DISSERTATION

CYANOBACTERIA BIOFERTILIZER SOLUBILIZES SOIL PHOSPHORUS AND ALTERS SOIL MICROBIAL COMMUNITIES

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ABSTRACT

CYANOBACTERIA BIOFERTILIZER SOLUBILIZES SOIL PHOSPHORUS AND ALTERS SOIL MICROBIAL COMMUNITIES

Cyanobacteria are oxygenic, photosynthetic bacteria that can also fix atmospheric nitrogen. Cyanobacterial fertilizer is currently being developed so that it can be produced on-farm without fossil fuel inputs to replace other N sources. Cyanobacterial fertilizer may also be beneficial to improving P availability if cyanobacteria are effective at solubilizing phosphorus. This dissertation consists of three sections which relate to the use of cyanobacteria as biofertilizer. In chapter 1, the objective was to discern P solubilization differences between the cyanobacteria *Anabaena* sp. and Mammoth P (MP) for potential use in conventional and organic production systems, using two different organic P sources (bone meal and rock phosphate). In chapter 2, the objective was to determine the effect of different organic fertilizers on the soil microbial community at two different soil depths within different cropping systems over a 2-yr period. Ultimately, the objective of the last study (chapter 3) was to compare the effect of different P and Fe concentrations on cyanobacterial growth and N fixation in order to better understand their interaction.

The objective of the first study was to evaluate P solubilization and activity of *Anabaena sp*. and Mammoth P using two different organic P sources (bone meal and rock phosphate) under laboratory conditions. Treatments were arranged in a full factorial design with three replications, three organic P sources [bone meal (BM), rock phosphate (RP) and control (No P added)], three P solubilizing microbial (PSM) treatments [control (No PSM), Cyanobacteria (Cyano), Mammoth

(MP)] and six sampling dates over 56 days. There were no significant differences observed in pH across treatments over the 56-day incubation ($p=0.05$), except in the No P control where the Cyano treatment reduced pH. The Cyano treatment had greater water-soluble P and Olsen-extractable P concentrations under the No P control and RP treatments compared with the No PSM control and MP treatments. In terms of Olsen P concentration, the MP treatment of the BM source was greater than the No PSM control and equal to the Cyano treatment. Results suggest that the Cyano treatment solubilized more P than MP, and thus may be an effective strategy for improving future soil P availability to plants, especially in light of future dwindling rock phosphate reserves. Organic fertilizer amendments are used primarily to increase nutrient availability to plants grown under organic or low synthetic input cropping systems, but fertilizer choice can affect soil microorganisms differently. The objective of the second study was to determine the effect of different organic fertilizers, including a non-traditional cyanobacteria-based fertilizer, on soil microbial communities of two different soil types and at two depths. Fertilizers were applied to the surface and sub-surface of certified organic cucumber and peach orchard plots, and soils were collected after harvest at two depths (0-2.5cm and 2.5-7.5cm). Soils were assayed for microbial ester-linked fatty acids (EL-FAMEs). Cyanobacteria and Neptune hydrolyzed fish emulsion fertilizers significantly impacted the soil community structure in the cucumber plots compared to control, blood meal, feather meal, and non-hydrolyzed fish emulsion fertilizers. Cyanobacteria and Neptune hydrolyzed fish emulsion fertilizers increased total microbial biomass and shifted the microbial community towards a greater relative abundance of Gram-negative bacteria, Gram-positive bacteria, and actinomycetes compared to other treatments. Within the peach orchard, soil microbial communities were differentially affected by fertilizer choice in the first year and at the deeper depth (2.5-7.5 cm). Specifically, plots receiving poultry manure supplemented with

cyanobacteria fertilizer had greater microbial biomass and relative abundances of fungi and arbuscular mycorrhizal fungi than plots receiving poultry manure alone. These specific shifts in biomass and community structure could be beneficial to organic vegetable and fruit production, as increased bacterial and actinomycete biomass could stimulate decomposition and nutrient turnover for vegetables, and increased fungal and AM fungal biomass could provide greater soil C storage, nutrient cycling, and symbiotic relationships with orchard trees. Furthermore, this study demonstrated that cyano-fertilizer could reduce grower reliance on commercial organic fertilizers or reduce application rates of manure when used as a supplemental fertilizer, and thus should be further explored and used by growers as a self-sufficient means to benefit soil microorganisms, plant production, and the environment.

The abundance of cyanobacteria may be limited by the availability of nutrients, such as iron (Fe) and phosphorus (P), both of which are required for nitrogenase synthesis, and P is also important in energy transfer. Iron is a very important compound that can bind proteins which affect the light response of cyanobacteria. Therefore, the objective of the third study was to evaluate effects of different P and Fe concentrations on *Anabaena sp.* cyanobacterial growth and N fixation. This study was conducted under laboratory conditions, the treatments were arranged in a Complete Randomized Design with two replications. In the first experiment, four P concentrations [Control ($7.6*10^{-4}$ M P), 5% ($3.8*10^{-5}$ M P), 25% ($1.9*10^{-4}$ M P), and 50% ($3.8*10^{-4}$ M P)] were evaluated for three weeks. Control was based on nutrient concentrations in Allen-Arnon media, and other treatments were percentages of the Allen-Arnon concentrations. In the second experiment, *Anabaena sp.* cyanobacteria were grown in media containing varying combinations of P and Fe concentrations [5% and 50%P with 100% Fe ($1.8*10^{-5}$ M Fe based on Allen-Arnon media), 5 and 50%P with 10% Fe ($1.8*10^{-6}$ M Fe), and 5 and 50%P with 1% Fe ($1.8*10^{-7}$ M Fe)]. The optical

density (OD) and Total Kjeldahl Nitrogen (TKN) concentrations were significantly lower in the 5% P treatment in the first experiment. In the second experiment, the OD and TKN were lowest in the treatment with the highest Fe concentration. In conclusion, the high P concentration (50-100%), and low Fe treatment exhibited the best growth for the cyanobacteria. Cyanobacterial fertilizer has been shown to solubilize soil P and to alter soil microbial communities, but care must be taken to optimize the nutrient media to maximize growth and N fixation.

In conclusion, based on this study, Anabaena sp. cyanobacteria have the capability to increase P availability in soil. The novel use of cyanobacteria, such as Anabaena sp., may have a significant role to play in tackling the future scarcity of plant-available P. Also, organic fertilizer decisions can influence soil microbial communities even in short periods of time (<1 yr). This study demonstrated that cyano-fertilizer could reduce grower reliance on commercial organic fertilizers or reduce application rates of manure or compost when used as a supplemental fertilizer. In addition, the high P concentration (50-100%) resulted in the best growth of the cyanobacteria in AA media under laboratory conditions. In addition, high Fe levels reduced OD and TKN. Therefore, using lower concentrations of P and Fe in the AA medium will be a great benefit to the growers who are growing cyanobacteria on-site by reducing cost when they use lower concentrations of P and Fe in the AA medium.

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DEDICATION

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Overview

As the global population has grown rapidly, the need for a more sustainable approach to food production has become more obvious. Organic agriculture has expanded to meet the growing demand for healthy and safe food production globally as well as ensuring long-term sustainability and addressing environmental problems connected with the use of agrochemicals such as soil erosion, water logging, the buildup of salts and toxic materials, and loss of nutrients and organic residues. Therefore, biological fertilizers have become alternatives to chemical fertilizers to meet the nutrient demands of crops (Mahdi et al., 2010). The agricultural revolution has focused on improving soil fertility through best management practices, yet the development of new inputs seems to depend on the optimization of (micro)biological processes (Canellas and Olivares, 2014) for enhancing nutrient availability, including N and P.

Nitrogen (N) is often the most limiting nutrient in agriculture and the largest and most costly input for crop production (Alvarez et al., 2021). Until now, there are limited N fertilization options for organic farmers. Organic fertilizers such as fish emulsion, manure, and compost are the primary sources of N fertilizer for organic or low input cropping systems, but they can be expensive and difficult to transport (Yoder and Davis, 2020; Sterle et al., 2021). However, there is another fertilizer option being developed that allows growers to produce N on-farm, in the form of living cyanobacteria. Cyanobacterial fertilizer, or cyano-fertilizer, can be a good source of N for organic growers due to its low residual NO_3-N compared to fish emulsion, its effectiveness compared to other organic fertilizers (Yoder and Davis, 2020), and its ability to be produced on-farm (Barminski et al., 2016; Wolde et al., 202

Cyanobacteria are phototrophic, N-fixing bacteria found in nearly every habitat on earth (Abed et al., 2009). Cyanobacteria can play a major role in agriculture by contributing to different functions such as inoculants for reinforcing soil fertility as well as enhancing soil structure in addition to improving crop yield because they perform oxygenic photosynthesis, and they have the unique ability to fix N from the atmosphere while also solubilizing P and increasing the P content in plant (Asmamaw et al., 2019; Afkairin et al., 2021). The application of cyanobacterial biofertilizers has been shown to reduce N_2O and NH_3 emissions as compared to solid organic fertilizers, such as blood and feather meals (Toonsiri et al., 2016; Erwiha et al., 2020). This result can also lead to improved soil fertility and plant growth because of increased organic matter content and enzymatic activities (dehydrogenase and nitrogenase) (Dhuldhaj and Pandya, 2017; Kumar et al., 2015). In addition, cyanobacteria produce phytohormones (Wenz et al., 2019), and auxin additions in fertilizers have been shown to be positively correlated with β-carotene concentration in lettuce (Sukor et al., 2021)

Soil P availability tends to be low in most soils due to the majority of soil P being sorbed or sequestered in various soil phases. To achieve long-term sustainability as global rock phosphate supplies are depleted, it will be essential to either increase P fertilizer use efficiency or enhance the dissolution of already present soil-borne P mineral phases. Micro-organisms may play a critical role in enhancing P availability through solubilization and improvement of soil-plant P relationships.

Microbial community fatty acid profiling can provide a good understanding of how microbial biomass, community composition, and potentially microbial activity and processes are affected by fertilization practices or soil depth. A better understanding of microbial community responses to diverse organic fertilizers is needed, as studies are scarce and often focused on manures. Very little

is known about how organic fertilizers, such as fish emulsion and new fertilizers, including cyano-fertilizer, affect soil microbial communities. In addition, because organic fertilizers are sometimes applied at or below the soil surface, organic fertilizers may have differential effects on soil microbial communities depending on soil depth.

Although it is clear that cyanobacteria require both P and Fe for optimal growth and N fixation, and both are required for the synthesis of nitrogenase. what remains unclear is the interaction of P and Fe in achieving optimum cyanobacterial conditions. The high P concentration with low Fe concentration exhibit the best growth for the cyanobacteria or the opposite. this point must be taken into consideration to optimize the nutrient media to maximize growth and N fixation.

This dissertation consists of three studies which relate to the use of cyanobacteria as biofertilizer. In chapter 1, the objective was to discern P solubilization differences between the cyanobacteria *Anabaena* sp. and Mammoth P (MP) for potential use in conventional and organic production systems, using two different organic P sources (bone meal and rock phosphate). In chapter 2, the objective was to determine the effect of different organic fertilizers on the soil microbial community at two different soil depths within different cropping systems over a 2-yr period. The objective of the last study (chapter 3) was to compare the effect of different P and Fe concentrations on cyanobacterial growth and N fixation in order to better understand their interaction. My hypotheses are as follows:

1- Cyanobacteria will increase P availability in soil as compared to MP or a control.
2- Cyano-fertilizer will significantly affect the biomass and structure of soil microbial communities in both the cucumber plots and peach orchard.

3- Cyano-fertilizer and Neptune hydrolyzed fish emulsion fertilizer will significantly impact the soil community structure and biomass in the organic vegetable system.

4- The high P concentration and low Fe treatment will exhibit the best growth for the cyanobacteria.

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Chapter 1. SOLUBILIZATION OF ORGANIC PHOSPHORUS SOURCES BY CYANOBACTERIA AND A COMMERCIALLY AVAILABLE BACTERIAL CONSORTIUM.[1]

1.1 Introduction

Agricultural cropping systems depend on phosphorus (P) fertilization provided by organic or inorganic sources to sustain crop yields. Some P applied to crops is lost from the plant-soil system through crop removal, erosion, runoff, and leaching. As a result, agricultural scientists have focused on enhancing P fertilizer use efficiency (Saeid, 2018). The agricultural revolution has focused on improving soil fertility through best management practices, yet the development of new inputs seems to depend on the optimization of (micro)biological processes (Canellas and Olivares, 2014) for enhancing nutrients, including N and P.

After N, P availability is required in the largest quantity for plant growth and yield, and plays a key role in how soil microorganisms store and utilize energy. However, obtaining P from soils for plant or micro-bial growth can be challenging. Soils contain on average ~ 400–1000 mg kg^{-1} total P, found both in mineral and organic forms, yet the portion available for plant uptake is approximately 1–2.5% of total P (Adnan et al., 2017). Between 20 and 80% of soil organic P is inaccessible due to fixation or adsorption to clay soil particles (Abdi et al., 2014; Halajnia et al., 2009). In addition, mineral phase precipitation reactions with Ca and Mg in alkaline soils, and Fe

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and Al in acidic soils, force P into relatively inaccessible inorganic pools. As a result, available soil P concentrations rarely exceed 0.1 mg kg^{-1} (Adnan et al., 2017). Because of this, producers rely on P fertilizers produced from mined rock phosphate to produce optimum crop growth conditions.

Phosphorus fertilizer use has supported crop yields to help feed many people. However, there are concerns regarding dwindling global P supplies associated with reaching peak P mining reserves in the near future (Cordell and White, 2011, 2014). As the main P fertilizer source, humans are reliant on the use of rock phosphate, a non-renewable resource used to mobilize P more rapidly in the environment than the natural P cycle (e.g. lithosphere-hydrosphere-biosphere cycling; Cordell and White, 2011). This conundrum of reliance on mined rock P has stimulated research to investigate how ecosystems can be anthropogenically manipulated to enhance existing soil-P dissolution and avail- ability. In particular, scientists are aware that some microorganisms can convert insoluble P into soluble forms, through phosphate solubilization via enzymatic reactions, thus increasing P availability to plants (Khan et al., 2007). Thus, it may be theorized that utilizing these P-solubilizing microorganisms in agroecosystems may play a vital role in increasing soil P availability.

Phosphate solubilizing microorganisms (PSMs) play an important role in achieving improved use of P resources and facilitating biogeochemical P cycling in natural and agricultural ecosystems. PSMs aid the soil biome by converting insoluble P to soluble forms through acidification, chelation, exchange reactions, enzymatic cleavage reactions, and polymeric substance formation (Delvasto et al., 2006). Using PSMs as biofertilizers to facilitate the solubilization of a wide range

of P com- pounds would not only reduce dependence on a limited resource, but may also improve crop yield (Nautiyal et al., 2000).

