

<https://doi.org/10.37501/soilsa/121491>

Effect of combined application of organic and inorganic phosphatic fertilizers on dynamic of microbial biomass in semi-arid soil

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Abstract

Received: January 23, 2019
Accepted: February 6, 2020
Associated editor: J. Wyszokowska

Keywords

Biogenic waste compost
CO₂ emission
Farmyard manure
Phosphorus availability
Sugar cane filter cake

A silt loam soil was amended with finely ground biogenic waste compost (BWC with C/N 12), farmyard manure (FYM with C/N 19) and sugar cane filter cake (SFC with C/N 14) mixes with inorganic phosphorus fertilizer and also applied in the form of enriched inorganic phosphorus fertilizer. Each organic amendment was applied at the rate of 0.5 mg C g⁻¹ soil, and incubation was carried out at 25°C and 50% water holding capacity for a period of 90 days. Soil samples were collected on days 0, 30, 60 and 90 of incubation and analyzed for microbial indices (microbial biomass C and microbial biomass P) by fumigation extraction technique and biochemical (phosphatase and dehydrogenase activity) parameters. The significant increase was recorded in cumulative respiration and microbial indices after application of amendments compared to unamended soil (control) due to the availability of easily decomposable organic carbon and P by organic amendments. Application of all amendments increased MBC, MBP and available P by 13%, 28% and 14% respectively with greater effect with inorganic P-enriched SFC and FYM followed by BWC. Microbial indices and enzyme activity showed the same temporal pattern with maximum increase on day 0 and 30. CO₂-C 24 h⁻¹ emission was also higher in the soils amended with organic sources compared to the unamended soil. It is concluded that use of organic amendments enhanced phosphorus bioavailability by increasing microbial activity and enzymatic activity.

1. Introduction

Soils of Pakistan are generally deficient in organic matter and available forms of certain major plant nutrients especially phosphorus. Phosphorus is one of the most limiting nutrients for crop production (Maranguit et al., 2017). Despite its wide distribution, phosphorus availability is low in soils due to the formation of precipitates with Ca and Mg or fixation with other soil components (Malik et al., 2013). In semi-arid areas of Pakistan, the problem of phosphorus is worst because of high soil pH, calcareous soils, low rainfall and water shortage which results in inefficient use of chemical fertilizers. Maintaining better crop by using organic fertilizer sources is a way to manage nutrient shortage in such areas. Microorganisms are highly sensitive and quick in response to the changes in soil conditions particularly in response to organic amendments. Therefore, soil microbial biomass estimation has been extensively used as a tool in studies on nutrients cycling and crop productivity (Schneider et al., 2016). Soil microbes have

the ability to store a substantial amount of P and then maybe slowly released back to the soil (Docampo et al., 2010). Upon the depletion of available carbon, turnover of microbial biomass starts and the N and P contained in microbial biomass become available to plants (Malik et al., 2012). Organic manures addition provides nutrients and additional C which helps in production of great microbial biomass (Sun et al., 2014). Organic amendments promote the activity of soil enzymes like dehydrogenase and phosphatase which helps in organic matter decomposition and P mineralization (Nie et al., 2013). The effectiveness of organic sources however depends on their concentration and mineralization rate (Zhang et al., 2013). Several studies have referred to the use of organic sources like farmyard manure, biogenic waste compost can increase bioavailability of soil nutrients and enhance the efficiency of chemical fertilizers but the combined use of organic fertilizers with inorganic fertilizers, especially with the technique of enrichment of inorganic P with organic sources, still needs to be explored. The main aim of this study was to under-

stand the dynamics of microbial biomass, phosphorus availability (Olsen P) and enzyme activity (phosphatase and dehydrogenase) in soils amended with three organic sources (compost, farmyard manure and sugar cane filter cake) when enriched with inorganic phosphorus source.

