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Detection of microbial activity in some organic amendments

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ABSTRACT

Aims: In this paper we conducted a laboratory experiment to assess the biological characters and maturity level of three organic materials: filter cake (FC), spent mushroom substrate (SMS) and fermented grass (FG), through microbial activity and phytotoxicity.
Methodology and results: Each sample was determined for physicochemical parameters, microbial activity and phytotoxicity. Microorganism population was counted by spreading plate method and microbial activities were tested by measuring fluorescein diacetate (FDA) hydrolysis and dehydrogenase activity (DHA). The phytotoxicity evaluation by seed germination was tested on *Hibiscus sabdariffa* (Roselle), *Abelmoschus esculentus* (Okra) and *Oryza sativa* (Rice). The results showed that all substrates have suitable physicochemical values that could be used as soil amendments. These organic matters are a rich source of microorganisms (>10^7 CFU/g), especially plant growth promoting bacteria (ranging from 10^4 to 10^6 CFU/g). The high value of FDA (ranging from 1.609 to 1.621 mg/g) and DHA activity (ranging from 153.95 to 179.92 µg/g) suggested a high degree of microbial activity in the organic amendment samples. Combining with germination index values in seed germination, most GI values indicate non-toxicity of those substrates, with the exception of fermented grass in germination of *H. sabdariffa*.
Conclusion, significance and impact of study: The result from this study proposes that these substrates can be considered as potential amendments to support soil property.

Keywords: *Abelmoschus esculentus, Hibiscus sabdariffa*, microbial activity, organic amendments, *Oryza sativa*, phytotoxcity

INTRODUCTION

Organic matter constitutes only a small proportion in soil composition but plays important roles in soil fertility. As well as providing nutrients for plants and habitation for microorganism, the presence of soil organic matter improves soil's physical, chemical and biological properties (Bot and José, 2005). Intensive agriculture, which typically involves continuous cropping, removal or burning all residues and the over use of fertilizers and pesticides, leads to loss of soil organic matter, thus changing soil characteristics (Sultana, 2011). Lack of soil organic matter decreases water-holding capacity and aggregation, increases bulk density and negatively affects microbial activity (Larney and Angers, 2012).

Application of organic amendments has been used as an effective and economic tool for recovery or maintaining soil health. This method has been shown to enhance soil biological activities as well as improving physical and chemical properties (Silva and Hue, 2000; Diacono and Framcesco, 2010; Adugna, 2016). Moreover, reuse of organic wastes as soil amendments have the advantage in environmental pollution prevention. However, maturity of these organic substrates should be investigated as some negative effects in the soil-plant system have long been reported (Iglesias Jiménez and Perez Garcia, 1989). Some undesirable compounds such as high salts, heavy metals, pathogens and toxins cause adverse effects on plant growth (Tam and Tiquia 1994; Ferreras *et al*., 2006). The quality of organic amendments depends on their origin and varies by time. Organic matter decomposition in natural habitats is mainly performed by microbial activity (Burns, 1982). Then, total microbial activity is a good measure for estimation of organic matter turnover. Hydrolysis of fluorescein diacetate (FDA) and dehydrogenase activity (DHA) have been proposed as popular methods to determine total microbial activity due to their accuracy and simplicity with a range of environmental samples (Schnurer and Rosswall, 1982; Trevors, 1984; von Mersi and Schinner, 1991; Adam and

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Duncan, 2001; Gaspar *et al*., 2001). Determination of enzyme activity is mostly conducted in soil samples to assess soil fertility (Franzlubbers and Haney, 2006), soil degradation or remediation processes (Pascual *et al*., 2000).

There is currently little information about enzyme activity in organic amendments. Further, there is no completely universal method for evaluating phytotoxicity in soil amendments because their phytotoxicity can arise from many factors. Seed germination is the most general biological method to determine phytotoxcity of compost (Marthur *et al*., 1993; Tiquia *et al*., 1996; Warman, 1999; Selim *et al*., 2012) because it provides direct and reliable information. However, using a single parameter on single plant species was not sensitive enough to detect immaturity of organic matter (Warman, 1999).

The objective of this research is to assess the quality of various organic amendments through microbial activity and phytotoxicity. The result is expected to be useful to farmers and producers on the potential of organic amendments in agricultural application.

