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3 **Long-term application of Jatropha press cake promotes seed yield by enhanced accumulation of soil**
4 **organic carbon, microbial biomass and enzymatic activity in soils of semi-arid tropical wastelands**

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Abstract

Jatropha curcas is a potential biofuel crop and press cake is an important co-product obtained during biodiesel production. In order to assess the long-term effects of application of *Jatropha* press cake on soil quality parameters and to evaluate its efficacy as an organic manure, a field trial was carried out in degraded wasteland of Gujarat, India with four treatments chosen on the basis of equivalents amounts of N supply through urea, *Jatropha* press cake, farmyard manure (FYM) and half dose of urea. The activities of various exocellular enzymes involved in nutrient cycling, microbial biomass carbon, respiration, fluorescein diacetate (FDA) hydrolysis and the influence of chemical composition of the organic manures on these parameters were studied following seven continuous years of application of nutrient amendments. Degradation of the phorbols, toxins present in press cake, was also studied. Except for acid phosphomonoesterase, enzyme activities were higher in FYM compared to other treatments. Lignin content was higher in FYM while the ligno-cellulosic fraction was higher by 45% in press cake as compared to FYM. Notably, carbon accumulation in soil was higher per unit of applied carbon in press cake treated soil. Ratio of microbial biomass carbon to soil organic carbon was higher in press cake amended soil. Phorbol esters were degraded to negligible amounts. The increase in yield of *Jatropha* following improved enzyme activities, microbial carbon and FDA hydrolysis owing to press cake application in erstwhile poor soils unequivocally establishes the scope of *Jatropha* press cake in reclaiming unfertile wastelands sustainably.

Keywords: *Jatropha* press cake, soil enzymes, microbial biomass, carbon sequestration, phorbol ester, ligno-cellulose

1. Introduction

Jatropha curcas is a potential biofuel crop for marginal lands in arid and semi-arid regions as it can contribute to the global fuel supply, while effecting the socio-economic upliftment of marginal landholders [1, 2]. In the present scenario, our assessment reveals that the cost of standalone *Jatropha* biodiesel production is relatively higher than the price of conventional high-speed fossil diesel [1]. Cultivation of *Jatropha* on marginal lands cannot be sustainable unless the product and the by-products are economically viable entities, while at the same time; the processes along the entire value chain are ecologically benign. Preparation of quality *Jatropha* biodiesel and its use to achieve cleaner emissions have been amply demonstrated [1, 3], but to make it more economically viable, the value-addition of its by-products is of profound importance towards reducing the overall cost of biodiesel production. Soap, glycerin, recovery of the catalyst (KOH) as potassic fertilizer, use of shells as solid fuel (briquette) or their gasification to generate electric power are the various ways by which one

can efficiently use the by-products, thereby reducing the cost of biodiesel production [1, 3, 4, 5]. *Jatropha* press cake is one of the main by-products obtained during biodiesel production from *Jatropha curcas* because for every metric ton of biodiesel manufactured, three times the amount of *Jatropha* press cake is produced [1]. However, the press cake obtained during oil expulsion, in spite of having high protein content, cannot be directly used as an animal feed owing to the presence of toxic constituents such as phorbol esters, trypsin inhibitors, glucosinolates, saponins, and phytates, etc. [6, 7, 8]. On the other hand, due to the presence of a high amount of nitrogen, besides containing phosphorus and potassium [9], several reports have suggested its use as an organic amendment either directly or after composting [10, 11, 12]. Although there are reports that clearly demonstrate the degradation of the toxic phorbols in soil under controlled conditions [13], reports of its degradation in the field has not been clearly demonstrated. Therefore, it is necessary to evaluate the degradation of phorbol esters in the soil if *Jatropha* press cake is to be used as an organic manure. Soil quality is a concept for examining and integrating relationships and functions among various biological (respiration, microbial biomass C, N, P etc.), chemical, biochemical (urease, acid and alkaline phosphomonoesterases, glucosidase etc.), and physical parameters (pH, hydraulic conductivity, infiltration rate, soil temperature etc.) in soils, which are important in the context of sustainable land use and management [14]. Although application of organic manure is a standard practice to replenish the nutrients [15] and maintain the soil quality, the *Jatropha* press cake, which is produced in large quantities, offers a good alternate substitute as an organic manure if it can be unequivocally proven that the latter improves soil quality without causing any adverse impact. The importance of plowing back *Jatropha* press cake and the practicality of utilizing it were also discussed earlier by us [1, 4, 16] wherein, a decentralized model for oil extraction or biodiesel production at the farm level was mooted for utilizing the cake as manure directly in the *Jatropha* plantation itself in a cost-effective manner. There are reports that evaluate the short-term effects of *Jatropha* press cake [17, 18], but the long-term effect of press cake application to soil on the biochemical (urease, arylsulphatase, glucosidase, acid and alkaline phosphomonoesterases, lipase) and biological properties (microbial biomass C, fluorescein diacetate hydrolysis, respiration, dehydrogenase activity) have not been thoroughly investigated in the field conditions. Recently, Wani et al. [19] have studied the effect of *Jatropha* press cake on yield of soybean and maize in a short term experiment to reveal the benefit of press cake as a source of plant nutrients under tropical conditions. However, they have not studied the effect of press cake on various soil biochemical and biological parameters which is of absolute importance in understanding the underlying mechanism by which press cake is beneficial in promoting soil quality. Soil enzymes and other microbial parameters not only play a role in nutrient cycling but are also

