

## Greenhouse gas emissions from a soil cultivated with wheat (*Triticum* spp. L.) and amended with castor bean (*Ricinus communis* L.) or *Jatropha curcas* L. seed cake: A greenhouse experiment

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### ABSTRACT

Cultivation of plants, such as castor bean (*Ricinus communis* L.) or *Jatropha curcas* L. is set to increase in future as their seeds are used to extract oil for biofuel production. *Ricinus communis* seed cake (RSC) and *J. curcas* seed cake (JSC) were applied to soil cultivated with wheat (*Triticum* spp. L.) while plant growth and emissions of the greenhouse gases, CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O were monitored. Application of RSC or JSC had no significant effect development of the wheat plants. Emissions of CO<sub>2</sub> and N<sub>2</sub>O increased in soil amended with JSC or RSC, especially when larger amounts were applied, but had no effect on emissions of CH<sub>4</sub>. It was found that application of seed cakes increased emissions of CO<sub>2</sub> and N<sub>2</sub>O, but did not stimulate plant growth.

**Keywords:** biofuel; decomposition of seed cake; emissions of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O; mineral N; soil characteristics

Fossil fuel prices were increasing over the last years so alternative energy sources were promoted to lessen our dependence on a limited resource. The cultivation of *Jatropha curcas* L. and *Ricinus communis* L. or castor bean was promoted recently as their seeds can be extracted for oil that can be converted in biofuel (Alguacil et al. 2012). After the extraction of the seeds for oil an organic N-rich waste product remains that is non-edible, but that could easily be applied to soil (Anandan et al. 2005).

Application of degradable organic waste to soil often increases emissions of greenhouse gasses (GHG), such as CO<sub>2</sub>, N<sub>2</sub>O and methane (CH<sub>4</sub>) (Chiaradia et al. 2009). The organic material is mineralized increasing emissions of CO<sub>2</sub> and CH<sub>4</sub> is produced when anaerobic micro-sites are formed in soil. Mineralization of N-rich organic material also increases the concentration of NH<sub>4</sub><sup>+</sup> in soil,

which, when oxidized contributes to the emissions of N<sub>2</sub>O. Application of organic material increases microbial activity and this reduces O<sub>2</sub> concentrations in soil. Anaerobic micro-sites are formed, which might increase N<sub>2</sub>O and CH<sub>4</sub> emissions.

In the study reported here, the effect of *Jatropha curcas* (JSC) and castor bean (RSC) seed cake on plant growth, soil characteristics, the emission of GHG and net the global warming potential (GWP) of the GHG was studied in soils cultivated with wheat (*Triticum* spp. L.). Soil was amended with urea, JSC or RSC at 75, 150 and 300 kg N/ha. The objective of the study was to investigate how different application rates of JSC or RSC affected wheat growth and emissions of GHG. It was hypothesized that JSC or RSC would stimulate wheat growth while reducing emissions of N<sub>2</sub>O compared to the urea-amended soil.

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## MATERIAL AND METHODS

**Seed cake.** The JSC was obtained from Biocombustibles de Guatemala, S.A. (Guatemala) and RSC from Aceites Torres Barriga (State of Oaxaca, Mexico). The oils were extracted from *J. curcas* seeds with a combination of mechanical and chemical techniques and the remaining JSC used in this study (Shah et al. 2005). The oil was extracted from *R. communis* by heating with sodium hydroxide. The amount of ricin in RSC was below the detection limit and the JSC contained 0.93 mg/kg phorbol ester.

**Area description and soil sampling.** The sampling site was located in Otumba in the State of México (19°42'N, 98°49'W). The 0–15 cm top-layer of three plots (approximately 0.5 ha each) was sampled with a 7 cm diameter auger (Eijkelkamp, NL). In each plot, 30 soil cores were taken, pooled so that three soil samples were obtained and characterized. The sandy loam soil (Typic Fragiudepts) with pH 8.9 and electrolytic conductivity (EC) 1.23 dS/m, had a total C content of 29.1 g C/kg soil and a total N content of 2.41 g N/kg and water holding capacity (WHC) of 567 g/kg dry soil. The field-based replications ( $n = 3$ ) were maintained in the greenhouse experiment so as to avoid pseudo-replication.