2. Materials and methods

2.1 Soil collection

A surface (0–15 cm) soil sample was collected in bulk from the University Research Farm (URF) Koont, Chakwal, Pakistan (latitude 32° 40' 4.0836" N and longitude 72° 30' 47.7900" E). The field moist soil was hand-picked to remove straw particles, stones and larger soil animals (earthworms etc.) then passed through a 2 mm sieve and homogenized. The homogenized soil was pre-incubated for 10 days at 25°C after moisture adjustment to 50% of soil water holding capacity. A subsample of the soil was air dried, ground and homogenized for physico-chemical analysis (Table 1). The particle size distribution was measured by Hydrometer method (Gee and Bauder 1986). Water holding capacity was measured by the preparation of saturated soil paste (Anderson and Ingram, 1993). EC and pH were measured using 1 : 2 soil/water ratio (Peters et al., 2003). Total organic carbon (TOC) was measured by Walkley-Black method (Nelson and Sommers 1982). Soil available P was determined by extracting 0.5 M NaHCO₃ with soil at pH 8.5 (Olsen and Sommers 1982). Total Ca and Mg were determined after digestion with mixture of HNO₃/HClO₄ (2 : 1) after AAS (Ryan et al., 2001).

Table 1.

Physico-chemical properties of Koont soil (Semi-arid) used in the experiment (n=3).

Property Texture	Soil Silt loam
Sand (%)	11.6
Silt (%)	52
Clay (%)	36.4
WHC (%)	27
pH _{1:2.5}	8.1
EC _{1:2.5} dS m ⁻¹	0.12
Organic Carbon (mg g ⁻¹)	4.7
Total N (mg g ⁻¹)	0.4
Total P (mg g ⁻¹)	0.58
Total K (mg g ⁻¹)	14.5
Soil available P (mg kg ⁻¹)	2.3
Total Ca (mg g ⁻¹)	0.9
Total Mg (mg g ⁻¹)	9.4

2.2 Organic materials

Three organic sources were used in this study including compost, farmyard manure (FYM) and sugar cane filter cake (SFC). Biogenic waste compost (BWC) was collected from compost production unit at PMAS-Arid Agriculture University Rawalpindi, Farmyard manure was collected from commercial FYM selling

place and sugar cane filter cake was collected from Sargodha sugar mill, Sargodha, Pakistan. Organic material samples were air dried and sieved. Organic sources (0.25 mg C g⁻¹ soil) were mixed with inorganic P (TSP) (10 mg P₂O₅/kg soil) and incubated for seven days for enrichment treatments. Organic sources were analysed for following chemical analysis EC and pH was measured using 1 : 2 soil/water ratio (Peters et al., 2003). Total organic carbon (TOC) was measured by Walkley-Black method (Nelson and Sommers 1982). Organic materials were digested in 1.2 : 1 H₂SO₄/H₂O₂ at 360°C for determination of Total P and Total N and were measured by Kjeldahl method (Table 2). The content of heavy metal of organic fertilizers were determined by atomic absorption spectroscopy (AAS) and presented in Table 4.

Table 2.

The chemical composition of compost, farmyard manure and sugar cane filter cake (n=3).

Properties	Sugar cane Filter Cake	Farmyard manure	Compost
pH (1 : 2)	7.4	6.8	8.1
EC (1 : 2 dS m ⁻¹)	15.3	9.8	12.8
TOC (g kg ⁻¹)	121.8	108.4	118.6
Total N (g kg ⁻¹)	8.9	9.4	6.2
Total P (g kg ⁻¹)	4.7	4.1	3.6
Total K (g kg ⁻¹)	12.8	11.3	10.3
Ca (g kg ⁻¹)	81.4	72.4	65.9
Mg (g kg ⁻¹)	9.3	8.2	7.5
Fe (g kg ⁻¹)	15.4	13.7	12.4
Mn (mg kg ⁻¹)	412.5	367.1	334.0
Zn (mg kg ⁻¹)	389.7	346.8	315.6
Cu (mg kg ⁻¹)	278.6	247.9	225.6
Total C/N	13.7	11.5	19.1
Total C/P	25.9	26.4	32.9