very sensitive to management practices and have been widely used as indicators of changes in soil quality resulting from management practices [20, 21, 22, 23, 24]. Thus, our aim was to evaluate whether long-term application of *Jatropha* press cake in degraded soils under semi-arid conditions could positively influence the soil physico-chemical and biological properties, without any negative implications on soil quality parameters vis-à-vis application of other organic amendment viz., farmyard manure (FYM) and mineral fertilizer. The specific objectives of the study were i) to quantitatively determine phorbol degradation under field conditions following press cake application ii) to examine and elucidate the influence of various nutrient management practices on the extracellular soil enzyme activities and biological parameters in *Jatropha* plantations iii) to study whether the improvement in various soil quality parameters leads to enhanced productivity.

2. Materials and methods

2.1 Study area, experimental design and soil sampling

The experiment was started in Neswad village (Altitude-314 ft, N 21° 30' 494'' E 072° 02' 185'') of Bhavnagar district, Gujarat, India in the year 2007. Initially, the land was cleared of the existing vegetation. The average annual rainfall in the region was 579 mm during the period of study. The region has mean maximum and minimum temperature of 35.3 and 18.7° C, respectively. The soil is classified as loamy mixed calcareous hyperthermic lithic ustorthents. In order to evaluate the efficacy of *Jatropha* press cake in comparison to organic manure (FYM) and mineral fertilizer (urea), an experiment was designed on degraded wastelands with four treatments chosen on the basis of N supply. The treatments were *Jatropha* press cake applied at 3.89 t ha⁻¹ (having an average of 3.6% N content) equivalent to 140 Kg N ha⁻¹; an equivalent dosage of 100% N applied through mineral fertilizer, urea, which served a control; farmyard manure (FYM) applied at 18.7 t ha⁻¹ (having an N content of 0.75%) and finally a reduced N dose of 50% through mineral fertilizer (70 kg N urea ha⁻¹). However, the above dose was reduced in subsequent years from July 2011 onwards to 100 kg N ha⁻¹ through urea and an equivalent dose of N was applied through press cake and FYM in the respective treatments. The amount of urea applied in the 50% N dose treatment was also reduced accordingly. Higher dosages of N applied in the initial years was to ensure the successful establishment of the *Jatropha curcas* plantation in degraded soil with inherent low soil fertility. Also, application of 140 kg N ha⁻¹ led to intense above-ground vegetative growth which resulted in lodging of plants in later years, prompting us to reduce the dose. In addition, P as P₂O₅ at 50 kg ha⁻¹ was uniformly applied to all the treatments through a single superphosphate until 2011. Thereafter, its application was reduced to 10 Kg ha⁻¹ based on findings of another study that the application of P successively over the years did not significantly increase the yield in *J. curcas* (unpublished data). Potassium at 25 kg K₂O

ha⁻¹ was applied annually through muriate of potash. Native soil without *J. curcas*, located outside the experimental block was also studied for comparative purpose; however, the data was not included in the statistical analysis. A half dose of N through urea, as well as a full dose of P, K and organic manures were applied annually in the month of June/July and the remaining half dose of mineral fertilizer was applied a month later. The plantation was irrigated during the non-rainy periods (December to May) with 4.4 ha cm of water. The experiment consisted of five blocks with four treatments per block. Each treatment within the block constituted a plot with five plants each with spacing of 3 m X 3 m in a randomized block design. The individual treatment plots were separated by ridges to avoid cross contamination. Soil samples (0-20 cm) were collected randomly from three plants of each treatment plot within a block. The samples were pooled to obtain a composite sample for each plot. In this way, a total of 20 samples were collected for all the treatments. For time management purpose, soil sampling was done in two phases, i.e., soil samples were collected in the months of November 2013 to analyse phorbol ester content and in January 2014 for studying all other physico-chemical and biological characteristics. The soil samples were immediately sieved (< 2 mm) and stored at 4° C prior to analysis. All the enzymatic and microbial parameters were analyzed within 2 weeks of sample collection while the other physico-chemical analyses were completed within 8 weeks.

2.2 Analysis of proximate carbon fractions of press cake and FYM

Lignin, cellulose, acid detergent soluble substances (ADSS) and ash content of press cake and FYM were determined according to Van Soest and Wine [25] as modified by Fioretto et al. [26]. C and N contents of press cake and FYM were determined by Vario cube CHNS analyzer (Elementar Analysensysteme GmbH).

