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COMPARATIVE USE OF ORGANIC SUBSTRATES AND MINERAL FERTILIZERS IN GROWING MAIZE (*ZEA MAYS* L.) FOR MAINTAINING SOIL FERTILITY IN RAINFED AREA OF PAKISTAN

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Abstract

At present Pakistan has 22 m ha of arable land out of which 4.59 million hectares are rainfed. About 1.8 million hectares of Pothowar Plateau comprises of Fatehjang¸ Murree¸ Attock and Jehlum. The topography of this region is uneven and sloppy lands are confronted with loss of fertility due to erosion problem. The rainfed area contributes about 24% in total crop yield of Pakistan. The fertility status of these soils ranges from low to very low. So, it is very important to adopt management strategies for nutrients replenishment in this area. Effects of mineral fertilizers, leguminous straw (chickpea) and leaf litter incorporated in an alkaline soil from Pakistan (0.80% C_{org}, 3.94 µg g^{-1} soil, $NaHCO_3$ extractable P 2.32 µg g^{-1} NO_3-N, pH 8.2, EC_e 0.56 dS cm^{-1}, 18% clay, 6% $CaCO_3$) under growing maize condition were studied in a 100-day pot experiment. Mineral fertilizer, plant residues (leguminous straw and non-leguminous) and leaf litter were added in five different treatments including a non amended control to the soils prior to cultivation of maize. The treatments were (1) untreated control, (2) 100 kg/ha N (Urea) and 120 kg/ha P_2O_5 (diammonium phosphate), (3) 1% chickpea residue, (4) 1% maize straw, (5) 1% leaf litter. The shoot and root dry matter in treatment 3 and 4 was three to two times higher than in the control. Similarly, addition of mineral fertilizers and chickpea residue increased root biomass significantly. Total C and N contents in maize shoot and root were double in treatment 2 and 3 followed by treatments 4 and 5 as compared to control. Microbial biomass (C_{mic}, N_{mic}, P_{mic}) contents increased significantly after addition of leguminous straw (chickpea residue) and mineral fertilizers followed by maize straw and leaf litter. Maximum increase of C_{mic} was observed in treatment where leguminous straw was added followed by maize straw and leaf litter.

Introduction

The decomposition of leguminous and non-leguminous residues by soil micro-organisms is a pivotal soil function leading to the release of nutrients for plant growth (Swift *et al.*, 1979). However, our knowledge of interactions between plant growth and microbial decomposition of plant litter is very much restricted, due to severe methodological problems: (1) the measurements of microbial biomass and activity in the presence of living plants and (2) the differentiation between the growing plant and decomposing litter. However, the fumigation-extraction method with pre-extraction (Mueller *et al.*, 1992; Mayer *et al.*, 2003; Muhammad *et al.*, 2006), allows a reliable estimation of the microbial biomass in the presence of living roots (Joergensen, 2000). The recovery of non-decomposed plant material by the simple sieving procedure of Magid & Kjaergaard (2001) and Magid *et al.*, (2004) might be able to replace the litter-bag technique, which has important drawbacks in determining the decomposition of plant residues by soil organisms, the change of the microclimate and especially a reduced contact between plant residues and soil colloids (Fruit *et al.*, 1999; Knacker *et al.*, 2003).

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Leguminous plants are important for improving soil fertility all over the world, including arid tropical countries (Wichern *et al.,* 2004; Muhammad *et al.,* 2006). These are increasingly important plants for use as green manure in stockless organic agriculture (Müller *et al.*, 2003).

Plant growth stimulates microbial growth and activity in the rhizosphere *via* exudates and a large variety of other rhizodeposits (Joergensen, 2000; Mayer *et al.*, 2003), changing the mineralization of native soil organic matter (Dormaar, 1990; Kuzyakov, 2002). Consequently, also the decomposition of freshly incorporated plant residues must be altered in the rhizosphere of actively growing plants. Therefore, the present study was carried out to prove the following hypothesis: (1) The comparison of mineral fertilizers with decomposed plant residues and leaf litter. (2) Their effect on microbial activity and biomass in the rhizosphere.