2.3 Incubation

The pre-incubated soil was added to incubation jars at the rate of 600 g jar⁻¹ on an oven-dry weight basis. Eight treatments were applied as follows: i) control (no amendment), ii) phosphorus fertilizer (TSP) 22.6 mg/kg of soil iii) compost (4.6 g kg⁻¹ of soil) with phosphorus fertilizer (iv) farmyard manure (3.8 g kg⁻¹ of soil) with phosphorus fertilizer (v) sugar cane filter cake (2.8 g kg⁻¹ of soil) with phosphorus fertilizer (vi) P-enriched compost (vii) P-enriched farmyard manure (viii) P-enriched sugar cane filter cake. Organic amendments were applied at the rate of 1% w/w basis. The experiment was laid out according to completely randomized design (CRD) with four replications. Soil respiration was measured by trapping the evolved CO₂ from each jar during the incubation on day 1, 2, 3, 5, 7, 10 and 14, and after that on a weekly basis until the end of the incubation period. Soil samples were collected on day 0, 30, 60 and 90 from each incubation jar and were analysed for microbial parameters. A glass beaker (100 cm³) containing 20 cm³ 1M NaOH was placed inside each incubation jar to preserve the evolved CO₂ from the soil samples to measure

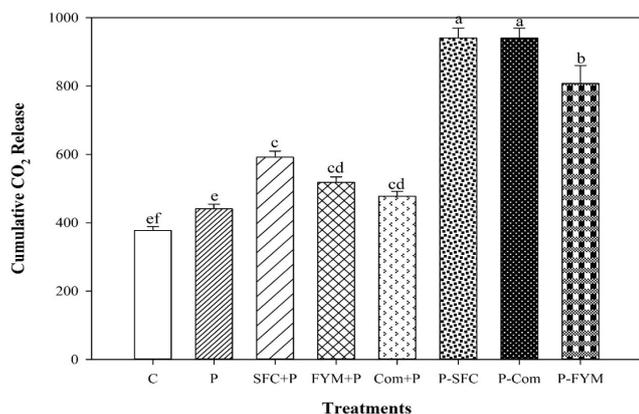


Fig. 1. Cumulative respiration in 90 days (mg CO₂-C g⁻¹ soil) from soil amended with compost, farmyard manure (FYM) and sugarcane filter cake (SFC) (n=3) C (Control), P (inorganic P fertilizer alone) and P-SFC represents Phosphorus enriched with C, P-FYM represents Phosphorus enriched with FYM and P-Com representing Phosphorus enriched with compost.

CO₂ emission by titration and Infrared gas analyser. Microbial biomass carbon (MBC) was measured by using fumigation extraction (Vance et al., 1987), microbial biomass phosphorus (MBP) was determined using fumigation-extraction technique (Joergenson et al., 1995). Soil available phosphorus was measured colorimetrically by 0.5 M NaHCO₃ using UV/VIS spectrophotometer at 450 nm wavelength. The alkaline phosphatase activity was measured by using *p*-nitrophenyl by UV/VIS spectrophotometer at 450 nm wavelength (Alef, 1995). The dehydrogenase activity (DHA) was evaluated by determination of reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to triphenylformazan (TPF μg g⁻¹) by UV/VIS spectrophotometer at 630 nm wavelength (Alef, 1995).

2.4 Statistical analysis

Cumulative respiration was analysed by one-way ANOVA and rest of the data was analysed by two-way ANOVA. Figures were made using software Sigma plot 12.0. The treatment means were compared by Tukey test at 5% level of significance.

3. Results

3.1 CO₂ emission

Soil respiration is assessed by CO₂ emission which results from organic matter decomposition. Thus, soil respiration rate indicates the magnitude of decomposition occurring at a given time in a particular soil. Organic amendments enhanced the CO₂ emission from amended soils compared to unamended soil as biomass of microorganisms increased immediately by the addition of easily decomposable organic C by the amendments (Kara et al., 2006). Maximum CO₂ (32 μg g⁻¹ soil) emission was recorded from the soil treated with P-enriched SFC, which can be explained by the presence of more C in SFC compared to FYM and compost.

