Effect of bacterial biofertilizers, native arbuscular mycorrhizal fungi and soil amendments on soil and grain phosphorus availability of flooded rice in dry zone, Sri Lanka

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Abstract— Rice is considered as the most important food crop in Sri Lanka and most of the Asian countries. Over the past three decades, rice farmers have become increasingly dependent on synthetic chemical phosphorous fertilizers, mainly triple super phosphate (TSP) as a source of phosphorous (P). However, dramatically increasing costs, serious environmental and health issues attached to chemical fertilizers, forced researchers to develop supplementary or alternate sources of P for rice. Hence, this study was done to evaluate the potential use of biofertilizers and natural soil amendments as alternatives to chemical P fertilizers in rice (*Oryza sativa* L.) farming in the dry zone of Sri Lanka. A field trial was carried out at Ranpathwela in Anuradhapura, North Central Province, Sri Lanka, during the *Yala* season in 2016. The experiment was designed as follows: CON: control; AMF: AMF inoculants (2 Mg ha^{-1}); RAMF: ERP (153.3 kg ha^{-1}) with the AMF inoculants (2 Mg ha^{-1}); MC: mixed microbial culture (5 kg ha^{-1}); RMC: ERP (153.3 kg ha^{-1}) with a mixed microbial culture [consortium of *Azospirillum* sp., *Pseudomonas* sp. and *Bacillus* sp. (5 L ha^{-1})]; BC: biochar (6 Mg ha^{-1}); CP: standard compost (10 Mg ha^{-1}) and IF: inorganic synthetic fertilizer (125 N kg ha^{-1}, 62.5 P$_2$O$_5$ kg ha^{-1} and 50 K$_2$O kg ha^{-1}). Three indigenous and two improved rice varieties were used. The experiment comprised of 24 plots and three replicates. Soil, roots and grains of rice were analyzed for phosphorus. Some other elements were shown to have an effect on plant available soil P such as aluminum and iron, were also estimated. It was revealed that significant differences ($p<0.05$) in variety, treatment and V x T interaction was observed in plant available soil phosphorus. The highest mean of available soil P was observed in TRSP2 x AMF (14.64 ± 0.12 mg kg^{-1}) followed by TRSP3 x MC (8.87 ± 0.001 mg kg^{-1}) interactions. The multifunctional microbial consortium in amended soil, including *Azospirillum* sp., *Pseudomonas* sp. *Bacillus* sp. and arbuscular mycorrhizal fungi, were capable of potentially increasing soil phosphorus. Furthermore, the addition of rock phosphate did not always make a difference in soil P availability for rice farming systems.

Keywords—Triple super phosphates, Eppawala rock phosphate, rice, phosphorus, arbuscular mycorrhizae, phosphorus solubilizing bacteria

I. INTRODUCTION

Phosphorus (P) is an essential macronutrient for proper growth and development of plants (Cordell et al., 2009). In tropical regions, plant – available soil P is often limited (Sharma et al., 2013). The P level in soil is about 0.05% (w/w), and plants can only use up to 0.1% of this phosphorus (Sharma et al., 2013). Orthophosphate P ions, usually H$_2$PO$_4^-$ and HPO$_4^{-2}$ is present in the soil solution as inorganic phosphorus (Hinsinger, 1998). The soluble inorganic P for plants absorption is limited because of its fixation with cations forming insoluble phosphates (Sharma et al., 2013). Phosphorus is fixed by cations such as Ca^{2+} to form a calcium phosphate [Ca$_3$(PO$_4$)$_2$] complex. Furthermore, with Al^{3+} and Fe^{3+} in acidic soils, aluminum phosphate (AlPO$_4$) and ferrous phosphate (FePO$_4$) are formed respectively (Vassilev et al., 2006; Bünemann et al., 2018). It was reported that around 75–90% of soil applied chemical phosphate fertilizers are rapidly converted into such metal-cation phosphates and are insoluble, hence difficult to uptake by plant roots (Grant et al.,

2004; Vassilev et al., 2006; Rivaie et al., 2008). Therefore, the regular application of these P fertilizers is needed and it creates long-term undesirable environment and health impacts (Walpola and Yoon, 2012).

Different phosphorus fertilizers are used by farmers includes, triple super phosphate (TSP), single super phosphate (SSP), ammonium phosphate (AP) and rock phosphate (RP). Triple super phosphate (TSP) is the most common P fertilizer used in rice farming in the North Central Province (NCP) of Sri Lanka. Generally, TSP is produced with the addition of concentrated phosphoric acid to a ground phosphate rock. Triple super phosphate contains approximately 17–23% of P and 44–52% P$_2$O$_5$ (Bandara et al., 2008). Triple super phosphate and AP are more soluble than RP (Withers et al., 2005).

Eppawala rock phosphate (ERP) is a locally available natural P resource of igneous origin containing about 28% to 40% of

P_2O_5 (Dahanayake, 1988). Rock phosphate can be used as an alternative for imported TSP, after the improvement of water solubility (Pitawala et al., 2003). The rock phosphate was successfully introduced to rice cultivation in acid sulphate soils in Mekong delta, Vietnam mainly because of the high effectivity of rock phosphate addition in acid sulphate conditions (Chien et al., 1990). Furthermore, different microbial processes have been used to improve microbial organic acid production and to solubilize rock phosphate (Sabah et al., 2016).

Rice grown in acidic soils in Sri Lanka ERP could be used as a source of P due to its increased solubility in acidic soils (Ratnayake et al., 2018). The amounts of P derived from compost amendments are relatively low (below 10 g kg^{-1}) (Hargreaves et al., 2008). *Pseudomonas* spp. *Agrobacterium* spp. and *Bacillus circulans* are considered as efficient P solubilizing bacteria (Bhattacharyya and Jha, 2012). The fungi which could solubilize P are mainly *Alternaria*, *Aspergillus*, *Trichoderma*, *Cephalosporium*, *Penicillium* etc. (Srinivasan et al., 2012; Sharma et al., 2013). Furthermore, organic acids, siderophores, protons, hydroxyl ions and CO_2 are produced by P solubilizing microorganisms (Sharma et al., 2013). These substances can chelate cations or reduce the pH and release phosphorus by substitution of H^+ for Ca^{+2} (Illmer and Schinner, 1995; Goldstein, 2000; Seshachala and Tallapragada, 2012).

