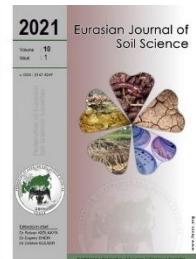




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Phosphorus mineralization in response to organic and inorganic amendment in a semi-arid pasture soil

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Abstract

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Phosphorus deficient soil was amended with compost (C) (organic source of phosphorus) and inorganic P (KH_2PO_4 as inorganic phosphorus) at different rates and incubated for 28 days. Six treatments were used including i) Control ii) Inorganic P (0.79 mg per 30 g of soil sample) iii) 100 % C (0.13 g) iv) 75% C (0.1 g) + 25% P (0.2 mg) v) 50% C (0.065 g) + 50% P (0.4 mg) vi) 25% C (0.03 g) + 75% P (0.6 mg). Soil respiration was recorded using Infra-red CO_2 gas analyzer. MBC was determined by using fumigation extraction method. Resin P and MBP extraction was carried out by anion exchange membranes and was determined colorimetrically. P pools were determined by using DeLuca method. Cumulative respiration microbial biomass significantly increased in organic amended soil with higher increase in soil emended with 75% C +25% P rate followed by 50% C and 50% P rate. It was concluded that compost amended with high inorganic P stimulated the formation of P labile pools which supply long term slow release of P for plants and microbes.

Keywords: Compost, microbial dynamic, phosphorus pools, pasture soil.

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Introduction

Phosphorus is one of the essential nutrients which directly affect crop production and crop quality (Maranguit et al., 2016) also phosphorus is non-renewable resource which highlights the importance of phosphorus for sustainable agriculture (Horta et al. 2018). Only 10-20% of applied P is taken up by the plants because majority of applied P is quickly fixed or precipitated as poorly available P resulting in the formation of large unavailable P bank (Khan et al., 2016). Organic amendments increase soil fertility by increasing organic matter contents and supply of essential crop nutrients (Partey et al., 2014). Incorporation of organic materials to soil cause rapid increase in microbial activity and growth (Schneider et al., 2016). Organic amendments are considered to mobilize P in the soil by releasing organic acids which replace P from the fixation sites in the soil complex. Increase in P availability depends upon the type of organic amendments added to soil and their C:P ratio. Soil microbes play key role in transformation of P by decomposing and providing P back in the soil system in the form of microbial biomass (Brennan et al., 2013). Moreover, microbes can also stimulate and enhance P availability by releasing organic molecules during the decomposition of organic materials which ultimately block the sorption sites for P (Damon et al., 2014). Combined application of inorganic P fertilizer and organic sources such as crop residues, organic manure and organic wastes have the potential to stimulate soil microbial biomass synthesis and labile microbial metabolites which is labile P pool and this pool is protected in soil against fixation (Mackay et al., 2017). A better understanding of the relationship between type of organic amendment, microbial activity and changes in soil P pools is important for a better management of soil P. Present study was conducted to



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determine the effect of organic and inorganic P sources on P pools and microbial activity in a pasture soil with low P availability. Here, we tested whether the changes in P pools induced by organic amendment is applicable or not. Compost was used as organic source. Compost was used as amendment because of nutrients they have for soil, microbes and plants. Compost have high Organic C which increase soil organic matter and stimulate soil microbial biomass and other crop nutrients necessary for soil fertility and crop productivity (Sun et al., 2014).

Material and Methods

Experimental Setup

Silt loam soil was collected from 0 to 15 cm depth in Urrbrae, South Australia (Longitude 138°38'3.2" E, Latitude 34°58'0.2"S). This site is in a semi-arid area and has a Mediterranean climate with cool, wet winters. The hot, dry summers can be interrupted by short, heavy rainfall events. Soil was collected along a transect in three 2 x 2 m plots which were at least 10 m apart. In each sampling plot, after removal of plants and surface litter, five samples of topsoil (0–15 cm) were taken and sieved to less than 2 mm followed by air-drying in a fan-forced oven at 40°C. Soil had following properties: 22% sand, 60% silt, 18% clay, maximum water holding capacity (WHC) 371 g kg⁻¹, pH (1:5) 6.3, electrical conductivity (EC) (1:5) 143 µS cm⁻¹, total organic C 17 g kg⁻¹, total organic N 1.5 g kg⁻¹, bulk density 1.3 g cm⁻¹, available P 10 mg P kg⁻¹ and available N 15 mg N kg⁻¹ (Table 1).

