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Impacts of Biochar-Based Controlled-Release Nitrogen Fertilizers on Soil Prokaryotic and Fungal Communities

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Abstract: Controlled-release Nitrogen Fertilizers (CRNFs) are an effective fertilization technique by minimizing nutrient loss and making Nitrogen (N) available to plants as they grow. Biochar-based CRNF (BCRNF) technologies have been demonstrated very promising in increase of corn yield. Despite the beneficial effects of BCRNFs, their impacts on prokaryotic and fungal soil communities are not well evaluated. Different formulations of BCRNF were developed to investigate their effects on corn productivity. We analyzed the soil microbes and their functional potential under different BCRNF regimes using amplified V_3–V_4 region of 16s rRNA for determining prokaryotic, and ITS genes for fungal communities. The soil prokaryotic diversity was similar across the treatments, with differences in prokaryotic genera with relative abundance of 0.1% or less in the soil ($p < 0.05$). In contrast, the fungal community diversity was different only for unfertilized soil. It had a high relative abundance for *Aspergillus*. Genus level comparison showed that Pseudofabraea was higher in Bioasphalt-based BCRNF compared to other treatments. Moreover, the N-fixing communities in soil were also similar across the treatments. At genus level, *Microvirga*, *Azospirillum*, and *Methyloprofundus* were highest in no-fertilizer control. The functional potential predictions using PICRUSt2 portrayed a consistent N-cycling functions across the treatments. However, the predicted gene functions related to nitrous-oxide reductase (*nosZ*) and hydroxylamine reductase (*hcp*) were significantly lower in soil receiving BCRNF containing biosolid. Overall, BCRNF treatments previously identified to increase corn yield displayed minimal shifts in the soil microbial communities. Thus, such novel fertilization would enable increased crop yield without affecting soil communities leading to sustainable crop production.

Keywords: Nitrogen; controlled-release fertilizer; biochar; corn; soil microbiome; microbial functions

Citation: Das, B.K.; Rubel, R.I.; Gupta, S.; Wu, Y.; Wei, L.; Brözel, V.S. Impacts of Biochar-Based Controlled-Release Nitrogen Fertilizers on Soil Prokaryotic and Fungal Communities. *Agriculture* **2022**, *12*, 1706. https://doi.org/10.3390/agriculture12101706

Academic Editor: Thomas F. Ducey

Received: 6 September 2022
Accepted: 13 October 2022
Published: 16 October 2022

1. Introduction

Soil microbes are affected by fertilizer applications intended to increase the yield in cropland systems [1]. Nitrogen (N) is an essential nutrient for crop growth and health, therefore requiring intensive application of N fertilizers. Common N fertilizers can easily be lost through leaching [2], denitrification [3], and volatilization [4]. Additionally, reapplication and excessive use of N fertilizer led to environmental degradation [5]. Control-release Nitrogen Fertilizers (CRNFs) are an alternative to address these problems and reduce fertilizer reapplication as water-soluble N is released gradually [6]. Furthermore, such fertlizers are also known to promote microbial biomass in the soil [7].

Common components used in CRFs are minerals such as zeolite clinoptilolite [8,9], or biodegradable polymers [10–12]. However, some of these particles may lead to deposition of harmful undecomposable residues in the soil. Biochar-based Controlled-Release Nitrogen fertilizers (BCRNFs) are made by mixing nitrogenous compounds with solid particles such as biochar, bio-asphalt, and other solids [13]. Hence, they are not only degradable

but promote the recycling of the bio-based waste. The use of such BCRNFs improves N availability in soil [14,15], and has been linked to numerous benefits, including managing heavy metals in soil by adsorption [16]. Several new solid matrices have been developed to be mixed with N fertilizer for slow and efficient release. Previous studies of BCRNFs have demonstrated very promising increased corn yield and some other crops [15,17–20]. However, the addition of BCRNFs in soil may cause shifts in the microbial community due to changes in the soil properties [7,21,22].

Biochar has been associated with alterations in soil properties. Its degradation has been associated with improvement in plant root colonization, increased phosphate solubilizing bacteria, and reduced bacterial plant pathogens [23]. Furthermore, culture-dependent methods showed that biochar is responsible for reshaping microbial community structure [24,25]. The soil microbiome has profound impacts on soil chemical properties, plant health and crop productivity, as reviewed recently [26–28]. In addition, biochar amended soil showed increased crop yield, improved soil quality and reduced N_2O emissions [29]. An investigation of soil bacterial community using terminal restriction fragment length polymorphism (tRFLP) and 454-pyrosequencing revealed that the addition of biochar enhances bacterial N-cycling [23]. In addition, microbial diversity increased in soil from Karst ecosystem amended by biochar based fertilizer along with increase in soil fertility [30]. However, deeper sequencing of microbial communities in high yielding BCRNFs has not been reported to date. A rigorous study using Next-Generation Sequencing to map the prokaryotic and fungal communities is needed to solidify the benefits of these BCRNFs in agricultural systems.

Understanding the relationship between crop productivity, N fertilizer release, and soil microbial communities is needed to inform the selection of fertilizers for both high yield and improved soil health [31]. A recent evaluation of novel BCRNFs for sustainable corn production revealed that two of the three novel formulations had significantly better yields of corn (Rubel et. al., in preparation, Table S1, Tukey Test * $p < 0.05$, 3 replicates each) [15,17–20]. This project explored how different formulations of BCRNFs affected the prokaryotic and fungal communities associated with corn growth. Additionally, we sought to characterize differences in the N-cycle-related functional potential of the various bacterial communities. We hypothesized that (i) soil microbial community composition would differ across various fertilizer treatments, (ii) N-fixing soil genera would be affected by BCRNFs, and (iii) the N-cycling related functional potential of the soil bacterial community would differ across various BCRNF treatments. To inform these hypotheses, the bacterial and fungal soil microbiomes following treatment with various experimental BCRNF were analyzed.

