

# Addition of Coffee Residue to Acid Soil Enhanced Microbial Biomass Phosphorous Accumulation and Increased Ryegrass Yield

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## Abstract

The majority of phosphorous (P) in most acid soils remains in insoluble forms and unavailable to plants. This study aimed to find out the effect of coffee residue amendment on the microbial biomass P accumulation, P availability and its uptake by Italian ryegrass in a low P supplying acid soil. Three levels of P *viz.*, 0, 30 and 80 kg ha<sup>-1</sup> with or without coffee residue were tested in this study. Addition of coffee residue combined with inorganic P significantly influenced the soil microbial biomass C and P, available P, dry matter yield, P content and uptake by Italian ryegrass. The highest microbial C and P, available P, dry matter yield and P uptake by ryegrass were obtained from 80 kg P with coffee residue addition. In contrast, phosphorous use efficiency (PUE) was highest in the plants treated with 30 kg P with coffee residue achieving 78.5% higher PUE over control. Dry matter yield and P uptake by rye grass was significantly and positively correlated with the decrease in microbial biomass P in soil indicating the contribution of this P fraction to the biosynthesis of dry matter and P uptake. The results suggest that organic materials like coffee residue of wide C: P ratio along with low doses of inorganic P could increase the P availability, uptake and use efficiency of plant in low P supplying acid soil.

## Keywords

Coffee Residue, Italian Ryegrass, Acid Soil, Microbial Biomass P, P Uptake

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## 1. Introduction

Phosphorus (P) has been identified as one of the most deficient essential nutrients in many agricultural crop production systems after nitrogen (N) [1, 2]. It must be applied either as organic or inorganic forms for optimal crop production in soils with low available P [3]. Nutrient inputs in the production systems have been increased for high yielding crops to sustain the growing population all over the world for last few decades [4]. There is an increasing awareness for the efficient use of P fertilizer because of the limits of global rock phosphate reserves [5-7], and to prevent the adverse effects on water quality through the transfer of

soil and fertilizer P to surface water [8].

The fertilizer P use efficiency (PUE) of crops is very low and ranges from 10 to 30% [8-10] due to the strong retention of phosphate ions by reactive soil components especially in low pH soil [11]. The remaining 70 to 90% P becomes part of the soil pool and is available for the crop over the following years [12]. This pool contributes to future crop production, increasing the efficiency of fertilizer by improving crop recovery in the following year of application. Recovery of applied P fertilizers by field crops is very inefficient [13]. Since water soluble P fertilizers are continually displaced through leaching, bulk water movements and aerial erosion, adjacent terrestrial and marine environments are susceptible

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to eutrophication due to nutrient enrichment [14-16]. This situation can lead to habitat loss and a decline in biodiversity [17]. Thus, it makes environmental and financial sense to increase the PUE of crop production by reducing fertilizer P inputs whilst maintaining crop yields and quality.

The fertilizer use efficiency of a crop production system (yield per unit input of fertilizer) is determined by the genetic characteristics of the crop plant and by the environment where the crop is grown [18]. Fertilizer use efficiency can thus be increased by modifying either the environmental condition or by growing varieties with enhanced nutrient use efficiency [8, 15, 19]. The P use efficiency can also be improved significantly by the integrated (organic and inorganic) application of P [20]. Organic fertilization can increase the availability of P in soil, due to the influence on chemical, physical and biological soil properties [11]. The amount of available soluble P depends on pH, extent of contact between the precipitated P and the soil solution, the rate of dissolution and diffusion of solid phase P, time of reaction, organic matter content, temperature and type of clay present [8, 21].

Although inorganic fertilizers are readily available, they are slowly converted to unavailable forms due to precipitation [22]. During early growth stages, plants may utilize the readily available form, while they compete for the slowly available forms in the later stages of growth. Hence, microbial biomass P can play a vital role to supply the slowly available forms since this fraction is highly labile and constitute as much as 20% of the total soil P [23]. In a low pH soil, if water soluble chemical fertilizers like triple superphosphate (TSP) or single superphosphate (SSP) are added, they will undergo some changes and will be fixed as Fe, Al or Mn phosphate which will not be available to the growing plants at any stage of growth [10]. Under these circumstances, if water soluble inorganic fertilizers are added with some organic materials like coffee residue of wide C: P ratio containing very low amounts of P compared to C, most of the added P is likely to be converted to microbial pool which can then be slowly released to the available pool at the later stages of plant growth and can increase the P use efficiency of crops. Here in this study different level of inorganic P was applied in combination with coffee residue to (i) determine the dynamics of soil microbial biomass, dry matter yield and P concentration of Italian ryegrass, (ii) examine the relationships between soil microbial biomass P and plant P uptake, and (iii) determine the P use efficiency of Italian ryegrass in low P supplying soil.