**Cultivation of wheat in the greenhouse.** Nine treatments were applied to each soil sample ( $n = 3$ ): (1) uncultivated unfertilized soil; (2) soil cultivated with wheat, but not fertilized; (3) soil cultivated with wheat and fertilized with urea so that 150 kg N/ha was added; (4), (5), (6) soil was mixed with JSC at three concentrations so that 75, 150 and 300 kg N/ha was applied considering the mineral N in the seed cake and that 86% of the organic N was mineralized within the experimental period (Ruíz-Valdiviezo et al. 2013); (7), (8), (9) soil mixed with RSC so that plants were fertilized with 75, 150 and 300 kg N/ha considering the mineral N in the seed cake and that 97% of the organic N was mineralized within the experimental period (Ruíz-Valdiviezo et al. 2013).

Eighty-one sub-samples of 3.25 kg soil were added to PVC tubes (length 50 cm and diameter 16 cm) filled at the bottom with 7 cm of gravel topped up with 3 cm sand. Five wheat (cv. Temporalera M87) seeds were planted 3 cm deep in each of the 81 PVC tubes. During the experiment that started the 24<sup>th</sup> of April 2012, 500 mL water was added to each column every seven days. The amount of water applied was such that no leaching occurred. At grain maturity, i.e. 97 days, the entire soil column was removed from the PVC tube and plant roots and shoots characterized.

**Emissions of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O.** The GHG emissions were sampled for 45 days and overall 16 samples were taken. On each of the 16 sampling days, a cylindrical PVC chamber (length 50 cm and  $\varnothing$  16 cm) fitted with two sampling ports was placed on the PVC tube and air-tied sealed with Teflon tape. After each gas sampling, the PVC chamber was removed from the PVC tube. Details of the sampling technique can be found in Aguilar-Chavez et al. (2012), while details of the settings of the GC and the standards used to calculate the flux of the GHG in Ruíz-Valdiviezo et al. (2010).

**Seed cake and soil analysis.** The techniques used to measure pH, EC, the organic C, total N, WHC and the particle size distribution can be found in Ruíz-Valdiviezo et al. (2010). The JSC and RSC were characterized for total C and N and their composition, i.e. soluble fraction (mostly proteins and soluble hydrocarbons), (hemi)cellulose and lignin (Table 1) (Ruíz-Valdiviezo et al. 2010).

**Statistical analysis.** Emission of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O was regressed on elapsed time, i.e. after 0, 15 and 30 min, using a linear model forced to pass through the origin, but allowing different slopes (production rates). The sample at time 0 accounted for the atmospheric CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O. The total emissions of CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub> over the cultivation period of 45 days were calculated by linear interpolation of data points between each successive sampling event and numerical integra-

Table 1. Some characteristics of *Jatropha curcas* L. and *Ricinus communis* L. seed cake

Seed cake	Soluble fraction	Hemicellulose	Cellulose	Lignin	Polyphenols	Ash	C organic	Total N
(g/kg dry matter)								
<i>Ricinus communis</i>	269	383	269	24	0.134	55	411	41.7
<i>Jatropha curcas</i>	532	174	143	98	0.085	53	430 <sup>a</sup>	38.0

tion of underlying area using the trapezoid rule (Aguilar-Chávez et al. 2012).

Significant differences between plant characteristics as a result of the different treatments were determined by analyses of variance (ANOVA) and based on the least significant difference (SAS Institute 1989). Significant differences between treatments for production of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O were determined using PROC MIXED considering repeated measurements (SAS Institute 1989).

