Emission of CO_2, CH_4 and N_2O and dynamics of mineral N in soils amended with castor bean (*Ricinus communis* L.) and piñón (*Jatropha curcas* L.) seed cake

V.M. Ruíz-Valdiviezo[1], L.D. Mendoza-Urbina[2], M. Luna-Guido[1], F.A. Gutiérrez-Miceli[2], M.R. Cárdenas-Aquino[1], J.A. Montes-Molina[2], L. Dendooven

[1]Laboratory of Soil Ecology, ABACUS, Cinvestav, México
[2]Department of Plant Biotechnology, Technological Institute of Tuxtla Gutiérrez, Tuxtla Gutiérrez, Chiapas, México

ABSTRACT

Extraction of oils from castor bean (*Ricinus communis* L.) and *Jatropha curcas* L. to produce biofuel is set to increase. The produced seed cake could be applied to soil as it is nutrient rich, but might affect soil functioning. Seven soils from Chiapas, México, were amended with seed cake of both plants while CO_2, CH_4 and N_2O emissions and mineral N concentrations were monitored in an aerobic incubation. The concentration of phorbol esters in the *J. curcas* seed cake (JSC) was 0.993 mg/g while no ricin was detected in the *R. communis* seed cake (RSC). Application of JSC increased CO_2 emission 2.5-times, N_2O 12.6-times and CH_4 18.4-times compared to the unamended soil, while RSC CO_2 emission 2.1-times, N_2O 21.3-times and CH_4 2.3-times. On average, 66% of the 88 mg organic N added with JSC was mineralized and 83% of the 101 mg organic N of the RSC within 56 days. It was found that *J. curcas* and castor bean seed cake increased CO_2, CH_4 and N_2O emission and mineral N in soil, without inhibiting soil microbial activity.

Keywords: biofuel; decomposition of organic residues; greenhouse gas emissions; soil characteristics

The cultivation of *Jatropha curcas* L. for the production of bio-diesel has increased in recent years (Fairless 2007). After the extraction of seeds for oil, an organic-rich waste product remains that could easily be applied to degraded soil (Ramachandran et al. 2007). However, *J. curcas* seed cake (JSC) contains organic components, such as phorbol esters, that might inhibit plant growth and biological functioning of the soil (Goel et al. 2007).

Ricinus communis L. also known as castor bean, is grown in temperate and tropical parts of the world for its oil, which is used extensively for medicinal and industrial purposes, i.e. biodiesel (Da Silva César and Batalha 2010). India is the world's largest producer of castor seed with an annual production of 590 × 10^6 kg and approximately 400 × 10^6 kg castor seed cake (RSC) remains after extraction of the oil (Lima et al. 2011). The RSC is rich in N, so it could also easily be used as organic fertilizer (Ramachandran et al. 2007), but might contain toxic compounds, e.g. ricin and ricinine, which might limit its use in agricultural practices (Anandan et al. 2005).

The emission of greenhouse gases from soil (GHG) is important as they contribute to global warming (Shang et al. 2011). Land use change and agricultural practices contributes significantly to the CO_2, CH_4 and N_2O emissions (Singh et al. 2010). Application of organic residues to soil, such as RSC and JSC, generally improves soil structure,

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Table 1. Some characteristics of the seven soils of Chiapas (Mexico) used

Soil	EC (dS/m)	pH	Organic C	Total N	WHC	Sand	Loam	Clay	USDA texture
			(g/kg)						
Flores Magón	0.93	7.1	13.2	1.3	880	650	130	220	Sandy clay loam
Suchiapa	0.54	8.1	20.8	1.4	850	670	190	140	Sandy loam
Tuxtla Gutiérrez	0.93	7.6	16.9	1.5	870	520	200	280	Sandy clay loam
Villa Acala	0.67	8.5	18.7	1.9	880	490	210	300	Sandy clay loam
Villa Hermosa	1.27	6.3	5.0	0.6	850	650	210	140	Sandy loam
Villa Morelos	0.31	5.8	5.0	0.4	840	680	270	50	Sandy loam
Zapotillo	0.43	6.4	20.8	1.4	870	500	310	190	Loam

EC – electrolytic conductivity; WHC – water holding capacity; USDA – Soil classification

but might increase emissions of GHG (Anis et al. 2010).

