Research reports from undergraduate students receiving support from the Shackouls Honors College via the Summer Research Fellowship

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Name: McKenna Alden

Faculty Mentor: Dr. Adam Skarke

Major: Environmental Geosciences

Department: Geosciences

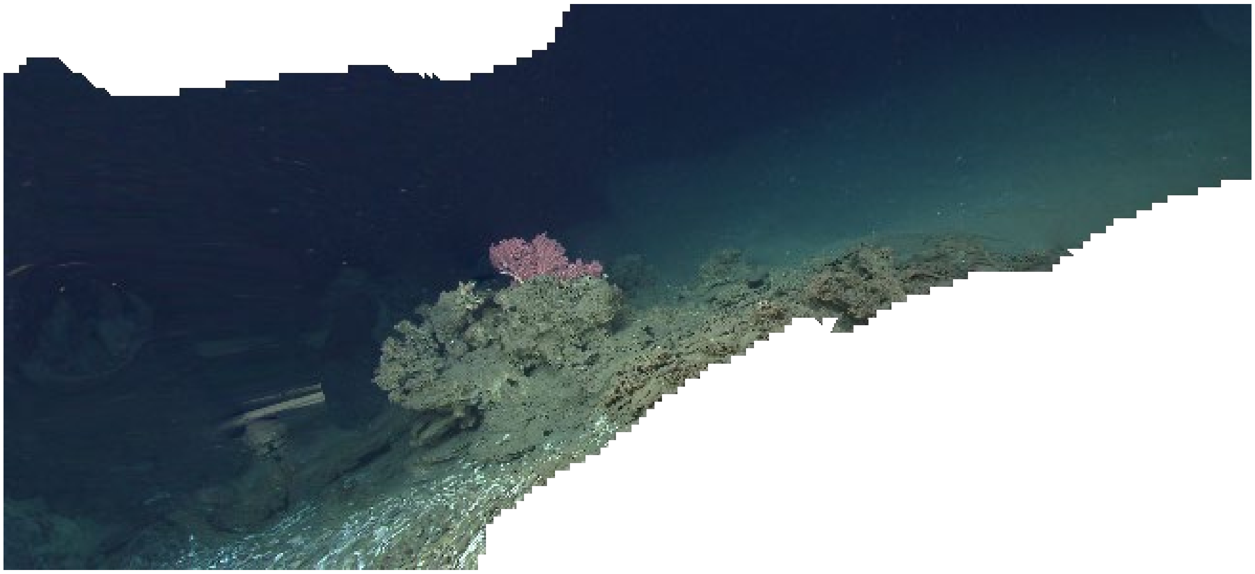
High-Resolution Mapping of Deep-Sea Benthic Environments Through Orthorectification of Oblique ROV Video Data