Phosphorus solubilizing microorganisms also produce acids such as sulfuric, nitric, and carbonic acids (Kim et al., 1997). However, it has been reported that the efficiency of releasing P from inorganic acids and chelating substances in soil is less than that of the organic acids (Kim et al., 1997). Further, arbuscular mycorrhizal fungi (AMF) effectively extend plant roots, aiding crop P nutrition by increasing the volume of soil where plants can absorb phosphates (Srinivasan et al., 2012).

Soil organic matter (SOM) is considered a major organic P source in soil. The soil organic P can be reached to 30–50% of the total P and is mainly found as inositol phosphate (Rodríguez and Fraga, 1999). According to Halvorson et al. (1990), organic P solubilization is the continuous dissolution of Ca-P compounds. Arbuscular mycorrhizal hyphae have the potential of increasing the root absorbing surface area (Read and Perez-Moreno, 2003). Considering the necessity of practicing sustainable agricultural practices, the present study was planned to assess the potential of biofertilizers, including AMF and natural soil amendments such as compost, biochar and ERP to replace TSP in rice (*Oryza sativa* L.) farming in the dry zone of Sri Lanka.

II. MATERIALS AND METHODOLOGY

The field trial was carried out at Ranpathwela in Anuradhapura, North Central Province Sri Lanka during the *Yala* season in 2016 (8º 23´30.8" N, 80º 39´03.2"E). Mean

annual precipitation was around 1750 mm (Eriyagama et al., 2010), with a dry period from May to September (Jayasena et al., 2011). The mean annual temperature is 30-35 ^{0}C. The soil type of the field is reddish brown earths (Alfisols) (Wijesundara et al., 2013).

A. Field plot experimental design

The experimental field comprised of 24 10 m x 3 m plots. Plots were prepared as rows and separated by 45 cm double bunds, minimizing the plant nutrient movement from one plot to the another. Ridges of 45 cm height and width were also made around the plot. Each plot was divided equally into 5 subplots for 5 different rice varieties. One treatment was applied in all 5 subplots to minimize mixing of added amendments.

The treatments were CON: control (no application of soil amendments and biofertilizers); AMF: AMF inoculants (2 Mg ha^{-1}); RAMF: ERP (153.3 kg ha^{-1}) with AMF inoculants (2 Mg ha^{-1}); MC: mixed microbial culture (5 kg ha^{-1}); RMC: ERP (153.3 kg ha^{-1}) with mixed microbial culture [mixed culture of *Azospirillum* sp., *Pseudomonas* sp. and *Bacillus* sp. (5 L ha^{-1})]; BC: biochar (6 Mg ha^{-1}); CP: standard compost (10 Mg ha^{-1}) and IF: inorganic synthetic fertilizer (125 N kg ha^{-1}, 62.5 P_2O_5 kg ha^{-1} and 50 K_2O kg ha^{-1}). Five rice varieties and three replicates were used randomly.

B. Preparation of biofertilizers and organic amendments prior to field application

Arbuscular mycorrhizal fungi inoculums were prepared using trap cultures. Soil with root fragments of herbs were collected from the upper layer (0-15 cm) of the organically managed rice field in Anuradhapura district and used as an indigenous AMF inoculum. Arbuscular mycorrhizal fungi colonized roots from a trap plant culture was used as a source of native AMF inoculum.

Azospirillum sp. was isolated from the rhizosphere soil of the rice field in Anuradhapura. Nfb semi-solid medium was inoculated with 0.1 ml of soil suspension and was incubated at 37 ^{0}C for 72 hours (Dobereiner, 1992). Isolated colonies were streaked on the plates of malate agar medium containing 0.1% NH_4Cl to get pure colonies and stored at -20 ^{0}C (Rodrignuez-Caceres, 1982). Then the isolated *Azospirillum* sp. was adjusted to 10^8 CFU mL^{-1} approximately in nutrient broth (NB) medium and used for *Azospirillum* inoculum. *Pseudomonas fluorescens* strains were isolated from the rhizosphere soil, from Anuradhapura and Eppawala rock phosphate deposits in Sri Lanka, following standard procedures using King's B agar medium (KB) (Cody et al., 2008). Potassium solubilizing *Bacillus* spp. were also isolated by serial dilution method using modified Aleksandrov medium.

C. Soil and plant sampling methods for chemical analysis

Soil samples of 500 g were collected 2 days prior to harvest from each subplot at a depth of 0-15 cm. Subsamples of soil were collected using a 2 m x 2 m sampling grid, excluding the edges in each experimental plot and composite samples were made. Collected soil was air-dried, gently crushed, passed through a 2 mm sieve and stored for subsequent soil analysis. Rice plant samples were also collected immediately after harvest. Plants were selected from each subplot chosen at random within the 1 m^2 field.

D. Soil pH

The soil pH was measured in soil suspension [soil/water ratio of 1:2.5 (w/v)] using the digital pH meter (Hanna Instruments Inc., USA) (Anderson and Ingram, 1993). Ten grams of soil was transferred into a 50 ml beaker and 25 ml of distilled water was added. After one hour, soil pH was measured regularly by inserting a glass pH-electrode into the saturated soil suspension.

E. Available phosphorus concentration in soil

Plant available soil P was measured with 0.5 mol L^{-1} $NaHCO_3$ (pH 8.5) followed by molybdenum blue colorimetric procedure (Olsen et al., 1954; Olsen and Sommers,1982). For the determination of available soil P, 2.5 g of sieved soil was measured and transferred into a 250 ml conical flask. Then 50 ml of 0.5 M, $NaHCO_3$ solution was added to samples and kept on the shaker for 30 minutes under 210 rpm. Afterwards 10 ml of the filtrate was transferred into a 50 ml volumetric flask and 2 ml of 2.75 M. H_2SO_4 with ammonium vanda-molybdate, the coloring agent was added. Phosphorous content was detected using the Atomic Absorption Spectrophotometer (Model No. UV/1800 APC) under the wavelength of 882 nm.