2.3 Analysis of soil physico-chemical, biochemical and microbial parameters

Soil pH and electrical conductivity (EC) were measured in 1:2 slurry of soil: water. Gravimetric water content was determined in 10 g portions of soil by drying at 105° C until a constant weight was achieved. Organic carbon (C_{org}) of the soil was determined according to the Walkley and Black procedure as described in Nelson and Sommers [27]. The total nitrogen of the soil was determined according to a modified Kjeldahl method using salicylic acid [28], while available P was determined by Olsen's method [29]. Basal respiration was determined by the alkali adsorption technique of Anderson [30] following ten days of incubation of field moist soil. Metabolic quotient was calculated as the ratio of basal respiration to mg microbial biomass carbon. Microbial biomass carbon (C_{mic}) was estimated by the fumigation–extraction method according to Vance et al. [31] using a K_c factor of 0.45. Soluble C in the K₂SO₄ extracts was analyzed using a LiquiTOC analyzer (Elementar Analysensysteme GmbH). The moisture content of the samples were adjusted to 50% water holding capacity

and equilibrated for 72 h at room temperature prior to analyses of the microbial biomass C. Aryl sulphatase, acid and alkaline phosphomonoesterase and glucosidase activities in the field moist soil were assayed according to Tabatabai [32] and lipase activity in soil was measured according to Margesin [33] using 4-nitrophenol as the standard. Urease, dehydrogenase and FDA activities were measured according to Kandeler and Gerber [34], Von Mersi and Schinner [35], and Schnürer and Roswall [36] respectively.

2.4 Analysis of phorbol esters

Phorbol ester in fresh samples of *Jatropha* press cake, oil and press cake amended soil was determined according to Makkar et al. [37] using phorbol- 12 myristate 13 acetate as standard. Briefly, 2g of sample was taken in a 50 ml capacity plastic centrifuge tube and sonicated using OscarUltrasonic cleaner (Model -ME109) at 240 W for 3 min following addition of 15 ml of methanol (100%). The contents were centrifuged at room temperature for 8 min. The supernatant was collected in a round bottom flask. The extraction step was repeated twice. The supernatants were pooled and concentrated using a vacuum evaporator till dryness and then quantitatively extracted with methanol. The extracts were centrifuged at 17000 x g for 10 min at room temperature, appropriately filtered through 0.2µm filter and used for high performance liquid chromatography (HPLC) which was carried out using Waters 2695 separation unit coupled with Waters 2996 photodiode array detector equipped with a reverse phase C18 column (Phenomenex , Luna 5 µm, 250 mm 4.6 mm i.d.), Waters 600 HPLC quaternary pump, Waters inline degasser and empower software. The column temperature was controlled at 25°C and the flow rate was 0.8 mL min⁻¹. The solvents used were 1.75 ml o-phosphoric acid (85%) in 1 L distilled water (A) and acetonitrile (B). All solvents were filtered and degassed by a Waters inline degasser. The gradient used was as follows: 0–20 min, 60% A and 40% B; 20–28 min, 25% A and 75% B; 28–33 min, 100% B; 33–37 min, 100% C and then the column was adjusted to the starting condition (60% A and 40% B). The phorbol esters (four peaks) appeared between 31 and 35 min. The peaks were integrated at 280 nm, and the results were expressed as equivalent of phorbol-12-myristate 13-acetate (Sigma), whose peak appeared at 39 min. Each sample was analyzed in triplicate.

2.5 Statistical analysis

Statistical analyses were carried out using Origin 9.0 and Excel 2010. The data are represented as mean ± standard deviation of 5 replicates unless otherwise mentioned. Data were log transformed wherever necessary in order to satisfy the assumptions of normality and variance and then were subjected to analysis of variance (ANOVA). A randomized block design (RBD) procedure was employed in Excel for ANOVA involving 4 treatments and 5 replications or blocks and significant differences between treatments means were analyzed

with least significant difference (LSD) at $p < 0.05$. Pearson's correlation coefficients were obtained using Origin 9.0 to examine the relationship between various measures of pH, total N, organic matter content, and the size and activity of the soil microbial community against the activity of assayed exocellular enzymes.

3. Results

3.1 Effect of urea, Jatropha press cake and FYM on soil physico-chemical and microbial parameters

The soil pH in the treatments ranged from 6.40 to 7.57 and varied significantly with treatments. Jatropha press cake application over the years (2007-2014) did not significantly alter the soil pH as compared to different doses of mineral fertilizer (MF). However, application of FYM resulted in a significant increase ($p < 0.05$), with a recorded pH of 7.57. The soil electrical conductivity (EC) also followed a similar trend to that of soil pH with cake amended soil being at par with urea-amended soils, while FYM amended soil showed higher EC values (0.32 dS m^{-1}). The native soil had a pH and EC of 6.97 and 0.14 dS m^{-1} , respectively. Application of Jatropha press cake significantly increased ($p < 0.01$) C_{org} , available P and total N content in the soil as compared to the control and 50% urea treatments (**Table 1**).

