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Impact of Chemically Diverse Organic Residue Amendment on Soil Enzymatic Activities in a Sandy Loam Soil

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Abstract: To monitor soil biological quality, it is of paramount importance to assess how chemically diverse organic residue amendments reciprocate to organic matter. The present incubation study aimed to evaluate the effect of organic residue amendments varying widely in their biochemical composition on the dynamics of soil enzymatic activity. The changes in the pattern of soil enzymatic activity have been monitored over a period of 63 days using a total of eleven different crop residues. The enzyme activity (dehydrogenase, fluorescein diacetate hydrolysis, acid phosphatase, alkaline phosphatase and phytase) in soils amended with chemically diverse organic residues were significantly higher as compared to the control. It was further observed that the enzymatic activities in *Azadirachta indica*, *Avena sativa* and *Lens culinaris* continued to be higher up to 28 days after their incorporation (DAI). Our study showed that plant residues varying in different cellulose and hemicellulose contents influenced the enzymatic activities as well as functional diversity of soil microbial communities.

Keywords: residue quality; soil microbes; incubation study; biochemical parameters; organic amendments

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1. Introduction

The ability of organic amendments to boost soil health and increase crop productivity makes their use in agriculture a common practise. In fact, organic amendments of various origins and compositions can provide the soil with essential nutrients and increase the amount of organic matter in the soil, both of which have positive effects on the soil's overall health [1]. Soil and crop management practices, such as residue management, tillage, crop rotation, is considered to be important global issues for enhancing soil quality and in attaining sustainability in agricultural production [2,3]. To work out residue decomposition response in soils, physical factors of soil or residue and biological components serve as the most suitable tools or functional traits [4,5]. The incorporation of organic materials in soil played a vital role in the maintenance of soil quality; most of the soil processes are driven through soil microorganisms, thereby releasing important plant nutrients through solubilization, the mineralization of organic and inorganic pools in soil and the stabilization of soil aggregates, and thus, it is beneficial for soil conservation and crop production [6,7]. In recent years, biological properties of soil have been considered the sensitive indicators for a quick response to alteration in the management systems [8]. Among the biological activities, enzymatic activities are considered the best indicators of specific biochemical reactions derived primarily from soil microbes as they participate in nutrient cycling and scale up the decomposition of organic amendments [9–11].

The microbial community and its functions are detected by changes in soil enzymatic activity [12]. They indicate modifications in soil biochemical processes and SOM dynamics ascribed to human-induced variation in abiotic and biotic components in soil [13,14]. The quantification of soil enzymatic activity in important nutrient cycling (C, N and P), and oxidation–reduction processes have been utilized extensively as a possible indicator for evaluating the impact of land use changes and management practices on soil health [15–17]. The amount of each soil enzyme varies primarily depending on different soil types containing different contents of the organic matter and intensity of the biological activities of soil [18]. Amongst various enzymes, the important soil enzymes include acid and alkaline phosphatases, amylases, arylsulphatases, β-glucosidases, cellulases, chitinases, soil dehydrogenases, proteases and ureases released from plants [19], animals [20], organic compounds and microorganisms [21] and soil [22,23]. Soil enzymes may also be utilised as an essential indicator of the soil quality as they are significantly correlated with soil properties [24].

Organic amendments, such as crop residues, green manure and livestock manure, have been identified as one of the most cost-efficient and successful sustainable agricultural practises for increasing SOM content [25]. Nutrient release and rate of decomposition of organic amendments is variably influenced by the C/N ratio and their biochemical composition [26]. For example, various organic residues differ in total N, C/N ratio, cellulose, hemicelluloses, lignin, polyphenol content resulting in differences in enzymatic activities and changes in functional quality of soil [27]. Organic residues having a low C/N (<20) show greater N mineralization compared to the residues having wide C/N ratios which cause N immobilization [28]. Additionally, some researchers have reported that chemically resistant residues, such as those high in lignin, degrade more slowly than residues with low lignin and high N concentrations, increasing soil organic matter and enhancing soil C stabilisation [29]. Wang et al. [30], in a study, observed that overall, diverse crop residue quality generated distinct expressions of the structure and function of the soil microbial community. Similarly, Domínguez et al. [31] reported that diversifying crop residues as an energy source for microbes will enhance enzymatic activities in the soil carried out by earthworms and finally augment the decomposition of organic matter. In addition to the quality and composition of crop residues, the organic matter decomposition is influenced by intrinsic soil conditions [32,33]. According to Mathew et al. [32], carbon-rich clayey soils in tropical humid regions had a better biomass production capacity than sandy soils, which resulted in increased plant C stocks and C transfer to soils. In addition, because of their tendency to aggregate, capacity to offer physical protection and capacity for mineral adsorption of C constituents, clayey soils also support substantial C stocks [32,34]. Furthermore, fine textured soils typically take longer to mineralize SOC and crop residues than coarse textured soils due to the fact that clay can physically shield soil's organic C and may restrict microbial access to it [35,36].

Presently, limited information is available in the literature on the role of crop residue heterogeneity on the dynamics of different enzymatic activities in soil. However, studies have investigated the effects of organic residue amendments on soil enzymatic activities, but most of these studies have focused on specific residue types or narrowly defined chemical compositions [37,38]. To fully comprehend the influence of organic residues on soil enzymatic activities, it is crucial to consider the diverse range of residues commonly available in agricultural systems. These residues exhibit variations in chemical composition, including cellulose, lignin and nitrogen content. Therefore, keeping in view the above-said, emphasis of the present study was undertaken to evaluate the changes in various soil enzymatic activities in soils amended with different types of plant residues having different biochemical composition. We hypothesized that crop residues differing widely in biochemical composition would have a differential effect on the enzymatic activity.