MATERIALS AND METHODS

Three organic substrates were selected in this study: filter cake (FC), spent mushroom substrate (SMS), and fermented grass (FG). Spent mushroom substrate is degraded for at least three months. As a control in this trial, a kind of normal soil taken from a paddy field in Khon Kaen province in the north-east region of Thailand was also used.

Determination of organic substrates' properties

All substrates were tested for some physicochemical properties. The pH value was measured in extract solution (sample: water = 1:5). Electrical conductivity (EC) was determined in a 1:5 suspension by conductivity meter. The moisture content of each sample was determined by measurement of fresh weight and dry weight (after oven drying at 105 °C for 48 h). The moisture content was calculated as per the following equation (1):

Moisture content = (Initial weight – Final weight)/Initial weight *100 (1)

Nutrient contents in these substrates were determined by standard methods. Total N was analyzed using the Kjeldahl method and measured by colorimetry. Total phosphorus was measured by the wet oxidation and yellow molybdo vanado phosphoric acid method. Total potassium was assayed by wet oxidation and flame photometer. The Walkley and Black method was used for organic matter analysis.

Assessing of total microorganism and plant growth promoting bacteria population

Each substrate was assessed for the following: total microorganisms (TM), nitrogen fixing bacteria (NFB), phosphorus solubilizing bacteria (PSB) and potassium solubilizing bacteria (KSB). Ten grams of organic substrate were placed in a sterilized flask containing 90 mL distilled water and shaken at 150 rpm for one hour. Microbial population was serially diluted and counted by the spreading plate method on specific media. Nutrient agar and nitrogen free medium were used for detecting total microorganisms and nitrogen fixing bacteria, respectively. After spreading, the plates were incubated at 30˚C for 1-2 days and number of colonies that grew on these two media was recorded. PBS and KBS populations were determined by spreading on NRIPB (Nautiyal, 1999) and Aleksandrov medium (Hu *et al*., 2006), respectively; they were then incubated at 30 °C for 4-5 days. The colony with halo zone was determined as PSB and KSB.

Fluorescein diacetate (FDA) hydrolysis activity

In this paper, FDA hydrolysis activity was measured by the modified method of Adam and Duncan (2001). One gram of non-autoclaved sample (fresh weight, sieved < 2 mm) was placed in a falcon tube and mixed with 7.5 mL potassium phosphate buffer pH 7.6, followed by adding 0.1 mL of 1000 mg/mL FDA to start the reaction. Blanks were added with 0.1 mL of acetone. The test tubes were shaken well by hand before placing in a shaker-incubator at 30 °C for 30 min. After shaking, 7.5 mL of chloroform/methanol (2:1 v/v) was added immediately to stop the reaction. All tubes were centrifuged at 6000 rpm for 15 min. The supernatant was kept and centrifuged at 10000 rpm for 5 min. The autoclaved samples, known as the control, were prepared similarly. The OD values were measured at 490 nm on a spectrophotometer. The amount of fluorescein released was calculated using the standard curve of fluorescein standard solution.

Dehydrogenase activity (DHA)

DHA was determined using INT, 2-(p-Iodophenyl)-3(p-nitrophenyl)-5-phenyl tetrazolium chloride or p-idonitrotetrazolium violet (von Mersi and Schinner, 1991). A mixture consisting of 1 g of substrate (moist weight, sieved < 2 mm), 1.5 mL TrisHCl buffer (1M, pH 7.0) and 2 mL 0.5% INT solution was incubated at 40 °C in the dark for 1 h. For blank samples, distilled water was added instead of INT. After incubation, 10 mL of extract solution (N, N-dimethylformamide: ethanol = 1: 1) was added to all test tubes, then mixed well and kept in the dark for 30 min. The control was carried out in the same way with autoclaved substrates. The INT formazan (INTF) extraction was filtered using Whatman No. 5 filter paper. The color of INTF was measured by spectrophotometer at 490 nm. These absorption values were calculated as the mg INTF g^{-1} sample based on a standard curve of INTF (Sigma Chemical Co., 2018).