Table 1. Physico-chemical properties of semi-arid pasture soil

Property	Soil
Texture	Silt loam
Sand (%)	22
Silt (%)	60
Clay (%)	18
Bulk Density (g/cm ⁻³)	1.3
Water Holding Capacity (g/kg)	378
pH _{1:5}	6.3
EC _{1:5} µS/cm	143
Organic Carbon (g/kg)	17
Available N (mg/kg)	15
Total N (g/kg)	1.5
Available P (mg/kg)	10
Total P (mg/kg)	371

Soil was incubated prior to experiment for seven days to avoid the flush of microbial activity and water holding capacity was adjusted at 50%. After pre-incubation of unamended soil six treatment were added i) Control ii) P fertilizer in KH₂PO₄ form at the rate of 0.79 mg for 30 g soil iii) 100% compost (0.13 g for 30 g soil) iv) 75% compost (0.1 g for 30 g soil) and 25% P (0.2 mg) v) 50% compost (0.065 g for 30 g soil) and 50% P (0.4 mg) vi) 25% compost (0.03 g for 30 g soil) and 75% P (0.6 mg) (Table 2).

Table 2. Concentration of Compost, Inorganic phosphorus (P) and Nitrogen (N) added in 30 g of soil

Treatments	Organic Addition	Inorganic P	N
Control			
Inorganic P		0.790 mg	1.300 mg
100% C	0.130 g	0.200 mg	1.300 mg
75%C+25%P	0.100 g	0.400 mg	1.300 mg
50%C+50%P	0.065 g	0.600 mg	1.300 mg
25%C+75%P	0.030 g		1.300 mg

(C= Compost) (Inorganic P in the form of KH₂PO₄) (N in the form of KNO₃)

Nitrogen was added with each treatment (1.3 mg N for 30 g soil) in the form of KNO₃. Soil was sampled at day 0 (taken approximately 3 hours after amendments addition), 14 and 28 after amending the soil. Each treatment was replicated four time and sampling time was arranged in completely randomized design. After thoroughly mixing the amendments and adjusting the water holding capacity up to 50%, 30 g soil was filled in PVC cores having diameter of 3.7 cm and 5 cm long with the net bottom having mesh size of 0.75 µm (Australian filter specialist) and bulk density was adjusted to 1.4 g cm⁻³. Cores to be sampled were placed in 1 liter jar with air tight lids and equipped with septum for respiration measurement. Centrifuge tubes containing 10 ml RO water were placed along with each core inside the jar to maintain the humidity. Jars and remaining cores were incubated at 25±1 for 28 days. On day 14, samples were collected for determination of

chemical and biological properties. Then the cores to be sampled on 28th day were placed in the jar for measurement of respiration. Soil respiration was measured on days 1, 2, 3, 5, 8, 11, 14, 17, 20 and day 23 by using Infra-red CO₂ gas analyzer. After each measurement lids were open and headspace was refreshed by using a fan. The CO₂ evolved from each sample was calculated as difference between the initial and the final CO₂ concentrations for each measurement period. Infra-red gas analyzer was calibrated by using known amount of CO₂ injected to the glass jars same to the samples used. Soil samples were stored in the cold room at 4°C before determining chemical and biological analysis. P pools were measured in samples from day 0, 14 and 28 days. Compost was analyzed for pH total organic carbon (TOC), Total P and Total N (Table 3).

Table 3. Chemical composition of Compost used as organic source of phosphorus

Compost	Sample Reading
Total C (g/kg)	213.48
Total N (g/kg)	6.63
Total P (g/kg)	4.51
C/N	32.49
C/P	44.74

Analysis of organic material

Walkley-Black method was used to determine the total organic C in the compost (Nelson and Sommers, 1996). Total P was determined colorimetrically after the digestion of 0.5 g compost and cow manure in 6:1 HNO₃:HClO₄ mixture (Westerman, 1990).

