

Assessing extracellular enzymatic activity in the soil on addition of root biomass with different biochemical composition

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Biochemical quality of a biomass determines the rate of its decomposition, which ultimately affects the relative microbial communities. Root biomass constitutes a significant fraction of total plant biomass and can play an important role in affecting microbial activity and extracellular soil enzymes when roots have different biochemical composition. To address the pivotal action of biochemical quality, a short-term incubation study was carried out to determine the effect of heterogeneity of root biomass on the functional characteristics of enzyme activities in the soil. Root biomasses of six crops with contrasting chemical composition (*Oryza sativa*, *Zea mays*, *Gossypium hirsutum*, *Glycine max*, *Cajanus cajan* and *Sesamum indicum*) were applied alone as well as in different combinations to a sandy-loam soil incubated under controlled environment condition for 63 days. The enzyme activity peaked on the 42nd day, which ranged from 11.85 to 16.85 μg triphenylformazan and from 9.84 to 13.67 μg *p*-nitrophenol $\text{g}^{-1} \text{h}^{-1}$ for dehydrogenase and alkaline phosphatase respectively. Among the different root biomasses and their combinations, maximum soil enzyme activity was found for *Z. mays* (T2) followed by *G. max* (T4) and *G. max* + *O. sativa* (T9). The roots of *Oryza sativa* (T3), *Cajanus cajan* (T6) and *Cajanus cajan* + *Oryza sativa* (T10) with high contents of hemicellulose and low contents of cellulose showed low enzymatic activity. Cellulose and hemicellulose contents in root biomass are mainly responsible for the enhanced functional diversity of soil microbial communities as well as enzyme activities.

Keywords: Biochemical composition, enzymatic activity, root biomass, soil microbial communities.

THE decomposition of plant residues in soils is not only regulated by environmental factors, but also by their biochemical composition^{1,2}. Therefore, the residue heterogeneity helps in stimulating various microbial communities which significantly affect the extracellular soil enzymes³. However, the decomposition of plant residues has been much better studied for above-ground than for below-ground components⁴.

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Root biomass and C-rich bio-macromolecules (celluloses, hemicelluloses) facilitate the synthesis of extracellular enzymes in the rhizosphere by accelerating the microbial population⁵⁻⁷. The quantity of root residue returned to the soil influences the soil organic matter content, but biochemical heterogeneity and soil enzymes among residues become important when other factors are kept constant.

Currently, soil enzymes are considered as ‘sensors’ of soil quality since they are relatively simple to measure, sensitive to environmental stress and respond rapidly to changes in the microbial status and physico-chemical conditions in the soil^{8,9}. Extracellular enzyme activities can also be directly affected by factors such as temperature, moisture, pH, nutrient availability and biochemical properties of the litter or root biomass^{9,10}.

Despite the huge amount of experimental work on residue decomposition in the soil, little is known about the impacts on the heterogeneity of root biomass, i.e. effect of incorporated biomass having different compositions on the dynamics of enzyme activities. The present study, therefore, was undertaken to relate the broad range of biochemical compositions of root biomass of annual crops to soil enzyme activities and C-mineralization rate in a sandy-loam soil under laboratory conditions. The novelty of the present study is to estimate the impact of biochemically differential root biomass composition of annual crops on soil enzymes for identification of probable indicators.

Materials and methods

Experimental set-up

This study was undertaken in the laboratory of the Department of Soil Science, Punjab Agricultural University (PAU), Ludhiana, India. The soil used in the study was of Typic Ustipsamment type, collected from the experimental farm of PAU (30°56'N, 75°52'E) in the Indo-Gangetic plains of northwestern India. Sampling was done from eight soil cores (0–0.15 m), homogenized, partially air-dried, ground and sieved (<2 mm mesh) to make a composite sample of approximately 50 kg. Table 1

shows the physical and chemical characteristics of the sampled soil.

