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Effect of Dry and Flooded Rice as Cover Crops on Soil Health and Microbial Community on Histosols

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Abstract

Soil loss due to subsidence is a major concern in the Everglades Agricultural Area (EAA) of South Florida. Summer is typically the fallow season in the EAA, and soil loss due to oxidation and erosion is significant. Flooding and cover cropping are common practices being adopted to conserve soil, reduce weed pressure, and enhance soil health in the EAA. Cover crops also increase the microbial biomass which are the key drivers of soil function. The objective of this study was to determine the effect of (i) fallow, (ii) dry rice as a cover crop, (iii) flooded fallow, and (iv) flooded rice as a cover crop on soil health indicators and microbial community and diversity within the EAA. Baseline (pre-planting) soil samples were collected from all fields before the application of different treatments and post-harvest soil samples were collected after rice was cut and tilled into the soil surface. Microbial community composition was determined using 16S rRNA gene amplicon and fungal ITS gene amplicon sequencing. Soil bulk density decreased, and cation exchange capacity (CEC) increased in all farming practices including fallow fields. Results showed flooded fallow, flooded rice, and rice planting increased maximum water holding capacity (MWHC) and soil protein and decreased total potassium (TK). Bulk soil microbial communities responded surprisingly quickly to the applied treatments. Taxonomic composition of prokaryotic and fungal communities at the phylum level revealed visible shifts in microbial communities in response to the treatments. Instead of leaving field fallow, planting rice or flooding is a better strategy to improve soil health.

Keywords: soil health, organic matter, microbial community, prokaryotic, fungal community

1. Introduction

The Everglades Agriculture Area (EAA) of southern Florida, USA consists of Histosols, which have 80-90 % organic matter (OM) (Morris et al., 2004; Wright and Hanlon, 2009). In the early 1900s, this area was artificially drained for cultivation. The organic matter exposed to the surface continues to decompose at faster rate by heterotrophic aerobic microorganisms compared to anaerobic microbial decomposition in flooded soil (Tate and Tarry, 1980). This process of soil decaying is called subsidence (Wright and Hanlon, 2009). From 1924 to 2019, the rate of subsidence in the EAA has been examined and reported multiple times. The average rate of subsidence from 1924-1967, 1968- 2009, and 2010 to 2019 was reported to be an average of 2.8 cm yr^{-1}, 1.4 cm yr^{-1}, and 0.64 cm yr^{-1}, respectively (Bhadha et al., 2020). If an effective soil conservation method is not adopted soon EAA soil will be too shallow for agricultural use or without soil. Soil conservation is a major concern in EAA for sustainable agriculture. Several management practices such as flooding, increasing water table, crop rotation, and cover cropping can be adopted to slow down the subsidence rate and also to build up organic matter in the soil.

Flooding or raising the water table results in the reduction of aerobic decomposition of organic matter which slows down the subsidence rate (Bhadha and Schroeder, 2017). Besides the reduction of soil subsidence, flooding also reduces nutrient depletion, and insect pests' population (Cherry et al., 2015). Crop diversification including cover crop or crop rotation is another option to manage subsidence. Sugarcane, rice, and winter vegetables are commonly grown in the EAA. After harvesting sugarcane and winter vegetables in the summer,