Soil Analysis

Hydrometer method was used to determine the particle size distribution (Gee and Bauder, 1986). Soil saturated paste was prepared to determine water holding capacity (Anderson and Ingram, 1993). Soil water content was measured by 24 h drying at 105 °C. Soil pH was measured by 1:5 soil:water suspension. Total organic carbon was determined by using Walkley-Black method (Nelson and Sommers, 1996). Microbial biomass carbon was determined by using fumigation extraction of Vance et al. (1997). Available P (Resin P) and microbial biomass phosphorus extraction was carried out by anion exchange membranes following Kouno et al. (1995) and was determined colorimetrically described by Murphy and Riley (1962). P pools were determined by using DeLuca method (Biologically-Based Phosphorus Extraction Method) (DeLuca et al., 2015). CaCl₂-extractable P was measured by dissolving 1.11 g CaCl₂ in 800 ml DI water. To extract Citric acid associated P, 2.1 g citric acid was dissolved in 600 ml DI water. Enzyme solution was prepared for extracting phosphatase associated P. Reagent A and reagent B were prepared for extracting HCl associated P.

Statistical Analysis

For each sampling time there were four replications of each treatment. Cumulative respiration was analyzed by one-way ANOVA and rest of the data was analyzed by one-way ANOVA. Means were compared by Tukey Test at 5% level of significance.

Results

Cumulative Respiration

Compared to un-amended control all the treatments increased the cumulative respiration with the greatest increase in 75% C+25% P amended soil unlikely in soil amended with 100% C. Possible reason behind this is that compost was already degraded and soil was also P deficient that's why maximum respiration was recorded from soil amended with 75% C+25% P (Figure 1). In comparison to organic P sources, cumulative respiration was lower under inorganic P. Overall, organic amendment increased the soil respiration compared to un-amended control and soil treated with inorganic fertilizer. Maximum cumulative respiration was observed under the treatments where more organic C was added in the soil.

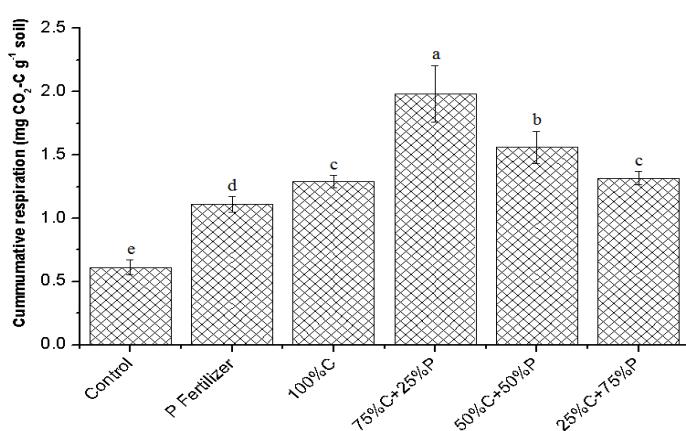


Figure 1. Cumulative respiration in response to inorganic P and compost amendments during 28 days of incubation period

Microbial biomass

Microbial biomass C (MBC) was higher in all soils amended with organic and inorganic fertilizer compared to un-amended control. Under all the treatments of compost, microbial biomass C was higher at day 0 with maximum increase in soil amended with 75% P+25% C followed by 100% C (Figure 2). MBC changed over time, in P inorganic fertilizer MBC increased up to day 14. Under, 75% C+25% P amended soil MBC significantly increased after day 14. In microbial biomass carbon soil amended with 100% and 75% Compost performed better than other treatments because of the maximum concentration of organic C present in compost. Microbial biomass P (MBP) was higher in all compost amended soils compared to P fertilizer alone and un-amended soil with the highest increase in 75% C+25% P amended soil (Figure 3). Microbial biomass P significantly changed with time, it was higher at 0 and significantly decreased up to day 14 in all the compost amended soils. In P fertilizer alone and un-amended control MBP remained same at day 0, 14 and 28 also there was no significant difference between P fertilizer alone and un-amended control.