Six representative plants species (main crops or cover crops) of agricultural systems in India, including two Poaceae (maize, rice), two Fabaceae (soybean, pigeon-pea), one Malvaceae (cotton) and one Pedaliaceae (sesame) were selected for the study (Table 2). The root biomass was sampled using the excavation method, separated manually, dried and cut into 1 cm length for the study at the start of senescence during the growing season for the root system mass. A sub-sample of each was also dried at 60°C, ground and used for chemical analysis (macro- and micro-nutrients; determined using ICAP elemental analysis) (Table 3). Cellulose and hemicellulose contents were estimated by proximate analysis using neutral digestion method¹¹.

The experiment was laid out in a completely randomized design with three replicates per treatment. The roots were added at the rate equivalent to 1.0% (except control), either alone or in a combination of 1:1 (w/w dry-weight basis) in polypropylene pots containing 400 g soil. The 11 treatments consisted of an unamended soil (control) and different root biomasses either used alone or in combinations (Table 2). The base of the pot was tapped firmly to allow the contents to settle, moistened to field capacity (20% w/w) and maintained in a moist air-circulating chamber at 30°C throughout the 63-day duration of the experiment.

Measurement of soil enzymatic activities and C mineralization

Soil sampling was done from the treatments at time intervals of 1, 3, 7, 14, 21, 28, 42 and 63 days after incubation (DAI), and stored at 4°C immediately for subsequent

analysis of enzyme activity (dehydrogenase (DHA), alkaline phosphatase (ALP), basal soil respiration (BSR)) to avoid minimum loss after collection.

DHA activity was determined using the method of Camina *et al.*¹². DHA was quantified from 1 g of soil sample using 0.2 ml of 3% triphenyltetrazolium chloride (TTC) and 0.5 ml of 1% glucose solution and incubated at 30°C for 24 h. Next, the triphenyl forazan (TPF) produced was extracted with 10 ml ethanol and determined spectrophotometrically (485 nm) after 2 h of refrigeration. Each DHA activity measured was the mean of three replicates, and expressed as $\mu\text{g TPF g}^{-1} \text{h}^{-1}$.

ALP was assayed on the basis of *p*-nitrophenol (PNP) release after the cleavage of *p*-nitrophenyl phosphate (PNPP) by alkaline phosphomonoesterase. Briefly, ALP activity was measured in 1 g air-dried soil sample incubated in 0.2 ml toluene, 4 ml modified universal buffer (MUB, pH = 11) and 1 ml of 0.05 M PNPP solution at 37°C for 1 h. The addition of 1 ml of 0.5M CaCl₂ and 4 ml of 0.5 M NaOH helped stop the reaction. Finally, the soil suspension was filtered and the filtrate was analysed for the estimation of PNP content on a spectrophotometer at 420 nm (ref. 13). The activity of alkaline phosphomonoesterase was expressed as $\mu\text{g PNP g}^{-1} \text{h}^{-1}$.

BSR of the samples was determined by trapping the evolved CO₂ in NaOH and the unreacted alkali in the CO₂ traps was then back-titrated with 1 N HCl¹⁴. The CO₂ absorbed in the traps was titrated at 1, 3, 7, 14, 21, 28, 42 and 63 days of NaOH placement. In this method, CO₂ evolved from each sample was calculated as the difference between the initial CO₂ concentration and that after each measurement period. BSR was calculated based on cumulative CO₂ evolution over a 30 day period.

Soil organic carbon (SOC) was analysed by the Walkley–Black chromic acid wet oxidation method¹⁵ using a diphenylamine indicator. SOC content after oxidation was determined by titration of the excess potassium dichromate using a 0.1 M solution of Mohr's salt.

Statistical analysis

All the data were analysed using analysis of variance (ANOVA) with IRRISTAT version 5.0. Least significance difference (LSD) at 0.05 level of probability was used to test the significance of differences among treatment means. Relationships between the measured parameters, DHA, ALP, BSR, organic carbon (OC) among different root biomasses and the combination of different species were determined by means of correlation tests (Pearson correlation coefficients), and significance was compared at a level of probability (*P*) of <0.05** and <0.01*.