farms in EAA are either left fallow, flooded fallow, or planted with rice. Flooded rice after sugarcane harvest is a good crop rotation practice as it helps to reduce soil oxidation (Bhadha et al., 2021). Growing cover crops in the fallow season between sugarcane and winter vegetables not only retain soil nutrients but also add organic matter. Cover crops are becoming more popular during the fallow season because they sequester carbon, reduce soil erosion, and increase microbial community and diversity (Bacq-Labreuil et al., 2019). Planting leguminous cover crops may result in greater soil nitrogen (N) due to N_2-fixation (Gabriel et al., 2012). Nonlegume cover crops, on the other hand, maybe more successful in increasing soil organic matter and minimizing N leaching due to their higher biomass (Sainju et al, 2003). Previous research has found that cover crops boost the number of saprophytic and mycorrhizal fungi (Bacq-Labreuil et al., 2019). Muhammad et al (2021) showed that planting cover crops increased bacteria and fungi biomass by 7-13% compared to no cover crop. The larger fungal/bacteria ratio indicates that fungi were more influenced by cover crops than bacteria. Cover crop residue provides essential nutrients for microbial growth and development (Tao et al., 2017), whereas microbial communities decompose cover crop residue and add nutrients for main crops. Microbial compounds released following the decomposition of crop residue are the main antecedents of soil organic matter (Cotrufo et al., 2013). Soil organic matter enhances the soil absorption complex and nutrient retention, which is good for plant development (Drost et al., 2020). Besides enhancing soil health, the microbial community is capable of protecting host plants from pathogens and increasing tolerance to environmental stress (Rillig, 2004). Microbial community and activity are influenced by cover crop species and soil type (Muhammad et al., 2021). Integrative assessment of how soil's physical, chemical, biological properties and microbial community are affected by common land management practices in the EAA is essential to fully understand soil loss via subsidence. However, there is limited information available on the effect of flooding and growing rice as a cover crop on soil physical and chemical properties and microbial community and diversity in the EAA. Therefore, the objectives of this study were to: i) evaluate the impact of cultivating flooded rice and dry rice as a cover crop, leaving the field fallow and alternative practices such as flooded fallow on soil health of histosols; and ii) investigate the microbial community and diversity.

2. Materials and Method

2.1 Soil Sampling

This study was conducted on Histosols within the EAA during the summer of 2021. Four commonly used farming practices including fallow, dry rice as a cover crop, flooded fallow, and flooded rice as a cover crop were included in this study. Each farming practice was conducted on a separate field and soil samples were collected form each filed as randomized complete design. Diamond variety of rice was seeded at 100 kg ha^{-1}. Irrigation was not applied to dry rice whereas in flooded rice field, flooding was initiated 3 weeks after planting. Eight random baseline soil samples (referred to in this study as pre) were collected in May 2021 before planting rice or flooding from each field. The second set of eight soil samples (referred to post) were collected in September 2021 after rice was cut and tilled into the soil surface or the flooded field was drained out. Post soil samples were collected at the same spot or as close as possible to the pre-soil samples to minimize error due to variability in field conditions.

2.2 Soil Analyses

Air-dried pre-and post- soil samples of particle size less than 2 mm were analyzed for soil health indicators, including soil pH, bulk density (BD), maximum water holding capacity (MWHC), organic matter (OM), cation exchange capacity (CEC), active carbon (AC), soil protein (SP), Mehlich-3 phosphorus (M3P), Mehlich-3 potassium (M3K), total phosphorus (TP), total potassium (TK), and total Kjeldahl nitrogen (TKN). Soil pH was determined using Accumet AB250 pH meter with a 1:10 soil to water ratio. Bulk density was calculated by dividing soil mass by a fixed core volume. Maximum water holding capacity was determined based on the saturation procedure described in Jenkinson and Powlson (1976). Organic matter content was calculated based on the loss on ignition method. Soil samples were placed in a muffle furnace at 550 ˚C to combust organic material. The weight of ashed samples were measured after bringing to room temperature. Soil OM content was calculated as the difference between dry weight and ashed weight on a percentage basis (Amgain et al., 2021a). Cation exchange capacity was estimated using the ammonium acetate method (Sumner and Miller, 1996). Active carbon was determined based on potassium permanganate ($KMnO_4$) oxidizable carbon using 0.02 M $KMnO_4$ for mineral soils, in which approximately 2.5 g of soil was reacted with 20 ml of 0.02 M $KMnO_4$ for exactly two minutes, filtered and the supernatant solution was then analyzed using Thermo Scientific Genesys 30 spectrophotometer at 550 nm (Schindelbeck et al., 2016). Soil protein was determined by using a sodium citrate extraction method (Schindelbeck et al., 2016) under autoclaving with high temperature and pressure. The extracted protein was quantified by using the Thermo pierce colorimetric bicinchoninic acid assay (BCA) as

calibrated against protein standards of known concentration. The color development was read by using Thermo Scientific Genesys 30 spectrophotometer at 550 nm. Soil available P and K were determined using Mehlich -3 extraction method and then analyzed using Agilent 5110 inductively coupled plasma-optical emission spectrometer (ICP-OES) (Santa Clara CA). Total P was determined by ashing samples for at least 5 hours (not to exceed 16 hours) at 550 ^{o}C in a muffle furnace followed by extraction with 6 M HCL and analyzed using ICP-OES. TKN was determined using the digestion method followed by colorimetric determination (EPA method 351.2).

