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Carbon Mineralization Potential and Enzymatic Activity in Soil Amended with Vinasses

ศักยภาพการปลดปล่อยคาร์บอนและกิจกรรมเอนไซม์ในดินที่บ่มด้วยน้ำกากส่า

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ABSTRACT

A laboratory incubation was conducted to evaluate the effects of applied vinasses by determination of C mineralization, soil respiration and enzymatic activity. Two soil types from sugarcane planted area were incubated for 12 weeks. The rate of vinasses input was 60 % of soil moisture at field capacity. The results indicated that vinasses added to soil stimulated the activity of soil microorganisms resulting in an increase of dehydrogenase and urease activities. There was little net C mineralization over the entire incubation time. Net C mineralization in sandy clay loam soil with fresh vinasses and with nutrient supplemented vinasses were accounted for 1.65 and 2.05 % of added organic C. While in sandy loam soil with both type of vinasses resulted in smaller mineralization with the net C mineralization 1.69 and 0.91% of added organic C after 12 weeks of incubation respectively. Vinasses added to soil increased soil chemical properties such as pH and EC due to its contains high amount of cations and anions such as K, Na, Ca, SO_4^-.

บทคัดย่อ

ประเมินอิทธิพลของการใช้น้ำกากส่าต่อการปลดปล่อยคาร์บอน การหายใจของจุลินทรีย์ และกิจกรรมของเอนไซม์โดยการบ่มดินสองชนิดเป็นเวลา 12 สัปดาห์ ปริมาณน้ำกากส่าที่เติมเท่ากับ 60 เปอร์เซ็นต์ของความจุความชื้นสนาม ผลการทดลองพบว่าการบ่มดินด้วยน้ำกากส่าช่วยกระตุ้นกิจกรรมของจุลินทรีย์ดิน ส่งผลให้กิจกรรมเอนไซม์ดีไฮโดรจีเนสและยูรีเอสเพิ่มสูงขึ้นกว่าดินที่ไม่เติมน้ำกากส่า ทุกกรรมวิธีทดลองมีการปลดปล่อยคาร์บอนเกิดขึ้นเพียงเล็กน้อยตลอดระยะเวลาการบ่ม ซึ่งการปลดปล่อยคาร์บอนสุทธิในดินร่วนเหนียวปนทรายที่บ่มด้วยน้ำกากส่าดิบและน้ำกากส่าเติมธาตุอาหารมีค่าเท่ากับ 1.65 และ 2.05 เปอร์เซ็นต์ของปริมาณอินทรีย์คาร์บอนที่เติมลงไปทั้งหมด ขณะที่ดินร่วนปนทรายมีการปลดปล่อยคาร์บอนสุทธิจากการการเติมน้ำกากส่าดิบและน้ำกากส่าเติมธาตุอาหารเท่ากับ 1.69 และ 0.91 เปอร์เซ็นต์ ตามลำดับ และการเติมน้ำกากส่าทำให้คุณสมบัติดิน เช่น pH และ EC เพิ่มขึ้นเนื่องจากน้ำกากส่ามีองค์ประกอบที่เป็นแคตไอออนและแอนไอออน เช่น K Na Ca SO_4^- ในปริมาณสูง

Key Words : Soil Carbon Mineralization, Vinasses, Soil Enzymatic Activity
คำสำคัญ : การปลดปล่อยคาร์บอน น้ำวีแนส กิจกรรมเอนไซม์ในดิน