## 2. Material and Methods

### Soil and coffee residue

The acid soil used in the experiment was collected from 0-15

cm layer of a fallow land of Madhupur Upazila, Tangail, Bangladesh (24.6119° N, 90.0315° E). The soil had never received any fertilizer amendment and was bare during soil sampling. After sampling, the soil was air dried (20°C), the undecomposed plant materials were removed carefully and subsequently sieved to 2 mm. Soil samples were incubated aerobically at 25°C and 40% water holding capacity (WHC) for 10 days to allow the soil microbial population to stabilize, minimizing the effects of soil handling and preparation. The used coffee residue applied in this experiment was obtained from a café, Dhaka City, Bangladesh. The physico-chemical properties of acid soil and coffee residue are presented in Table 1.

**Table 1.** Physico-chemical properties of soil and coffee residue used in the experiment.

Physico-chemical properties	Soil	Coffee residue
USDA soil order	Oxisol	ND
Texture	Loam	ND
Bulk density (g cm <sup>-3</sup> )	1.15	ND
Moisture content (%)	2.2	8.4
pH <sub>water</sub> (1:5)	5.1	7.62
Organic C (%)	3.4	63.3
Total N (%)	0.37	2.6
Total P (%)	0.04	0.10
C: N ratio	9.2	24.4
C: P ratio	89.5	633
Olsen P (µg g <sup>-1</sup> )	3.5	ND
Ammonium N (mg kg <sup>-1</sup> )	6.1	ND
Nitrate N (mg kg <sup>-1</sup> )	14	ND
Colwell P (mg kg <sup>-1</sup> )	31	ND
Ca (%)	0.17	0.41
Mg (%)	0.03	0.19
S (%)	0.06	ND
K (%)	0.02	2.12
Mn (%)	0.0004	0.01
Al (%)	6.1	0.53
Fe (%)	9.5	0.22
Zn (mg kg <sup>-1</sup> )	35	ND
Cu (mg kg <sup>-1</sup> )	6.2	ND
Lignin (%)	ND	26.3
Cellulose (%)	ND	29.4
Hemi-cellulose (%)	ND	11.2

ND: Not determined

### Pot experiment

Freshly collected coffee residue was incorporated to the soil at the rate of 10 t ha<sup>-1</sup> (Oven dry basis) and then equivalent of 4.5 kg oven dried soil was placed in each Wagner pot (0.02 m<sup>2</sup>). Three levels of P viz., 0, 30 and 80 kg ha<sup>-1</sup> were applied. Phosphorous was applied as Ca(H<sub>2</sub>PO<sub>4</sub>) along with basal dressing of N and K @ 200 kg ha<sup>-1</sup> from NH<sub>4</sub>NO<sub>3</sub> and K<sub>2</sub>SO<sub>4</sub>, respectively. The experiment was laid out following completely randomized design with five replicates. One hundred Italian ryegrass (*Lolium multiflorum* Lam) seeds were sown uniformly in each pot. The pots were incubated in a glasshouse maintaining the temperature at 25±1°C for 120 days. Adequate moisture was maintained by regular addition

of deionized water. Soil samples were collected at 7, 15 and 45 days after sowing (DAS) for microbial biomass C, P and available P determination. Plants (shoot and root) were harvested at 45 DAS, and cleaned, washed with deionized water, air dried, and then finally oven dried at 70°C for 96 hours before weighing. The samples were finely ground, packed in polyethylene bags, labeled and stored for chemical

analyses. Soil and plant parameters *viz.*, soil microbial biomass C and P, available P (Olsen) concentration in soil, shoot and root dry weight, shoot and root P concentration, P uptake by shoot and root were analyzed.

Phosphorous uptake, utilization and use efficiency were calculated following the below mentioned formulae

$$\text{Phosphorus Uptake Efficiency (PUPE)} = \frac{\text{P in plant (kg)}}{\text{P in soil (kg)}} \quad (1)$$

$$\text{Phosphorus Utilization Efficiency (PUTE)} = \frac{\text{Yield (kg)}}{\text{P in plant (kg)}} \quad (2)$$

$$\text{Phosphorus Use Efficiency (PUE)} = \frac{\text{Yield (kg)}}{\text{P in soil (kg)}} \quad (3)$$

Fertilizer P uptake, utilization and use efficiency were also calculated following the below mentioned formulae

$$\text{Fertilizer P Uptake Efficiency (FPUPE)} = \frac{(\text{P in fertilized plant} - \text{P in control plant})}{\text{P applied}} \quad (4)$$

$$\text{Fertilizer P Utilization Efficiency (FPUTE)} = \frac{(\text{Yield of fertilized pot} - \text{yield of control pot})}{(\text{P in fertilized pot} - \text{P in control pot})} \quad (5)$$

$$\text{Fertilizer P use Efficiency (FPUE)} = \frac{(\text{Fertilized pot yield} - \text{control pot yield})}{\text{P applied}} \quad (6)$$