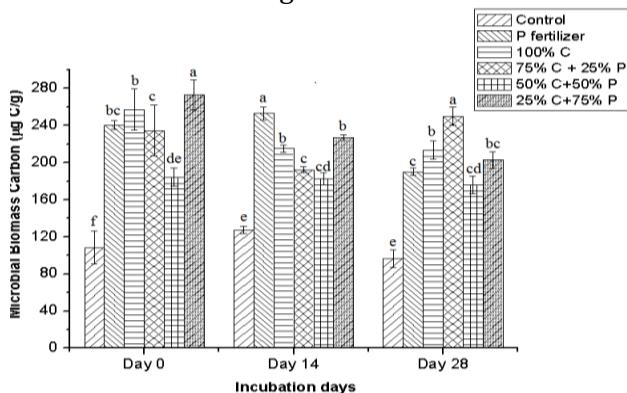


Figure 2. Microbial biomass carbon in response to inorganic P and compost amendments during 28 days of incubation period

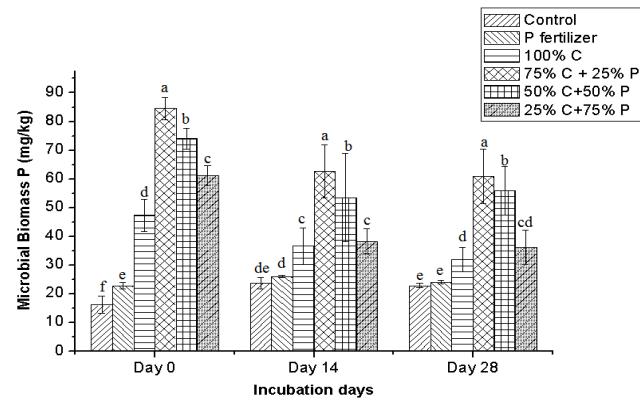


Figure 3. Microbial biomass phosphorus in response to inorganic P and compost amendment during 28 days of incubation period

Available N

Available N was significantly higher in all the amended soils compared to un-amended control with maximum increase in 75% C+25% P followed by 50% C + 50% P amended soil (Table 4). All the organic and inorganic amendments were significantly higher than un-amended control. There was no significant difference in available N between these 75% C+25% P and 50% C + 50% P soils throughout the experiment. Little change was observed in available N throughout the experiment as available N was observed higher throughout the experiment days and it was higher at day 0 and decreased a bit at day 14 and 28.

Table 4. Available N in response to inorganic P and compost amendment

Treatment	Available N (mg/kg)		
	Day 0	Day 14	Day 28
Control	0.27F	0.38F	0.31F
Inorganic P	4.52C	4.34CD	3.93D
100 % Compost	5.84BC	5.21BC	4.87BC
75% C + 25% P	7.44A	6.96A	6.83A
50% C + 50% P	7.13A	6.82B	6.74B
25% C + 75% P	6.97B	6.88A	6.81A

Available P

Resin (Available) P was significantly higher throughout the incubation time in soils amended with compost compared to P fertilizer alone and un-amended control with maximum in soil amended with 50% C+50% P followed by 75% compost + 25% P (Figure 4). Available P changed significantly throughout the study period from day 0 to day 28. Available P was highest at day 0 and decreased up to day 28 in all the treatments. There was significant difference in all sampling dates under P fertilizer alone amended soil. Organic P mixed with inorganic P had significant effect on P availability under all application rates.

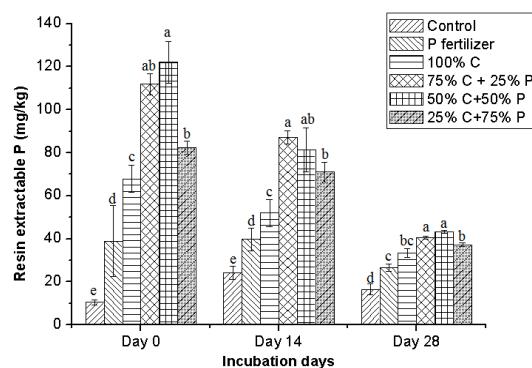


Figure 4. Available / Resin phosphorus in response to inorganic P and compost amendments during 28 days of incubation period

P pools

In the un-amended soil P pools increased in the order Citrate-extractable P (46.8%)> HCL-extractable P_i (38.8%) > CaCl₂-extraxtable P (7.3%) Phosphatase-extractable P (6.9%). Under all the amended treatments concentrations of Citrate-extractable P, HCL-extractable P_i, CaCl₂-extraxtable P_i, Phosphatase-extractable P increased by 2-3 folds compared to un-amended control. Maximum P pools were recorded in soil treated with inorganic P fertilizer which may have through mineralization (Table 5). Among all the amended treatments concentration of P pools were higher in soil amended with 75% organic and 25 inorganic P application rate. Maximum percentage of P pools was in order HCL-extractable P_i (40.4%), Citrate-extractable P (29.24%), Phosphatase-extractable P (18.35%), CaCl₂-extraxtable P (11.92%). Concentrations of P pools significantly decreased from day 0 to day 28 under all the treatments.