Materials and Methods

Soil sample preparation: An arable loamy soil was collected at 0-15 cm depth from Rawalpindi and sieved (< 2 mm sieve). A greenhouse pot experiment with 5 replications was conducted with the following five treatments: (1) control maize plants and without organic substrate, (2) + 100 kg N/ha (Urea) and 120 kg P_2O_5/ha (diammonium phosphate), (3) freshly chopped chickpea residues (*Cicer arietinum*) + growing maize, (4) no leguminous straw + growing maize plants (*Zea mays* L.), and (5) Leaf litter + growing maize plants. The experiment was carried out for 100-day in a greenhouse.

Each treatment consisted of 8 kg soil (on an oven-dry basis), filled in pots of 25 cm height and adjusted to 50% water holding capacity by watering from the top and also adjusted to 1.3 g cm^{-3} bulk density by pressing the soil with a wooden hammer. In the two chickpea and maize residue treatments, 200 g of freshly chopped materials were mixed as 5 cm pieces into 08 kg soil before filling into the pots. In the maize plant treatments, 4 seeds of maize were sown at a depth of 3 cm on 24 April, 2006, two weeks after filling the soil into the pots, so that the decomposition of organic substrates and leaf litter did not interfere with the germination of the plants. After emergence of the maize plants, two plants were screened out, so that each pot maintained two healthy plants up to maturity. At the beginning, moisture was maintained at 50% of the soil water holding capacity twice a week and adding the water lost regularly. The moisture was reduced according to the requirement of the growing plants.

Sampling of soil and plant material: Watering of the pots was stopped before 3 days (with plants) or 5 days (without plants) before sampling. Sampling was done on four days replicate-wise. In each pot, shoots of the maize plants above the soil surface were removed, dried, weighed, and stored for further analysis. A soil sample of approximately 1.1 g was taken with a soil corer from the centre of each pot. After dry matter determination, the soil was sieved (<2 mm) by carefully crumbling it between the fingers to remove maize roots and plant residues. Soil adhering to roots and residues on top of the sieve were carefully removed from the roots and passed through the sieve. Separated maize roots and chickpea, maize and leaf litter residues were removed, dried, weighed, and stored for further analysis. The soil passed through the sieve was air-dried and stored for the analysis of soil organic C and total N etc.

Determination of Soil microbial biomass: Microbial biomass C, biomass N and biomass P were estimated by the fumigation-extraction method (Brookes *et al*., 1982; Brookes *et al*., 1985; Vance *et al*., 1987) using the pre-extraction procedure (Mueller *et al*., 1992 as modified by Mayer *et al*., 2003; Muhammad *et al*., 2006).

For pre-extraction, 4 x 60 g soil (for microbial biomass C and biomass N) and 6 x 5 g soil (for microbial biomass P) were transferred into 250 ml or 100 ml plastic bottles and 150 ml or 50 ml demineralised water were added, respectively. The bottles were shaken for 20 min at 200 rev. min^{-1}. The soil suspension was poured through a 2 mm sieve and 200 ml demineralised water were used to rinse roots and organic particles remaining on the sieve. Roots and organic particles were rejected. After stirring with a glass-stick, the suspension was allowed to sediment for at least 30 min. Roots and organic particles appearing at the surface of the suspension were removed using tweezers. The suspension was poured into a folded filter paper (Schleicher & Schuell 595½, Dassel, Germany; 240 mm diameter for 60 g soil and 150 mm for 5 g soil).

For estimating microbial biomass C and biomass N, two of the pre-extracted 60-g portions were fumigated for 24 h at 25°C with ethanol-free $CHCl_3$. In addition to the usual fumigation procedure, 3 drops of liquid $CHCl_3$ were added directly to the soil samples in the filter paper. The fumigants were removed before the soil, including the filter paper, was extracted with 200 ml 0.5 *M* K_2SO_4 by 30 min horizontal shaking at 200 rev min^{-1} and filtered (Schleicher & Schuell 595 ½). The non-fumigated portions plus filter paper were extracted similarly at the time fumigation commenced. Organic C in the extracts was measured as CO_2 by infra-red absorption after combustion at 850°C using a Dimatoc 100 automatic analyzer (Dimatec, Essen, Germany). Microbial biomass C was E_C / k_{EC}, where E_C = (organic C extracted from fumigated soil) – (organic C extracted from non-fumigated soil) and k_{EC} = 0.45 (Wu *et al.*, 1990, Joergensen, 1996). Total N in the extracts was measured as activated NO_2^* by chemoluminescence detection (Dima-N, Dimatec) after combustion at 850 °C. Microbial biomass N was E_N / k_{EN}, where E_N = (total N extracted from fumigated soil) - (total N extracted from non-fumigated soil) and k_{EN} = 0.54 (Brookes *et al.*, 1985; Joergensen & Mueller, 1996).