#### *Analytical procedures*

Soil pH was determined at a soil-to-water ratio of 1:5 with a glass electrode. Organic carbon (OC) content of oven-dried (70°C) soil and coffee residue was measured by dry combustion at 1050°C using a TOC-analyzer (Shimadzu TOC-5000). Total N concentration was determined by Kjeldahl method [24]. Total and available P concentrations were determined following standard methods as described by Rayment, Lyons [25]. The shoot and root P uptake were calculated by multiplying the P concentration (%) with their corresponding shoot and root dry weight and divided by 100. Soil microbial biomass C was determined by the fumigation extraction (FE) method with 0.5 M K<sub>2</sub>SO<sub>4</sub> as the extractant according to [26]. The microbial biomass C (B<sub>C</sub>) was calculated from the equation: B<sub>C</sub>=2.22×E<sub>C</sub> where, E<sub>C</sub> is the organic C extracted from the fumigated sample minus organic C extracted in the unfumigated samples. The extracted organic C was analyzed by a total organic C analyzer (Shimadzu TOC-5000). Soil microbial biomass P was also determined by fumigation extraction method [27, 28]. Three portions of pre-incubated moist soil (equivalent to 2.5 g oven dry weight) were taken and each of them was extracted with 50 mL 0.5 M NaHCO<sub>3</sub> (pH 8.5) after different pretreatment *i.e.*, the first after fumigation, the second after un-fumigation and the third after the addition of 25 μg P g<sup>-1</sup> soil as KH<sub>2</sub>PO<sub>4</sub> to the

extractant. P contents were determined and the microbial biomass P was calculated by a K<sub>EP</sub> value of 0.40 [27].

#### *Statistical analyses*

The statistical analyses were performed using the software package IBM SPSS, version 20 (SPSS IBM, 2010). For all tests, the level of significance was carried out at p< 0.05. The normality testing of the data was conducted using the Kolmogorov-Smirnov goodness of fit test and equality of variances was tested using the modified Leven's test. A one-way analysis of variance (ANOVA) was performed to observe whether the nutritional quality parameters and P use efficiency of Italian rye grass was affected by different levels of inorganic P fertilization along with coffee residue. Multiple comparisons among the different treatments were done using a Scheffé test.

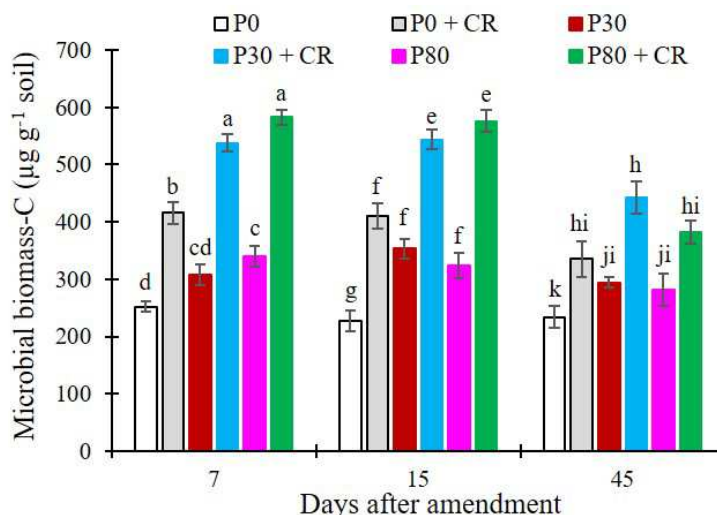
## 3. Results and Discussion

### *Soil microbial biomass C*

Addition of coffee residue in combination with different levels of inorganic P showed a significant influence on the accumulation and dynamics of soil microbial biomass C at different DAS in soil (Figure 1). Microbial biomass C increased rapidly initially after the amendment of coffee residue and inorganic P and peaked at 7 DAS in all most all the

treatments except the soils treated with 30 kg P alone and in combination with coffee residue. After 7 DAS, the biomass C decreased gradually up to 15 DAS and then decreased rapidly and reached to its minimum value at 45 DAS in all the treatments except the control where no amendments were

added. The biomass C increased with increasing levels of inorganic P application and was maximum (583  $\mu\text{g g}^{-1}$  soil) at 7 DAS by the application of 80 kg P along with coffee residue and the minimum (227  $\mu\text{g g}^{-1}$  soil) biomass C was found at 15 DAS where neither coffee residue nor P were applied.

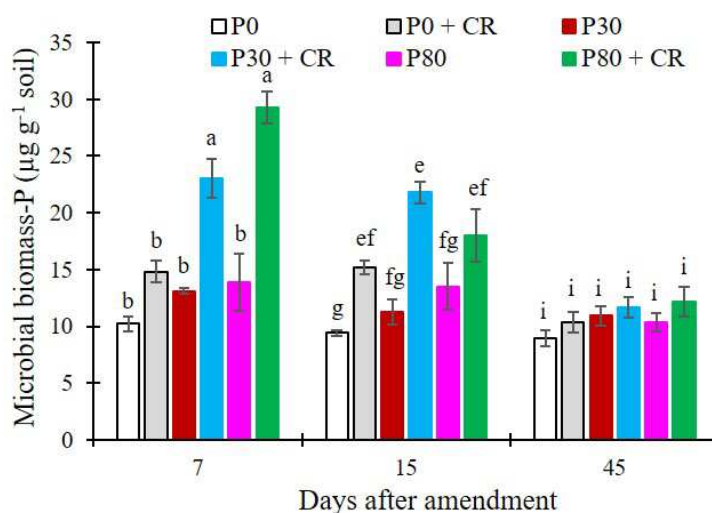


**Figure 1.** Effect of different levels of inorganic P with or without coffee residue addition on the soil microbial biomass C content (values are mean  $\pm$  standard error, N = 5). Letters above columns are different if the values are significantly different ( $P < 0.05$ ).

