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Mineralización de C y N e identificación de los microorganismos involucrados en la biodegradación de residuos de plantas en diferentes suelos mediante la prueba ^{13}C-DNA SIP

Tesis que presenta

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Resumen

Un buen manejo de la materia orgánica del suelo es muy importante, ya que contribuye a aumentar la productividad de las plantas y reducir la dependencia de los fertilizantes inorgánicos, así como mejorar la fertilidad y las características físicas, químicas y biológicas del suelo. Los residuos de las plantas son la fuente primaria de materia orgánica del suelo. Por lo tanto, un manejo apropiado de materia orgánica del suelo requiere un mejor conocimiento de las dinámicas de descomposición de los residuos derivados de plantas con la finalidad de conocer la liberación de los nutrimentos durante el proceso de biodegradación y la absorción de la planta, así como evitar la pérdida de los nutrimentos del suelo. Todo lo anterior, aunado a la demanda de servicios y alimentos que la población requiere. Sin embargo, en la mayoría de los estudios de descomposición de residuos se determinan las dinámicas de (C) y (N), pero hay poco conocimiento acerca de los microorganismos activos involucrados en la biodegradación. Así, en una primera etapa de la investigación, se evaluó el efecto de la incorporación de hojas de *Jatropha curcas* sobre las dinámicas de carbono (C) y nitrógeno (N), en suelos provenientes de cinco sitios experimentales del estado de Chiapas durante una incubación aerobia de 56 días. Asimismo, se evaluaron las dinámicas de C y N en suelos adicionados con harinas de *Jatropha curcas* y harinas de *Ricinus communis*; y también se determinó la mineralización del C y N en el proceso de descomposición de residuos de maíz y su fibra neutro detergente (formada principalmente de celulosa y hemicelulosa) en un suelo agrícola y un suelo salino-alcalino del exlago de Texcoco. En estos tres experimentos las evidencias demuestran que la adición de materia orgánica de origen vegetal a los suelos agrícolas, o bien, al suelo salino-alcalino, modifica las dinámicas de carbono y nitrógeno, así como las emisiones de gases con efecto de invernadero. Se concluyó que al adicionar una fuente de C extra al suelo aumenta la producción de CO_2 comparado con el tratamiento control durante la incubación aerobia de 56 días, con lo que se tiene evidencia de la presencia de microorganismos capaces de utilizar los componentes (e.g. glucosa, celulosa, hemicelulosa) de los residuos de las plantas en las condiciones donde se establecieron los experimentos. Basándose en lo

anterior, en una segunda etapa se diseñó una cámara de crecimiento para marcar de plantas (maíz, como planta modelo) con ^{13}C mediante el establecimiento de la técnica *'$^{13}CO_2$ pulse-labelling'* (Experimento 4); y los resultados mostraron que el contenido de ^{13}C en la parte área de la planta fue 35 atom% ^{13}C y 14 atom% ^{13}C en las raíces. Considerando que el contenido de ^{13}C en los residuos de maíz fue alto comparado con el contenido de ^{13}C de otras especies de plantas reportadas en la literatura. Un suelo agrícola fue adicionado con residuos de maíz con ^{13}C para estudiar los microorganismos involucrados en su descomposición y a partir de la dinámica de producción de CO_2 durante una incubación aerobia de 56 días, se seleccionaron los días 0, 1, 3, 7 y 14 para la extracción del DNA total del suelo. Después, el DNA pesado (marcado con ^{13}C) y el DNA ligero (no marcado) fueron separados mediante una ultracentrifugación con gradientes de densidad en CsCl a 45,000 rpm a 20 °C durante 40 h. El DNA marcado con ^{13}C fue de buena calidad y se logró amplificar un fragmento del rDNA 16S mediante la reacción de PCR. Los amplicones fueron clonados y secuenciados, y en un análisis de las secuencias se identificaron microorganismos degradadores de celulosa por ejemplo pertenecientes al género *Sphingomonas*. En conclusión, la combinación de las técnicas isotópicas *'$^{13}CO_2$ pulse-labelling'* y ^{13}C-DNA-SIP permitió identificar a los microorganismos involucrados en la biodegradación de residuos de plantas, como el maíz y así la funcionalidad de las poblaciones microbianas en el suelo pudo ser determinada.

Abstract

A good management of the Soil Organic Matter is very important as it contributes to enhance plant productivity, reducing dependency on inorganic fertilizers and improves soil fertility and physical, chemical and biological soil characteristics. Plant residues are the primary source of soil organic matter. Therefore, proper soil organic matter management requires a better understanding of plant residue decomposition dynamics with the purpose of determining the release of nutrients during the biodegradation process and plant uptake, as well as prevent loss of soil nutrients, is a global concerning as the food is required in a better quality and quantity. However, in most studies on decomposition of plant residues, the dynamics of carbon (C) and nitrogen (N) are determined but there is a little knowledge about the microorganisms involved in their biodegradation. In the fist stage of the investigation, we evaluated the effect of the incorporation of leaves of *Jatropha curcas* in soils from five experimental sites of Chiapas on the dynamics of C and N during an aerobic incubation of 56 days. In a second experiment, we evaluated the dynamics of C and N in soils amended with *Jatropha curcas* and *Ricinus communis* seed cakes. Thereafter, we determined the mineralization of C and N in the decomposition of maize residues and the neutral detergent fraction (NDF) (composed mainly of cellulose and hemicellulose) in an agricultural soil and a saline-alkaline soil of the former Lake Texcoco in Mexico. The results of these experiments showed that amending with plant organic matter to agricultural or saline-alkaline soils modifies the C and N dynamics and greenhouse gases emission. Thus, it was found that amending a source of extra C to soil increases the production of CO_2 compared with control treatment during an aerobic incubation of 56 days, predicting that plant residues (e.g. glucose, cellulose, hemicellulose) were biodegraded in these conditions by soil organisms. On the basis of these data, in the second stage, we designed a growth chamber of ^{13}C-enriched plants (maize, as a model plant) by $^{13}CO_2$ pulse-labelling technique. Our results showed that the ^{13}C enrichment in the plant shoot was 35 atom% and 14 atom% in the roots. Considering that the maize plants that were pulse labelled were highly enriched with ^{13}C compared with % ^{13}C enrichment of other plant species reported in the literature. An agricultural soil (as a model soil) was amended with ^{13}C labelled maize

residue to study the microorganisms involved in decomposition. The production of CO_2 was monitored in an aerobic incubation of 56 days taking samples at 0, 1, 3, 7, 14 and 28 days to extract total DNA from soil microcosm. Thereafter, the light (unlabelled) and heavy DNA fraction (^{13}C-labelled) were separated by density gradient ultracentrifugation using CsCl at 45,000 rpm and 20 °C for 40 h. The ^{13}C-labelled DNA was of good quality and the 16S rDNA was amplified by PCR. The amplified DNA could be cloned and sequenced and an analysis of the 16S rDNA identified known degraders of cellulose, i.e. *Sphingomonas*. In conclusion, the combination of $^{13}CO_2$ pulse-labelling technique and ^{13}C-DNA SIP approach allowed elucidate microorganisms involved in the degradation of crop residues such as maize, so that the functionality of bacterial populations in soil could be determined.

Introducción

La evaluación mundial de la degradación antropogénica de los suelos ha demostrado que cada año se provoca una pérdida de entre 5 y 7 millones de hectáreas de suelos cultivables, debido sobre todo a la erosión, la destrucción de la cubierta vegetal, el sobrepastoreo, la quema de vegetación, la desertificación, la salinización, la compactación física y el avance y crecimiento de las ciudades en un suelo fértil (FAO, 2012). Estos efectos suelen traducirse en una disminución de los nutrimentos en el suelo y en la productividad de las cosechas, y por ende, problemas socioeconómicos para los agricultores. En la actualidad, las prácticas agrícolas y pecuarias a menudo resultan en la sobreexplotación del suelo, sin una atención especial por mejorar la fertilidad de los suelos deteriorados, los cuales constituyen la base para una seguridad alimentaria continua (FAO, 2012). De esta manera, a fin de satisfacer las futuras necesidades mundiales de alimentos, es necesario cultivar nuevas variedades con características deseables, tal que tengan un potencial de rendimiento mayor. Por ello, este problema puede solucionarse si la descomposición de la materia orgánica puede optimizarse. Según datos de la FAO (2012) para un desarrollo agrícola sustentable, la conservación de los recursos naturales, especialmente de la Materia Orgánica del Suelo (MOS), el producto de la descomposición de los compuestos orgánicos adicionados al suelo e indicadores de calidad del suelo, se consideran medidas claves para la recuperación de los suelos deteriorados. Por lo tanto, la adición de los residuos de cosechas se ha convertido en una estrategia importante para mejorar la fertilidad y mantener la sustentabilidad del suelo.

Diversos autores (Bertrand *et al.,* 2006; Paterson *et al.,* 2006; Baldock, 2007; Ruiz-Valdiviezo *et al.,* 2010; Mohammadi *et al*., 2011) han publicado que la descomposición de la materia orgánica de origen vegetal en diferentes ecosistemas juega un papel importante en el ciclo del carbono, debido a que este proceso puede contribuir a mantener la materia orgánica en el suelo, mejorar las propiedades químicas, físicas y biológicas e incrementar la disponibilidad y sustentabilidad de las cosechas. Sin embargo, aún no se tiene un conocimiento claro de los mecanismos y los

microorganismos involucrados en la descomposición de residuos de plantas y liberación de los nutrimentos al suelo.

La técnica de pulsos marcados con $^{13}CO_2$ y la prueba de isótopos estables (DNA SIP; por sus siglas en inglés) ofrecen potencial para a) estudiar la ruta metabólica que siguen los fotosintatos de plantas marcadas con ^{13}C a llegar a determinados grupos de microorganismos en la rizósfera, b) elucidar los ciclos de los nutrimentos (^{13}C y ^{15}N), y c) determinar las interacciones de los organismos dentro de los ecosistemas (Vandenkoornhuyse *et al.,* 2007; Dumont *et al.,* 2011).

La prueba DNA SIP es un método utilizado para marcar microorganismos no cultivables en muestras ambientales o directamente en el campo usando sustratos enriquecidos con un isótopo estable (e.g., ^{13}C). Después del consumo del sustrato, las células de los microorganismos que consumen el sustrato se enriquecen con el isótopo. Así, los biomarcadores moleculares enriquecidos, como ácidos grasos fosfolipídicos (PLFA, por sus siglas en ingles), DNA y RNA; pueden ser analizados mediante técnicas moleculares y analíticas, y de este modo, usados para la identificación y caracterización de los organismos que incorporan el sustrato isotópico (Neufeld *et al.,* 2007).

Esta investigación propone utilizar la combinación de las técnicas isotópicas *'$^{13}CO_2$ pulse-labelling'* y ^{13}C-DNA SIP para determinar las dinámicas de C y N en diferentes suelos adicionados con diferentes materiales de origen vegetal, como hojas de *Jatropha curcas*, harinas de *Jatropha curcas* y *Ricinus communis*, residuos de plantas de maíz marcadas con ^{13}C y sin marcar, fracción fibrosa ((hemi)celulosa) de maíz marcada con ^{13}C y sin marcar; así como determinar los microorganismos que están presentes y metabólicamente activos.

Esta tesis contiene cinco artículos científicos producto de la investigación doctoral, de los cuales dos han sido aceptados, uno ha sido enviado para su revisión y dos están en preparación.

Estado del Arte

1. ¿Qué es el suelo?

El suelo es un cuerpo natural tridimensional que cubre la corteza terrestre, está compuesto por materiales orgánicos y minerales, y tiene la capacidad de soportar una gran diversidad de plantas, animales y microorganismos. Generalmente está formado por varias capas denominadas horizontes.

De acuerdo con Brady y Weil (2004), el suelo tiene cinco funciones básicas. Primera, sirve como sustrato, aporte de nutrimentos y soporte para las plantas. Segunda, regula los volúmenes de agua distribuidos en el medio. Tercera, recicla materiales de desecho. Cuarta, es hábitat de gran cantidad de insectos, arácnidos y microorganismos. Quinta, soporta al hombre y sus grandes obras de ingeniería.

Este sistema complejo que constituye el suelo, característicamente heterogéneo espacial y temporalmente, alberga una gran riqueza de especies vegetales, animales y microbianas. El suelo es un ambiente muy apropiado para el desarrollo de los microorganismos, tanto eucariotas (algas, hongos, protozoos) como procariotas (bacterias y arqueas). También encontramos virus y bacteriófagos. Todos estos organismos establecen relaciones entre ellos en formas muy variadas y complejas, y también contribuyen a las características propias del suelo por su papel en la modificación de las fases sólidas, líquidas y gaseosas. Los microorganismos desempeñan funciones de gran importancia en relación con los procesos de edafogénesis; ciclos biogeoquímicos de elementos como el carbono, el nitrógeno, oxígeno, el azufre, el fósforo, el hierro y otros metales; fertilidad de las plantas y protección frente a patógenos; degradación de compuestos xenobióticos, etc. (Alexander, 1994).

2. Descomposición de la materia orgánica de origen vegetal en suelos

La descomposición de la MOS es considerada una fuente sustancial para la disponibilidad de nutrimentos en el suelo. La incorporación de estiércol animal y otros materiales orgánicos en el suelo es reconocida por tener efectos benéficos en los procesos físicos y químicos del suelo, así como en las propiedades biológicas. Los residuos de plantas son la fuente primaria de materia orgánica para los microorganismos en el suelo. De acuerdo al NRCS (2000), la MOS proviene de diferentes fuentes, humus, residuos de plantas frescas y exudados de plantas. Estas fuentes están vinculadas por procesos químicos y bioquímicos y difieren en la velocidad de descomposición con una disminución en las velocidades en el siguiente orden: exudados de plantas > residuos frescos > humus. Estos componentes son la fuente energética de las comunidades de organismos heterotróficos. Así, debido a la relevancia que tiene la MOS en el mejoramiento de la calidad del suelo, es importante conocer bien cómo ésta se descompone y cómo los nutrimentos de residuos de plantas son liberados; asimismo, qué factores afectan la descomposición. Los procesos de descomposición de los residuos derivados de plantas son afectados por factores identificados y no identificados, y por la disponibilidad de los nutrimentos; los microorganismos del suelo, las propiedades físicas, la calidad de los residuos de cosechas, la exudación de las raíces y el "priming effect" de la rizósfera, son algunos de los factores determinados (Bertrand *et al.,* 2006; Paterson *et al.,* 2006; Ruiz-Valdiviezo *et al.,* 2010). Los factores ambientales como textura, humedad y temperatura del suelo son muy importantes porque pueden modificar la velocidad de descomposición debido a sus efectos en la actividad microbiana. La calidad de los residuos de plantas incorporadas al suelo determina tanto la velocidad de descomposición, como las dinámicas de los nutrimentos (Gnankambary *et al.,* 2008). También, la calidad del residuo, es decir, la composición química, la relación carbono/nitrógeno (C/N) y la relación carbono/fósforo (C/P), así como la concentración de los compuestos simples y complejos, han mostrado ser importantes debido a que modifican la velocidad de descomposición. La composición típica de los residuos de plantas verdes es: celulosa (45%), hemicelulosa (20%), lignina (20%), proteínas (8%), azúcares y almidones (5 %), y grasas y ceras (2 %) (Vanlauwe *et al.,* 1997).

Cuando los residuos derivados de las plantas son incorporados al suelo, sus componentes orgánicos están sujetos a la descomposición microbiana, por ello, ésta se considera un proceso biológicamente estimulado (Lee *et al.,* 2011). La velocidad de descomposición es determinada por tres factores principalmente: microorganismos, ambiente físico (temperatura, humedad, textura y niveles de oxígeno) y la calidad de los residuo de plantas (relación C/N y otras propiedades químicas) (Coûteaux *et al.,* 1995; Vanlauwe *et al.,* 1997; Poll *et al.,* 2008). Así, los agentes físicos, químicos y biológicos transforman los compuestos orgánicos complejos en compuestos orgánicos e inorgánicos simples y de fácil asimilación por las plantas, mejorando las características físicas, químicas y biológicas del suelo tal como se muestra en la Figura 1. Estas actividades influyen positivamente en la productividad del suelo y en la producción de las cosechas mediante el mejoramiento de la estructura del suelo y del ciclo de los nutrimentos. La composición y la estructura del residuo de las plantas cambia durante la descomposición (Dresboll *et al.,* 2006). Los productos finales de la descomposición del residuo de planta son dióxido de carbono, agua, energía, biomasa microbiana, nutrimentos inorgánicos y resíntesis de compuestos orgánicos como humus, compuestos fenólicos, celulosa, hemicelulosa y lignina (Baldock, 2007). El humus tiene un efecto muy importante en las propiedades del suelo, como oscurecimiento del suelo, agregación del suelo y estabilidad del agregado y sirve como una fuente de almacenamiento de N, P y otros nutrimentos (Stumpe *et al.,* 1997).

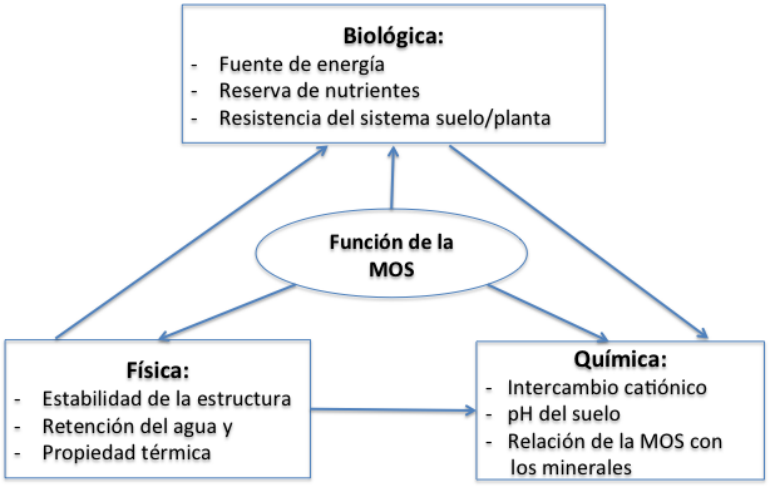


Figura 1. Función de la MOS en el mejoramiento de la calidad del suelo.