2. Materials and Methods

2.1. Biochar-Based Controlled-Release Fertilizers Preparation

Three experimental BCRNFs: S1, S2, and S3, were prepared using methods described previously (Rubel et al., in preparation [20]). N fertilizer was reconstituted with Biochar and other bioingredients. They were then pelletized into manageable particles and coated with a biodegradable layer of polylactic acid. Each formulation had different proportions of sawdust Biochar (BC), Bioasphalt (BA) and other substances (Table S2). Biosolids were obtained from a local cheese factory (Bel Cheese Manufacturing Plant, Brookings, SD, USA). Rice Starch (Glutinous Rice Flour, Erawan Marketing Company Ltd., Bangkok, Thailand) and Bentonite (Be) were used as binder substances. The particle size of BC and Rice starch was less than 0.425 µm (Mesh 40). For coating of the solid matrix, Granular Polylactic acid (PLA) pellets (Solutions of Consequences LLC, Grand Rapids, Michigan, USA) were used [17,18].

The moisture contents of the Biochar, Rice starch, Biosolids, and PLA pellets as recorded right before sample preparation were determined to be 2.90%, 12.29%, 86.44%, and 0.33%, respectively. AS (21-0-0, 99% pure) (Carolina Biological Supply Company, NC, USA), was used as N source, and adjusted to 2.4 g in each treatment. All processing and

fertilizer preparation was done with distilled water and at room temperature (23 ± 2 °C). Formulation S1 had the highest concentration of biochar and was referred to as "Biochar". BCRNF with biosolid (S2) was referred to as "Biosolid", and S3 contained bio-asphalt and was labeled as "Bioasphalt".

2.2. Evaluation of BCRNFs with Corn

Soil from a local field under corn cultivation (Table S3) was supplemented with one of the three experimental BCRNFs S1, S2, and S3, ComCRF (Osmocote® Smart-Release®, composition listed in Table S4), AS, and a negative control (No-fertilizer). Soil was placed into pots (diameter = 6 inches, height = 40 inches) and corn seeds were added. The crops were harvested 23 weeks later, and the yield was significantly higher for formulations "Biochar" and "Biosolid", with biosolid yielding best results (Rubel et. al., *in preparation*) [15,20]. Bulk soil samples were collected after harvest.

2.3. DNA Extraction and Sequencing

Bulk soil samples collected from the trial described above were used in this study to examine the impact of these novel BCRNFs on soil microbes. DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany). Two extractions were done for each soil sample and the extracts were pooled before aliquoting for sequence analysis. The DNA extracts were then split for 16S rRNA (V_3 to V_4) and ITS1-5 fungal gene sequencing. For prokaryotic 16S rRNA gene extracts were labeled as B1-B21, and F1-F21 for fungal ITS1-5. The samples were sent to Novogene for sequencing using the Illumina platform (NovaSeq 6000 PE25). Raw sequence reads were cleaned using Novogene's standard protocol. This included assigning reads to samples using barcodes, and barcodes and primer sequences were removed. After trimming, reads less than 60 bp, containing N (undetermined bases) > 10%, and with a Qscore [32] of 5 or less across 50% of total bases were removed. High-quality sequences were used for bioinformatic analysis using QIIME2 [33].

2.4. Sequence Data Analysis

The sequences obtained were further analyzed using QIIME2 [33]. The paired-end sequences were demultiplexed and merged to create artifacts for denoising. DADA2 was used for denoising both 16S rRNA and ITS1 sequences [34]. For the 16S rRNA gene, paired-end sequences were truncated to 210 bases, denoised and rarefied to 60,855 reads, whereas for fungal ITS1 they were truncated to 200 bases before merging and rarefied to 85,182 reads. The feature sequences obtained from 16S rRNA sequence data were classified using Naïve Bayes Classifier using full-length sequences [35–39], and using the recent SILVA 138 database released in December 2019 [40]. The fungal ITS were classified using the UNITE Database [41] released on May 10, 2021 for QIIME2 [42]. The OTU Tables obtained after sequence analysis [43] and representative sequences were used to study the core-diversity of the microbial communities [44–46]. The OTU table and taxonomic data were exported for downstream analysis and visualizations on R [47] and RStudio [48]. The OTU tables were reorganized using the R packages: "*dplyr*" [49], "*tidyr*" [50], and "*reshape2*" [51]. Nitrogen fixing community analysis was performed by filtering using a list of bacterial genera known to have nitrogen fixing members from a recently created *nif* gene-based database [52]. Furthermore, functional predictions were made using PICRUSt2 (v2.1.4) [53]. The statistical analysis and graphical designs were made using "*ggplot2*" [54], "*ggpubr*" [55], "*RColorBrewer*" [56], *viridis*" [57], "*plotly*" [58], and "*heatmaply*" [59]. The statistical comparisons were made either using Wilcoxon test or Kruskal—Wallis test.

3. Results

3.1. Taxonomic Diversity

For the community analyses in the soil receiving various treatments, we obtained a total of 3,589,697 raw reads of prokaryotic 16S rRNA gene, and 3,329,274 raw reads of ITS1 gene. After initial quality filtering, 99.97% and 99.98% reads were retained for

downstream analysis of prokaryotic and fungal communities, respectively. After analyzing the sequences using QIIME2 and microbiomeanalyst.ca, a total of 2909 OTUs were used to survey the prokaryotic communities, and 1352 OTUs for the fungal communities.