F. Total phosphorus concentration in rice grains

Total P concentrations of rice grains were estimated using the dry-ashing method. For the determination of total P in a grain sample, 1.0 g of ground sample was collected in a porcelain crucible and placed in a muffle furnace at 550 ^{0}C temperature for 5 hours and cooled. Afterwards the grain samples were taken out from the furnace and 5 ml of 5 N HCl was added and mixed well (Lu, 2000). the grain samples were filtered, coloring reagent was added to the flask and read for P detection using Atomic Absorption Spectrophotometer (Model No. UV/1800 APC) under 410 nm wavelength.

G. Plant available soil aluminum and iron

Soil available concentrations of elements were quantified after being digested by wet acid digesting method and examined for soil available Al and Fe contents using Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES) (Thermo Scientific ICAP7400 DUO).

H. Data analysis

Treatments were arranged in completely randomized design in three replicates. Statistical analyses were performed as two- factor factorial design using the MINITAB statistical software package (MINITAB 17.1.0 version). The two-way ANOVA was applied and the significant differences among the means were tested. The Tukey's test was used to conduct the pairwise comparisons for the significant treatments. The significance level (α) 0.05 was used for all the statistical tests. Pearson correlation analysis was done in order to find the correlation among different variables.

III. RESULTS AND DISCUSSION

A. Soil pH

The soil in the experimental plots was slightly acidic before the addition of soil amendments and biofertilizers. Initially, the soil pH was 6.35 and the increase of the pH upon submergence of soil was evident. After 100 days of growth of rice, mean soil pH values changed slightly towards neutral range (6.46 -7.85).

Compared to the other rice varieties used, the increase of soil pH during the rice growth was greater in TRSP2 variety with and without amendments or biofertilizer additions (Fig. 1). It has been reported that the pH range of soil 6.0 -7.5 is the most productive (Rowell, 1994). The pH range of all amended soil positively influenced the soil P availability (Abreu et al., 2005). Furthermore, it was revealed that a significant difference ($p < 0.05$) was observed among different rice varieties (V), treatments (T) and variety and treatment interactions (V x T). The highest mean pH was observed in TRSP2 x CON (7.85 $\pm$ 0.01) followed by TRSP2 x RMC (7.34 $\pm$ 0.01), TRSP2 x CP (7.18 $\pm$ 0.03), TRSP2 x MC (7.17 $\pm$ 0.06) and TRSP2 x RAMF [(7.03 $\pm$ 0.02) Fig. 1].
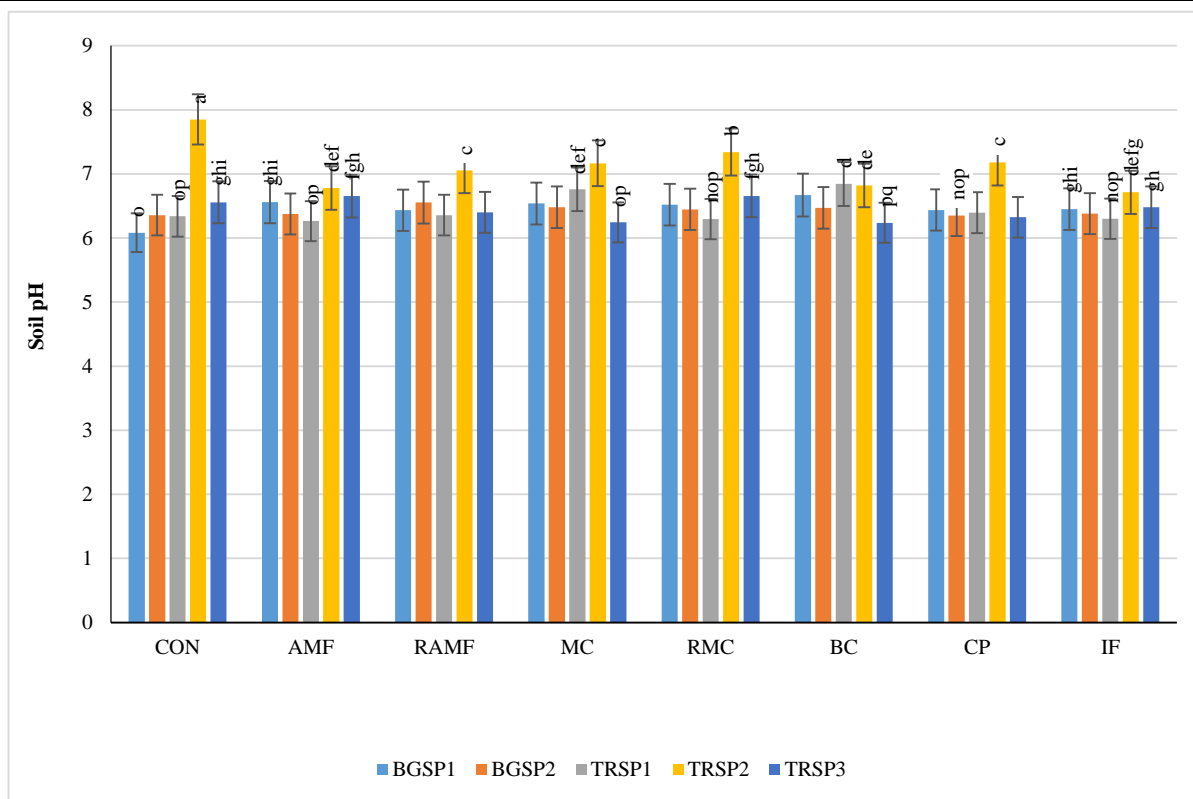
Fig. 1: Mean soil pH with the amended biofertilizers, natural amendments and synthetic fertilizer applications and growth of the different tested rice varieties. Error bars indicate 95% confidence intervals. Means denoted as same letters do not differ at p< 0.05.

(CON: No biofertilizer or amendment addition; AMF: Addition of AMF; RAMF: Rock phosphate + AMF addition; MC: Addition of mixed bacterial culture; RMC: Rock phosphate + mixed microbial culture; BC: Addition of biochar; CP: Addition of compost; IF: Addition of recommended dose of synthetic fertilizer).