La clasificación de los componentes orgánicos en los residuos está basada en su tamaño molecular, solubilidad y constituyentes primarios, como se muestra en el Cuadro 1. Cuando los residuos de las plantas se incorporan al suelo, algunos componentes se consumen rápidamente, mientras otros se consumen lentamente. Compuestos simples como la sacarosa, los aminoácidos y los compuestos fenólicos de bajo peso molecular son de rápida y fácil descomposición, mientras que los compuestos poliméricos como celulosa, hemicelulosa y lignina son de descomposición lenta (Berg y McClaugherty, 2003). Frecuentemente, la velocidad de descomposición de los residuos de las plantas es alta, inicialmente debido a que los compuestos simples o solubles son utilizados por un gran número de microorganismos. Una descomposición rápida de los residuos vegetales lleva a la pérdida de masa del residuo. Esto es debido a la lixiviación de los componentes orgánicos simples y la liberación de CO_2. La lixiviación es la pérdida de los nutrimentos y la incompleta descomposición de los componentes, causada por movimientos de agua durante el proceso de descomposición.

La liberación de CO_2 es el resultado de la actividad microbiana en condiciones aerobias, y por el contrario, en condiciones anaerobias pueden ser producidos ácidos orgánicos o metano pueden ser producidos en lugar de CO_2 (Laanbroek, 2010).

Cuadro 1. Composición representativa de los residuos de maíz, frijol y trigo (g kg^{-1} peso seco).

Componentes	Maíz	Soya	Trigo
Componentes solubles	293	557	288
Hemicelulosa	268	90	184
Celulosa	284	222	361
Lignina	56	119	141
Ceniza	93	64	84
Nitrógeno	10	22	9
Carbono	449	440	460

Fuente: Jensen *et al.,* 2005; Avilés-Marín *et al.,* 2011

La descomposición de los residuos de plantas ha sido estudiada por medición de diferentes parámetros, tales como evolución de la producción de dióxido de carbono (CO_2) o respiración, la liberación de nutrimentos y el contenido de carbono del residuo. Cuando los residuos de plantas son descompuestos, las poblaciones microbianas crecen rápidamente, que se observa como un incremento en la liberación de CO_2 en el suelo. Así también, mediante la evolución de CO_2, se determina la velocidad de mineralización del carbono en los residuos. La liberación de nutrimentos durante la descomposición es interesante, particularmente en términos de disponibilidad de los nutrimentos para los microorganismos del suelo, así como para las plantas (Corbels *et al.,* 2003; Baggie *et al.,* 2005). De igual forma, la respiración del suelo es también un buen indicador para evaluar las actividades respiratorias de las raíces de las plantas. Estos procesos son acelerados o retardados por factores ambientales debido a sus efectos en las actividades metabólicas de las plantas y los microorganismos (Hu *et al.,* 2006). Algunos estudios (Hobbie, 1996; Osono *et al.,* 2003; Marschner *et al.,* 2005) han mostrado que la temperatura, la disponibilidad de agua y el pH son factores clave que afectan la velocidad de respiración del suelo. De acuerdo a Nikliska y Klimer (2007), la temperatura tiene un efecto substancial en la respiración y en la pérdida de los residuos de plantas, ya sea directa o indirectamente a través de la perdida de agua. Los componentes liberados durante la descomposición son también afectados por las propiedades de los residuos de plantas. La velocidad de respiración de los residuos de plantas con una relación C/N alta es menor que para los residuos de plantas con una relación C/N baja debido a que los residuos con una relación de C/N alta

no contienen suficiente N para satisfacer los requerimientos de los microorganismos del suelo. Además, estos residuos contienen grandes cantidades de compuestos de carbono de difícil degradación tales como celulosa o lignina. La velocidad de respiración de una mezcla de residuos de plantas (mezcla de residuos con relación C/N alta o baja) es frecuentemente más alta que la velocidad de respiración encontrada en los residuos con relación C/N alta, mientras la liberación de N es mayor. Aún así, no se conoce bien acerca de la velocidad de respiración y algunos mecanismos de liberación de nutrimentos en el proceso de descomposición de los residuos de plantas en suelos no son muy claros.

2.1 Factores que afectan la descomposición de los residuos de plantas

2.1.1 Propiedades de los residuos de las plantas

Las principales propiedades químicas y físicas de los residuos que determinan la velocidad de descomposición y la liberación de nutrientes se discuten en la secciones siguientes.

2.1.1.1 Propiedades químicas

Muchas de las propiedades químicas de los residuos son buenos indicadores para la evaluación de la velocidad de la degradación de los residuos derivados de las plantas. Los residuos típicamente consisten de tres fracciones, las cuales son diferentes con respecto a su velocidad de descomposición: 1) azúcares y aminoácidos de fácil descomposición; 2) compuestos de lenta descomposición, como celulosa y hemicelulosa; y 3) material recalcitrante, como la lignina (Van Veen *et al.,* 1997; Talbot *et al.,* 2012). Varios estudios han revelado que la concentración inicial de N (Baitilwake *et al.,* 2012), P (Soon y Arshad, 2002), lignina (Talbot *et al.,* 2012), polifenoles (Zibilske y Bradford, 2007) y carbono soluble (Justes *et al.,* 2009) son buenos indicadores de la calidad del residuo y la velocidad de su descomposición.

El contenido inicial de N de los residuos de las plantas es uno de los factores claves en la aceleración o inhibición de la descomposición del residuo, de tal forma que éste determina la dinámica de la biomasa microbiana en la mineralización de los residuos (Heal *et al.,* 1997). Consecuentemente, el contenido de N en los residuos es positivamente correlacionado con el porcentaje de mineralización.

Por otro lado, la relación C/N en el suelo es de aproximadamente 12 y la de los microorganismos es de aproximadamente 8, aunque estos valores dependen del tipo de suelo y especie vegetal. Mientras la relación óptima C/N para el crecimiento microbiano es alrededor de 25, la relación C/N de los residuos derivados de las plantas está en el rango de 20 a 500 y depende de la madurez de la planta y la especie (Cadisch y Giller, 1997). De acuerdo a Baldock (2007), los residuos de las plantas con una relación C/N alta (> 40) son mineralizados más lento que los residuos con una relación C/N menor de 40. De aquí que, los materiales derivados de plantas con una relación C/N baja satisfacerán los requerimientos de N de la población microbiana del suelo, en consecuencia el N extra será mineralizado y disponible para la absorción de la planta. Normalmente, los residuos de plantas de la familia *Poaceae* como trigo, avena y cebada tienen una relación C/N alta y la familia de leguminosas o *Fabacea* como, altramuz, algarroba, soya y frijol chino tiene una relación C/N baja. El estudio conducido por Soon y Arshad (2002) ha revelado que la velocidad de descomposición de pajas de tres cultivos fue en el orden de: guisante > canola > trigo con contenido de N en los residuos de 7.09, 7.04 y 5.06 mg g^{-1} y relación C/N de 66, 71 y 97, respectivamente. Por otro lado, Abera *et al.* (2012) reportaron que la relación C/N en los residuos de leguminosas (*Phaseolus vulgaris* L.; *Cajanus cajan* L.) adicionados a suelos tropicales es un indicador importante, concluyendo que el ajuste de la relación C/N (mezclado residuos con diferente calidades) es indispensable para mantener un equilibrio entre el N liberado, el N requerido por las plantas y el N utilizado por las comunidades microbianas durante las etapas tempranas de las cosechas.

Por otro parte, la relación lignina/carbohidratos afecta la velocidad de degradación de los residuos de planta (Heal *et al.,* 1997; Duncan y Schilling, 2010). La lignina juega un papel importante

en la estructura de la pared celular de las plantas y en la resistencia a la descomposición microbiana. Herman *et al.* (1997) reportaron que la descomposición de la materia orgánica y la mineralización del N disminuirá cuando la concentración de lignina y la relación C/N se incrementan. Así, en una etapa posterior de la descomposición del residuo cuando los compuestos de fácil descomposición son agotados, los microorganismos degradadores de lignina dominarán y regularán el curso de la degradación (Berg y McClaugherty, 2003). De aquí que, actualmente, varios autores han estudiado la descomposición de lignina en diferentes tipos de suelos, así como los posibles microorganismos responsables de su descomposición (Kabuyah *et al.,* 2012).

Las partes de las plantas con diferente composición bioquímica muestran diferentes dinámicas de mineralización de C. Generalmente, el contenido de carbono orgánico de la mayoría de las plantas es cerca de 40%, mientras que la mayor parte del carbono retornará a la atmósfera como CO_2 y cerca 20-32 % restante se incorpora como materia orgánica del suelo (Davidson y Janssens, 2006). De acuerdo a Hadas *et al.* (2004), la velocidad de descomposición de residuos de maíz, trigo y arroz, y la cantidad de N inmovilizado en la biomasa microbiana en las primeras etapas de la descomposición se incrementa dependiendo de las concentraciones del carbono soluble y disponible, encontrando que estos se descomponen en los primeros días en un experimento de incubación.

Así también, la influencia de la concentración inicial de los polifenoles y la relación polifenoles/N de los residuos de las plantas en la pérdida de masa y la liberación de N han sido estudiadas (Reddy *et al.,* 2008; Matos *et al.,* 2011). De acuerdo a Silva *et al.* (2008), la relación polifenol/N y el contenido de polifenoles en diferentes especies de leguminosas fueron correlacionados negativamente con la liberación de N. Asimismo, Seneviratne *et al.* (2009) encontraron que el carbono orgánico del suelo disminuyó con el incremento de la relación polifenoles/N en diferentes fracciones del suelo, lo que sugiere la presencia de una respuesta negativa de los polifenoles en el crecimiento microbiano; concluyendo que esta propiedad química es un buen indicador de la mineralización de N.

2.1.1.2 Propiedades físicas

Las propiedades físicas de los residuos de las plantas y su contacto con el suelo tienen un efecto pronunciado sobre los procesos de inmovilización y mineralización del N (Bending y Turner, 2004). La reducción del tamaño de partícula incrementa el área de superficie de contacto disponible para la colonización por los microorganismos, permitiendo una mayor distribución de los residuos en el suelo. Por lo tanto, los residuos de tamaño pequeño se descomponen más rápido que los que tienen un tamaño grande. Bhupinderpal-Singh *et al.* (2006) mostraron en un experimento donde se incorporó residuos de canola *(Brassica napus)* de diferentes tamaños, que el tamaño de partícula tuvo un efecto significativo sobre la inmovilización del N mineral (NH_4^+, NO_3^-), pero no tuvo efecto en la velocidad de mineralización del carbono. A su vez también encontraron que el tamaño de partícula de la canola no tuvo efecto sobre el contenido de C y N microbiano durante la incubación.

2.1.2 Efectos de los factores ambientales en la descomposición

2.1.2.1 Propiedades del suelo (arcilla, aireación, pH)

La arcilla es uno de los componentes de la textura del suelo que permite determinar la aireación y drenaje en el suelo, y significativamente afecta la velocidad de descomposición de los residuos. La arcilla es definida como partículas del suelo con un tamaño menor de 0.002 mm. La concentración de arcilla ha sido correlacionada positivamente con el tamaño de agregación y formación de agregados mientras ésta es correlacionada negativamente con el potencial de mineralización de N (Sylvia *et al.,* 2005). Las arcillas juegan un papel importante en el C orgánico del suelo, agua y retención de nutrimentos por cubrimiento de los residuos de las plantas y las reacciones químicas entre minerales (negativamente cargados) y cationes tales como el amonio (NH_4^+), de este modo se reduce la pérdida de masa del residuo. Epstein *et al.* (2002) concluyeron que la velocidad de descomposición de MOS se incrementó cuando el contenido de arcilla en el suelo disminuyó debido a una baja de los niveles de oxigeno en el suelo y la acumulación de MOS fue positivamente

relacionada con la concentración de arcilla en el suelo. Otro estudio en laboratorio encontró que después de la incubación, el incremento en el contenido de arcilla y la temperatura tuvo un efecto positivo sobre la mineralización de N de residuos de cosechas en el suelo (Roy *et al.,* 2011).

Por otro lado, una aireación adecuada del suelo acelera la descomposición de los residuos de las plantas y el crecimiento de los microorganismos. Suficiente oxígeno estimula a los organismos del suelo a convertir los compuestos orgánicos a inorgánicos. Bacterias y hongos son los dos principales degradadores de residuos de plantas. Las bacterias consisten de organismos aerobios o anaerobios; ambos grupos son capaces de metabolizar moléculas poliméricas como lignina, celulosa y hemicelulosa; sin embargo, las poblaciones microbianas crecen rápidamente y la descomposición es mayor en condiciones aerobias, debido a que la energía producida del metabolismos aerobio es más alto que en el metabolismo anaerobio (Madigan *et al.,* 2003). Johnson *et al.* (2006) observaron que durante las primeras etapas de la descomposición de residuos en suelos inundados, el crecimiento de plantas de arroz (*Oryza sativa L.*) puede ser inhibido por la inmovilización de nutrimentos o por la producción de ácidos orgánicos potencialmente tóxicos, y concluyeron que una aireación suficiente del suelo puede mitigar los efectos negativos de la producción de ácidos orgánicos y metano.

Debido a su efecto sobre la actividad microbiana, el pH del suelo influye en los procesos de descomposición de residuos de las plantas. También puede afectar la solubilidad de los nutrimentos y cambiar la composición de la comunidad microbiana. El ciclaje de los nutrimentos disminuye o se detiene si las poblaciones microbianas se ven afectadas negativamente (Nierop *et al.,* 2005). La actividad microbiana es mayor en un suelo con pH neutro. Los resultados de un experimento en invernadero con diez tipos de suelos realizado por Marschner *et al.* (2005) mostraron que la composición de la comunidad microbiana fue afectada más fuertemente por el pH que por las otras propiedades del suelo. Por lo tanto, la capacidad de supervivencia y competitividad de especies microbianas son alteradas por el pH del suelo. En términos de la función de los residuos vegetales en los cambios del pH del suelo, el grado de descomposición de los residuos de las plantas, el patrón de liberación de aniones y cationes e inmovilización por microorganismos afectarán el pH del suelo.

Además, los residuos de las plantas con altas concentraciones de nitrógeno orgánico, como las proteínas y los aminoácidos, producirán una mineralización alta de amonio y/o nitrato en el suelo. La amonificación produce iones hidroxilos (OH^-) e incrementa el pH del suelo, mientras la nitrificación produce protones H^+ acidificando el suelo como se ilustra en las reacciones químicas (1) y (2). Rukshana *et al.* (2011) demostraron que el cambio de pH del suelo después de la adición de residuos de plantas fue dependiente del tipo de residuo de planta y el pH inicial del suelo.

Amonificación:

$$R\text{-}CO\text{-}NH_2 + 3H_2O \rightarrow 2NH_4^+ + \mathbf{2OH^-} + CO_2 \quad (1)$$

(R = cadena de carbonos con longitud indefinida)

Nitrificación:

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + \mathbf{2H^+} \quad (2)$$

A pesar de que la actividad celulítica es inhibida en el suelo con un pH alto (Horikoshi, 1999); en el grupo de trabajo del Dr. Luc Dendooven, Luna-Guido *et al.* (2003) y Conde *et al.* (2005), quienes adicionaron rastrojo de maíz uniformemente marcado con ^{14}C y su fracción detergente neutra (en su mayoría (hemi)celulosa a suelos con diferentes valores de pH (7.7, 8.2, 9.5, 9.8 y 10) y contenido de sales (1.2, 3.2, 24.6, 32.7 y 56 dS m^{-1}), encontraron la presencia de una posible actividad celulolítica en este ambiente extremo. Sin embargo, los mecanismos por los cuales los materiales derivados de plantas afectan el pH del suelo al que son adicionados no son completamente conocidos.

2.1.2.2 Temperatura y humedad

La temperatura y la humedad son factores físicos importantes que afectan la velocidad de descomposición de los residuos, ya que influyen directamente sobre la actividad microbiana en el suelo. Es conocido que la actividad microbiana aumenta con el incremento de la temperatura con un

óptimo de 30 a 45 ºC, pero la relación entre la actividad microbiana y la temperatura depende de las especies microbianas. La respiración microbiana se correlaciona positivamente con la temperatura y depende de la capacidad de adaptación de los organismos a su hábitat del suelo (Cookson *et al.,* 2002; Berg y McClaugherty, 2003). Coûteaux *et al.* (2002) llegaron a la conclusión de que, en condiciones aeróbicas, la velocidad de descomposición de los residuos de *Glomeris marginata* incrementó cuando la temperatura aumentó de 15 a 23 ºC y además de que se encontró un aumento de liberación de N y reducción de la velocidad de acumulación de MOS.

Además de la temperatura del suelo, la humedad del suelo también tiene un fuerte efecto sobre la descomposición de los residuos. La humedad adecuada acelera la tasa de descomposición y el crecimiento de microorganismos debido a que el agua es requerida para la descomposición de residuos vegetales. Sin embargo, los niveles altos de humedad producirán condiciones anaeróbicas y pueden obstaculizar el proceso de descomposición.

2.1.2.3 Propiedades de la rizósfera: Exudación de las raíces

La exudación de las raíces es muy importante, ya que además de producir compuestos de fácil descomposición, estimulan la descomposición de los residuos de las plantas. La exudación de las raíces es parte del rizodepósito, el cual es la principal fuente de C orgánico en la rizósfera debido al crecimiento de las raíces de las plantas. Paterson *et al.* (2006) afirman que los compuestos orgánicos secretados por las raíces afectan la velocidad de la descomposición de los residuos y la liberación de nutrimentos debido a la influencia en la actividad y la abundancia de las poblaciones degradadoras en el suelo. Los exudados también contribuyen a una mayor estabilidad de los agregados y los exudados solubles de las raíces pueden actuar como un eslabón entre las partículas de arcilla (Hütsch *et al.,* 2002). La liberación de sustancias orgánicas de las raíces de las plantas es crucial en las interacciones planta-microorganismo, las cuales tienen fuertes efectos sobre la actividad y estructura de la comunidad microbiana, y por lo tanto en la liberación de nutrimentos (Lynch y Whipps, 1990; Marschner *et al.,* 2001).