Results and discussion

Figure 1 shows the chemical compositions of different root biomasses used in the study, which were analysed to

Table 1. Initial physico-chemical characteristics of the experimental soil

Parameters	Value
pH	7.6
EC (dS ⁻¹)	0.28
OC (g kg ⁻¹)	4.2
Available N (kg ha ⁻¹)	87.2
Available P (kg ha ⁻¹)	12.2
Available K (kg ha ⁻¹)	84
Sand (%)	64.4
Silt (%)	24.3
Clay (%)	11.3
Texture	Sandy loam
Zinc (ppm)	1.40
Copper (ppm)	0.94
Iron (ppm)	4.20
Manganese (ppm)	3.33

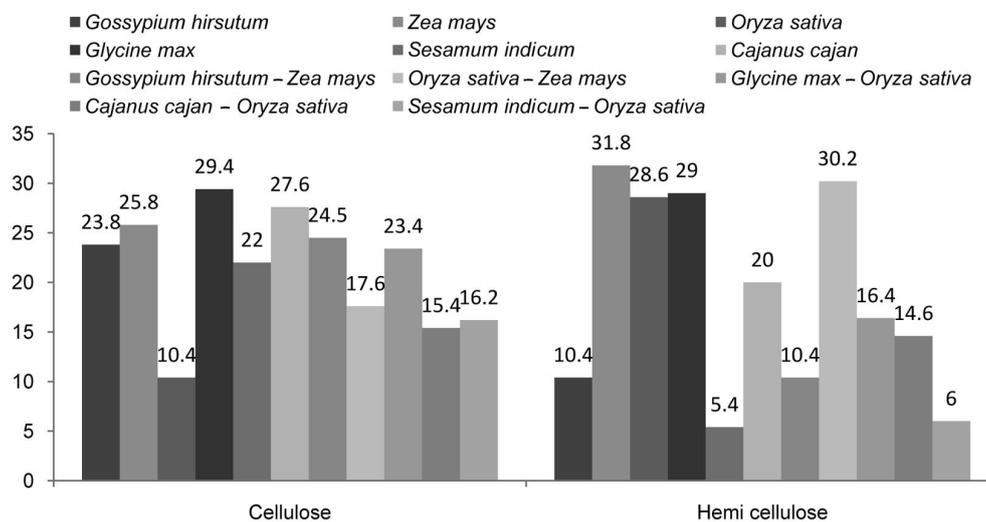
*pH, water: soil :: 2:1; EC, Electrical conductivity; OC, Organic carbon; N, KMNO₄ oxidizable nitrogen; P, Olsen phosphorus; K, Ammonium acetate exchangeable potassium.

Table 2. Treatments used in the study and the respective crop descriptions

Treatment	Latin name	English name	Family
T0	–	Control	–
T1	<i>Gossypium hirsutum</i>	Cotton	Malvaceae
T2	<i>Zea mays</i>	Maize	Poaceae
T3	<i>Oryza sativa</i>	Rice	Poaceae
T4	<i>Glycine max</i>	Soyabean	Fabaceae
T5	<i>Sesamum indicum</i>	Sesame	Pedaliaceae
T6	<i>Cajanus cajan</i>	Pigeon pea	Fabaceae
T7 (=T1 + T2)	<i>G. hirsutum</i> + <i>Z. mays</i>	Cotton + maize	Malvaceae + Poaceae
T8 (=T2 + T3)	<i>Z. mays</i> + <i>O. sativa</i>	Maize + rice	Poaceae + Poaceae
T9 (=T3 + T4)	<i>G. max</i> + <i>O. sativa</i>	Soyabean + rice	Fabaceae + Poaceae
T10 (=T3 + T6)	<i>C. cajan</i> + <i>O. sativa</i>	Pigeonpea + rice	Fabaceae + Poaceae
T11 (=T3 + T5)	<i>S. indicum</i> + <i>O. sativa</i>	Sesame + rice	Pedaliaceae + Poaceae