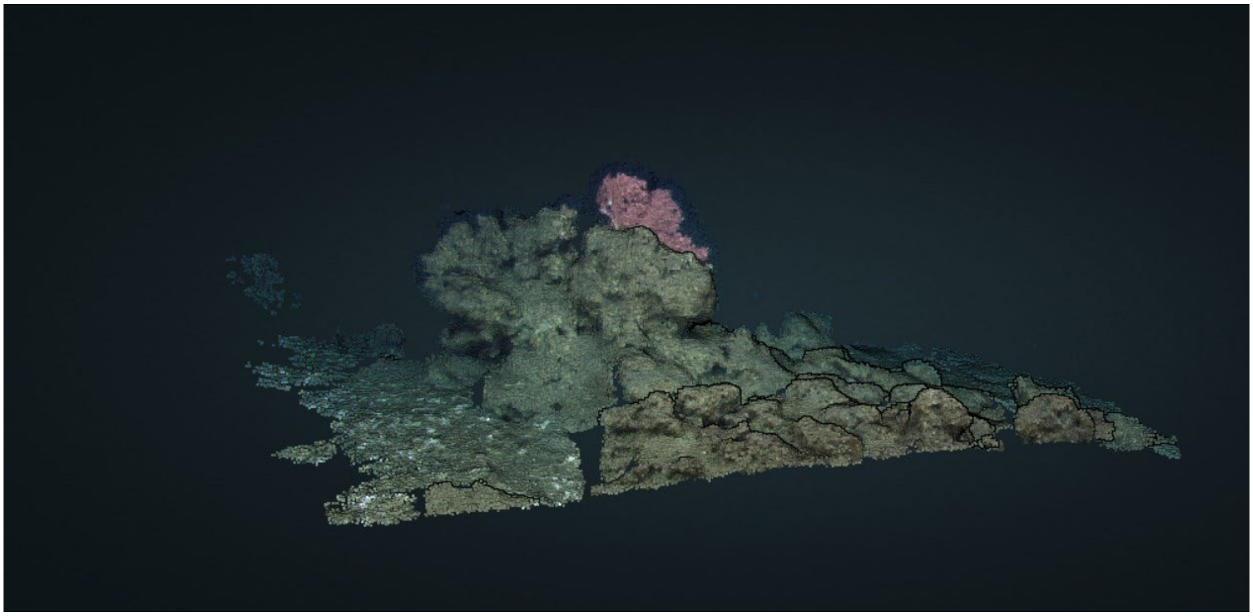
Introduction

The development of remotely operated vehicles (ROVs) has enabled extensive research in deep-sea benthic environments and resulted in the generation of vast quantities of publicly archived ROV video data. These video data are potentially an immensely valuable resource for the oceanographic research community. However, for scientists not directly involved in their collection, the amount time and effort required to review tens to hundreds of hours of video in order to determine its potential value to their research objectives is often prohibitive. Accordingly, the goal of this project was to develop a system to generate geospatially referenced, high-resolution seafloor image maps through orthorectification of commonly collected oblique ROV video data. As a proof of concept, a seafloor map of the Veatch Canyon methane seep site was generated with video data acquired during an exploratory dive of the ROV Deep Discoverer aboard NOAA Ship Okeanos Explorer (EX1304L1 Dive 13). Specifically, oblique ROV camera video frames were extracted and then orthorectified in a single map using associated vehicle position and attitude records. The geospatial representation of seafloor features in the resulting map was evaluated relative to that of coincident spatial datasets collected by the autonomous underwater vehicle Sentry including side scan sonar, multibeam bathymetry, multibeam backscatter, and normal oriented still camera photomosaic surveys. Results indicate that the presented ROV video data orthorectification approach yields maps that are consistent with those surveys in terms of geospatial positioning of seafloor features and, in some cases, of higher resolution. Maps resulting from the presented method will enable end users to rapidly understand what environmental features were observed during an ROV dive as well as the spatial relationship between them. Standardized mapping of new and archived ROV video data in this manner holds great potential to enhance accessibility and utilization of those data by the broader oceanographic research community.

Methods

I used VLC Media Player to create pictures out of individual frames of NOAA archival ROV data. Then I put those pictures into WebODM, an open-source drone mapping program, to create an orthomosaic and 3-D model of the images. Because the videos are taken at an oblique angle each picture within the mosaic has to be "orthorectified", which is a technique that corrects the image distortion that would appear if all the images were put together without rectification. We then georeferenced the location of the pictures and the position of the ROV to create an overhead map of the area that was surveyed. Below is an orthophoto and a 3-D representation that was created through this process.





Future Work

Future work would likely focus on how we can pilot ROVs in a way that makes this process more accurate. There are a couple changes that could be made to the way that ROVs are currently operated that would make this process more accurate. These include getting more accurate positional and geographical data from ROVs by including new types of navigation systems, collection of ROV camera tilt and zoom parameters during dives and surveying specific areas with a static camera to ensure more continuous seafloor coverage and video frame overlap.

Name: Surabhi Gupta 27^h January 2023

Faculty Mentor: Dr. Adam Skarke

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Identification of seafloor gas seeps in sonar data to develop a machine learning detection database

INTRODUCTION

Seafloor gas seeps, which discharge methane gas into the ocean, are found on the continental margins globally. They are an important component of the global marine biogeochemical cycle but their quantity and distribution are not well understood. They contribute to ocean acidification and deoxygenation and indicate locations susceptible to underwater landslides, which can potentially generate hazardous tsunami waves. Furthermore, they are biodiversity hotspots in the deep-sea, harboring unique species, and a demonstrated energy production resource. Hence, it is important to identifying where gas seeps occur in the world. The current method used to discover them is manual visual detection of seep gas bubble plumes in sonar data by trained individuals, which is costly, time consuming, and inconsistent. Thus, there is a need for a more consistent, efficient, and cost-effective system to identify seeps features The aim of this project is to create a sonar image database with labeled seep plumes to directly support the development of a machine learning based system to automatically detect gas seeps in sonar data. The development of such a system will address many of the shortcomings of the current manual approach, In the fall semester of 2022, I worked on developing the aforementioned database.

BACKGROUND

Methane seeps are locations where methane gas is released from seafloor sediments into the ocean. This menthane is generated from the decomposition of organic material mixed in seafloor sediments. The gas is initially trapped in an ice-like solid material called hydrate, which exists in in the open space between seafloor sediments grains. When warmed or exposed to reduced pressures, this hydrate material breaks down releasing methane into the ocean.