2.1.3 Efecto de imprimación (EI) o *'priming effect'*

El efecto de imprimación fue descubierto por Löhnis (1926) quien estudió la descomposición de residuos de plantas leguminosas en el suelo, encontrando un aumento significativo de la mineralización del N por adición del residuo orgánico y fresco al suelo. Y no fue sino hasta el año 1953 cuando el término *'priming effect'* fue introducido por Bingemann *et al.* (1953). Actualmente, el efecto de imprimación se define como los cambios bruscos y rápidos en la sustitución de la materia orgánica del suelo, causados por un tratamiento comparativo moderado del mismo, como la adición de un sustrato, su manipulación del suelo o incluso el aumento o disminución de la humedad.

La adición de distintas sustancias al suelo, no sólo puede causar una aceleración en la mineralización, efecto de imprimación positivo (Fig. 2), sino también una reducción o inmovilización del carbono o nitrógeno adicionado, efecto de imprimación negativo (Kuzyakov *et al.,* 2000). Existe otro tipo de E.I., denominado efecto de imprimación aparente, el cual es un fenómeno complejo, que está relacionado con el recambio de los nutrimentos derivados del suelo. Este recambio parece ser similar al observado en el EI verdadero (positivo y negativo). Sin embargo, el proceso para un EI aparente no es un recambio en la materia orgánica del suelo. El mecanismo de este puede ser una incompleta descomposición del carbono y nitrógeno durante el experimento (Kuzyakov *et al.,* 2000).

Los métodos para cuantificar el *'priming effect'* están basados en la determinación del $^{13}CO_2$ o $^{14}CO_2$ y $^{12}CO_2$, así como $^{15}NO_3^-$ incorporando compuestos marcados isotópicamente como isótopos radioactivos (^{14}C y ^{15}N) e isótopos estables (^{13}C) (Kuzyakov *et al.,* 2000; Haichar *et al.,* 2007).

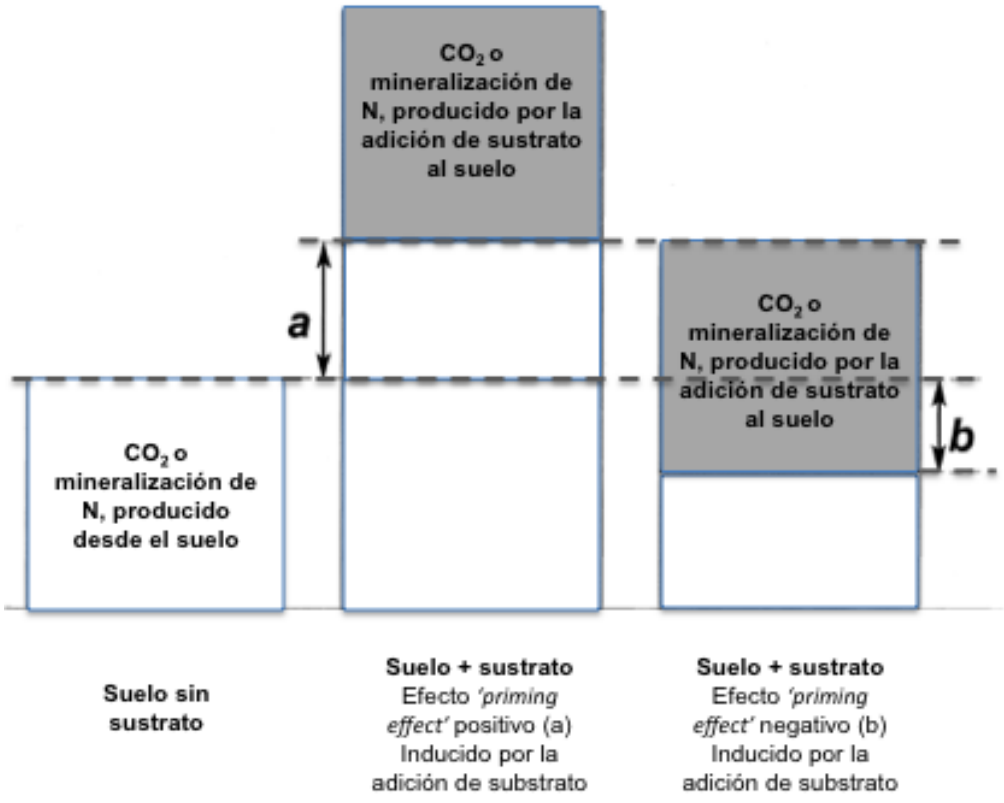


Figura 2. Esquematización del *'priming effect'* (Modificado de: Kuzyakov *et al.,* 2000)

3. Métodos moleculares aplicados en estudios de ecología microbiana del suelo

El análisis de las poblaciones microbianas en hábitats naturales como el suelo, es uno de los pilares de la investigación actual sobre el funcionamiento de los ecosistemas naturales. En la microbiología tradicional del suelo, los datos de los microorganismos se han obtenido por análisis de la biomasa microbiana, es decir, células en cultivos líquidos o colonias obtenidas en placas; asimismo estos cultivos han sido estudiados empleando métodos de bioquímica celular, biología molecular (basados en DNA o RNA) y fisiología. Sin embargo, tales métodos han tenido fuertes limitaciones debido a que sólo se puede tener acceso a una pequeña fracción de la microbiota del suelo por medio del cultivo directo en placas (van Elsas y Boersma, 2011), ya que sólo una cantidad de bacterias en el suelo, usualmente estimada alrededor del 1 % del número total de células observadas por cuenta

directa, pueden ser cultivadas en medios de cultivo en el laboratorio (Skinner *et al.,* 1952; Hirsch *et al.,* 2010).

Actualmente, la disponibilidad de métodos moleculares basados en la extracción de ácidos nucleicos directamente del suelo, han revolucionado los estudios de la microbiota del suelo, permitiendo un mayor conocimiento de la diversidad, el funcionamiento y las interacciones microbianas en el suelo (Su *et al.,* 2012). Estos métodos se basan en el análisis de marcadores moleculares como los ácidos grasos de fosfolípidos (PLFA; por sus siglas en inglés) y los ácidos nucleicos (DNA, RNA). Ambos tipos de marcadores se encuentran presentes en todas las células, que se pueden extraer directamente de muestras de suelo sin necesidad de realizar cultivos previos y además permiten diferenciar distintos grupos de microorganismos. El DNA metagenómico o DNA ambiental, es el nombre que se le otorga al DNA total que es extraído de muestras ambientales tales como suelos, sedimentos y aguas. La amplificación de la región que codifica el rRNA (rDNA 16S) y el análisis bioinformático de su secuencia nos indica la presencia, abundancia y diversidad de las comunidades microbianas sometidas a diferentes perturbaciones o estrés en el suelo (Hirsch *et al.,* 2010). Así, también el empleo de ácidos nucleicos se ha implementado rápidamente debido a la gran cantidad de información que proporciona y al fácil análisis de estos biomarcadores, especialmente tras el gran desarrollo y aumento de nuevos métodos moleculares durante los últimos años, principalmente en la combinación de diferentes estudios metagenómicos y transcriptómicos (Daniel, 2005; van Elsas y Boersma, 2011). En el Cuadro 2 se muestra una comparación de los diferentes métodos moleculares empleados en la actualidad, en estudios de ecología molecular de suelos.

4. Metodología de isótopos estables ^{13}C- DNA SIP

La prueba de isótopos estables (DNA SIP) es una herramienta poderosa para la identificación de microorganismos activos que asimilan determinados sustratos de C y nutrimentos, incorporándolos en la biomasa celular (Dunford y Neufeld, 2010). Brevemente, esta técnica consiste en incubar la muestra de suelo en presencia de un sustrato marcado con un isótopo estable (no radiactivo), seguido

de la identificación de los microorganismos de la comunidad que han incorporado dicho sustrato a sus ácidos nucleicos, mediante el análisis de rDNA 16S metagenómico u otro gen específico de interés (Nogales, 2005). Por lo tanto, a diferencia de los estudios metagenómicos convencionales, la metagenómica funcional basada en la técnica DNA SIP no sólo nos permite analizar las comunidades microbianas, sino que también nos permite determinar una relación directa entre la identidad y función de las comunidades microbianas.

Consecuentemente, está técnica independientemente del cultivo, ha sido una importante metodología para la asignación de la función metabólica de las diversas comunidades que habitan en una amplia gama de ambientes terrestres y acuáticos (Radajewski *et al.,* 2000). Así, la técnica SIP, ha sido utilizada junto con las técnicas convencionales de identificación para saber cuáles son las comunidades activas o que están realizando su función del ecosistema: "¿Qué están haciendo?" (Fig. 3) (Chen y Murrell, 2010).

En estudios previos SIP se han identificado los microorganismos involucrados en la degradación de metanol, metano, benzoato, naftaleno, silicato, fenantreno, benceno, fenol y herbicidas marcados con ^{13}C (Radajewski *et al.,* 2000; Friedrich, 2006; Neufeld *et al.,* 2007; Whiteley *et al.,* 2007). Aunque, los sustratos marcados con ^{13}C, han sido los más utilizados para SIP, la técnica también se ha establecido para sustratos marcados con ^{15}N (Cadisch *et al.,* 2005; Buckley *et al.,* 2007; Cupples *et al.,* 2007; Addison *et al.,* 2010). Los lípidos, los ácidos nucleicos y las proteínas han sido empleados como biomarcadores (Evershed *et al.,* 2006; Neufeld *et al.,* 2007; Jehmlich *et al.,* 2008).

Cuadro 2. Comparación de métodos moleculares en estudios de ecología microbiana en suelos (Fuente: van Elsas y Boersma, 2011)

Método	Reproducibilidad	Interpretación de resultados	Ventajas	Desventajas	Principales desafíos	Comentarios
Cultivo en placa	Media	Información limitada sobre poblaciones activas *in situ* debido a un recuento anormal	Permite analizar más a fondo las colonias, incluidas las características metabólicas o la secuencia completa del genoma	Baja resolución, carece de representación. Morfotipos difíciles de distinguir	Sólo para los microorganismos cultivables (sólo el 1% de la comunidad)	El cultivo basado en el análisis fundamental como apoyo para las observaciones basadas molecularmente
Extracción de ácidos nucleicos del suelo	Alta	Obtención instantánea de la microbiota existente en forma de moléculas portadoras de información	Fácil acceso a los genes de la comunidad microbiana del suelo	Tiende a un muestreo incompleto y sesgado	La integridad química y la pureza del DNA del suelo puede limitar los análisis	Los ácidos nucleicos son la base de todo el trabajo molecular: los sesgos deben reducirse
PCR/qPCR	Alta	Representación de los organismos o genes amplificados y/o cuantificados	Técnicas de rutina de alta sensibilidad, permiten la detección y/o cuantificación	Varios sesgos de PCR y artefactos, incluyendo la inhibición	Sólo las especies con abundancia >0,1-1% son visibles	Método clave para la detección molecular del suelo
Huellas genéticas (DGGE, TGGE,T-RFLP, SSCP, RISA, LH-PCR) -filogenéticas -funcionales	Alta	Representación instantánea de la diversidad microbiana (dominante) y de la comunidad constituida, bajo diferentes niveles de sensibilidad	Fáciles comparaciones entre las muestras, la posibilidad de obtener diferentes marcadores de una misma muestra	Solo por arriba de 1000 se acede a la comunidad deseada	Muchas dificultades debido a la naturaleza de las técnicas de separación	DGGE se ha convertido en un método de rutina. Hay que tener cuidado con las interpretaciones, debido a los sesgos
Librerías de clonas	Media	Contabilización de tipos de secuencias dominantes en la comunidad	Fácil censo de los genes blancos en la comunidad, permiten hacer estimaciones sobre la diversidad	Preparación laboriosa de la muestra	Errores debido al sesgo de la clonación y la PCR	Bueno, pero visión limitada de diversidad de genes/organismos
Prueba de isótopos estables	Alta	Información directa sobre la incorporación del compuesto marcado en los miembros de una comunidad: se destacan las bacterias activas	Proporciona información sobre la comunidad activa. La relación entre la estructura y la función puede ser elucidada	Problemas de oportunistas enmascarando los datos	Se basa en la actividad de los microorganismos, que puede ser muy baja	Método apreciado para describir las actividades *in situ*
Microarreglos	Media	Información paralela sobre diversidad, a niveles filogenéticos o funcionales	Rendimiento muy alto, acerca de la información directa de las secuencias	Sólo los genes se encuentran con chip	Problemas debido a hibridaciones cruzadas con secuencias de baja similitud	Permite un análisis de alto rendimiento a través de hábitats
Secuenciación de alto rendimiento: -metagenoma -metatranscriptoma	Media	Grandes cantidades de información sobre los miembros totales y activos de la comunidad a nivel de secuencia	Análisis de alto rendimiento, todo en uno. Alto potencial para los estudios comparativos	Los métodos son propensos a errores	Interpretaciones erróneas debido a artefactos o errores	Método de elección en muchos estudios. Una vez más, se advierte en la interpretación de los datos

Con respecto a los ácidos nucleicos, DNA SIP y RNA SIP son métodos establecidos (Radajewski *et al.,* 2000; Manefield *et al.,* 2002; Neufeld *et al.,* 2007; Whiteley *et al.,* 2007), pero mRNA SIP sólo se ha reportado una vez (Dumont *et al.,* 2011).

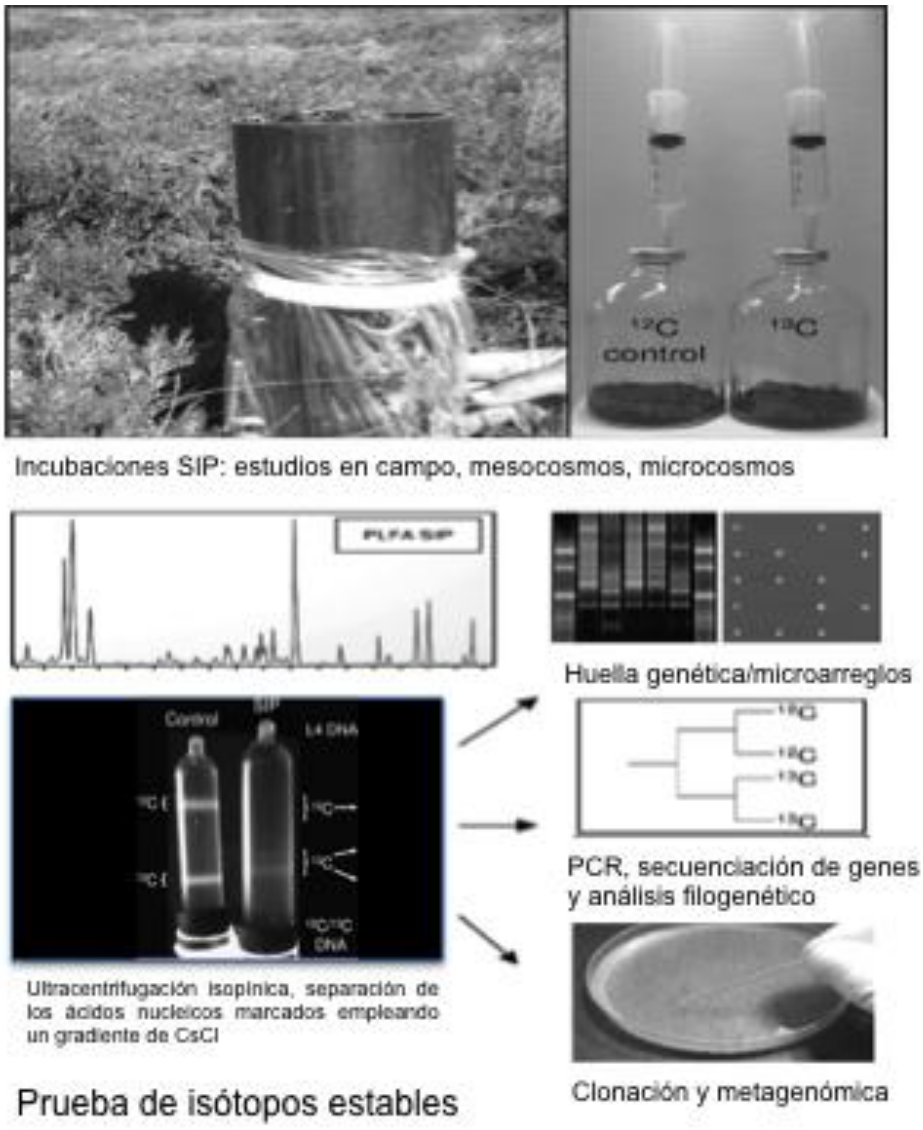


Figura 3. Etapas de la metagenómica funcional basada en la metodología ^{13}C-DNA SIP (Modificada de: Neufeld *et al.,* 2007).

5. Marcaje isotópico de plantas mediante la técnica *'$^{13}CO_2$ pulse-labelling'*

Las técnicas isotópicas empleadas en el pasado usaron compuestos marcados con ^{14}C, debido a que este tipo de compuestos eran fácilmente disponibles y proporcionaban un nivel alto de sensibilidad (Merckx *et al.,* 1986). Sin embargo, este tipo de compuestos son radiactivos y por lo tanto producen serios riesgos ambientales y operacionales, además que se requieren costos elevados para su eliminación y manipulación, por lo tanto, han sido limitados a estudios en laboratorio. Recientemente avances en el uso de isótopos estables han eliminado estos inconvenientes, y las plantas han sido marcadas con $^{13}CO_2$ por varias razones, incluyendo estudios en fisiología de plantas, fijación de CO_2 y seguimiento de ^{13}C en las comunidades microbianas de la rizósfera (Bromand *et al.,* 2001; Butler *et al.,* 2003; Hafner *et al.,* 2012; Hannula *et al.,* 2012). Por otra parte, el ^{13}C es considerado mejor al ^{14}C en estudios de marcaje o enriquecimiento, debido a que la diferencia fotosintética y metabólica es menor con ^{13}C que con ^{14}C (Svejear *et al.,* 1990).