2.3 DNA Extraction, 16S rRNA Gene Amplicon Library Preparation and Sequencing

Soil samples were placed on ice until returning to the lab and thereafter stored at -80 ℃ until further processing. Approximately 5 g soil samples were homogenized by grinding using mortar and pestle. DNA was then isolated from 0.25 g (wet weight) samples using the DN easy Power Soil kit (Qiagen, Germantown, MD) following manufacturer's instructions. Samples were treated by bead beating twice for 30 seconds at 4.0 m/s in a FastPrep-24 bead beater (MP Biomedical, Irvine, CA). DNA was dissolved in 50 µl molecular biology grade water and stored at -20 ℃ before use. Quality and quantity of DNA was assessed by UV/Vis spectroscopy using a NanoDrop ND-1000 (Thermo Scientific, Waltham, MA) and yielded DNA concentrations between 109.65 to 213.82 ng/µl with 260/280nm ratios > 1.7 from all samples. DNA samples were stored at -80 ℃ until use.

PCR amplifications, library preparations and DNA sequencing were conducted at the University of Illinois Chicago genome research core. PCR products for 16S rRNA gene amplicon libraries were generated following Earth Microbiome Project protocols (Thompson, 2017 #591). The V4-V5 region of the 16S rRNA gene was amplified using primers 515F-Y (Parada, 2016 #428) and 926R (Quince, 2011 #1542). Fungal ITS gene fragments were amplified using primers ITS1F and ITS2R (White et al., 1990). Barcoded libraries were prepared, pooled and sequenced on an Illumina MiniSeq instrument using the 250 cycle MiSeq Reagent kit v3 (Illumina, San Diego, Ca).

Amplicon sequences were demultiplexed on the instrument. Dada2 with default settings implemented in the QIIME 2 package was used to remove barcodes, quality filter ($Q \geq 30$), trim reads, merge reads, and screen for chimera (Callahan, 2016 #1283) (Bolyen, 2019 #1346). This yielded between 15,612 and 21,765, as well as 14,150 and 78,130 non-chimeric high-quality sequences per sample for 16SrRNA genes and fungal ITS genes, respectively. Amplicon sequence variants (ASV) table was generated with dada2 and taxonomic assignments were made with qiime feature-classifier using sklearn algorithm against the Silva database version 138 (Quast, 2012 #1284).

Data were rarefied to even depth of 15,000 (16S rRNA genes) and 14,000 (fungal ITS genes) sequences per sample. Bray-Curtis and weighted Unifrac distance matrices were calculated using "qiime diversity core-metrics-phylogenetic" command in Qiime 2. Shannon Index, Observed_OTUs, Chao1 alpha diversity metrics were calculated using phyloseq V1.30.0 (McMurdie, 2013 #2081) in R version 3.6.3 (R Core Team, 2020 #1579).

2.4 Statistical Analysis

Effects of different farming practices on soil health indicators were analyzed using the generalized linear mixed models (GLIMMIX) method (SAS version 9.4, SAS Institute Inc.) as a randomized complete design. Since each farming practice was conducted on a separate field, each farming practice was analyzed separately and compared pre vs post soil samples. Means were separated using Tukey–Kramer multiple-comparison procedure when the F test was significant at $p \leq .05$.

For microbial data, Principal coordinate analyses (PCoA) and distance-based redundancy analysis (dbRDA) were run by using the PCoA and capscale functions in vegan, respectively, using the vegan package (https://CRAN.R-project.org/package=vegan {Oksanen, 2019 #1577}) implemented in R. Significance of differences between treatments and time point were tested by the adonis function with 999 permutations in vegan. Pearson correlations and ANOVA comparison of means were conducted in base R.

3. Results

3.1 Impact on Soil Physical Properties

Soil BD ranged between 0.69 and 0.73 g cm^{-3} and 0.53 and 0.58 g cm^{-3} for pre and post soil samples, respectively (Figure 1 a). All farming practices showed a significant decrease in BD. The maximum water holding capacity of pre and post soil ranged from 173 to 183 % and 182 to 199 %, respectively (Figure 1 b). Maximum water holding capacity was higher in post-harvest soil samples compared to pre-planting/flooding soil samples in all farming practices except for the field that was left fallow. Fallow field was the only farming practice that showed

a decrease in OM, all other farming practices remained statistically similar and showed no change between pre and post soil samples.