*Soil microbial biomass P*

Fertilization of inorganic P with or without coffee residue significantly influenced the microbial biomass P at 7 and 15 DAS but no significant variation was found at 45 DAS (Figure 2). Microbial biomass P followed almost similar pattern like microbial biomass C. A sharp increase in biomass P was observed as soon as the inorganic P and coffee residue amendment were added and peaked at 7 DAS in all most all

the treatments except the soils amended with coffee residue only and control soil. Thereafter, the biomass P began to decrease gradually up to 15 DAS and then decreased sharply and reached to its lowest value at 45 DAS in all the treatments. The biomass P was maximum (29.22  $\mu\text{g g}^{-1}$ ) at 7 DAS by the application of 80 kg P along with coffee residue and the minimum (8.98  $\mu\text{g g}^{-1}$ ) biomass P was recorded at 45 DAS where no coffee residue and P was applied.



**Figure 2.** Effect of different levels of inorganic P with or without coffee residue addition on the soil microbial biomass P content (values are mean  $\pm$  standard error, N = 5). Letters above columns are different if the values are significantly different ( $P < 0.05$ ).

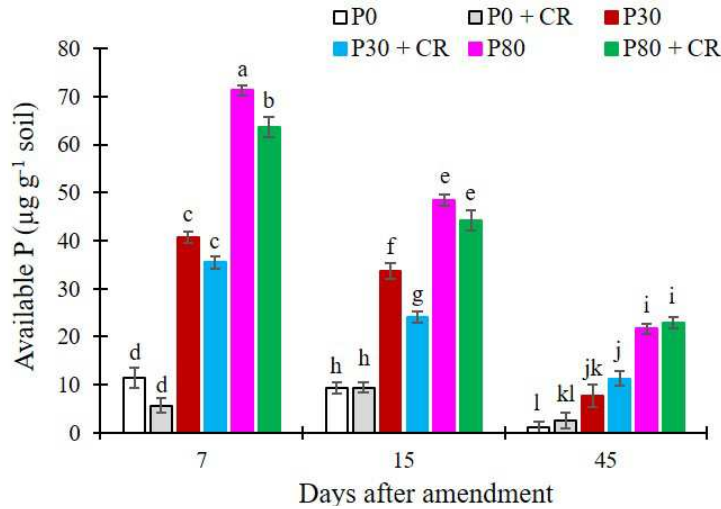
*Soil available P*

Different levels of P with or without coffee residue had a significant influence on the available P content of soil at different

DAS (Figure 3). Available P content increased rapidly just after inorganic P and coffee residue addition and reached to its peak at 7 DAS in all the treatments. Available P increased gradually with

the increased levels of P application. A sharp decrease in available P was observed between 7 and 15 DAS, and then gradually decreased up to 45 DAS. The highest available P ( $71.28 \mu\text{g g}^{-1}$

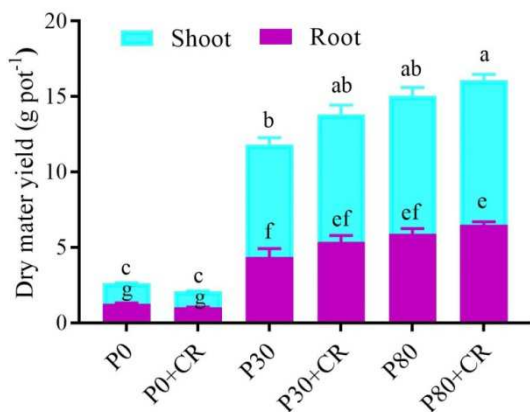
soil) was noticed at 7 DAS when the soil was fertilized with 80 kg P only and the lowest ( $1.11 \mu\text{g g}^{-1}$  soil) was noticed at 45 DAS where no coffee residue and P was applied.



**Figure 3.** Effect of different levels of inorganic P with or without coffee residue addition on the soil available P content (values are mean  $\pm$  standard error, N = 5). Letters above columns are different if the values are significantly different ( $P < 0.05$ ).

*Dry matter yield*

The application of coffee residue and different levels of inorganic P had a significant influence on the dry matter yield of Italian ryegrass. The highest shoot (16.1 g) and root dry matter yield (6.51 g) were observed when the soil was fertilized with coffee residue in combination with 80 kg P and these differed significantly with the same parameters obtained from the other treatment combinations including control (Figure 4). Whereas, the lowest shoot (2.1 g) and root dry matter yield (1.1 g) were found when the soil was amended with only coffee residue (Figure 4).