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Introduction

Sugarcane is processed to produce sugar, and molasses, the by product is fermented to produce ethanol for value addition. After ethanol extraction by distillation the remaining material is known as vinasses (molasses from alcohol wastewater). Vinasses has high organic matter, N, P and K content (Wilkie et al. 2000). Vinasses is also a problematic effluent for the sugar industry, due to its high volume (12-13 L/l ethanol), high biological oxygen demand (BOD, 30-40 g/l) and chemical oxygen demand (COD, 60-100 g/l) (Eugenia et al.1995). The simplest approach to utilize vinasses is to use it as soil amendment which returns most of the mineral back into the soil (Eugenia at el.1995). Application of vinasses improved cane yield and sucrose percentage in cane compared to non fertilizer and conventional fertilization (Yang-Rui Li et al. 2006). However, the advantage of vinasses application depends on many factors such as climatic conditions, soil type, drainage, cropping system or characteristics of vinasses. Tejada et al. (2007) reported that fresh beet vinasses, a soil amendment, increased monovalent cations and fulvic acid content in soil, leading to degradation of soil structure, increased soil bulk density and exchangeable sodium percentage (ESP) which lead to soil anaerobic condition and hence decreased soil microbial biomass, soil respiration and soil enzymatic activity. The presence of small pores reduced microbial accessibility of organic material to decompose, causing physical protection of C and reduction in N mineralization (Van Veen and Kluikman, 1990). According to Tate III (2002) oxygen concentration in soil affects the metabolic status of the enzyme-producing cells. Inubushi and Acquaye (2004) have shown that biogeochemical processes in flooded paddy soil ecosystems were microbially mediated. Vinasses, the organic by products from industrial processes represent an important source of nutrients, especially for organic fertilization. Nelson and Oades. (1998) and Barzegar et al. (2002) reported that the influence of organic matter on soil properties depends on amount, type and size of the added organic materials.

In the past, physical and chemical properties have been used to evaluate the effect of the application of different organic matter source to soil. However, such properties change very slowly and require many years to provide any significant results. In contrast, microbiological and biochemical properties are very responsive and provide immediate and precise information on small changes occurring in soil (Dick & Tabatabai, 1993). Microbial biomass is shown to be a sensitive indicator of differences in sustainable cropping systems, the toxicity of pollutants and the degradation of organic compounds (pesticides and industrials chemicals) can be monitored by following changes in the soil microbial biomass (Inubushi and Acquaye, 2004; Weaver et al. 1994). According to Ryan et al. (2002), C sequestration in soil indicated different effect of organic fertilizers and amendments on soil pools and processes, and therefore on soil fertility, all these depending on climatic conditions, soil type, cropping system and the characteristics of the organic matter. Thus the soil ecosystem in sugarcane plantation area is different due to factors, e.g., soil type, soil texture, drainage (dry or flood) and cultivation practices. Properties and quantity of the applied vinasses, soil type and soil condition affect not only soil

microbiological, biochemical, chemical properties, but also plant nutrients. These factors lead us to undertake the present study. The objectives of the study were to evaluate the effects of fresh and nutrient supplemented vinasses applied to soils on the C mineralization, soil respiration, soil enzymatic activities and soil chemical properties.

Materials and methods

Soil samples

The two major types of soil in sugarcane plantation, sandy loam (SL) and sandy clay loam (SCL) were collected from Phukieo district, Chaiyaphum province. The samples were taken, 0-15 cm from the soil surface. Composite soil samples, were thoroughly mixed and dried at room temperature. Soil were sieved by using 2 mm mesh sieve. Soil samples were analyzed for the soil characteristics, i.e., pH, EC, clay content, total N, total organic carbon and exchangeable cations. The data were presented in *Table 1.*

Vinasses samples

Two types of vinasses were used in the experiment, fresh vinasses were sampled in the ethanol production plant, while nutrient supplemented vinasses were collected from mixing tank. All samples were kept at 4 °C for 1-3 days before incubation and stored at -20 °C for further use as reported by Tejada et al. 2007. Initial characteristic of vinasses, i.e., pH, EC, specific gravity, dry matter, total N, P, K, Ca, Mg, Na, Fe, Cu, Mn, Zn, BOD, COD, total sugar and protein were analyzed. The data were presented in *Table 2.*