Multibeam sonars are instruments mounted on research ships for the purpose of mapping the seafloor. The sonar emits a fan shaped beam of sound, which reflects off the bottom, mapping a "swath" of the seafloor (Fig 1). Any targets below the ship within this fan shaped beam are imaged by the sonar (Fig 2). Seep bubble plume create particularly strong sonar targets because the difference in acoustic impedance between seawater and methane results in a particularly strong reflection from the bubbles. There are other features that can appear similar to seeps in sonar data such as fish schools and acoustic noise artifacts. Thus, careful review of sonar data is necessary to make sure only seep images are used for training detection software.

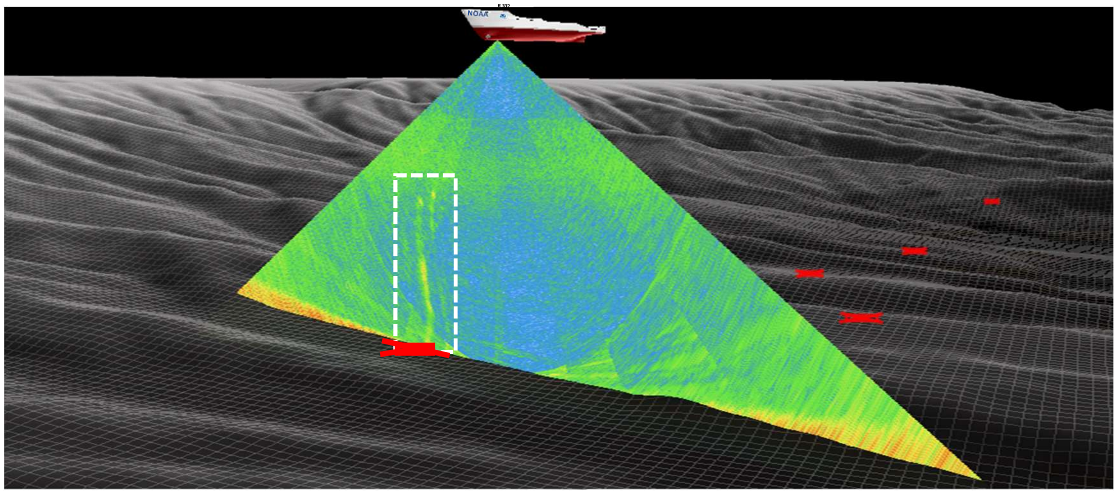

Fig. 1 Collection of multibeam sonar water column data with a research vessel. Red targets indicate identified seafloor seep locations.

RESEARCH OBJECTIVE

The objective of my research project was the creation of a sonar image database with labeled seep bubble plumes to support the development of a machine learning software system to automatically detect and segment gas seeps in sonar data.

METHODOLOGY

In collaboration with Dr. Skarke, I developed and then applied the following workflow to identify seeps in sonar images (Fig 2), label their presence or absence for each image, and draw bounding boxes (Fig 2) to segment the portion the images with seeps in them:

- Water column sonar data in *.wcd format is converted to *.MAT format through Sonar2MAT software to make it accessible for processing with Matlab software.
- We developed a MATLAB code to create sonar profile images (*.tif format) from the *.MAT files. Initial phase of the project involved a lot of troubleshooting to find the most efficient way to process the data using MATLAB.
- Sonar images were manually interpreted, by myself, to detect seep plume targets.
- Seep targets are segmented and labeled with rectangular bounding boxes (Fig 2).
- Processed image database with bounding box data is exported to collaborators for use in training and validation of automated seep detection model.

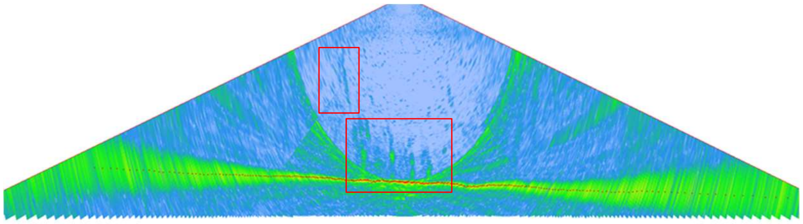

Fig. 2 Methane seep bubble emerging from seafloor imaged in multibeam sonar water column profile. Red boxes indicate plumes labelled in MATLAB.

RESULTS

We have labelled and classified the presence or absence of seeps in over 155,000 sonar images so far. Additionally, we have collaborated with computer engineering colleagues to develop a machine learning framework for seep identification from the identified and labelled seep database I worked on throughout the fall semester. We have submitted abstract for two conferences upcoming in February 2023 and they have been accepted. I plan to present the work and its future outcomes at these conferences through a poster presentation. I also plan to present the research in Spring Undergraduate Research Symposium.