A specific PSM, cyanobacteria, may be used as a biofertilizer because they perform oxygenic photosynthesis and have the ability to fix atmospheric N (Chamizo et al., 2018; Cameron and Julian, 1988). Within the context of P, cyanobacteria have also been shown to solubilize less soluble P forms such as calcium phosphate [Ca3(PO4)2], ferric phosphate (FePO4), aluminum phosphate (AlPO4) and hydroxyapatite [(Ca5(PO4)3. OH)] in soil, sediments, or in pure culture, thus increasing P availability to plants (Yandigeri et al., 2011).

Another specific PSM, Mammoth PTM, a relatively new microbial biostimulant developed by research scientists at Colorado State University, combines *Citrobacter freundii*, *Enterobacter cloacae*, *Pseudomonas putida*, and *Comamonas testosterone* bacteria. It has been reported that Mammoth P (MP) has the ability to solubilize P faster than any single bacterial strain alone (Baas et al., 2016), with other studies reporting that MP increased *Cannabis sativa* yield by 16.5%, as well as led to increased plant height and basal stem area (Conant et al., 2017). Thus, PSM (either cyanobacteria or MP) may be a novel means by which producers can improve soil P availability.

Soil P availability tends to be low in most soils due to the majority of soil P being sorbed or sequestered in various soil phases. To achieve long-term sustainability as global rock phosphate supplies are depleted, it will be essential to either increase P fertilizer use efficiency or enhance Micro- organisms may play a critical role in enhancing P availability through solubilization and improvement of soil-plant P relationships. Returning to the premise that cyanobacteria and MP can both increase P availability, our objective was to discern P solubilization differences between

the cyanobacteria *Anabaena* sp. and MP for potential use in conventional and organic production systems, using two different organic P sources (bone meal and rock phosphate).

1.2. Materials and methods

1.2.1 Soil collection and analysis

Soil was collected from the Agricultural Research, Development and Education Center (ARDEC), Colorado State University (40°36′36.9″N 104°59′38.2″W). The upper 5 cm of soil was removed to avoid any surface accumulated P, and then soil was collected by hand from the 5–15 cm depth and placed in 19 L buckets. The soil, classified as a fine, smectitic, mesic Aridic Argiustoll of the Nunn series (NRCS, 1980), was transported to the laboratory and air-dried. The soil was ground to pass through a 2-mm sieve, after which soil properties were characterized (Table 1.1).

Soil texture (sandy clay loam) was determined using the hydrometer method (Western States Program, 1998). Plant available P was determined via the Olsen-P extraction (Olsen et al., 1954) and analyzed colorimetrically. Total P was determined after 4 M HNO_3 digestion (Bradford et al., 1975). Soil pH and electrical conductivity (EC) were determined by shaking soil with deionized water (1:1 w/w) for two hours prior to assessment (Thomas, 1996; Rhoades, 1996). Total carbon and nitrogen were analyzed using a LECO Tru-Spec CN analyzer (Leco Corp., Saint Joseph, MI, USA). Soil water holding capacity was deter- mined by make a plug of glass wool about 1 cm long and placing it in the bottom of a 1 cm diameter translucent plastic tube, weighing the tube with glass wool in it, then filling the tube with air dry soil to 1 cm from the top, and reweighing the tube. Then, water was added drop by drop until the wetting front reached the halfway point. After equilibrating overnight, the test tube was weighed again. The wet soil down to about 1 cm above

the wetting front was removed and placed in a soil moisture tin. The weight of the tin plus wet soil was recorded, and then the tin was placed in an oven at 105 °C overnight. The soil was then removed from the oven, cooled, and the weight of the tin plus oven dry soil was recorded. The water holding capacity was determined as: the dissolution of already present soil-borne P mineral phases.

1.2.2 Fertilizer analyses and P solubilizing organisms

The cyanobacteria *Anabaena* sp., which was originally cultured from sediment collected from Richard's Lake in Fort Collins, CO, USA, was used in the study. The culture was maintained in Allen Arnon medium in the laboratory (Allen and Arnon, 1955) (Barminski et al., 2016), and was used as a liquid cyanobacterial fertilizer (Cyano). Optical density (OD) was used to estimate bacterial growth, and OD_{750nm} of the culture was measured using a Hach DR3900 spectrophotometer (Hach Company, USA). Biomass was calculated using the following standard curve (Wenz et al., 2019):

$$\text{Biomass (mg L}^{-1}) = 981.5\ (OD_{750nm}) - 3.3245 \quad (2)$$

The *Anabaena* sp. culture was blue-green in color and had long, straight filaments with vegetative cells about 2–8 μm in diameter, oval heterocysts a bit larger than the vegetative cells, and a few akinetes. Mammoth$_{TM}$ P (MP), a product that contains a consortium of four bacterial taxa (described above) produced by Growcentia Inc. (Fort Collins, CO, USA) (Baas et al., 2016) was also evaluated. Both cyanobacteria and MP were analyzed for total macro and micronutrients via ICP-AES (Soltanpour et al., 1996) (Table 1.2).

1.2.3 Soil incubation procedure

Fifty g of air-dried soil was mixed with either BM or RP at the equivalent of 56 kg P ha^{-1}, plus a control (no P added). Within each treatment, a control (No PSB), Cyanobacteria (Cyano), or Mammoth P (MP) were applied at 8.8 mL. All mixtures were placed in 1 L mason jars, water content was adjusted to 60% water-filled pore space with distilled water and mixed well. Jars were incubated at 25 °C for 56 days, opening the jars for one hour once per week to maintain aerobic activity. At 0, 3, 7, 14, 28, and 56 d, soils were destructively analyzed for pH and Olsen P as previously described. Soils were also analyzed for water-soluble P by placing 2 g of soil into a 50 mL centrifuge tube. Twenty mL of distilled water (DI) were added to the tubes, the tubes were shaken for 1 h, centrifuged, and then the liquid was filtered through 0.45 μm membrane filters. A modified ascorbic-acid method was used to determine water soluble P content at 882 nm (Rodriguez et al., 1994).

1.2.4 Statistical analysis

Data were analyzed using R Version 3.5.3 and RStudio Version 1.0.153. (The R Foundation for Statistical Computing; Wickham et al., 2018). The experimental units were arranged in a full-factorial design with 9 (i.e., 3*3) treatments, six destructive sampling dates, and three replications. Analysis of variance (ANOVA) and pairwise comparisons of means were obtained from mean square errors to determine whether main effects or interactions were significant ($P < 0.05$). Tukey adjusted pairwise comparisons were used to illustrate differences between treatments (Lenth et al., 2019).

1.3 Results and discussion

1.3.1 Soil pH

In general, there was no change in soil pH during the 56-day incubation period between treatments (Fig. 1.1). Overall, these findings are similar to Natesan and Shanmugasundaram (1989) and Cameron and Julian (1988) who reported that cyanobacteria did not reduce pH yet increased P-solubilization (similar to our findings, discussed below). In addition, Yandigeri et al. (2011) reported no pH change in a medium containing soluble or insoluble phosphate sources. The lack of soil pH change in the current study is supported by Banet et al. (2020), who used the same soil as in the current study. The authors reported that the soil contained approximately 9% calcium carbonate, and thus would have a relatively large buffering capacity to resist change in pH. Opposite to the above findings, Roychoudhury and Kaushik (1989) found that a pH reduction occurred in a medium containing rock phosphate and cyanobacteria, apparently due to the liberation of organic acids. In addition, Vaishampayan et al. (2001) found that soil pH decreased with time after cyanobacteria application, as explained by the formation of organic acids as a result of decomposition of old cells. Singh (1961) mentioned that cyanobacteria addition neutralized the pH of Uttar Pradesh soil (i. e., an alkaline soil).

1.3.2 Water-soluble phosphorus

Water-soluble P concentrations, as a function of treatment and time, are shown in Fig. 2. In the control and RP treatments (Fig. 1.2A and B), water-soluble P was greater in the Cyano treatment from day zero to seven as compared to other treatments, after which no significant difference in water–soluble P was observed among treatments. In the BM treatment (Fig. 1.2 C), the Cyano had greater water-soluble P when compared to the No PSM control and MP treatments on day zero through day 14, after which no significant treatment effects were observed. Within the No P

Control, RP, and BM treatments, the MP treatment had essentially the same water-soluble P as the control.

The observed cyanobacteria P solubilization over time was similar to findings of Yandigeri et al. (2011) who used the same genus of cyano- bacteria as in the current study. Those authors found that P solubilization increased when cyanobacteria were in the presence of either tricalcium phosphate (TCP) or RP, likely due to the microbial release of phthalic acid. Phthalic acid has also been shown to reduce pH (Yandigeri et al., 2011), yet this did not occur in the current study potentially due to soil buffering capacity. Regardless, phthalic acid might be synthesized from the tricarboxylic acid cycle through the intermediate proto- catechuic acid. Roychoudhury and Kaushik (1989) reported similar findings when cyanobacteria were applied to TCP and RP. In the present study, the BM treatment with cyanobacteria had the highest water- soluble P concentrations among all treatments. This may have been due to BM being more soluble than RP, as suggested by Jeng et al. (2006). Similarly, Kahiluoto and Vestberg (1998) reported that plant P uptake from BM was greater than from an apatitic source, likely due to solubility differences. Nelson and Janke (2007) explained that the primary P mineral in BM is calcium-deficient hydroxyapatite, which is more soluble than RP. Although BM P solubility was increased in the presence of Cyano, it is important to note that there were essentially no significant differences in water-soluble P between MP and the control (Fig. 1.2).

1.3.3 Olsen extractable phosphorus

Olsen extractable P concentrations, as a function of treatment and time, are shown in Fig. 3. In the No P control on day 0, 14, and 56, the Cyano treatment had a greater Olsen extractable P concentration as compared to other treatments (Fig. 3A). In the RP treatment (Fig. 1.3B), Olsen P

was significantly greater (>3-fold higher) in the Cyano treatment compared to the No P control and MP on day zero, although after that timepoint Olsen P concentrations remained essentially the same regardless of treatment. In the BM treatment (Fig. 1.3C), both Cyano and MP treatments had greater Olsen P concentrations as compared to the No PSM Control up until day seven, and the Cyano treatment continued to contain greater Olsen P concentrations as compared to the Control through day 14; (Fig. 1.3C). On days 28 and 56 there were no significant differences among treatments.

In general, when significant differences were present, the Cyano treatment usually resulted in greater Olsen P concentrations compared to the No PSM control and MP treatment for all P sources (Fig. 1.3). Mandal et al. (1992) reported that cyanobacterial addition to soil resulted in an increase in Olsen P and a decrease in other P fractions (except for the Ca-bound P), due to the decomposition of cyanobacterial biomass. However, Adnan et al. (2017) found that Olsen P concentrations increased following addition of a consortium of phosphate solubilizing bacteria (*Pseudomonas*, *Pantoea*, *Mycobacterium*, *Bacillus*, *Rhizobia*, *Burkholderia*, *Arthrobacter* and *Enterobacter*) to organic and mineral P sources; this consortium included *Pseudomonas* and *Enterobacter* which were also included in the MP consortium. However, since MP did not result in observations similar to Adnan et al. (2017), perhaps their results were related to the other PSB not present in MP (e.g., *Pantoea, Mycobacterium*, *Bacillus*, *Rhizobia*, *Burkholderia*, and *Arthrobacter*).

In this study, cyanobacteria *Anabaena* sp. enhanced plant availability of soil P. Microbiologists and soil scientists have a responsibility to society for finding ways of making soil P more available to crops, in light of its status as a non-renewable resource. Other researchers have investi- gated

the ability of PSM, such as bacteria, fungi, actinomycetes, cyanobacteria, and mycorrhizae to solubilize insoluble phosphate soil mineral phases (Sharma et al., 2013). More importantly, biofertilizers are also renewable as compared to mined rock phosphate. Thus, cyanobacter may play a crucial future role in augmenting P supply to crops by increasing soil P availability through the exploitation of natural processes. Furthermore, in addition to P solubilization, biofertilizers may also play a role in biological nitrogen fixation, decomposition of organic wastes, etc. Biofertilizers may play multi-functional roles in improving crop productivity, as well as enhancing food security, in future agricultural production systems.

1.4. Conclusions

In conclusion, we successfully utilized cyanobacteria *Anabaena* sp. to increase P availability in soil. Our hypothesis regarding Cyano treatment effects on water-soluble and Olsen P concentrations as compared to MP or a control was supported by the results of this study. The novel use of cyanobacteria, such as *Anabaena* sp., may have a significant role to play in tackling the future scarcity of plant available P. An evaluation of the potential to scale-up cyanobacteria application under field applications is warranted to evaluate its large-scale impact on P solubilization and plant uptake.

Tables

Table 1.1. Physical and chemical properties of soil used in the organic P solubilization study.

Parameter	Value
Sand (%)	54.3
Silt (%)	30.7
Clay (%)	15.0
Texture	Sandy clay loam
pH	8.09
EC (ds m^{-1})	2.95
Total C (%)	1.92
Total N (%)	0.10
Total P (mg kg^{-1})	446
Olsen P (mg kg^{-1})	2.01
Water holding capacity (%)	17.5

Table 1.2. Cyanobacteria and a commercially available bacterial consortium (Mammoth P) total nutrient concentration.