Incubation experiment

The soil-vinasses mixture was incubated under controlled environment conditions for measuring C mineralization. The rate of vinasses input was 60% of soil moisture at field capacity (soil moisture at field capacity of sandy loam and sandy clay loam soil was 24.55% and 32.42% respectively). There were nine sets and four replicates of each treatment and sampling date, 8 sets of soil samples were taken at 0, 1, 2, 3, 4, 6, 9, 12 weeks. The glass bottles were open to maintain the aerobic condition for the soils. Another set for gas sampling were closed with airtight seals using septum rubber. The vinasses were homogeneously mixed with the soil samples and rapidly weighed 350 g and placed in glass bottle with an inner diameter of 80 mm. A control treatment (soil without addition of vinasses) was included to determine C mineralization from native soil organic matter. The initial weights of the bottles were recorded, soil moisture content was adjusted using distilled water. The incubation was carried out in the dark at 25 ± 1°C for 90 days.

Soil sampling and analytical determinations

Samples from 24 bottles at a time was used for analysis (destructive sampling). The incubated soil was mixed and analyzed for dehydrogenase activity, urease activity, total organic carbon, dissolved organic carbon, pH, EC and total N. Soil pH was determined in distilled water with a glass electrode (soil : H_2O ratio 1:2). Soil electric conductivity was determined in distilled water with a glass electrode (soil : H_2O ratio 1:5). Soil organic carbon was determined by oxidizing organic matter in soil samples with $K_2Cr_2O_7$ in sulfuric acid (96%) for 30 min, and measuring the concentration of Cr^{3+} formed (Nelson and Somers, 1975). Soil total N was determined by the Kjeldahl method (Keeney and Nelson, 1982). The activity levels of soil enzymes

were measured. Dehydrogenase activity was measured by colorimetric determination of 2,3,5-triphenylformazan (TPF) produced by the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) (Casida et al., 1964). Urease activity was determined by determination of ammonia released after incubation of soil sample with urea solution for 2 h at 37 °C (Tabatabai and Bremner, 1972). The quantity of evolved CO_2 was determined by titrating the residual NaOH by 0.2 M HCl after precipitation $NaCO_3$ by $BaCl_2$ solution. The net amount of CO_2 produced from the incubated soil-vinasses mixture was determined by the difference between CO_2 evolved from the vinasses amended soil and CO_2 evolved from the control soil. The cumulative C mineralization for all treatment was expressed for % mineralization C as :

$$\frac{[(CO_2\text{-C in amended soil}) - (CO_2\text{-C in control soil})]}{\text{added OC}} * 100$$

Statistical analysis

Analysis of variance (ANOVA) was performed using the IRRISTAT v. 5.0 software package using the four replicated data for each treatment.

Results

Physical and chemical properties of two soil types used are as described in Table 1. The initial characteristics of sandy loam soil were; pH 4.9, EC 0.3 mS m^{-1}, TOC and total N were 2.15 and 0.04 % respectively, while pH, EC, TOC and total N of sandy clay loam were 7.9, 1.2 mS m^{-1}, 2.71 and 0.12 % respectively. Vinasses used in these experiment were concentrated fresh and nutrient supp. vinasses with 35 and 31 % brix respectively, with acidic (4.3 and 4.1 respectively. Total N were 5.39 and 14.99 %, TOC were 13.45 and 10.85 % in fresh vinasses and nutrient supp. vinasses respectively (Table 2).

Table 1 Chemical characteristics of soils prior the experiment.

Soil characteristics	Sandy loam	Sandy clay loam
pH	4.9	7.9
EC (dS m^{-1})	0.3	1.2
Clay (%)	6	26
Total N (%)	0.04	0.12
Total organic carbon (%)	2.15	2.71
Available P (mg kg^{-1})	81	57
Exchangeable K (mg kg^{-1})	37	157
Exchangeable Ca (mg kg^{-1})	66	8860
Exchangeable Mg (mg kg^{-1})	24	1923
Exchangeable Na (mg kg^{-1})	741	185

Table 2 Characteristics of vinasses used in the experiment.