2. Materials and Methods

2.1. Experimental Site

A laboratory study has been conducted using sandy loam soil (*Typic Ustochrept*, USDA classification) collected from the experimental field of the PAU, Ludhiana located in the Indo-Gangetic plains of north-western India. Soil samples (0–15 cm depth) were randomly collected from eight different locations within the field, mixed thoroughly to make a composite sample of approximately 50 kg. The sample was then partially air-dried, cleaned, sieved (<2 mm), brought to field capacity and stored in the bag. The soil under study was normal in reaction and electrical conductivity (pH 8.0 and electrical conductivity 0.4 dSm^{-1}), medium in organic carbon 4.1 g kg^{-1} [39], $KMnO_4$-oxidizable N of 9.7 mg kg^{-1} [40] and low in available P 3.1 mg kg^{-1} [41] and available K 31 mg kg^{-1} [42].

2.2. Experimental Details

A total of eleven different amendments with their respective crop description having different C/N ratio were selected, to study their effect on soil enzymes and are given in Table 1. The experiment was laid down in a complete randomized design (CRD) with three replications of each treatment. The crop residues were collected, washed, dried, ground and stored for the proximate analysis. On dry weight basis, 500 g soil was taken in the polypropylene pots od f one litre capacity, and moistened to its field capacity. Crop residues were added at the rate of 1% to these pots and were mixed thoroughly with the soil. During the course of incubation period, moist air was continuously circulated in the chamber at 30 °C. The soil sampling from each treatment were done at the time intervals of 1, 3, 7, 14, 21, 28, 42, 63rd days after incubation (DAI) and stored at 4 °C immediately for the subsequent analysis of enzymatic activities {(dehydrogenase (DHA), fluorescein diacetate (FDA), acid phosphatase (ACP), alkaline phosphatase (ALP), phytase (PA)} to avoid minimum losses after collection.

Table 1. List of the treatments used for the study and respective crop residue description.

Treatments	Latin Name	English Name	Family
T_0	-	Control	-
T_1	*Melia azedarach*	Dek	*Meliaceae*
T_2	*Azadirachta indica*	Neem	*Meliaceae*
T_3	*Populus alba*	Poplar	*Salicaceae*
T_4	*Avena sativa*	Oat	*Poaceae*
T_5	*Zea mays*	Maize	*Poaceae*
T_6	*Triticum aestivum*	Wheat straw	*Poaceae*
T_7	*Oryza sativa*	Rice straw	*Poaceae*
T_8	*Hordeum vulgare*	Barley	*Poaceae*
T_9	*Lens culinaris*	Masar	*Fabaceae*
T_{10}	*Saccharum officinarum*	Sugarcane Trash	*Poaceae*
T_{11}	*Brassica juncea*	Mustard	*Brassicaceae*

2.3. Proximate Analysis of Organic Residues

The total organic carbon (C) and total nitrogen (N) content of the crop residues were estimated by dry combustion method using Vario EL CHN elemental analyser (Heraeus Elementor EL, Hanau, Germany). The proximate analysis was done to estimate the cellulose, hemicellulose and lignin fractions of the residues [43]. Neutral digestion was performed with 1 g of plant residues and 100 mL of neutral detergent solution. The acid digestion was performed using 100 mL of detergent solution acid per gram of the residues at 150 °C in the digester block for 1 h. The samples were then vacuum filtered using crucibles with the repeated washings of hot distilled water and acetone (10–20 mL) and allowed to be dried for 12 h at 105 °C. The hemicellulose content was evaluated from the difference between the percentage of total neutral fiber and the percentage of acid detergent fiber. The cellulose content of the acid fiber was obtained after digestion with 12 M H_2SO_4 for 3 h and the left-over material remained after cellulose quantification was burned in a muffle furnace at 500 °C for

3 h for the determination of lignin content. The anthrone colorimetric method was used to measure total soluble sugar (TSS) content as described by [44] and the starch (ST) content was measured using a method with 3,5-dinitrosalicylic acid (DNS) method as described by [45].