Mixed microbial culture (MC and RMC) added treatments showed an increase of pH with the growth of different rice varieties. Decarboxylation of anionic organic matter by microorganisms could have been increased the pH (Yan et al., 1996). Nitrogen mineralization by soil microorganisms subsequently produced OH^- from organic ligands, also explain the increase of soil pH (Mkhabela and Warnan, 2005). Rock phosphate decreases exchangeable Al^{+3} in soil resulting an increase in soil pH to neutral range which enhance the plant available phosphorus (van Der Heide, et al., 1992; Abreu et al., 2005). The soil pH values of rock phosphate added treatments in this study (RAMF and RMC) were higher compared to the other treatments – for example, in TRSP2 x RMC (7.34 ± 0.01) and TRSP2 x RAMF (7.05 ± 0.02).

Soil pH has been increased in biochar amended subplots (Fig. 1). Biochar increases the cation exchange capacity (CEC), pH buffering capacity and adsorb Al^{+3} and H^+ of the soil (Nigussie et al., 2012). The addition of biochar increases soil pH was also reported in acidic soil (Zhang et al., 2010; Butnan et al., 2015). According to Mukherjee et al. (2011), combination of soil and biochar may result in different pH buffering capacities. Soil pH was increased by 0.11% for each $Mg\ ha^{-1}$ of biochar applied after 0.5 year of application (Carvalho et al., 2013). Incorporation of biochar into soils was most effective on P availability, because with the increases of pH in

soil, decreases the P adsorption onto the Fe and Al oxides (Carvalho et al., 2013).

It was also reported that compost increases soil pH, while NPK chemical fertilizer decreases soil pH (Warren Fonteno, 1993; Liu et al., 2010). The decrease of soil pH by addition of NPK fertilizers could due to leaching of basic cations, such as Ca^{+2}, Mg^{+2} while acidic Al^{+3} ions entering into the soil solution (Whalen et al., 2000). Additionally, nitrification of added N fertilizer resulted the generation of H^+ ions leading to soil pH decline (Liu et al., 2010).

B. Plant available phosphorus in soil

It was revealed that significant differences (p<0.05) in variety, treatment and V x T interaction was observed in plant available soil phosphorus. The highest mean of available soil P was observed in TRSP2 x AMF (14.64 ± 0.12 mg kg^{-1}) followed by TRSP3 x MC (8.87 ± 0.001 mg kg^{-1}) TRSP2 x RMC (6.59 ± 0.01 mg kg^{-1}) BGSP2 x CON (6.25 ± 0.18 mg kg^{-1}) and TRSP2 x MC [(6.01 ± 0.002 mg kg^{-1}) Fig. 2]. It was reported that the optimal range of soil available P for proper rice growth in Sri Lankan soils is 10 – 20 mg kg^{-1} (Kendaragama et al., 2006). The results of the present study revealed that the available P concentrations were lower than the optimal range of soil available P in the experimental plots
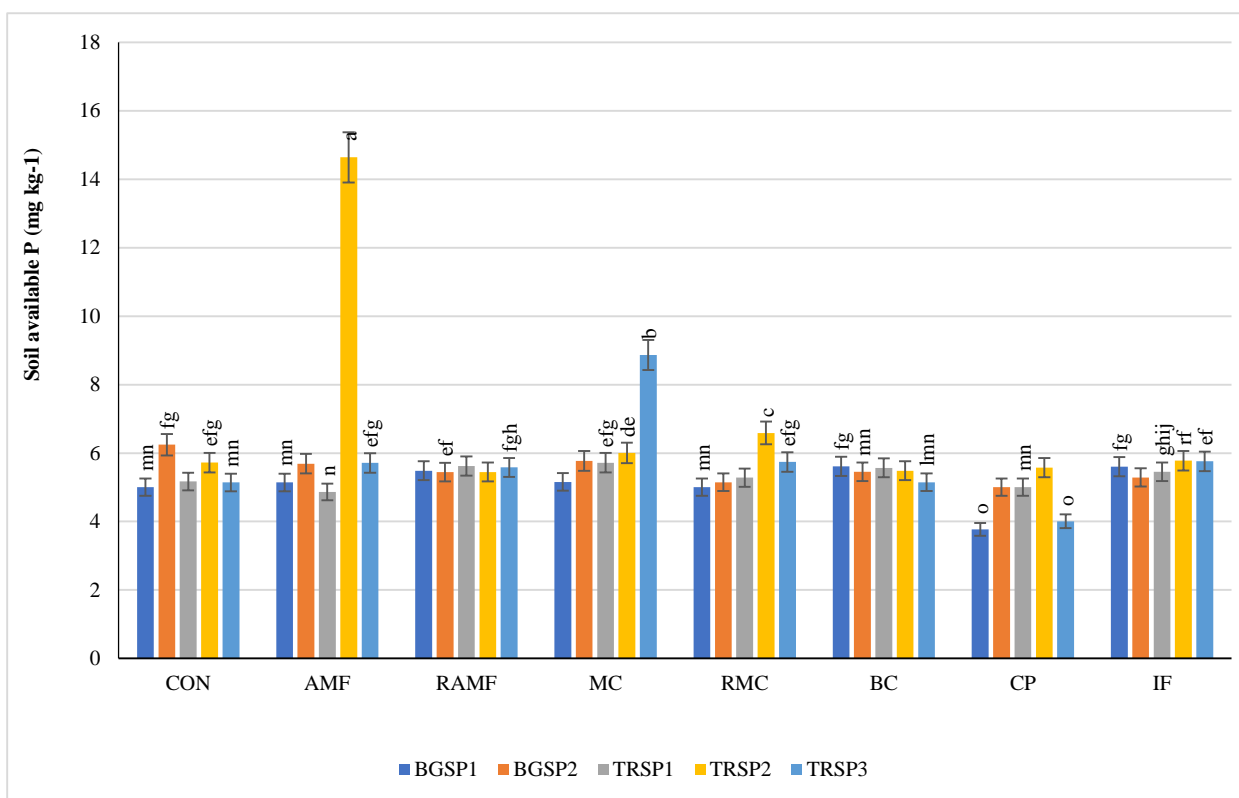
Fig. 2: Mean plant available soil P (mg kg-1) with the amended biofertilizers, natural amendments and synthetic fertilizer applications and growth of the different tested rice varieties. Error bars indicate 95% confidence intervals. Means denoted as same letters do not differ at p< 0.05.