Para un exitoso seguimiento del ^{13}C durante la descomposición, el material de la planta en estudio deberá ser similar entre las partes áreas y la raíz de la planta, y deberá tener una huella $\delta^{13}C$ que después de la incubación sea significativamente diferente de la huella inicial del suelo y otros residuos de la planta (Moore-Kucera y Dick, 2008). Uno de los medios para llegar a este propósito es por medio de las exposiciones continuas a $^{13}CO_2$, pero requieren de cámaras de crecimiento sofisticadas y costosas con controles para todos los factores de crecimiento (v.g. CO_2, O_2, temperatura, luz, humedad) (Johnson *et al.,* 2002). Un método más económico para enriquecer plantas es mediante la técnica *'$^{13}CO_2$ pulse-labelling'*, en donde las plantas son expuestas a $^{13}CO_2$ por períodos cortos, y el resto del tiempo las plantas se mantienen en una atmósfera con concentraciones de CO_2 similares a las del ambiente y bajo condiciones fácilmente manejables (Moore-Kucera y Dick, 2008).

En el grupo de trabajo del Dr. Luc Dendooven diseñamos una cámara de enriquecimiento para plantas (Figura 4) (Ruíz-Valdiviezo *et al.,* 2012 *en proceso*), en la que se aplicó la técnica *'$^{13}CO_2$ pulse-labelling'*. En dicho experimento se lograron enriquecer las partes áreas (35 atom% ^{13}C), asimismo como las raíces (14 atom% ^{13}C) de plantas de maíz; así mismo este material marcado sirvió como modelo para aplicar de manera exitosa la metodología SIP para estudiar los microorganismos que participan en la biodegradación de residuos de plantas en el suelo.

Figura 4. Cámara de enriquecimiento con ^{13}C de plantas mediante la técnica *'$^{13}CO_2$ pulse-labelling'* (Fuente: Ruíz-Valdiviezo *et al.,* 2012 *en proceso*).

6. Aplicación de la prueba ^{13}C-DNA SIP en estudios de biodegradación de residuos de plantas marcadas con ^{13}C en suelos

Con anterioridad se mencionó que la prueba de isótopos estables ofrece potencial para estudiar los microorganismos que puedan estar involucrados en procesos o ciclos biogeoquímicos en el suelo. Uno de los procesos biogeoquímicos más importante en este ambiente es la descomposición de los residuos de plantas por medio de bacterias u hongos, cuya naturaleza bioquímica (dependiendo

de la especie, o el sistema de cultivo) y forma en que se incorpora (sujeto al tipo de suelo y prácticas agrícolas) son esenciales para mantener la fertilidad y estructura del suelo, así como proporcionar nutrimentos para cultivos de plantas, de esta manera, son una parte central del ciclo de C para los ecosistemas, como los suelos (Todorovic *et al.,* 2010). Sin embargo, poco se conoce acerca de los microorganismos involucrados en su biodegradación. Por lo tanto, la relación entre los microorganismos, la materia orgánica y los parámetros ambientales son de especial importancia para una mejor evaluación de la dinámica del carbono en los ecosistemas terrestres (Singh *et al.,* 2010).

A pesar de la amplia aplicación de la técnica SIP en ecología de suelos, son pocos los trabajos enfocados a identificar las poblaciones microbianas involucradas en la descomposición de materia orgánica vegetal: probablemente debido a que se requiere un alto contenido de ^{13}C o ^{15}N en las plantas enriquecidas, para incorporar el isótopo en los biomarcadores moleculares (Radajewski *et al.,* 2003). Inicialmente, la técnica PLFA SIP se utilizó para seguir la pista del flujo de ^{13}C de residuos de plantas poco enriquecidos a los ácidos grasos de los fosfolípidos presentes en las membranas de los microorganismos que asimilan ^{13}C. De esta manera, la aplicación de este método reveló que en suelos cultivados con arroz, un subgrupo de la comunidad microbiana utilizó los residuos de arroz (Murase *et al.,* 2006). Sin embargo, el análisis de los PLFA frecuentemente proporciona menor información filogenética de los microorganismos que los análisis basados en los ácidos nucleicos. Por otro lado, Haichar *et al.* (2007) aplicaron ^{13}C-Celulosa al suelo e incubaron por 90 días mediante ^{13}C-DNA SIP, y después de llevar a cabo la secuenciación y el análisis filogenético, se encontraron que la mayoría de las secuencias pertenecen a microorganismos cultivables o no cultivables que poseen la habilidad para degradar celulosa, como *Dyella*, *Mesorhizobium* sp., *Sphingomonas* sp., *Dyella*, *Mesorhizobium* sp. no cultivable y Deltaproteobacterium no cultivable.

Recientemente, Lee *et al.* (2011) reportaron un caso donde se emplea la metodología de ^{13}C-DNA SIP para estudios de las comunidades bacterianas involucradas en la descomposición de callos de arroz marcados con ^{13}C en condiciones aerobias; encontrando después del análisis molecular del ^{13}C-DNA la presencia de diversos grupos de bacterias pertenecientes a las clases: Actinobacteria,

Bacilli, gamma-Proteobacteria, Chloroflexi, Sphingobacteria, Flavobacteria, Clostridia, Acidobacteria, Cyanobacteria y Candidate, con lo que se puede inferir que la adición de callos promueve el crecimiento de bacterias que consumen materia orgánica en el suelo.

En el grupo de trabajo del Dr. Luc Dendooven se realizó un experimento en el que se incorporó maíz marcado uniformente con ^{14}C y su fracción detergente neutro para determinar la actividad celulolítica en un suelo salino-alcalino. Sin embargo, en dicho experimento debido a los riesgos de usar isótopos radiactivos no fue posible la identificación de las comunidades degradadoras de residuos de plantas. Es así, como inició el planteamiento de esta investigación.

7. Sitios de estudio empleados en esta investigación

En esta investigación se utilizaron diferentes suelos del Estado de Chiapas, un suelo extremo salino-alcalino del exlago de Texcoco y un suelo agrícola del municipio de Otumba en el Estado de México. Es importante mencionar que a los suelos que fueron empleados en este estudio, se les adicionó residuos derivados de plantas, como hojas, rastrojo y harinas (tanto de *Jatropha curcas* como de maíz). Además, otro aspecto importante por el que se utilizó el suelo del exlago de Texcoco, se debe a que año con año se incrementa la superficie agrícola que se saliniza por el uso inadecuado de fertilizantes, o bien, por el uso ineficiente de aguas de riego, por lo que probablemente en pocos años, grandes extensiones con suelos agrícolas o salinos serán más deteriorados y cuando eso suceda, no habría información de cómo recuperarlos.

7.1 Suelos del estado de Chiapas

En el estado de Chiapas (México), han sido cultivadas 10, 225 ha., con *Jatropha curcas* en 23 municipios distribuidas en cinco regiones geográficas: Depresión Central, Fronteriza, Sierra, Istmo-Costa y Fraylesca (Unión de Sociedades Bioenergéticas Chiapas, S.C. de R.L. de C.V.). La idea del proyecto en Chiapas fue emplear las semillas de *Jatropha* para la producción de biodiesel y establecer

el cultivo en suelos marginales o degradados; las características de estos suelos pueden ser aprovechadas debido a que la planta es resistente a la sequía y tiene el potencial de crecer fácilmente en suelos degradados y áridos, y por ende, ayuda a prevenir la erosión y aumentar la materia orgánica en el suelo (Francis *et al.,* 2005).

Por consiguiente, fueron muestreados los suelos de cinco municipios del Estado de Chiapas con características contrastantes. Los sitios experimentales se localizan en la región Central y la región "*Fraylesca*" y se caracterizan por presentar climas cálido-húmedos y subcálido-subhúmedos con lluvias en verano. La temperatura oscila entre 21.5 y 26.2 ºC, y la precipitación anual es de 800 a 1273 mm. Estos suelos son del tipo Regosol, lo que nos indica que son pobres en nutrimentos y muy susceptibles a la erosión (Soil Survey Staff Classification System, 1999).

7.2 Suelos del Estado de México

7.2.1 Suelo del exlago de Texcoco, Texcoco

De acuerdo con Dendooven *et al.* (2010), el exlago de Texcoco se encuentra al este de la Ciudad de México. Geográficamente se encuentra entre los meridianos 98º 56´ y 99º 01´ O y entre los paralelos 19º 27´ y 19º 32´ N (Fig. 5). El área se encuentra a una altitud promedio de 2240 metros sobre el nivel del mar (msnm) rodeada por montañas formadas por rocas andesíticas, basálticas y riolíticas.

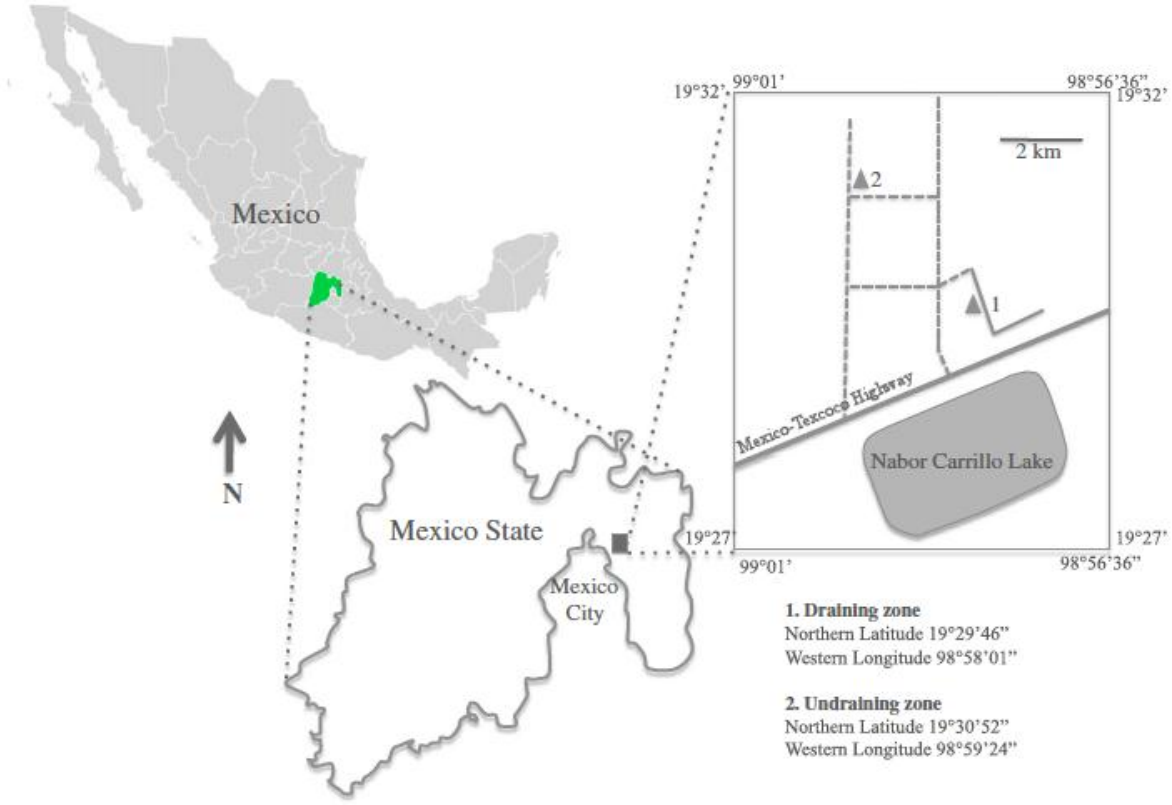


Figura 5. Localización del suelo salino-alcalino (Fuente: Dendooven *et al.,* 2010)

El exlago de Texcoco está dentro de la Cuenca del Valle de México, que se formó durante el Terciario al quedar obstruida la salida natural de las corrientes de agua, convirtiéndose así en una cuenca cerrada (Gutiérrez y Ortiz, 1999). En las partes bajas de la misma se encontraban los lagos de Texcoco, Chalco, Xochimilco, Xaltocan y Zumpango (Rivera, 1975), todos ellos últimos vestigios de un lago mucho mayor que al final de la época glacial probablemente formaba un gran cuerpo de agua poco profundo (Del Valle, 1983). El lago recibió el aporte de sales de origen volcánico al estar rodeado de los volcanes Xitle, Xico, Teutli, Chichinatzin, Papayo, Popocatépetl e Iztaccíhuatl (algunos activos recientemente). A pesar de los beneficios que los bosques y los lagos aportaban, existían problemas de inundación en las zonas pobladas de las partes más bajas durante la Colonia. Para resolver dichos inconvenientes se proyectó el secamiento artificial del lago de Texcoco durante el siglo XVIII. Cuando se desarrollaron las obras de drenaje no se consideraron las intensas tolvaneras que se generarían en el periodo de sequía, ni la aparición de sedimentos salino-sódicos (Gutiérrez y Ortiz, 1999).

En los años 70´s, se inició un proyecto hidráulico y ecológico para resolver los problemas de contaminación ambiental y para la recuperación de los suelos, su forestación y en menor medida utilizarlos como un suelo cultivable. El área del exlago se empezó a irrigar con las aguas residuales para drenar las sales y mejorar la aireación en la zona radicular. El sistema de drenaje implementado fue variado a lo largo de los años y de acuerdo a las necesidades específicas de la zona y se ha logrado reducir sustancialmente el contenido de sal y pH (de conductividad electrolítica (CE) > 50 dS m^{-1} y el pH> 10 hasta CE <10 dS m^{-1} y pH < 8,5) (Luna-Guido *et al.,* 2000). Así también, Kao et al*.,* (2006) y (Sullivan *et al.,* 2006) propusieron que la inundación con las aguas residuales pueden adicionar nutrimentos para la vegetación pionera.

Consecuentemente, a partir de 1970 en los suelos drenados del exlago de Texcoco se encuentran plantas como *Distichlis spicata* (L.), *Suadera nigra, Tamarix* spp*., Eruca sativa, Brassica campestris, Eragrostis obtusiflora, Bouteloua, Muhlenbergia, Hordeum y Cynodon* (Luna-Guido *et al*., 2000).

7.2.2 Suelo del municipio de Otumba en el Estado de México

De acuerdo con Méndez-Bautista et al. (2010), el sitio de Otumba (Latitud Norte 19º 41' 36' Longitud Oeste 98º 43' 24') está localizado en el estado de México. El suelo de este sitio es del tipo arcilloso, calizo y rocoso de origen sedimentario. Y se ha utilizado para la siembra de cultivos de temporal.

Justificación

La materia orgánica derivada de plantas se ha utilizado comúnmente para la recuperación de los microorganismos y características del suelo. El conocer los efectos de diferentes tipos de materia orgánica de origen vegetal en ambientes salinos-alcalinos y suelos agrícolas degradados, nos permitirá comprender el tipo de dinámicas de descomposición, así como la factibilidad de su recuperación en el menor tiempo posible. Del mismo modo, se ignora o no hay conocimiento claro de los microorganismos involucrados y metabólicamente activos en la biodegradación de residuos de plantas.

Hipótesis

La adición de residuos de plantas modificará las poblaciones microbianas en diferentes suelos degradados de agricultura y un suelo salino-alcalino y por ende, las dinámicas de C y N.

La combinación de la técnica *'$^{13}CO_2$ pulse-labelling'* y la prueba de isótopos estables ^{13}C-DNA SIP permitirán identificar los microorganismos involucrados en la biodegradación de residuos de plantas en diferentes tipos de suelos.

Objetivo General

Investigar las dinámicas de C y N durante la biodegradación de residuos de plantas en diferentes tipos de suelos, y analizar las poblaciones microbianas involucradas en el proceso de biodegradación de estos materiales marcados con ^{13}C empleando la prueba ^{13}C-DNA SIP.

Objetivos Particulares

Determinar las dinámicas de C y N en los diferentes suelos adicionados con residuos de plantas durante una incubación aerobia de 56 días.