2.4. Enzymatic Activity in the Soil

Dehydrogenase (DHA) was quantified from 1 g of soil sample using 0.2 mL of 3% triphenyltetrazolium chloride (TTC) and 0.5 mL of glucose solution (1%) and incubated at 30 °C for 24 h. Then, triphenyl foramazan (TPF) was extracted with 10 mL ethanol and determined spectrophotometrically after 2 h refrigeration at 485 nm. Each value of DHA activity was the mean of three replicates and was expressed as µg TPF formed/g/h [46]. The activity of acid (ACP) and alkaline phosphatase (ALP) was determined on the release of p-nitrophenol (PNP) after the cleavage of p-nitrophenyl phosphate (PNPP) by acid and alkaline phosphomonoesterase. Briefly, ACP and ALP activity was measured in the 1.0 g air-dried soil sample incubated in the 0.2 mL toluene, 4 mL Modified Universal Buffer (MUB) of pH = 4, 11 (for ACP and ALP respectively) and 1 mL of 0.05 M PNP solution at 37 °C for 1 h. The addition of 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH led to a stop in the reaction. Finally, the soil suspension was filtered, and filtrate was analysed for the estimation of p-nitrophenol content on a spectrophotometer at 420 nm [47]. The activity of acid and alkaline phosphomonoesterase was expressed as µg PNP released/g/h. Phytase activity was estimated by [48] method. To determine phytase activity, 1 g of the soil was transferred in which 4 mL NaOAc buffer solution (pH 4.5) and 1 mL 1µM sodium phytate solution were added to the tubes. After incubation at 37 °C for 1 h, the reaction was terminated with the addition of 0.5 mL of 10% trichloroacetic acid, and the hydrolysed product was measured using Olsen method. Phytase activity was expressed as µg Pi released/g soil/h. The activity of fluorescein diacetate (FDA) hydrolysis was measured using 2 g of soil treated with 60 mM potassium phosphate buffer (pH 7.6) and 0.2 mL solution of FDA (1000 mg mL^{-1}). The samples were incubated for 20 min in the incubator at 30 °C. After incubation, the reaction was stopped by the addition of 15 mL of chloroform: methanol in 2:1 ratio immediately to terminate the reaction. The supernatant was filtered after centrifugation at 2000 rpm for 10 min, and the colour intensity of fluorescein present in the filtrate was measured at 490 nm on a spectrophotometer [49].

2.5. Statistical Analysis

The least significance difference (LSD) among the treatment means was calculated at 5% level of probability by using Analysis of variance (ANOVA), carried out using IRRISTAT version 5.0.

3. Results

The chemical composition of all the organic residues used in the study are given in the Table 2. The carbon content of different residues varied from 27.3% to 44.6% and the N content ranged from 0.39% to 4.82% with C/N ratio ranging from 9 to 106. The cellulose (CEL) content of the residues ranged from 17.6% to 45.4%, hemicellulose (HEM) content ranged from 7.80% to 23.5% and Lignin (LIG) contents ranged from 9.50% to 30.6% (Table 2).

Effect of Organic Residues on Enzymatic Activity

Plant residues significantly increased the activities of DHA, FDA, APA, APP and PA at all the sampling dates. DHA ranged from 15.7 to 19.1, 9.13 to 18.9 and 21.7 to 57.4 µg TPF g^{-1} h^{-1} for tree residues, cereals and others, respectively. T1, T4 and T9 showed maximum DHA activity. There was a significant increase in DHA of up to 28 DAI, and it decreased thereafter (Figure 1). Among tree species, *Azadirachita* sp. showed significantly higher activities at the 28th DAI, followed by *Populus alba* and *Melia azedarach*. Among cereal residues, *Avena sativa* showed significantly higher DHA followed by *Zea mays*. Among other plant residues species, *Lens culinaris* showed significantly higher DHA at the 28th DAI.