CON: No biofertilizer or amendment addition; AMF: Addition of AMF; RAMF: Rock phosphate + AMF addition; MC: Addition of mixed bacterial culture; RMC: Rock phosphate + mixed microbial culture; BC: Addition of biochar; CP: Addition of compost; IF: Addition of recommended dose of synthetic fertilizer).

except TRSP2 x AMF (Fig. 2). However, P deficiency was not observed in any rice plant in any of the treatments. However, Kendaragama et al. (2006), reported that around 44% of rice growing soils in Sri Lanka, the available soil P concentration is less than 10 mg kg^{-1}, while the rest have higher P concentrations, and the mean P concentration was approximately 13 mg kg^{-1}.

Furthermore, it is estimated that a rice crop with an average yield of 6 Mg ha^{-1} removes 20 kg ha^{-1} P per season (Amarawansha and Indrarathne, 2010). When a rice plant absorbs P from the soil, the available soil P content is reduced (Amarawansha and Indrarathne, 2010). Phosphates fixed by Fe^{+2}, Al^{+3} and Ca^{+2} in soils is a major cause of low soil phytoavailable phosphorus (Pampolino et al., 2018). Approximately 70–90% of P in the soil is fixed and hence, not available to be absorbed by plants (Ladha et al., 2003). Biochar and compost showed high affinity for Al^{+3} and Fe^{+2} hence, reduce P fixation. This helps long term chelation of Al^{+3} and Fe^{+2} by biochar and compost instead of P (Major et al., 2010). Then P will become readily available for use by crops and considered as a sustainable strategy to replace conventional P fertilizers. However, these characteristics could be varied widely with the parent biomass types and production conditions of both biochar and compost (Mukherjee et al., 2011).

Soil bacteria, mainly *Pseudomonas* and *Bacillus*, can solubilize organic P by secreting enzymes, which include phosphohydrolases, phytases and phosphonatases (Ladha et al., 2003). Soil P get solubilized by rhizosphere microbial consortium by releasing the phosphatase enzyme, organic acids, and siderophores (Shenoy et al., 2001). Furthermore, organic matter can improve soil P availability by adsorbing the phosphorus fixing cations (Brady and Weil, 2008). However, the soil P availability was not substantially increased with the addition of rock phosphate compared to MC and RAMF treatments (Fig. 2).

C. Plant available aluminum concentration in soil

Plant available Al concentration in soil significantly varied (p< 0.05) in rice varieties and the interactions (Fig. 3). Among the interactions, the highest available soil Al concentration was observed in BGSP1 x RAMF (8.68 ± 0.09 mg kg^{-1}) followed by BGSP2 x CON (8.49 ± 0.06 mg kg^{-1}), BGSP2 x CP (7.21 ± 0.02 mg kg^{-1}), BGSP2 x AMF [(6.28 ± 0.17 mg kg^{-1}) Fig. 3]. Low soil available Al was observed in IF amended treatment with all 5 rice varieties (Fig. 3). If the soil is acidic and contained high concentration of inorganic P, insoluble Al-P complexes will be formed reducing both available soil Al and P (Zhang and Matsumoto, 2005). Phosphorous reported
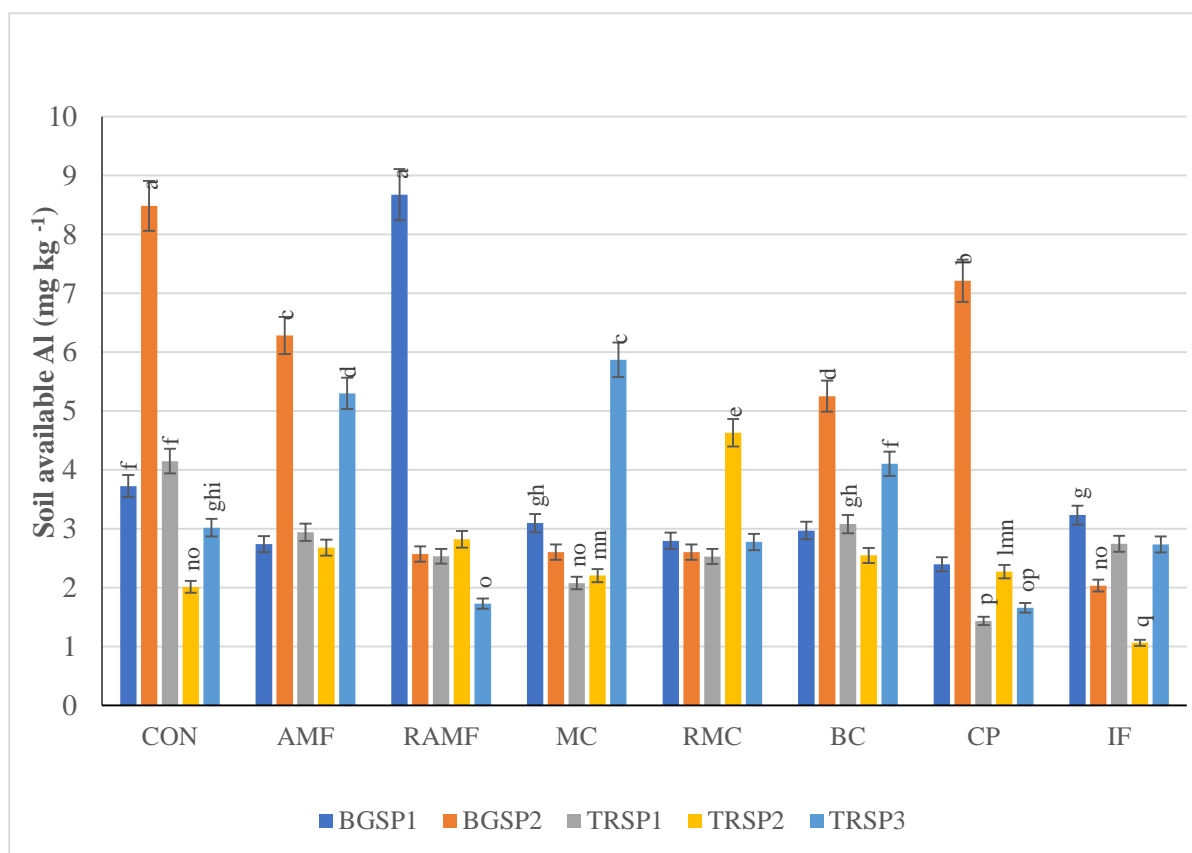
Fig. 3: Mean plant available soil Al (mg kg-1) with the amended biofertilizers, natural amendments and synthetic fertilizer applications and growth of the different tested rice varieties. Error bars indicate 95% confidence intervals. Means denoted as same letters do not differ at p< 0.05.