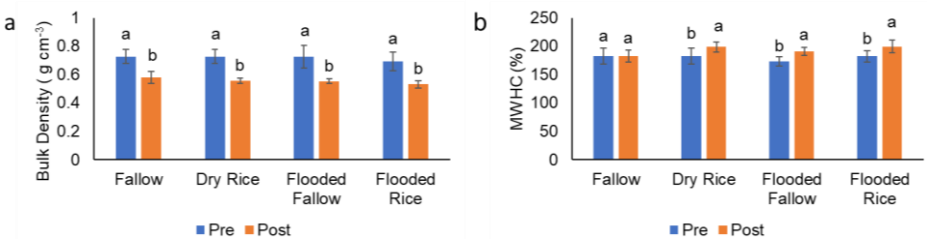

Figure 1. Effects of farming practices on physical soil health indicators. a. bulk density and b. maximum water holding capacity. Means sharing a common letter within each farming practice are not significantly different at p = 0.05 significance level

3.2 Impact on Soil Chemical Properties

All soil samples had neutral to alkaline soil pH (6.9 to 7.8). For flooded fallow, the pH of soil samples before and after flooding was statically identical. The pH of the soil increased significantly in fallow and dry rice but declined in flooded rice (Fig 2a). Organic matter content of pre-planting and post-harvest soil samples ranged from 75.7 to 77.6 and 71.1 to 80.6 %, respectively (Figure 2b). Cation exchange capacity of pre-planting soil samples ranged from 72 to 78 cmolc kg^{-1} and post-harvest soil samples ranged from 138 to 156 cmolc kg^{-1} (Figure 2c). All farming practices increased cation exchange capacity. Total Kjeldahl nitrogen (TKN) of pre and post soil samples ranged from 14611 to 18749 mg kg^{-1} and 12481 to 14993 mg kg^{-1}, respectively (Figure 2d). TKN concentration of flooded fallow and flooded rice farming was statically similar, whereas TKN concentration was reduced for fallow and dry rice. Total phosphorus ranged from 1706 to 2296 mg kg^{-1} and 1517 to 1923 mg kg^{-1} for pre and post soil samples (Figure 2e). The only farming practice that decreases TP was a fallow field. There was no significant difference in TP between pre and post soils for dry rice, flooded fallow, and flooded rice. Total potassium ranged from 540 to 606 mg kg^{-1} for pre soil samples and 303 to 653 mg kg^{-1} for post soil samples (Figure 2f). All farming practices significantly decreased TK except fallow. The field that had been kept fallow, there was no significant difference in TK between pre and post soil samples. Mehlich-3 phosphorus of pre and post soil samples ranged from 174 to 210 mg kg^{-1} and 168 to 201 mg kg^{-1}, respectively (Figure 2g). No significant difference in pre and post M3P was observed for dry rice and flooded rice fields, whereas M3P was increased in a fallow field and decreased in the flooded fallow field. Mehlich-3 potassium ranged from 307 to 386 mg kg^{-1} for pre soil samples and 180 to 476 mg kg^{-1} for post soil samples (Figure 2h). There was a significant decrease in M3P in the fallow field, whereas M3K increased in flooded fallow. Mehlich3 potassium remained statistically similar for dry rice and flooded rice.