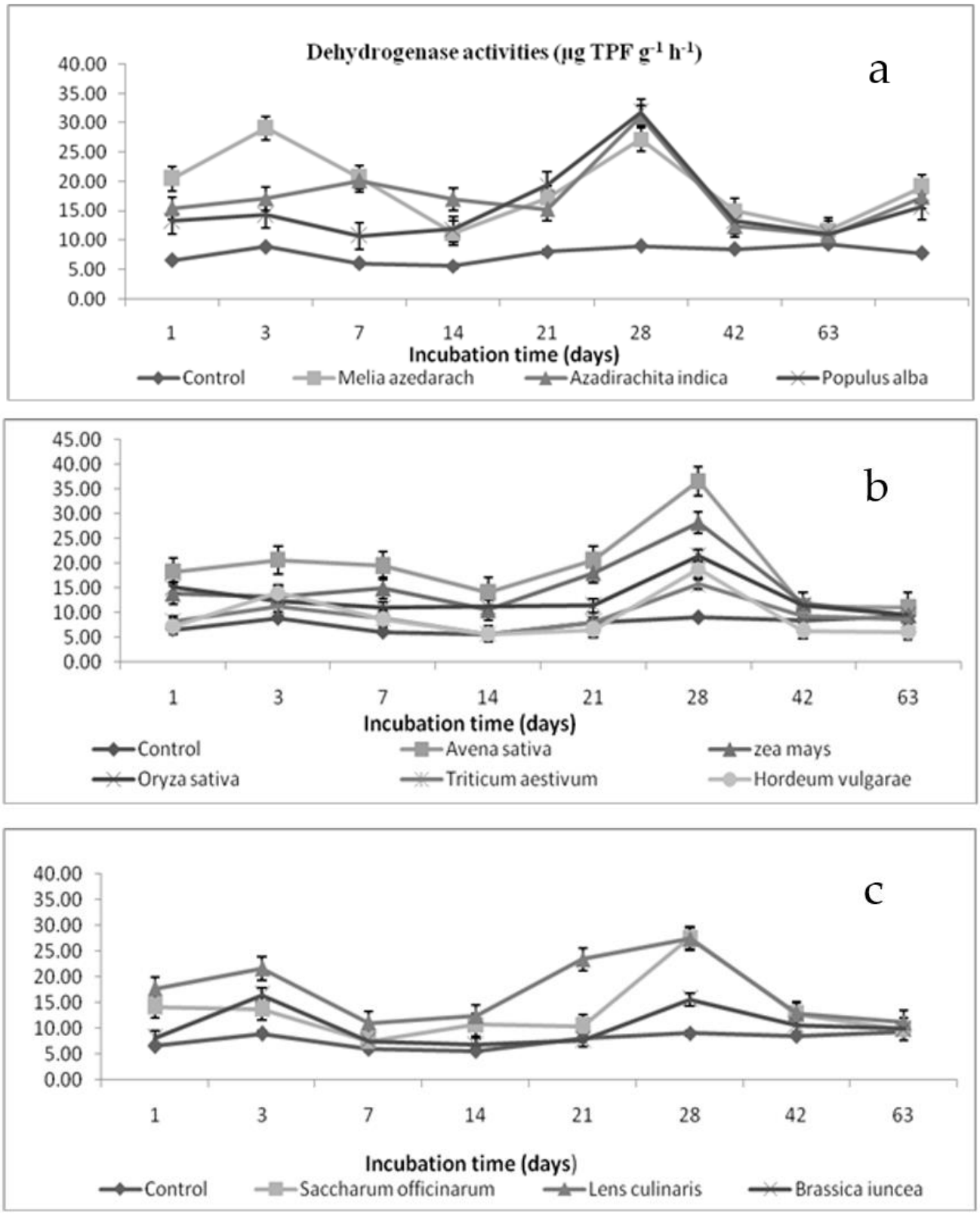

Figure 1. Changes in dehydrogenase activity (µg TPF g^{-1} h^{-1}) as influenced by tree litters (**a**), cereals (**b**), other crop (**c**) residues. Error bars denote standard deviation.

Table 2. Biochemical analysis of different crop residues.

Crop Residues	CEL (%)	HEM (%)	LIG (%)	TSS (mg/L)	ST (mg/L)	C (%)	N (%)	C/N
Tree residues								
Melia azedarach	17.6	7.80	9.50	0.58	0.17	44.6	4.82	9
Azadirachta indica	39.6	16.5	23.9	0.55	0.14	39.8	2.19	18
Populus alba	45.4	10.8	30.3	0.46	0.11	27.3	0.912	30
Cereals								
Avena sativa	27.1	21.3	14.8	0.84	0.16	42.0	1.32	32
Zea mays	39.5	22.5	18.9	0.78	0.12	38.2	0.62	62
Triticum aestivum	38.5	20.7	15.0	0.79	0.10	38.3	0.81	48
Oryza sativa	35.1	23.5	18.2	0.67	0.17	42.0	0.39	106
Hordeum vulgare	33.7	20.1	30.6	0.70	0.08	35.6	0.71	50
Other crop residues								
Lens culinaris	18.4	22.3	11.1	0.66	0.18	34.1	1.71	20
Saccharum officinarum	23.9	20.3	20.0	0.62	0.11	37.3	0.914	41
Brassica juncea	21.4	15.6	19.5	0.60	0.10	40.5	1.18	34

CEL—Cellulose; HEM—Hemicelluloses; LIG—Lignin; TSS—Total Soluble Solids; ST—Starch; C—Total Carbon; N—Total Nitrogen.

Fluorescein diacetate (FDA) activity ranged from 0.356 to 0.436, 0.254 to 0.306 and 0.308 to 0.380 µg fluorescein released/g dry soil/20 min for the residues of trees, cereals and the other plant species, respectively. Among plant residues, *Populus alba*, *Triticum aestivum* and *Saccharum officinarum* residues showed maximum activity (Figure 2). Among tree residues, *Melia azedarach* showed significantly higher FDA activities at the 7th DAI than the other residues species. In the case of cereal residues, *Triticum aestivum* showed significantly higher FDA activity, which was closely followed by *Avena sativa* and was least in the case of *Zea mays*. The *Saccharum officinarum* residue showed significantly higher FDA activity on the 7th DAI compared to other residues species.

The activity of APA and APP varied significantly among the various treatments and showed highest phosphatase activity up to the 14th and 21st DAI in *Azadirachita indica, Avena sativa* and *Lens culinaris*, respectively; thereafter, a marked decrease was observed (Figures 3 and 4). Among the tree species, *Azadirachita indica* showed maximum APA and APP enzymatic activity followed by *Melia azedarach* and *Populus alba.* Among the cereals, *Avena sativa* showed significantly higher APA and APP activity than the others. Among other plant residue species, *Lens culinaris* showing a significantly higher increase in the phosphatase activities than the others.

The values of phytase activity (PA) ranged from 11.2 to 12.5, 10.8 to 13.5 and 10.3 to 11.7 µg P hydrolysed/g/h in the soils amended with the residues of trees, cereals and the others, respectively. Initially, a slow increase in PA activity was noticed up to the 14th DAI, which increased markedly at the 28th DAI (Figure 5). Among the tree species, *Melia azedarach* showed significantly higher activity than other species. The significantly higher PA was observed in *Avena sativa* followed by *Zea mays*. Among the residues of other plant species, a significantly higher increase in PA activity at the 28th DAI was observed in *Lens culinaris* followed by *Saccharum officinarum*.