Although the JSC and RSC are nutrient rich, little information exists on how this organic waste product might affect mineral N and emission of GHG. Soil sampled from seven sites in Chiapas (Mexico) was amended with JSC and RSC. The objective of this study was to investigate the effect of RSC and JSC on mineral N in soil and GHG emissions in seven soils from Chiapas that were cultivated with *J. curcas* and castor bean for biofuel production.

MATERIAL AND METHODS

Sampling sites. Soil was collected from seven different locations in Chiapas (México). Details of the sampling sites and climatic conditions can be found in Ruíz-Valdiviezo et al. (2010).

Soil and seed cake sampling. Soil was collected from the 0–15 cm layer of five 500 m^2 plots at seven sampling sites on February 20, 2011. In each plot 25 soil cores were sampled with a 7 cm stony soil auger diameter (Eijkelkamp, Nl) and pooled. As such, 35 soil samples were obtained, i.e. from five plots at seven sites (n = 35). The soil sampled was characterized and used to study dynamics of C and N (Table 1). This field based replication was maintained in the incubation study.

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 *Ricinus communis* by heating with sodium hydroxide. The JSC and RSC was characterized (Table 2) and details of the techniques used can be found in Ruíz-Valdiviezo et al. (2010). The ricin in RSC was measured in the laboratory of biochemistry at National Autonomous University of Mexico (Mexico) (Kanamori-Kataoka et al. 2011). The concentration of phorbol esters in JSC was determined at the University of Hohenheim (Stuttgart, Germany) (Makkar et al. 1997).

Experimental set-up, treatments and incubation experiments. The soil was sieved separately (< 5 mm) and adjusted to 40% water holding capacity (WHC) by adding distilled water. The soil (1 kg per plot) was pre-incubated in drums (soil from each site in one drum) containing a jar with 1 L distilled water to avoid desiccation and 250 mL 1 mol/L NaOH to trap evolved CO_2.

Table 2. Some characteristics of *Jatropha curcas* L. and *Ricinus communis* L. seed cake

Seed	Organic C	Total N	Soluble[a]	Hemicellulose	Cellulose	Lignin	Polyphenols	Ash
	(g/kg dry matter)							
Jatropha curcas	430[b]	38.0	532	174	143	98	0.085	53
Ricinus communis	411	41.7	269	383	269	24	0.134	55

[a]soluble fraction is the neutral detergent fraction and contains mostly easily decomposable carbohydrates and proteins; [b]mean of three replicates (n = 3)

Table 3. Emissions of CO_2, CH_4 (mg C/kg dry soil day) and N_2O (mg N/kg dry soil day) and concentrations of NH_4^+, NO_2^- and NO_3^- (mg N/kg dry soil) from seven soils of Chiapas (Mexico) left unamended (control) or amended with seed cake of castor bean (*Ricinus communis* L.) (RSC) or piñón (*Jatropha curcas* L.) (JSC)

Soil	Emission of CO_2				Emission of CH_4				Emission of N_2O			
	control	JSC	RSC	SE	control	JSC	RSC	SE	control	JSC	RSC	SE
Flores Magon	11.1	23.3	22.1	3.1	–0.118	–0.042	–0.031	0.020	0.61	0.97	1.53	0.51
Suchiapa	10.5	26.7	23.4	3.5	–0.127	0.055	–0.026	0.019	0.09	5.48	11.51	0.92
Tuxtla Gutierrez	13.3	26.7	23.4	3.5	–0.118	0.028	–0.065	0.015	0.05	0.73	2.00	0.14
Villa Acala	6.4	25.1	18.0	2.2	–0.136	–0.053	–0.057	0.021	0.02	4.50	2.59	0.36
Villa Hermosa	10.2	24.8	22.5	3.3	–0.012	0.017	–0.019	0.006	0.38	1.54	4.21	0.49
Villa Morelos	7.7	23.3	19.6	2.7	–0.006	–0.006	–0.016	0.005	0.04	0.36	0.86	0.06
Zapotillo	12.3	28.0	24.6	2.9	–0.051	–0.029	–0.028	0.006	0.00	1.25	2.49	0.20
SEE	1.8	3.6	3.1		0.023	0.010	0.008		0.17	0.55	0.59	
	concentration of NH_4^+				concentration of NO_2^-				concentration of NO_3^-			
	control	JSC	RSC	MSD	control	JSC	RSC	MSD	control	JSC	RSC	MSD
Flores Magon	3.9	2.6	12.9	12.2	0.2	0.5	0.4	0.7	17.1	121.3	95.2	63.2
Suchiapa	ND	11.4	16.2	13.4	0.3	1.9	1.3	1.4	20.2	69.8	143.6	53.6
Tuxtla Gutierrez	7.3	12.3	10.5	26.9	0.1	1.6	0.5	1.9	7.2	50.6	60.8	79.6
Villa Acala	10.4	ND	23.7	10.5	0.1	0.7	2.0	3.1	47.2	79.1	94.6	99.7
Villa Hermosa	4.3	9.8	22.3	17.6	0.5	0.3	0.6	0.8	9.5	23.0	60.8	35.0
Villa Morelos	13.4	17.6	18.1	11.4	0.4	0.7	0.7	0.4	11.3	93.0	100.3	46.7
Zapotillo	0.2	6.2	15.1	13.5	0.4	0.6	0.2	0.6	24.5	81.3	131.1s	26.6
MSD	13.4	15.5	21.4		0.9	1.9	1.7		23.8	50.1	88.3	