3.2. Prokaryotic Diversity across the Treatments

The V_3 to V_4 regions of 16S rRNA gene aligned with 43 bacterial and 5 archaeal phyla. Actinobacteria, Proteobacteria, Chloroflexi, Firmicutes, and Acidobacteriota were the top 5 bacterial phyla, whereas Crenarchaeota was the prominent Archaeal phylum (Figure 1A). The highest number of prokaryotic phyla (44) was found in the No-fertilizer treatment, whereas the least (35) were in the soil before planting, and soil with AS fertilizer (Table S5). However, the highest number of genera and species was seen in Bioasphalt treated soil, possibly due to the unique but unknown nature of the bioasphalt component added (Table S2). Despite such observed differences, the alpha diversity index (Shannon) of all the treatments did not vary significantly (Figure S1A). Beta-diversity derived using the Bray–Curtis method showed that the prokaryotic communities clustered in different groups (Figure 1B,C), with treatments No-fertilizer, ComCRF, and Bioasphalt in one cluster, and Biosolid, AS, Biochar, and time-zero-soil, in another. The soil prokaryotic communities for one of the replicates for comCRF treatment were random and close to AS-fertilized soil. Statistical comparisons using the Wilcoxon test revealed that several prokaryotic taxa varied among the treatments which gave the highest yield (Table S6). *Methylocaldum tepidum* and uncultured *Spartobacteria* were higher in soil with Biochar compared to Biosolid formulation. Several least abundant prokaryotic species belonging to 24 different phyla had a significantly different abundance across treatments ($p < 0.05$) (Figure S2).

3.3. Fungal Diversities across the Treatments

Analysis of the ITS1 sequences revealed 5 fungal phyla with an abundance of over 1%. Ascomycota dominated in each treatment type (Figure 2A), occupying the highest proportion in soil with Bioasphalt formulation. Moreover, the number of phyla across treatments was similar. The Biosolid formulation had a high number of unique taxa at each classification level (Table S7). In contrast, commercial fertilizer had a low number of families, genera, and species. The Shannon index for fungal diversity was significantly lower in soil that did not receive any fertilizer compared to other treatments (Figure S1B). The Bray–Curtis distances of the fungal communities for all treatments clustered together and randomly, except for the soil before planting corn, i.e., time-zero (Figure 2B). Moreover, 77.88% to 87.07% of the fungal genera were unidentified using the UNITE database, last updated in May 2021 [41,60]. Four of the 34 genera had near 0% abundance across the treatments. A heatmap of the filtered genera showed that only a few known fungal genera dominated the sample treatments (Figure 3). Intriguingly, all N-treated soils other than Bioasphalt had highly similar fungal communities. *Aspergillus*, *Curvularia*, and Pseudofabraea, were highest in No-fertilizer, Time-zero, and Bioasphalt treated soil, respectively (Figure 3). *Aspergillus* and *Mauginiella* were significantly different in comCRF. The *Bannoa* genus had a significantly different abundance in biochar compared to biosolid treated soil. A detailed list of fungal genera varying across the treatments is given in Table S8.