Element	Cyanobacteria	Mammoth P
	----------mg L^{-1}----------	
Al	0.291	0.196
B	0.416	0.444
Ba	0.225	0.287
Ca	12.49	114.07
Cd	0.001	0.004
Co	0.001	0.001
Cu	<0.005	<0.005
Fe	2.657	0.230
K	146.9	212.1
Mg	14.33	60.97
Mn	0.292	0.32
Mo	0.049	0.012
Na	126.3	22.71
Ni	<0.015	0.003
P	<0.500	<0.500
Pb	0.011	0.015
S	0.019	0.145
Se	0.029	0.003
Si	2.750	27.53
V	0.001	0.005
Zn	1.450	0.893

Figures

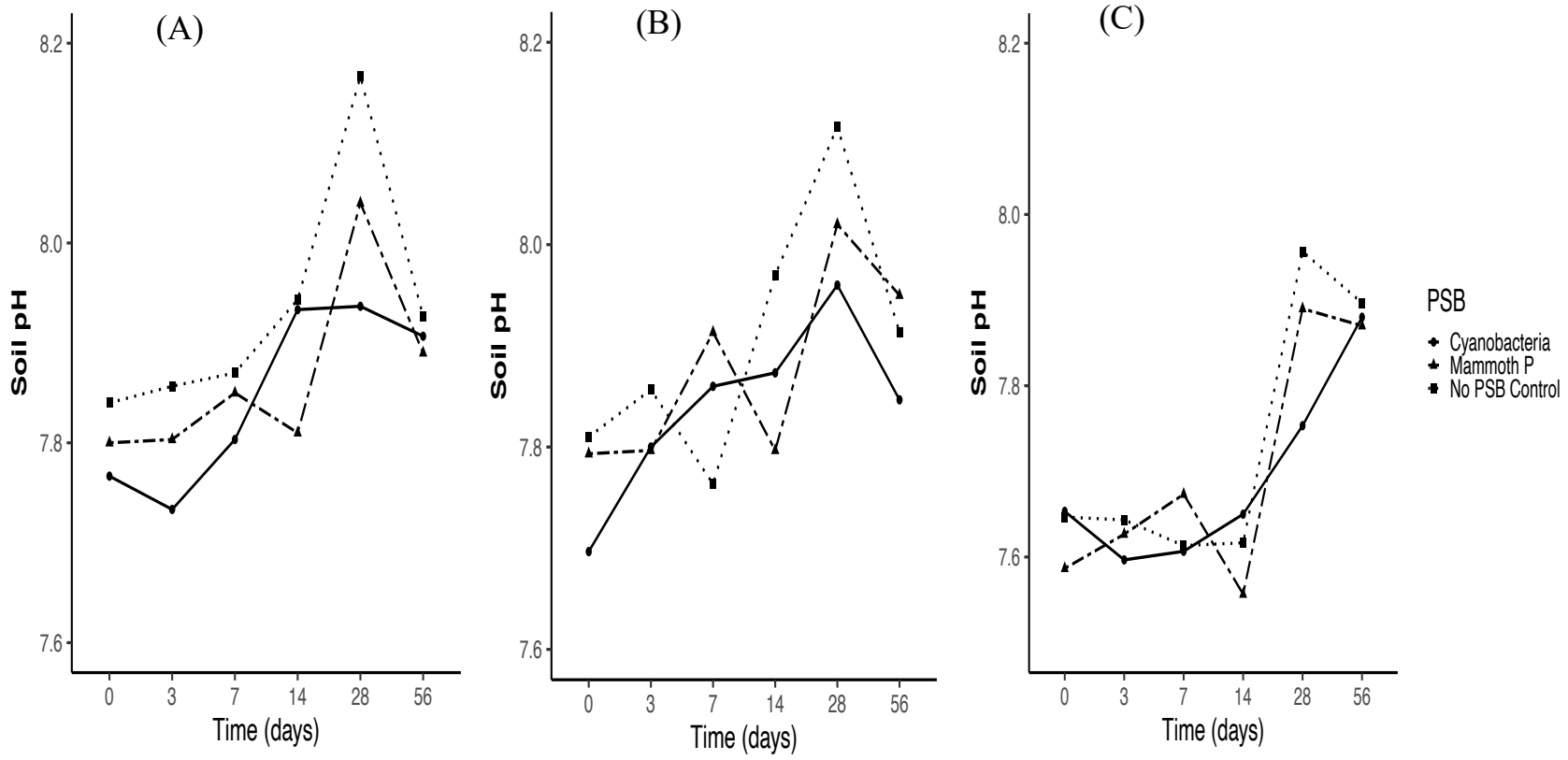

Fig. 1.1. Soil pH during incubation experiment in (A) No P control, (B) rock P, and (C) bone meal. In (A), (B), or (C), there were no significant soil pH differences between cyanobacteria, Mammoth P, and the no PSB control ($p>0.05$) based on Tukey adjusted pairwise comparisons. Thus, statistical analyses are not shown.

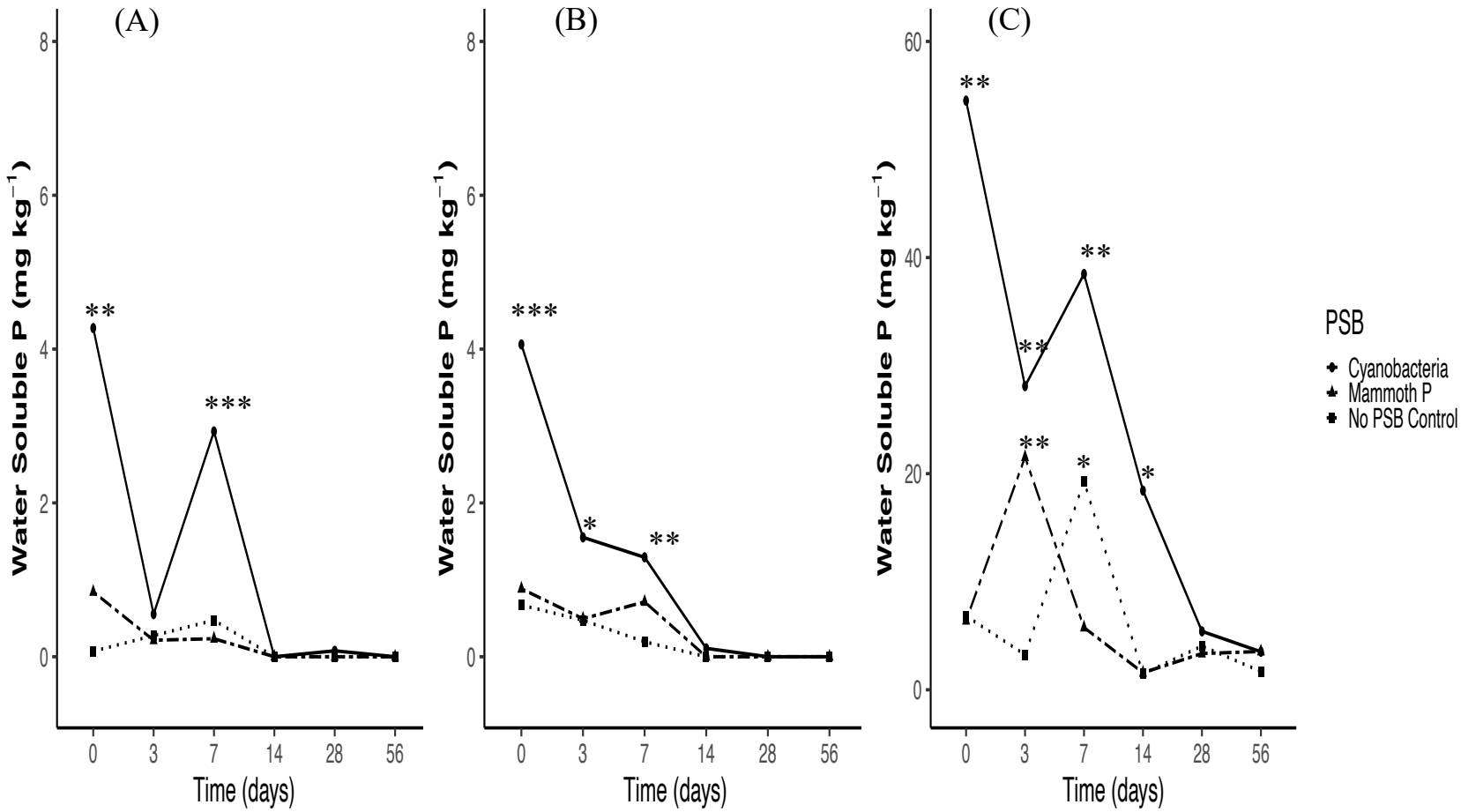

Fig. 1.2. Water-Soluble P as a function of time in (A) No P control, (B) rock P, and (C) bone meal treatments during the 56-day incubation experiment. Statistically significant differences from control are indicated by ** $P < 0.01$, *** $P < 0.001$ or ns $P > 0.05$.

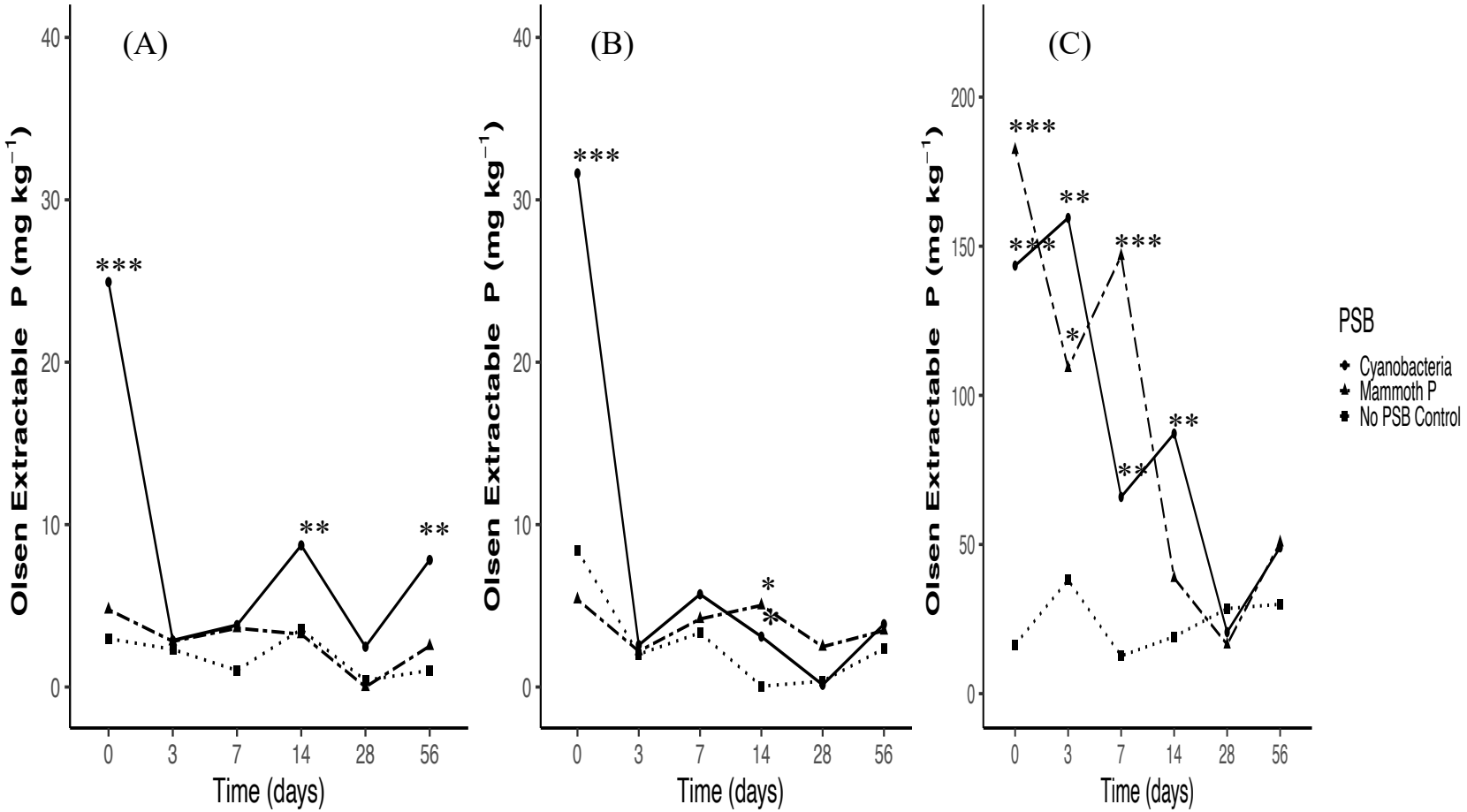

Fig. 1.3. Olsen Extractable P as a function of time in (A) No P control, (B) rock P, and (C) bone meal treatments during the 56-day incubation experiment. Statistically significant differences from control are indicated by ** $P < 0.01$, *** $P < 0.001$ or ns $P > 0.05$.

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Chapter 2. SOIL MICROBIAL COMMUNITY RESPONSES TO CYANOBACTERIA VERSUS TRADITIONAL ORGANIC FERTILIZERS

2.1. Introduction

Fertilization is a worldwide management practice used to improve nutrient availability to plants. Organic and inorganic fertilizer applications to soil can also alter structure and biomass, enhance soil microbial activity, alter the microbial community structure, and ultimately improve soil health (Marschner et al., 2003; Tian et al., 2017; Zhen et al., 2014). Many studies have shown that soil microbial biomass and enzyme activities increase when organic fertilizers are applied to soil (Crecchio et al., 2001; Ebhin Masto et al., 2006; Kaur et al., 2005; Parham et al., 2002; Peacock et al.,2001) Studies in grassland plots have determined that the soil microbial community had been affected by the applications of manure and/or inorganic fertilizers over a long period of time (nearly 100 years) (Kidd et al., 2017). Commonly, organic fertilizers have a great influence on soil microbial communities. For instance, in an 8-month field trial, manure applications increased microbial biomass carbon (C), soil respiration, enzyme activities, and N mineralization (Zhang et al., 2018). Microbial changes may be due to the addition of organic C to the soil in manure form, which benefits saprophytic soil microorganisms, in addition to providing nutrients such as N, P, and K (Zhang et al., 2018). However, in short-term (less than 1 year) fertilizer experiments, others found no significant effects on soil microbial communities despite the existence of fertilizer inputs (Crecchio et al., 2001; Franco-Otero et al., 2011; Marschner et al., 2003).

Organic fertilizers such as fish emulsion, manure, and compost are the primary sources of N fertilizer for organic or low input cropping systems, but they can be expensive and difficult to

transport (Yoder and Davis, 2020). However, there is another fertilizer option being developed that allows growers to produce N on-farm, in the form of living cyanobacteria. Cyanobacterial fertilizer, or cyano-fertilizer, can be a good source of N for organic growers due to its low residual NO_3-N compared to fish emulsion, its effectiveness compared to other organic fertilizers (Yoder and Davis, 2020), and its ability to be produced on-farm (Barminski et al., 2016; Wolde et al., 2020).

Cyanobacteria are prokaryotic oxygenic phototrophs found in nearly every habitat on earth (Abed et al., 2009). Cyanobacteria play a major role in agriculture by contributing to different functions such as inoculants for reinforcing soil fertility as well as enhancing soil structure in addition to improving crop yield because they perform oxygenic photosynthesis, and they have the unique ability to fix N from the atmosphere while also solubilizing P and increasing P content in plant (Afkairin et al., 2021; Asmamaw et al., 2019). The application of cyanobacterial biofertilizers has been shown to reduce N_2O and NH_3 emissions as compared to solid organic fertilizers, such as blood and feather meals (Erwiha et al., 2020; Toonsiri et al., 2016) . This result led to improved soil fertility and plant growth because of the increased organic matter content and enzymatic activities (dehydrogenase and nitrogenase) (Dhuldhaj and Pandya, 2017; Kumar et al., 2015). In addition, cyanobacteria produce phytohormones (Wenz et al., 2019), and auxin additions in fertilizers have been shown to be positively correlated with β-carotene concentration in lettuce (Sukor et al., 2021; Holik and Vranová, 2019).