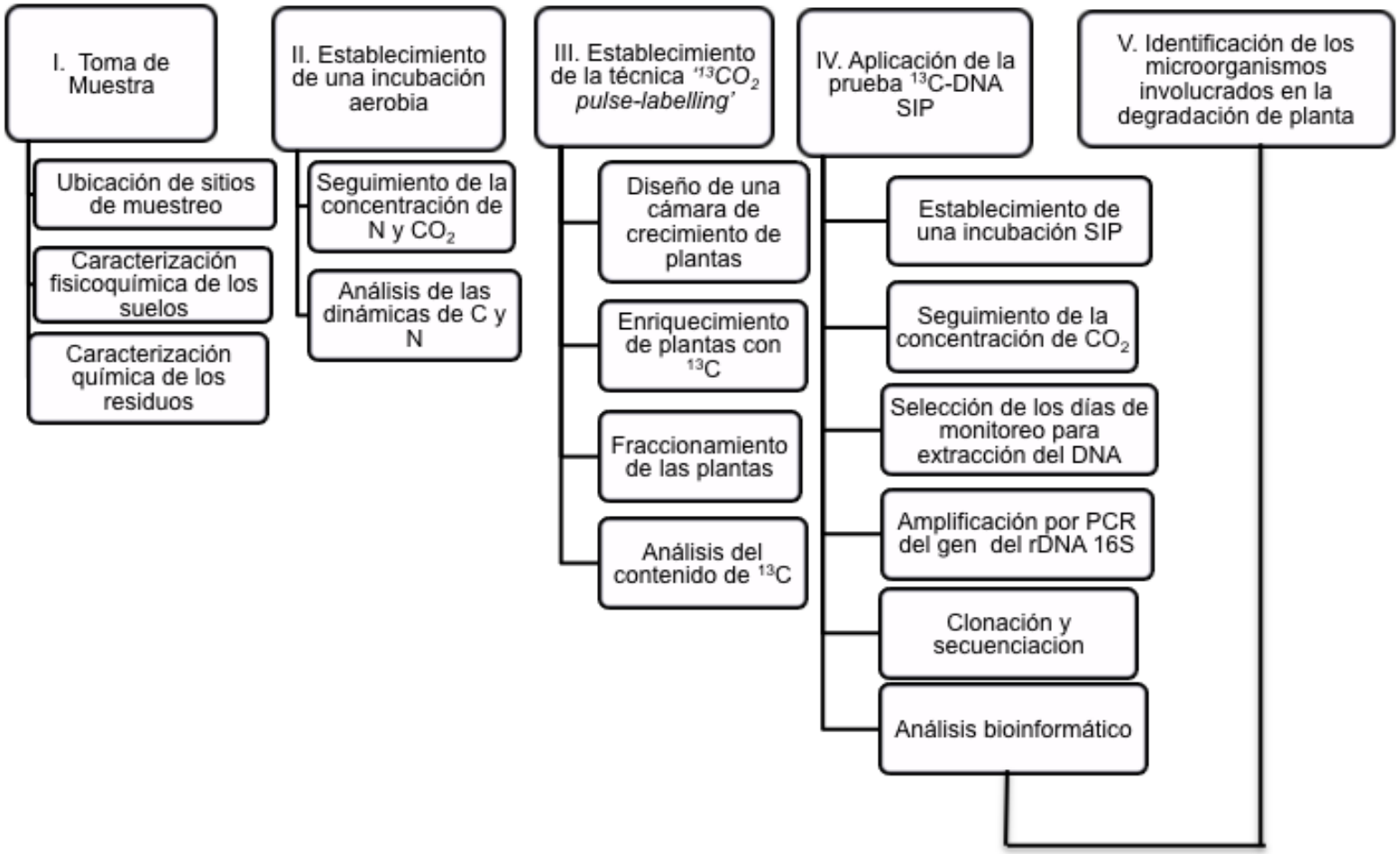
Establecer la técnica *'$^{13}CO_2$ pulse-labelling'* para el enriquecimiento de plantas con ^{13}C.

Identificar los microorganismos involucrados en la biodegradación de los materiales marcados con ^{13}C en los diferentes tipos de suelos mediante la prueba de isótopos estables ^{13}C-DNA SIP.

Materiales y Métodos

Con el objeto de evitar la reiteración de información, los materiales y métodos se encuentran reportados en el apartado correspondiente en cada artículo. La estrategia experimental de todo el Proyecto Doctoral está descrita en un diagrama en la siguiente página.

ETAPAS DE LA ESTRATEGIA EXPERIMENTAL[a]



[a] Si considera importante conocer el diseño experimental, el diseño de tratamientos, las variables evaluadas, la metodología y los resultados relevantes. Consultar el artículo de interés según corresponda en el índice.

Resultados y Discusión

Con el objeto de evitar la reiteración de la información, los resultados y discusión se encuentran en el apartado correspondiente en cada artículo.

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Greenhouse gas emissions and C and N mineralization in soils of Chiapas (México) amended with leaves of *Jatropha curcas* L.

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ABSTRACT

Jatropha curcas L. will be cultivated in large parts of the central highlands of Chiapas (México) so that its seeds can be extracted for biofuel. Little is known how the cultivation of *J. curcas*, which contains phorbolesters, might affect soil processes. Soil was sampled at five locations and amended with leaves of *J. curcas* while dynamics of ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) and emissions of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O), well known greenhouse gasses, were monitored. If we considered no priming effect, then between 12 and 31% of the C added with *Jatropha* leaves mineralized within 56 days. However, the concentration of mineral N (sum of NH_4^+, NO_2^- and NO_3^-) did not increase in the *Jatropha*-amended soils compared to the unamended soils. The mean CO_2 emission rate increased significantly 3.7 times when *Jatropha* leaves were added to the different soils. The N_2O emission was low in the unamended soil and remained <5 μg N_2O-N kg^{-1}. Application of *J. curcas* leaves increased the N_2O emission rate significantly in two soils, but not in the other three. Oxidation of CH_4 occurred in each of the unamended soils with the fastest decrease generally found within the first day. Application of *Jatropha* leaves had no significant effect on oxidation of CH_4, except in one soil. It was found that application of *Jatropha* leaves did increase emission of CO_2, did not affect the soil mineral N content and had only an increasing effect on emission of N_2O and oxidation of CH_4 in some soils.

1. Introduction

The use of biomass to provide energy has been fundamental in the development of civilization. Biomass contributes significantly to energy production and its importance is likely to increase in the near future (Achten et al., 2008). In this context, *Jatropha curcas* might be a sustainable source of biofuel as its seeds contain up to 35% oil that are easily converted to biodiesel (Janaun and Ellis, 2010). Additionally, when marginal land is planted with *J. curcas* deteriorated soil might be restored as the soil organic matter will increase, while having a positive effect on the surrounding ecosystems (Francis et al., 2005). *J. curcas* is drought-resistant well adapted to arid and semi-arid conditions, easily cultivated and often used to prevent soil erosion. This shrub or small tree originating from Central- and South-America is now widely planted worldwide in the (semi-arid) tropics (Fairless, 2007). Like other *Jatropha* species, *J. curcas* is a succulent that sheds its leaves during the dry season (Heller, 1996).

Organic matter in soil is important for soil structure and cation exchange capacity, and provides nutrients for crops upon decomposition (Bronick and Lal, 2005). The organic matter input to soil has been shown to be critical for improving soil quality and optimizing nutrient availability and water efficiencies, and ultimately crop production in agroecosystems (Sinaj et al., 2001; Tschakert et al., 2004). An understanding of organic matter dynamics and a prediction of nutrient release are thus important. Incorporating crop residues is a way to maintain or increase soil organic matter and predicting its decomposition is important to synchronize crop growth and nutrient (N, P) availability (Raiesi, 2006). The chemical composition of the organic material applied, which can be characterized by techniques developed by Van Soest (1963) and Van Soest and Wine (1967), will affect its decomposition, and thus the nutrients available for crops.

In the state of Chiapas, 10,225 ha have already been planted with *J. curcas* in 23 municipalities by '*Union de Sociedades Bioenergéticas Chiapas, S.C. of C.V's R.L.*' In Chiapas, *J. curcas* is often used to border fields and has been used in traditional medicine and in other parts of the world to manufacture soaps (Kumar and Sharma, 2008). It has been proposed to further expand this area and approximately 200,000 ha are suitable to be planted with *J. curcas* in Chiapas alone

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Table 1
Location, climate and land use at the experimental sites used for the study of the decomposition of leaves of *Jatropha curcas* in Chiapas.

Experimental site	Geographical coordinates	Type of soil[a]	Use of soil	Climate[b]	Temperature and annual rainfall	Slope (%)	Altitude (m)
Zapotillo	16°06′32″N 92°57′05″W	Regosol	Agriculture	ACx	21.5 °C, 1273 mm	Steep, 25–30	1012
Acala	16°33′27″N 92°49′42″W	Vertisol	Agriculture	ACw	26.2 °C, 1000 mm	Nearly level, <5	412
Villaflores	16°13′39″N 93°17′44″W	Regosol	Agriculture	ACw	23.7 °C, 1150 mm	Nearly level, <5	574
Cintalapa	16°32′31″N 93°55′05″W	Regosol	Agriculture	ACx	24.5 °C, 800 mm	Nearly level, <5	632
Suchiapa	16°37′06″N 93°05′28″W	Cambisol	Agriculture	ACw	26 °C, 990 mm	Nearly level, <5	491

[a] Soil classification USDA (Soil Survey Staff, 1999),
[b] Climate ACx = semiwarm subhumid climate with rains in summer; ACw = warm subhumid climate with rains in summer.

while in other states in the North of Mexico cultivation of this plant is considered. The idea is to use the seeds for biofuel, while at the same time vegetate marginal lands in the state. The question remains how the plant and decaying leaves will affect dynamics of C and N in soil and especially greenhouse gas emissions (GHG), i.e. nitrous oxide (N_2O), methane (CH_4) and carbon dioxide (CO_2) (Searchinger et al., 2008) as *J. curcas* leaves contain phorbolesters that might affect soil processes (Becker and Maktar, 2008). Land-use change due to the cultivation of *J. curcas*, i.e. changes in soil organic C content and GHG emission, might be as important as the production of biofuel itself (Melillo et al., 2009).

In the central and fraylesca regions of Chiapas, five soils were sampled where *J. curcas* has been planted recently and amended with or without leaves of *J. curcas*. Soil was incubated aerobically at 40% of water holding capacity (WHC) and 22 ± 2 °C for 56 days, while emissions of N_2O, CO_2 and CH_4 and dynamics of NH_4^+, NO_2^- and NO_3^- were monitored. The objective of the study was to investigate the effect of leaves of *J. curcas* on dynamics of C and N in soil sampled at five locations in Chiapas (México).

2. Material and methods

2.1. Sampling sites

Soil was collected from five different locations in Chiapas (México) (Table 1). The experimental sites located in the Central Depression and '*Fraylesca*' are characterized by a hot humid climate (Aw) with rains in summer and sub-hot sub-humid climate (ACw) with rains in summer. The mean annual temperature fluctuates from 21.5 to 26.2 °C and the annual rainfall from 800 to 1273 mm. This type of climate is common in tropical regions of Mexico. The altitude varied from 412 m in Acala to 1012 m in Zapotillo. The soils are regosols (Soil Survey Staff classification system, 1999), poor in nutrients and very susceptible to erosion. The crops cultivated in the area are mainly maize and bean and they are not irrigated. The amount of fertilizer applied depends on the crop and the local farmer but generally ranges from 100 to 150 kg N ha^{-1}.

2.2. Soil and leaves sampling

Soil was collected from the 0–15 cm layer of three plots at the five selected sampling sites on 10th of February 2009. In each plot, ten soil cores were sampled with a stony soil auger diameter 7 cm (Eijkelkamp, Nl), and pooled so that at each sampling site three soil samples were obtained. As such, 15 soil samples were obtained, i.e. from three plots at five sites (n = 15). The soil sampled was characterized and used to study dynamics of C and N. This field based replication was maintained in the incubation study.

At the Acala municipality, 3 kg green undamaged leaves were collected from ten *J. curcas* trees. The leaves were air-dried and ground in a laboratory mill to 20 mesh (0.84 mm diameter). The dried samples were analyzed for total N and C, neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, polyphenol and ash content in triplicate (Table 2).

2.3. Experimental set-up and treatments

Soil samples were taken to the laboratory and treated as follows. The soil was sieved separately (<5 mm), characterized, and adjusted to 40% water holding capacity (WHC) by adding distilled water. Forty-two sub-samples of 25 g soil from each of the 15 samples (five soils in three replicates) were added to 120 ml flasks. Twenty-one sub-samples were amended with 1 g leaf-C kg^{-1} soil and 21 were left unamended. Three flasks were chosen at random from each soil samples amended with or without leaves and extracted for inorganic-N with 100 ml 0.5 M potassium sulphate (K_2SO_4). The samples were shaken for 60 min and filtered through Whatman No. 42 paper® to provide zero-time samples. The remaining flasks were placed in 940 ml glass jars containing a vessel with 10 ml distilled water (H_2O) to avoid desiccation and a vessel with 20 ml 1 M sodium hydroxide (NaOH) to trap carbon dioxide (CO_2) evolved. The jars were sealed and incubated at 22 ± 2 °C for 56 days. An additional 18 jars without soil, but containing a vessel with 10 ml distilled H_2O and one with 20 ml 1 M NaOH, were sealed and served as controls to account for the CO_2 trapped from the atmosphere. After 1, 3, 7, 14, 28 and 56 days, three jars were selected at random from each treatment. The flasks were opened, the vessel with 1 M NaOH removed, stoppered and the soil was treated as described for zero-time samples. All remaining jars were opened, aerated for 10 min to avoid anaerobic conditions, resealed and further incubated.

Additionally, emissions of CO_2, N_2O and CH_4 were measured. Twenty-four sub-samples of 10 g soil of the 15 soil samples (five sites in triplicate) were added to 120 ml serum glass bottles. Twelve sub-samples were amended with 1 g leaf-C kg^{-1} soil and 12 were left unamended. Twelve additional bottles without soil were sealed and served as controls to account for the N_2O, CH_4 and CO_2 in the atmosphere. The serum bottles were closed with a Teflon stopper and sealed with an aluminium cap 20 mm tear-off seal. Three serum bottles were selected at random, the headspace analyzed for CH_4 and N_2O to account for the CH_4 and N_2O in the atmosphere and the soil extracted for inorganic N as described before. After 1, 3 and 7 days, three serum bottles were selected at random and the headspace analyzed for CO_2, N_2O and CH_4. The serum bottles were opened and the soil extracted for inorganic N as described before.

Soil was incubated at 22 ± 2 °C although higher soil temperatures have been registered in the semi-arid regions of Mexico

Table 2
Chemical composition of *Jatropha curcas* L. leaves.

Organic-C (g kg^{-1})	Total N (g kg^{-1})	Soluble[a] (g kg^{-1})	Lignin (g kg^{-1})	(Hemi)cellulose (g kg^{-1})	Polyphenols (g kg^{-1})	Ash (g kg^{-1})
437 (6)[b]	34 (2)	564 (17)	111 (5)	262 (14)	6.9 (0.3)	102 (2)

[a] Soluble fraction calculated as (1000 – (NDF ‰)).
[b] Values between parenthesis are the standard deviation of nine replicates.

Table 3
Some characteristics of soil at the five experimental sites used for the study of the decomposition of leaves of *Jatropha curcas* in Chiapas (Mexico).

	EC[a] (dS m^{-1})	pH	Organic C (g kg^{-1} soil)	Total N (g kg^{-1} soil)	WHC[b] (g kg^{-1} soil)	Clay (g kg^{-1} soil)	Silt (g kg^{-1} soil)	Sand (g kg^{-1} soil)	Textural classification
Zapotillo	0.67	6.4	22.8	1.9	878	182	132	686	Sandy loam
Acala	0.81	8.2	22.0	2.0	895	253	326	421	Loam
Villaflores	0.24	8.2	9.4	0.6	848	54	126	820	Loamy sand
Cintalapa	0.34	6.7	5.0	0.5	850	55	78	867	Loamy sand
Suchiapa	0.58	5.6	19.8	2.4	879	140	382	478	Loam

[a] EC: Electrolytic conductivity.
[b] WHC: Water holding capacity.

during certain parts of the day. As this study is part of a larger investigation into dynamics of C and N in soil, the same incubation temperature was used so that different ecosystems could be compared.

2.4. Chemical analyses

Soil pH was measured in 1:2.5 soil-H_2O suspension using a glass electrode (Thomas, 1996). The electrolytic conductivity (EC) was determined in a saturated solution extract as described by Rhoades et al. (1989). The WHC was measured on soil samples water-saturated in a funnel and left to stand overnight. Soil particle size distribution was determined by the hydrometer method as described by Gee and Bauder (1986). The organic C in sludge and soil was measured in a total organic carbon analyzer TOC-V_{CSN} (SHIMADZU, USA). Total N was measured by the Kjeldahl method using concentrated H_2SO_4, K_2SO_4 and HgO to digest the sample (Bremner, 1996). The NH_4^+, NO_2^- and NO_3^- in the K_2SO_4 extracts were determined colorimetrically on a San Plus System–SKALAR automatic analyzer (Mulvaney, 1996).

The headspace of the serum vials was analyzed for CO_2 and N_2O on an Agilent Technology 4890D gas chromatograph fitted with an electron capture detector (ECD). A HP-PLOT Q 30 m column with the temperature of the detector, injector and oven at 225, 100 and 35 °C, respectively, was used to separate the CO_2 and N_2O from the other gases. The carrier gas N_2 was flowing at 6 ml min^{-1}. The CH_4 of the vials was analyzed on an Agilent Technology 4890D gas chromatograph fitted with a flame ionization detector (FID). A PORAPAK Q 80/100 12′ × 1/8″ × 0.085″ column with the detector temperature at 310 °C, injector at 100 °C and oven at 32 °C was used to separate the CH_4 from the other gases. The carrier gas He was flowing at 25 ml min^{-1}.

Each day that the headspace of the serum bottles was sampled, standard concentrations of CH_4, CO_2 and N_2O were injected and analyzed. A standard curve for each of the gases was determined and used to calculate the concentrations of CH_4, CO_2 and N_2O.

The *Jatropha* leaves were fractionated according to the Van Soest method (Van Soest, 1963; Van Soest and Wine, 1967). Hot extraction with Neutral Detergent Solution (NDS) removed the 'soluble' part of the leaves, leaving a Neutral Detergent Fibre residue containing most of the 'cell wall constituents'. A second hot extraction with acid detergent solution (ADS) removed the acid labile components of the cell wall, leaving an acid detergent fibre (ADF) residue consisting of cellulose- and lignin-like product. Cold extraction with 72% sulphuric acid (H_2SO_4) dissolved cellulose-like products, leaving a lignin-like residue. Ash content was determined after ashing in a muffle furnace at 600 °C. The Folin-Denis method was used for the extraction of total extractable polyphenols of the leaves with 50% methanol at 80 °C using tannic acid as standard (Anderson and Ingram, 1989).