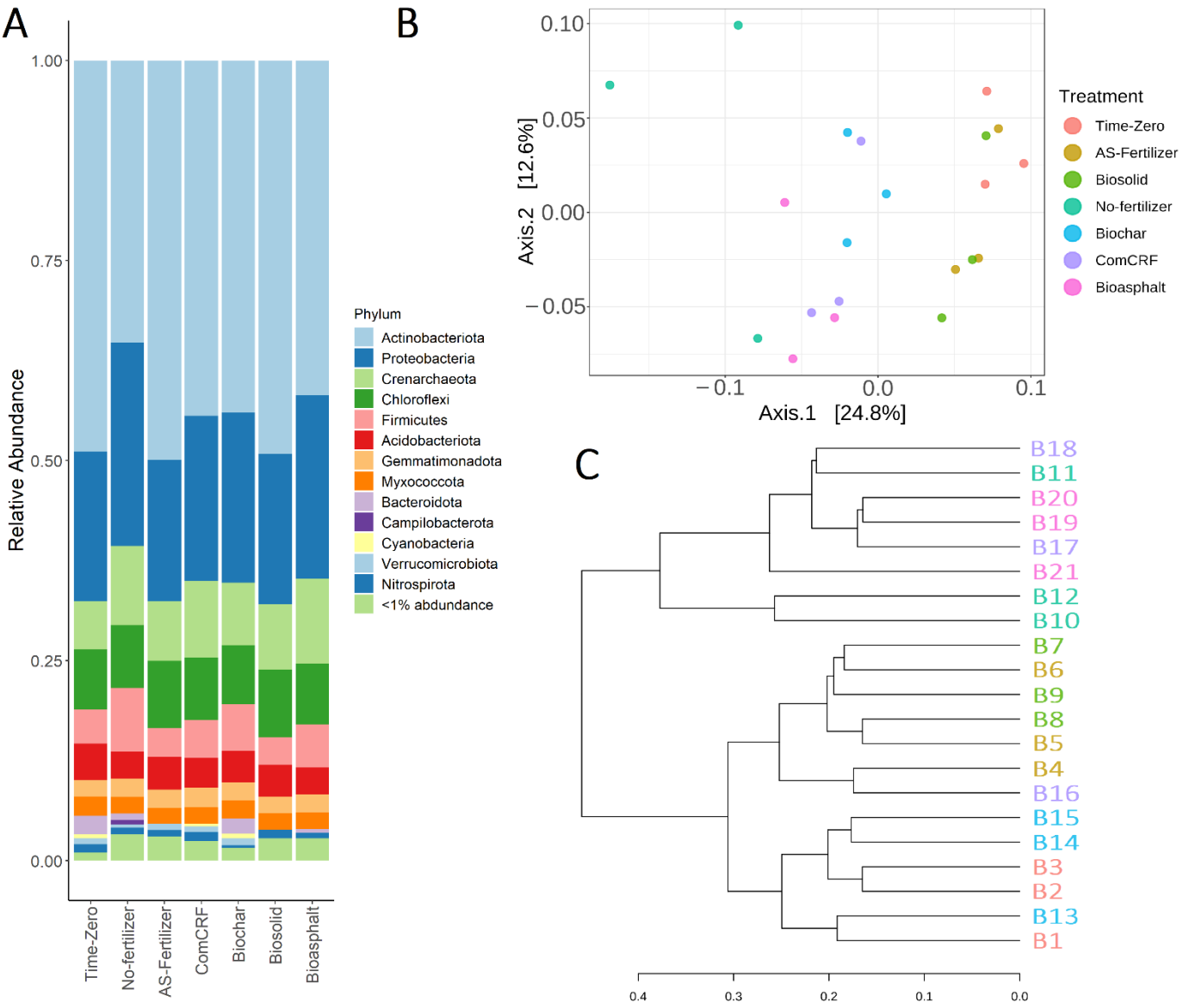

Figure 1. The prokaryotic community in soil at phylum level (**A**), Beta-diversity [PERMANOVA] F-value: 1.8188; R-squared: 0.43804; *p*-value < 0.001 (**B**), and hierarchical clustering using "*Ward*" clustering algorithm and Bray-Curtis Index as distance measure (**C**).

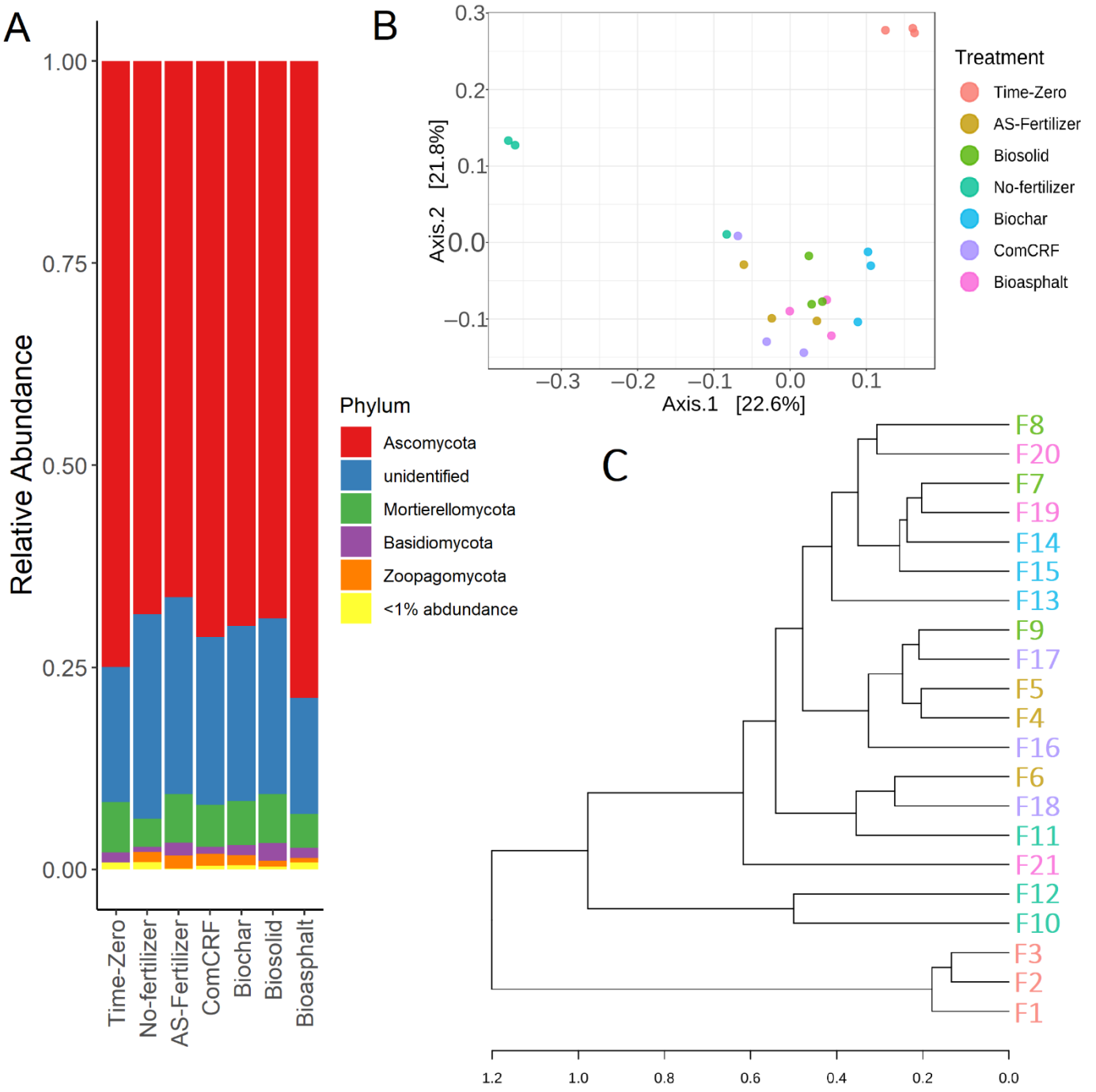

Figure 2. The fungal community in soil at phylum level (**A**), Beta-diversity [PERMANOVA] F-value: 2.7183; R-squared: 0.5381; *p*-value < 0.001 (**B**), and hierarchical clustering using "*Ward*" clustering algorithm and Bray–Curtis Index as distance measure (**C**).