Fatty acid methyl ester (FAME) profiling is an effective method to characterize microbial community biomass and composition, including bacterial and fungal components, and to assess rapid changes in microbial communities in response to soil amendments (Schutter and Dick, 2000). There are two methods to measure the FAMEs – Phospholipid Fatty Acid Analysis (PLFA) and

Ester-Linked Fatty Acid Methyl Ester (EL-FAME), and both provide information on microbial community and biomass composition in soil and in relation to soil management (Li et al., 2020). Direct extraction of microbial fatty acids applied in the EL-FAME method provides a relatively simple and rapid analysis, relative to the PLFA method, and has the ability to distinguish microbial communities that are different in biomass and structure among different environments, soil types, and management practices (Schutter and Dick, 2000; Stromberger et al., 2007). Since fatty acids and phospholipids are degraded rapidly by endogenous and exogenous phospholipases upon cell death, they are reliable measures of viable cell biomass (Balkwill et al., 1988; Peacock et al., 2001). Additionally, fatty acids and phospholipids can be used to identify broad physiological groups of bacteria (Guckert et al., 1991; Peacock et al., 2001; Vestal and White, 1989). These methods have detected differences in microbial community composition among rhizospheres of different crop species, cultivars, and soil type (Schutter et al., 2001; Steger et al., 2003). It has been demonstrated that when the structure of the microbial community is more stable, there are important effects on the rates of denitrification, nitrification, and N fixation (Hsu and Buckley, 2008). Also, the structure of the community and soil microbial biomass are good indicators of soil health and quality, and cropland management practices can change those parameters due to their sensitivity (Bending et al., 2002).

Microbial community fatty acid profiling can provide a good understanding of how microbial biomass, community composition, and potentially their activity and processes are affected by fertilization practices or soil depth, although very few studies have been conducted to date. For example, Peacock et al. (2001) used the PLFA method to determine the effect of inorganic N fertilizer and dairy manure application on microbial communities and biomass composition. They found that manured surface soils were enriched with bacterial mono-unsaturated (Gram-negative)

and eukaryotic markers. Microbial communities also differ in composition and biomass as a function of depth within a soil type (Amador and Atoyan, 2012). For example, Gram-negative bacteria, fungi, and protozoa were less abundant at higher depths, while Gram-positive bacteria and actinomycetes were more abundant with depth (Fierer et al., 2003). A better understanding of microbial community responses to diverse organic fertilizers is needed, as studies are scarce and often focused on manures. Very little is known about how organic fertilizers, such as fish emulsion and new fertilizers, including cyano-fertilizer, affect soil microbial communities. In addition, because organic fertilizers are sometimes applied at or below the soil surface, organic fertilizers may have differential effects on soil microbial communities depending on soil depth. Therefore, the aim of this study was to determine the effect of different organic fertilizers on the soil microbial community at two different soil depths within different cropping systems over a 2-yr period.

2.2 Materials and Methods

2.2.1. Experimental Design and Soil Sampling

Soil samples were collected from a certified organic cucumber field and a certified organic peach orchard, both in Colorado. The first location (L1) was at the Colorado State University Horticultural Research Center (4300 E County Road 50, 80524) in Fort Collins, Colorado, USA. The second location (L2) was at Ela Family Farms in Hotchkiss, Colorado, USA. Each location represented a unique experiment with different treatments, and both experiments used a randomized complete block design (RCBD).

The first experiment was in a cucumber plot previously described by Wickham (2016), with a soil classified as fine, smectitic, mesic, Aridic Argiustoll of the Nunn series (USDA NRCS, 1980). Prior to 2015, plots at this location were planted to buckwheat cover crop and received an annual

application of manure which was broadcast and then tilled in, following certified organic practices. The fertilizer treatments applied in 2015 were as follows: unfertilized Control, Blood meal 12-0-0 (Down to Earth, Inc., Eugene, OR) surface applied or sub-surface applied, Feather meal 12-0-0 (Down to Earth, Inc., Eugene, OR) surface applied or sub-surface applied, hydrolyzed fish emulsion 2-4-1 (Neptune's Harvest, Gloucester, MA), Alaska$_{TM}$ non-hydrolyzed fish emulsion 5-1-1 (Planet Natural, Bozeman, MT), and cyano-fertilizer. The cyano-fertilizer (*Anabaena cylindrica*) was originally cultured from sediment collected from Richard's Lake in Fort Collins, CO, USA (Wolde et al., 2020). It was grown on-farm using methods described by Barminski et al., (2016) and had an average 23.3 ppm (23.3 mg N/kg) or <1% N by weight (Total Kjeldahl N). This study included eight treatments replicated four times in a randomized complete block. This included a control and three types of fertilizer applications applied at 76 kg N ha^{-1}: solid fertilizers (blood meal, feather meal) surface banded near the drip irrigation line, liquid fertilizers (hydrolyzed fish emulsion, non-hydrolyzed fish emulsion, and cyano-fertilizer) applied through the drip irrigation system, and solid fertilizers (blood meal, feather meal) sub-surface banded 5 cm deep near the drip irrigation line.

The second experiment took place in a peach orchard previously described by Sterle et al, (2021) with a soil type of Mesa loam which is classified as a mesic Typic Calciargid (Natural Resources Conservation Service, 2008). The following fertilizer treatments were applied for two years during 2014-2015: 100 kg N ha^{-1} of Richlawn$_{TM}$ 5-3-2 dried poultry manure (HM) (Platteville, CO), 100 kg N ha^{-1}of dried poultry manure + 25 kg N ha$^{-1\ of}$ cyano-fertilizer (HM+C), and 75 kg N ha^{-1} of dried poultry manure + 25 kg N ha^{-1} as cyano-fertilizer (LM+C). Poultry manure was applied to the plots by broadcasting within the tree rows but not in the alleys between the trees. Manure was not incorporated. The cyano-fertilizer was applied separately from manure, in liquid form through

the micro-sprinkler irrigation system. These treatments were replicated five times in a randomized complete block design.

After harvest of either cucumbers (August 2015) or peaches (August 2014 and 2015), soil samples were collected from each location by compositing ten 0-2.5cm deep cores and ten 2.5-7.5cm deep cores from within each plot; samples were not taken within 30 cm of each plot border to minimize edge effects. After sampling, soil samples were stored in coolers and transported to the laboratory, homogenized by hand, placed into 50 ml sterile polypropylene centrifuge tubes, and stored at -80 ^{o} C until further use.

2.2.2. Ester-Linked Fatty Acid Methyl Ester (EL-FAME) Analysis

Prior to EL-FAME extraction, soil moisture was determined gravimetrically. Ten g of soil were weighed, dried in an oven at 105^{o} C for 24 hours, and weighed again. Community structure was determined from frozen soil samples by EL-FAME (Schutter and Dick, 2000). The whole-cell fatty acids including phospholipids, glycolipids, and neutral lipids were extracted from the soil. Each soil sample was placed into a 50-ml glass centrifuge tube, and Reagent 1 (0.2 N KOH prepared in methanol) was added to each tube. Then, samples were extracted for one hour in a 37^{o}C water bath, during which time, samples were vortexed for 10 seconds every 10 minutes. After that, Reagent 2 (1.0 M acetic acid) was added to neutralize the pH. Then Reagent 3 (10 mL hexane) was added to each tube to extract fatty acids into the organic phase (hexane) and vortexed for 20 seconds. Then all tubs were centrifuged at 480 x g (Sorvall HS-4 swinging bucket motor) for 20 minutes at 4^{o} C to separate the hexane and aqueous phases. By using a Pasteur pipet, two-thirds of clean hexane supernatant was removed from each tube and transferred into new glass tubes (17 x 100 mm). Samples were prepared for gas chromatography (GC) by evaporating the hexane solvent under N_2 gas. The dried EL-FAME residue was then re-dissolved by adding100 μL of hexane

containing 0.10 μg μL^{-1} nonadecanoic acid methyl ester (C 19:0) as an internal standard. All samples were stored at –20° C prior to analysis.

EL-FAME extracts were analyzed by gas chromatography-mass spectrometry (GC-MS) with a Trace GC coupled to a Thermo TSQ8000 Evo mass spectrometer (Thermo Scientific), equipped with a Phenomenex ZB-5HT Inferno GC column (30m x 0.25mm x 0.25 um). The GC inlet was set at 285 °C, and the oven temperature was programmed at 60 °C for 2 mins, a ramp of 15 °C per min to 330 °C, and then held at 330 °C for 10 mins. Peaks were identified based on mass spectral and retention time matching to a 37 FAME mixture (Sigma) and a bacterial acid methyl ester mixture (Sigma). Additional FAMEs were detected based on mass spectral matching to the NIST Mass Spectral Library (v14, www.nist.gov) (Denef et al., 2009; Frostegard et al., 1993; Gomez et al., 2014). According to Schutter and Dick, (2000) and Stromberger et al, (2007), the EL-FAME biomarkers were attributed to the following microbial groups. Gram-positive bacteria (i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0), Gram-negative bacteria (16:1ω7c, 17:1ω7, 17:0cy, 18:1ω7c, 18:1ω8, and 19:0cy), actinomycetes (10Me16:0, 10Me17:0, and 10Me18:0), fungi (18:2ω6,9c), and arbuscular mycorrhizal (AM) fungi (16:1ω5c). Total bacterial biomass was determined from the sum of biomarkers of Gram-positive and Gram-negative bacteria, actinomycetes, as well as the general bacterial fatty acids 15:0 and 17:0. Total microbial biomass was the sum of all bacterial and fungal EL-FAMEs. Stress indicators were signified by the ratio of 17:0cy to its precursor 16:1ω7c (Stress 1), and by the ratio of 19:0 cy to its precursor 18:1ω7c (Stress 2) (Grogan DW, 1997).

2.2.3. Statistical analysis

Data were analyzed using R Version 3.5.3 and RStudio Version 1.0.153. (The R Foundation for Statistical Computing)(Wickham et al., 2018). We used a mixed model that considered treatment,

depth and their interaction as fixed effects, and block and block × treatment as random effects to account for the complete block design and the spatial correlation between soil samples taken at consecutive depths in the same plot. Multiple comparisons of soil microbial community groups across organic fertilizer treatments were significant ($P<0.05$), and Tukey adjustment pairwise comparisons were used to identify variables that differed significantly among treatments (Lenth et al., 2019). Soil microbial community EL-FAME data were analyzed by Principal Components Analysis (PCA), after normalizing the data from nmole g^{-1} soil using the PC-ORD statistical package (MjM Software, Gleneden Beach, OR, 1999). Communities were analyzed separately for each location, using the PC-ORD version 6 statistical package (McCune and Mefford, 2012).

2.3. Results

2.3.1. Microbial community structure

In the cucumber experiment, cyano-fertilizer and Neptune hydrolyzed fish emulsion resulted in a different soil microbial community structure compared to microbial communities of control soil and soil receiving other organic fertilizer treatments, with the PCA analysis explaining 54.6% of the variance (Fig. 2.1). Changes in microbial community structure were consistent between the two soil depths. Differences in community structure were largely attributed to fertilizer-induced shifts in several EL-FAMEs, as indicated by the PCA analysis. EL-FAMEs with negative eigenvector coefficients for PC 1, and therefore associated with communities from cyanobacteria or fish emulsion fertilized soil, included biomarkers for actinomycetes (10Me17:0 and 10Me18:0) and Gram-negative bacteria (17:1ω7, 17:0cy, 18:1ω8) (Table 2.1). Conversely, the EL-FAME with the highest positive eigenvector coefficient for PC 1 was 18:1ω9 (fungi). Differences in community structures in soil fertilized with cyanobacteria or fish emulsion versus communities in

all other soil treatments were statistically significant, according to multi-response blocked permutation procedure (MRBP) analysis conducted in PC-ORD.

In the peach orchard, soil microbial community structure was affected by the different fertilizers in 2014 but not in 2015. In 2014, microbial community structures in several plots receiving the LM+C treatment (75kg N ha^{-1} as dried poultry manure + 25kg N ha^{-1} as cyano-fertilizer) differed from the other microbial communities, with the PCA analysis explaining 67.4% of the variance along PC1 and 2 (Fig 2). EL-FAMEs associated with several LM+C communities included 16:1ω5 (AM fungi), 18:1ω9 (fungi), and i14:0 (Gram-positive bacteria), based on positive eigenvector coefficients for PC1 and/or PC2 (Table 2.2). Pairwise comparisons among the fertilizer treatments in 2014 by MRBP analysis in PC-ORD showed that the lower application rate of dried poultry manure with cyano-fertilizer treatment (LM+C) resulted in a soil microbial community structure significantly different from communities under the other fertilizer treatments.

2.3.2. Microbial community biomass

Total microbial EL-FAME biomass in the top layer of soil (0-2.5 cm depth) of the cucumber experiment ranged from 24 to 73 nmol EL-FAMEs g^{-1} soil (Table 3). In contrast, microbial EL-FAME biomass was lower in deeper soil layer (2.5-7.5 cm depth), ranging from 9 to 38 nmol g^{-1} soil, and there was no significant difference among treatments in the deeper depth. Within the 0-2.5 cm depth of soil, cyano-fertilizer and Neptune hydrolyzed fish emulsion were the only organic fertilizers that increased microbial EL-FAME biomass compared to the control. Total microbial, Gram-positive bacterial, and Gram-negative bacterial EL-FAME biomass were ~2.5×, ~2.4×, and 3-4× greater in surface soil receiving either cyano-fertilizer or Neptune hydrolyzed fish emulsion than in control soil (Table 2.3). Neptune hydrolyzed fish emulsion and cyano-fertilizer also

increased actinomycete EL-FAME biomass by an order of magnitude in surface soil compared to the control, although the effect was not statistically significant for the cyano-fertilizer.

The addition of cyano-fertilizer to poultry-manure fertilized peach orchard soil affected microbial EL-FAME biomass in soil at deeper depth (2.5-7.5 cm) in 2014. Total microbial EL-FAME biomass was 1.6× greater in the LM + C treatment (75kg N ha^{-1} dried poultry manure + 25kg N ha^{-1} cyano-fertilizer) compared to HM soil receiving 100kg N ha^{-1} dried poultry manure (Table 2.4). Biomass of EL-FAMEs associated with Gram-negative bacteria, fungi, and AM fungi were 1.5×, 1.7×, and 2× greater in subsurface soils fertilized LM+C compared to HM treatment, respectively (Table 2.4). There was no significant effect of fertilizer treatment on Gram-positive bacterial or actinomycete EL FAME biomass. Overall, the peach orchard soil had greater EL-FAME biomass compared to EL-FAME biomass in the cucumber soil.