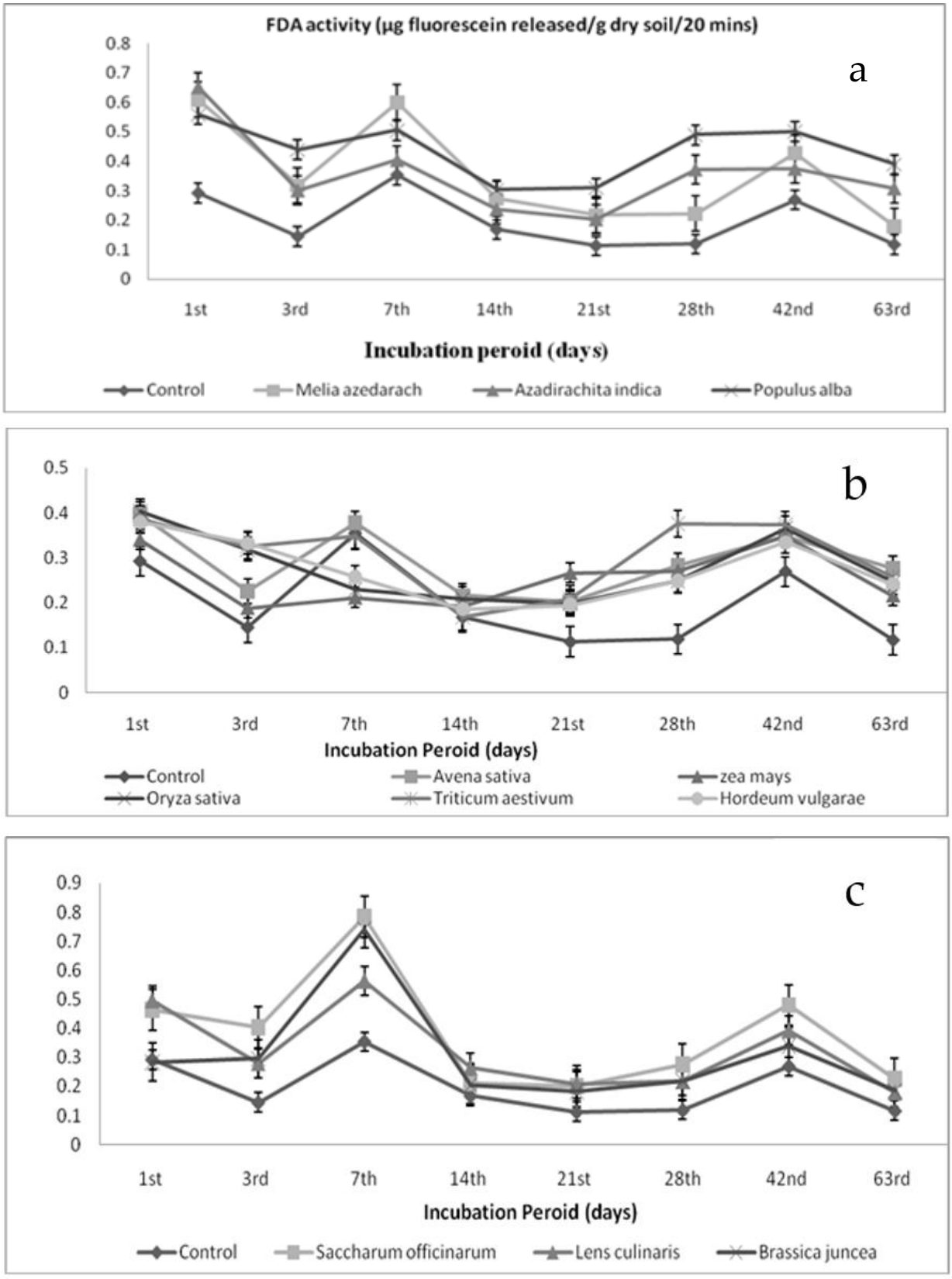

Figure 2. Changes in FDA activity (µg flourescein g^{-1} 20 min^{-1}) as influenced by tree litters (**a**), cereals (**b**), other crop residues (**c**). Error bars denote standard deviation.