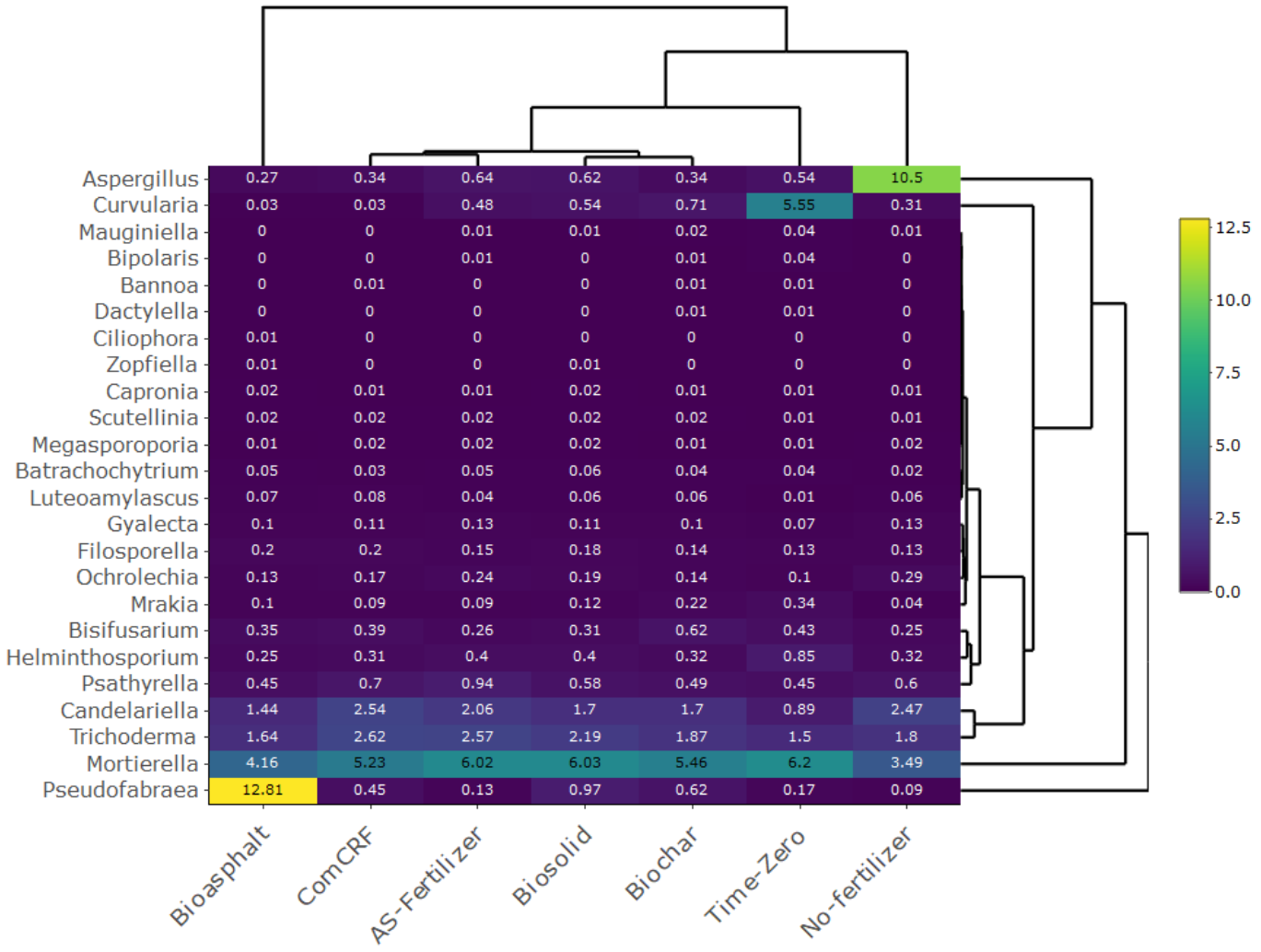

Figure 3. Heatmap showing the distribution of fungal genera across the treatments using "*hclust*" hierarchical clustering algorithm.

3.4. The N-Fixing Bacterial Diversity

On average 5.97% of the total bacterial communities belonged to N-fixing genera (Figure 4A). Across the treatments, predominance and diversity were highest in No-fertilizer, where 8.33% of the total bacterial genera contained *nif* gene clusters, and the least were in the AS-fertilizer with 4.69%. Statistically, none of the treatments had significantly unique N-fixing genera as determined by the Wilcoxon test ($p < 0.05$). The top 3 genera in each treatment were *Microvirga*, *Skermanella*, and *Bradyrhizobium* (Figure 4B,C). *Azospirillum* and *Methyloprofundus* were distinctly higher in abundance in No-fertilizer compared to other treatments. Similarly, *Hyphomicrobium* and *Mesorhizobium* were high in Bioasphalt. The biological replicates for No-Fertilizer varied in N-fixing community composition, mirroring diversity for overall prokaryotic diversity (Figure 1C). According to the hierarchical clustering, No-fertilizer was associated with distinct or different communities. Biochar and Bioasphalt clustered together with comCRF. Time-zero soil and Biosolid had similar N-fixing genera (Figure 4C).