Phytotoxicity assay

In this study, the toxicity of three organic substrates was assessed through the seed germination test (Tam and

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Tiquia, 1994). The substrates were extracted in distilled water (1:10 w/v) and shaken for 30 min, then filtered. Three plant species, roselle (*Hibiscus sabdariffa*), okra (*Abelmoschus esculentus)* and rice *(Oryza sativa*), were selected. Seed surface was sterilized by soaking in 6% Clorox solution for 5 min and rinsed three times in distilled water. The treated seeds, ten seeds per dish, were placed in sterile petri plates lined with tissue paper. Then, 5 mL of extract solution was added to the plates and incubated in the dark for seven days. For the control, sterilized distilled water was added. Further, a solution of chemical fertilizer, distilled water = 1: 50, was used as a positive control. After seven days, the number of germinated seeds and seedling length (root length and shoot length) were recorded.

Relative growth index (RGI) is categorized as follows: (1) 0<RGI<0.8: inhibition; (2) 0.8<RGI<1.2: no significant effect; (3) RGI>1.2: stimulation root growth (Young *et al*., 2012).

$$\text{RGI} = \frac{\text{Mean of root length in sample}}{\text{Mean of root length in control}}$$

Germination index (GI) was based on the germination rate (GR) and RGI using the following equation (2):

$$\text{GR (\%)} = \frac{\text{Mean of germinated seed in sample}}{\text{Mean of germinated seed in control}} \times 100$$

GI (%) = RGI x GR (2)

The maturity of substrates was assumed when GI > 80% (Iglesias-Jiménez and Pérez-García, 1992).
Vigor Index (VI) = Germination percentage x Seedling length (3)

Experimental design and data analysis

All experiments were arranged by completely randomized design with three replications. Analysis of variance (ANOVA) was used to analyze the data using Statistic 10 and Microsoft Excel software. The significant differences between treatments were determined by a Least Significant Difference (LSD) test at P < 0.05%.

RESULTS AND DISCUSSION

Organic amendment's properties

Physicochemical analysis of three organic amendments and soil are shown in Table 1. All substrates were slightly alkaline, had low salinity and high OM. The pH of the three substrates ranged from 7.47-7.82. According to Moore (2001), a pH range of 5.5 – 8 is suitable for plant growth and most soil biological processes, including nutrient cycling and microbial activity. Electrical conductivity (EC) is one of most noticeable chemical features of organic amendments. The EC value indicates the amount of salt in an amendment that may negatively affect on plant growth or soil salinity. In this test, SMS had the highest electrical conductivity (1.57 dSm^{-1}), followed by others in the following order: SMS > filter cake > fermented grass. These low EC values reflected no negative effects on soil EC. Moisture of all substrates varied in range from 10.73% to 22.05%. Deepesh *et al*. (2014) reported that moisture content in good quality organic amendment is around 50–55 %. The moisture and OM of the tested soil was extremely low (2.22%, 0.67%) due to its sandy texture. Most soils in the northeast region of Thailand are poor in OM as well as fertility.

Table 1 also presents information about nutrient content in the three substrates and soil. Generally, organic matter can be used as fertilizers or conditioners depending on their nutrient content. All substrates in this test were low in nutrient availability but were rich sources of organic carbon. Filter cake got the highest amount of nutrient elements (N 2.45%, P 1.86% and K 0.58%) and the lowest C/N ratio (8:1) in comparison with the other two. Nutrient content in SMS and fermented grass was quite low (ranging from 0.14% to 0.75%). The critical C/N ratio (59:1) in SMS was the consequence of high OM amounts (74.9%). The C/N ratio is one important index to assess the decomposition rate and the type of nitrogen released during the decomposition of OM. Some research suggests the ideal C/N ratio for OM decomposition is between 20 to 30 (Marthur *et al*., 1993; Epstein, 1998).