## RESULTS

### Characterization of the JSC, RSC and wheat.

The organic C and total N were similar in both seed cakes (Table 1). The soluble fraction (mostly proteins and soluble carbohydrates), however, was twice as large in the JSC than in the RSC, and the lignin content four times, while the hemicellulose and cellulose content was lower in JSC than in RSC.

Plant height, plant fresh weight, root dry weight, number of ears and ear fresh weight were significantly affected by treatment, but not the other plant characteristics (Table 2).

**Greenhouse gas emissions.** The CO<sub>2</sub> emissions rates were larger just after application of the JSC and RSC, and in soil amended with JSC after 7, 31 and 35 days (Figure 1a). The CO<sub>2</sub> emission rate was significantly affected by treatment (Table 3). Cultivating soil with wheat and applying urea had no significant effect on the CO<sub>2</sub> emission rates. However, the application of RSC or JSC significantly increased the CO<sub>2</sub> emission rate compared to the uncultivated soil with significantly larger

production in the JSC-amended soil than in the RSC-amended soil.

The soil was both a sink for CH<sub>4</sub> (negative CH<sub>4</sub> emission rates) and a source (Figure 1b). The mean CH<sub>4</sub> emission rate was not affected by treatment as was the CH<sub>4</sub> emitted over the 45 days period (Table 3).

In soil amended with large amounts of JSC and RSC, N<sub>2</sub>O emission rates increased between days 5 and 25 (Figure 1c). Cultivating soil with wheat and applying urea had no significant effect on the N<sub>2</sub>O emission rates (Table 3). Application of RSC or JSC, however, increased significantly the N<sub>2</sub>O emission rate compared to the uncultivated soil, except when 75 kg N/ha JSC was applied. The N<sub>2</sub>O emitted over the 45 days period increased also with increased application of RSC or JSC.

The contribution of CH<sub>4</sub> to the GWP of the GHG was highest (nearly 15%) in the unfertilized soil (uncultivated and cultivated with wheat) (Table 3). This dropped to < 4% when urea, JSC or RSC were applied to soil. Application of large amounts of JSC or RSC further decreased the contribution of CH<sub>4</sub> in GWP of the GHG emissions.

## DISCUSSION

**Plant characteristics.** It has been reported that organic material application stimulates normally plant growth and can increase crop yields. Application of seed cake might affect plant growth in different ways. First, mineralization of the seed cake might increase nutrient content (N, P) in soil thereby stimulating plant growth. Second, seed cake

Table 2. Characteristics of wheat (*Triticum* spp. L.) cultivated in unfertilized soil or fertilized with urea at 75, 150 or 300 kg N/ha derived from *Jatropha curcas* (L.) seed cake (JSC) or castor bean (*Ricinus communis* L.) seed cake (RSC) application and kept in the greenhouse for 97 days

	Control	Urea	JSC (kg N/ha)			RSC (kg/N ha)			MSD	F value	P value
			75	150	300	75	150	300			
Plant height (cm)	63 <sup>Ba</sup>	67 <sup>AB</sup>	69 <sup>A</sup>	68 <sup>AB</sup>	64 <sup>AB</sup>	64 <sup>AB</sup>	66 <sup>AB</sup>	64 <sup>AB</sup>	5.6	2.55	0.0266
Plant fresh weight (g)	5.7 <sup>B</sup>	9.0 <sup>AB</sup>	10.2 <sup>A</sup>	10.1 <sup>A</sup>	10.9 <sup>A</sup>	8.2 <sup>AB</sup>	9.8 <sup>AB</sup>	10.7 <sup>A</sup>	4.1	3.55	0.0028
Root dry weight (g)	1.6 <sup>B</sup>	2.8 <sup>AB</sup>	3.2 <sup>A</sup>	2.6 <sup>AB</sup>	2.6 <sup>AB</sup>	1.7 <sup>AB</sup>	2.5 <sup>AB</sup>	1.7 <sup>AB</sup>	1.5	2.68	0.0172
Number of ears	6.4 <sup>B</sup>	8.2 <sup>AB</sup>	7.8 <sup>AB</sup>	8.1 <sup>AB</sup>	9.2 <sup>A</sup>	7.0 <sup>AB</sup>	7.1 <sup>AB</sup>	7.6 <sup>AB</sup>	2.5	2.32	0.0358
Ear fresh weight (g)	7.9 <sup>B</sup>	9.5 <sup>AB</sup>	9.8 <sup>AB</sup>	10.6 <sup>AB</sup>	11.2 <sup>A</sup>	9.3 <sup>B</sup>	10.1 <sup>AB</sup>	12.2 <sup>A</sup>	2.7	4.40	0.0005