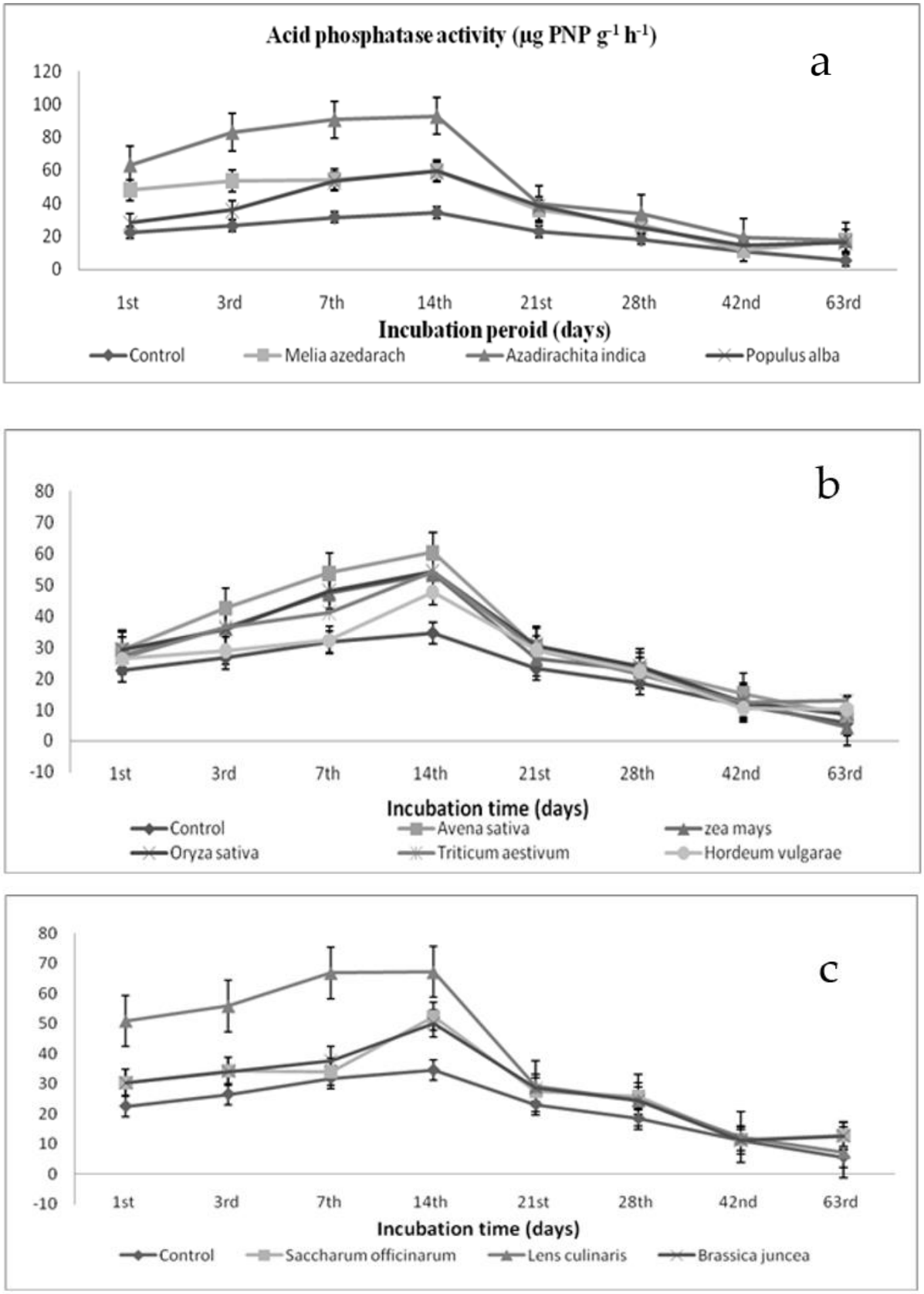

Figure 3. Changes in acid phosphatase activity(µg PNP g^{-1} h^{-1}) as influenced by tree litters (**a**), cereals (**b**), other crop residues (**c**). Error bars denote standard deviation.

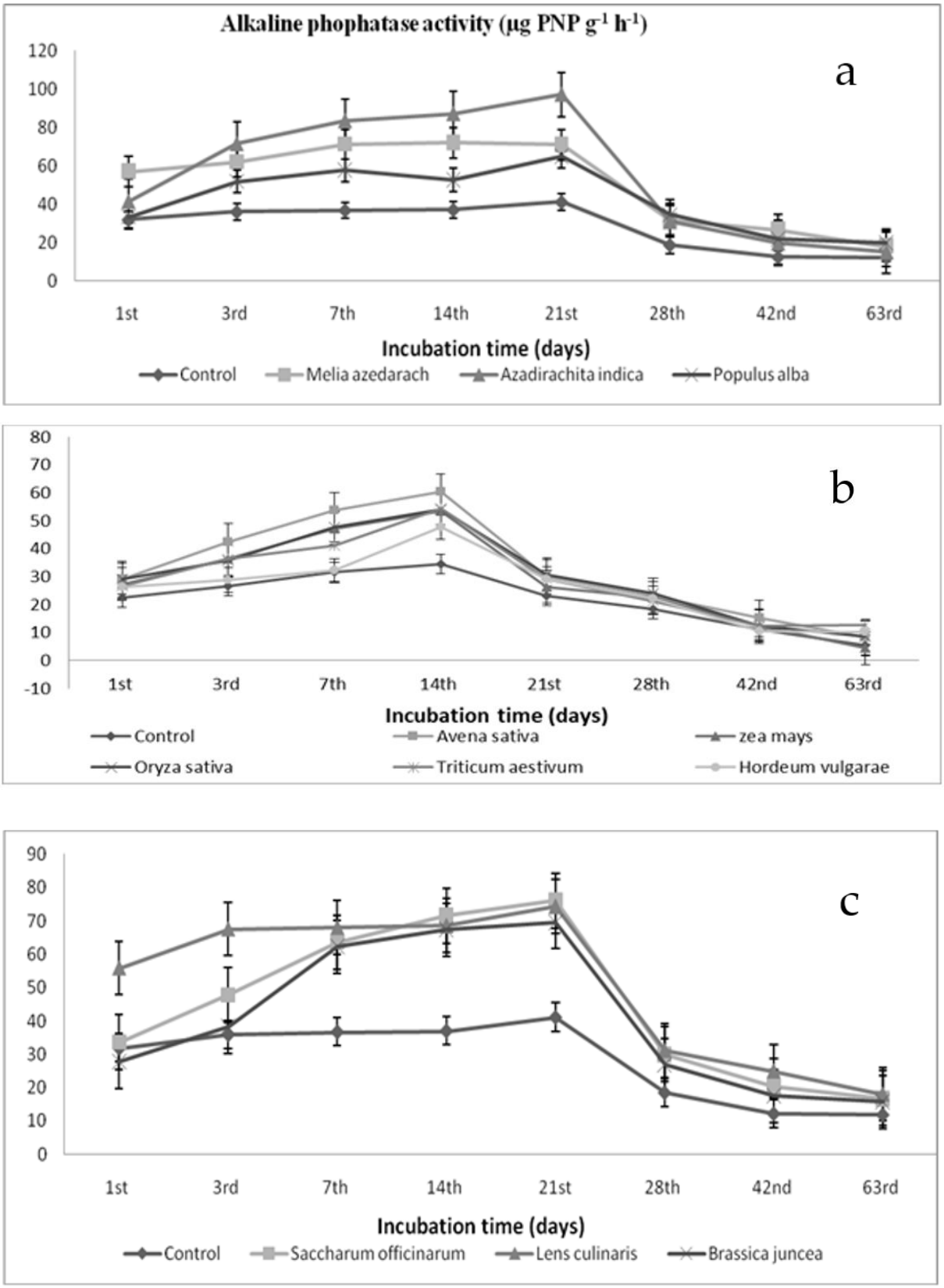

Figure 4. Changes in alkaline phosphatase activity (µg PNP g^{-1} h^{-1}) as influenced by tree litters (**a**), cereals (**b**), other crop residues (**c**). Error bars denote standard deviation.