Characteristics	Fresh vinasses	Nutrient supp. vinasses
Brix at 20°C (%)	35	31
pH	4.3	4.1
EC (dS m^{-1})	26	31
Dry matter (g kg^{-1})	270	244
Total N (g kg^{-1})	5.36	14.99
Total P (g kg^{-1})	0.59	3.32
Total K (g kg^{-1})	16.89	19.20
Total organic C (%)	13.45	10.85
Na (g kg^{-1})	3.80	3.85
Ca (g kg^{-1})	8.58	5.90
BOD (g L^{-1})	275	105
COD (g L^{-1})	406	323
Specific gravity (kg L^{-1})	1.17	1.14
Glucose (%)	6.20	7.32
Sucrose (%)	15.69	14.54
Fructose (%)	2.51	1.02
Total sugar (%)	24.41	22.88
Protein (% dry matter)	12.8	9.49

Carbon mineralization

Soil microbial respiration by measuring carbon dioxide emission is a direct indicator of microbial activity, and indirect reflection of availability of organic material. Our results indicated that there were significant differences among soil type and vinasses type on soil respiration (CO_2-C) (the data was not shown here. In both soils without vinasses gave lowest soil respiration throughout the incubation period. Fresh vinasses added to both soil types and nutrient supplemented vinasses added to sandy clay loam were clearly increased soil respiration with a range of 1.461 and 1.641 mg CO_2-C g^{-1} dry soil over 12 weeks of incubation. In sandy loam soil with nutrient supplemented vinasses, soil respiration sluggishly increased much slower than other soils with vinasses *(Figure 1)*. There was little net C mineralization over the entire incubation time (*Figure 2*). The patterns of C mineralization for sandy clay loam soil with fresh vinasses and sandy clay loam soil with nutrient supplemented vinasses were quite similar, they gradualy mineralized through the whole incubation period which mineralized C accounted for 1.65 and 2.05 per cent of the added organic C. While sandy loam soil with fresh vinasses and sandy loam soil with nutrient supplemented vinasses resulted in very low C mineralization in the first 3 weeks with negative value compared to soil without vinasses incorporated. Then there was little C mineralization till 12 weeks, but lower than sandy clay loam with the net C mineralization 1.69 and 0.91 per cent of added organic C (*Figure 2*).

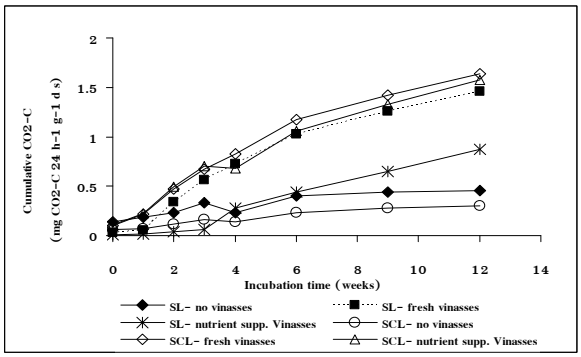

Figure 1 Cumulative CO_2-C of soil amended with and without vinasses

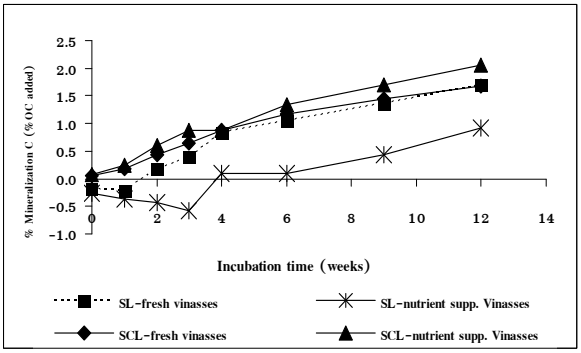

Figure 2 C mineralization of soil amended with vinasses

Soil enzymatic activities

Dehydrogenase activity has been proposed as a measure of overall microbial activity. Vinasses amended soil stimulated dehydrogenase activity with the significant differences from the initiation of incubation (*Figure3*). Sandy clay loam soil with fresh vinasses and nutrient supplemented vinasses resulted the highest dehydrogenase activity at the first week with 6.49 and 5.46 mg of TPF g^{-1} h^{-1}, slightly decreased and became stable till the end of incubated time with 0.558 and 0.13 mg of TPF g^{-1} h^{-1}, respectively; while sandy clay loam soil without vinasses, the dehydrogenase activity seemed to be linear with a low range of 0.51-0.96 mg of TPF g^{-1} h^{-1}. Dissimilarly, in sandy loam soil with fresh vinasses and nutrient supplemented vinasses showed high dehydrogenase activity compared with sandy loam soil without added vinasses, but in sandy loam