<<Table 1>>

<<Table 2>>

<<Figure 1>>

These parameters were lower as compared to FYM, wherein the highest amounts of C_{org} (3.04%), available P (13.2 mg kg^{-1}) and total N (0.14%) contents were observed. Urea application resulted in ca. 50% improvement in C_{org} when compared with native soil, while the extent of improvement was more than threefold using organic treatment. Further, carbon sequestration was higher in press cake amended soil per unit of carbon applied. No significant difference was observed among the treatments for the soil C:N ratio. The C_{org} , available P, total N and C:N ratio of the native soil were 0.71%, 1.71 mg kg^{-1} , 0.03% and 20 respectively. As expected, a statistically higher accumulation ($p < 0.01$) of C_{mic} was observed in organic treatments (press cake and FYM) as compared to soils treated with different doses of mineral fertilizer. However, among the organic treatments, press cake and FYM were statistically at par with respect to the each other (**Table 1**). Further, there was a strong linear correlation ($R = 0.835^{***}$) between C_{mic} and C_{org} (**Fig. 1**). Basal respiration, as measured by an alkali adsorption technique, was significant ($p < 0.05$) in press cake amended soils ($0.53 \pm 0.14 \mu\text{g CO}_2\text{-C g}^{-1} \text{ dry soil h}^{-1}$) as compared to all other treatments. On the other hand, $q\text{CO}_2$ was significantly higher ($p < 0.05$) in control (urea 100% N) as compared to FYM but was at par with the other treatments indicating relatively higher stress in Urea treatments. Further, FYM amended soil had the lowest $q\text{CO}_2$ of $0.84 \pm 0.48 \mu\text{g CO}_2\text{-C mg}^{-1} C_{\text{mic}} \text{ h}^{-1}$ but

was at par with press cake treatment (**Table 1**). Basal respiration and $q\text{CO}_2$ of native soil was $0.08 \pm 0.06 \mu\text{g CO}_2\text{-C g}^{-1} \text{ dry soil h}^{-1}$ and $0.80 \pm 0.03 \mu\text{g CO}_2\text{-C mg}^{-1} \text{ C}_{\text{mic}} \text{ h}^{-1}$ respectively. As compared to control and T4 treatment, both the organic amendments significantly increased ($p < 0.01$) FDA hydrolysis while dehydrogenase activity was higher in FYM treated soil, which are indicators of the overall microbial metabolic activity (**Table**

1).3.3 Proximate analysis of Jatropha press cake and FYM

The proximate carbon fractions of press cake and FYM showed that the former contained 20% more ADSS as compared to the latter. Further, the lignin content was higher in the FYM while the cellulose content was higher in the cake (**Table 2**). Moreover, the lignin+cellulose content, was higher in the cake by ca. 45% over the FYM.

<<Table 3>>

<<Table 4>>

The treatments had a highly significant ($p < 0.01$) influence on the various exocellular enzymes that were assayed (**Table 3**). Application of Jatropha press cake and FYM resulted in a significant increase ($p < 0.05$) in soil enzymatic activities as compared to both the doses of MF and the order of increase was Control = urea 50% N < press cake < FYM for arylsulphatase, glucosidase, urease, and alkaline phosphomonoesterase. However, in the case of acid phosphomonoesterase, the increase in activity was in the order Control = urea 50% N < FYM < press cake. Lipase activity was not significant in the experiment. Further, the enzymatic activities when expressed as a ratio per mg C_{mic} , were significantly lower in Jatropha press cake as compared to the other treatments (**Table 3**). Except for acid phosphomonoesterase, the activities of all other enzymes viz., urease, glucosidase, arylsulphatase and alkaline phosphomonoesterase correlated significantly and positively with pH, C_{org} , total N, C_{mic} . On the other hand, a non-significant inverse correlation ($R=-0.141$; $p=0.554$) was observed in the case of acid phosphomonoesterase activity, with pH (**Table 4**). Glucosidase activity was the only one that had a significant and positive correlation with all the microbial, physical and biochemical properties that were evaluated. Further, the FDA activity was the only microbial parameter that significantly correlated with all the enzymatic activities. In general, the enzymatic activities as well as microbial properties of the native soil were comparable to mineral fertilizer application in most of the cases but were lower than Jatropha press cake and FYM treatments. Among the extracellular soil enzymes that were assayed, there was an absence of correlation between acid phosphomonoesterase and other enzymatic activities studied. Further, an inverse non-significant correlation with urease activity was also observed. However, a strong and significant correlation ($p < 0.05$) was observed among the other extracellular enzymes that were studied, except between urease and lipase (**Table 5**). The $\text{C}_{\text{mic}}:\text{C}_{\text{org}}$ ratio was 0.0207, 0.0140, 0.0142, 0.0117 in Jatropha press cake, control, farmyard manure and

urea 50% N, respectively. Further the ratio was significantly higher ($p=0.0046$) in press cake as compared to the other treatments. Data for the last two consecutive years revealed marked improvement in seed yield following the long-term application of *Jatropha* press cake to the soil, which was followed by that obtained under FYM treatment. The seed yield obtained under control conditions during this period was 20-26% lower than press cake treatment, while in comparison to FYM treatment, it was 13-14% lower (**Table 6**).

<<Table 5>>

<<Table6>>

<<Figure 2>>

3.3 Analysis of phorbol esters in press cake amended soil

Analysis of phorbol esters in the soil revealed that there was negligible presence (0.001 mg g^{-1}) of these compounds indicating their degradation within four months of press cake application (**Fig. 2**). However, the presence of the same was established in fresh samples of *Jatropha* oil (1.4 mg g^{-1}) and press cake (0.04 mg g^{-1}).