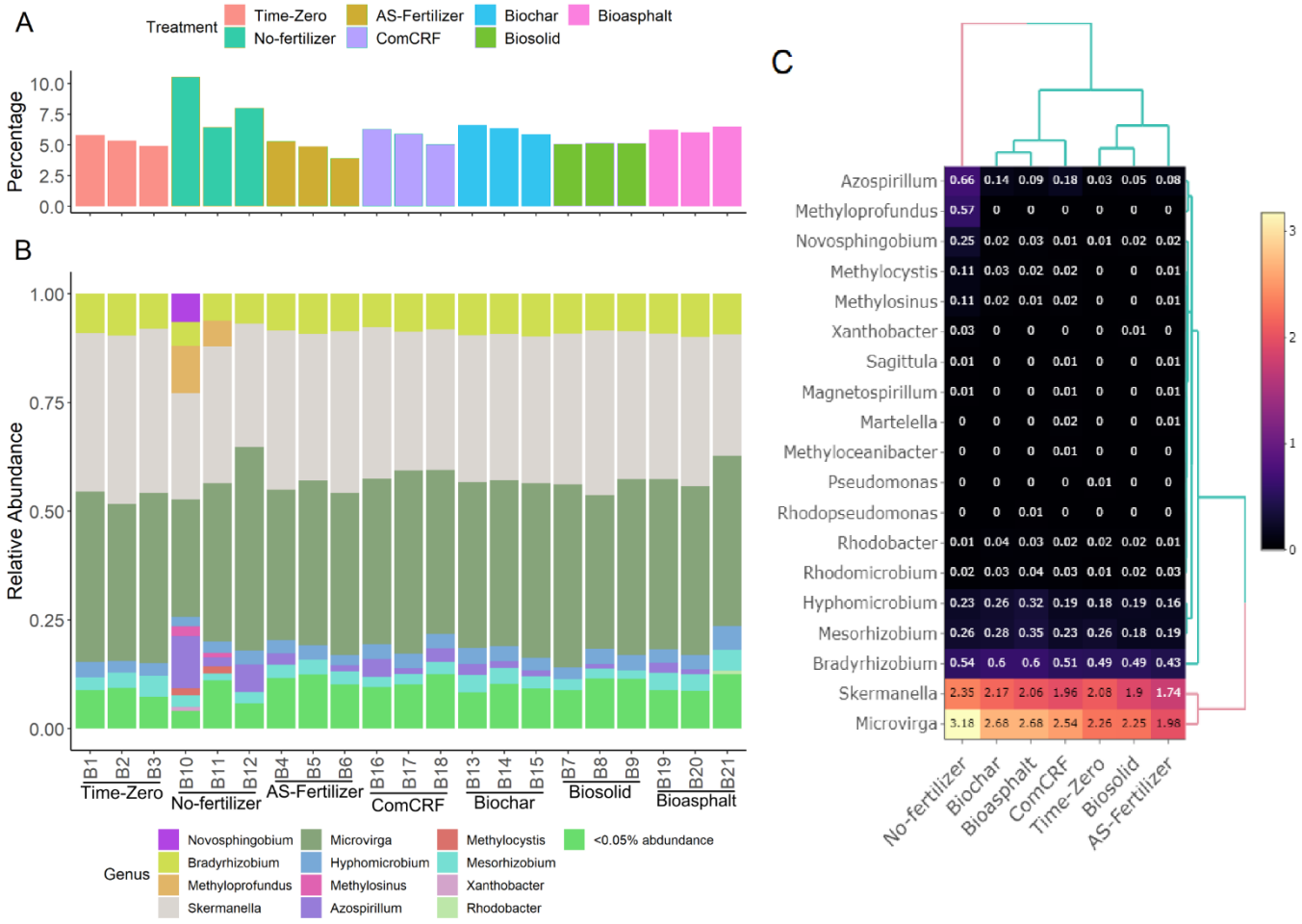

Figure 4. Distribution of Nitrogen-fixing genera across the treatments. Percentage of total OTU associated with nitrogen fixation (**A**); stacked bar plot showing distribution of N-fixing genera in each replicate (**B**), and heatmap showing distribution of genera and "*hclust*" hierarchical clustering of the treatments (**C**).

3.5. The Functional Potential of Soil Bacteria in Each Treatment

The functional predictions from PICRUSt2 showed that the bacterial N-fixing potential was not very high, while N incorporation through glutamine synthetase (*glnA*) was. To study the distribution of each N cycle-related gene function, a "percentize" normalization was used to generate a heatmap (Figure 5A). The highest proportion of N cycle functional potential was seen in glutamate synthase, carbonic anhydrase, and glutamine synthetase. Comparatively, nitrogenase (*nifH*) function was highest in No-fertilizer, whereas it was low in soil receiving biosolid formulation and initial soil. However, the gene functional potential was significantly different in soil that did not receive any fertilizer for 13 different gene functions. NMDS analysis showed that the soil not receiving fertilizer was placed far from other treatments in the NMDS scale (Figure 5B). The time-zero soil had significantly high ferredoxin-nitrate reductase (*narB*). In contrast, No-fertilizer soil had significantly low *narB* gene function (Figure 5C). Among the treatment types, Biosolid had a significantly different nitrous-oxide reductase (*nosZ*) (Figure 5D) and hydroxylamine reductase (*hcp*) (Figure 5F), both involved in denitrification. The treatment that received AS-fertilizer had significantly low carbonic anhydrase (*cah*) functional potential (Figure 5E).