Future work will involves refinement of the workflow for image labeling and segmentation to improve efficiency. Potential implementation of additional image segmentation to make the identified targets more accurate is under consideration. We will begin labeling targets that are look like seeps- such as fish schools which give false positive results. I plan to work on the project further as there are still thousands of images which need to be manually identified and labeled to train and validate the machine learning model.

Name: Tajinder Singh

Faculty Mentor: Dr. Ryan A. Folk

Major: Microbiology and Environmental Sciences

Department: Biological Science and Plant & Soil Sciences

P-LDH/BC as a slow-release fertilizer: effects on plant growth, yield, nutrient uptake, plant-microbe symbiosis, soil microbial community, and soil health.

During the Fall of 2022, I worked on the experimental trial stage of the project to test the efficiency of P-LDH/BC as a slow-release phosphorous fertilizer in comparison to standard industry fertilizer. The fertilizer was tested in a greenhouse experiment on green beans (*Phaseolus vulgaris)* in a double factor treatment design. The study consisted of 3 different treatments (industry standard, biochar, P-LDH/BC) each applied at 3 different rates (0x, 1x, 2x) relative to recommended application rates based on soil test results, giving 9 unique treatments. Each of the 9 treatments was replicated 3 times to give a total of 27 pots.

In terms of experimental measurements, plant height and chlorophyll content were recorded every week for the 6 weeks of study. On November 20th, at the end of the study, destructive sampling was done to collect soil, beans, plant, and nodule tissues separately for final measurements. The beans and plant tissues were dried overnight in an oven while the soil and nodule tissues were stored in ethanol for further processing. Starting January 10th, the plant and bean samples were ground in a Willey mill and further processed in a smaller mill to obtain sample sizes of < 10 mm. These were then ashed in a furnace, digested with hydrochloric and nitric acid, and analyzed for macro and micro-nutrient composition using an Inductive Couple Plasma-Mass Spectrometer. The resultant data were studied using linear mixed models and graphical tools were used to generate figures. Starting April 10th, genomic DNA was extracted from the ethanol preserved soil and nodule tissues, using an extraction protocol developed in-house. They were amplified using PCR targeting 16S and ITS loci to measure bacterial and fungal diversity respectively. Amplicons were quantified using a Qubit fluorometer and further analyzed by imaging on agarose gels. On June 10th, they were finally sent off to the external contractors at Michigan State University for sequencing on MiSeq platform and the sequencing results were received on August 1st .

Results

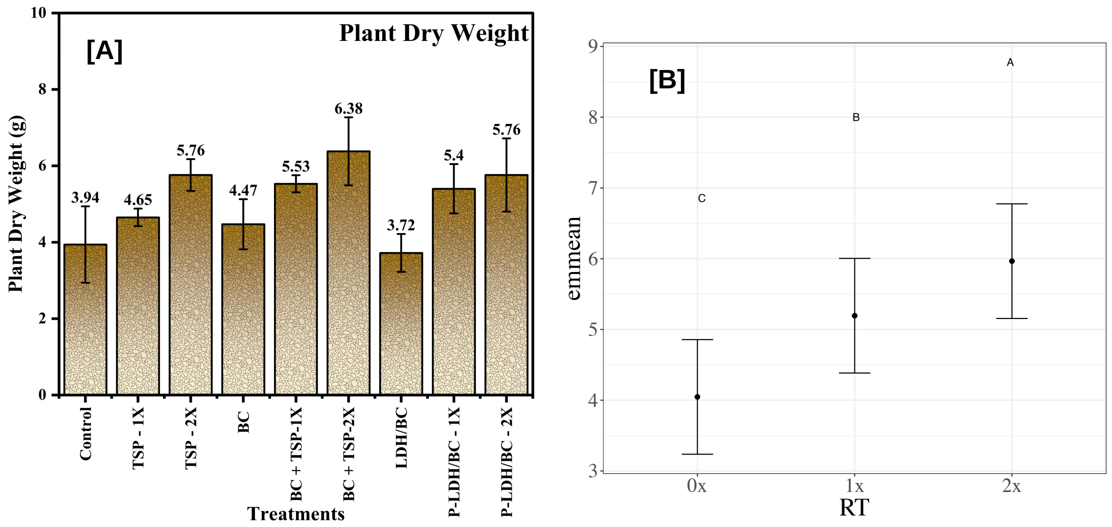

Fig 1: a) Raw results for plant dry weight. b) Effect plot from a linear mixed model; this specific effect plot expresses the relative effect of the application rate on plant dry weight in terms of standardized effect sizes.