For estimating microbial biomass P, the first two 5-g portions were fumigated as described above, extracted with 100 ml 0.5 *M* $NaHCO_3$ (pH 8.5) by 30 min horizontal shaking at 200 rev min^{-1}, centrifuged for 15 min, at (2000 *g*) and filtered (Schleicher & Schuell 595 ½). Two non-fumigated portions were extracted similarly at the time fumigation commenced. The remaining two portions were extracted after addition of 25 µg P g^{-1} (0.5 ml KH_2PO_4) in the same way as non-fumigated samples. Phosphate was measured by photo-spectrometry at 882 nm as described by Joergensen *et al.*, (1995).

Results and Discussion

Neither the addition of leaf litter, nor the sole maize growth led to significant changes in any of the soil microbial properties analysed in comparison to the control soil (Table 1). In contrast to this, the combination of growing maize plants and chickpea residues increased the contents, in most cases highly significant, of microbial biomass C (3 times), biomass N (4 times), and biomass P (4.5 times). These differences led to a significant decrease in the microbial biomass C/N ratio and the microbial biomass C/P ratio as well. The amount of the maize shoot C was more than doubled in treatment 4 with chickpea residues than without in treatment 3 (Table 2). In these five maize treatments, the mean shoot C/N ratio was 92 and the mean shoot C/root C ratio was 5.1 without any significant treatment effects.

Table 1. Quantitative microbial biomass in alkaline arid soil after 100-day of experiment.

	Microbial biomass C	Microbial biomass N	Microbial biomass P	Microbial biomass C/N	Microbial biomass C/P
	(µg g $^{-1}$ soil)				
Maize	99c	13c	09c	7.6a	10.0a
NP+ Maize	166b	30b	20b	5.5b	6.3c
Chickpea residues+ Maize	264a	50a	40a	5.3b	6.5c
Maize residues+ Maize	158b	33b	27b	4.8c	4.3d
Leaves litter+ Maize	106c	20c	15c	5.3b	7.1b

Different letters within a column indicate a significant difference (P <0.05, Tukey/Kramer, n = 7)

Table 2. Organic C in maize shoot and root after termination of experiment and ratios shoot organic C/N and shoot/root C.

	Shoot C (g pot^{-1})	Shoot C/N	Root C (g pot^{-1})	Shoot/ root C
Maize	20.8 b	94 b	4.2 b	5.4 a
NP + Maize	45.5 a	86 b	9.0 a	5.2 a
Chickpea residue + Maize	46.9 a	83 c	8.6 a	5.4 a
Maize residue + Maize	45.6 a	88 b	9.1 a	5.0 a
Leaf litter + Maize	46.8 a	111 a	10.5 a	4.5 a

Different letters within a column indicate a significant difference (P < 0.05, Tukey/Kramer, n = 7)

Phenol in the form of phosphatase release was highest in chickpea rhizosphere as compared to other amendments (Table 3). Phophatase release in treatment 3 was 3 times stronger as compared to control. Similar effect was observed for shoot dry weight. Maximum shoot dry weight was in chickpea residue amended treatment followed by maize, fertilizer and leaf litter treatments respectively.

Plant remains > 2 mm increased significantly in the order chickpea residues < maize residues < leaf litter (Table 4). Only 26% of the chickpea residues could be recovered as plant remains > 2 mm in treatment 3. The two POM fractions 63–400 µm and 400–2000 µm comprised 20% and 8% of soil organic C. Some amounts of maize straw and leaf litter and maximum chickpea residues were transferred into these two fractions according to the significant differences between the four amendment treatments and the control. The differences were small in all other cases, except treatment with chickpea residues.

The effects of chickpea and maize residues and leaf litter and fertilizers effect maize plant growth and soil microbial properties strongly positive because all amendments add considerable amounts of easily available substrate to soil, increasing the microbial turnover. This constancy supports the hypothesis of a biological space in soil maintaining very stable amounts of microbial biomass (Nannipieri *et al*., 1978). In accordance with this, Chen *et al.*, (2003) did not measure an increase in substrate-induced respiration at the end of a 56-day micro-pot experiment with growing wheat plants after the amendment of 20 mg alfalfa g^{-1} soil, but they measured a strong increase in microbial biomass N, indicating a method-specific reaction on the experimental conditions. In the leguminous and non-leguminous residues and fertilizer treatment of the present experiment, the four soil microbial indices varied in a considerably smaller range and increased on average by roughly 200-300% in comparison to the sole maize treatment, with indication of a positive change in the microbial community on the basis of the nearly constant gross ratios microbial biomass C/N, microbial biomass C/P ratio.

