderiales
Anaerolineales
Campylobacterales
Pasteurellales
Tepidiformales
Desulfuromonadales
Desulfovibrionales
Rhizobiales
Eggerthellales
Acidithiobacillales
Gemmatales
Rhodospirillales
Unclassified Planctomycetes
Unclassified Vicinamibacteria
Chromatiales
Caulobacterales
Oscillatoriales
Pseudonocardiales
Rhodobacterales
Desulfobacterales
Unclassified Bacteria
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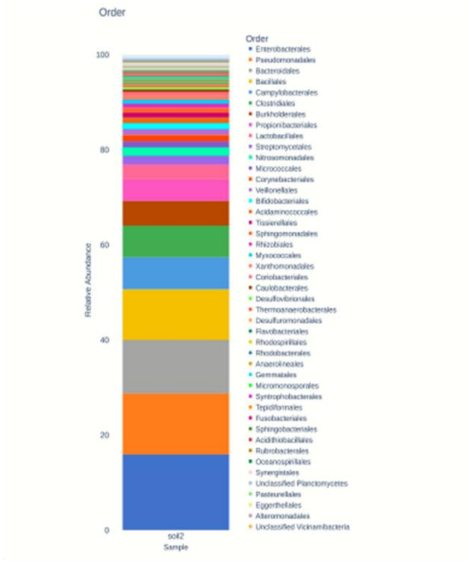
Syntrophobacterales
Flavobacteriales
Gemmatimonadales
Rubrobacteriales
Thermomicrobiales


Order
Relative Abundance
Sample
soil2
Order
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Pseudomonadales
Bacteroidales
Bacillales
Campylobacterales
Clostridiales
Burkholderiales
Propionibacteriales
Lactobacillales
Streptomycetales
Nitrosomonadales
Micrococcales
Corynebacteriales
Veillonellales
Bifidobacteriales
Acidaminococcales
Tissierellales
Sphingomonadales
Rhizobiales
Myxococcales
Xanthomonadales
Coriobacteriales
Caulobacterales
Desulfovibrionales
Thermoanaerobacterales
Desulfuromonadales
Flavobacteriales
Rhodospirillales
Rhodobacterales
Anaerolineales
Gemmatales
Micromonosporales
Syntrophobacterales
Tepidiformales
Fusobacteriales
Sphingobacteriales
Acidithiobacillales
Rubrobacteriales
Oceanospirillales
Synergistales
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Pasteurellales
Eggerthellales
Alteromonadales
Unclassified Vicinamibacteria


Class
Relative Abundance
Sample
soil1
Class
Actinobacteria
Bacilli
Bacteroidia
Clostridia
Gammaproteobacteria
Betaproteobacteria
Deltaproteobacteria
Alphaproteobacteria
Negativicutes
Anaerolineae
Epsilonproteobacteria
Tepidiformia
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Planctomycetia
Acidithiobacillia
Unclassified Planctomycetes
Vicinamibacteria
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Chloroflexia
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Nitriliruptoria
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Sphingobacteriia
Phycisphaerae
Ignavibacteria
Mollicutes
Spirochaetia
Verrucomicrobiae
Opitutae
Unclassified Armatimonadetes
Tissierellia
Limnochordia
Oligoflexia
Dehalococcoidia
Thermoleophilia
Candidatus Babeliae


Class
Relative Abundance
Sample
soil2
Class
Gammaproteobacteria
Bacilli
Bacteroidia
Actinobacteria
Clostridia
Betaproteobacteria
Epsilonproteobacteria
Alphaproteobacteria
Negativicutes
Deltaproteobacteria
Tissierellia
Coriobacteriia
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Flavobacteriia
Planctomycetia
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Fusobacteriia
Sphingobacteriia
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Rubrobacteria
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Nitrospira
Verrucomicrobiae
Deinococci
Chloroflexia
Acidobacteriia
Nitriliruptoria
Unclassified Firmicutes
Mollicutes
Spirochaetia
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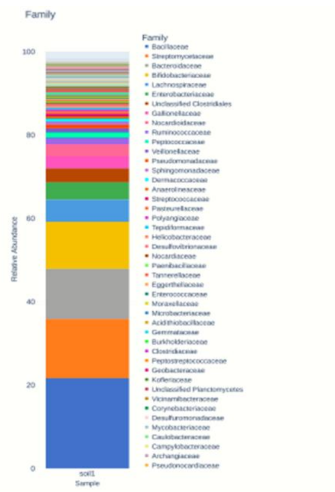
Dehalococcoidia