The plant dry weight was observed to increase with the increase in rate of phosphorous in the treatments. There were no observed statistically significant effects observed due to the different treatments. A similar effect to plant dry weight was observed in the bean samples, where bean weight increased with the increase in rate but remained constant across all three treatments at each rate. These results help establish that the P-LDH/BC performed as well as standard industry fertilizer in terms of dry weight for both the beans and plant sample. Harvest weight is often related to yield, so this observation helps support that P-LDH/BC is predicted to have a yield effect similar to that of standard industry fertilizer, and would not have any negative economic impact for crop production if used as an alternative eco-friendly fertilizer.

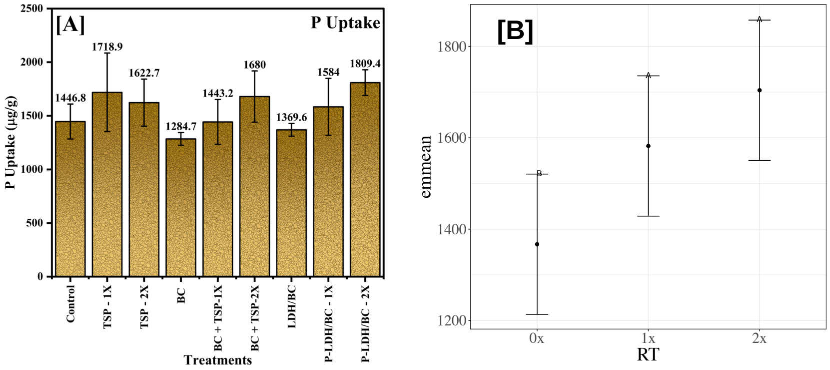

Fig 2: a) Plant phosphorous uptake. b) Linear mixed model effect plot, showing the effect of application rate.

The phosphorous uptake in plants were also observed to increase with the increase in rate of phosphorous in the treatments. There were no statistically significant effects observed due to the different treatments. A similar effect was observed in the bean samples, where the uptake increased with the increase in rate but remained constant across all three treatments at each rate. This result similarly helps to establish that the P-LDH/BC performed as good as standard industry fertilizer for phosphorous uptake in both the beans and plant sample.

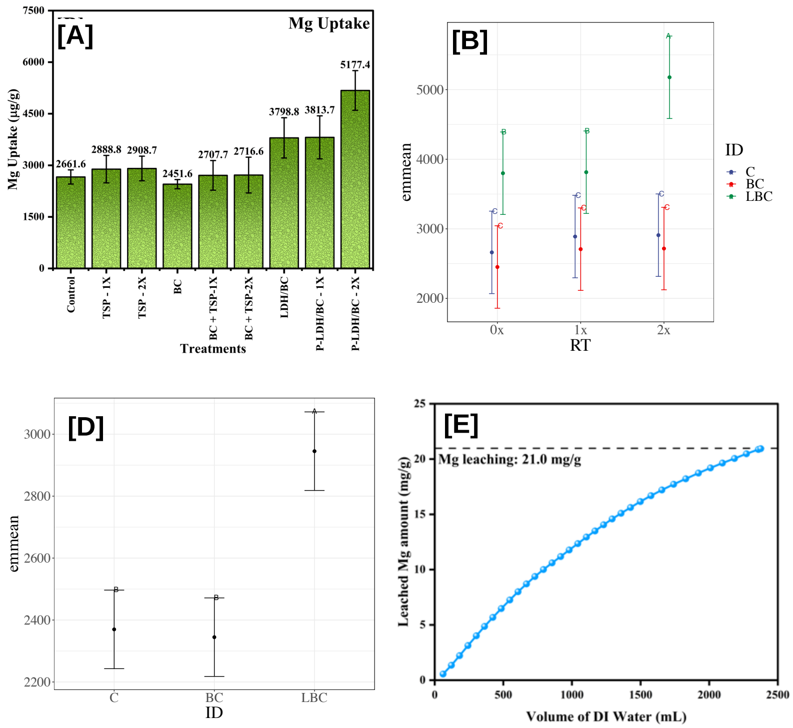

Fig 3: a) Plant magnesium uptake. b) Linear mixed model effect plot for application rate by the three treatments (in blue, red, and green). c) Bean magnesium uptake. D) Linear mixed model effect plot for magnesium uptake per treatment. e) Column study result for magnesium leachate.

A different trend was observed for magnesium uptake as opposed to phosphorous. Magnesium uptake varied across the treatments, with P-LDH/BC having a higher rates of magnesium uptake while uptake was lower and similar for both Biochar and the industry standard. A contrasting result for magnesium could be due to the nature of the P-LDH/BC structure, as it is a double hydroxide of magnesium and iron, some of which was shown to be leaching into the soil in a column study experiment conducted on the material. Another factor would be due to better uptake capacity of the plants induced by microbial activity in rhizosphere and roots, a hypothesis that would require an in-depth look at the MiSeq microbial data to determine the functional groups of microbes present in the soil and if they could be driving higher uptake with enzyme activity. Higher amounts of magnesium being available in the soil from P-LDH/BC should increase microbial activity in the soil, as magnesium is often considered an important co-factor for enzyme activity.