2.4. Discussion

2.4.1. Microbial Community Responses to Fertilizers

Organic fertilizers can have significant short-term and long-term impacts on soil microbial communities. In this study, the microbial community structure and biomass was sensitive to different organic fertilizers in both annual vegetable and perennial tree fruit systems, although not consistently each year. Arancon et al. (2005) found that short-term increases in microbial biomass occurred when vermicompost was applied. In our study, total microbial biomass and relative abundance and biomass of bacteria, actinomycetes, fungi, and AM fungi were influenced in the short-term by organic fertilizer type in one or both experiments. Schutter et al.(2001) noted a greater fungal biomass under conditions with greater C availability and moisture. In addition, increased fungal biomass has also been recorded in situations with plant residues and greater

soil moisture(Frey et al., 1999; Wienhold et al., 1998). Yevdokimov et al (2008) noticed an increase in fungal biomass with greater application rates of N and concluded that this response was due to the fungi being more sensitive to N than to C. Kimura and Asakawa, (2005) found that compost addition to paddy soils resulted in a shift in microbial community structure, specifically increased amounts of actinomycetes and Gram-positive bacteria. On the other hand, in a conventionally cultivated soil, actinomycete fatty acid biomarkers were in greater abundance than in an organically managed soil, which may be due to the role of actinomycetes as decomposers of nutrient-poor C compounds and may thus appear more active in the growing season when N becomes limited in the soil(Lundquist et al., 1999; MacKenzie and Quideau, 2010) . In contrast, others reported low fungal biomass when compared to microbial biomass, and no effect on fungal biomass by different types of organic fertilizer such as vermicompost or manure (Lazcano et al., 2012).

Cyanobacteria have been reported to influence the microbial community of tropical alluvial soil under both unsterilized and sterilized conditions (Ranjan et al., 2016). In this study, the cyano-fertilizer and Neptune hydrolyzed fish emulsion increased microbial biomass and altered microbial community structure as compared to other fertilizers and the control. Cyano-fertilizer and Neptune hydrolyzed fish emulsion are liquid treatments that contain abundant soluble C and N; in addition, the cyano-fertilizer contains living cyanobacteria that can fix C and N. Other studies have also reported that organic manure and biofertilizer organic nutrient management (ONM), chemical nutrient management (CNM), and integrated nutrient management (INM) increase soil microbial biomass shortly after application (Dinesh et al., 2010). Organic fertilizers generally provide C, N, and energy to soil microbes for their growth and reproduction, and organic fertilizer effects are typically longer lasting than chemical fertilizer effects (Zeng et al., 2007), although this study

demonstrates that certain organic fertilizers (e.g, cyano-fertilizer and Neptune hydrolyzed fish emulsion) can result in short-term increases in soil bacterial, fungal, and total microbial biomass more so than other organic fertilizers.

Murate and Nishida. (1987) reported that N-fixing cyanobacteria produce heterocyst cells containing characteristic glycolipids; the chemical structures of major components of *Anabaena cylindrica* are 3,25-Dihydroxyhexacosanyl α-D-glycopyranoside and 3,25,27-Trihydroxyoctacosanyl α-D-glycopyranoside (Gunstone and Harood, 1994). In general, the fatty acids composition of planktonic *Anabaena* contain 14:0, 16:0, 16:1(*cis*-), 18:0, 18:2, 16:1 ω9, and 18:1 ω 7 (Gugger et al., 2002; Li and Watanabe, 2001; Willers et al., 2015). If cyanobacteria survived in soil after irrigation, these organisms may have contributed to EL-FAMEs measured in this study, and subsequently microbial community structural differences among fertilizer treatments (Tables 1 and 2). However, the presence of cyanobacteria alone does not fully explain microbial community changes, as soil microbial biomass and structure responded similar between cyano-fertilizer and Neptune hydrolyzed fish emulsion in the cucumber experiment.

2.4.2 Microbial community groups under two different depths in cucumber and peach soils

To our knowledge, we are the first to report that cyano-fertilizer or Neptune hydrolyzed fish emulsion can affect the biomass and structure of soil microbial communities, and this effect was statistically significant at the 0–2.5 cm depth in cucumber planted soil and the 2.5-7.5 cm depth in peach orchard soil. Overall, cyano-fertilizer increased total microbial biomass in both locations' soils (cucumber plots and peach orchard), with the increase being attributed mainly to bacteria and actinomycetes in the cucumber soil, and fungi and AM fungi in peach orchard soil. The effect on microbial biomass was consistent despite large differences in overall microbial biomass between the cucumber and peach orchard soils, where biomass differences were presumably because of

differences in soil type and management (annually cropped and tilled system in cucumber production vs perennial and no-till system in peach orchard production). The difference in microbial biomass between the two experiments is in agreement with Girvan et al. (2003) who found that the microbial biomass in arable soil was mainly influenced by soil type and short-term management. Furthermore, effects of cyano-fertilizer in the peach orchard soil were more heterogenous across plots than in the cucumber soil and inconsistent over time, as evidenced by the less distinguished separation of microbial community EL-FAME profiles in 2014 and lack of significant effects in the second year. This more subtle effect of cyano-fertilizer in the peach orchard soil could be due to the lack of soil mixing (because of no-till), a relatively high microbial biomass that made smaller changes difficult to detect, and non-uniform growth of fungi and AM fungi in soil.

Bardgett et al. (1997) support our finding that microbial biomass decreased with soil depth, particularly in the cucumber soil. Similarly, Ekelund et al.(2001) reported that bacterial abundance decreased with soil depth. Microbial biomass and CO_2 respiration were greater in the top 5 cm depth of the soil and decreased in the bottom 15 cm depth with change in the diversity due to the primary source of nutrient input in grasslands being above ground (Griffiths et al., 2003). In addition, Van Leeuwen et al. (2017) pointed out that land use exerts strong effects on soil microbes in the topsoil and that microbial biomass and activity decrease with soil depth. The shallow soil depth (0-2.5 cm) had greater microbial biomass compared with the deeper depth (2.5-7.5 cm) in the cucumber experiment. Within the peach orchard, however, there was no consistent pattern of microbial biomass being greater in the surface than in the subsurface depth, because the cyano-fertilizer addition in the LM+C treatment stimulated microbial biomass to such an extent in the first year, that the depth effect was mostly negated. This may have occurred if the lower

application rate of poultry manure allowed more of the cyano-fertilizer to move deeper into the soil, so that it had a greater effect on microbial communities at the deeper depth than observed in the HM+C treatment. Peacock et al. (2001) reported that manure application increased microbial biomass in the 5-10 cm depth due to the movement of soluble C below the 0-5 cm depth. Griffiths et al.(2003) found that microbial community was changed in the 5-10 and 10-15 cm depths during the summer due to rhizosphere effects, such as increased root exudation at the height of plant growth.

2.5. Conclusion

When possible, it would be beneficial to choose organic fertilizers that not only increase crop yield but also promote soil microbial biomass, as this could promote sustainable management of agroecosystems. This study demonstrated that organic fertilizer decisions can influence soil microbial communities even in short periods of time (<1 yr). Bacteria and actinomycetes were the most sensitive to cyano-fertilizer and Neptune hydrolyzed fish emulsion in the annually cropped cucumber system, whereas fungi and AM fungi responded positively (in the first year) to cyano-fertilizer in the perennial peach orchard. These specific shifts in biomass and community structure could be beneficial to organic vegetable and fruit production, as increased bacterial and actinomycete biomass could stimulate decomposition and nutrient turnover for vegetables, and increased fungal and AM fungal biomass could provide greater soil C storage, nutrient cycling, and symbiotic relationships with orchard trees. Furthermore, this study demonstrated that cyano-fertilizer could reduce grower reliance on commercial organic fertilizers or reduce application rates of manure when used as a supplemental fertilizer. Either scenario benefits the grower by reducing dependence on commercial fertilizers, conserving primary fertilizer sources, and reducing environmental impacts associated with organic fertilizer production, transportation, and excessive

application. Thus, cyano-fertilizer technology and application should be further explored and used by growers, due to its potential impact on soil microbial biomass and fungi for improved soil health in addition to its benefits to plant production and the environment.

Tables

Table 2.1. Eigenvector coefficients of microbial ester-linked fatty acid methyl esters (EL-FAMEs) for principal components (PC) axes 1 and 2 in the cucumber experiment, as shown in Fig 1.

		Eigenvector Coefficient	
Microbial Group	EL-FAME	PC1	PC2
Actinomycetes	10Me16:0	-0.574	-0.032
	10Me17:0	-0.970	0.025
	10Me18:0	-0.934	0.036
Fungi	18:1ω9	0.653	0.570
	18:2ω6	0.200	-0.568
AM Fungi	16:1ω5	-0.169	-0.665
Gram-negative Bacteria	16:1ω7	-0142	-0.536
	17:1ω7	-0.824	0.068
	17:0cy	-0.904	0.015
	18:1ω7	-0.080	-0.206
	18:1ω8	-0.899	0.013
	19:0cy	-0.412	0.223
Gram-positive Bacteria	i14:0	0.259	-0.368
	i15:0	0.001	-0.668
	a15:0	0.279	-0.025
	i16:0	-0.156	-0.156
	i17:0	-0.725	-0.001

	a17:0	-0.755	-0.024
Non-specific	13:0	-0.937	0.043
	14:0	-0.835	0.414
	15:0	-0.965	0.021
	16:0	0.836	0.107
	18:0	-0.245	0.273

Table 2.2. Eigenvector coefficients of microbial ester-linked fatty acid methyl esters (EL-FAMEs) for principal component (PC) axes 1 and 2, in the peach experiment in 2014 as shown in Fig 2.

		Eigenvector Coefficient	
Microbial Group	EL-FAME	PC1	PC2
Actinomycetes	10Me16:0	-0.103	0.697
	10Me17:0	-0.634	0.587
	10Me18:0	-0.970	-0.017
Fungi	18:1ω9	0.436	-0.300
	18:2ω6	-0.235	-0.706
AM Fungi	16:1ω5	0.430	0.695
Gram-negative Bacteria	16:1ω7	-0.459	-0.607
	17:1ω7	-0.883	0.115
	17:0cy	-0.101	-0.482
	18:1ω7	0.123	-0.230
	18:1ω8	-0.678	0.437
	19:0cy	-0.442	0.331
Gram-positive Bacteria	i14:0	0.623	-0.688
	i15:0	-0.427	-0.606
	a15:0	0.029	-0.948
	i16:0	0.283	-0.868
	i17:0	-0.833	-0.181
	a17:0	-0.436	-0.658

Non-specific	13:0	-0.965	0.135
	14:0	-0.976	0.029
	15:0	-0.959	-0.021
	16:0	0.889	0.108
	18:0	-0.737	-0.403

Table 2.3. Soil microbial biomass and community composition (0-2.5 cm depth) in a certified organic cucumber field receiving different organic fertilizer treatments. Within the 0-2.5 cm soil depth, fertilizer treatment means with the same letter are not significantly different at $P < 0.05$ using Tukey's honest significant difference (HSD) test. There were no significant differences among fertilizer treatments in the 2.5-7.5 cm depth.

Treatment[1]	Depth	Total Microbial Biomass	Gram+ Bacteria	Gram- Bacteria	Fungi	Actinomycetes
		----------nmol g^{-1}----------				
A	0-2.5	37.3 bc	5.02 ab	7.34 bc	12.7 a	0.60 bc
	2.5-7.5	28.6	4.21	4.85	8.71	1.46
BA	0-2.5	24.2 c	3.34 b	3.38 c	8.33 a	0.74 bc
	2.5-7.5	18.5	1.86	2.87	4.90	0.00
BB	0-2.5	39.8 bc	5.73 ab	6.61 bc	14.0 a	0.59 bc
	2.5-7.5	22.6	3.41	3.59	9.21	0.69
C	0-2.5	27.8 c	4.50 ab	4.44 c	9.80 a	0.39 bc
	2.5-7.5	28.5	4.58	5.21	9.03	0.95
CY	0-2.5	68.1 a	10.5 a	15.1 ab	17.7 a	3.12 ab
	2.5-7.5	38.2	6.41	8.81	9.98	2.35
FA	0-2.5	31.6 c	5.29 ab	6.16 bc	10.9 a	0.00 c
	2.5-7.5	9.03	1.23	1.52	3.19	0.00
FB	0-2.5	26.5 c	3.85 b	5.33 bc	9.74 a	0.00 c
	2.5-7.5	16.3	2.73	2.43	5.52	0.00

N	0-2.5	73.0 a	11.1 a	17.5 a	20.2 a	4.23 a
	2.5-7.5	38.5	6.33	8.36	10.2	2.59

[1]Fertilizer treatments are indicated as follows: A= Alaska non-hydrolyzed fish emulsion, BA= Blood meal surface applied, BB= Blood meal sub-surface applied, C= Control, CY= Cyano-fertilizer, FA= Feather meal surface applied, FB= Feather meal sub-surface applied, N= Neptune hydrolyzed fish emulsion.

Table 2.4. Soil microbial biomass community composition in a certified organic peach orchard receiving different organic fertilizer treatments in 2014. Within the 2.5-7.5 cm depth, fertilizer treatment means with the same letter are not significantly different at $P < 0.05$ using Tukey's honest significant difference (HSD) test. There were no significant differences among treatments in the 0-2.5 cm depth.

Treatment[1]	Depth	Total Microbial Biomass	Gram+ Bacteria	Gram- Bacteria	Fungi	AM Fungi	Actinomycetes
			---------- nmol g^{-1}----------				
HM+C	0-2.5	531	92.0	117	175.0	46.2	23.9
	2.5-7.5	368 ab	56.0 a	78.7ab	118 ab	43.3 ab	27.7 a
LM+C	0-2.5	472	92.0	106	154	48.8	24.9
	2.5-7.5	490 a	71.8 a	108a	162 a	64.9 a	33.7 a
HM	0-2.5	499	87.6	117	162	45.2	22.5
	2.5-7.5	321 b	51.1 a	71.2b	94.5 b	32.5 b	25.4 a

[1]Fertilizer treatments are indicated as follows: HM+C= 100kg N ha^{-1} as dried poultry manure + 25kg N ha^{-1} of cyano-fertilizer, LM+C= 75kg N ha^{-1} as dried poultry manure + 25kg N ha^{-1} as cyano-fertilizer, HM= 100kg N ha^{-1} of dried poultry manure.