SE – standard error of the estimate ($P < 0.05$); MSD – minimum significant difference ($P < 0.05$); SEE – standard error of the estimate ($P < 0.05$); ND – not determined

After one week, three different treatments were applied to the soil samples. In a first experiment, 18 sub-samples of 50 g soil from each plot sampled at each site were added to 100 mL-glass flasks. Six sub-samples of soil were amended with 175 mg JSC, 225 mg RSC or left unamended. The amount of seed cake applied was 1 g C/kg. The 100-mL glass flasks were separately placed in 1-L glass jars containing a 25-mL flask with distilled water to avoid desiccation and a 25-mL glass flask with 20 mL 1 mol/L NaOH to trap the evolved CO_2. All flaks were closed air-tight and incubated at 22 ± 2°C in the dark for 56 days. Additionally, 18 1-L glass jars, but without soil accounted for the CO_2 trapped form the air. After 0, 3, 7, 14, 28 and 56 days, a glass jar from each treatment, plot sampled at each site was selected and opened. The 25 mL glass flask with the NaOH was removed, air-tight sealed and stored pending analysis. The soil in the 100 mL glass flask was removed and extracted for mineral N with 200 mL 0.5 mol/L K_2SO_4.

In a second experiment, 12 sub-samples of 10 g soil from each plot sampled at each site were added to 110 mL-glass flasks. Four sub-samples were amended with 35 mg JSC, 45 mg RSC or left unamended. All the 110-mL glass flasks were sealed air-tight with a suba-seal, closed with an aluminium seal and incubated in the dark for 7 days. Additionally, 12 110-mL glass flasks, but without soil were incubated and accounted for the CH_4 and N_2O in the atmosphere. After 0, 1, 3 and 7 days, a glass flask from each treatment and plot sampled at each site was selected at random and the headspace analyzed for N_2O and CH_4.

The techniques used to characterize soil, determine mineral N, analyze CH_4, CO_2 and N_2O emitted during the aerobic incubation and details of the statistical analysis can be found in Ruíz-Valdiviezo et al. (2010).

RESULTS

Seed cake characteristics. The soluble fraction was higher in JSC than in RSC and the lignin and hemicellulose content were lower in the first than in the latter (Table 2). The concentration of phorbol esters in JSC was 0.993 mg/g. No ricin was detected in RSC.

CO_2, CH_4 and N_2O emission and mineral N. Application of RSC increased CO_2 emission 2.1-fold (mean of all soils) compared to the unamended soil, while application of JSC increased it 2.5 times ($P < 0.05$) (Table 3). Application of JSC and RSC generally increased CH_4 and N_2O emission in soils. Approximately 6.1% of the organic N in JSC was emitted as N_2O and 14.1% of the organic N in RSC after 7 days.

Application of JSC and RSC increased the concentrations of NH_4^+, NO_2^- and NO_3^- after 56 days (Figure 1, Table 3). The largest increase was generally found when RSC was applied to soil, although large variations were observed between soils. The increase in mineral N ($NH_4^+ + NO_2^- + NO_3^-$) in the JSC-amended soils (average of all soils) was 58.5 mg N/kg dry soil and 83.9 mg N/kg dry soil in the RSC-amended soil. As such, 66.4% of the organic N added with JSC (88 mg N) and 83.1% added with RSC (101 mg N) was mineralized within 56 days.