4.0 Discussion

4.1 Influence of nutrient amendments on soil physical properties

The present experiment was carried out with the primary objective of evaluating the efficacy of *Jatropha* press cake in improving the soil quality by investigating the soil physico-chemical, biochemical and microbiological properties. As observed from the data, press cake amendment to the soil did not alter the soil pH as compared to control and urea 50% N treatment. The increase in pH of soil fertilized with FYM might be primarily attributed to relatively higher pH of the FYM ($\text{pH}=7.5$) itself. This might also be partly explained by a corresponding release of hydroxyl ions following decomposition of FYM [38]. Continuous addition of press cake and FYM over the period resulted in a gradual accumulation of nitrogen in soil relative to control. Soil sampled previously from the same experiment revealed that the cation exchange capacity (CEC) in press cake amended soil was the highest (45 cmol kg^{-1}) while being at par with FYM amended soil, and was 22% higher than control (personal communication with DR Chaudhary).

4.2 Influence of nutrient amendments on soil biochemical parameters and phorbol ester degradation in press cake amended soils

The enzymes assayed in this study play a crucial role in C, N, S and P cycling in soils and are more sensitive to changes in soil properties. Hence, their measurements are largely utilized as indicators of the management's effect on soil health and biological fertility [39]. The increasing activity of these enzymes under organic treatments as compared to different doses of mineral fertilizer implies a higher rate of turnover of C, N, P and S

in these treatments [40]. Similar increases in enzymatic activities have been observed in soils under organic management [41, 42]. The positive linear correlation observed between C_{org} and the activity of the assayed enzymes (except acid phosphomonoesterase) was the reason for significantly higher enzyme activities in the organic amended soils as compared to those in urea fertilized soils. Among the organic treatments, the FYM treatment, having the highest C_{org} , exhibited the highest enzymatic activity. A similar relationship between organic C content and the activity of assayed enzymes was also observed by others [40, 43, 44, 45] and this may be attributed to better stability of enzymes in the presence of higher organic carbon [46]. The inverse correlation found between acid phosphomonoesterase and pH of soil might be the reason for the significant increase in activity of this enzyme in press cake amended soil as compared to the FYM and other treatments. However, the lower activity of this enzyme in urea-amended soils in spite of their lower pH may be due to the presence of relatively lower amounts of C_{mic} and C_{org} as compared to the press cake treatment. In addition, there was a lack of significant correlation between acid phosphomonoesterase as well as other assayed enzymes, except for, FDA. Thus, heightened acid phosphomonoesterase activity as well as an increase in microbial biomass can be used as a signature for assessing press cake application in at least neutral to near neutral soils. A lack of any significant lipase activity in any of the treatment, more particularly in press cake treatment, implies that there is no effect of trace oil (usually ca. 6-8% in *Jatropha* press cake). Higher urease activity in organic treatments as compared to urea treatments in our experiment is probably due to its strong correlation with soil organic matter and microbial biomass [47, 48, 49, 50]. Phorbol esters are toxins present in press cake, which may have deleterious effect on soil health if they persist for longer time durations. Our studies, in open field conditions, unequivocally prove that they are degraded within a four months' time after the press cake application and also do not have any adverse effect on soil microbial parameters.

4.3 Influence nutrient amendments on soil organic carbon accumulation and biological parameters and its effect on yield

The soil organic carbon was improved by *Jatropha* cultivation irrespective of the type of nutrient management practice that was adopted. Notably, the organic carbon that was accumulated in soil per unit of carbon applied was much higher through the *Jatropha* press cake, in spite of it being applied in lower quantity as compared to FYM. Further, we believe that the shade beneath the dense canopy of *Jatropha* [51] offered a favourable soil microclimate that might have prevented rapid turnover of the added carbon. In addition, soil temperatures of the plantation with plants in deciduous state were found to be up to two degrees celsius lower as compared to fallow

land. The influence of soil temperature and moisture on soil organic carbon transformation and turnover has been reported [52].

A significant positive linear correlation between C_{org} and C_{mic} has been reported by several workers [23, 53, 54, 55]. Although our results broadly agreed to the same correlation pattern, an intriguing observation was that the press cake and FYM treated soils were at par with respect to C_{mic} accumulation, in spite of a significantly lower C_{org} in the former. This trend of higher C_{mic} accumulation in press cake treated soil was also consistently noticed in our earlier periodic observations (data not shown). Further, the increase in C_{mic} was concurrent with the increased FDA and dehydrogenase enzyme activities in soil, which are indicators of overall microbial activity. There is a general belief that C_{mic} declines in managed agricultural systems as opposed to unmanaged ones [56] which is predominantly on account of decrease in soil organic matter in the former. Increase in C_{mic} is not uncommon in field experiments [49, 57, 58, 59] and has been attributed to the presence of readily available nutrients, pH, different crop residues, contribution from root litter, root exudates, reduced or no tilling [23, 49, 58, 60]. But not many researchers have elaborated on the importance of the substrate quality in contributing to the higher C_{mic} observed in field experiments, although it is well documented in literature that, the prevailing environmental conditions, substrate composition, and their quality have profound influences on microbial utilization of C and nutrients in the substrate [56, 61, 62, 63, 64, 65, 66]. The proximate C analysis of press cake and FYM revealed that although the lignin content was lower in the press cake, the cellulose content was higher as compared to the FYM. It is well known that the presence of a high N content of the substrate results in the rapid decomposition of labile C and N compounds as well as non-lignified cellulose [62, 67] and turnover of C, while ligno-cellulosic fractions as well as lignin are recalcitrant and are slowly degraded [26]. Thus, we conjecture that the bulk of the cellulosic fraction of press cake might be in the form of lignocellulose and this together with a high amount of ADSS fraction and higher leaf and root litter formation might be responsible for the sustained higher C_{mic} in press cake amended soils. The advantage of press cake over the other treatments in increasing the leaf and root biomass was evident even during observations in the early stages of this experiment (2.5 years old), wherein it was found that application of press cake led to 21.2 and 9.2% increase in dry root biomass accumulation and 14.7 and 8.9% increase in dry leaf litter fall over control and FYM, respectively. Higher microbial biomass in press cake amended soil, as deduced by FAME analysis, was also reported by Chaudhary et al. [17] in microcosm experiments carried out over a short period of 60 days. Additionally, a significantly higher $C_{mic}:C_{org}$ ratio in press cake amended soils, which is supposed to be a