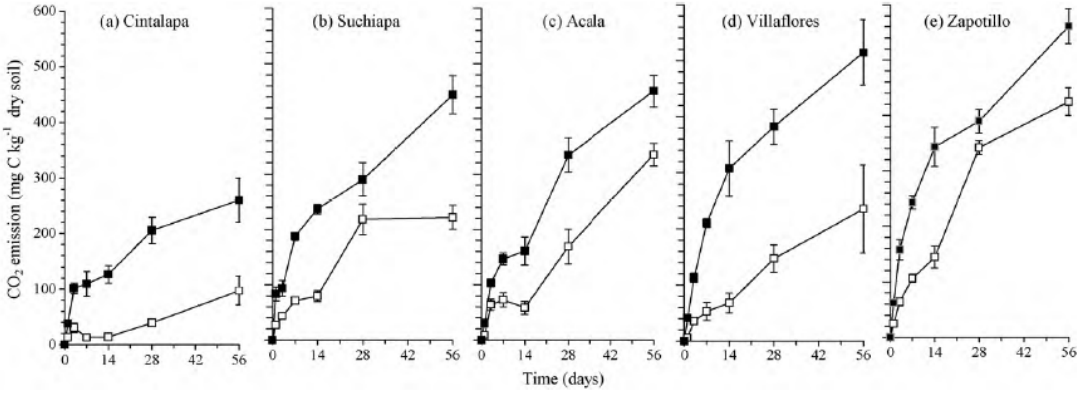

Fig. 1. Emission of CO_2 (mg C kg^{-1} dry soil) from unamended soil (□) and soil amended with leaves of *Jatropha curcas* (■). Bars are ± 1 standard deviation.

Table 4
The effect of the application of *Jatropha curcas* leaves on CO_2, CH_4 and N_2O emission rates and concentrations of NH_4^+, NO_2^- and NO_3^- in five soils of Chiapas (Mexico).

Leaves	Soils					
	Cintalapa	Suchiapa	Acala	Villaflores	Zapotillo	
Mean concentration of NH_4^+ (mg N kg^{-1} dry soil)						MSD[a]
Without	11.6 A a	3.4 A c	2.4 A c	4.0 A c	6.3 A b	1.7
With	7.8 B a	3.6 A c	2.8 A c	3.6 A c	6.1 A b	1.7
MSD	1.7	0.7	0.5	0.8	1.5	
Mean concentration of NO_2^- (mg N kg^{-1} dry soil)						MSD[a]
Without	0.1 B b	0.6 A a	0.4 B ab	0.2 A b	0.8 A a	0.4
With	0.3 A c	0.5 A bc	1.2 A ab	0.2 A c	1.4 A a	0.7
MSD	0.1	0.4	0.5	0.1	0.6	
Mean concentration of NO_3^- (mg N kg^{-1} dry soil)						MSD[a]
Without	16.9 A c	12.3 A c	35.5 A b	17.5 A c	42.2 A a	5.6
With	17.5 A b	16.5 A b	31.2 B a	15.8 A b	36.0 B a	10.6
MSD	3.6	14.4	3.1	5.9	5.9	
CO_2 emission rate after 56 days incubation (mg C kg^{-1} dry soil day^{-1})						SEE[b]
Without	1.66 B c	4.87 B b	5.99 B b	4.49 B b	8.71 B a	0.84
With	5.55 A c	9.09 A b	9.14 A b	10.94 A a	11.82 A a	0.50
SEE	0.48	0.63	0.53	0.90	0.80	
N_2O emission rate after 7 days incubation (μg N kg^{-1} dry soil day^{-1})						SEE[b]
Without	−0.15 B a	−0.73 A a	−0.44 A a	0.23 A a	0.13 A a	0.51
With	1.20 A a	−0.49 A c	0.40 A abc	−0.02 A bc	1.05 A ab	0.59
SEE	0.47	0.30	0.59	0.71	0.62	
Emission of CH_4 after 1 day incubation (μg C kg^{-1} dry soil day^{-1})						SEE[b]
Without	−2.1 A a	−8.2 A b	−1.8 A a	−5.4 A ab	−5.4 A ab	2.1
With	−3.2 A a	−7.7 A b	−1.0 A a	−2.6 A a	−13.6 B c	2.0
SEE	1.3	2.4	1.4	1.5	3.1	

[a] MSD: Minimum significant difference ($P < 0.05$).
[b] SEE: Standard error of the estimates.

2.5. Statistical analysis

Cumulative production of CO_2 and emission of N_2O an CH_4 was regressed on elapsed time using a linear regression model which was forced to pass through the origin but allowed different slopes (production rates) for each treatment. This approach is supported by theoretical considerations that no N_2O, CH_4 and CO_2 is produced at time zero and the CH_4, N_2O and CO_2 in the atmosphere was accounted for in the control without soil.

Significant differences between the soil characteristics as a result of the different treatments were determined by analysis of variance (ANOVA) and based on the least significant difference using the General Linear Model procedure (PROC GLM, SAS Institute, 1989). This procedure can be used for an analysis of variance (ANOVA) for unbalanced data, i.e. when data are missing. Significant differences between treatments for emission of CO_2 and N_2O and production of CH_4 within a day were determined using PROC MIXED considering repeated measurements (SAS Institute Inc., 1989).

The relationships between the different soil properties (emission of CO_2, N mineralization, electrolytic conductivity, pH, water holding capacity, and organic C, clay, silt and sand content) were visualized by principal component analysis (PCA). Variables were auto-scaled prior to PCA (Sena et al., 2002). The number of components was determined by the Eigenvalue-one criterion (Kaiser, 1960). Moreover, a scree test (Cattell, 1996) was performed to corroborate primer results, only principal components with Eigenvalues >1 and that explain >20% of the total variance were retained. A VARIMAX rotation was performed to enhance interpretability of

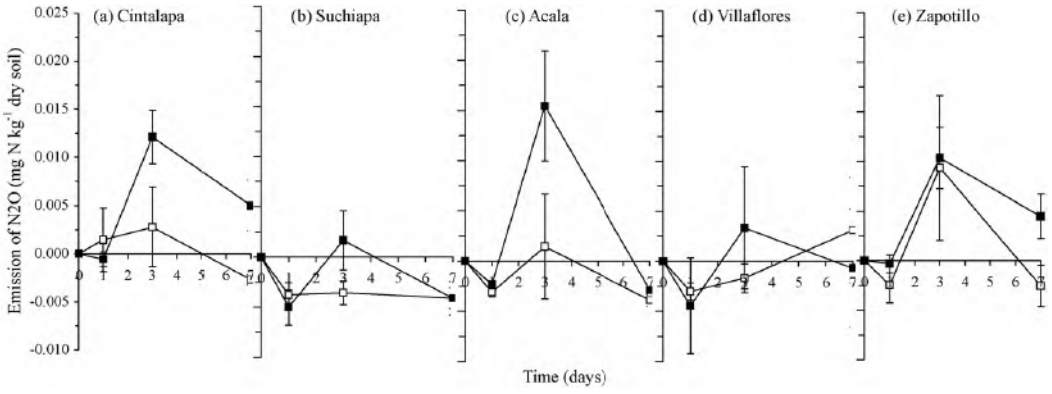

Fig. 2. Emission of N_2O (mg N kg^{-1} dry soil) from unamended soil (□) and soil amended with leaves of *Jatropha curcas* (■). Bars are ± 1 standard deviation.

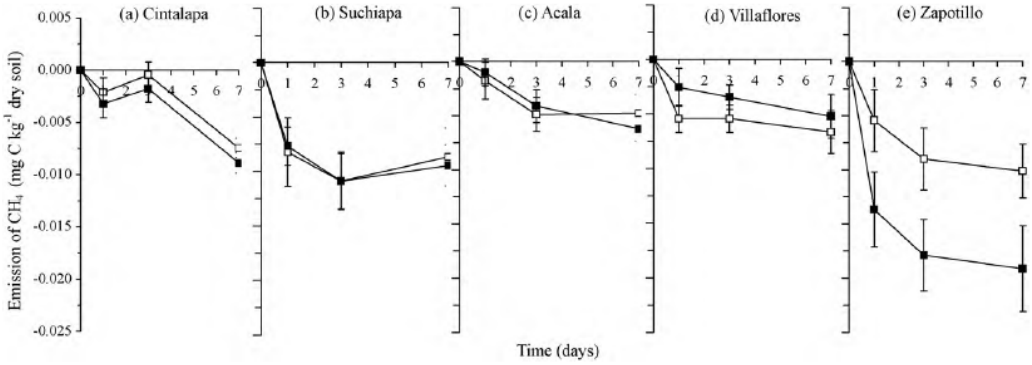

Fig. 3. Emission of CH_4 (mg C kg^{-1} dry soil) from unamended soil (□) and soil amended with leaves of *Jatropha curcas* (■). Bars are ± 1 standard deviation.

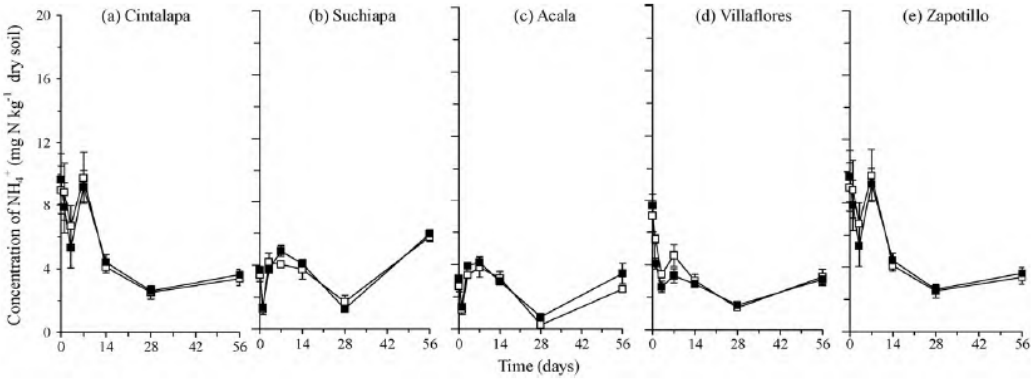

Fig. 4. Concentration of NH_4^+ (mg N kg^{-1} dry soil) in unamended soil (□) and soil amended with leaves of *Jatropha curcas* (■). Bars are ± 1 standard deviation.

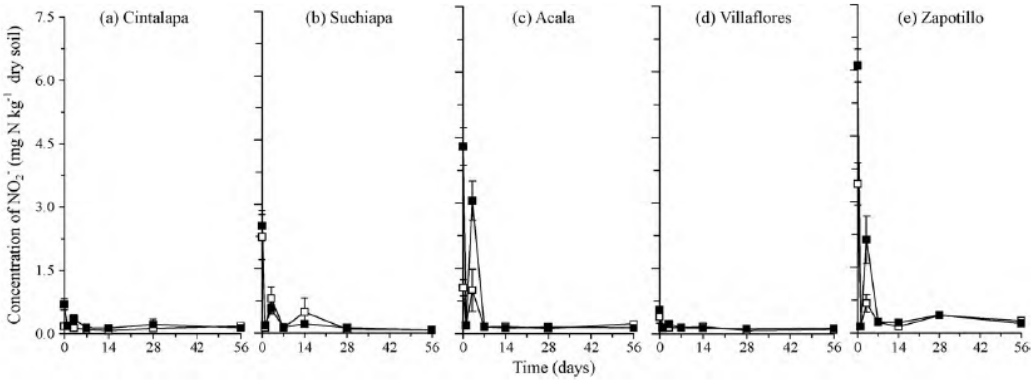

Fig. 5. Concentration of NO_2^- (mg N kg^{-1} dry soil) in unamended soil (□) and soil amended with leaves of *Jatropha curcas* (■). Bars are ± 1 standard deviation.

the uncorrelated components (Flury and Riedwyl, 1988). PCA often reveals previously unsuspected associations among variables and thereby allows interpretation that would not be possible otherwise (Johnson and Wichern, 1998). The matrix of 5 columns (sites) and 9 lines (variables) was used for principal component analysis. All analyses were performed using the SAS statistical package (SAS Institute, 1989).

3. Results

3.1. Soil and leaves characteristics

The chemical composition of the *J. curcas* leaves is given in Table 2. The N content was 34 g kg^{-1} and the C content 437 g kg^{-1}. The soluble fraction was 564 g kg^{-1}, the lignin content 111 g kg^{-1}

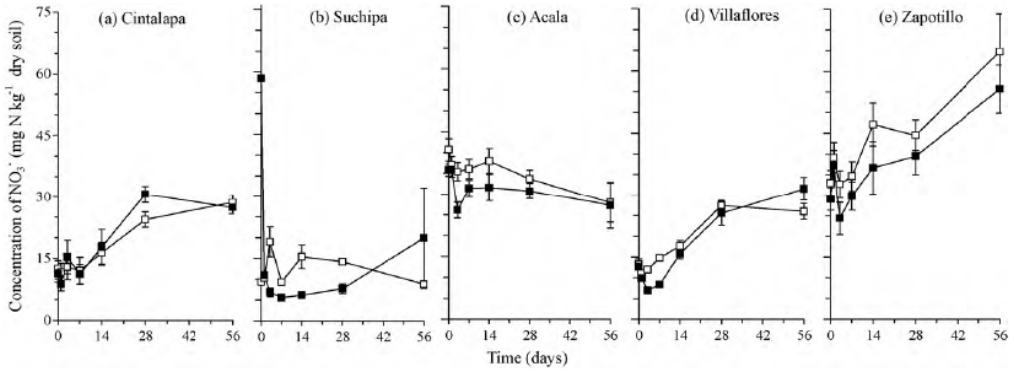

Fig. 6. Concentration of NO_3^- (mg N kg^{-1} dry soil) in unamended soil (□) and soil amended with leaves of *Jatropha curcas* (■). Bars are ± 1 standard deviation.

and the (hemi)cellulose 262 g kg^{-1}. The amount of polyphenols was 6.2 g kg^{-1} and the ash content 102 g kg^{-1}.

The loamy, sandy loam or loamy sand soils were moderately alkaline to acid (Table 3). The organic C content ranged from 5.0 g kg^{-1} to 22.8 g kg^{-1} with little variation in WHC between the soils and EC < 1 dS m^{-1}. The total N content ranged from 0.5 g kg^{-1} to 2.4 g kg^{-1}.

3.2. Emission of CO_2, CH_4 and N_2O

The CO_2 emission rate was significantly different between the unamended soils ($p < 0.0001$) (Fig. 1, Table 4). It was highest in the Zapotillo soil and lowest in the Cintalapa soil. The CO_2 emission rate increased significantly 3.7 times (mean of all soils) when *Jatropha* leaves were added to the different soils ($p < 0.0001$).

The emission of N_2O was low in the unamended soils and remained < 5 μg N_2O-N kg^{-1} within 7 days (Fig. 2). Application of *J. curcas* leaves had no effect on the N_2O emission rate except in the Cintalapa soil where it increased significantly ($p = 0.0051$) (Table 4).

The concentration of CH_4 decreased over time in the unamended soils with the fastest decrease generally found within the first day ($p < 0.05$) (Fig. 3). The fastest decrease in the concentration of CH_4 within a day was found in the Suchiapa soil and the lowest in the Villa Acala soil (Table 4). The decrease in the concentrations of CH_4 within a day was not affected significantly by the application of *Jatropha* leaves, except in the Zapotillo soil ($p = 0.0117$) (Table 4).

3.3. Mineral N

The concentration of NH_4^+ was largest in the Cintalapa soil and lowest in Villa Acala soil (Fig. 4, Table 4). Application of *Jatropha* leaves had no significant effect on the concentration of NH_4^+, except in the Cintalapa soil where it decreased significantly as compared to the unamended soil ($p < 0.0001$).

The concentration of NO_2^- decreased sharply within first day in the unamended and soil amended with leaves, increased generally at day 3 and decreased again thereafter (Fig. 5). Application of *Jatropha* leaves had no significant effect on the concentration of NO_2^-, except in the Cintalapa and Villa Acala soils where it increased significantly compared to the unamended soil ($p \leq 0.0023$).

The concentration of NO_3^- increased significantly over time in the unamended soil except in the Suchiapa and Villa Acala soils ($p < 0.05$) (Fig. 6). The application of *Jatropha* leaves had no significant effect on the concentration of NO_3^- except in the Villa Acala and Zapotillo soils where it decreased significantly compared to the unamended soil ($p \leq 0.0409$) (Table 4).

3.4. Principal component analysis

The PCA analysis separated the different soils (Fig. 7). The Acala and Suchiapa soils can be found in the lower right quadrant. In these soils the highest amounts of leaves were mineralized, i.e. Acala 31% and Suchiapa 22% soils, and these soils were characterized by a

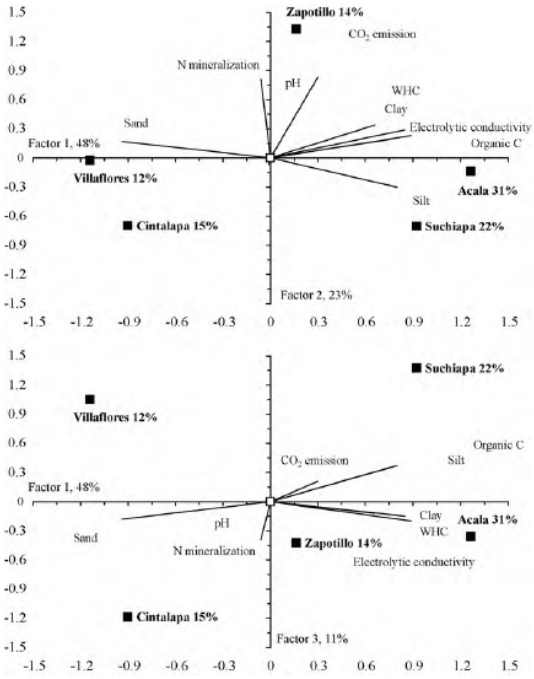

Fig. 7. Principal component analysis (PCA) performed on soil properties data after a varimax rotation with sand, clay, silt and organic C content, N mineralization, emission of CO_2, WHC (water holding capacity), pH and electrolytic conductivity. The two axes explained 71% of the variation.

positive PC1, i.e. a high WHC, EC, clay, silt and organic C content. These soils were characterized by a negative PC2, i.e. a low C and N mineralization. The Villaflores and Cintalapa soils can be found in the lower left quadrant. In these soils, the amounts of leaves mineralized were lower, i.e. Villaflores 12% and Cintalapa 15% soils, and these soils were characterized by a negative PC1, i.e a low WHC, EC, clay, silt and organic C content. The Villaflores and Cintalapa soils were also characterized by a negative PC2, i.e. a low C and N mineralization. The Zapotillo soil can be found in the higher right quadrant. In this soil 14% of leaves were mineralized and this soil was characterized by a small positive PC1and a positive PC2, i.e. a high C and N mineralization.