DISCUSSION

Characteristics of JSC and RSC. Organic N content of seed cakes used in this study was within the range of 22.4 to 54.6 g N/kg for oil seed cakes of *Ricinus* and *Jatropha* (Devappa et al. 2010). Devappa et al. (2010) reported a C:N value of 8.6 for JSC and Müller et al. (2006) 8.5 for RSC.

The soluble fraction in JSC and RSC was similar to values reported by Martín et al. (2010), while the hemicellulose content was lower for seed cakes of Neem, Moringa, Trisperma and Ricinus (from 3 to 68 g/kg dry seed cake). The high hemicellulose and cellulose content of RSC was similar to that found in palm kernel and olive and due to the husks contained in the cake (Ramachandran et al. 2007). Ricin was not detected in the defated RSC. Barnes et al. (2009) reported that the glycoprotein ricin degraded as a result of heating with sodium hydroxide. The concentration of phorbol esters in JSC was 0.93 mg/g and higher than reported by Saetae et al. (2011) (0.73 mg/g). Concentrations depend on variety, cultivation technique, plant maturity and oil extraction method.

C and N mineralization of JSC and RSC amended to soil. If no priming effect was considered, than 64% of the C added with JSC and 49% added with RSC mineralized within 56 days. The decomposition of JSC and RSC was high at the onset of the experiment and decreased thereafter. A larger CO_2 emission at the onset of the experiment has also been observed by Müller et al. (2006), and can be attributed to the mineralization of the easily decomposable fraction, e.g. extractable polysaccharides. Once this fraction is mineralized, CO_2 emission drops as only more recalcitrant organic material remains. Consequently, the CO_2 emission from the JSC-amended soil was higher than RSC-amended soil as the soluble fraction was nearly twice as high in JSC than in RSC.

Approximately 66.4% of organic N in the JSC was mineralized and 83.1% in RSC within 56 days. As such, large amounts of the organic N in the seed cake will become available as nutrient in soil quickly (Müller et al. 2006, Ramachandran et al. 2007). The difference in N mineralized between the two seed cakes might be due to different factors. First, the total N content of the RSC was larger than in JSC so more mineral N will be released. Second, more of the organic C in the JSC was mineralized so more N will be immobilized by the soil microorganisms reducing the mineral N available in soil.

Both seed cakes are excellent organic N fertilizers, but as reported before they contain some metabolites that might be of environmental concern. The phorbol ester content of JSC used in this experiment was low compared to values reported (0.73 to 3.85 mg/g) (Makkar et al. 1997, Devappa et al. 2010, Saetae et al. 2011). Even if some phorbol esters would remain in the JSC, their toxicity in soil is debatable. Devappa et al. (2010) reported that they are biodegraded rapidly and their degraded products appear to be innocuous. No ricin was found in the RSC so it can be easily applied to soil as organic fertilizer, but it might be worthwhile to analyse RSC for ricin before it is applied routinely to soil.

Emission of N_2O and CH_4. The emission of N_2O increased when JSC and RSC were applied to soil with the largest increase found in the latter. Zou et al. (2005) also reported an increase in N_2O emissions (17%) when rapeseed cake was added to rice paddies. The amount of N mineral-