reliable soil microbial parameter for describing changes in managed ecosystems [68] further substantiates how *Jatropha* press cake application improves soil quality.

The ratios of enzyme activities to biomass carbon were also relatively lower for most of the enzymes in *Jatropha* press cake which might suggest that the enzyme production in press cake is limited by the recalcitrant nature of the substrate. This view is supported by Amin et al. [69] who have recently reported a decrease in specific activities of measured enzymes during decomposition of maize roots as compared to leaf residues and ascribed it to the presence of higher ligno-cellulosic fractions of the roots besides other chemical features of the wall constituents. Further, lower enzymatic activities does not necessary reflect lower efficiency [70] and we hypothesize that higher efficiency of the enzymes in *Jatropha* press cake amended soil might have resulted in more resource allocation to increase biomass by some group of microorganisms for ecological or competitive advantage over others.

The metabolic quotient (qCO_2), another stress indicator and a variable that evaluates the efficiency of soil microbial populations in utilizing C compounds [54, 71], was least in FYM, which was however, at par to press cake treatment, and significantly different from MF treatments. The higher qCO_2 in mineral fertilizer amended plots can be explained by their relatively lower pH resulting from fertilizer induced acidification [58]. Further, a lower qCO_2 has been ascribed to increased fungal presence [72] which might have been the case in the press cake and FYM treatments due to the presence of the predominant recalcitrant C fractions. Thus, the absence of any adverse effect on soil microbial biomass and biochemical activity establishes the fact that the application of press cake is safe without any negative implications on soil health. Similar results were drawn by Chaudhary et al. [17] who found a significantly lower ratio of saturated to monounsaturated fatty acid methyl esters (FAMES), an indicator of lower stress, in press cake amended soils. Further, it has been reported that nutrient availability and productivity of agro ecosystems depend on the size and activity of the microbial biomass [73]. Thus, we believe that the increased microbial biomass in press cake amended soil, along with the increased enzymatic activities observed even after a time lag of 6 months following press cake application, connote sustained processes towards nutrient solubilization that might have nourished the plants with an adequate nutrient supply along the entire fruiting period. This is important since *Jatropha* has a continuous asynchronous flowering and fruiting pattern (June-July to January-February) and availability of nutrients to the plants until the end of fruiting season increases yield. The higher seed yield obtained due to application of press cake as compared to other treatments corroborates our findings. A similar increase in yield with a concomitant increase in C_{mic} as well as $C_{mic}:C_{org}$ ratio has also been reported by [74] in soybean. It might be noted that the

reduction in the application of mineral fertilizer by half, reduced the yield significantly by 14-21%, but the soil health parameters were numerically superior as compared to full inorganic N application (control). Taking note of the improved soil microbial and biochemical parameters due to the application of mineral fertilizer at a lower rate as well as the organic amendments, especially the press cake, it is imperative that an integrated nutrient management strategy be employed to optimize the quantities of inorganic fertilizers and organic manure inputs for achieving higher yields in a sustainable manner [58, 75]. Although this has been envisaged for further studies.

5.0 Conclusions

Application of *Jatropha* press cake directly (without composting) improved the soil physical, chemical, biochemical and microbial properties by increasing these parameters relative to MF. Although the C addition to soil through FYM treatment was more than two fold higher than through cake application, the accumulation of organic carbon per unit of C addition in press cake amended soil was approximately 54% higher. The higher C_{mic} to C_{org} ratio along with other favourable soil health indicators through press cake application validates its potential of increasing soil organic matter build up and enhanced soil fertility in the long run. The study also unambiguously establishes that the toxic phorbol esters are degraded and do not deter microbial activity. All of the evaluated parameters due to press cake application favourably contributed towards increased *Jatropha* productivity. Thus, we believe that use of *Jatropha* press cake would increase the soil organic matter in semi-arid tropical regions where the accumulation of latter is low due to rapid turnover rates. Therefore, we suggest an integrated nutrient management strategy using *Jatropha* press cake and mineral fertilizers for achieving sustainable, higher seed yield. The enhanced soil fertility and biological activity as evident by increased organic carbon and enzymatic activity mediated by soil microbes in the erstwhile poor soils unequivocally establishes the scope of *Jatropha* press cake in reclaiming unfertile wastelands.

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Conflict of interest

The authors declare that they do not have any conflict of interest.