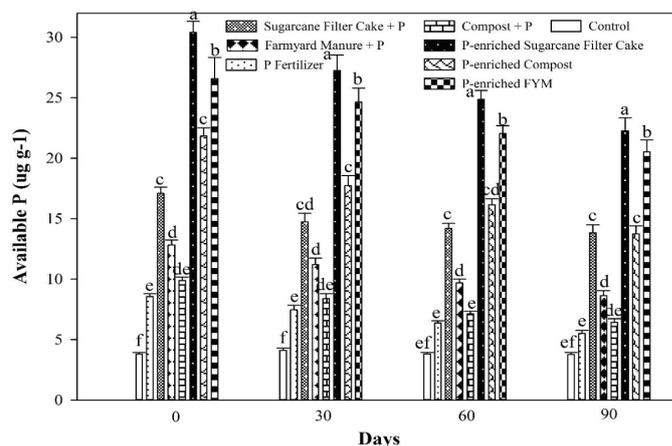


Fig. 2. Soil available P (Olsen P) in 90 days under soil amended with compost, farmyard manure and sugarcane filter cake (n=3).

Cumulative respiration was lowest in unamended control (Fig. 1). Cumulative respiration strongly increased with the addition of organic sources. Highest cumulative respiration was recorded under soil amended with P-enriched SFC and P-enriched Compost. Maximum CO₂ released was recorded in the compost amended soil, which can be explained by the presence of strongly decomposable complex of organic molecules in compost (Table 2) (Fig. 1). A positive relation was observed between the soil respiration and C added by the amendments.

Table 3.

The amount of Organic C, Total N and Total P added with amendments at the rate of 0.5 mg C g⁻¹ soil

Element	Compost (mg kg ⁻¹ soil)	Farmyard manure (mg kg ⁻¹ soil)	Sugarcane filter cake (mg kg ⁻¹ soil)
Organic C	1,283	1,023	1,565
Total N	67	113	101
Total P	37	41	54

3.2 Soil available phosphorus

The content of soil available P was lowest in unamended control soil and increased with organic and inorganic P addition rate. Available P was higher throughout the incubation period in soils amended with compost, farmyard manure and sugar cane filter cake by 14% compared to P fertilizer alone and unamended soil with maximum increase in soil amended with P-enriched sugar cane filter cake (30.4 μg g⁻¹) at day 0, which was five to six-fold higher than unamended control (Fig. 2). Available P was highest at day 0 and decreased in all the treatments on day 30, 60 and 90, which can be ascribed to P. Decrease microbial uptake of available P over time and can be due to following reasons: decreased phosphatase activity, organic P pools formation, mineralization of

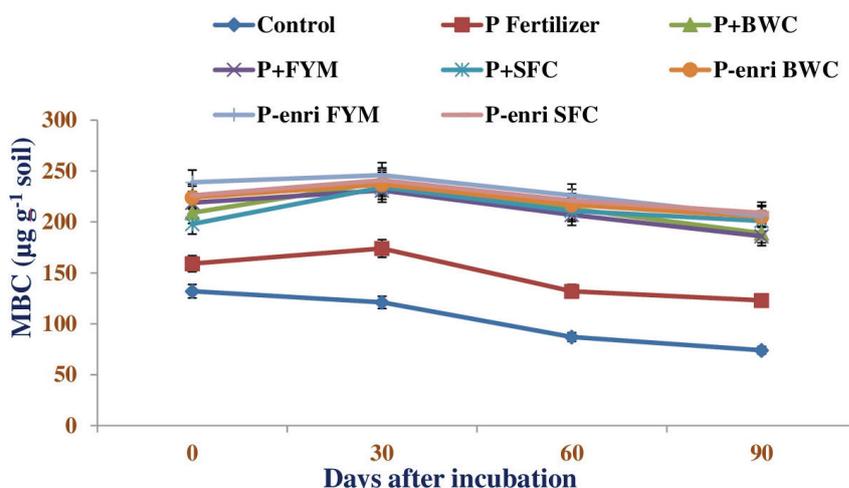
organic P and P fixation (Malik et al., 2012). A slight increase in P availability was observed on day 60 in amended soils, which can be attributed to microbial turnover. Throughout the incubation days, maximum P was recorded in soils amended with P-enriched organic sources, which leads us to conclude that enrichment of inorganic P with organic P sources can act as the best source for the constant provision of P.