4. Discussion

4.1. Characteristics of the Jatropha curcas leaves

The total N content of the Jatropha leaves was 34 g kg^{-1} dry plant material and within the range of 14 to 43 g kg^{-1} reported for species of genus *Acacia*, *Prosopis*, *Mimosa*, *Castanea*, *Quercus* and *Fagus* (Sariyildiz and Anderson, 2005; Herrera-Arreola et al., 2007) and similar to those reported for *Jatropha* leaves in India (Chaudhary et al., 2008). The C:N ratio of 12.9 for *Jatropha* leaves was at the lower end of values reported by Sariyildiz and Anderson (2005) indicating that they were rich in N. Sariyildiz and Anderson (2005) reported C:N values ranging from 16.3 to 19.9 for three deciduous tree species, while Teklay et al. (2007) found values ranging from 11.9 to 21.3. The polyphenols concentration of 6.9 g kg^{-1} for leaves of *J. curcas* was within the range of 5.0 g kg^{-1} to 11 g kg^{-1} reported for leguminous species (Thomas and Asakawa, 1993; Kachaka et al., 1995). The lignin content of *Jatropha* was 111 g kg^{-1} and within the range of 20 g kg^{-1} dry leaves to 125 g kg^{-1} dry leaves reported by Vanlauwe et al. (1997) for leaves of *Leucaena*, *Dactyladenia* and *Flemingia*, but lower than values found by Sariyildiz and Anderson (2005) for *Castanea sativa*, *Quercus robur* and *Fagus sylvatica*. The (hemi)cellulose content of *Jatropha* leaves was 262 g kg^{-1} and lower than values of 364 g kg^{-1} for *Acacia tortuoso* leaves and 380 g kg^{-1} for *Prosopis* spp. leaves (Reyes-Reyes et al., 2003).

4.2. C mineralization of J. curcas leaves amended to soil

Decomposition rates of the leaves were higher at the onset of the experiment and decreased thereafter. This larger emission of CO_2 at the onset of the experiment has also been observed by Franzluebbers et al. (1994), Wirén-Lehr et al. (2002) and Reyes-Reyes et al. (2003). It is attributed to the rapid decomposition of the extractable polysaccharides in the leaves (Trinsoutrot et al., 2000).

If we considered no priming effect (Kuzyakov et al., 2000), then between 12 and 31% of the C added with *Jatropha* leaves mineralized within 56 days. Similar values were reported by Teklay et al. (2007) for plant species from Ethiopia. They reported that 10, 15, 17 and 42% of the total C of leaves from *Cordia africana*, *Albizia gummifera*, *Milletia ferruginea* and *Croton macrostachyus*, respectively, mineralized within 30 days. Different plant characteristics, e.g. soluble fraction, polyphenol and lignin content or C:N and lignin:N ratio, are known to affect their decomposition. The soluble fraction is easily decomposable (Sakala et al., 2000) and can stimulate the decomposition of the (hemi) cellulose (Vanlauwe et al., 1994).

The rather high lignin content of the *Jatropha* leaves might have reduced their mineralization. Both lignin and polyphenols decrease decomposition of organic residues and are sources of phenolic units for the formation of humus (Handayanto et al., 1994; Stevenson and Cole, 1999). A higher concentration of lignin implies not only more recalcitrant material, which resists rapid microbial decomposition, but also the formation of a complex phenyl-propanol structure, which often encrusts the cellulose-hemicellulose matrix preventing its decomposition (Sanger et al., 1996). Polyphenols form complexes with proteins and bind on microbial enzymes, organic N in leaves, and soluble forms of organic N released from the leaves thereby inhibiting the growth of a number of microorganisms (Palm and Rowland, 1997; Trinsoutrot et al., 2000).

Other factors, such as C:N and lignin:N ratio, have been suggested as reliable indicators to predict rate of decomposition (Berg and Staaf, 1981; Enríquez et al., 1993; Thomas and Asakawa, 1993; Handayanto et al., 1994; Moretto et al., 2001). The lignin:N ratio of 3.3 of *Jatropha* leaves was lower than values of 5.4 to 7.9 for green leaves of *Castanea sativa*, *Quercus robur* and *Fagus sylvatica* reported by Sariyildiz and Anderson (2005), so the mineralization of the *Jatropha* leaves should be higher. It has to be remembered that phorbol esters and other toxins might have affected the mineralization rates of the leaves and resulted in lower degradation rates.

The amount of leaves mineralized within 56 days was different between the soils and ranged between 12% in the Villaflores soil and 31% in the Acala soil, as has often been reported (e.g. Saggar et al., 1999; Gnankambary et al., 2008). Differences in the amounts of C mineralized between the soils can be explained by differences in soil characteristics, such as pH (Saggar et al., 1999), CEC, soil structure (Amato and Ladd, 1992), clay content (e.g. Van Veen et al., 1985), specific surface area of the clay, the nature of the clay mineral (Saggar et al., 1996). The PCA indicated that a high WHC, clay, silt and organic C, content favoured mineralization of the leaves, i.e. in the Acala and Suchiapa soils, while the sand content reduced it as in the Villaflores and Cintalapa soils although less outspoken in the Zapotillo soil (Fig. 7).

4.3. N mineralization in J. curcas leaves amended soil

The rate of decomposition and the amount of N-mineralization from organic material determines the short-term benefits of tree residues for plant nutrition (Jensen, 1997). However, the dynamic of nitrogen in natural ecosystem is affected by ecological and biological conditions. It has been shown that the rate of nitrogen mineralization differs among species having different leaf composition (Lupwayi and Haque, 1998) and different soil types (Sariyildiz and Anderson, 2005).

The amount of N mineralized in our study varied between soils but was generally low and addition of *Jatropha* leaves had no significant effect on the mineral N in soil. The rate of litter decomposition is related to the initial concentration of N (Witkamp, 1966), which is recognized as the main factor limiting the growth of decomposer populations (Berg and Staaf, 1987). Decomposition rates are affected by nutrient and lignin content of litter (Moorhead et al., 1996). Ratios such as C:N, lignin:N, polyphenol:N, cellulose:N, (lignin + polyphenol):N have been correlated with the N-mineralization or N accumulation from different organic residues (Constantinides and Fownes, 1994; Kachaka et al., 1995; Janssen, 1996; Handayanto et al., 1997). As suggested for organic material in other ecosystems (Melillo et al., 1982; Taylor et al., 1989; Berg et al., 1996; Teklay et al., 2007), the lower decomposition rate of *Jatropha* leaves could be related to the low lignin-to-N ratio. However, although characteristics of the leaves are important in the release of N, soil characteristics, such as soil texture and structure (Ladd et al., 1993; Seneviratne et al., 1999), soil N content (Trinsoutrot et al., 2000), pH (e.g. Saggar et al., 1999), cation exchange capacity (Amato and Ladd, 1992), sodicity (Nelson et al., 1996), clay content (e.g. Van Veen et al., 1985), specific surface area of the clay, and the nature of the clay mineral (Saggar et al., 1996), and initial soil fertility also affect N release (Seneviratne et al., 1999; Loranger et al., 2002).

4.4. Emission of N_2O and CH_4

The emission of N_2O from the soils in our study were low. Nitrification and denitrification are the processes that most contribute to emissions of N_2O from soil (Bateman and Baggs, 2005; Menendez et al., 2008) and they are controlled by different factors, such as management practices, inorganic or organic fertilization and soil water regime (Firestone et al., 1980; Zou et al., 2007; Ellert and Janzen, 2008). Addition of *Jatropha* leaves had no effect on emission of N_2O except in one soil. The amount of N mineralized was low in all soils independent of application of *Jatropha* leaves so little N cycling occurred, i.e. nitrification, nitrifier-denitrification and denitrification (Wrage et al., 2001). Consequently emissions of N_2O were low (Stevenson and Cole, 1999).

In our study, CH_4 was oxidized quickly in all soils and no production occurred. Soils are known sinks and sources of CH_4 (Le Mer and Roger, 2001). Production of CH_4 is related to anaerobic conditions and soil organic matter in rice field soils (Garcia et al., 1974; Wassmann et al., 1998; Yao et al., 1999), peat soils (Magnusson, 1993; Moore and Dalva, 1993) and river sediments (Gebert et al., 2006). Microbial oxidation of methane occurs naturally in soils (Hanson and Hanson, 1996). It is affected by water and nutrient content, methane flow and soil temperature (Maurice and Lagerkvist, 2004). Agricultural systems usually are normally not a large source or sink of CH_4 (Chan and Parkin, 2001). They are only sources of CH_4 after application of manure or other organic materials (Johnson et al., 2007), but application of *J. curcas* leaves had no significant effect on the emission of CH_4.

Cultivating *J. curcas* trees to extract oil from their seeds to be used as biofuel, will increase soil organic matter content as a result of litter deposition thereby reclaiming deteriorated and marginal lands (Fairless, 2007). Increased soil organic matter content will improve soil structure, water infiltration and gas diffusion (Bronick and Lal, 2005). As such, the number and size of the anaerobic microsites will decrease reducing denitrification and production of CH_4, while favouring oxidation of CH_4. The nutrients status of the soil, i.e. mineral N, will increase as the *J. curcas* leaves have a low C:N ratio (Jensen, 1997). Although that increased soil organic matter C indicates that more C will be sequestered from the atmosphere in the soil, increased N cycling might increase emission of N_2O. This increased emission of N_2O might ultimately negate the increased C sequestered in soil effect as the global warming potential of N_2O is 310 times that of CO_2 (Li et al., 2005; IPCC, 2007). However, C will also be trapped in the plant biomass for an extended period of time and reduced machinery and fertilizer use will reduce the global warming potential of the *J. curcas* ecosystem (West and Marland, 2002).

5. Conclusion

The amount of *J. curcas* leaves decomposed varied between the soils studied, but was often low. Oxidation of CH_4 occurred in all soils and application of *J. curcas* leaves did not inhibit the activity of methanotrophs and/or stimulate emission of CH_4. Although the C:N ratio of the leaves was low, no increase in mineral N was found in the soils amended with the leaves and their effect on emissions of N_2O was low when added to soil. It appears that planting of *J. curcas* would not inhibit dynamics of C and N in soil, and might increase organic matter in deteriorated soils.

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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

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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 seed cake of *J. curcas* was 0.993 mg/g while no ricin was detected in *R. communis. Ricinus communis* and *J. curcas* seed cake increased emission of CO_2, N_2O and CH_4 compared to the unamended soil. *Jatropha curcas* seed cake increased mineral N with 76 and *R. communis* with 98 mg N/kg soil. 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 seed cake; emissions of CO_2, CH_4 and N_2O; mineral N; soil characteristics

The cultivation of *Jatropha curcas* L. for the production of bio-diesel has increased in recent years (Francis et al. 2005). It was though that > 23 million acres might be planted in India and China alone by 2010 (Fairless 2007). After the extraction of the oil, an organic-rich waste product remains that is non-edible, but that could easily be applied to degraded soil (Ramachandran et al. *2*007). However, seed cake of *J. curcas* contains organic components, such as trypsin inhibitors, curcin, tannins, saponins, phytates and phorbol esters, that might inhibit plant growth and biological functioning of the soil (Goel et al. *2*007, Achten et al. *2*008). Phorbol esters are the most active compounds in *Jatropha* seed and seed cake with concentrations ranging from 2 to 3 mg/g in the first and from 2 to 8 mg/g in the latter (Makkar et al. 1997).

Ricinus communis L. also known as "castor bean" is grown in temperate and tropical parts of the world for its oil, which is extensively used for medicinal and industrial purposes. Additionally, its oil can be used for the production of biodiesel (César and Batalha 2010). India is the world's largest producer of castor seed with an annual production of 590×10^6 kg. Approximately 400×10^6 kg castor seed cake remains after extraction of the oil (Lima et al. *2*011). The castor bean seed cake is non-edible, but rich in N, so it could also easily be used as organic fertilizer (Ramachandran et al. *2*007). However, the seeds also contain toxic components, such as ricin and ricinine, which might limit the use of seed cake in agricultural practices (Anandan et al. *2*005).

The emission of greenhouse gases from soil (GHG) is important as they contribute to global warming (Shang et al. *2*011). Land use change and agricultural practices contributes significantly to the emission of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) (Singh et al. *2*010). Application of organic residues to soil, such as castor bean and *Jatropha* seed cake, generally improves soil structure, increases crop production by providing plant nutrients and suppresses soil borne diseases (Anis et al. *2*010). However, the application of organic residues might increase the emissions of CO_2, CH_4 and N_2O (Fangueiro et al. *2*010).

Although the seed cake of both *J. curcas* and castor bean are nutrient rich, little information exists

on how this organic waste product might affect the biological functioning of a soil (Hayashi 2012), mineral N and emission of GHG. Soil sampled from seven sites in Chiapas (Mexico) where *J. curcas* and castor bean will be cultivated industrially for biofuel production was amended with seed cake of both plants. The emissions of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) and dynamics of mineral N (ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) were monitored in an aerobic incubation experiment. The objective of this study was to investigate the effect of seed cake of *J. curcas* and *R. communis* on mineral N in soil and GHG emissions.

MATERIAL AND METHODS

Sampling sites. Soil was collected from seven different locations in Chiapas (México) (Table 1). The sampling sites located in the Central Depression are characterized by a hot humid climate (Aw) with rains in summer and sub-hot sub-humid climate (ACw) with rains in summer. The mean annual temperature fluctuates from 21.5 to 26.2°C and the annual rainfall from 800 to 1273 mm. This type of climate is common in the tropical southern regions of Mexico. The altitude varied from 412 m in Villa Acala to 1012 m in Zapotillo. The soils are Regosols, poor in nutrients and very susceptible to erosion (Soil Survey Staff classification system 1999).

Soil and seed cake sampling. Soil was collected from the 0-15 cm layer of five 500 m^2 plots defined at seven sampling sites on 20th of February 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 7 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 seed cake of *J. curcas* was obtained from '*Biocombustibles de Guatemala, S.A.'* (Guatemala) and seed cake of castor bean 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 seed cake used in this study (Forson et al. *2*004, Shah et al. *2*005). The oil was extracted from *Ricinus communis* by heating with sodium hydroxide. The seed cakes were characterized in triplicate (Table 2). The ricin in the seed cake of castor bean was measured in the laboratory of biochemistry at the National Autonomous University of Mexico, UNAM (Mexico) as described in Kanamori-Kataoka et al. (2011). The concentration of phorbol esters in the seed cake of *J. curcas* was determined at the 'Institute of Animal Production in the Tropics and Subtropics of the University of Hohenheim (Stuttgart, Germany)' as described by Makkar et al. (1997).

The *J. curcas* and *R. communis* seed cake was fractionated according to the Van Soest method (Van Soest 1963, Van Soest and Wine 1967). Hot extraction with neutral detergent solution (NDS) removed the 'soluble' part of the seed cake, leaving a neutral detergent fibre residue containing most of the 'cell wall constituents'. A second hot extraction with acid detergent solution (ADS) removed the acid labile components of the cell wall, leaving an acid detergent fibre (ADF) residue consisting of cellulose- and lignin-like product. Cold extraction with 72% sulphuric acid (H_2SO_4) dissolved cellulose-like products, leaving a lignin-like residue. Ash content was determined after ashing in a muffle furnace at 600°C. The Folin-Denis method was used for the extraction of total extractable polyphenols of the seed cake with 50% methanol at 80°C using tannic acid as standard (Anderson and Ingram 1989).

Experimental set-up and treatments. Soil samples were taken to the laboratory and treated as follows. The soil was sieved separately (< 5 mm), characterized and adjusted to 40% water holding capacity (WHC) by adding distilled water. The soil was conditioned in drums containing a jar with 1 l distilled water to avoid desiccation and 250 mL 1 mol/L NaOH to trap evolved CO_2 for seven days.

After one week, three different treatments were applied to the different soil samples. Soil was amended with 3.5 g *J. curcas* fresh seed cake/kg soil, 4.5 g fresh castor bean cake/kg soil or left unamended. The amount of seed cake applied was such that 1 g C /kg soil was added.

Incubation experiment to measure mineral N in soil and emissions of CO_2. Eighteen sub-samples of 50 g soil (three treatments for six measurements over time (day 0, 3, 7, 14, 28 and 56 days)) from each plot (n = 5) sampled at each site (n = 7) were added to 100 mL-glass flasks. Six sub-samples of soil (six measurements over time) were amended with 175 mg *J. curcas* fresh seed cake, 225 mg fresh castor bean cake or left unamended. All the 100-mL glass flasks with the sub-sample of soil were placed in a 1-L glass jar 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 containing a 25 mL flask with distilled water and a 25 mL glass flask with 20 mL 1 mol/L NaOH, but without soil were also incubated and accounted for the CO_2 trapped form the air. After 0, 3, 7, 14, 28 and 56 days, a glass jar from each treatment (n = 3), plot (*n* = 5) sampled at each site (*n* = 7), was selected at random 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 the soil extracted for mineral N with 200 mL 0.5 mol/L K_2SO_4 (Ruíz-Valdiviezo et al. 2010).

Incubation experiment to measure emissions of N_2O and CH_4. Twelve sub-samples of 10 g soil (three treatments for four measurements over time (day 0, 1, 3, and 7 days)) from each plot (*n* = 5) sampled at each site (*n* = 7) were added to 110 mL-glass flasks. Four sub-samples of soil (four measurements over time) were amended with 35 mg *J. curcas* fresh seed cake, 45 mg fresh castor bean cake or left unamended. All the 110-mL glass flasks 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 also incubated and accounted for the CO_2 in the atmosphere. After 0, 1, 3 and 7 days, a glass flask from each treatment (n = 3), plot (*n* = 5) sampled at each site (*n* = 7), was selected at random and the headspace analyzed for N_2O and CH_4. Details of the techniques used to measure N_2O and CH_4 with a gas chromatograph can be found in Ruíz-Valdiviezo et al. (2010).