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Table 1

Influence of nutrient amendments on soil physical, chemical and microbial properties in *Jatropha* plantation. Values represented are mean $\pm$ S.D of 5 replicates; values followed by different alphabets in the rows are significantly different at $P < 0.05$ using LSD. Note native soil was not part of the experimental design and hence not included in data analysis and provided only for comparative purpose.

Parameter	Treatment				
	Urea (100% N)	Jatropha press cake	Farmyard manure	Urea (50% N)	Native soil
Soil EC (dS m ⁻¹)	0.17 $\pm$ 0.03 a	0.26 $\pm$ 0.04 b	0.32 $\pm$ 0.03 c	0.20 $\pm$ 0.07 a	0.14 $\pm$ 0.01
Soil pH	6.40 $\pm$ 1.05 a	6.67 $\pm$ 0.73 a	7.57 $\pm$ 0.07 b	6.63 $\pm$ 0.78 a	6.97 $\pm$ 0.01
Available P ^a (mg kg ⁻¹)	1.8 $\pm$ 1.3 a	11.1 $\pm$ 1.8 b	13.2 $\pm$ 1.2 b	1.7 $\pm$ 1.1 a	1.6 $\pm$ 1
Organic C (%)	1.05 $\pm$ 0.17 a	2.32 $\pm$ 0.26 b	3.04 $\pm$ 0.41 c	1.07 $\pm$ 0.14 a	0.71 $\pm$ 0.24
Total N (%)	0.04 $\pm$ 0.01 a	0.10 $\pm$ 0.02 b	0.14 $\pm$ 0.02 c	0.06 $\pm$ 0.01 a	0.03 $\pm$ 0.01
C/N	26 $\pm$ 7 a	24 $\pm$ 3 a	22 $\pm$ 1 a	19 $\pm$ 2 a	20 $\pm$ 6
Biomass C ^b	148 $\pm$ 62 a	489 $\pm$ 187 b	437 $\pm$ 121 b	125 $\pm$ 44 a	151 $\pm$ 10
Basal respiration ^c	0.24 $\pm$ 0.11 a	0.53 $\pm$ 0.14 b	0.34 $\pm$ 0.12 a	0.21 $\pm$ 0.08 a	0.08 $\pm$ 0.06
Metabolic quotient ^d	1.72 $\pm$ 0.59 b	1.20 $\pm$ 0.54 ab	0.84 $\pm$ 0.48 a	1.68 $\pm$ 0.49 b	0.80 $\pm$ 0.03
FDA hydrolytic activity ^e	22 $\pm$ 7 a	61 $\pm$ 6 b	57 $\pm$ 10 b	24 $\pm$ 5 a	36 $\pm$ 7
Dehydrogenase activity ^f	49 $\pm$ 25 a	95 $\pm$ 25 a	114 $\pm$ 54 b	60 $\pm$ 16 a	85 $\pm$ 15

^a Back transformed log values

^b $\mu\text{g C g}^{-1}$ dry soil

^c $\mu\text{g CO}_2\text{-C g}^{-1}$ dry soil h^{-1}

^d $\mu\text{g CO}_2\text{-C mg}^{-1} \text{C}_{\text{mic}} \text{h}^{-1}$

^e $\mu\text{g fluorescein released g}^{-1}$ dry soil 2h^{-1}

^f $\mu\text{g INTF released g}^{-1}$ dry soil

Table 2

Characteristics of deoiled cake and FYM. Values represented are mean $\pm$ S.D of 3 replicates except for C:N which are presented as means.

Characteristics	Organic substrate	
	Deoiled Cake	FYM
ADSS (%)	51.5 $\pm$ 0.2	40.3 $\pm$ 1.3
Cellulose (%)	37.9 $\pm$ 0.6	15.1 $\pm$ 2.0
Lignin (%)	8.8 $\pm$ 0.7	15.6 $\pm$ 1.1
Lignin + cellulose (%)	46.8 $\pm$ 0.2	30.7 $\pm$ 0.8
Ash content (%)	1.7 $\pm$ 0.2	29.0 $\pm$ 0.6
Total Nitrogen (%)	3.6 $\pm$ 0.2	1.1 $\pm$ 0.1
Total Carbon (%)	42.4 $\pm$ 0.2	21.6 $\pm$ 0.25
C:N ratio	11.8	18.9

Table 3

Influence of different nutrient amendments on potential extracellular soil enzymatic and specific activities (expressed per unit of microbial biomass carbon) in Jatropha plantation. Values represented are mean $\pm$ S.D of 5 replicates; values followed by different alphabets in the rows are significantly different at $P < 0.05$ using LSD. Note native soil was not part of the experimental design and hence not included in data analysis and provided only for comparative purpose.