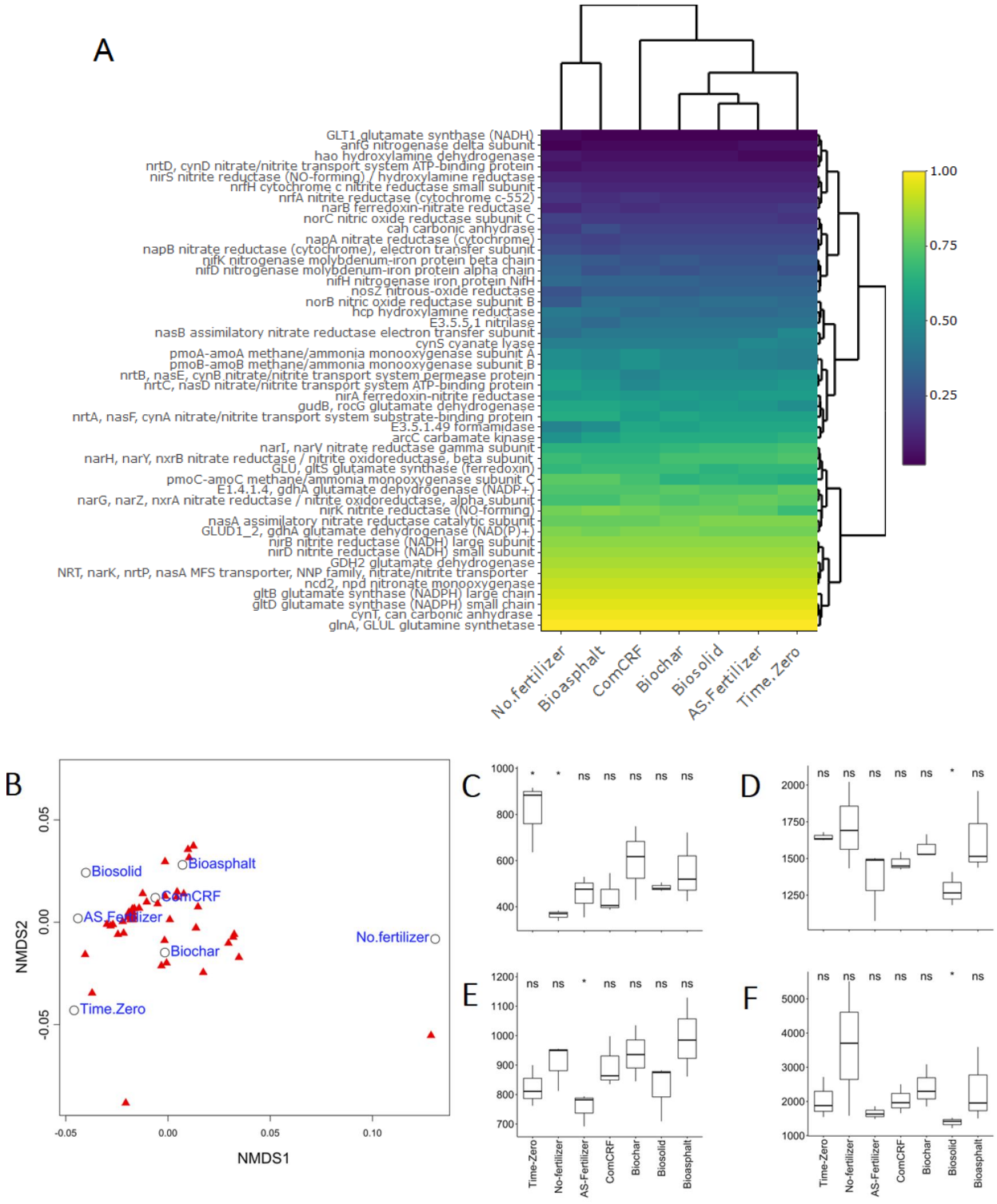

Figure 5. Functional predictions of bacterial gene associated with the nitrogen cycle. Heatmap showing N-cycle functional predictions using "*hclust*" algorithm across the treatments (**A**); Non-metric Dimensional Scaling (NMDS) showing the distribution of treatments (**B**); N-cycle functional potential in the treatments by specific gene functions, ferredoxin-nitrate reductase (*narB*) [EC:1.7.7.2] (**C**); nitrous-oxide reductase (*nosZ*) [EC:1.7.2.4] (**D**); carbonic anhydrase (*cah*) [EC:4.2.1.1] (**E**); and hydroxylamine reductase (*hcp*) [EC:1.7.99.1] (**F**). [*$p < 0.05$, ns = not significant].

4. Discussion

Biochar-based Controlled-release Nitrogen Fertilizers (BCRNFs) mixed with either biosolid or biochar have shown promising results in increasing the yield of corn (Rubel et al., in preparation) [18,20]. In this study, we compared the soil prokaryotic and fungal communities in novel BCRNFs with those of commercial controlled-release fertilizer and controls. We found that the prokaryotic communities in the highest-yielding novel BCRNF, biosolid, did not have a unique composition for soil prokaryotic or fungal communities. They had common bacterial taxa known to be found in healthy soil types across the globe [61]. Therefore, despite increasing the yield, our results show that novel BCRNFs do not cause significant shifts in soil microbial community.

4.1. BCRNFs Do Not Cause Major Shifts in the Soil Prokaryotic Communities

We found that the soil prokaryotic composition did not differ significantly across different types of BCRNFs. This could be because each treatment received the same amount of N, except the No-Fertilizer control. Prior research showed that bacterial communities are less sensitive to N fertilizer than fungal communities [62]. In addition, soil prokaryotic communities were spread over 44 phyla in soil without the fertilizer, but the soil receiving AS had only 35 phyla. Soil prokaryotic community diversity has been reported to decrease with fertilizer application [63]. Alternatively, the addition of N causes selection pressure and eliminates some prokaryotic communities, leading to a decrease in diversity [64]. Actinobacteria, Proteobacteria, and Acidobacteriota were amongst the top bacterial phyla, and are known to be common in soil across the globe [61,65,66]. Among the archaeal community, Crenarchaeota were abundant in all the treatments. They are known to be ammonia oxidizing archaea [67], and have been reported to be present in high numbers [68]. Although the 16S rRNA gene is widely used to capture prokaryotic communities, it is highly dependent on amplification efficiency. To minimize bias in the analyses, we had three replicates for each treatment, and also used a recently updated classification for all bacterial OTUs [40].