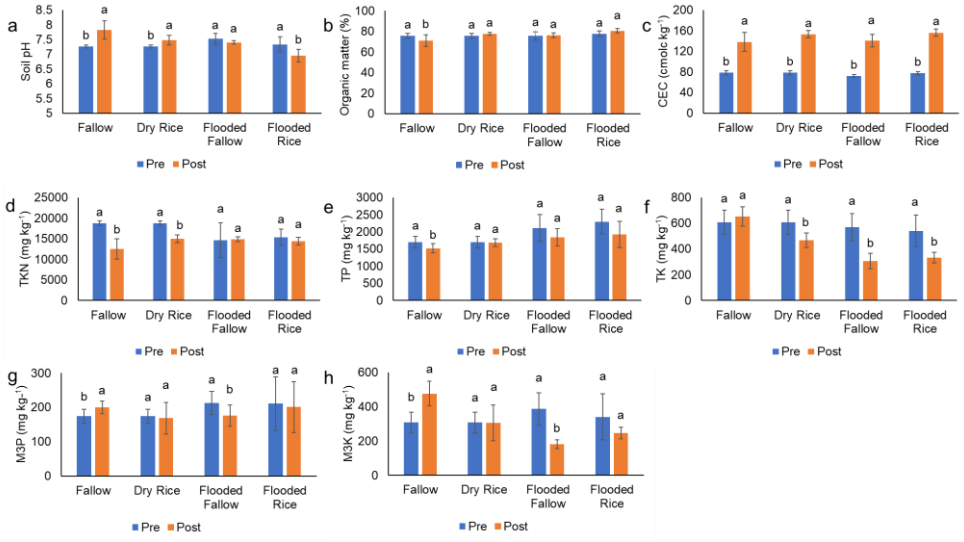

Figure 2. Effects of farming practices on chemical soil health indicators. a. soil pH, b. organic matter, c. cation exchange capacity (CEC), d. total Kjeldahl nitrogen (TKN), e. total phosphorus (TP), f. total potassium (TK), g. Mehlich-3 phosphorus (M3P), and h. Mehlich-3 potassium (M3K). Means sharing a common letter within each farming practice are not significantly different at p = 0.05 significance level

3.3 Impact on Soil Biological Properties

Active carbon ranged from 27909 to 29553 mg kg^{-1} and 28437 to 29004 mg kg^{-1} for pre and post soil samples, respectively (Figure 3a). Active carbon remained statistically similar for all farming practices. Although statistically similar, flooded rice and flooded fallow had increased AC, whereas fallow and dry rice had decreased AC. Soil protein ranged from 286 to 322 mg kg^{-1} for pre soil samples and 350 to 456 mg kg^{-1} for post soil samples (Figure 3b). Dry rice, flooded fallow, and flooded rice farming practices increased soil protein, whereas it remained statistically similar for fallow.

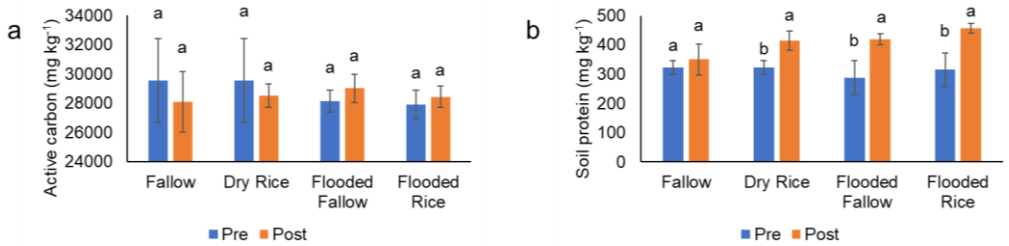

Figure 3. Effects of farming practices on biological soil health indicators. a. active carbon and b. soil protein. Means sharing a common letter within each farming practice are not significantly different at $p = 0.05$ significance level

3.4 Impact on Microbial Community and Diversity

To test if and how EAA soil microbial communities respond to the different treatments, prokaryotic and fungal communities were analyzed via amplicon sequencing of 16S rRNA- and fungal ITS genes, respectively. Surprisingly, principal Coordinate Analyses of weighted Unifrac distance matrices revealed significant differences between pre- and post-treatment in prokaryotic and fungal communities even within the relatively short timeframe (Figure 4). The same analysis based on Bray-Curtis distance matrices did not reveal significant differences (data not shown). Differences between weighted Unifrac and Bray-Curtis-based results suggest that the changes in both types of communities are predominantly caused by changes among closely related taxa.

Although the number of replicates was not sufficient to draw clear conclusions about the effects of each treatment on the microbial communities, alpha diversity measures indicated that the flooded fallow treatment could potentially reduce prokaryotic diversity (Figure 5). In contrast, Observed ASVs, Chao-1, and Shannon index all indicated a reduced fungal diversity in all but the flooded fallow treatment (Figure 5). Taken together, these data suggest that prokaryotic and fungal communities responded differently to the imposed treatments.