Table 1: Some physicochemical characteristics in three organic amendments in comparison with normal soil.

	pH	EC dSm^{-1}	Moisture %	TN %	TP %	TK %	OM %	OC %	C/N
FC	7.47 ± 0.02c	0.52 ± 0.06b	10.73 ± 1.02 c	2.45a	1.86a	0.58a	34.30b	18.5b	8:1c
FG	7.82 ± 0.03a	0.26 ± 0.03c	19.61 ± 0.81^{b}	0.75b	0.14c	0.20c	33.14c	16.56c	22:1b
SMS	7.72 ± 0.02b	1.57 ± 0.06a	22.05 ± 1.99a	0.72c	0.21b	0.68b	74.9.1a	42.49a	59:1a
Soil	7.09 ± 0.04d	0.05 ± 0.003d	2.22 ± 0.32^{d}	0.05d	0.11d	1.62d	0.67d	0.4d	8:1d

Different small letters indicate significant differences (p<0.05) between substrates. Values are the mean ± standard deviation of three replications (Standard deviation <10%); EC: electrical conductivity; FC: filter cake; SMS: spent mushroom substrate; FG: fermented grass; TN: total nitrogen; TP: Total phosphorus; TK: Total potassium; OM: organic matter; OC: organic carbon; C/N: organic C/total N

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A low C/N ratio of organic amendments may limit the growth of microorganism, which in turn decreases decomposition rates (Berg and McClaugherty, 2003). Meanwhile, a high amount of carbon compounds required strong activity of the microbial population; therefore, microorganisms use soil nitrogen for their growth and cause immobilization of soil nitrogen (Lucas *et al*., 2014). A high number of organic particles need a long time to degrade. This allows organic matter to remain in the soil longer, consequently, supporting soil structure and other biological activities. Some research demonstrates that organic amendments, which are rich in cellulose, can enhance fungal activity and improve soil structure by soil aggregate formation (Lucas *et al*., 2014). Moreover, high nitrogen material should be added to reach appropriate C/N ratios before using these substrates in soil.

Microbial activity

Microbial activity was determined using both direct (total microorganism and PGPB population) and indirect methods (enzyme activity).

The results in Table 2 reveal differences in the microbial community among substrates. Generally, all substrates were abundant in microbial populations as well as PGPB. Fermented grass had the highest population ($P<0.01$) of total microorganisms ($4.47x10^7$ CFU/g), nitrogen fixing bacteria ($1.08x10^6$ CFU/g) and potassium solubilizing bacteria ($1.84x10^6$ CFU/g). SMS and filter cake had similar populations of total microorganisms ($1.62x10^7$ CFU/g and $1.26x10^7$ CFU/g, respectively) and nitrogen fixing bacteria ($8.13 x10^5$ CFU/g and $7.77x10^5$ CFU/g, respectively). SMS was the richest source of PSB ($2.3 x10^5$ CFU/g). Soil tested in this experiment was too poor in the number of both PSB and KSB ($1.2x10^2$, $<10^2$ CFU/g, respectively).

The FDA and DHA activity of all samples are presented in Figure 1. All amendments had high enzyme activities in both FDA and DHA activity. Most microorganisms, including fungi, bacteria, some protozoa and algae, were involved in FDA hydrolysis activity (Schnurer and Rosswall,1982).

Table 2: Total microorganism and PGPB populations in three organic substrates and normal soil.

	TM	NFB	PSB	KSB
Substrates	CFU/g			
FC	$1.26x107^b$	$7.77x105^c$	$8.02x104^b$	$1.01x105^c$
SMS	$1.62x107^b$	$8.13 x105^c$	$2.3 x105^a$	$2.07x104^b$
FG	$4.47x107^a$	$1.08x106^b$	$5.43 x104^c$	$1.84x106^a$
Soil	$5.7x106^c$	$1.54 x106^a$	$1.2x102^d$	$<102^d$

Different small letters indicate significant differences ($p<0.05$) among substrates. Values are the mean of three replications FC: filter cake; SMS: spent mushroom substrate; FG: fermented grass; TM: total microorganism; NFB: nitrogen fixing bacteria; PBS: phosphorus solubilizing bacteria; KSB: potassium solubilizing bacteria.