Figures

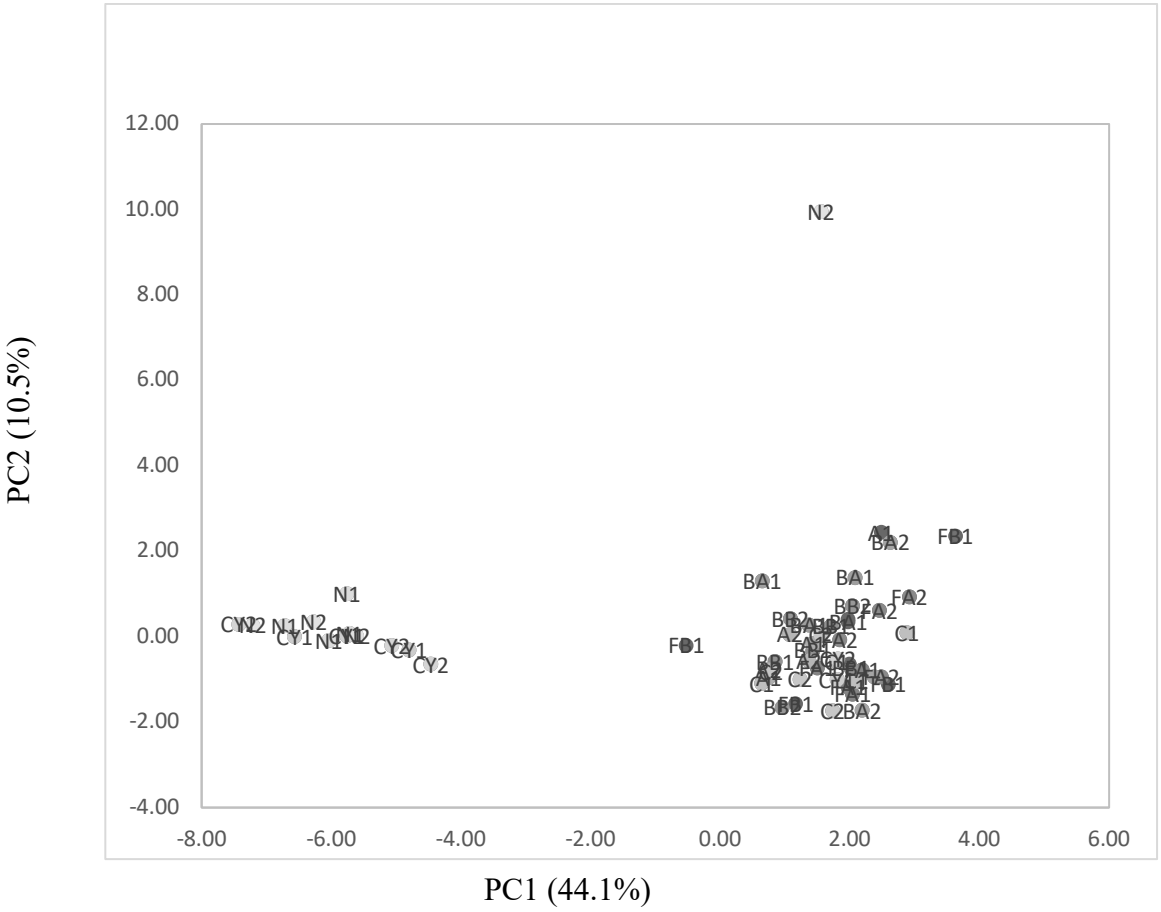

Fig 2.1. Principal components analysis (PCA) of soil microbial community EL-FAMEs from the 2015 cucumber experiment, sampled at two different depths: 0-2.5 cm depth (1) and 2.5-7.5 cm depth (2). Fertilizer treatments are indicated as follows: A = Alaska non-hydrolyzed fish emulsion, BA = Blood meal surface applied, BB = Blood meal sub-surface applied, C = Control, CY = Cyano-fertilizer, FA = Feather meal surface applied, FB = Feather meal sub-surface applied, and N = Neptune hydrolyzed fish emulsion. The percent variance explained by each PC is shown in parentheses.

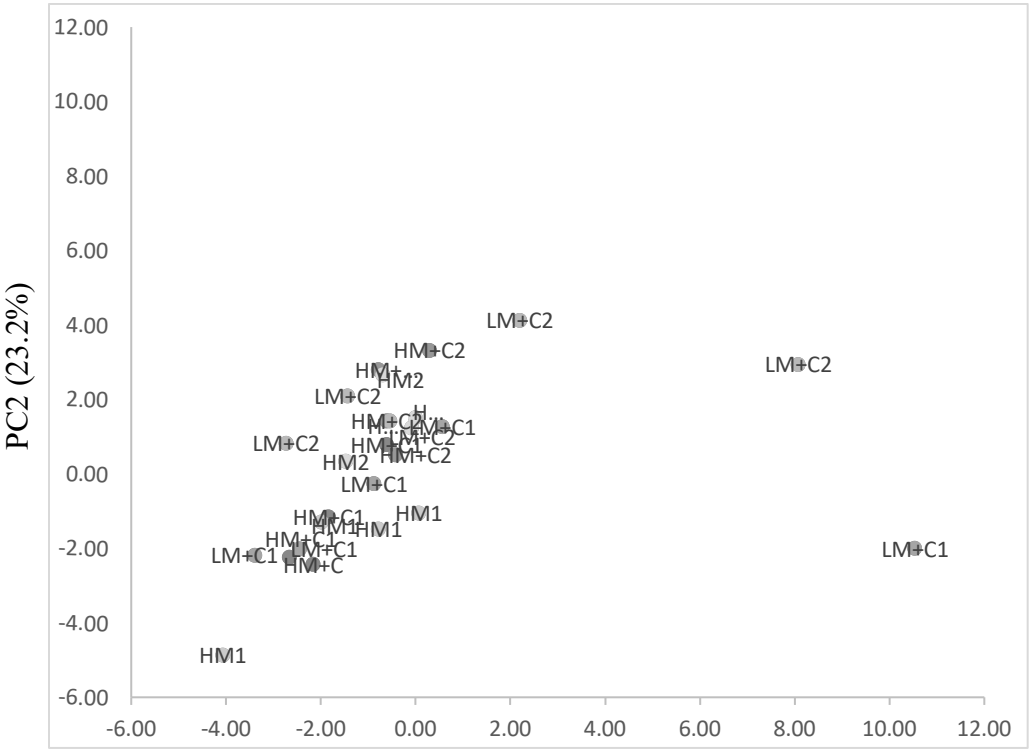

Fig 2.2. Principal components analysis (PCA) of soil microbial community EL-FAMEs from the 2014 peach orchard experiment, sampled at two different depths: 0-2.5 cm depth (1) and 2.5-7.5 cm depth (2). Fertilizer treatments are indicated as follows: HM+C = 100kg N ha^{-1} of dried poultry manure + 25kg N ha^{-1} of cyano-fertilizer, LM+C = 75kg N ha^{-1} as dried poultry manure + 25kg N ha^{-1} of cyano-fertilizer, and HM = 100kg N ha^{-1} of dried poultry manure. The percent variance explained by each PC is shown in parentheses.

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Chapter 3. OPTIMIZING PHOSPHORUS AND IRON CONCENTRARIONS TO MAXIMIZE CYANOBACTERIA GROWTH AND NITROGEN FIXATION

1- Introduction

Cyanobacteria are among the most diverse and widely distributed prokaryotes and are commonly found in productive lakes worldwide. These lakes are defined as eutrophic lakes that are generally shallow, turbid, and support abundant aquatic plant growth (Kotak et al., 2000). Cyanobacteria represent a unique group of ancient organisms with tremendous environmental significance throughout time for several reasons, including oxygenic photosynthesis, fixation of atmospheric nitrogen (N), and production of toxins (Whitton and Potts, 2007). In addition, these bacteria play an invaluable role in freshwater ecosystems because of their abilities to produce oxygen via oxygenic photosynthesis and convert atmospheric N to the biologically available form – ammonium (Crawford, 2008). Their ubiquitous distribution and ability to dominate phytoplankton communities are related to a complex interaction among many factors, including the concentration and ratio of nutrients such as P, N, and Fe, the ability of certain genera to fix atmospheric N_2, buoyancy control, and minimal grazing by zooplankton (Kotak et al., 2000).

N-fixing cyanobacteria are O_2-evolving photoautotrophs, and they depend upon light energy, water, and CO_2. Nutritional requirements of this group of microorganisms are relatively simple, during photosynthesis they utilize solar energy and several essential nutrients (C, N, P, S, K, Fe, etc.) to synthesize their biomass compounds and to multiply their cells; thus, cyanobacteria need specific quantities of those essential elements to be capable to produce biomass (Singh et al., 2006). A deficiency of one of the elements will limit growth (Liebig's law of the minimum) (Markou et al., 2014). Nutrients, in particular Fe, strongly influence cyanobacteria dynamics, P (or orthophosphate), and N (in the forms of ammonia, nitrite, and nitrate) (Chirico et al., 2020).

Phosphorus and Fe are the major limiting nutrients for N-fixation by cyanobacteria. These nutrients play a major role in phytoplankton communities (Fernández-Juárez et al., 2019), and both are required for the synthesis of nitrogenase (Kuffner and Paul, 2001).

Phosphorus is required in cellular synthesis of nucleic acids and membrane phospholipids, as well as for energy transfer through tri- and bi-phosphorylated nucleotides. In aquatic environments, P is biologically available as orthophosphate (Shun et al., 1994). Primary production of phytoplankton in freshwater systems is usually controlled by P availability. It is considered an integral part of the metabolism of all life forms (Correll, 1998).

Iron plays an important role in the growth and physiological processes of cyanobacteria. Iron is required for photosynthetic electron transport proteins such as ferredoxin and cytochrome, DNA RNA, and chlorophyll synthesis. In addition, it is considered a component of the iron-molybdenum cofactor that catalyzes the nitrogenase enzyme (Barminski et al., 2016). Cyanobacteria have the ability to acquire and transport Fe with specific Fe-uptake mechanisms, including low molecular weight compounds called siderophores, which are extracellular Fe-binding compounds that comprise part of the high-affinity Fe transport system in cyanobacteria (Kuffner and Paul, 2001).

Although it is clear that cyanobacteria require both P and Fe for optimal growth and N fixation, what remains unclear is the interaction of P and Fe in achieving optimum cyanobacterial growth and N-fixation. Therefore, the objective of this study was to compare the effect of different P and Fe concentrations on cyanobacterial growth and N fixation to better understand their interaction and ultimately determine P and Fe requirements for optimal growth and N fixation.

2- Materials and Methods

2.2. Experimental design

Research was conducted to compare the performance of *Anabaena* sp., grown in Allen-Arnon media (Allen and Arnon, 1955), with different concentrations of P and Fe. A xenic culture of *Anabaena* sp. was originally cultured from sediment collected from Richard's Lake in Fort Collins, CO, USA (Nubel et al.,1997), and was used in the study. The culture was maintained in Allen-Arnon medium in the laboratory (Barminski et al., 2016; Afkairin et al., 2021), and was used in liquid form. The *Anabaena* sp. culture was blue green in color and had long, straight filaments with vegetative cells about 2–8 μm in diameter, oval heterocysts a bit larger than the vegetative cells, and few akinetes (Afkairin et al., 2021).

In the first experiment, cyanobacteria were grown in media containing four concentrations of P: Control—100% Allen-Arnon [($7.6*10^{-4}$ M P), 50% ($3.8*10^{-4}$ M P), 25% ($1.9*10^{-4}$ M P), and 5% ($3.8*10^{-5}$ M P)] and the experiment was replicated twice in Allen-Arnon medium.

In the second experiment, cyanobacteria were grown in media varying concentrations of P and Fe, including two P concentrations and three Fe concentrations for a total of six treatments: -

5% P /100% Fe ($1.8*10^{-5}$ M Fe based on Allen-Arnon media)

50%P/100% Fe ($1.8*10^{-5}$ M Fe).

5% P/ 10% Fe($1.8*10^{-6}$ M Fe).

50%P/10% Fe ($1.8*10^{-6}$ M Fe)

5 % P/1% Fe ($1.8*10^{-7}$ M Fe).

50%P/1% Fe ($1.8*10^{-7}$ M Fe).

Both experiments were carried out in the laboratory and set up as a CRD. Cyanobacteria were grown for three weeks in 500-mL Erlenmeyer flasks. Flasks were agitated at 150 rpm on a G10

Gyrotary orbital shaker (New Brunswick Scientific Co., Inc., Edison, NJ, USA) under a 2500-lux grow light (Growl LED Inc., Wildomar, CA, USA) that operated with a 12-h/12-h light/dark cycle.

2.2. Experimental analysis

Optical density (OD) was used to estimate cyanobacterial growth, and the optical density (OD) at 750 nm of the culture was measured using a Hach DR3900 spectrophotometer (Hach Company, USA). Triplicate subsamples from each flask were analyzed to measure OD at 750 nm. OD was measured day 1, day 7, day 14, and day 21. For each experiment, the Allen-Arnon medium that cyanobacteria were cultured in was used as the control. The culture pH was measured weekly on days 7, 14, and 21. On day 21, one subsample from each flask was taken to determine total Kjeldahl N (TKN), using a HACH Simplified TKN instrument (TNT 880, Hach Company, USA).

2.3. Statistical analysis

Data were analyzed using R Version 3.5.3 and RStudio Version 1.0.153 (The R Foundation for Statistical Computing; Wickham et al., 2018). The experimental units were arranged in a factorial two-way analysis of variance (ANOVA) with two replications in the first experiment and three replications in the second experiment. The ANOVA and pairwise comparisons of means were obtained from mean square errors to determine whether main effects or interactions were significant ($P < 0.05$). Tukey adjusted pairwise comparisons were used to evaluate differences between treatments (Lenth et al., 2019).

3- Results and Discussion

The 100% P level had the highest OD while the 5% P treatment had the lowest OD in weeks 1 and 2 (Fig.3.1). However, by week 3, both the 5% and 25% P treatment levels were significantly lower in OD than the 50% P treatment and 100% P treatment. There was no change in the pH between the treatments except the 5% P treatment in the first week (Fig 3.2). TKN increased when P concentration increased, in 50% P, and 100% P compared to the 5% P had the lower TKN level, however there were not significantly different between the treatments in cultures of cyanobacteria (Fig. 3.3). P is a critical nutrient for cyanobacteria growth limiting factor for cyanobacteria, specifically in natural environments where P can be limiting (Markou and Georgakakis, 2011). Lower P concentration is related to low cell densities due to the increase in carbohydrate and a decrease in protein content (Grobbelaar, 2004). This is consistent with our finding that the growth of cyanobacteria was significantly lower in the 5% P treatment than the 100% P treatment (Fig 1).