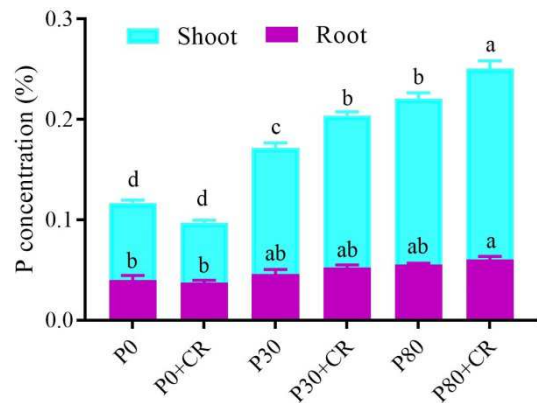


**Figure 4.** Effect of different levels of inorganic P with or without coffee residue addition on the dry matter yield of Italian ryegrass (values are mean  $\pm$  standard error, N = 5). Letters above columns are different if the values are significantly different ( $P < 0.05$ ).

*Shoot and root P concentration*

Significant variation in shoot and root P concentration of Italian ryegrass were observed due to the addition of different

levels of P in combination with coffee residue. The maximum shoot P (0.251%) and root P (0.060%) concentrations were detected from the plant treated with 80 kg P along with coffee residue and were significantly different from other treatments (Figure 5). The lowest shoot and root P concentrations (0.097%) and (0.037%) were determined from the coffee residue amended soil without any inorganic P fertilization.

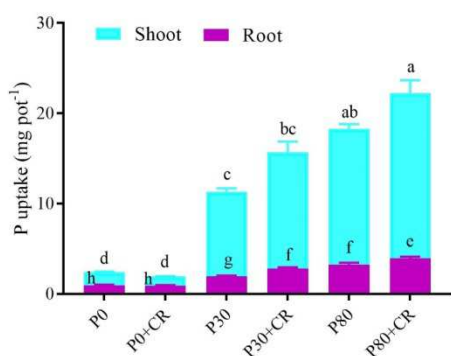


**Figure 5.** Effect of different levels of inorganic P with or without coffee residue addition on the P concentration of Italian ryegrass (values are mean  $\pm$  standard error, N = 5). Letters above columns are different if the values are significantly different ( $P < 0.05$ ).

*Shoot and root P uptake*

Results reveal that application of different levels of inorganic P in combination with coffee residue exerted a significant influence on shoot and root P uptake by Italian ryegrass. Both shoot ( $18.29 \text{ mg pot}^{-1}$ ) and root ( $3.94 \text{ mg pot}^{-1}$ ) P uptake were maximum in plants grown on soil amended with coffee residue along with 80 kg P and the minimum for both cases were found from the plant amended with coffee residue only (Figure 6).





**Figure 6.** Effect of different levels of inorganic P with or without coffee residue addition on P uptake by Italian ryegrass (values are mean  $\pm$  standard error, N = 5). Letters above columns are different if the values are significantly different ( $P < 0.05$ ).

**Table 2.** Phosphorus uptake, utilization and use efficiency of Italian Ryegrass as affected by the addition of different levels of inorganic P with or without coffee residue (mean values  $\pm$  standard error, N = 5).

P levels with or without coffee residue	Phosphorus uptake efficiency (PUPE)	Phosphorus utilization efficiency (PUTE)	Phosphorus use efficiency (PUE)	% increase over control
P0	0.124 $\pm$ 0.01f	1106 $\pm$ 68a	135 $\pm$ 8b	-
P0 +CR	0.218 $\pm$ 0.04b	1093 $\pm$ 47a	147 $\pm$ 6b	4.44
P30	0.162 $\pm$ 0.01d	1044 $\pm$ 18a	169 $\pm$ 7b	25.18
P30 +CR	0.257 $\pm$ 0.01a	888 $\pm$ 51b	241 $\pm$ 56a	78.51
P80	0.149 $\pm$ 0.01e	821 $\pm$ 28bc	139 $\pm$ 8b	2.96
P80 +CR	0.204 $\pm$ 0.01c	728 $\pm$ 35c	227 $\pm$ 10a	68.14

Means with different letter (s) are significantly different from other treatments according to Scheffé-test ( $p < 0.05$ )

#### Phosphorous utilization efficiency (PUTE)

Phosphorous utilization efficiency is the kg of dry matter yield divided by kg of P in plant, measure the efficiency of P in plant is utilized for producing economic yield. Application of P significantly decreased the PUTE of Italian ryegrass in soil compared to control. The PUTE decreased with the increased level of P application. The PUTE was maximum (1106) when no P and coffee residue was applied, and it was minimum (728) when the soil was amended with coffee residue along with 80 kg P (Table 2).