3.3 Microbial indices

In the current study, MBC increased from the very start with the addition of all organic amendments, and the highest increase was recorded in soil amended with P-enriched FYM followed by P-enriched SFC and P-enriched Compost respectively (Fig. 3). The immediate increase was recorded at day 0 in soils amended with organic sources compared to unamended control by 14% with the maximum in soil amended with P-enriched SFC followed by FYM and Compost. No significant difference was observed in MBC within the organic sources, all of which had strongly enhanced MBC indicating the availability of easily decomposable C in the organic sources. Combination of inorganic P and organic P sources stimulated microbial growth.

MBP increased at the start of the incubation and highest value ($17.63 \mu\text{g g}^{-1}$) was recorded on day 30. This increasing trend and maximum contents of MBP on day 30 can be explained by the addition of more P in the form of inorganic and organic P combined application (Fig. 4). After day 30, a declining trend was recorded, which might be due to the depleting and washing out of these mineralizable substances during the middle and the final days of incubation period. A similar trend of increase and decrease in MBP were reported by (Sinegani and Mahohi, 2009) during the incubation period in soil amended with organic sources. Biomass C and P were lower on day 90 compared to day 0 which represents the microbial turnover that could release nutrients for plant uptake in later phases. All the organic amendments increased MBP by 28% compared to unamended control with the maximum increase in MBP by soils amended with P-enriched FYM.

Fig. 3. Microbial biomass carbon in 90 days under soil amended with compost, farmyard manure and sugarcane filter cake (n=3).



3.4 Enzymatic activity

In accordance with CO_2 emission, the maximum of dehydrogenase activity (DHA) ($29 \mu\text{g g}^{-1}$ soil) was at the start of the incubation period and then gradually declined till the end period of incubation (Fig. 5), which indicates the reduction in microbial demand after immediate increase in dehydrogenase activity after amendment addition (Malik et al., 2012). Addition of organic amendments increased the DHA in the soil but the unusual trend was observed by P fertilizer alone and unamended control. Significant dehydrogenase activity was recorded in control compared to soil amended with inorganic P + organic sources. The increase in DHA were higher in soil amended with SFC followed FYM. Unlike other microbial parameters, soil amended with farmyard manure showed the higher DHA in the later stages, which can be due to the presence of more N contents and less easily mineralizable N compounds in farmyard manure than in the other two organic amendments. Phosphatase activity was significantly higher throughout the incubation period in all the soils treated with organic amendments compared to unamended control and P fertilizer alone, with the highest increase in soil treated with P-enriched SFC followed by P-enriched compost and P-enriched FYM respectively (Fig. 6). The highest activity was recorded on day 30 which can be ascribed due to an increase in microbial biomass and P demand by microbes.

4. Discussion

This study confirmed that combined application of organic and inorganic sources enhances soil respiration, microbial biomass, enzyme activity and nutrient availability especially in soil amended with inorganic P-enriched organic sources. P enriched organic sources strongly increased soil respiration at the start of the study and stimulated microbial growth. Organic amendments, particularly SFC and FYM, provided enough nutrients to microbes and contained a higher concentration of nutrients than compost.

Fig. 4. Microbial biomass phosphorus in 90 days under soil amended with compost, farmyard manure and sugarcane filter cake (n=3).