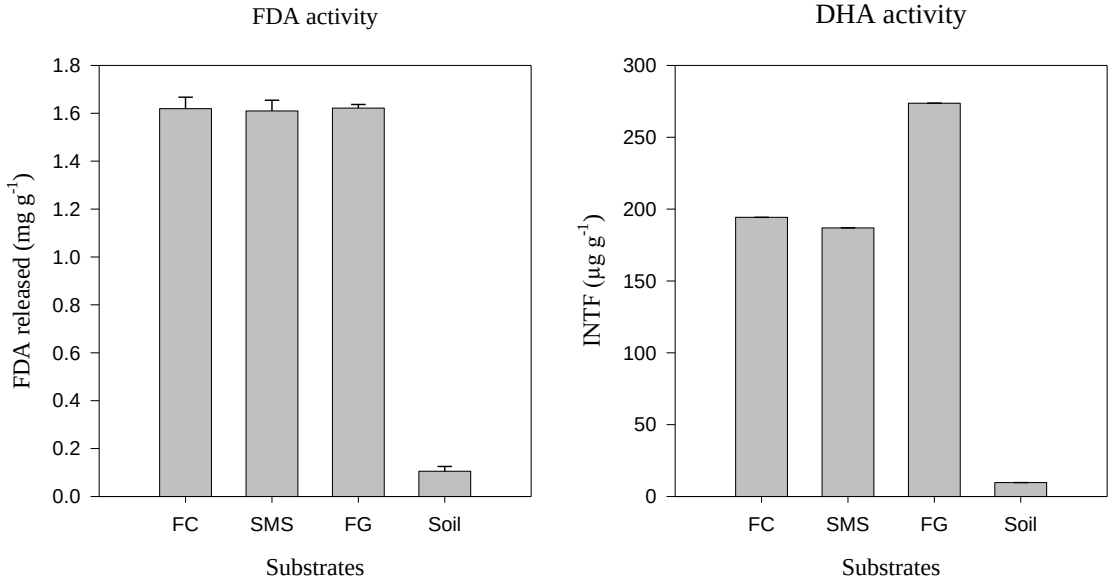

Figure 1: FDA and DHA activity in three organic substrates and normal soil.

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FDA is catalyzed by various microbial enzymes such as proteases, lipases and esterases. In this test, FDA hydrolysis in three organic substrates was similar and ranged from 1.609 to 1.621 mg/g. Sánchez-Monedero *et al*. (2008) found the significant difference between FDA hydrolysis and microbial biomass in soil added with various organic amendments regardless of the origin, composition and degree of stability of the organic amendments. Meanwhile, the highest DHA ($P<0.01$) among the three amendments were found in fermented grass (273.64 µg/g). The DHA in SMS and filter cake were not significantly different but had high values (186.90 µg/g, 194.22 µg/g, respectively). Wolińska *et al*. (2012) demonstrate the main role of DHA enzymes in OM decomposition through high correlation coefficients of enzymatic activities and total organic carbon. However, the C/N ratio is the main factor affecting microbial activity, which explains why fermented grass had the highest DHA. The tested soil presented the lowest amount of FDA and DHA (0.105 mg/g, 9.65 µg/g).

Seed germination

The Germination Index (GI) indicates the presence of phytotoxins that influence both seed germination and root elongation (Emino and Warman, 2004). The effects of different organic amendments' extraction on each kind of seed germination displayed dissimilar patterns (Table 3). Differences of GI and VI in *A. esculentus* and *O. sativa* responded by seedling growth parameters (root length or shoot length); however, in *H. sabdariffa,* these differences were mainly due to reduced germination. Filter cake and SMS showed promotion in seed germination of *A. esculentus* (RGI >1.2, GI >80%) and no inhibition effect on the seed germination of *O. sativa* and *H. sabdariffa* (0.8<RGI<1.2, GI >80%). Extraction of filter cake and SMS did not significantly affect either root elongation or the germination rate of *O. sativa* and *H. sabdariffa* in comparison with the control. Meanwhile, the promoting activity of these substrates on the GI of *A. esculentus* was completely due to stimulation of root growth (RGI ranging 1.48-1.88); in turn, their GI was significant higher ($P<0.01$) than other treatments. On the other hand, fermented grass had no effect on the seed germination parameters of *A. esculentus* in comparison with the control. However, this substrate also showed root elongation promotion of *O. sativa* (RGI ~ 1.2), but inhibited germination rates (73.33%) of *H. sabdariffa*. The negative impact on the GI of *H. sabdariffa* was also found in soil extraction. This may be due to the sensitivity of *H. sabdariffa* with some unknown components in fermented grass and soil. Soil extraction did not reveal inhibition on GI or VI, while chemical solution totally inhibited germination parameters of all seed (Table 3).