MSD – least significant difference ( $P < 0.05$ ). <sup>a</sup>Values are expressed per plant. Values within the row with the same letter are not significantly different ( $P < 0.05$ )

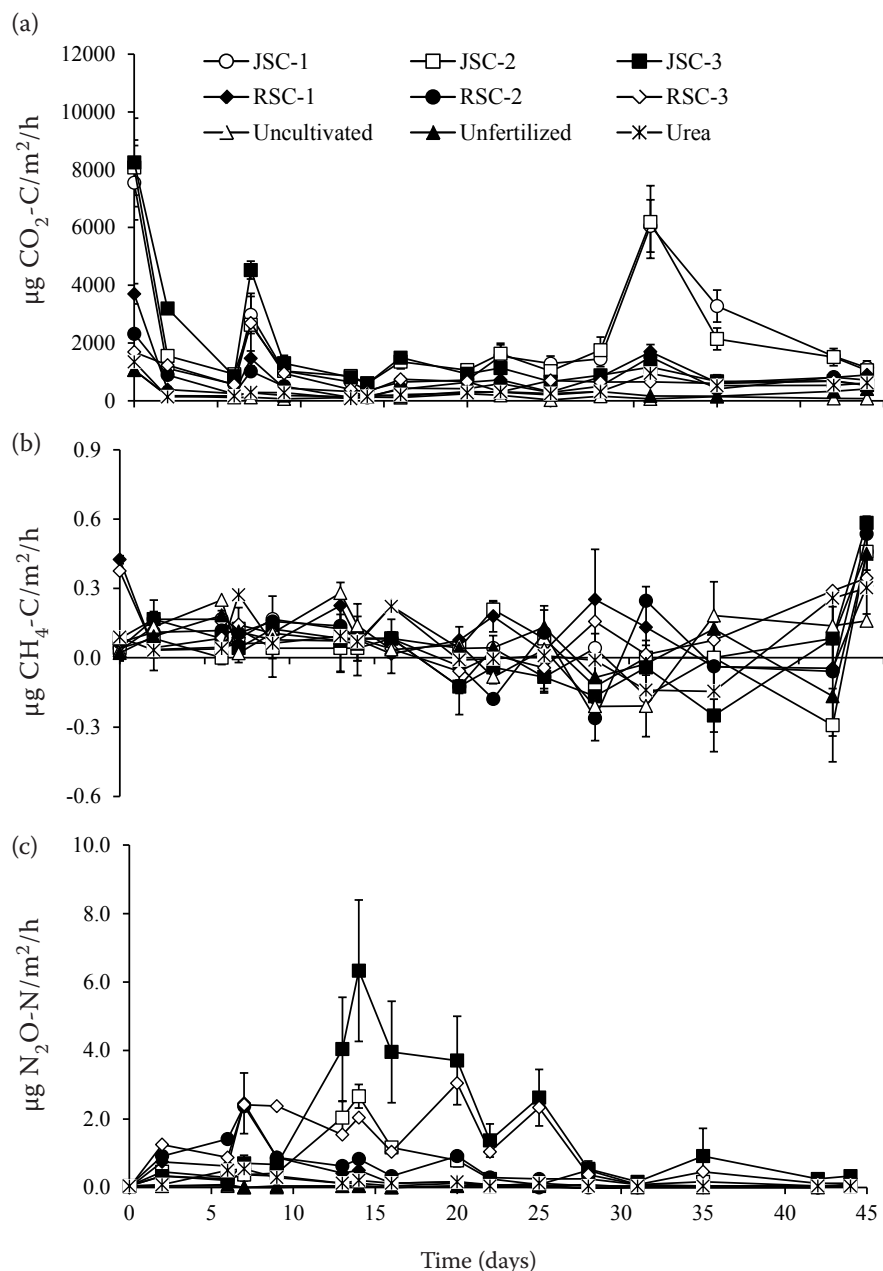


Figure 1. (a) The  $\text{CO}_2$ ; (b)  $\text{CH}_4$  and (c)  $\text{N}_2\text{O}$  emission rate for uncultivated soil, soil cultivated with unfertilized wheat (*Triticum* spp. L.), soil cultivated with wheat and fertilized with 150 kg urea-N/ha, amended with castor bean (*Ricinus communis* L.) seed cake (RSC) at 75 (RSC-1), 150 (RSC-2), and 300 kg N/ha (RSC-3), or amended with *Jatropha curcas* L. seed cake (JSC) at 75 (JSC-1), 150 (JSC-2), and 300 kg N/ha (JSC-3). Bars are  $\pm$  one standard deviation

might improve soil structure and aeration thereby facilitating plant growth and increasing plant yield. However, seed cake might also inhibit plant growth as it contains metabolites (phorbol esters, ricin) that might be toxic. In this study, application of JSC or RSC had no significant effect on plant development. It can be assumed that application of urea-N and the lower amounts of JSC or RSC provided enough

N for the crops, so that higher application rates of JSC or RSC had no further effect on crop growth.

**Greenhouse gas emissions.** Just after application of JSC or RSC, the emission of  $\text{CO}_2$  increased as the added organic material was mineralized. Although the oils were extracted from the seed cake, enough easily decomposable organic material remained to stimulate the emission of  $\text{CO}_2$ . The amount of

Table 3. CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O emission rate and cumulative emission of CH<sub>4</sub>, N<sub>2</sub>O and sum of the greenhouse gases (GHG) (CO<sub>2</sub> equivalent g/ha) from soil left bare, cultivated with wheat (*Triticum* spp. L.) but not fertilized or fertilized with urea at 75, 150 or 300 kg N/ha derived from *Jatropha curcas* (L.) seed cake (JSC) or castor bean (*Ricinus communis* L.) seed cake (RSC) application. Greenhouse gas emissions were measured for 45 days