Table 5. Effect of inorganic P and compost on phosphorus pools in semi-arid pasture soil

Treatments	Citric acid-extractable P (µg/g)	HCL-extractable P (µg/g)	Phosphatase-extractable P (µg/g)	CaCl ₂ -extractable P (µg/g)
Control	125 B	106 E	23 E	17 D
P fertilizer	130 A	216 A	78 A	63 A
100% C	80 E	125 D	42 C	15 D
75% C + 25% P	114 C	141 C	35 D	37 C
50% C + 50% P	128 A	163 BC	61 B	43 B
25% C + 75% P	102 D	175 B	59 B	44 B
P-value	< 0.05	< 0.05	< 0.05	< 0.05

Discussion

Results of this study showed that inorganic P and application of P with organic amendment like compost resulted in immediate increase in labile P pools compared to un-amended soil. Generally, compost where applied at the rate of 75% C+25% P and 50% C+50% P greatly stimulated the microbial activity and growth also production of P pools and maximum microbial activity was observed under soil amended within organic P and 75% C and 25% P and 50% C and 50% P. Results and findings are being discussed based on amendment composition, forms and percentage of P added with organic amendment. Organic amendment i.e. compost significantly increased soil respiration, microbial biomass C and microbial biomass P which can be explained by the addition of high amount of C and P in the form of compost ([Malik et al., 2013](#)). Application of amendment also resulted in significant increased concentrations of both labile and non-labile P pools at day 0 which can be explained by the presence of soluble P in organic amendment. In this short period of experiment time increase in non-labile P pool (HCL-extractable P_i) can be due to immediate sorption or precipitation but there could be other possible reason that this non-labile P forms can also come from organic amendments ([Schmidt et al., 2011](#)). Different amount of inorganic P was added in compost, compost resulted in smaller increased concentrations of P pools compared to where inorganic P alone was applied, this smaller increase compared to inorganic P can be explained by the strong decomposition of compost and compost may contain complex substances and compounds like lignin and other macromolecules ([Zameer et al., 2010](#)). After the addition of compost formation of labile P pools were increased and this P pools formation was stronger with time. This formation of P pools can be explained that microbial activity and growth was higher and it increased over time and organic P pools can also be formed even when the microbial activity and growth is not increased ([Malik et al., 2013](#)). Microbial biomass C was highest at day 0 and decreased gradually over time which indicated decline in the presence of easily available decomposable organic C and this can be correlated with soil respiration in which amendments immediately increased the CO₂ evolution and after that gradually decreased and at certain point soil respiration was stable and did not changed based on the daily recorded measurement which was also due to not presence of easily decomposable organic C.

By comparing the treatments having low organic P concentration with treatments having high organic P concentration treatments it was observed that having low amount of organic P amendments significantly increased the soil respiration but that increase was lower than the increase induced by treatments having higher amount of organic P added concentrations. Similarly, microbial biomass C was enhanced by treatments having high amount of organic P and this can be explained by the addition of high amount of organic P in the form of compost resulted in high soluble P and easily decomposable organic C. Though, inorganic P addition maintained the available P. A great difference was observed in the formation of P pools between inorganic P treated soil and high P treatments with organic amendments which was clearly due to the low amount of presence of soluble P in inorganic P treatments compared with organic additions.

Conclusion

Results confirmed that organic amendment (compost) applied with inorganic P enhanced the soil respiration, microbial biomass C, Resin P and available P compared to inorganic P applied alone (due to presence of more decomposable organic C and microbial activity). Organic amendment was found less prone to sorption and precipitation compared to inorganic P because all the labile P forms were higher on all sampling days compared to un-amended soil and P fertilizer alone. P pools were also enhanced by both organic and inorganic amendments compared to un-amended control. Under compost amendment soil especially amended with high organic C concentrations along with inorganic fertilizer concentrations stimulated more microbial activity and growth and P pools formation which was due to addition of large amount of water soluble and easily decomposable C and P. Results confirmed that addition of organic P sources in the form of compost in addition to inorganic source can behave as a constant source of nutrients to microbes and plants and enhance the bioavailability of P to plants and microbes.

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