Taxonomic composition of prokaryotic and fungal communities at the phylum level revealed visible shifts in microbial communities in response to the treatments. In prokaryotic communities the combined relative abundance of taxa below 1.0% relative abundance, grouped as “Other”, increased in all but the fallow treatment from between 5.1% and 5.8% to 6.7% and 9.3% post-treatment. Conversely, Actinobacteriota decreased in all but the fallow treatment from 17.2% - 20.8% to 14.5% - 17.0%. The only other substantial pre-post difference appeared in the increased relative abundance of Chloroflexi in the fallow treatment from 14.0% to 19.3%. Pre-treatment fungal communities were highly dominated by Ascomycota ranging between 78.2% to 81.6%, accompanied by less than 15% combined other identified fungal phyla (Basidiomycota, Mortierellomycota, Rozellomycota, and other phyla below 1.0% relative abundance). Relative abundance of Ascomycota consistently decreased in all four treatments, with the fallow treatments showing the largest declines. Conversely, all treatments showed an increase of unidentified fungal taxa from between 9.9% to 16.2% pre-treatment to between 20.0% and 43.2%, likely representing poorly studied thus far uncultivated taxa. Notably, Rozellomycota increased in relative abundance in all four treatments from below 1.0% pre-treatment to 1.3% in the dry rice, 2.4% in the flooded rice, and 7.9% in the fallow treatment.

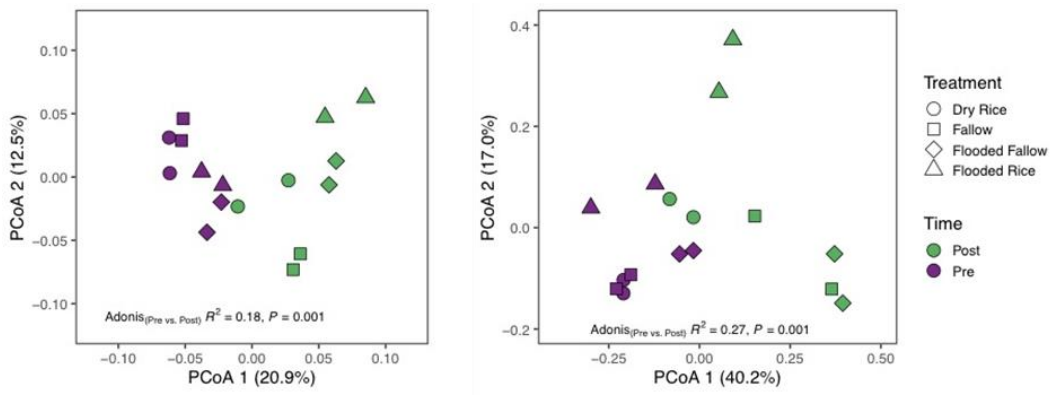

Figure 4. Principal coordinate analyses of prokaryotic (left) and fungal (right) microbial communities to study treatments based on weighted Unifrac distances of 16S rRNA genes and fungal ITS genes, respectively. Adonis test results for differences between communities before and after treatments are given

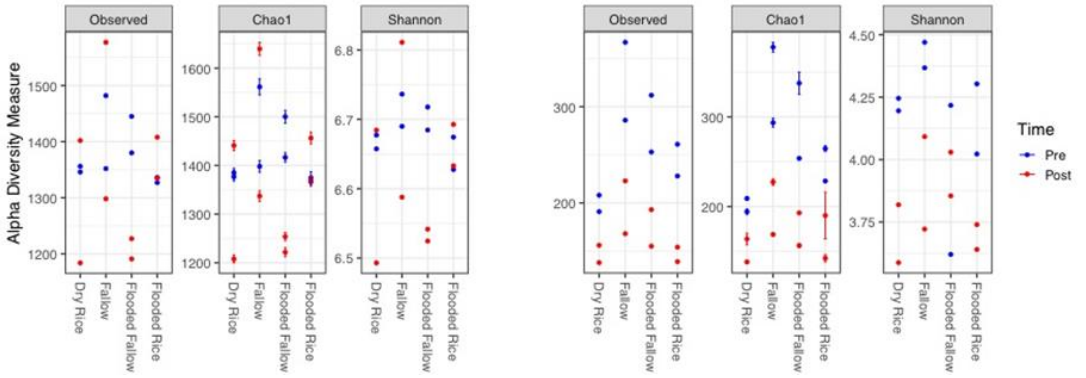

Figure 5. Responses of alpha diversity in prokaryotic (left) and fungal (right) communities depending on treatments