Treatment	Emission rate			Cumulative emission		
	CO <sub>2</sub> -C	N <sub>2</sub> O-N	CH <sub>4</sub> -C	N <sub>2</sub> O	CH <sub>4</sub>	GHG
	(µg/m <sup>2</sup> /h)			(g CO <sub>2</sub> equivalent/ha over 45 days) <sup>a</sup>		
Bare unamended soil	242 <sup>Eb</sup>	0.029 <sup>F</sup>	0.138 <sup>A</sup>	0.159 <sup>C</sup>	0.024 <sup>A</sup>	0.183 <sup>C</sup>
Wheat cultivated soil	321 <sup>E</sup>	0.046 <sup>F</sup>	0.088 <sup>A</sup>	0.137 <sup>C</sup>	0.024 <sup>A</sup>	0.161 <sup>C</sup>
Wheat cultivated soil applied with 150 kg urea N/ha	333 <sup>DE</sup>	0.258 <sup>DEF</sup>	0.096 <sup>A</sup>	0.658 <sup>C</sup>	0.018 <sup>A</sup>	0.676 <sup>C</sup>
Wheat and soil amended with 75 kg JSC-N/ha	2038 <sup>B</sup>	0.217 <sup>EF</sup>	0.117 <sup>A</sup>	0.523 <sup>C</sup>	0.020 <sup>A</sup>	0.543 <sup>C</sup>
Wheat and soil amended with 150 kg JSC-N/ha	2238 <sup>B</sup>	0.900 <sup>BCD</sup>	0.058 <sup>A</sup>	2.577 <sup>BC</sup>	0.012 <sup>A</sup>	2.589 <sup>BC</sup>
Wheat and soil amended with 300 kg JSC-N/ha	2792 <sup>A</sup>	1.763 <sup>A</sup>	0.100 <sup>A</sup>	7.557 <sup>A</sup>	0.005 <sup>A</sup>	7.562 <sup>A</sup>
Wheat and soil amended with 75 kg RSC-N/ha	963 <sup>C</sup>	0.796 <sup>CDE</sup>	0.133 <sup>A</sup>	1.506 <sup>BC</sup>	0.031 <sup>A</sup>	1.537 <sup>BC</sup>
Wheat and soil amended with 150 kg RSC-N/ha	763 <sup>CD</sup>	1.008 <sup>BC</sup>	0.113 <sup>A</sup>	2.435 <sup>BC</sup>	0.018 <sup>A</sup>	2.453 <sup>BC</sup>
Wheat and soil amended with 300 kg RSC-N/ha	1117 <sup>C</sup>	1.504 <sup>AB</sup>	0.129 <sup>A</sup>	5.668 <sup>AB</sup>	0.035 <sup>A</sup>	5.702 <sup>AB</sup>
Minimum significant difference ( $P < 0.05$ )	200	0.321	0.050	4.940	0.059	4.940