Table 3. Phosphatase release during 100-day of the pot experiment in the form of phenol (mg kg^{-1}).

	Phenol (Phosphatase) (mg kg^{-1} hr^{-1})	Shoot dry weight (g pot^{-1})
Maize	45 d	21 c
NP + Maize	60 c	38 b
Chickpea residue + Maize	106 a	55 a
Maize residue + Maize	78 b	40 b
Leaf litter + Maize	60 c	24c

Different letters within a column indicate a significant difference ($P < 0.05$, Tukey/Kramer, $n = 7$)

Table 4. Organic C content in plant remains particulate organic matter (POM) of different sizes.

	POM-C 63–400 µm	POM-C 400–2000 µm	Plant remains > 2 mm
Control (maize)	1050 a	390 a	-
NP + Maize	1100 a	390 a	90 c
Chickpea residues + Maize	1010 a	410 a	460 b
Maize straw + Maize	1400 a	450 a	980 a
Leaves litter + Maize	1150 a	405 a	350 b

Different letters within a column of a specific block indicate a significant difference ($P < 0.05$, Tukey/Kramer, $n = 7$)

In contrast to the microbial biomass indices, the amount of shoot C and root C, i.e., the C input into soil showed not only a 60% increase, but revealed more than two times larger yields in the maize plus chickpea and other amendments treatment in comparison to the sole maize treatment. It can be assumed that this increase in maize growth by the addition of residues and fertilizers was mainly due to the release of nutrients during decomposition. However, the C/N ratio in the shoot biomass did not differ in the two maize plant treatments, indicating that N was not the only limiting nutrient for maize growth. Also the ratio shoot C/root C was identical in the four maize growth treatments of the present pot experiment, but this ratio was apparently shifted towards shoot production in comparison to the field situation, probably due to sufficient water supply (Richner *et al.*, 1996; Liedgens & Richner, 2001).

Only 26, 30 and 40% of the chickpea and maize residues and leaf litter added were recovered as particulate organic matter > 2 mm, indicating nearly complete to partial decomposition during the experiment. This fast turnover is in contrast to many other studies undertaken using the litter-bag approach. After two years of field incubation in nylon nets, the remaining alfalfa C of a mixture with a red-earth extremely low in organic matter varied in a depth-specific way between 28 and 19% (Rovira & Vallejo, 2000). In this nearly C-free soil material, a certain part of the added organic material might have been used to build up soil organic matter. In an incubation experiment at constant 20 ºC, roughly 60% of alfalfa residues disappeared from litter-bags placed on the soil surface within 40 days (Dalias *et al.*, 2003). However, some studies reporting faster turnover of plant residues even in litter bags support our findings. Bross *et al.*, (1995) carried out a field experiment with litter-bags and measured a decrease in alfalfa residues between 90% and 96% during summer in Michigan, USA. Annual decomposition rate coefficients (-r, $C_{t1} = e^{-r} C_{t0}$, C = amount of C, t1 = 1 year) of 4.61 and 5.12 could be measured in the present sole alfalfa residues

treatment and in the maize plus alfalfa residues treatment, respectively. This is consistent with Schomberg & Steiner (1999), who measured a decomposition rate coefficient of 4.4 in a field experiment. Consequently, mass losses of 74%, 70% and 60% are possible for chickpea, maize residues and leaf litter under the environmental conditions of the present pot experiment.

Conclusions

The original hypotheses, assuming an abundant supply of plant essential nutrients with the turnover of leguminous and non-leguminous residues is as good as mineral fertilizers. The release of nutrients with the decomposition of organic substrate mobilizes even native immobilized nutrients in the vicinity of rhizosphere. Organic substrates enriched the soil with nutrients and then supplied to the plants. However, the decomposition rate of chickpea was much faster than maize and leaf litter. The highest microbial indices were observed in chickpea residue as compared to non-leguminous substrates.

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