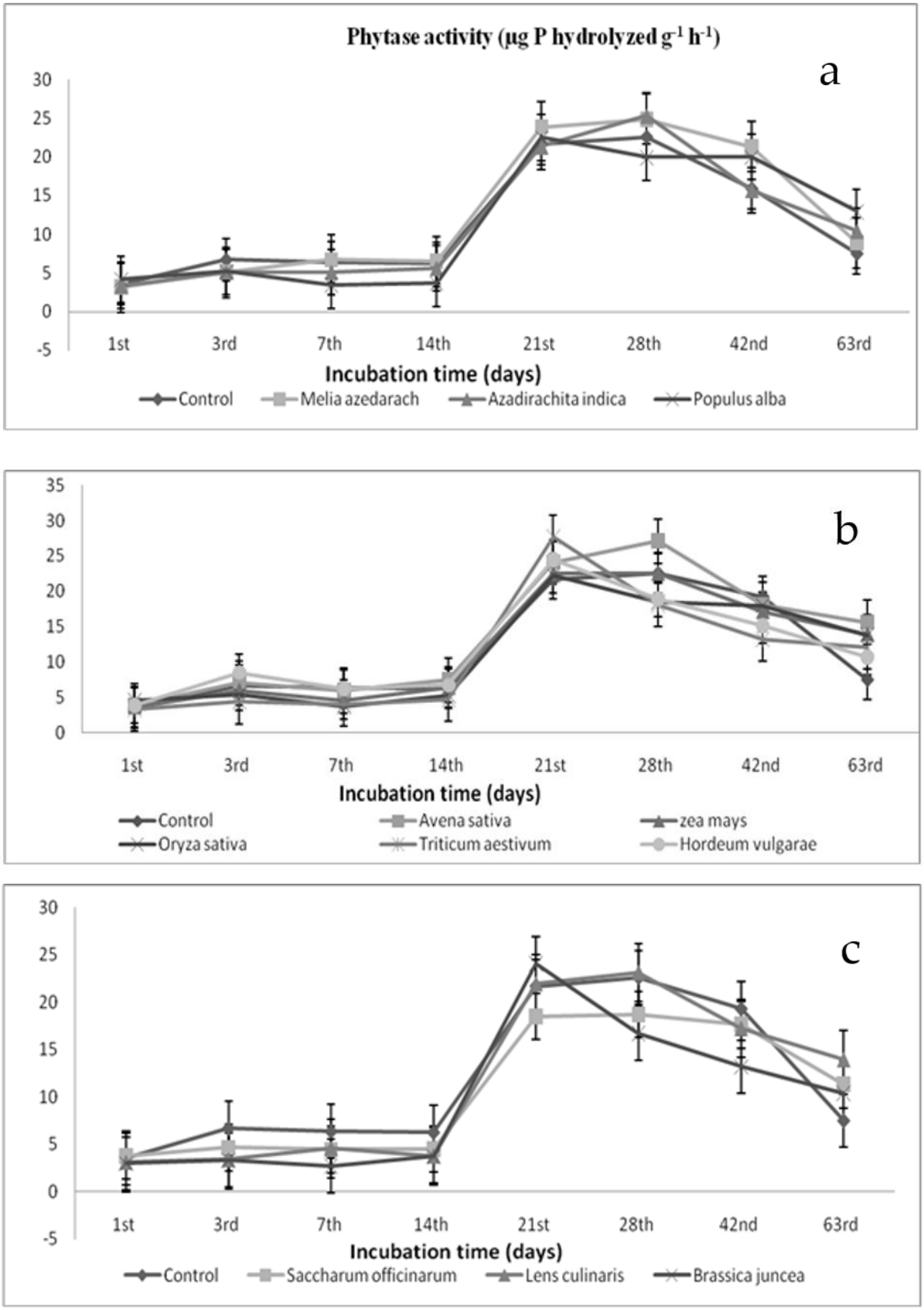

Figure 5. Changes in phytase activity (µg P hydrolyzed g^{-1} h^{-1}) as influenced by tree litters (**a**), cereals (**b**), other crop residues (**c**). Error bars denote standard deviation.