Table 3: Phytotoxicity effects of three organic amendments and soil on germination parameters of three kinds of seed.

	Root (cm)	Shoot (cm)	RGI	GP (%)	GI (%)	VI
Oryza sativa						
Control	6.13±0.14^{c}	3.33±0.21^{a}	1.00	96.67	100.00	945.33
FC	4.96±0.21^{d}	2.61±0.35^{b}	0.81	100.00^{a}	83.75	783.45
SMS	5.78±0.11^{cd}	3.19±0.34^{a}	0.94	90.00^{ab}	87.84	835.14
FG	7.29±0.76^{b}	3.35±0.35^{a}	1.18	100.00^{a}	123.03	1100.69
Soil	8.43±0.37^{a}	3.25±0.28^{a}	1.36	100.00^{a}	142.28	1207.59
CF	0.76±0.38^{e}	1.90±0.30^{c}	0.12	80.00^{b}	10.27	219.86
Hibiscus sabdariffa						
Control	4.00±0.73^{a}	5.61±0.88^{a}	1.00	90.00^{a}	100.00	960.67
FC	3.59±0.51^{a}	5.99±1.64^{a}	0.90	90.00^{a}	89.75	958.00
SMS	4.40±0.61^{a}	6.00±1.35^{a}	1.09	83.33^{ab}	101.77	962.96
FG	3.60±1.17^{a}	5.46±0.61^{a}	0.90	73.33^{bc}	73.33	737.95
Soil	3.63±0.16^{a}	5.66±0.30^{a}	0.91	76.67^{bc}	77.23	791.09
CF	1.61±0.90^{b}	2.65±0.88^{b}	0.43	66.67^{c}	29.88	316.05
Abelmoschus esculentus						
Control	2.18±0.32^{c}	1.81±0.37^{c}	1.00	100.00^{a}	100.00	398.67
FC	3.22±0.22^{b}	3.64±0.68^{a}	1.48	100.00^{a}	147.55	685.67
SMS	4.09±0.38^{a}	3.91±0.25^{a}	1.88	100.00^{a}	187.77	800.67
FG	2.42±0.53^{c}	2.45±0.18^{b}	1.1	100.00^{a}	110.86	486.33
Soil	2.03±0.19^{c}	1.44±0.21^{cd}	0.93	96.67^{a}	90.16	335.76
CF	0.00±0^{d}	0.83±0.09^{d}	0.00	0.00^{b}	0.00	0.00

Different small letters indicate significant differences ($p<0.05$) among substrates; RGI: Relative growth index; GP: germination percentage; GI: germination index; VI: vigor index; FC: filter cake; SMS: spent mushroom substrate; FG: fermented grass; CF: chemical fertilizer in water ratio 1:50

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The increase in GI and VI may come from PGPB activity within organic amendments. Some undesirable compounds such as high salts, heavy metals, pathogens and toxins caused adverse effects on plant growth (Tam and Tiquia 1994; Ferreras *et al*. 2006). Thus, GI >80 % is one of the main indexes to evaluate phytotoxicity of compost (Tam and Tiquia, 1994; Tiquia and Tam, 1998). However, GI is not sensitive enough to distinguish immature and mature substrates (Emino and Warman, 2004). The study of Saidi *et al*. (2009) shows that the most appropriate parameters to evaluate the maturity degree of compost include the C/N ratio < 15; $NH4^{+}$-N < 400 mg/kg; CO^{2}-C < 2000 mg CO2-C/kg; dehydrogenase activity < 1 mg TPF/g dry matter and germination index > 80%. Some studies find that GI has a positive correlation with NO^{3}- N, P and K content (Zayed and Atta, 2012) and a negative correlation with EC and carbon mineralization rates (Aslam *et al*., 2008). Until now, the correlation between enzyme activity and toxicity in organic compost has not been found. Microbial activity is a good indicator for the stability of organic matter decomposition rather than toxicity.

From the above results, we conclude that all three organic substrates are suitable to be soil amendments. Fermented grass and filter cake, with considerable amounts of nutrients and moderate C/N ratios, could be used directly on soils to support fertility, whereas SMS is a good source for maintaining OM and improving soil structure in the long term.

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