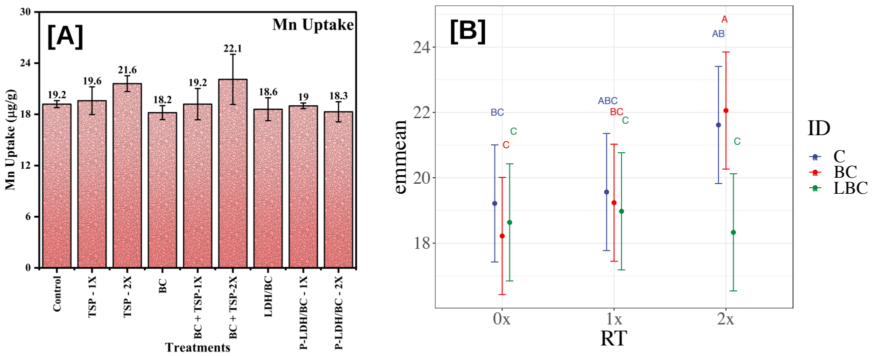

Fig 3: a) Plant manganese uptake. b) Linear mixed model effect plot showing manganese uptake per treatment rate by the three treatments (in blue, red, and green).

An interesting trend was also observed for manganese uptake: at 2x rate the uptake for P-LDH/BC was lower than biochar and the industry standard, which showed a similar uptake. Manganese uptake has previously been shown to be lower with higher magnesium availability. This could be consistent with the results discussed above, because the leachate study demonstrated additional magnesium available in the soil in the LDH/BC treatment. It has also been recently established in tomatoes and rice that higher fungal associations in the root reduces manganese uptake. This would require a closer look and a targeted analysis of the MiSeq data for the fungal communities present and their community structure.

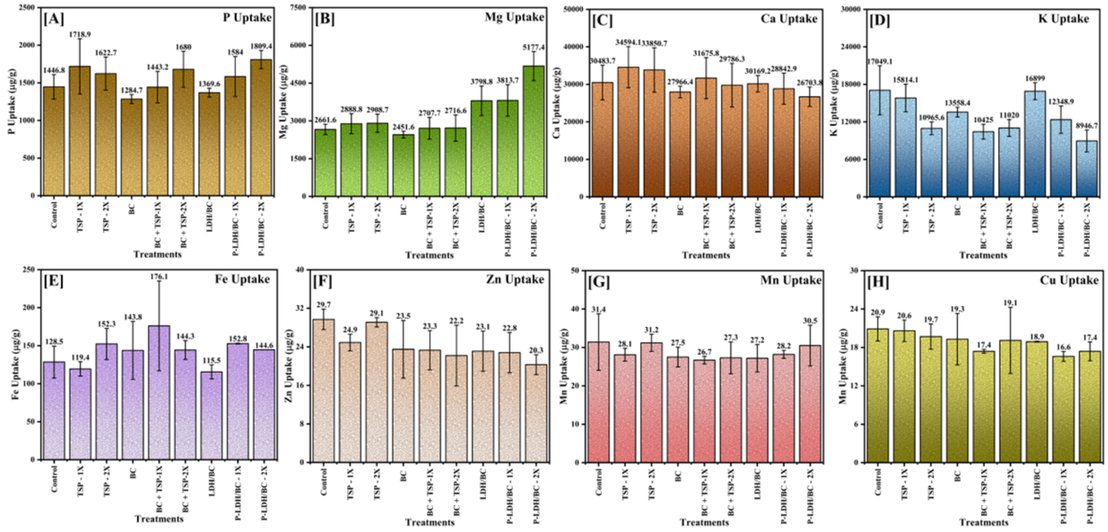

Fig 4: Comprehensive figure showing Macro and Micronutrient uptake in plant sample.