Table 3. Analysis of macro- and micro-nutrient concentrations in different treatments of root biomass

Root biomass	Zn (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)	N (%)	P (%)	K (%)
<i>G. hirsutum</i>	23.9	639	18.0	6.3	0.29	0.035	0.63
<i>Z. mays</i>	25.6	1285	28.0	7.8	0.33	0.037	0.83
<i>O. sativa</i>	145	4547	127	19.8	0.66	0.031	1.06
<i>G. max</i>	10.3	437	16.2	8.0	0.31	0.011	0.21
<i>S. indicum</i>	19.3	479	14.1	6.8	0.26	0.045	1.53
<i>C. cajan</i>	17.1	400	12.2	6.3	0.46	0.027	0.55
<i>G. hirsutum</i> + <i>Z. mays</i>	23.0	938	24.4	6.1	0.50	0.044	0.78
<i>O. sativa</i> + <i>Z. mays</i>	109	3165	97.7	34.1	0.47	0.037	0.96
<i>G. max</i> + <i>O. sativa</i>	118	2358	79.0	12.7	0.46	0.029	0.68
<i>C. cajan</i> + <i>O. sativa</i>	99.0	3091	77.1	9.4	0.61	0.035	0.80
<i>S. indicum</i> + <i>O. sativa</i>	101	2069	64.5	11.0	0.46	0.045	1.42

**Figure 1.** Cellulose and hemicellulose content (%) in different root biomasses.

identify the most significant differences among the species. The cellulose content of root residues ranged from 10.4 g kg⁻¹ for rice to 29.4 g kg⁻¹ for soybean, and hemicellulose content ranged from 5.4 g kg⁻¹ for sesame to 31.4 g kg⁻¹ for maize (Figure 1). The roots of Poaceae exhibited high variability in their chemical composition

when compared to those of species of other families which are all dicotyledons (Malvaceae, Fabaceae, Pedaliaceae). Conversely, the proportion of hemicellulose in Poaceae was higher than for other species, and the differences in composition among the roots of Poaceae species were lower. Results of the present study confirm our

Table 4. Changes in dehydrogenase ($\mu\text{g TPF g}^{-1} \text{ soil h}^{-1}$) activity among different root biomass treatments at various days of incubation

	1st	3rd	7th	14th	21th	28th	42th	63th
Control	2.51	4.22	8.05	6.45	6.98	4.61	7.59	8.15
<i>G. hirsutum</i>	3.49	4.31	8.74	8.11	10.31	3.68	11.85	8.72
<i>Z. mays</i>	3.85	4.01	10.36	9.56	10.55	5.03	13.77	11.60
<i>O. sativa</i>	5.36	5.90	9.09	7.50	9.91	4.13	12.62	9.59
<i>G. max</i>	3.86	4.71	9.24	7.19	10.66	5.03	14.35	10.82
<i>S. indicum</i>	4.28	4.87	8.98	7.44	7.31	4.78	12.11	9.51
<i>C. cajan</i>	4.50	7.18	5.74	7.99	7.63	4.44	12.18	9.22
LSD (0.05)	NS	NS	NS	NS	NS	NS	3.79	NS
<i>G. hirsutum</i> + <i>Z. mays</i>	4.98	4.40	8.23	7.92	6.84	3.85	8.41	8.22
<i>O. sativa</i> + <i>Z. mays</i>	6.48	5.65	8.50	6.71	7.47	4.54	12.21	8.91
<i>G. max</i> + <i>O. sativa</i>	7.66	4.97	8.17	7.82	7.62	3.61	13.55	10.50
<i>C. cajan</i> + <i>O. sativa</i>	4.91	5.90	9.97	6.90	9.48	3.72	11.50	8.62
<i>S. indicum</i> + <i>O. sativa</i>	4.22	5.56	6.53	8.41	7.12	3.24	12.22	10.37
LSD (0.05)	NS	NS	NS	NS	NS	NS	3.38	NS

hypothesis that there is a large variation in root biochemical composition among plant species grown under similar conditions, thereby having different decomposition rates. Roumet *et al.*¹⁶ reported that the root tissues of Fabaceae species are characterized by higher total N than those of Poaceae species.