#### Phosphorous use efficiency (PUE)

Phosphorous use efficiency is a measure of the economic yield produced per unit P in the soil. Phosphorous use efficiency was significantly influenced by the application of different levels of P in combination with coffee residue. It was higher in combined application of coffee residue and inorganic P compared to single application of P. Phosphorous use efficiency decreased with higher levels of P. The highest PUE was found in soil amended with coffee residue in combination with 30 kg P achieving 78.51% higher PUE over control (Table 2). The PUE in 80 kg P applied soil was lower than control soil indicating higher P fixation in this treatment in acidic soil. The PUE was 96% higher in soil

#### Phosphorous uptake efficiency (PUPE)

Phosphorous uptake efficiency is the ratio of kg P in plants and kg P in soil. It was significantly greater when coffee residue was applied with inorganic P compared to single application of P and increased with the higher levels of P up to 30 kg and then declined. The highest PUPE (0.257) was observed when the soil was amended with coffee residue along with 30 kg P but a rapid decline in PUPE was found when coffee residue was applied with 80 kg P. The lowest PUPE (0.124) was recorded from the absolute control treatment (Table 2).

amended with coffee residue + 30 kg P than 80 kg P applied soil without coffee residue (Table 2). The lowest value (135) was obtained from the soil which was neither amended with coffee residue nor fertilized by any P levels (Table 2).

#### Fertilizer P uptake efficiency (FPUPE)

Fertilizer P uptake efficiency was significantly influenced by the application of different levels of P along with coffee residue. The FPUPE decreased with increased rate of P. The maximum FPUPE (0.27) was obtained when the soil was amended with coffee residue along with 30 kg P and it was minimum (0.15) in soil which was fertilized with 80 kg P only (Table 3).

#### Fertilizer P utilization efficiency (FPUTE)

Fertilizer P utilization efficiency decreased gradually with increased levels of P application. The highest (1032) FPUTE was recorded in soil fertilized with 30 kg P only and the lowest (693) value was found in soil amended with coffee residue in combination with 80 kg P (Table 3).

#### Fertilizer P use efficiency (FPUE)

Application of coffee residue in combination with different levels of P had a significant influence on FPUE and decreased with increased P level. The FPUE was maximum (231) in soil fertilized with 30 kg P along with coffee residue and the minimum value (121) was found in soil fertilized with 80 kg P (Table 3).

**Table 3.** Fertilizer P uptake, fertilizer P utilization and fertilizer P use efficiency of Italian ryegrass as affected by the addition of different levels of P with or without coffee residue (mean values  $\pm$  standard error, N = 5).

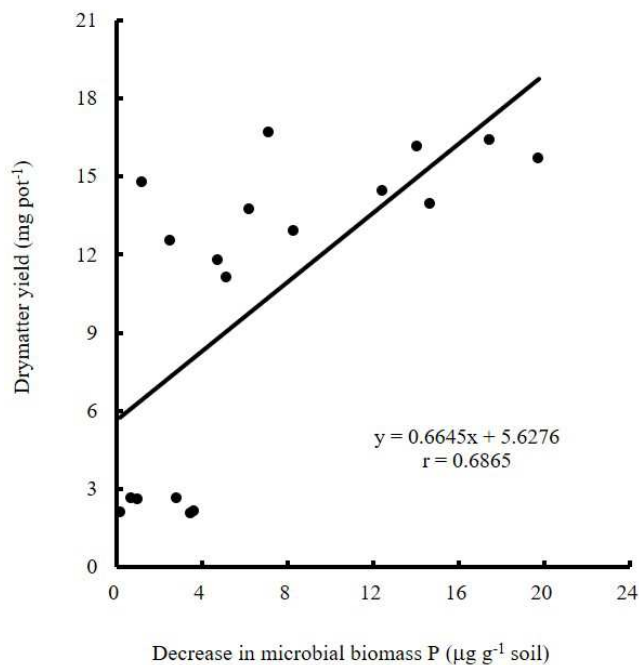
P levels with or without coffee residue	Fertilizer P uptake efficiency (FPUPE)	Fertilizer P utilization efficiency (FPUTE)	Fertilizer P use efficiency (FPUE)
P0	-	-	-
P0 +CR	-	-	-
P30	0.18 $\pm$ 0.01c	1032 $\pm$ 43a	184 $\pm$ 13b
P30 +CR	0.27 $\pm$ 0.02a	861 $\pm$ 60b	231 $\pm$ 21a
P80	0.15 $\pm$ 0.01c	779 $\pm$ 32bc	121 $\pm$ 9c
P80 +CR	0.21 $\pm$ 0.02b	693 $\pm$ 33c	141 $\pm$ 9c

Means with different letter (s) are significantly different from other treatments according to Scheffé-test ( $p < 0.05$ )

Relationships among soil microbial biomass P, dry matter yield and total P uptake by Italian rye grass