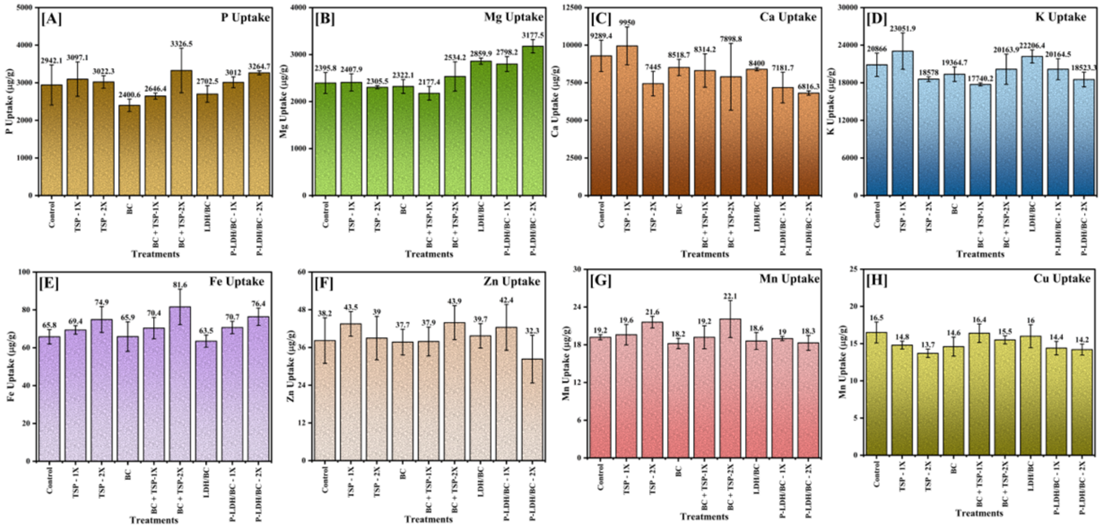

Fig 5: Comprehensive figure showing Macro and Micronutrient uptake in Bean sample.

Overall, P-LDH/BC was statistically shown to perform as well as standard industry fertilizer for all major macro and micro-nutrients tested, except magnesium and manganese which were discussed earlier. The results help to establish that using P-LDH/BC as an eco-friendly alternative fertilizer had no negative impacts on the nutrient uptake and content in green beans. The differences observed in magnesium showed an improved uptake, directly translating into a better bean quality for economic yield. The reduction in manganese uptake is desirable as manganese is toxic to plants in higher rates (often seen in acidic soils), and it is not often a limiting nutrient in nature. P-LDH/BC could help reduce manganese uptake by green beans in acid soils.

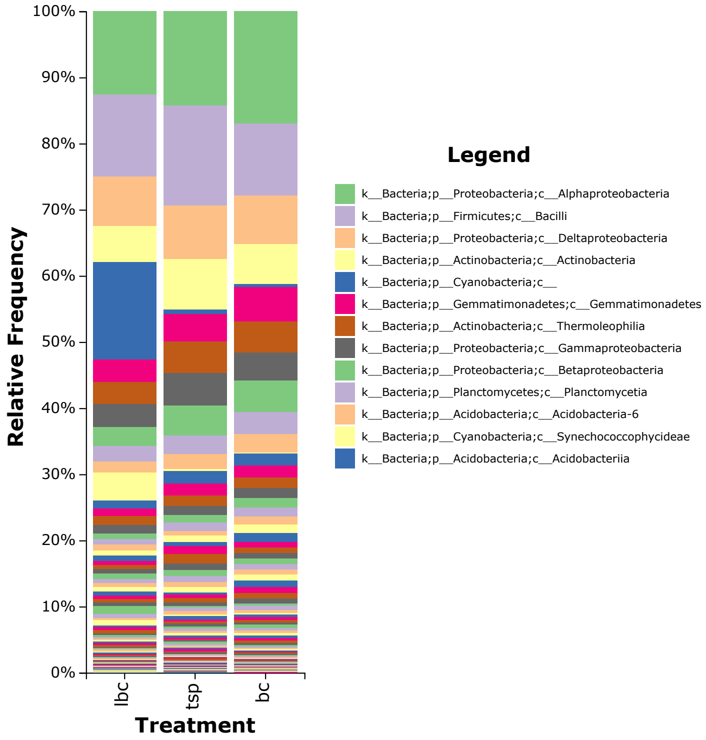

Fig. 6. Stacked barplot showing major bacterial groups (taxonomic classes, GreenGenes level 3) collapsed by treatment (x-axis). Bar thickness is proportional to relative frequency (y-axis). For instance, it can be seen that alphaproteobacterial were the most prevalent group overall, more so in bc (biochar).

Preliminary analyses have been executed for microbial diversity data. Fig. 6 shows a stacked bar plot identifying the most prevalent bacterial groups (taxonomic classes) per treatment. Overall makeup was similar across the treatment with some differences in prevalence. ANCOM analyses did not detect specific enrichment of any taxonomic group in any treatment or rate, suggesting similar overall taxonomic makeup across the experiment.