Dehydrogenase activity

The DHA activity measured among different root biomasses and their combinations varied significantly at day 42 (Table 4). The highest DHA activity was observed in T4 treatment (*G. max*), reaching $14.35 \mu\text{g TPF g}^{-1} \text{ soil h}^{-1}$, followed by T2 (*Z. mays*), T3 (*G. max* + *O. sativa*) and the lowest in treatment T0 (control). DHA is an important and one of the most sensitive bioindicators of soil fertility¹⁷. The higher enzyme activity in T4, T2, T3 can be due to the release of enzymes from roots sustained by root exudates, or as a result of greater microbial activity¹⁸. In compound treatments at day 42, addition of *G. max* and *Z. mays* significantly increased DHA activity by 61.1% in treatment T9 and 45.2% in treatment T8 respectively, higher compared to treatment T0. Some studies demonstrated that root decomposition could vary among species depending on their biochemical composition (e.g. cellulose, hemicellulose, polysaccharides, polyphenols and soluble fractions) and microbial community structure^{19,20}. The results also showed greater enzymatic activity with high holocellulose (hemicellulose + cellulose) content (Figure 1).

In each treatment, DHA activity increased with the progression of incubation, but differences were found to be greater in the treatments having high holocellulose content, especially with root biomass of *G. max* and *Z. mays* (Figure 1 and Table 4). During the first seven days, the addition of different root biomasses alone or in combinations increased DHA activity from 5.74 ± 0.09 to

$10.36 \pm 0.03 \mu\text{g TPF g}^{-1} \text{ h}^{-1}$, compared to the control ($8.05 \pm 0.01 \mu\text{g TPF g}^{-1} \text{ h}^{-1}$). However, after 28 days of incubation, significant increase in DHA activity (ranging from 11.50 ± 0.01 to $13.77 \pm 0.04 \mu\text{g TPF g}^{-1} \text{ h}^{-1}$) was observed compared to the control (reaching $7.59 \pm 0.04 \mu\text{g TPF g}^{-1} \text{ h}^{-1}$). A reasonable hypothesis could be that higher C:N ratio of root biomass leads to slow decomposition rate and hence less DHA than in the control soils studied.

Alkaline phosphatase activity

The ALP activity from root biomass varied significantly among the treatments both in terms of species used and incubation time. The ALP activity among treatments showed significant increase on day 7, ranging from 6.56 ± 0.03 to $9.20 \pm 0.02 \mu\text{g PNP g}^{-1} \text{ h}^{-1}$ over the control ($6.43 \pm 0.03 \mu\text{g PNP g}^{-1} \text{ h}^{-1}$), suggesting increased availability of substrate in the soil. The results were found to be consistent with several studies on the increase in ALP activity with amendment of any organic matter^{21,22}.