*Microbial biomass P and dry matter yield of Italian rye grass*

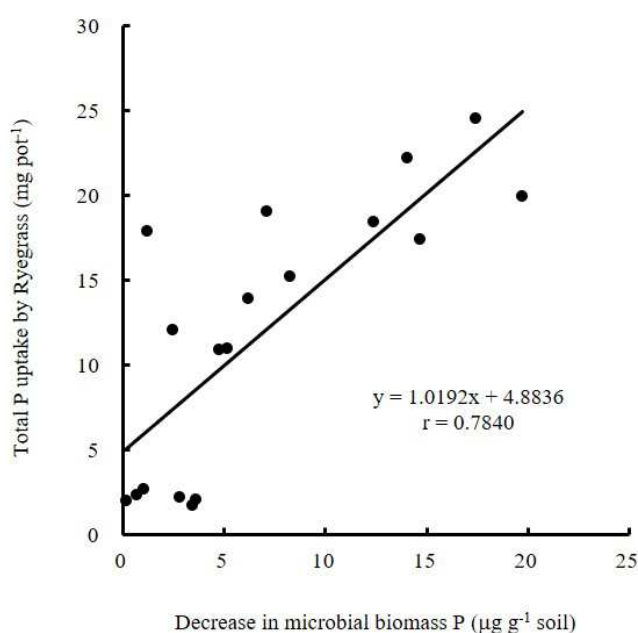
Dry matter yield of any plant species depends on many physiological and biochemical factors. Among them soil microbial biomass P may play an important role in increasing dry matter. The co-efficient  $r$  value ( $r = 0.6865$ ) clearly indicates that dry matter yield was significantly and positively correlated with the decrease in soil microbial P (Figure 7). The positive slopes indicate that microbial biomass P contributed to the increase in dry matter yield of ryegrass.

**Figure 7.** Relationship between dry matter yield of Italian ryegrass and decrease in soil microbial P in soil as influenced by the addition of different levels of inorganic P with or without coffee residue.

*Microbial biomass P and total P uptake by Italian rye grass*

Positive correlation between microbial P and total P uptake by ryegrass was also observed (Figure 8). The co-

efficient  $r$  value ( $r = 0.7840$ ) clearly shows that total P uptake by ryegrass was significantly and positively correlated with the decrease in microbial P. The  $r$  value and the slopes of this equation indicate that soil microbial biomass P certainly increased the total P uptake by ryegrass (Figure 8).

**Figure 8.** Relationship between total P uptake by Italian ryegrass and decrease in soil microbial P in soil as influenced by the addition of different levels of inorganic P with or without coffee residue.

Phosphate fixation and low availability is a major soil fertility constraint in acid soils containing large amounts of free iron and aluminium oxides. By comparing the P-sorption capacity of surface and subsurface soil samples, Guppy, Menzies [11] provided indirect evidence that soil organic matter can reduce the P-sorption capacity of such soils. This implies that for high P-fixing soils, i.e. oxide-rich soils derived from volcanic and ferro-magnesian rocks, management systems are capable of accumulating and maintaining greater amounts of calcium-saturated soil organic matter in the surface horizon would increase P availability from both organic and inorganic fertilizer sources. The chemical and nutritional benefits of organic matter are related to the cycling of plant nutrients and the ability of the soil to supply nutrients for plant growth.

Organic matter retains plant nutrients and prevents them leaching to deeper soil layers. Microorganisms are responsible for the mineralization and immobilization of N, P and S through the decomposition of organic matter [11]. Thus, they contribute to the gradual and continuous liberation of plant nutrients. Available nutrients that are not taken up by the plants are retained by soil organisms. Considering this concept, we used organic materials like coffee residue of wide C: P ratio (>700) to increase the P use efficiency by ryegrass. Our target was to biologically convert the fertilizer P to microbial pool which could be used as slow release fertilizer at the later stages of plant growth.

#### *Effect of inorganic P and coffee residue on microbial biomass C and P formation*

The initial increase in biomass C in coffee residue amended soils reflect the decomposition of the substrates during the early phase. Maximum biomass C at 7 DAS may be due to high rate of microbial C assimilation at the initial stage (Figure 1). The microbial assimilation of coffee residue-C is comparable to that found using straw amendments [29]. The supply of carbon and energy from the decomposition of coffee residue to sustain the soil microbial biomass was reduced by day 15 resulting in a significant decrease in biomass-C. On the other hand, the microbial biomass C was significantly higher in coffee residue amended soil than single application of inorganic P. This pattern of change in biomass-C for the coffee residue amended soil might be due to the gradual decomposition of coffee residue which provided a sustained supply of carbon and energy to maintain higher amounts of microbial biomass over an extended period. The results of our study are in good agreement with those of Cogle, Saffigna [30] who explained that the labile fractions of straw were rapidly decomposed during the early phase and the resistant fractions were mineralized in the later stages and produced the slowly metabolizing microbial biomass.