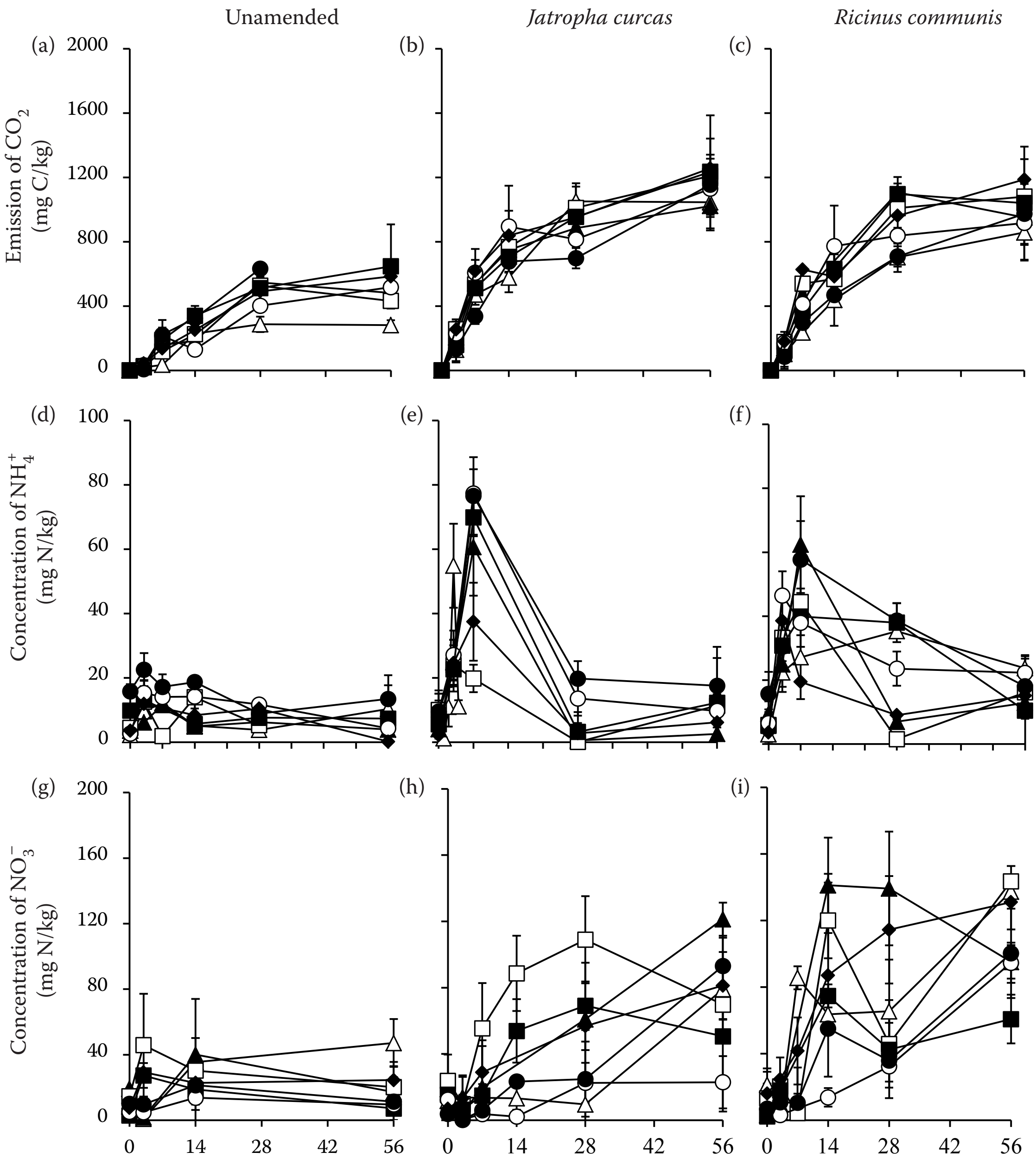

Figure 1. (a) Emission of CO_2 (mg C/kg dry soil) from unamended soil of Flores Magón (▲), Suchiapas (□), Tuxtla Gutiérrez (■), Villa Acala (Δ), Vista Hermosa (○), Villa Morelos (●) and Zapotillo (♦); (b) soil amended with *Jatropha curcas* L. seed cake (JSC) or (c) *Ricinus communis* L. seed cake (RSC); (d) concentration of NH_4^+ (mg N/kg dry soil) in unamended soil; (e) soil amended with JSC or (f) RSC; (g) concentration of NO_3^- in unamended soil; (h) soil amended JSC or (i) RSC. Bars are one STD (n = 5)

ized was larger in the RSC-amended soil than in JSC-amended soil so more nitrification occurred in the first than in the latter and N_2O is emitted when NH_4^+ is oxidized to NO_2^- and then to NO_3^- (Khalil et al. 2009).

Agricultural soils that are a sink for CH_4 can become a source when organic material is applied. Collins et al. (2011) found that CH_4 emissions after manure applications were higher than from unfertilized or fallow soil or when amended with urea or anaerobically digested fibre. Application of organic material will increase anaerobic micro-sites thereby stimulating emissions of CH_4. Application of JSC increased microbial activity more than when RSC was added, so emission of CH_4 increased more in the first than in the latter case.

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Corresponding author:

Prof. Luc Dendooven, ABACUS, Laboratory of Soil Ecology, Cinvestav, México D.F., México
phone: + 52 55 5747 3319, fax: + 52 55 5747 3313, e-mail: dendooven@me.com