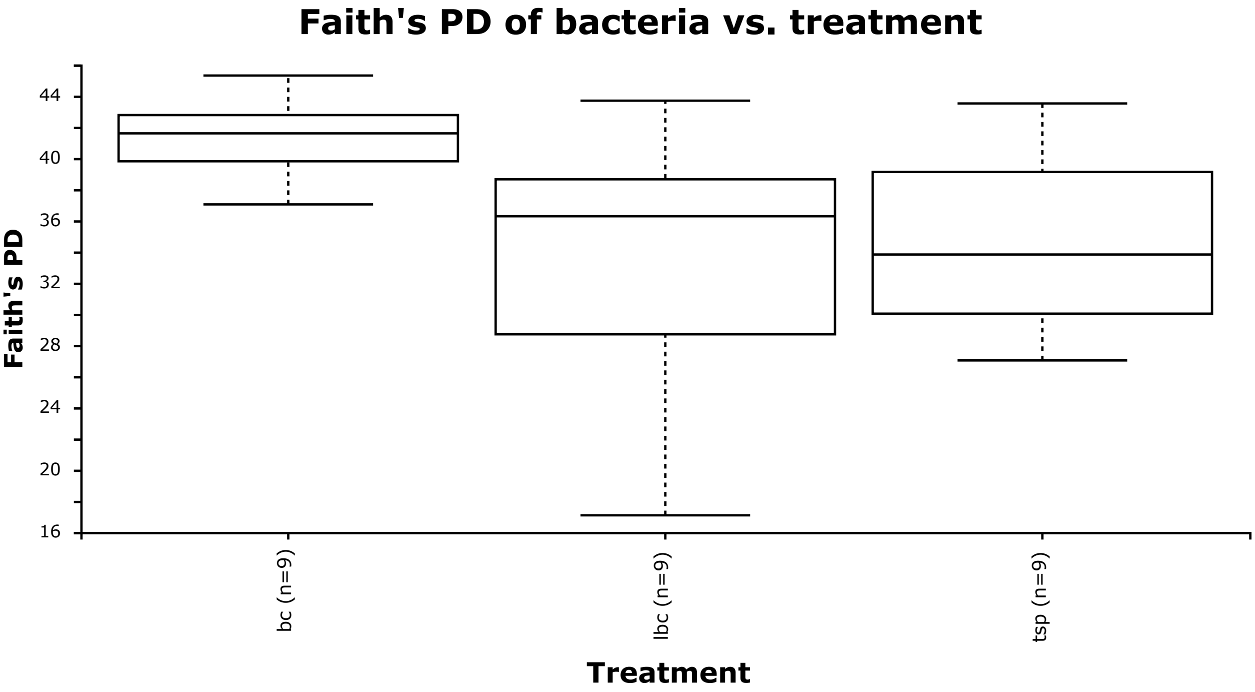

Fig. 7. Faith's PD (bacteria) per treatment. The groups were significantly different, with PD significantly higher in the biochar treatment.

Preliminary investigations of experimental effects on microbial diversity were also conducted. Both soil and nodule samples were sequenced, but nodules were similar across treatments, reflecting successful inoculation with rhizobia. Soil bacterial diversity was generally high across the samples, with the highest diversity seen in biochar (Fig. 7 shows Faith's PD, one of the statistics examined), and this difference was significant. This means that P-LDH/BC performs similarly to industrial standard fertilizer in terms of soil health, but a biochar difference is still biologically interesting. Several explanations, which will require further investigation, could be responsible for this. First of all, treatments were applied on a per-weight basis, meaning that the pure biochar treatment applied more organic material. Either the carbon itself or the increased soil surface area due to the material itself could promote bacterial community diversity. Second, ecological studies have often shown that nutrient amendments decrease biodiversity because nutrient-rich conditions tend to favor fewer, more aggressive species able to rapidly utilize resources. Regardless, these results suggest that P-LDH/BC may have a similar profile to industrial fertilizer in terms of soil health effects.

Future Outlook

The next step of the project would involve writing and publishing a manuscript to communicate the novel findings in nutrient uptake trends observed. This study is one of, if not the first to take an in-depth look at the use of P-LDH/BC complex as a phosphorous fertilizer complex. In the last decade, it has been shown effective for phosphorous remediation from polluted water sources, with only a suggestion of its potential use as a fertilizer, supported by short-term germination studies. This study further strengthens that claim by testing P-LDH/BC as a fertilizer and showing its performance as at-par if not better than standard industry fertilizer for plant growth and nutrient uptake & content.

Furthermore, the MiSeq data will be analyzed in-depth to investigate the effects on microbial diversity and community structure. It will be related with a new carbon and nitrogen analysis on the plant tissue, which is to be undertaken in the current academic year as part of a follow-up study. The carbon and nitrogen analysis will be an excellent ecological tool to understand metabolic efficiency of the plants, especially legumes like green beans, because carbon:nitrogen ratios are a standard measure of leaf economics and an index of forage/harvest quality. The findings of the carbon and nitrogen study along with the detailed analysis of the MiSeq data for microbial diversity and community structure will be communicated in a separate publishable manuscript, tentative prepared in the spring of next year.