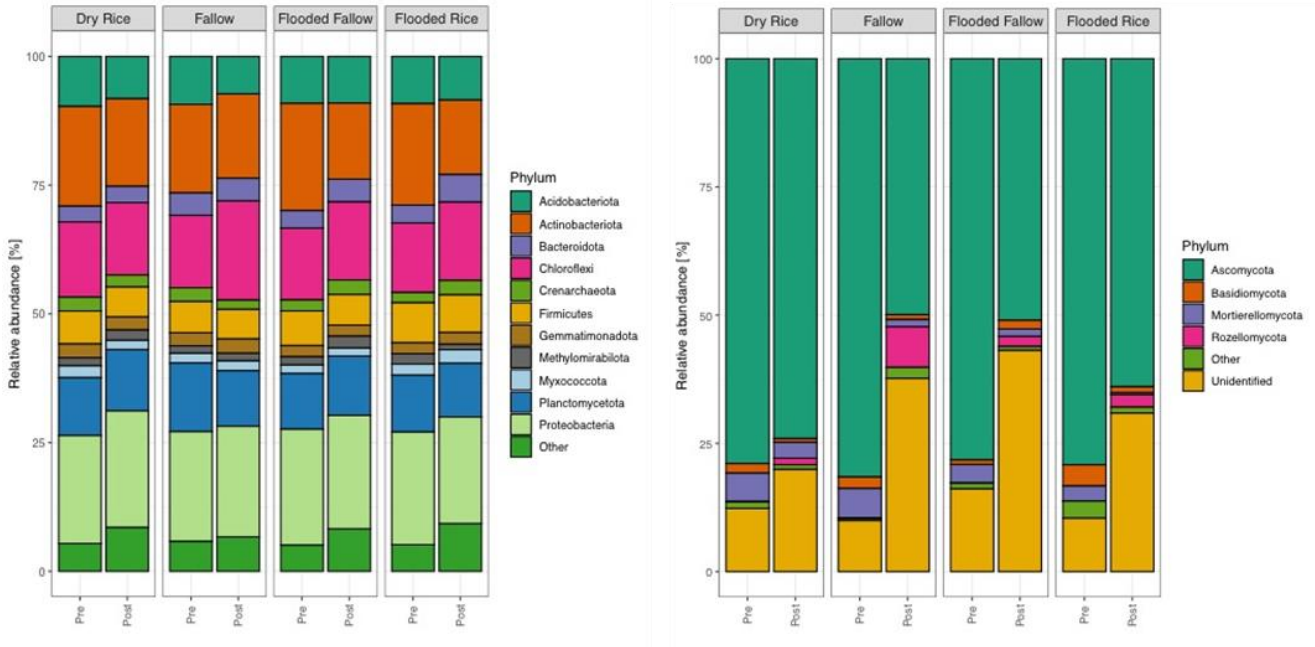

Figure 6. Phylum-level relative abundances of major prokaryotic (top) and fungal phyla (bottom) present in EAA soils pre- and post-treatment. Given are the relative abundances of phyla with relative abundances > 1.0%. All phyla with smaller relative abundances were grouped together as “Other”. Fungal ITS sequences that were confidently identified as fungal ITS sequences but could not be further classified were labeled as “Unidentified”