soil the dehydrogenase activity was at its peak at 3-4 weeks after application, it started slower than in sandy clay loam soil. At the end of incubation time the dehydrogenase activity in sandy loam were 0.14, 1.10 and 0.37 mg of TPF g^{-1} h^{-1} in sandy loam without vinasses, fresh vinasses and nutrient supplemented vinasses respectively *(Figure 3)*.

Urease activities were very low in all soils with and without vinasess over the first 4 weeks of incubation *(Figure 4)*. In both soils with nutrient supplemented vinasses, urease activities emerged rapidly to their peaks range from 6.57 and 9.08 mg NH_4-N g^{-1} 2 h^{-1} at 6 weeks. The nutrient supplemented vinasses tend to stimulate urease activity more than fresh vinasses in both soil type. At the end of incubation the activity of urease in sandy loam and sandy clay loam with and without vinasses were ranged from 0.03 to 4.36 mg NH_4-N $g^{-1}2$ h^{-1}.

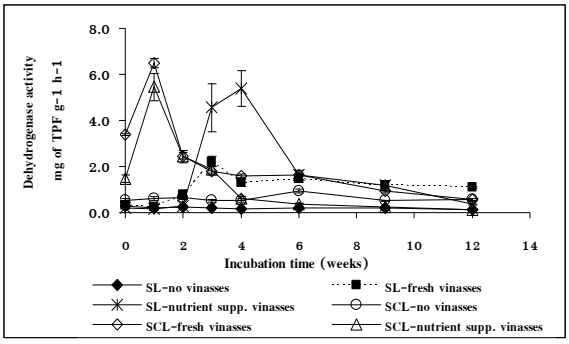

Figure 3 Dehydrogenase activity of soil amended with and without vinasses

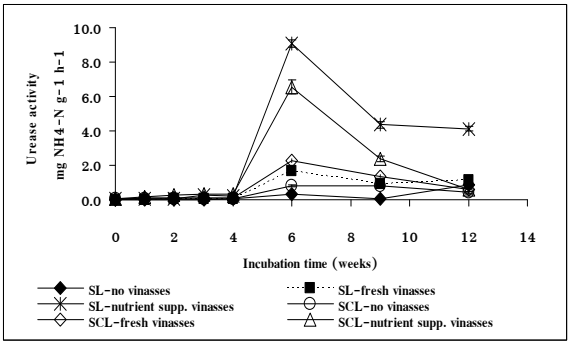

Figure 4 Urease activityof soil amended with and without vinasses

Changes of soil chemical properties

Vinasses was acidic (4.3 and 4.1 in fresh and nutrient supplemented vinasses) but after 12 weeks of incubated, soil amended with vinasses resulted the changed of pH *(Figure 5)*. Sandy loam soil without vinasses had its pH remained acidic through the whole incubation. Sandy loam soil with fresh vinasses and with nutrient supplemented vinasses, their soil pH kept the same as initial during 2 weeks of incubation, then started to increase to a range of neutral to higher from 4 weeks of incubation to the end. In sandy clay loam soil, vinasses decreased soil pH of the beginning. Sandy clay loam soil with fresh vinasses, nutrient supplemented vinasses and without vinasses, soil pH became a little above 7 in 1 weeks o incubation, and remained around neutral till the end of incubation *(Figure 5)*.