4. Discussion

Crop residues differed widely with regard to the chemical composition of their tissues, and these residues contribute differently to soil C inputs [50,51]. Liu et al. [51] concluded in an experiment that differences in chemical composition between various plant residues, and soil texture is the sole reason for the enhancement of residue degradation and SOC accumulation. The plant residues, compared with the unamended control, yielded a significant increase in the activities of different enzymes measured in this study. FDA and DHA activities in the soil amended with different plant residues showed higher activities on the 7th and 28th DAI compared to the control, respectively. It might be due to higher microbial activity during the mineralization of crop residues. The level of DHA in the soil can be used as an index of the total microbial activity. The additions of organic inputs to soil stimulate DHA activity because the added material may contain intracellular and extracellular enzymes [52]. Sekaran et al. [53] reported that applying various crop residues to the soil improved the dehydrogenase enzymatic activity in the soil. Dehydrogenases have a significant impact on a number of oxidative processes that dehydrate organic molecules [54]. In addition, a significant positive correlation between the SOC and DHA has also been studied [55]. Averaged over all the sampling dates, the maximum DHA was observed for *Avena sativa* amended plots followed by *Azadirachita indica*. The nature of plant residues (e.g., easily decomposable C fraction, cellulose, hemicelluloses, lignin) is reported to affect the enzymatic activity differently [56]. This is likely due to the increase in microbial activities that directly depend on labile C pools and stimulates autochthonous microbial activity in organic amended soils [57]. The rate of soil microbial population, growth and extracellular enzyme production of different crop residues having different chemical compounds change because of their different degradation rates [58]. Consistent with our study, other researchers [59,60] have also reported a decrease in DHA with time.

Unlike DHA, FDA hydrolysis peaked at the 7th DAI when the FDA production rate ranged between 0.211–0.784 µg fluorescein released/g dry soil/20 min. The increase in the FDA hydrolysis activity indicated an increase in C, and the nutrients release the soil from the added plant residues [11] since FDA is considered a comprehensive C cycling enzyme assay [61]. The hydrolysis of fluorescein diacetate (FDA), which affects the activity of various hydrolytic enzymes, is a gauge of the soil's overall microbial activity [62]. The FDA activity declined sharply thereafter, which may be due to the depletion of the easily available substrate. Averaged of all the sampling dates, residues of tree species exhibited the maximum FDA activity followed by other residues and was least in cereals. Zhao et al. [63] reported that FDA has been utilized to estimate the potential microbial activity of soil amended with a wide range of organic amendments. FDA has also been used to determine amounts of active fungi and bacteria and to locate acetyl esterases in living protist cells [64]. Cellulose is readily degraded by both fungi and bacteria. However, lignin is more recalcitrant, and its complete degradation is restricted to a selected group of fungi that produce the extracellular lignin peroxidases [65]. In addition, from different studies it is recognized that lignin depolymerization and the mineralization of subsequent diverse aromatics present in crop residues are the two steps in the biodegradation of lignin in nature [66]. Due to the above reasons, FDA activity was higher in those plant residues (e.g., *Populus alba*, *Triticum aestivum*, *Saccharum officinarum*), which have high cellulose and lignin content. These results can be attributed to the presence of higher cellulose and hemicellulose contents as well as low N content [67].

A significant increase in APA and APP activities were observed with the addition of different plant residues, which peaked at the 14th and 21st DAI, and thereafter, the enzymatic activities decreased markedly. Stegarescu et al. [68] in a similar study reported that the maximum increase in APA was between 7 to 10 days after the incorporation of plant residues, which was attributed by the increase in microbial respiration. Carlson et al. [69] reported in an experiment using organic amendments a significant decrease in the overall phosphatase activity over different time intervals, which suggest that the activity of enzymes is stimulated by organic amendments as phosphatase producing

microbial community is lost over time. In addition, the addition of organic amendments may stimulate microbial production of enzymes, such as dehydrogenase and phosphatase, to enhance organic matter decomposition and organic P mineralization [70]. The significant increase in phosphatase activity after the addition of different plant residues may be due to inherent high microbial activities [71]. Like phosphatase activities, PA is also related to the transformation and mineralization of organic P to inorganic P in soils. PA plays a crucial role in P cycling by catalysing the hydrolysis of the organic form of P in soil (i.e., phytate). In addition, P availability for plants and microbial uptake in soil is highly influenced by PA [72]. Sharma and Dhaliwal [73] reported a significant increase in phytase enzymes upon the application of organic amendment, such as rice straw compost. Similarly, Saikia et al. and Bera et al. [22,62] reported an increase in phytase activity in soils at days of vigorous growth by using green manures and crop residue as an organic amendment.

Enzymatic Activity Correlates Their Biochemical Composition of Plant Residues

The C/N ratio is usually considered a key factor in controlling decomposition and nutrient release and is considered to be a good indicator of litter decomposition rate in many ecosystems [74,75] as mineralization rates decrease with increasing C/N ratio [75]. Our incubation study showed that the lower C/N ratio and higher enzymatic activity influenced the mineralization process. The structural components and their proportions (soluble carbohydrates, cellulose, hemicelluloses, lignin, etc.) differ among plant residues and likewise break down at different rates, influencing microbial community structure [76]. High CEL and HEM is linked to rapid decomposition and high LIG content, high C/N ratio, and low total N are associated with a slower breakdown [77]. The present study also showed the slow enzymatic activity during the first week of incubation, which could possibly be due to the presence of recalcitrant forms of C, such as lignin [78,79].

5. Conclusions

The results from this study suggest that the increase in enzymatic activities with the addition of crop residues are strongly influenced by their biochemical composition (cellulose, hemicelluloses, lignin content) and their C/N ratio. The lower the C/N ratio, the more labile C pools will result in their high degradation and enzymatic activity. The quality of crop residues, and particularly their cellulose and hemicellulose content, will highly influence the soil enzymatic activities. Holocellulose (hemicelluloses + celluloses) was the responsible factor for explaining the variability of C mineralization and enzymatic activities in soil amended with different types of plant residues.

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