4. Discussion

The high pH of EAA soil may be associated with the mixing of limestone bedrock with the topsoil. Increased soil pH is a serious problem for EAA producers because it has the potential to reduce the bioavailability of micronutrients from the soils (Sims and Patrick, 1978). The decrease in bulk density after all treatments is associated with the loosening of soil over time. Previously soil was compacted due to the use of a heavy machine for planting and harvesting vegetables. Over time these soils lose due to roots of rice plants and weeds. Lower soil BD is preferable since it improves aeration, tilth, and reduces root constriction. Increase in OM contributed to better nutrient cycling, aggregate stability, and water holding capacity (Lehman et al. 2015). The increase in MWHC of flooded field is most likely due to the increased delivery of finer-sized material in the form of silt and clay by irrigation water. Those fine particles high in the organic matter have a larger surface area which increases the water holding capacity. Increase in MWHC is also associated with increase in the OM content of the soil. Bhadha et al. (2017) reported every 1% increase in OM equates to a 2.3% increase in soil water holding capacity of sandy soils. Growers can enhance the MWHC of their soils by using farming techniques that increase soil OM. Soil CEC is influenced by carbon content and fraction clay sized particles (Parfitt et al., 1995). A higher CEC (72 $cmolckg^{-1}$) value of Histosols in EAA may be associated with high soil carbon content (> 70%). An increase in soil CEC is considered a beneficial improvement since it has the ability to hold fertilizer and pesticides in the soil matrix for a longer. The quantity of AC in soil quantifies the amount of C that can be easily mineralized to CO_2 in a short period of time under ambient circumstances. In the EAA, soil loss due to oxidation is a concern, therefore farming practices that increase AC is not always a beneficial practice. The deduction in TKN after dry rice is attributed to N uptake by rice. With no fertilizer application, rice cultivation in the EAA solely depends on the soils and irrigation water for its nutritional needs. Reduction in TKN in the fallow field is associated with leaching and other forms of N loss. Although TKN was not significantly different in a flooded field, it has decreased, which is due to N uptake by rice and some of the loss was compensated by irrigation water. Although TKN decreased after flooding and rice planting, soil protein level increased. Soil protein is the quantity of organically bound N in soil OM that can be mineralized by microbial communities. Our findings showed that flooding or growing cover crops in EAA helps to increase soil protein levels, which is beneficial because maintaining microbially degradable N helps to meet the nitrogen demands of subsequent cash crops. A decrease in TK in rice cultivated field might be due to uptake by rice plants. A decrease in TP in the fallow field is associated with soil loss due to wind erosion and leaching following rainfall. In the flooded field, no change in TP may be due to the addition of P from irrigation, whereas in a dry rice field, leaching loss and wind erosion of sediment is minimum. Tootoonchi et al (2018) reported TP of EAA canal water ranged from 0.033 to 0.066 mg L^{-1} in the 2014 and 2015 rice growing season. In another study, Amgain et al (2021b) observed TP of canal water ranged from 0.01 to 0.8 mg L^{-1} from July through August 2019. Increase in plant available potassium (M3K) and phosphorus (M3P) in a fallow field may be due to the breakdown of recalcitrant to readily available forms. From a nutrient perspective increase in M3P and M3K is a good sign as it indicates nutrient availability for the subsequent cash crop.

Bulk soil microbial communities responded surprisingly quickly to the applied treatments. Pre-treatment prokaryotic communities were composed similar to a previous report from the EAA (Huang et al., 2021). Notably, both, PCoA and phylum-level relative abundances showed distinct changes in microbial communities in different treatments in relatively short periods of time. Although no direct link could be observed between specific soil physical and chemical properties and soil microbial communities in this study, at least in part due to the limited replication, soil moisture and pH were previously shown to be the predominant drivers associated with differences in microbial communities in EAA soils (Huang et al., 2021). Although, soil moisture was not determined directly here, divergence of microbial communities between fallow and flooded treatments may be indicative of shifts towards facultative or strict anaerobic microbial communities in flooded treatments, as well as a sufficient turnover of microbial communities within the treatment times for such changes to become detectable.

5. Conclusions

From a soil conservation standpoint, cultivating rice as a cover crop or flooding the land had positive effects on the soil physical and chemical priorities. Rice as a cover crop or flooding had reduced bulk density and increased soil protein and water holding capacity. Soil with a lower bulk density and a higher maximum water holding capacity is desirable because it promotes root development and reserves water for the dry season. Within the EAA, planting rice in flooded field or flooding the field during the fallow period is a good management practice to enhance soil health as it helps to reduce soil loss via oxidation. Furthermore, detailed characterization of rice cultivation and flooding treatments on soil microbial communities and activates may be warranted to interrogate

in more detail which roles the different microbial tax and activities play in the retention or degradation of soil organic matter and associated mineral nutrients.

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