CON: No biofertilizer or amendment addition; AMF: Addition of AMF; RAMF: Rock phosphate + AMF addition; MC: Addition of mixed bacterial culture; RMC: Rock phosphate + mixed microbial culture; BC: Addition of biochar; CP: Addition of compost; IF: Addition of recommended dose of synthetic fertilizer).

to be alleviated Al toxicity in plants such as wheat (Iqbal, 2013) and sorghum (Tan and Keltjens, 1990). Soil available Al concentrations was low in MC, RMC, BC and CP amended plots except the growth of few rice varieties (Fig. 3). It could be possible that with the amended biochar, soil pH gets increased and fixed Al by surface adsorption to the silicates. Therefore, the findings were consistent with Zhang and Matsumoto (2005), that the soil biochar application resulted the Al adsorption. Humic substances in mature compost also decreases Al concentration in acid soil through forming chelates (Zhang and Matsumoto, 2005). Humic substances in compost also decrease exchangeable Al through the chelation by functional group, especially carboxylate (Iqbal, 2013).

D. Plant available iron concentration in soil

Soil available Fe concentration varied significantly ($p < 0.05$) with the rice variety, treatment and V x T interactions. The highest soil available Fe was observed in BGSP2 x IF (1.38 ± 0.07 mg kg^{-1}) followed by TRSP3 x IF (1.31 ± 0.08 mg kg^{-1}), TRSP2 x BC (1.24 ± 0.01 mg kg^{-1}), TRSP2 x MC (1.21 ± 0.01 mg kg^{-1}) and BGSP1 x CP (1.18 ± 0.01 mg kg^{-1}) respectively (Fig. 4). Soil available Fe concentrations in organic amendments and biofertilizer added treatments were lower

than that of IF treatments (Fig. 4). Iron in the form of oxyhydroxides combines with SOC forming Fe-organic compounds (Lemanceau et al., 2009; Wang et al., 2010). Soil organic matter can effect on different physiochemical reactions that influence the iron availability for plants (Wang et al., 2010). The reduced environmental conditions in flooded rice fields and the addition of compost resulted the increase of Fe adsorption in complex forms to exchange sites (Lemanceau et al., 2009). Furthermore, build-up of SOC in soil converts adsorbed fractions to more plant accessible forms of Fe gradually in soil which further increase the plant uptake of micronutrients (Hansch and Mendel, 2009).

Reducing conditions promoting microbial Fe^{+3} reduction and hence the release of the more mobile Fe^{+2} from hydrologically connected soils (Grybos et al., 2007). Iron deficiency and an excess in the soil hinder several physiological functions in the rhizosphere and plants (Bashir et al., 2011). Soil microbial activities, such as acidification and secretion of Fe-chelating molecules influence the solubilization and absorption of Fe in soil (Marschner and Römheld, 1994). Furthrhe release of protons (H$^+$) in the rhizosphere via H$^+$- ATPase and phytosiderophore secretion in the root zone, increases the availability of Fe in soil (Nozoye et al., 2007).
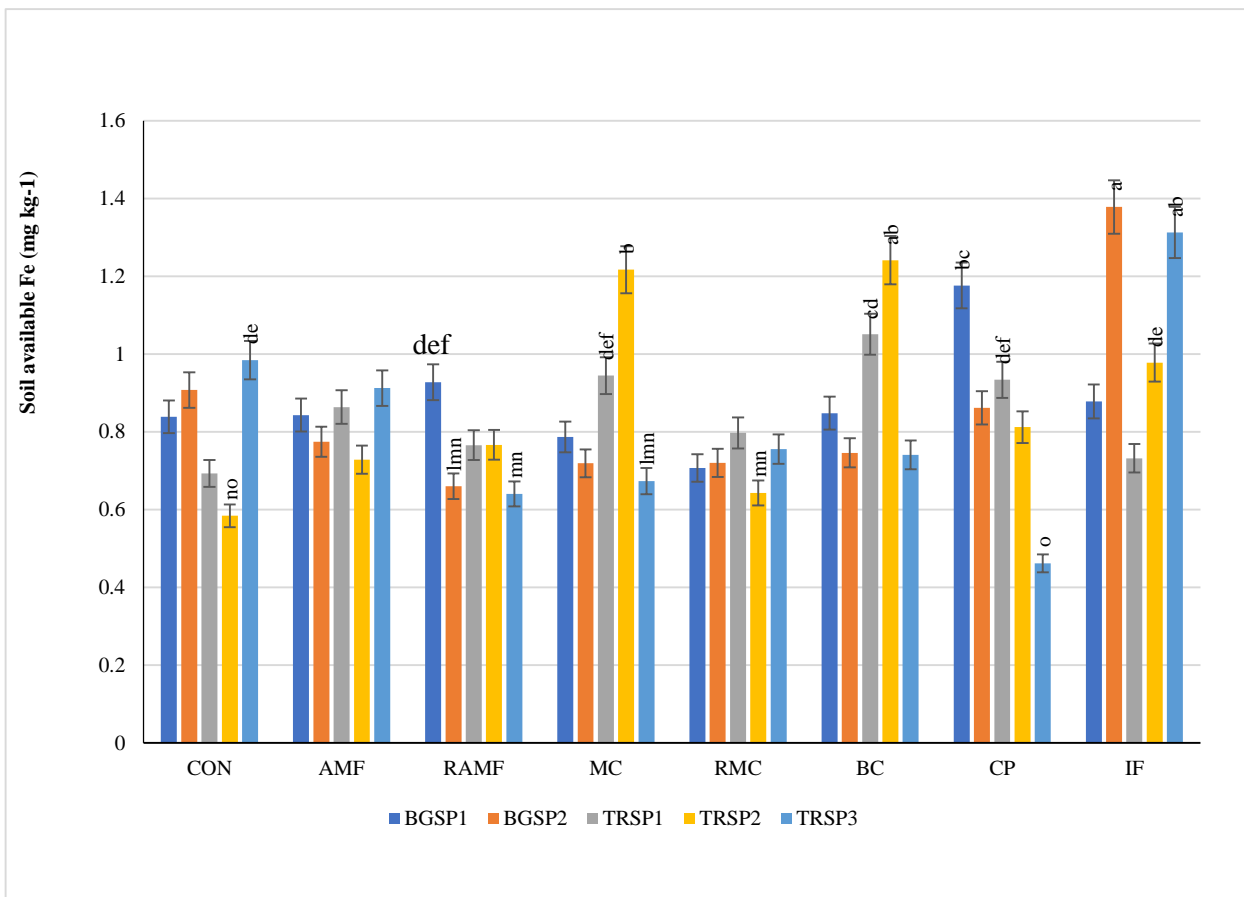
Fig. 4: Mean plant available soil Fe (mg kg-1) with the amended biofertilizers, natural amendments and synthetic fertilizer applications and growth of the different tested rice varieties. Error bars indicate 95% confidence intervals. Means denoted as same letters do not differ at p< 0.05.