The EC of vinasses was very high (26 and 31 dS m^{-1} for fresh vinasses and nutrient supplemented vinasses). With amended vinasses to both soils at the beginning of incubation, EC significantly increased from the initial EC of both soils *(Figure 6)*. After 12 weeks, EC of sandy loam soil with fresh vinasses and with nutrient supplemented vinasses were 1.2 and 1.7 dS m^{-1} which were much more than that of without vinasses, 0.04 dS m^{-1} . In sandy clay loam soil with fresh vinasses and with nutrient supplemented vinasses the EC were increased to 1.0 and 2.1 dS m^{-1} respectively while sandy clay loam soil without vinasses, its EC was 0.13 dS m^{-1} *(Figure 6)*.

Presence of high organic carbon content of vinasses, both soil type amended with vinasses resulted in increased TOC significantly compared to soil without vinasses. The TOC in soil tends to be

stable over the incubation time. After incubating for 12 weeks, sandy loam and sandy clay loam soil without vinasses gave TOC of 0.53 and 1.65% respectively, while the sandy loam with fresh vinasses, sandy loam with nutrient supplemented vinasses, sandy clay loam with fresh vinasses and sandy clay loam with nutrient supplemented vinasses increased TOC to 1.67, 1.62, 2.27 and 2.41% respectively *(Table 3)*.

Vinasses amendment showed significant increase of DOC in both soil type compared to control soil over the entire incubation time (Table 4). In both soil types incorporated with vinasses the content of DOC was highest in the first day and rapid decreased to the minimum content at 6 weeks after incubation followed by slightly increase and stabilizing till 12 weeks of incubation. After 12 weeks of incubation, the sandy loam with fresh vinasses, sandy loam with nutrient supplemented vinasses, sandy clay loam withfresh vinasses and sandy clay loam with nutrient supplemented vinasses gave 0.36, 0.73, 0.17 and 0.21% of DOC respectively compared with sandy loam and sandy clay loam without vinasses gave 0.01 % DOC and the data are presented in *Table 4*.

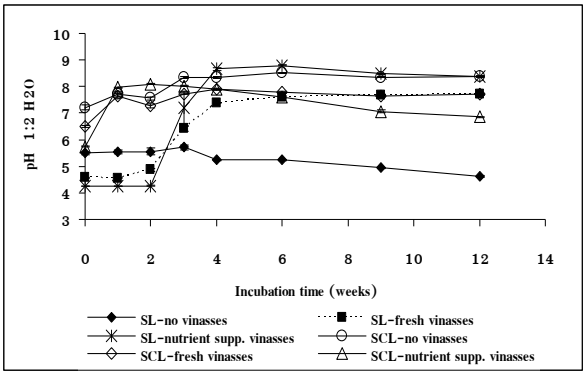

Figure 5 Soil pH amended with vinasses

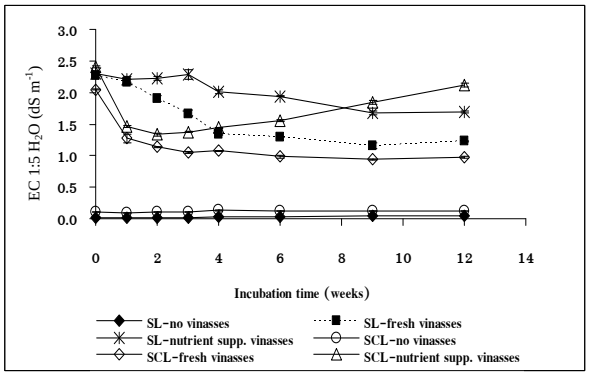

Figure 6 Electrical conductivity of soil amended with vinasses

Discussion

From the present study we found that the net C mineralization of amended soil were very little. This might be affected by concentration of vinasses and the amount of vinasses applied to soil. The vinasses used in the study was a concentrated vinasses originating from sugarcane molasses. Some researchers reported about effect of characteristics of vinasses on the potential of vinasess decomposition. As indicated by Sowmeyan et al.(2008), sugarcane molasses contained around 2% of melanoidins which the structure was still unknown but melanoidins have the antioxidant properties, they are toxic to many microorganisms and only 6-7% degradation of melanoidins has been achieved in the conventional anaerobic-aerobic effluent treatment process. Similarly to Parnaudeau et al.(2008) who indicated that vinasses from sugarcane contain melanoidins and lignin-derived phenolic compounds which can inhibit or reduce the activity of microorganisms. The amount of vinasses amended in this incubation experiment was 60% of soil moisture at field capacity equal to 4.62-7.96% of added carbon. These amounts of carbon in vinasses probably affected the activity of soil microorganisms to decompose