<sup>a</sup>the global warming potential of the gasses emitted was calculated considering the CO<sub>2</sub>-equivalent emission of 298 for N<sub>2</sub>O and 25 for CH<sub>4</sub> (IPCC 2007) emitted over a 45-day period; <sup>b</sup>values with the same letter within the column are not significantly different between the treatments, i.e. the columns ( $P < 0.05$ )

easily decomposable organic material was twice as high in JSC than in RSC. Consequently, the emission of CO<sub>2</sub> was larger from the JSC-amended soil than from the RSC-amended soil.

Cultivation of wheat had no significant effect on emission of CO<sub>2</sub>, although it has often been reported that it does (Lam et al. 2011). Root exudates might stimulate microbial activity and consequently emission of CO<sub>2</sub>.

Application of organic material to soil often increases emissions of N<sub>2</sub>O (Odlare et al. 2012). Mineralization of organic material releases ammonium (NH<sub>4</sub><sup>+</sup>), especially when the C-to-N ratio of the substrate is low, such as in the JSC and RSC. Ammonium is oxidized NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> thereby producing N<sub>2</sub>O. Additionally, increased microbial activity increases anaerobic micro-sites thereby stimulating denitrification.

The contribution of N<sub>2</sub>O to the GWP of the GHG was 75% in the uncultivated soil or the cultivated and unfertilized soil. Application of urea or seed cake increased the contribution of N<sub>2</sub>O and it became > 99% at the highest application rates of JSC

or RSC. The increase in N<sub>2</sub>O emissions when JSC or RSC was applied to soil was due to an increased N cycling (Li et al. 2005) and presumably not due to an increase in anaerobic conditions as only N<sub>2</sub>O emissions increased and not CH<sub>4</sub> emissions. Li et al. (2005) found that emission of N<sub>2</sub>O increased from arable soil when crop residue were added, but CH<sub>4</sub> emission was not affected.

An agricultural soil is often a sink for CH<sub>4</sub> (Levine et al. 2011), but not in the first weeks of this experiment. Organic material application and plant growth might stimulate CH<sub>4</sub> emissions from soil. In this study, however, application of JSC or RSC and maize did not affect emissions of CH<sub>4</sub>.

The contribution of CH<sub>4</sub> to the GHG emission is generally low and sometimes negative (Berglund and Berglund 2011), but this was not the case in this study. Arable soils can become a source of CH<sub>4</sub> when anaerobic micro-sites are formed, i.e. in the centre of aggregates. It has to be remembered that the soil was sieved before the column was packed so the soil structure was destroyed which might have favoured anaerobic conditions.



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