CON: No biofertilizer or amendment addition; AMF: Addition of AMF; RAMF: Rock phosphate + AMF addition; MC: Addition of mixed bacterial culture; RMC: Rock phosphate + mixed microbial culture; BC: Addition of biochar; CP: Addition of compost; IF: Addition of recommended dose of synthetic fertilizer).

E. Total phosphorus in rice roots

Statistical analysis revealed that total root P content showed significantly different (p< 0.05) with the rice variety, treatment and V x T interactions. The highest level of root P was observed in BGSP2 x RMC (8.73 ± 0.01 mg kg^{-1}) followed by BGSP2 x MC (8.01 ± 0.002 mg kg^{-1}), TRSP1x IF (7.01 ± 0.002 mg kg^{-1}), TRSP2 x MC [(6.87 ± 0.003 mg kg^{-1}) Fig. 5]. Furthermore, IF added treatment plots with different rice varieties were ranged from 4.73 ± 0.006 mg kg^{-1} to 7.01 ± 0.002 mg kg^{-1} of the TRSP1 x IF and TRSP2 x IF interactions respectively (Fig. 5). Arbuscular mycorrhizal fungi have the capability to boost the uptake and root compartmentalization of phosphate (Krishna and Bagyaraj, 1982). However, this phenomenon was not clearly showed in all rice varieties in the present study, because of the submerged conditions for rice growth.

F. Total phosphorus in rice grains

Statistical analysis revealed that the total P concentration of rice grains were significantly different (p< 0.05) in rice variety, treatment and V x T interactions. Among the interactions, the highest P concentration of rice grain was recorded in RMC x TRSP1(10.00 ± 0.91 mg kg^{-1}) followed by BGSP1 x CP (9.29 ± 0.02 mg kg^{-1}), BGSP1x IF (8.35 ± 0.05 mg kg^{-1}), BGSP2 x MC (7.69 ± 0.4 mg kg^{-1}), BGSP2 x IF (7.53 ± 0.06 mg kg^{-1}) and TRSP1 x MC [(7.47 ± 0.05 mg kg^{-1}) Fig. 6]. Phosphorus is accumulated into rice grains after 6 to 15 days of flowering (Allen and Mallarino, 2006). It was estimated that approximately 80% of the applied P fertilizer in the world is removed from fields at harvest, mostly in the grains of cereals (Wei et al., 2018). Furthermore, at maturity, P is mainly accumulated in grains in the form of phytic acid, an that cannot be digested by humans and other monogastric animals (Wei et al., 2018). This could be the reason of finding higher amounts of P in sewage which affect negatively on water quality and environment (Wei et al., 2018).
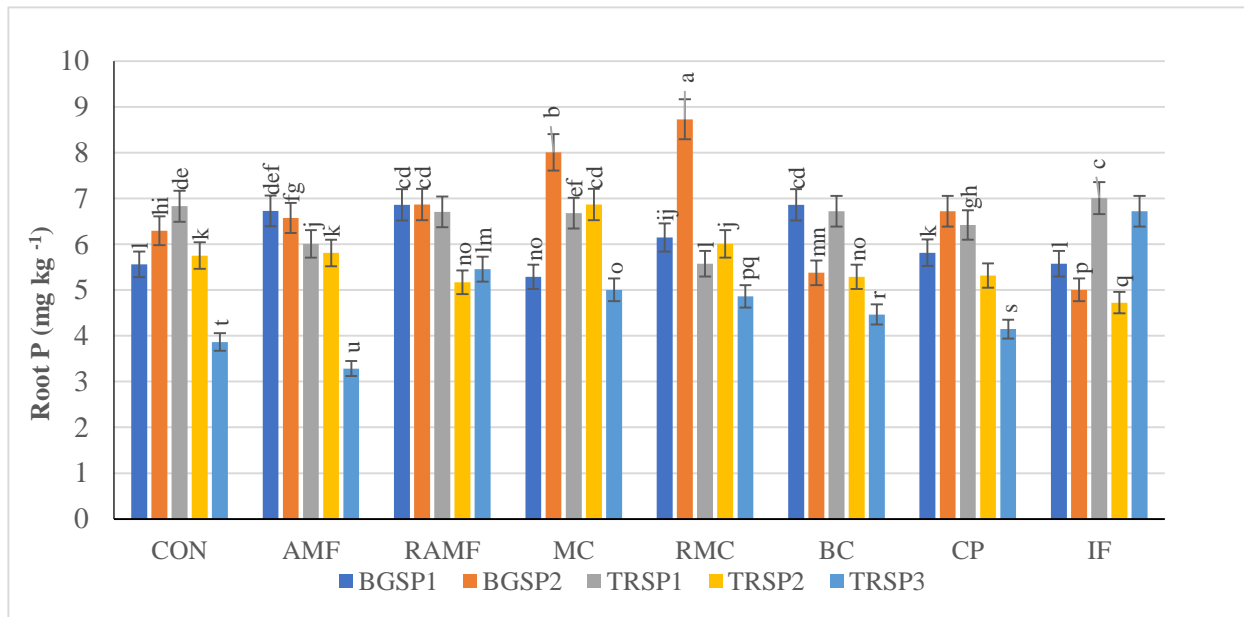
Fig. 5: Mean root total P (mg kg-1) the amended biofertilizers, natural amendments and synthetic fertilizer applications and growth of the different tested rice varieties. Error bars indicate 95% confidence intervals. Means denoted as same letters do not differ at p< 0.05.