vinasses resulting in the highest net C mineralization only 2.05 % of the added carbon. A comparable study done by Parnaudeau et al.(2008) which had input only 0.02% C resulted around 40 % of the net C mineralization.

Conclusions

Use of sugarcane vinasses as soil amendment can stimulate biological activities during a short term incubation as corresponded to the increase of soil respiration, soil enzymatic activity (dehydrogenase and urease activity). The activities of soil micro-organisms was occurred faster in sandy clay loam rather than sandy loam soil. Nutrient supplemented vinasses tended to increase the C mineralization and the activity of soil microorganisms compared to fresh vinasses. Applied vinasses increased soil chemical properties such as pH and EC due to its contains high amount of cations and anions such as K, Na, Ca, SO_4.

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Table 3 Total organic carbon in soils amended with and without vinasses

Soil type	Type of vinasses added	Total organic carbon (%)							
		0 day	1 wk	2 wk	3 wk	4 wk	6 wk	9 wk	12 wk
Sandy loam	Without vinasses	0.45	0.38	0.39	0.38	0.47	0.45	0.51	0.53
	Fresh vinasses	2.77	2.59	2.31	2.02	1.75	1.73	1.68	1.67
	Nutrient supp. vinasses	2.37	2.27	2.21	1.85	1.74	1.64	1.59	1.62
Sandy clay loam	Without vinasses	1.44	1.40	1.42	1.36	1.47	1.55	1.66	1.65
	Fresh vinasses	2.35	2.49	2.19	2.39	2.12	2.24	2.30	2.27
	Nutrient supp. vinasses	2.28	2.63	2.47	2.49	2.18	2.05	2.52	2.41
F-test	Vinasses	**	**	**	**	**	**	**	**
	Soil type	**	**	**	**	**	**	**	**
	Vinasses*Soil type	**	**	**	**	**	**	**	**
CV %		5.2	3.1	6.2	5.5	4.8	7.7	4.0	6.6
LSD.05	Vinasses*Soil type	0.1516	0.9198	0.1725	0.1451	0.1176	0.1867	0.1027	0.1679

Table 4 Dissolved organic carbon in soils amended with and without vinasses

Soil type	Type of vinasses added	Dissolved organic carbon (%)							
		0 day	1 wk	2 wk	3 wk	4 wk	6 wk	9 wk	12 wk
Sandy loam	Without vinasses	0.02	0.03	0.01	0.04	0.03	0.01	0.01	0.01
	Fresh vinasses	2.05	1.88	1.63	1.16	0.72	0.13	0.42	0.36
	Nutrient supp. vinasses	1.59	1.54	1.54	1.14	1.06	0.30	0.77	0.73
Sandy clay loam	Without vinasses	0.01	0.02	0.02	0.05	0.03	0.01	0.02	0.01
	Fresh vinasses	1.57	0.73	0.59	0.48	0.44	0.08	0.21	0.17
	Nutrient supp. vinasses	1.48	0.64	0.51	0.42	0.43	0.09	0.25	0.21
F-test	Vinasses	**	**	**	**	**	**	**	**
	Soil type	**	**	**	**	**	**	**	**
	Vinasses*Soil type	**	**	**	**	**	**	**	**
CV %		5.2	8.4	5.2	14.6	8.8	16.5	16.7	8.6
LSD.05	Vinasses*Soil type	0.8833	0.1027	0.5630	0.1208	0.5974	0.2519	0.7008	0.3230

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