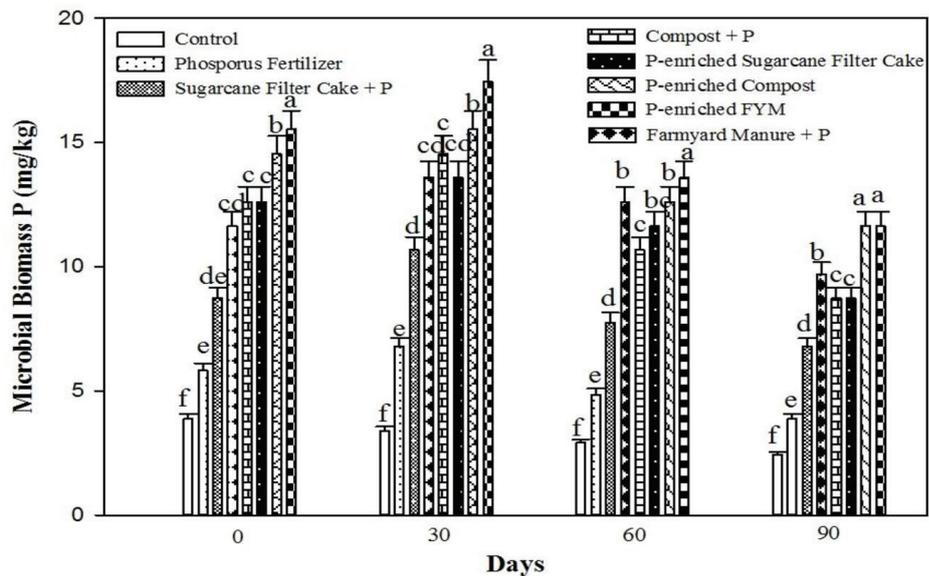


Fig. 5. Dehydrogenase activity in 90 days under soil amended with compost, farmyard manure and sugarcane filter cake (n=3).

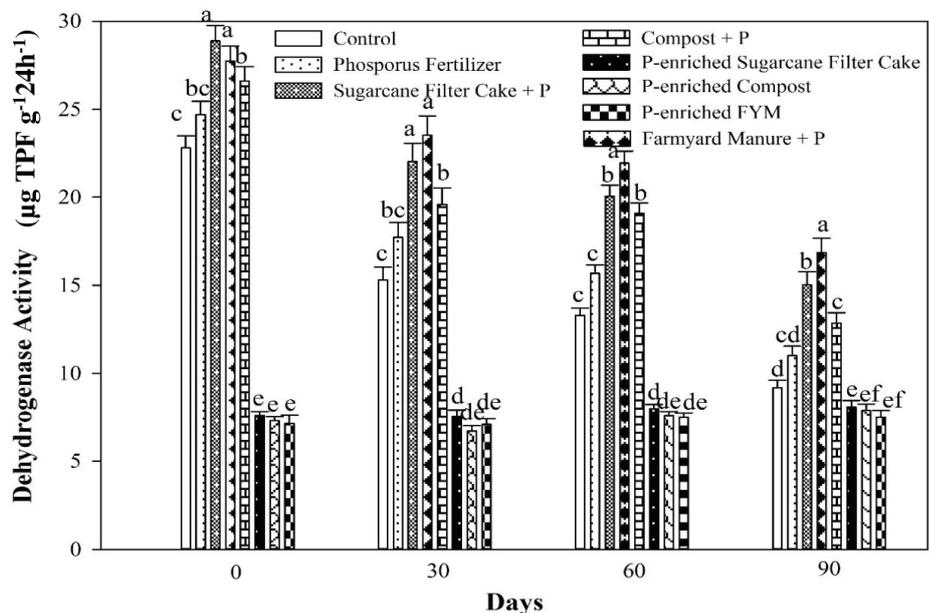
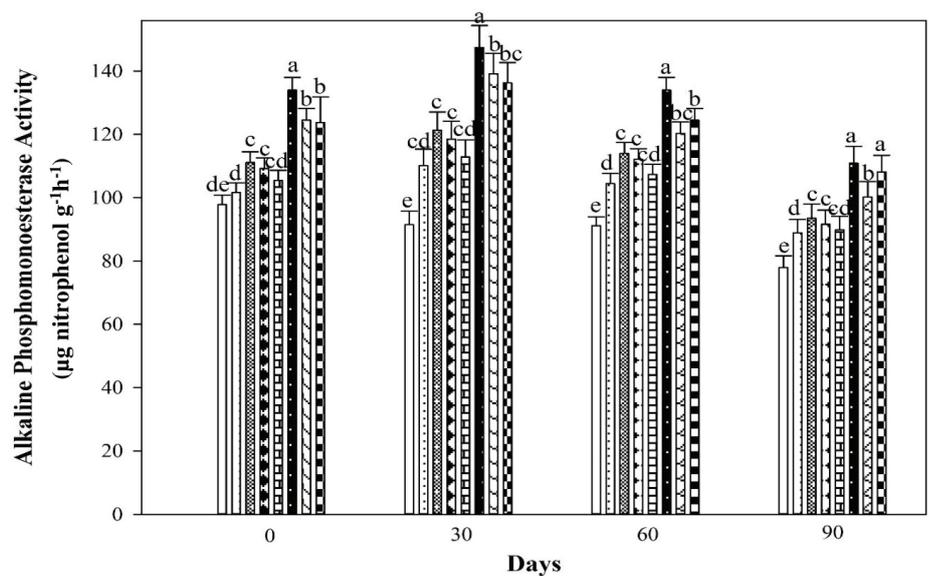