In the second experiment, there were no significant differences in OD among Fe treatments, regardless of P concentration. OD generally increased with time, as expected (Fig.4). OD of cyanobacteria was greatest in treatments with the higher P concentration (50%) and low to moderate Fe concentrations (1% Fe and 10% Fe) (Fig 3.4). The pH was significantly lower in the high Fe treatments, regardless of P level (Fig. 5). In addition, the 50% P treatment had significantly higher solution pH than the 5% P treatment. There were significant differences in TKN concentration among the treatments: the TKN was lower in the high Fe (100% Fe) treatment (Fig. 6). There was a significant negative correlation between Fe concentration and OD (r=-0.80), Fe and pH (r= -0.95), and Fe and TKN (r=-0.72) (Fig.7). There was a positive relationship between P concentration OD and P concentration and pH, although the correlations were not significant (Fig. 3.7). P concentration was negatively correlated with TKN, but not significantly (Fig.3.7) It

might be a great benefit to the growers who are growing cyanobacteria on-site by reducing cost when they used lower concentrations of P and Fe in the media AA medium.

4- Conclusions

In conclusion, we determined that the high P concentration (50-100%) resulted in the best growth of the cyanobacteria in AA media under laboratory conditions. There were positive relationships between P concentration with OD and pH. High concentrations of Fe reduced OD and TKN in this study. There was negative correlation between the Fe concentration and OD, pH, and TKN, indicating that as Fe concentration increases, culture OD, pH, and TKN concentration decrease. Further research is needed to determine whether the reduced growth and N fixation resulted from the low pH observed in the high Fe treatments.

Figures

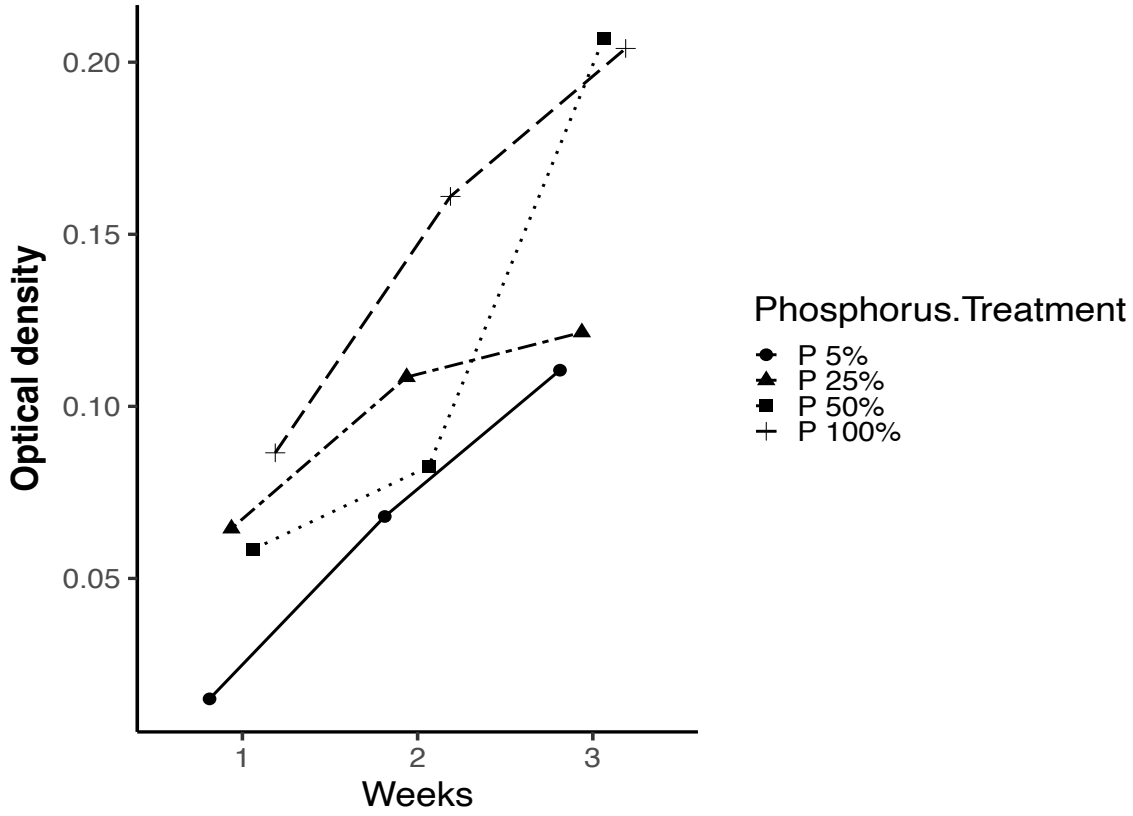

Fig 3.1. Optical density (OD) as influenced by P treatment and time. The P concentrations are relative to the Allen-Arnon solution. Error bars represent the standard error of the mean. Treatments within date with a common letter above the bars are not significantly different from one another ($p<0.05$) based on Tukey adjusted pairwise comparisons.

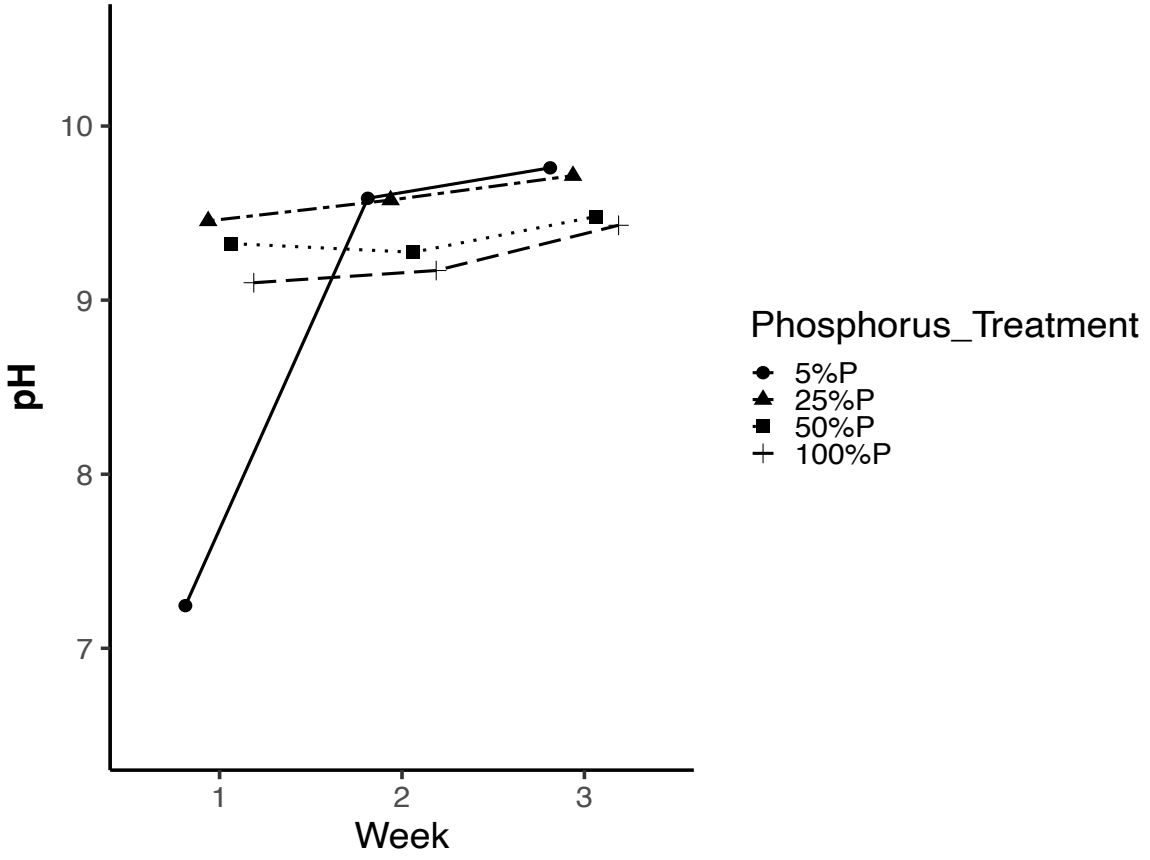

Fig 3.2. Culture pH between weeks and treatments. Error bars represent the standard error of the mean. There were no significant differences between the treatments ($P > 0.05$) based on Tukey adjusted pairwise comparisons

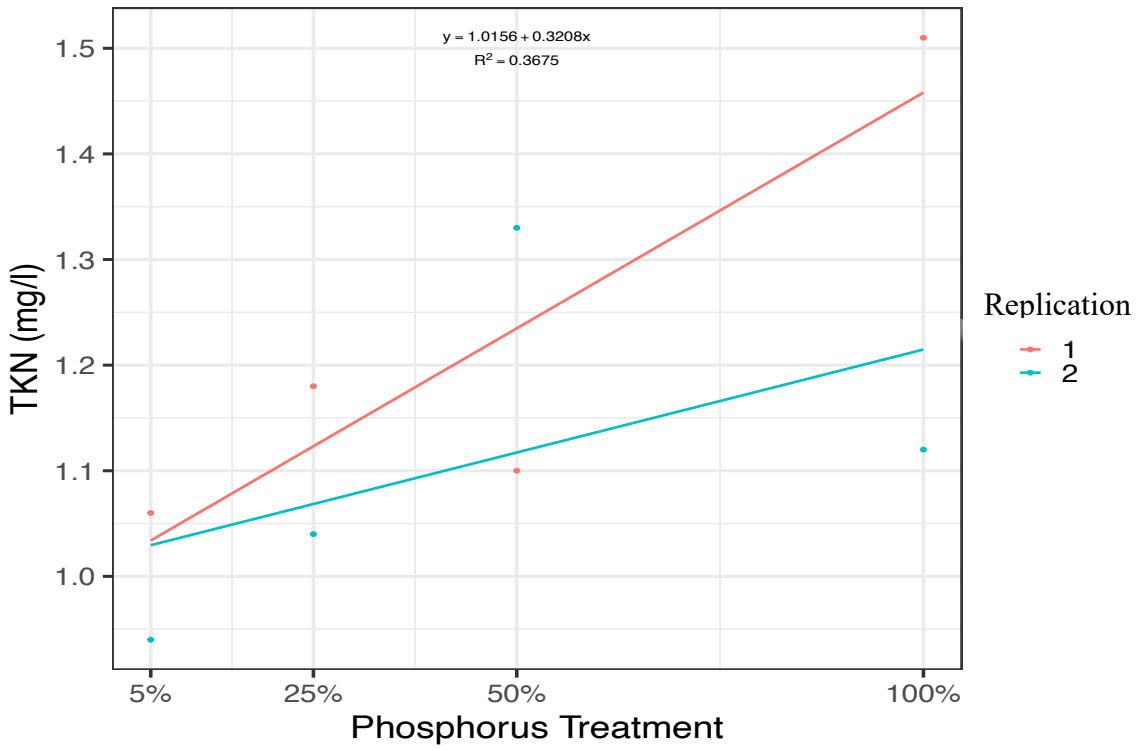

Fig 3.3. The total Kjeldahl nitrogen (TKN) concentration in culture of cyanobacteria as a function of P concentration (% are relative to Allen Arnon media). There were no significant differences between the treatments ($P > 0.05$; n=2) based on Tukey adjusted pairwise comparisons.

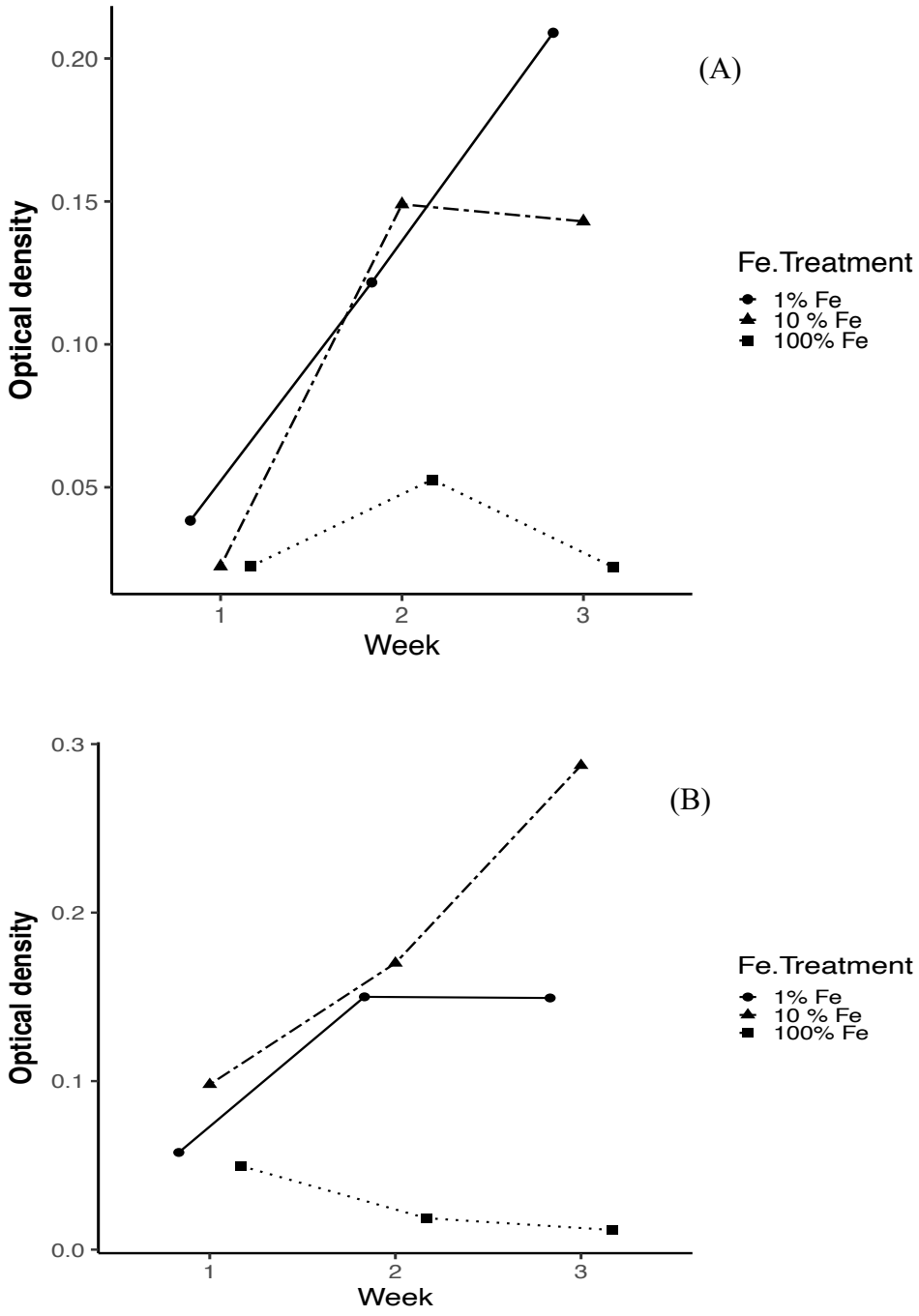

Fig 3.4. Optical density (OD) as influenced by P and Fe concentrations and time. The P and Fe concentrations are relative to the Allen-Arnon solution. (A) 5% P and (B) 50% P, there were significant different OD between treatments ($p<0.05$) based on Tukey adjusted pairwise comparisons. Thus, statistical analyses are not shown.