4.2. Fungal Diversity Remained Unchanged across the BCRNFs

The analysis of soil fungal communities revealed that BCRNFs did not cause differentiation in soil fungal composition. The fungal communities were also similar in the BCRNFs, except for one genus in Bioasphalt formulation which had a high proportion of fungi belonging to the Pseudofabraea. These fungi are known to cause a leaf-spotting disease in citrus plants [69], which could be a reason for the lower yield of corn. Only a couple of fungal genera were significantly different proportionally in No-fertilizer and time-zero soil (Figure 2). In contrast, the alpha diversity for fungi was least in the No-fertilizer treatment (Figure S1B). This could be due to the changes in soil chemical properties due to increased root exudates of plants to enhance fungal communities, observed in several previous experiments [70,71]. Furthermore, it could also be due to the sensitivity of the fungal community to nitrogen fertilizer [72]. Ascomycota were dominant in all treatments. Ascomycota are highly prevalent in soil [73,74], and they are known for their importance in N as well as carbon cycling [75]. Among the treatments, *Aspergillus* and *Curvularia* were significantly high in No-fertilizer and time-zero soil, respectively. A better estimate of the fungal diversity could not be achieved as a large proportion of reads were not successfully classified using the recently updated UNITE database [42]. This is due to the presence of a large unidentified fungal subkingdom.

4.3. N-Fixing Diversity and N-Cycle Functional Potential Were Different in Soil without Fertilizer

Classification using the *nif* cluster-based database [52] showed that *Microvirga*, *Skermanella*, and *Bradyrhizobium* dominated the N-fixing genera across the treatments (Figure 4B,C). *Microvirga* has been reported to be present in nodules of *Listia angolensis* (from Zambia) and *Lupinus texensis* (from Texas, USA) [76]. However, it should be noted that not all bacterial strains belonging to N-fixing genera fix nitrogen [77,78]. The N-fixer di-

versity was high in no-fertilizer, previously recorded in a study using pyrosequencing [79]. The functional potential related to only a few N-cycle-related gene functions varied significantly among the treatments. N-cycle-related functional potential had equal distribution in the novel BCRNFs, except *nosZ* and *hcp* which were low in biosolid (Kruskal–Wallis test, $p < 0.05$). Decrease in the *nosZ gene*s subsequently reduces the bacterial activity to transform N_2O to N_2. Similar results were obtained by Harter et al. showing reduction of greenhouse gas emissions in Biochar amended soil [29]. Furthermore, the glutamine synthetase (GS) gene was highly prevalent across all treatments (Figure 5A). GS enzyme activity has been found to increase when there were low ammonia concentrations or low N availability [80]. The functional prediction derivations using PICRUSt2 and KEGG databases gave us a clear screenshot of how the soil bacteria regulated the N-cycle in these artificial novel fertilizer regimes (Figure 5). The bioinformatics tool, PICRUSt2, has emerged as a cost-effective tool to estimate ecological functions and yields reliable comparisons [53]. These reports are fundamental to the understanding of shifts in soil prokaryotic and fungal communities in a greenhouse experiment using BCRNFs and form a basis for further exploration in field conditions.

5. Conclusions

The prokaryotic and fungal communities were distributed evenly in high-yielding formulations of novel BCRNFs. The results indicated that BCRNFs do not alter the soil microbial communities substantially, while promoting yield. This aids in soil conservation and would be essential for the management of soil and the improvement of agriculture practices [66]. Furthermore, nitrogen fixing bacteria were diverse in pots without any fertilizers most probably to compensate for the N needs of the corn. The highest crop-yielding BCRNF—biosolid, was predicted to have reduced bacterial functions for greenhouse gas emission and carbon utilization, potentially warranting environmental benefits of this biosolid based BCRNF. Overall, biosolid BCRNF seems to be a valuable alternative to nitrogen fertilization with minimal shift in the soil communities while maximizing the crop yield.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12101706/s1, Figure S1: Alpha diversity using Shannon index, Bacterial (A) and Fungal (B) asterisk represent comparisons made using Kruskal–Wallis test with $*p < 0.05$; Figure S2: Bubble plot showing prokaryotic species significantly different for at least one treatment. Multiple dots together signify same grouping across different phyla; Table S1: Corn yield and Biomass; Table S2: Fertilizer descriptions (wt. % amount with moistures); Table S3: Soil Properties; Table S4: Commercial fertilizer ingredients; Table S5: Taxonomic distribution of the prokaryotic community in soil for each treatment; Table S6: Taxa different in high yield compared to other treatments at lowest taxa; Table S7: Taxonomic distribution of the fungal community in soil for each treatment; Table S8: Genera of fungus significantly different for treatment method.

Author Contributions: Conceptualization, B.K.D., L.W., Y.W. and V.S.B.; methodology B.K.D., R.I.R., S.G., Y.W., L.W. and V.S.B.; validation, B.K.D., L.W., Y.W. and V.S.B.; formal analysis, B.K.D., R.I.R., L.W. and V.S.B.; investigation, B.K.D., R.I.R. and S.G.; resources, L.W..; data curation, B.K.D.; writing—original draft preparation, B.K.D.; writing—review and editing, B.K.D., R.I.R., Y.W., L.W. and V.S.B.; visualization, B.K.D.; supervision, L.W., Y.W. and V.S.B.; project administration, L.W., Y.W. and V.S.B.; funding acquisition, L.W., Y.W. and V.S.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the South Dakota Governor's Office of Economic Development (grant #: POC2020-04) and the USDA NIFA through the North Central Regional Sun Grant Center and Hatch Projects (No. 3AR652, 3AR689, and 3AH658) of the South Dakota Agricultural Experimental Station.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data is available on NCBI's Sequence Read Archive (SRA) database under BioProject: PRJNA854759.

Acknowledgments: We thank Salman Alanazi, Sikander Ameer, Abdus Sobhan, Abdulkarim Aldekhail from Agricultural and Biosystems Engineering, South Dakota State University, Brookings, SD 57006, USA for their help during the greenhouse trials.

Conflicts of Interest: The authors declare no conflict of interest.

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