Fig. 6. Alkaline phosphatase activity in 90 days under soil amended with compost, farmyard manure and sugarcane filter cake (n=3).



4.1 Soil respiration and microbial indices

Soil respiration is the principal mechanism of C transfer from the soil to the atmosphere and its measurement is the most traditional and popular technique to estimate the microbial activity in soil. The highest respiration rate was recorded during the start of the incubation period in both amended and unamended soils (Fig. 2), which explains the presence of an immense amount of decomposable C. A later decline was observed in CO₂ emission in late phases, which may be ascribed to the depletion of those decomposable substances during the second week of incubation period and for the rest of the study (Duong et al., 2009).

Microbial biomass carbon (MBC) has been considered as an estimation of the quantity of the microbial biomass (Bastida et al., 2008) and its estimation has proved very helpful in calculating the changes in microbial biomass in response to variations in soil management techniques (Achat et al., 2010). Increases in microbial biomass carbon after various organic amendments in the soil like farmyard manure, sugar cane filter cake and composts have been reported by several scientists in the past (Marschner et al., 2003; Bohme et al., 2005; Tejada et al., 2006). Lowest MBC was observed in unamended control. Microbial biomass carbon strongly increased after amendments addition with the maximum increase in soil amended with P-enriched SFC and P-enriched FYM followed by BWC amended soil. These findings are in line with (Malik et al., 2013) who also reported the small effect of compost compared to other organic amendments. After day 0 decrease in MBC was observed showing the depletion of available organic C. Positive relation was observed between the MBC and organic C added by the amendments.

The early growth of microbial biomass phosphorus could be due to the P from the amendments. The increase in MBP might be endorsed by the growth of microbes in response to organic amendments, which resulted in more assimilation of P into the microbes (Gichangi et al., 2009; Malik et al., 2013). In support to these results, Ayaga et al., (2006) reported increased microbial biomass after organic application to the soil which resulted in more demand for P and its subsequent incorporation into the microbial biomass or its associated pool of metabolites. Such microbial assimilated P would be released slowly on microbial turnover that can be taken up by plants.