Parameter	Treatments				
	Urea (100% N)	Jatropha press cake	FYM	Urea (50% N)	Native soil
Urease ^a	61 $\pm$ 57 a	130 $\pm$ 32 b	223 $\pm$ 42 c	94 $\pm$ 51 a	63 $\pm$ 20
Specific urease activity ^b	396 a	276 a	523 ab	764 b	416
Aryl sulphatase ^c	22 $\pm$ 8 a	43 $\pm$ 10 b	65 $\pm$ 12 c	23 $\pm$ 12 a	33 $\pm$ 15
Specific arylsulphatase activity ^d	151 b	91 a	156 b	174 b	220
Acid Phosphomonoesterase ^c	207 $\pm$ 47 a	356 $\pm$ 71 b	224 $\pm$ 34 a	219 $\pm$ 55 a	163 $\pm$ 30
Specific acid phosphomonoesterase activity ^d	1571 b	810 a	530 a	1918 b	1080
Alkaline phosphomonoesterase ^c	145 $\pm$ 74 a	334 $\pm$ 123 b	489 $\pm$ 37 c	210 $\pm$ 97 a	174 $\pm$ 17
Specific alkaline phosphomonoesterase activity ^d	955 ab	690 a	1175 b	1699 c	1151
Glucosidase ^c	111 $\pm$ 54 a	274 $\pm$ 83 b	338 $\pm$ 37 c	127 $\pm$ 29 a	122 $\pm$ 11
Specific glucosidase activity ^d	752 b	575 a	807 b	1071 c	811
Lipase ^e	28 $\pm$ 4 a	34 $\pm$ 5 a	37 $\pm$ 7 a	30 $\pm$ 1 a	32 $\pm$ 4
Specific lipase activity ^f	225 b	77 a	91 a	266 b	214

^a $\mu\text{g N}$ hydrolysed g^{-1} dry soil 2 h^{-1}

^b $\mu\text{g N hydrolysed mg}^{-1} \text{ biomass } 2 \text{ h}^{-1}$

^c $\mu\text{g nitrophenol released h}^{-1} \text{ g}^{-1} \text{ dry soil}$

^d $\mu\text{g nitrophenol released mg}^{-1} \text{ biomass h}^{-1}$

^e $\mu\text{g of nitrophenol released } 10 \text{ min}^{-1} \text{ g}^{-1} \text{ dry soil}$

^f $\mu\text{g nitrophenol released mg}^{-1} \text{ biomass } 10 \text{ min}^{-1}$

Table 4

Linear correlation coefficients (R)^a between physico-chemical, biochemical, microbial properties and the activity of soil enzymes.

Parameter	Aryl sulphatase	Acid phosphomonoesterase	Alkaline phosphomonoesterase	Glucosidase	Urease	Lipase
Organic C	0.860***	0.312 ^{NS}	0.877***	0.926***	0.840***	0.530*
Total N	0.851***	0.243 ^{NS}	0.880***	0.898 ***	0.834**	0.528*
pH	0.748***	-0.141 ^{NS}	0.782***	0.643**	0.755***	0.257 ^{NS}
Microbial Biomass C	0.751***	0.471*	0.794***	0.898***	0.684***	0.350 ^{NS}
FDA	0.717***	0.497*	0.750***	0.821 ***	0.629**	0.661**
Basal respiration	0.415 ^{NS}	0.459*	0.428 ^{NS}	0.507*	0.278 ^{NS}	0.610**
Available P	0.771***	0.481*	0.752***	0.853***	0.667**	0.578**
Dehydrogenase	0.411 ^{NS}	0.203 ^{NS}	0.477*	0.478*	0.407 ^{NS}	0.767***

^a Statistical significance shown: * p < 0.05, ** p < 0.01, *** p < 0.001, NS not significant

Table 5

Linear correlation coefficients (R)^a between the various soil enzymes studied in different fertilizer treatments in Jatropha plantation.

Parameter	Aryl sulphatase	Acid phosphomonoesterase	Alkaline phosphomonoesterase	Glucosidase	Urease	Lipase
Aryl sulphatase	1	0.064 ^{NS}	0.924***	0.891***	0.877***	0.503*
Acid phosphomonoesterase		1	0.061 ^{NS}	0.271 ^{NS}	-0.030 ^{NS}	0.101 ^{NS}
Alkaline phosphomonoesterase			1	0.946**	0.933***	0.535*
Glucosidase				1	0.832***	0.518*
Urease					1	0.443 ^{NS}
Lipase						1

^a Statistical significance shown: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS not significant

Table 6

Seed yield of *Jatropha curcas* under different nutrient management practices. Values represented are mean $\pm$ S.D of 5 replicates; values followed by different alphabets in the columns are significantly different at $P < 0.05$ using LSD.

Treatment	Seed yield (t ha ⁻¹)	
	2012-13	2013-14
Urea (100% N)	1.55 $\pm$ 0.31 b	1.27 $\pm$ 0.06 b
Jatropha Cake	2.08 $\pm$ 0.28 d	1.59 $\pm$ 0.09 c
FYM	1.79 $\pm$ 0.29 c	1.38 $\pm$ 0.18 b
Urea (50% N)	1.33 $\pm$ 0.38 a	1.00 $\pm$ 0.11 a

Figure captions

Fig. 1. Correlation between microbial biomass carbon (C_{mic}) and organic carbon (C_{org}) in various treatments of organic and mineral fertilizers in Jatropha plantation.

Fig. 2. HPLC chromatograms comparing the presence or absence of Phorbol esters represented by a cluster of peaks (redbar) at around 33 min RT in (A) Jatropha oil (B) de-oiled Jatropha cake (C) Cake amended soil. Note the absence of peaks in (C) indicating degradation of phorbol esters.