Family
Relative Abundance
Sample
soil1
Family
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Streptomycetaceae
Bacteroidaceae
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Lachnospiraceae
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Gallionellaceae
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Ruminococcaceae
Peptococcaceae
Veillonellaceae
Pseudomonadaceae
Sphingomonadaceae
Dermacoccaceae
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Streptococcaceae
Pasteurellaceae
Polyangiaceae
Tepidiformaceae
Helicobacteraceae
Desulfovibrionaceae
Nocardiaceae
Paenibacillaceae
Turneriellaceae
Eggerthellaceae
Enterococcaceae
Moraxellaceae
Microbacteriaceae
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Gemmataceae
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Clostridiaceae
Peptostreptococcaceae
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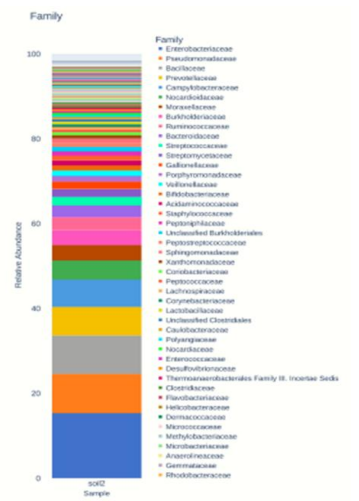
Campylobacteraceae
Archangiaceae
Pseudonocardiaceae


Family
Relative Abundance
Sample
soil2
Family
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Bacillaceae
Prevotellaceae
Campylobacteraceae
Nocardioidaceae
Moraxellaceae
Burkholderiaceae
Ruminococcaceae
Bacteroidaceae
Streptococcaceae
Streptomycetaceae
Gallionellaceae
Porphyromonadaceae
Veillonellaceae
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Xanthomonadaceae
Coriobacteriaceae
Peptococcaceae
Lachnospiraceae
Corynebacteriaceae
Lactobacillaceae
Unclassified Clostridiales
Caulobacteraceae
Polyangiaceae
Nocardiaceae
Enterococcaceae
Desulfovibrionaceae
Thermoanaerobacterales Family III. Incertae Sedis
Clostridiaceae
Flavobacteriaceae
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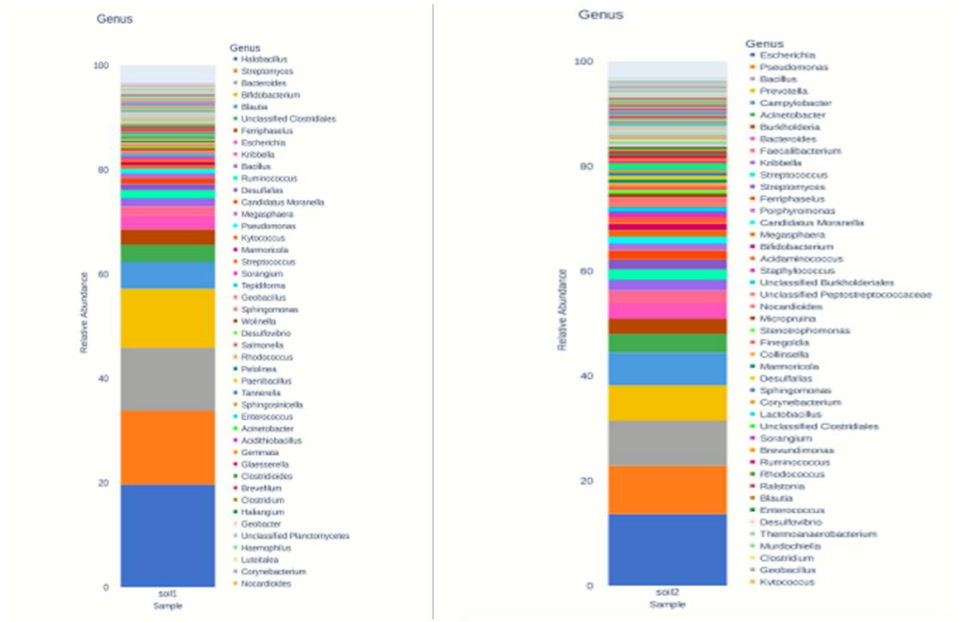
Dermacoccaceae
Micrococcaceae
Methylobacteriaceae
Microbacteriaceae
Anaerolineaceae
Gemmataceae
Rhodobacteraceae



Taxonomic diversity analysis revealed a complex and diverse bacterial community structure within the biodynamic preparations BD500 and BD501. Operational Taxonomic Units (OTUs) were assigned to multiple taxonomic levels, including phylum, class, order, family, genus, and species, indicating broad taxonomic representation across both preparations Figure 2. At the phylum level, the microbial communities of BD500 and BD501 were dominated by major bacterial groups commonly associated with organic- and mineral-based environments. Differences in the relative abundance of dominant phyla were observed between the two preparations, reflecting variation in microbial community composition influenced by the distinct preparation materials and processes. Further taxonomic resolution at the genus and species levels revealed the presence of both abundant and rare taxa. Several OTUs were assigned to bacterial genera known for their roles in nutrient cycling, organic matter decomposition, and soil health enhancement. A proportion of OTUs remained unclassified at lower taxonomic levels, suggesting the presence of novel or poorly characterized bacterial taxa.