4.2 Biochemical Parameters

Dehydrogenase activity plays a vital role in the decomposition of organic matter and thus is associated with processes of oxidative phosphorylation (Lakhdar et al., 2010). Significant dehydrogenase activity was recorded in control compared to the soil amended with P-enriched organic sources, which may come from the indigenous soil N and C or microbes could be N limited under P-enriched organic amended soils.

Alkaline phosphatase (APA) is believed as an indicator of P mineralization in the soil as it catalyses the hydrolysis of phosphate from organic monoesters required by the microorganisms and plants to maintain the cellular metabolism (Sinegani and Mahohi, 2009). The highest activity was recorded on day 30, which can be ascribed due to an increase in microbial biomass and increase in microbe P demand. These results are in line with findings of (Sinegani and Mahohi, 2009), who reported the increase in phosphatase activity in response to the organic amendments in soil during the early stages of the incubation period.

4.3 Organic Amendments

The stronger effect of organic amendments on the microbial activity, which was measured as soil respiration and enzymatic activity and MBC, can be explained by the supply of easily available nutrients. As the soil was alkaline so CO₂ from carbonates can also contribute to CO₂ release. Moreover, the fact that amendments had a similar effect on dehydrogenase activity, which is another measure of microbial activity and CO₂ suggesting that most of the CO₂ measured might come from microbial respiration. SFC and FYM had a significant effect on microbial activity, biomass C and P and biochemical parameters compared to compost, which can be explained by the presence of higher concentration of nutrients (Total organic carbon, Total N and Total P) in SFC and FYM than (Table 2). Soil amended with compost also increased microbial activity and biomass though to a lesser extent compared to SFC and FYM, which can be ascribed to fewer nutrients and organic material present in compost that is less prone to decomposability than SFC and FYM because, during the process of composting, available organic material was already decomposed and only recalcitrant compounds remained (Wang et al., 2004). In available P, the effect of compost was much lower than SFC and FYM, which can also be explained by the high compost pH and a very low fraction of total P present. This makes compost a long-lasting organic material and slow releaser of nutrients.

Table 4.
Heavy metal analysis of organic amendments samples (n=3)

Sample	As (mg kg ⁻¹)	Cd (mg kg ⁻¹)	Co (mg kg ⁻¹)	Cr (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Li (mg kg ⁻¹)	Pb (mg kg ⁻¹)	Se (mg kg ⁻¹)
Compost	ND	ND	ND	23.708	20.935	10277	16.49	ND	ND
Farmyard manure	ND	ND	ND	12.076	11.696	8033	17.78	ND	ND
Sugarcane filter cake	12.160	ND	ND	16.525	66.613	8352	14.09	ND	1.503

ND = Not detected

5. Conclusions

All the organic amendments strongly enhanced soil microbial (soil respiration, MBC, MBP) and biochemical properties (dehydrogenase activity and phosphatase activity) compared to unamended soil, especially the soils treated with inorganic P-enriched organic amendments, which resulted in maximum increase in all the parameters. This leads us to conclude that P enrichment technique with organic sources could be very useful for stable and constant source of P to soil microbes and plants. Microbial biomass can also release nutrients upon microbial turnover when organic C gets depleted from amendments. The detailed conclusions are as follows:

Biogenic waste compost and farmyard manure are quick sources of nutrients as they contain easily decomposable compounds compared to sugar cane filter cake as it contains complex organic compounds and high concentration of organic C and other nutrients.

Generally, soil amended with sugar cane filter cake gave significant results (due to the presence of more organic C and P and having low pH) in all parameters though farmyard manure and compost also behaved as permanent and stable source soil nutrients for soil microbes. Phosphorus requirement by soil and plant can be accomplished by adding combined organic and inorganic phosphorus sources and due to this we can also reduce the dependence on an inorganic P source to meet the soil and plant P requirement. For this purpose, inorganic P enrichment with organic P sources can be the best technique.

Acknowledgement

Shahab Ahmad Khosa and Khalid Saifullah Khan are highly grateful to Higher Education Commission, Pakistan for providing financial assistance to conduct this study.

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