The ALP activity showed an increasing trend with the period of incubation throughout the experiment. With progression of the incubation period, the activity in treatment T3 was higher compared to the other treatments up to 63 days throughout the experiment. Among the different root biomasses and compound treatments, higher ALP activity was recorded in T3 (*O. sativa*), T9 (*G. max* + *O. sativa*) and T10 (*C. cajan* + *O. sativa*) with values 14.0 ± 0.02 , 13.53 ± 0.01 and $13.51 \pm 0.01 \mu\text{g PNP g}^{-1} \text{ h}^{-1}$ at day 63 respectively, followed by T4, T6 and T11 (13.0 , 12.9 and $13.25 \mu\text{g PNP g}^{-1} \text{ h}^{-1}$ respectively). The lowest activity ($8.41 \mu\text{g PNP g}^{-1} \text{ h}^{-1}$) was recorded in T0 (control). Higher activity of phosphatase enzyme in *O. sativa* (paddy biomass) might be due to the organic acids produced during decomposition, which in turn increased SOC and nitrogen^{23,24}. Another reason for higher

Table 5. Changes in alkaline phosphatase ($\mu\text{g PNP g}^{-1} \text{h}^{-1}$) activity among different root biomass treatments at various days of incubation

	1st	3rd	7th	14th	21th	28th	42th	63th
Control	4.00	5.33	6.43	8.46	6.60	7.52	5.01	8.41
<i>G. hirsutum</i>	4.68	5.75	6.71	9.65	9.22	9.84	6.66	11.6
<i>Z. mays</i>	4.71	5.59	7.66	10.7	10.78	10.69	8.25	12.7
<i>O. sativa</i>	4.47	7.00	7.73	11.0	12.68	11.41	9.18	14.0
<i>G. max</i>	4.46	5.07	6.56	9.71	12.20	9.81	7.86	13.0
<i>S. indicum</i>	3.76	5.31	6.76	8.98	9.92	10.46	6.08	12.8
<i>C. cajan</i>	3.00	6.58	7.57	10.9	11.66	10.78	8.11	12.9
LSD (0.05)	NS	NS	0.94	1.54	2.30	1.05	1.95	2.93
<i>G. hirsutum</i> + <i>Z. mays</i>	3.52	6.17	7.44	10.0	9.44	10.37	6.40	10.37
<i>O. sativa</i> + <i>Z. mays</i>	3.90	5.99	7.34	10.3	11.51	12.25	7.51	12.16
<i>G. max</i> + <i>O. sativa</i>	4.34	6.14	7.91	10.3	10.05	13.67	8.78	13.53
<i>C. cajan</i> + <i>O. sativa</i>	4.21	6.67	9.20	11.5	10.68	12.19	10.12	13.51
<i>S. indicum</i> + <i>O. sativa</i>	3.69	6.50	7.78	11.5	9.53	12.10	8.63	13.25
LSD (0.05)	NS	NS	1.03	1.31	2.20	2.39	2.02	1.69

ALP activity at 63 days of incubation may be due to high amounts of microbial biomass produced during the decomposition of paddy straw by autochthonous microorganisms. ALP activity increases when there is equilibrated balance between added carbon and nitrogen²⁵. Addition of organic manure to the soil leads to enhanced SOC status and microbial biomass, which subsequently enhances soil enzyme synthesis and accumulation. Increase in soil enzyme activity with the application of organic manure soil was reported by Goyal *et al.*²⁶ and Dinesh *et al.*²⁷. In all the treatments, the mean enzymatic activity increased significantly from 7 to 63 days. The highest value of ALP was recorded at day 63 in all the treatments. There was significant decrease in enzyme activity from day 21 to day 28, which may be due to temporal variations. This has also been reported by Nannipieri *et al.*²⁸, who confirmed that temporal variations in the activity of these enzymes were due to their differential production rates, as influenced by the physiological age of different groups of microorganisms present in the soil. The activity of alkaline phosphatase (APPA) is also an important biological indicator of soil fertility^{29,30}. Like DHA, application of different types of root biomass positively influenced soil APPA. Maximum increase in APPA was observed up to 21 DAI in soil amended with different individual root biomasses, whereas it extended up to the 28 DAI under different combinations of root biomass. Among the root biomasses, rice and soybean showed highest increase (85–92%) in APPA at 21 DAI, and their combination (80%) at 28 DAI over control (Table 5). The possible reasons can be the synergistic effect of root residues in the mixture, which promote bacteria and fungi as they make available P by secreting enzyme ALP³¹. The organic P fraction in the soil organic matter is the main substrate for APPA to increase P supply in both natural and agricultural ecosystems. The inorganic fraction of soil P easily forms insoluble complexes with

cations, and/or incorporated into organic matter by microbes^{32,33}.