The physicochemical analysis of the biodynamic preparations revealed notable differences between BD500 and BD501, reflecting their distinct preparation processes. BD500 exhibited a slightly alkaline pH (7.6) with moderate electrical conductivity (2.64 dS m^{-1}), high organic carbon (27.87 g kg^{-1}), and substantial levels of nitrogen, phosphorus, potassium, calcium, and magnesium. These characteristics indicate that BD500 is a nutrient-rich preparation capable of supporting microbial growth and enhancing soil fertility. Such nutrient-enriched properties of cow dung-based biodynamic preparations have been previously reported to promote microbial proliferation, organic matter decomposition, and nutrient cycling in agricultural soils (Reganold and Wachter, 2016; Berendsen *et al.,* 2012). In contrast, BD501, being a silica-based preparation, is generally characterized by lower organic carbon and nutrient contents but a more alkaline pH, aligning with its role as a physiological stimulant rather than a direct nutrient source (Steiner, 1924; Fließbach *et al.,* 2007). These compositional differences underscore the complementary functions of BD500 and BD501 within biodynamic farming systems.

High-throughput sequencing of the 16S rRNA gene revealed that both preparations harbor diverse bacterial communities, although with distinct structures and compositions. OTU clustering and taxonomic annotation demonstrated the presence of multiple bacterial taxa across phylum, genus, and species levels. BD500, with its higher organic content, supported greater microbial richness and diversity as evidenced by alpha diversity indices including Shannon, Chao1, Simpson, and ACE. The rank-abundance profiles suggested a more even distribution of species in BD500, whereas BD501 exhibited a community dominated by fewer taxa, reflecting differences in nutrient availability and preparation methodology. Similar observations have been reported where organic-rich substrates promote microbial richness and evenness, enhancing functional redundancy and ecosystem resilience (Hartmann *et al*., 2015; Fierer *et al*., 2007). Taxonomic analysis revealed that the bacterial communities of both preparations were dominated by phyla such as Firmicutes, Proteobacteria, and Actinobacteria, which are commonly associated with nutrient cycling, organic matter degradation, and soil health maintenance. At the genus level, BD500 contained bacterial groups likely involved in nitrogen transformation and decomposition of complex organic matter, consistent with its high carbon and nitrogen content. A proportion of OTUs remained unclassified at lower taxonomic levels in both preparations, indicating the presence of novel or poorly characterized microbial taxa, which could contribute to

unique functional roles within the preparations. Such functional microbial diversity is essential for the ecological effectiveness of biodynamic preparations and has been emphasized in several studies highlighting the role of microbial consortia in promoting plant growth and soil fertility (Berendsen *et al.,* 2012; Caporaso *et al*., 2012).

Rarefaction and rank-abundance analyses confirmed that sequencing depth was sufficient, capturing most of the microbial diversity present in BD500 and BD501. The GC content of the sequences fell within the expected range for bacterial 16S rRNA genes, indicating high-quality sequencing data suitable for reliable downstream analysis. These findings demonstrate that both biodynamic preparations possess complex microbial communities that are likely to contribute to soil health, nutrient cycling, and plant physiological responses when applied in agricultural systems. Overall, the combined physicochemical and microbiological analyses highlight the complementary roles of BD500 and BD501 in biodynamic farming. BD500 provides nutrient-rich support for microbial growth, enhancing decomposition and nutrient availability, whereas BD501 likely acts as a physiological stimulant with a unique microbial profile adapted to mineral-rich environments. Together, these preparations may synergistically improve soil fertility, microbial diversity, and plant growth, supporting the sustainable management principles of biodynamic agriculture (Fließbach *et al*., 2007; Reganold and Wachter, 2016). The findings of this study provide valuable insights into the functional and ecological roles of biodynamic preparations, emphasizing their potential in promoting resilient and productive agroecosystems.

CONCLUSION

The present study provides a comprehensive analysis of the physicochemical and microbial properties of biodynamic preparations BD500 and BD501. BD500, derived from cow dung, exhibited high organic carbon and nutrient content, supporting a rich and diverse microbial community. In contrast, BD501, a silica-based preparation, demonstrated a more alkaline environment with lower nutrient content, yet harbored a distinct microbial profile. High-throughput sequencing revealed complex bacterial communities in both preparations, dominated by taxa involved in nutrient cycling, organic matter decomposition, and soil health maintenance. Alpha and beta diversity analyses, along with rarefaction and rank-abundance curves, confirmed sufficient sequencing depth and highlighted differences in microbial richness, evenness, and community structure between the two preparations. Taxonomic analyses indicated that both preparations possess functional microbial diversity capable of contributing to soil fertility and plant growth, with BD500 providing nutrient enrichment and BD501 likely serving as a physiological stimulant. Overall, the study demonstrates that BD500 and BD501 have complementary roles in biodynamic agriculture, combining nutrient provisioning and microbial stimulation to enhance soil health and ecosystem sustainability. These findings offer valuable insights into the mechanisms underlying biodynamic preparations and support their continued use in sustainable crop management practices.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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