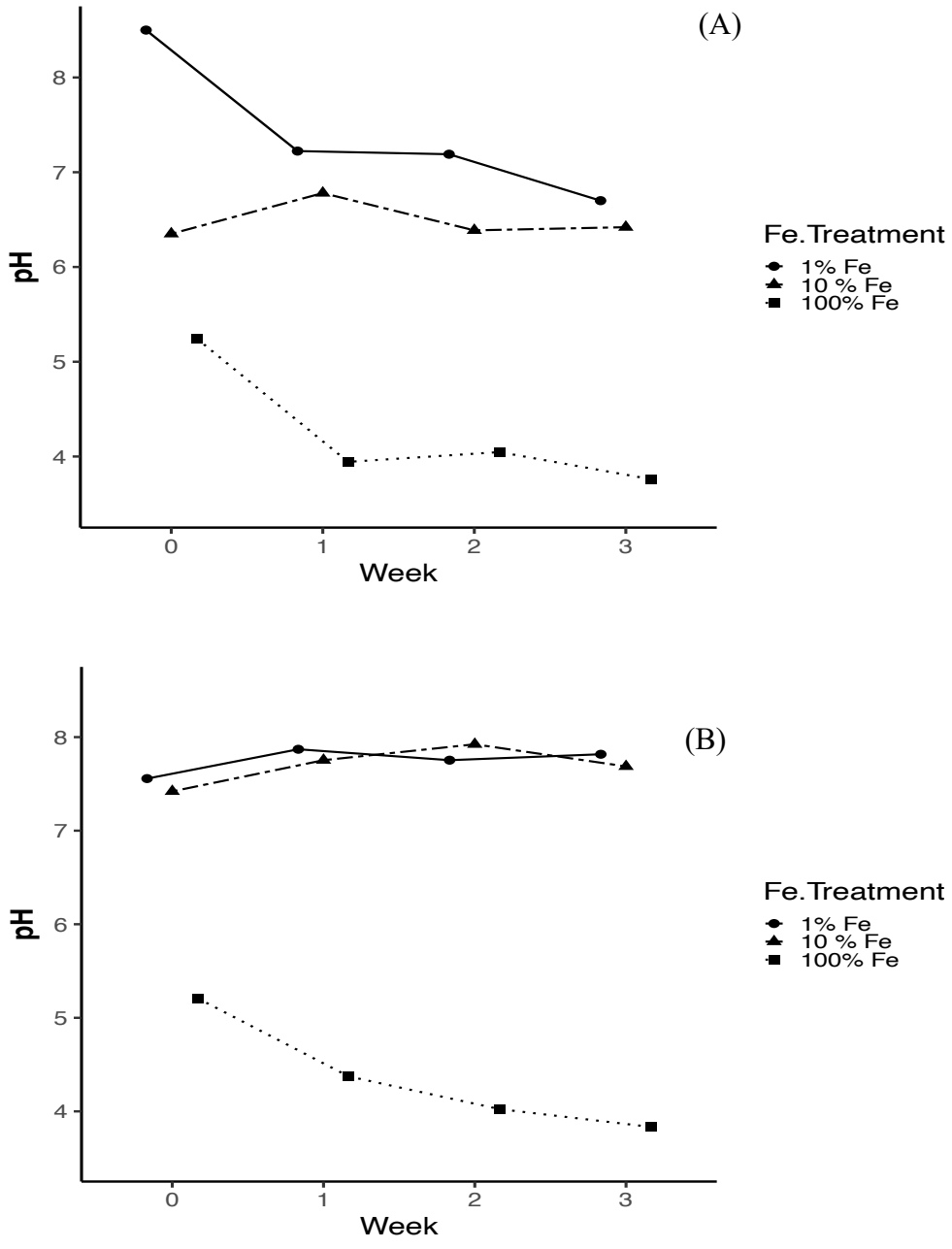

Fig 3.5. Culture pH as influenced by P and Fe concentrations and time (A) 5% P and (B) 50% P, there were significant different pH between treatments ($p<0.05$) based on Tukey adjusted pairwise comparisons. Thus, statistical analyses are not shown.

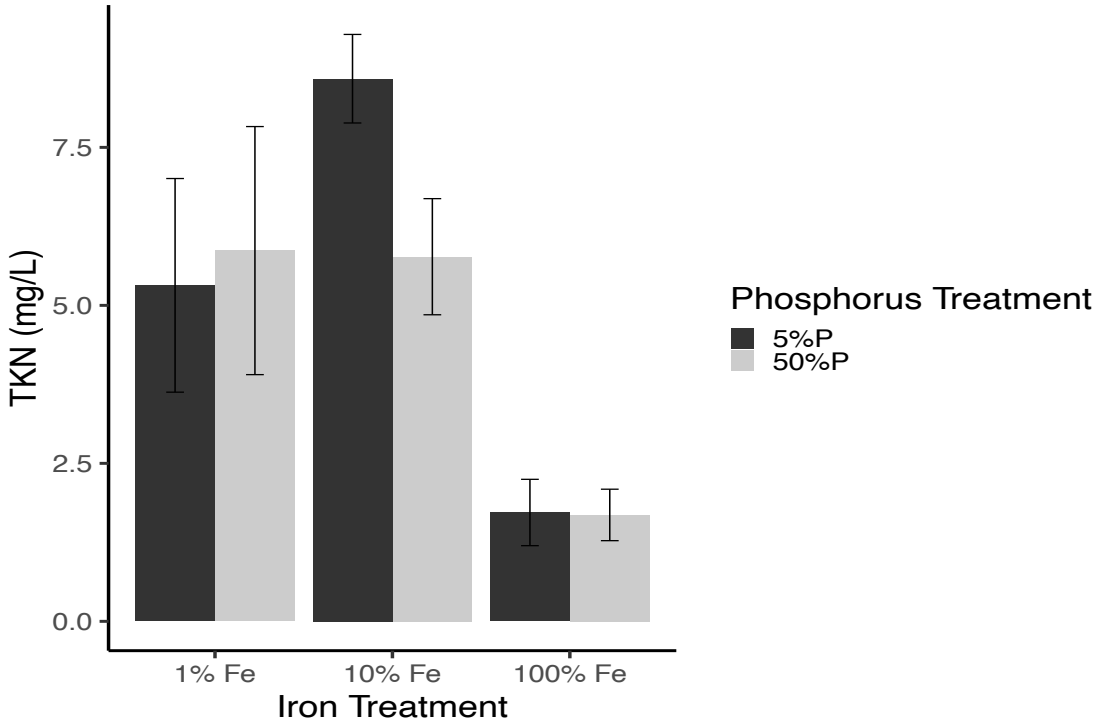

Fig 3.6. The total Kjeldahl nitrogen (TKN) concentration in cultures of cyanobacteria in (day 21) were significantly lower in 100% Fe compered to 10% Fe and 1% Fe concentration.

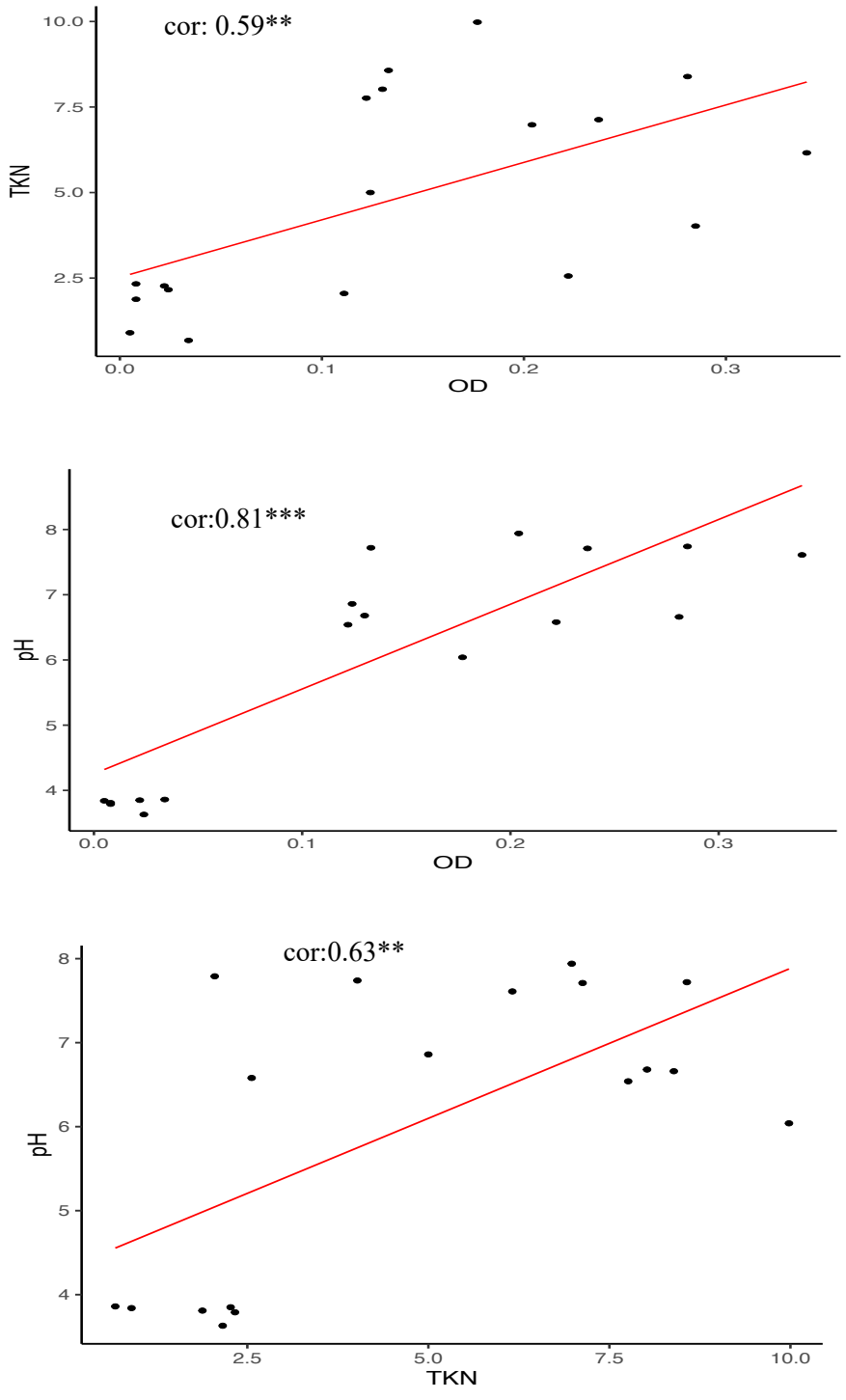

Fig 3.7. Scatter plots representing correlation coefficients of P concentration and Fe concentration with TKN, pH and optical density in the second experiment in day 21.

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Conclusions and future recommendations

Results of the study evaluating P solubilization and activity of *Anabaena sp*. and Mammoth P using two different organic P sources (bone meal and rock phosphate) under laboratory conditions indicate that the Cyano treatment solubilized more P than MP. Cyano treatment increased water-soluble and Olsen P concentrations as compared to MP or control. Based on this study, *Anabaena sp.* cyanobacteria have the capability to increase P availability in soil. The novel use of cyanobacteria, such as *Anabaena sp.*, may have a significant role to play in tackling the future scarcity of plant-available P. An evaluation of the potential to scale-up cyanobacteria application under field applications is warranted to evaluate its large-scale impact on P solubilization and plant uptake, and thus, to evaluate its effectiveness as a strategy for improving future soil P availability to plants, especially considering future dwindling rock phosphate reserves.

The study to determine the effect of different organic fertilizers, including a non-traditional cyanobacteria-based fertilizer, on soil microbial communities of two different soil types and at two depths, and thus should be further explored and considered by growers as a self-sufficient means to benefit soil microorganisms, plant production, and the environment. When possible, it would be beneficial to choose organic fertilizers that not only increase crop yield but also promote soil microbial biomass, as this could promote sustainable management of agroecosystems. This study demonstrated that organic fertilizer decisions can influence soil microbial communities even in short periods of time (<1 yr). Bacteria and actinomycetes were the most sensitive to cyano-fertilizer and Neptune hydrolyzed fish emulsion in the annually cropped cucumber system, whereas fungi and AM fungi responded positively (in the first year) to cyano-fertilizer in the perennial peach orchard. These specific shifts in biomass and community structure could be

beneficial to organic vegetable and fruit production, as increased bacterial and actinomycete biomass could stimulate decomposition and nutrient turnover for vegetables, and increased fungal and AM fungal biomass could provide greater soil C storage, nutrient cycling, and symbiotic relationships with orchard trees. Furthermore, this study demonstrated that cyano-fertilizer could reduce grower reliance on commercial organic fertilizers or reduce application rates of manure or compost when used as a supplemental fertilizer. Either scenario benefits the grower by reducing dependence on commercial fertilizers, conserving primary fertilizer sources, and reducing environmental impacts associated with organic fertilizer production, transportation, and excessive application. Thus, cyano-fertilizer technology and application should be further explored and used by growers, due to its potential impact on soil microbial biomass and fungi for improved soil health in addition to its benefits to plant production and the environment.

I determined that the high P concentration (50-100%) had the best growth of the cyanobacteria in AA media under laboratory conditions. I also found that high Fe levels reduced OD and TKN. The observed alterations for the growth in this study need future research to determine whether the reduced growth and N fixation are due to low pH or high Fe treatments. We highly recommended it use lower concentrations of P and Fe in the media AA medium to be a great benefit to the growers who are growing cyanobacteria on-site by reducing cost when they used lower concentrations of P and Fe in the media AA medium .Further research is needed to develop an improved AA media, specifically its P and Fe concentrations, to define the optimal concentrations for producing healthy cyanobacteria filaments and heterocysts for the cyanobacteria.

The use and benefits of cyano-fertilizer to crop production and soil health should be further explored by growers and agronomists, and outreach conducted to growers so that they are aware of the potential benefits of cyanobacterial biofertilizers. Furthermore, there is potential for cyano-

fertilizers to be a better source for solubilization P insoluble in soil and apply cyano-fertilizer under field application to evaluate its large-scale impact on P solubilization and plant uptake.