Basal soil respiration

BSR, measured in terms of CO₂ evolution and O₂ consumption by the soil is frequently used for the determination of microbial activity in the soil. The rate of soil respiration was significantly affected by different root residues and their combinations (Table 6). Similar to DHA and APPA, highest respiration was observed in soil amended with maize followed by soybean among different root residues. Cumulative BSR in control was 176 mg CO₂-C g⁻¹ soil day⁻¹, which increased to 492 and 486 mg CO₂-C g⁻¹ soil day⁻¹ in maize and soybean respectively. Application of crop residues varying in biochemical composition would be expected to increase the soil biological activity and promote microorganisms due to increased C and N in the soil depending on the carbon source in the residue^{34,35}. BSR as measured by CO₂ production was related mainly to holocellulose content of different root residues. Both maize and soybean roots contained maximum cellulose content. Among different mixtures of root residues, highest BSR was observed in legumes–cereals than cereal-based combinations due to higher content of cellulose in legumes compared to cereal residues. BSR in residue amended soils was very low during initial days of incubation which could be due to the potential of the residue to overcome the obstinacy of the native microorganisms in the soil, because it usually acts as a buffer against soil inoculants³⁶.

The present study showed the same pattern of decomposition of residues at varying rates, of which soybean and maize roots had high holocellulose content and showed greater enzymatic activity compared to the other root residues. Root biomasses from different plant species are known to differ in their cellulose and hemicellulose

Table 6. Changes in cumulative soil respiration as influenced by root biomass of different species and their combinations

	7th	14th	21th	28th	42th	63th
Control	111.8	126.0	138.1	148.3	165.6	172.8
<i>G. hirsutum</i> – <i>Z. mays</i>	286.9	349.3	384.3	421.0	453.8	478.6
<i>O. sativa</i> – <i>Z. mays</i>	307.1	367.6	400.6	436.7	469.2	500.1
<i>G. max</i> – <i>O. sativa</i>	297.0	355.7	395.0	431.9	463.0	491.3
<i>C. cajan</i> – <i>O. sativa</i>	324.5	384.5	424.4	464.4	502.1	529.4
<i>S. indicum</i> – <i>O. sativa</i>	283.3	336.4	375.2	412.6	445.6	470.1
LSD (0.05)	54.0	51.3	51.3	51.4	52.5	55.7
<i>G. hirsutum</i> – <i>Z. mays</i>	286.9	349.3	384.3	421.0	453.8	478.6
<i>O. sativa</i> – <i>Z. mays</i>	307.1	367.6	400.6	436.7	469.2	500.1
<i>G. max</i> – <i>O. sativa</i>	297.0	355.7	395.0	431.9	463.0	491.3
<i>C. cajan</i> – <i>O. sativa</i>	324.5	384.5	424.4	464.4	502.1	529.4
<i>S. indicum</i> – <i>O. sativa</i>	283.3	336.4	375.2	412.6	445.6	470.1
LSD (0.05)	51.4	50.9	51.0	50.5	51.5	52.5

Table 7. Changes in cumulative organic carbon content as influenced by root biomass of different species and their combinations