CON: No biofertilizer or amendment addition; AMF: Addition of AMF; RAMF: Rock phosphate + AMF addition; MC: Addition of mixed bacterial culture; RMC: Rock phosphate + mixed microbial culture; BC: Addition of biochar; CP: Addition of compost; IF: Addition of recommended dose of synthetic fertilizer).
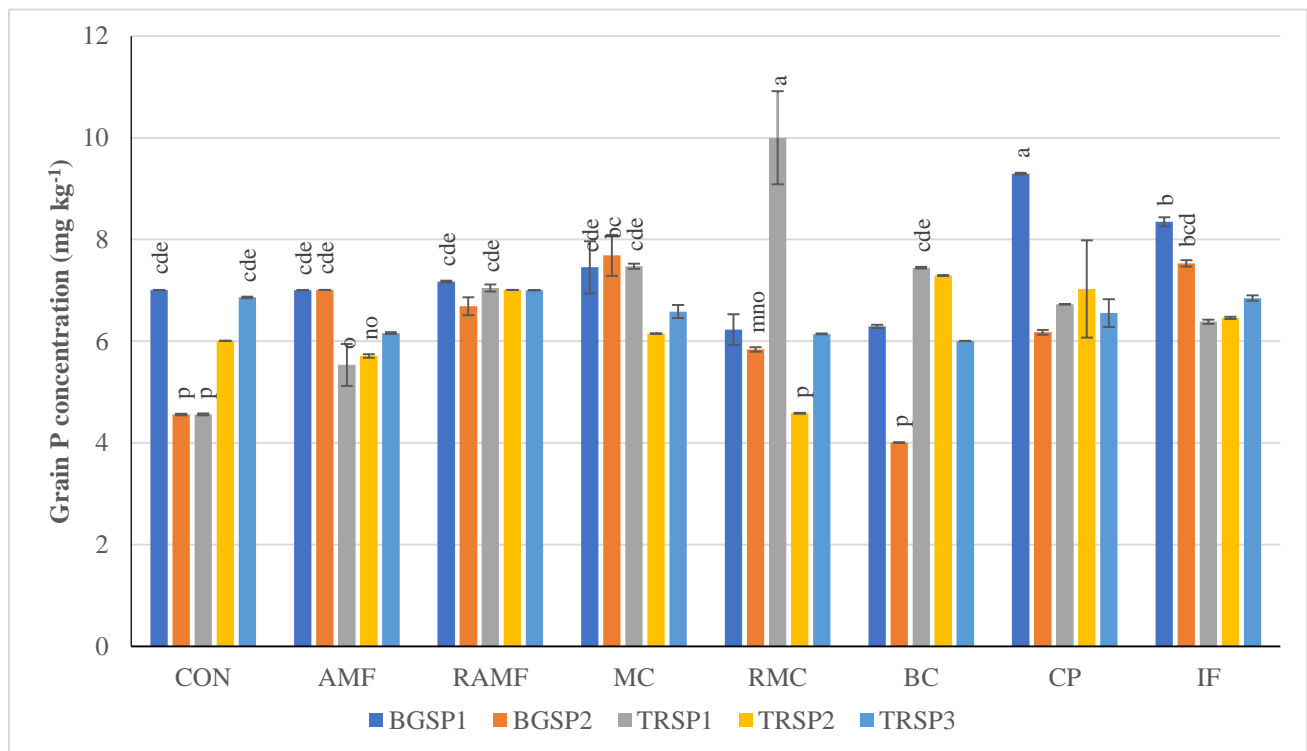

Fig. 6: Mean grain total P (mg kg-1) the amended biofertilizers, natural amendments and synthetic fertilizer applications and growth of the different tested rice varieties. Error bars indicate 95% confidence intervals. Means denoted as same letters do not differ at p< 0.05.

CON: No biofertilizer or amendment addition; AMF: Addition of AMF; RAMF: Rock phosphate + AMF addition; MC: Addition of mixed bacterial culture; RMC: Rock phosphate + mixed microbial culture; BC: Addition of biochar; CP: Addition of compost; IF: Addition of recommended dose of synthetic fertilizer).

IV. CONCLUSION

The multifunctional microbial consortium in amended soil, include *Azospirillum* sp., *Pseudomonas* sp. *Bacillus* sp. and arbuscular mycorrhizal fungi, capable of potentially increasing soil P. Furthermore, the addition of rock phosphate does not always make a difference in P availability in the soil for rice plants. However, rice soil management using biofertilizers, organic and synthetic fertilizers still do not have enough P when compared with ideal soil conditions in fields. Although not considered in this study, monitoring microbial cells and activity during the production and formulation of inoculants and identification of the most efficient microbes which influence on the supply of P is important to produce more effective biofertilizers for the rice farming in dry zone, Sri Lanka.

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