Changes in microbial biomass-P followed the pattern like that of biomass-C irrespective of treatments. The highest biomass P at 7 DAS might be due to high availability of C from coffee residue and P from chemical fertilizers at the initial stage (Figure 2). In the coffee residue amended soil, the marked increase in the microbial biomass-P induced by the decomposition of coffee residue and microbial assimilation of available P. It is well established fact that organic manure addition generally increases the microbial populations and soil biological activity [31], which is in accordance with the present study where coffee residue treated pots showed an increase in biomass P compared to the pots where no coffee residue were added. It has been reported that addition of organic manure could increase the

availability of secondary and micronutrients in the soil, which might also be responsible to increase the microbial population [23]. Moreover, increased microbial biomass P recorded in coffee residue treated pots might be due to the fact that coffee residue provided adequate biomass as a feed for the microbes and helped in increasing microbial population in the soil, as evident in this study.

#### *Effect of inorganic P and coffee residue on dry matter yield and P nutrition of Italian ryegrass*

Application of coffee residue and inorganic P had a significant influence on shoot and root dry weight, root and shoot P uptake by Italian ryegrass (Figures 4-6). As soil P level was raised, shoot and root P concentration, and shoot P uptake increased significantly, and the highest values were found in plants treated with 80 kg P along with coffee residue. Although inorganic P application improved growth and P nutrition of ryegrass with and without coffee residue addition but combined application of P with coffee residue had more pronounced effects rather than single P fertilization. The use of coffee residue improved soil organic matter, physical, chemical and microbial properties that ultimately affect the availability and P nutrition of plants [22, 32]. The findings of our study are in good agreement with the finding of Fageria, Baligar [19] who reported that application of P significantly influence the plant growth and P nutrition of rice. Phosphorus regulates the energy storage, transfers and triggers the meristematic activities in the developing roots and facilitates the uptake of N and other mineral nutrients. Combined application of coffee residue and P performed well compared to P application only, as developing roots were in close contact with P-enriched coffee residue amended soil. In addition, there was a less contact of applied P with soil colloids through coffee residue amendment, which are responsible for precipitation, fixation and retention of phosphorus fertilizer resultantly P availability to plants was improved [33]. Improvement in shoot and root P contents with increase in P application rate seems due to higher P contents in soil solution, which were available to plant.

#### *Effect of inorganic P and coffee residue on P use efficiency of Italian rye grass*

Phosphorus uptake efficiency (PUPE) was significantly higher in soil fertilized with inorganic P with coffee residue compared to without coffee residue. The PUPE increased with the increase in rate of P applied up to 30 kg and then declined with the increase in P level. Phosphorus use efficiency was higher in coffee residue amended soil with or without inorganic P compared to sole application of P (Table 2). Averaged over all the treatments, the soil incorporated with only coffee residue showed best PUE of



Italian rye grass. Integrated use of organic and inorganic P fertilization increased PUE by 30% in wheat compared to single application of inorganic P [33]. Application of P increased PUE up to 30 kg but decreased at higher P dose. Nonetheless, higher PUE at lower P level might be the result of intense root competition and thereby an efficient exploitation of applied P. Similarly, at higher P application rates plants used smaller proportion of fertilizer P due to the greater fixation that resulted in low PUE [33]. Higher available P in coffee residue amended soil compared with combined application of P and coffee residue might be due to fixed and unutilized P left over as microbial P in coffee residue amended soil [8]. Moreover, there were low chances for P adsorption in coffee residue amended soil because of less contact with soil. Shenoy and Kalagudi [8] reported that P retention in roots is improved enough levels, which may increase PUE at low P level. Therefore, higher P uptake by coffee residue addition resulted in higher PUE in Italian ryegrass in this study. Fertilizer P use efficiency of Italian ryegrass was also higher at coffee residue incorporated soil along with inorganic P compared to the soil without any coffee residue incorporation (Table 3). FPUE decreased with the increase in rate of P fertilization. It might be due to the higher PUE at low P level. Thus, the finding of our study is in good agreement with the finding of Yamoah, Bationo [20] who reported that fertilizer P use efficiency improved significantly, when integrated (organic and inorganic) source of P was used.

## 4. Conclusions

Results reveal that dry matter yield, P uptake and PUE of Italian ryegrass was increased when low levels of inorganic P was applied along with an organic material like coffee residue of wide C: P ratio. Our results clearly suggest that integrating coffee residue with low doses of inorganic P increased microbial assimilation of inorganic P which enhanced P availability to plants compared to application of P alone. As a result, greater amounts of fertilizer P were available to ryegrass over a longer period of time, especially at the later stages of growth increasing yield and PUE compared to single application of P. In addition, incorporation of extra carbon as coffee residue will help to improve organic matter content of soil. This study supports previous reports that the efficacy of commercial P fertilizers can be improved substantially by combined application of coffee residue and inorganic P and could contribute to solve the problem of P availability in P deficient low pH soils. This research has increased our understanding of how coffee residue can be used as an organic matter to improve P availability and use efficiency

of ryegrass. Additional work is now necessary to study the effect of coffee residue with low doses of inorganic P on plant growth and P uptake in various soil types and under field conditions.

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## Conflict of Interest

The authors declared that they have no conflict of interest.

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