	1st	3rd	7th	14th	21th	28th	42th	63th
Control				0.48		0.43		0.50
<i>G. hirsutum</i>	0.535	0.508	0.534	8	0.506	6	0.534	8
<i>Z. mays</i>	0.958	0.861	0.920	2	0.850	6	0.848	8
<i>O. sativa</i>	0.883	0.855	0.701	1	0.732	6	0.724	7
<i>G. max</i>	0.886	0.855	0.838	7	0.739	8	0.743	0
<i>S. indicum</i>	0.925	0.888	0.845	4	0.768	8	0.793	7
<i>C. cajan</i>	0.938	0.960	0.907	0	0.791	4	0.796	6
LSD (0.05)	0.069	0.093	0.074	2	0.054	0	0.083	4
<i>G. hirsutum</i> – <i>Z. mays</i>	0.824	0.812	0.770	4	0.676	5	0.743	7
<i>O. sativa</i> – <i>Z. mays</i>	0.945	0.858	0.861	3	0.804	4	0.799	1
<i>G. max</i> – <i>O. sativa</i>	0.853	0.786	0.832	1	0.699	2	0.724	7
<i>C. cajan</i> – <i>O. sativa</i>	0.919	0.770	0.891	1	0.755	8	0.737	4
<i>S. indicum</i> – <i>O. sativa</i>	0.876	0.809	0.809	7	0.699	1	0.773	3
LSD (0.05)	0.081	0.071	0.068	7	0.046	7	0.037	2

content, which in turn will affect the decomposition rate depending on the availability of C and N³⁷. Among different root biomasses, maize, soybean and maize–soybean combination treatments showed significantly higher DHA compared to other root biomass and their combinations. DHA at 42 DAI significantly increased by 81.4%, 89.1% and 78.5% in maize, soybean and their combination respectively, compared to control.

Organic carbon

In the soil system, OC is mainly contributed by root decomposition and crop residue incorporation³⁸. Biotic interactions in the biochemically different root biomasses have a profound effect on SOC content. Over the incubation period from 7 to 42 days, OC content showed increasing trend (0.701% to 0.920%) compared to control

Table 8. Pearson's correlation coefficient among the observed parameters

Parameters	Root biomass of different species				Root biomass of compound treatment system			
	DHA	APPA	BSR	OC	DHA	APPA	BSR	OC
DHA	1	0.759*	0.861*	0.849*	1	0.803**	0.698	0.675
APPA		1	0.819*	0.622		1	0.786**	0.636

DHA, Dehydrogenase; APPA, Alkaline phosphatase; BSR, Basal soil respiration; OC, Organic carbon.
* $P < 0.01$; ** $P < 0.05$.

(0.534%). The change in OC content during incubation study was found to be higher in maize followed by pigeon pea and least in the soil amended with cotton root biomass, whereas in their combinations, highest change in OC content was in rice–maize followed by sesame–rice and least in soybean–rice incorporated soils (Table 7). The increase in OC content in residue-treated soils can be attributed to the stimulation of growth and activity of microorganisms, resulting in higher production of microbial biomass^{39,40}. Higher organic content during the decomposition of different root residues in the present study can be related to specific types of rhizodeposits and plant residues, and therefore can affect carbon content in the soil^{41,42}.

Correlation of enzyme activities with soil respiration and soil organic carbon content

Both DHA and APPA assayed in the study were significantly and positively correlated with OC and BSR (Table 8). The values of correlation coefficient (r) for soil enzyme activities ranged from 0.759* to 0.938**. Significant correlations between enzyme activity and OC observed in this study suggest a general relationship between soil microbiological properties. Accumulation of root biomass improved the OC status of soils, which in turn was reflected in higher soil enzymatic activity^{43,44}. It is evident that soil enzymatic activity is strongly correlated with SOC content. The higher OC level can provide enough substrate to support higher microbial activity and hence higher enzyme production⁴⁵.

Conclusion

Soil enzymes activity is sensitive to changes occurring in soil processes due to addition of root biomass having different biochemical composition. Addition of different root biomasses increased soil microbial activity over unamended control. The present study suggests that decomposition of root biomass can be controlled by labile compound present in biochemically different mixtures of root biomass by a broad range of biochemical composition and chemical heterogeneity of the mixtures. Holocellulose content in different root residues was the sole

factor that explained the large variability in C-mineralization and enzymes activity in amended soil.

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