The techniques used to characterize the soil, determine the mineral N and analyze the CH_4, CO_2 and N_2O emitted during the aerobic incubation are also given in Ruíz-Valdiviezo et al. (2010).

Statistical analysis. Cumulative production of CO_2 and emission of N_2O an CH_4 was regressed on elapsed time using a linear regression model which was forced to pass through the origin but allowed different slopes (production rates) for each treatment. The CH_4, N_2O and CO_2 in the atmosphere were accounted for in the control without soil.

Significant differences in the mineral N between the soils, treatments and their interactions were determined by analysis of variance (ANOVA) and based on the least significant difference using the general linear model procedure (PROC GLM, SAS Institute 1989). Significant differences in emission of CO_2, N_2O and CH_4 between treatments, soils and their interaction were determined using PROC MIXED (SAS Institute Inc. 1989).

The relationships between the soil characteristics, GHG emissions and N mineralization were analyzed by principal component analysis (PCA) using the orthogonal/varimax rotation. Details can be found in Valdez-Pérez et al. (2011). The matrix of 35 columns (seven soils with five replicates) and 20 lines (soil characteristics (pH, EC, WHC, total N, organic C, clay content, sand content, silt content), emission of CO_2, CH_4 and N_2O, and mineralized N in unamended soil and soil amended with seed cake of *Jatropha* and *Ricinus*) was used for PCA. The PCA analyses were performed using the SAS statistical package (1989).

RESULTS

Soil and seed cakes characteristics. The EC in the loamy, sandy loam or sandy clay loam soil was low and the pH ranged from slightly alkaline to acidic (Table 1). The WHC was similar in all soils and organic matter was generally high except in the soil of Vista Hermosa and Villa Morelos where the organic C content was only 5 g/kg.

The C-to-N ratio was lower (10.0) in the castor bean than in the *J. curcas* seed cake (11.3) (Table 2). The soluble fraction was much higher in the *J. curcas* than in the castor bean seed cake. Consequently, the lignin and hemicellulose content were lower in the first than in the latter. The concentration of phorbol esters in the seed cake of *J. curcas* was 0.993 mg/g. No ricin was detected in seed cake of *Ricinus communis*.

Emission of CO_2, CH_4 and N_2O. The largest emission of CO_2 was detected in the unamended soil from Tuxtla Gutiérrez and the lowest in soil from Villa Acala after 56 days (Fig. 1a). Application of *R. communis* seed cake significantly increased emission of CO_2 2.1-fold (mean of all soils) compared to the unamended soil, while application of *J. curcas* seed cake increased it significantly 2.5 times ($P < 0.05$) (Fig. 1b, c). The CO_2 emission rate was significantly affected by treatment and soil, but not by the interaction between both ($P < 0.05$) (Table 3).

The lowest emission of CH_4 was detected in the unamended soil from Villa Acala and the highest in soil from Villa Morelos (Table 4). Application of *R. communis* seed cake significantly increased emission of CH_4 compared to the unamended soil, while application of *J. curcas* seed cake decreased it significantly ($P < 0.05$). The CH_4 emission rate was significantly affected by treatment, soil and the interaction between both ($P < 0.05$) (Table 3).

The largest emission of N_2O was detected in the unamended soil from Flores Magón and the lowest in soil from Zapotillo (Table 4). Application of *R. communis* seed cake increased emission of N_2O 21.3-fold (mean of all soils) compared to the unamended soil, while application of *J. curcas* seed cake increased it 12.6 times. The N_2O emission rate was significantly affected by soil and the interaction between soil and treatment, but not by treatment ($P < 0.05$) (Table 3).

Mineral N. The concentrations of NH_4^+ remained < 30 mg NH_4^+-N in all the unamended soils for the entire incubation (Fig. 2a). Application of *Jatropha* seed cake increased concentration of NH_4^+ sharply to > 50 mg NH_4^+-N in some soils within the first seven days, but amounts dropped < 25 mg NH_4^+-N in all soils at day 28 (Fig. 2b). Application of castor bean seed cake increased the concentration of NH_4^+ as did *Jatropha* seed cake, but the effect was less outspoken and the subsequent drop was often slower (Fig. 2c).

The concentrations of NO_2^- remained < 3 mg NO_2^--N in all unamended soils for the entire incubation (Fig. 2d). Application of *Jatropha* seed cake increased concentration of NO_2^- sharply to > 5 mg NO_2^- -N in some soils within the first 14 days, but amounts dropped < 3 mg NO_2^- -N in all soils at day 28 (Fig. 2e). Application of *Ricinus* seed cake increased the concentration of NO_2^- as did *Jatropha* seed cake, but the effect was larger and the subsequent drop was faster (Fig. 2f).

The concentrations of NO_3^- remained < 50 mg NO_3^--N in all unamended soils for the entire incubation (Fig. 2g). Application of *Jatropha* seed cake increased concentration of NO_3^- to > 50 mg NO_3^- -N in most soils after 56 day (Fig. 2h). Application of *Ricinus* seed cake had the same effect on the concentration of NO_3^- as did *Jatropha* seed cake, but the increase in the amount of NO_3^- was often larger in the first than in the latter (Fig. 2i).

Principal component analysis. Loading parameters obtained after VARIMAX rotation are given in Table 5. The value of PC1, which explained 34% of the variation, was highly variable between the soils (Fig. 3). The heavier soil of Villa Acala had high values for pH, total N, organic C, EC, WHC and mineralized N in the unamended soil (positive PC1), while the lighter soil of Villa Morelos had low values for these characteristics. Contrarily, the GHG emissions were high from the latter and low from the first. The PC2 value (loaded positively by CO_2 and N_2O emission from soil amended with *Ricinus* seed cake and CH_4 from *Jatropha*-amended soil), which explained 16% of the variation, was similar (small and negative) for all soils, except for the soil of Suchiapa. In the latter, the PC2 value was large and positive.

DISCUSSION

Characteristics of the *Jatropha curcas* and *Ricinus communis* seed cake. Organic N content of seed cakes used in this study was within the range (22.4 to 54.6 g N/kg) for oil seed cakes of the genus *Azadirachta, Laurus, Ricinus and Jatropha* (Gaind et al*. 2*009, Chaturvedi et al*. 2*010, Devappa et al*. 2*010, Ertas and Alma 2010). Devappa et al. (2010) reported a C:N value of 8.6 for *Jatropha* seed cake and Müller and von Fragstein und Niemsdorff (2006) 8.5 for castor bean cake. The C-to-N ratio was lower in the castor bean than in the *J. curcas* seed cake as reported also by Gaind et al. (2009).
The soluble sfraction of *Jatropha* seed cake was 674 g/kg and 324 g/kg for castor bean seed cake. Martín et al. (2010) reported values ranging from 530 g/kg to 900 g/kg for seed cakes of the genus *Jatropha, Azadirachta, Moringa, Aleurites and Ricinus.* The soluble fraction was much higher in *J. curcas* seed cake than in castor bean seed cake and was similar to values reported for *J. curcas* and *R. communis* seed cakes in Cuba (Martín et al*. 2*010). The lignin and hemicellulose content were lower in the *Jatropha* than in the *Ricinus* seed cake. The hemicellulose content of castor bean and *Jatropha* seed cakes were larger than values reported by Martín et al. (2010) 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 castor bean seed cake, which was similar to that found in palm kernel and olive was due to the husks contained in the cake (Ramachandran et al*. 2*007). Ricin was not detected in the defated *Ricinus*

communis seed cake as a result of heating with sodium hydroxide. Barnes et al. (2009) reported that the glycoprotein ricin degraded under these conditions.

The concentration of phorbol esters in the seed cake of *J. curcas* was 0.93 mg/g, which was lower than that reported in others studies (Makkar et al. 1997, Martínez-Herrera et al. 2006, Devappa et al. 2010). Saetae et al. (2011) reported that the phorbol ester content was 0.73 mg/g in *J. curcas* seed cake and depended on variety, cultivation technique, plant maturity and oil extraction method.

C mineralization of *J. curcas* and *R. communis* seed cakes amended to soil. If we considered no priming effect, then 64% of the C added with *Jatropha* seed cake and 49% of the C added with *Ricinus* seed cake mineralized within 56 days. The decomposition of seed cake was high at the onset of the experiment and decreased thereafter. A larger emission of CO_2 at the onset of the experiment has also been observed by Müller and von Fragstein und Niemsdorff (2006), and can be attributed to the mineralization of the easily decomposable fraction, e.g. extractable polysaccharides. Once this fraction is mineralized, emission of CO_2 drops as only more recalcitrant organic material remains. Consequently, the CO_2 emission from the *Jatropha*-amended soil was higher than from the *Ricinus* seed cake-amended soil as the soluble fraction was nearly twice as high in *Jatropha* than in *Ricinus* seed cake (Sakala et al. 2000). The soluble fraction is also known to stimulate the *decomposition* of the (hemi)cellulose (Vanlauwe et al. 1997).

N mineralization in *J. curcas* and *R. communis* seed cakes amended soil. Application of *J. curcas* seed cake increased the amount of mineral N with 76 mg N/kg soil and *R. communis* with 98 mg N/kg soil (mean of mineral N in all soils amended with *J. curcas* or *R. communis* seed cake at day 56 minus the mean of mineral N in all unamended soils at day 56). Nearly all the organic N in both seed cakes, i.e. 86% in the *Jatropha* and 97% in *Ricinus* seed cake, mineralized within 56 days. As such, nearly all of the organic N in the seed cake will become available as nutrient in soil (Müller and von Fragstein und Niemsdorff 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 *Ricinus* seed cake was larger than in *Jatropha* so more mineral N will be released. Second, more of the organic C in the *Jatropha* seed cake was mineralized so more N will be immobilized by the soil microorganisms reducing the mineral N.

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, the compound responsible for the toxicity in *Jatropha* seed, of the seed cake used in this experiment was low compared to values reported (0.73 to 3.85 mg/g) (Makkar et al. 1997, Martínez-Herrera et al. 2006, Devappa et al. 2010, Saetae et al. 2011). Even if some phorbol esters would remain in the *Jatropha* seed cake, 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 castor bean cake so it can easily be applied to soil as organic fertilizer, but it might be worthwhile to analyse castor bean cake for ricin before it is applied routinely to soil.

Emission of N_2O and CH_4. The emission of N_2O was higher from the soil amended with *Ricinus* seed cake than that amended with *Jatropha* seed cake. As mentioned before, the amount of N mineralized was larger in the first (98 mg N/kg soil, mean of all soils) than the latter (76 mg N/kg) so more nitrification occurred and N_2O is emitted when NH_4^+ is oxidized to NO_2^- and then to NO_3^- (Khalil et al. 2009).

All soils from Chiapas were sinks for CH_4, i.e. oxidation was larger than production. Soils can be a sink or a source for CH_4 although their contribution to the global warming potential of an agricultural system is small (Chan and Parkin 2001), except for rice cultivating systems (Qin et al. 2010). Agricultural soils that are a sink for CH_4 can become a source when organic material is applied (Amon et al. 2006). Collins et al. (2011) found that methane 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 *Jatropha* increased microbial activity more than when *Ricinus* was added, so emission of CH_4 increased more in the first than in the latter.

Principal components analysis and soil characteristics. The GHG emissions from the unamended soil were correlated, loaded negatively on PC1 and most accentuated in light soils (Villa Morelos and

Villa Hermosa). Fangueiro et al. (2010) reported similar results. The amount of mineralized N, however, showed opposite trends and was higher in heavy soils (Villa Acala and Tuxtla Gutiérrez). Increased organic matter mineralization, i.e. increased emission of CO_2, will increase emissions of N_2O as N cycling increases when organic matter decomposition increases (Dittert et al. 2005). Increased microbial activity will increase anaerobic micro-sites in soil and thus emissions of CH_4. Mineralization of *Jatropha* seed cake was higher in heavy soils, but no such effect was found for mineralization of *Ricinus* seed cake.

CONCLUSION

It was found that *R. communis* did not contain ricin and the phorbol ester content of *J. curcas* was low. Application of *R. communis* or *J. curcas* seed cake increased emission of CO_2, CH_4 and N_2O. The effect of *J. curcas* seed cake on emission of CO_2 was more outspoken than that of *R. communis*, while the effect of *R. communis* on emission of N_2O and CH_4 was larger than that of *J. curcas*. Nearly all the organic N in both seed cakes was mineralized within 56 days. Mineralization of *Jatropha* seed cake was higher in heavy soils, but no such effect was found for mineralization of *Ricinus* seed cake.

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Legends to the figures

Fig. 1. a) Emission of CO_2 (mg C/kg dry soil) from unamended soil of Flores Magón (▲), Suchiapa (□), Tuxtla Gutiérrez (■), Villa Acala (△), Vista Hermosa (○), Villa Morelos (●) and Zapotillo (◆), b) soil amended with *Jatropha curcas* L. seed cake or c) *Ricinus communis* L. seed cake incubated aerobically at 22^{o}C for 56 days. Bars are one STD (*n* = 5).

Fig. 2. a) Concentration of NH_4^+ (mg N/kg dry soil) in unamended soil of Flores Magón (▲), Suchiapa (□), Tuxtla Gutiérrez (■), Villa Acala (△), Vista Hermosa (○), Villa Morelos (●) and Zapotillo (◆), b) soil amended with *Jatropha curcas* L. seed cake or c) *Ricinus communis* L. seed cake incubated aerobically at 22^{o}C for 56 days. d) Concentration of NO_2^- (mg N/kg dry soil) in unamended soil, e) soil amended with *J. curcas* L. seed cake or f) *R. communis* L. seed cake, g) Concentration of NO_3^- (mg N/ kg dry soil) in unamended soil, h) soil amended with *J. curcas* L. seed cake or i) *R. communis* L. seed cake. Bars are one STD (*n* = 5).

Fig. 3. Principal component analysis with a matrix of 35 columns (seven soils with five replicates) and 20 lines (soil characteristics (pH, EC, WHC, total N, organic C, clay content, sand content, silt content), emission of CO_2, CH_4 and N_2O, and mineralized N in unamended soil and soil amended with seed cake of *Jatropha* and *Ricinus*). The PC1 explained 34% and PC2 16% of the variation.

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

a EC: Electrolytic conductivity, b WHC: Water holding capacity

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

Soil	C organic	Total N	Soluble	(Hemi)cellusose	Cellulose	Lignin	Polyphenols	Ash
	(g/kg)							
Jatropha curcas	430 [a]	38.0	674	174	143	98	0.085	53
Ricinus communis	411	41.7	324	383	269	24	0.134	55

[a] Mean of three replicates (n = 3)

Table 3. The effect of treatment (unamended soil or soil amended with seed cake of castor bean (*Ricinus communis* L.) or piñón (*Jatropha curcas* L.)), soil (seven soils of Chiapas, Mexico) and their interaction on emissions of CO_2, N_2O and CH_4

	Emission of CO_2		Emission of CH_4		Emission of N_2O	
	F value	P-value	F value	P-value	F value	P-value
Treatment	46.19	<0.0001	20.38	<0.0001	0.90	0.4067
Soil	3.51	0.0022	3.51	0.0028	3.28	0.0038
Treatment*Soil	0.61	0.8358	19.09	<0.0001	9.25	<0.0001

Table 4. Emissions of N_2O (mg N/kg) and CH_4 (mg C/kg) from seven soils of Chiapas (Mexico) left unamended (control) or amended with seed cake of castor bean (*Ricinus communis* L.) or piñón (*Jatropha curcas* L.)

	Emission of CH_4				Emission of N_2O			
Characteristic	Unamended	*Jatropha*	*Ricinus*	SEE [a]	Unamended	*Jatropha*	*Ricinus*	SEE
	(mg C/kg soil)				(mg N/kg soil)			
Flores Magon	-0.118	-0.042	-0.031	0.020	0.61	0.97	1.53	0.51
Suchiapa	-0.127	0.055	-0.026	0.019	0.09	5.48	11.51	0.92
Tuxtla Gutierrez	-0.118	0.028	-0.065	0.015	0.05	0.73	2.00	0.14
Villa Acala	-0.136	-0.053	-0.057	0.021	0.02	4.50	2.59	0.36
Villa Hermosa	-0.012	0.017	-0.019	0.006	0.38	1.54	4.21	0.49
Villa Morelos	-0.006	-0.006	-0.016	0.005	0.04	0.36	0.86	0.06
Zapotillo	-0.051	-0.029	-0.028	0.006	0.00	1.25	2.49	0.20
SEE	0.023	0.010	0.008		0.17	0.55	0.59	

[a] SEE: Standard error of the estimate at $P < 0.05$

Table 5. Rotated loadings on the principal components

Measurements	Principal components PC1	PC2	PC3
Eigenvalues	3.26	1.97	1.41
Proportions	0.22	0.13	0.09
	Rotated loading on retained components [b]		
Organic C	72 *	43 *	- 36
Total N	94 *	15	- 22
pH	84 *	35	17
Electrolytic conductivity (EC)	26	- 23	81 *
Clay	92 *	- 16	2
Sand	- 65 *	40 *	31
Loam	- 41 *	- 20	- 47
Water holding capacity (WHC)	73 *	- 35	- 43 *
CO_2 emission from the unamended soil	- 77 *	15	- 24
CO_2 emission from *Jatropha*-amended soil	23	- 3	- 23
CO_2 emission from *Ricinus*-amended soil	- 11	75 *	- 18
CH_4 emission from the unamended soil	- 84 *	- 35	- 4
CH_4 emission from *Jatropha*-amended soil	- 21	59 *	51 *
CH_4 emission from *Ricinus*-amended soil	- 69 *	5	11
N_2O emission from the unamended soil	61 *	- 35	- 36
N_2O emission from *Jatropha*-amended soil	1	0	- 55 *
N_2O emission from *Ricinus*-amended soil	6	13	- 8
Mineralized N in the unamended soil	- 27	0	68 *
Mineralized N in the *Jatropha*-amended soil	49 *	34	19
Mineralized N in the *Ricinus*-amended soil	10	87 *	15

[*] Parameters with significant loadings on the within column principal component

Fig. 1

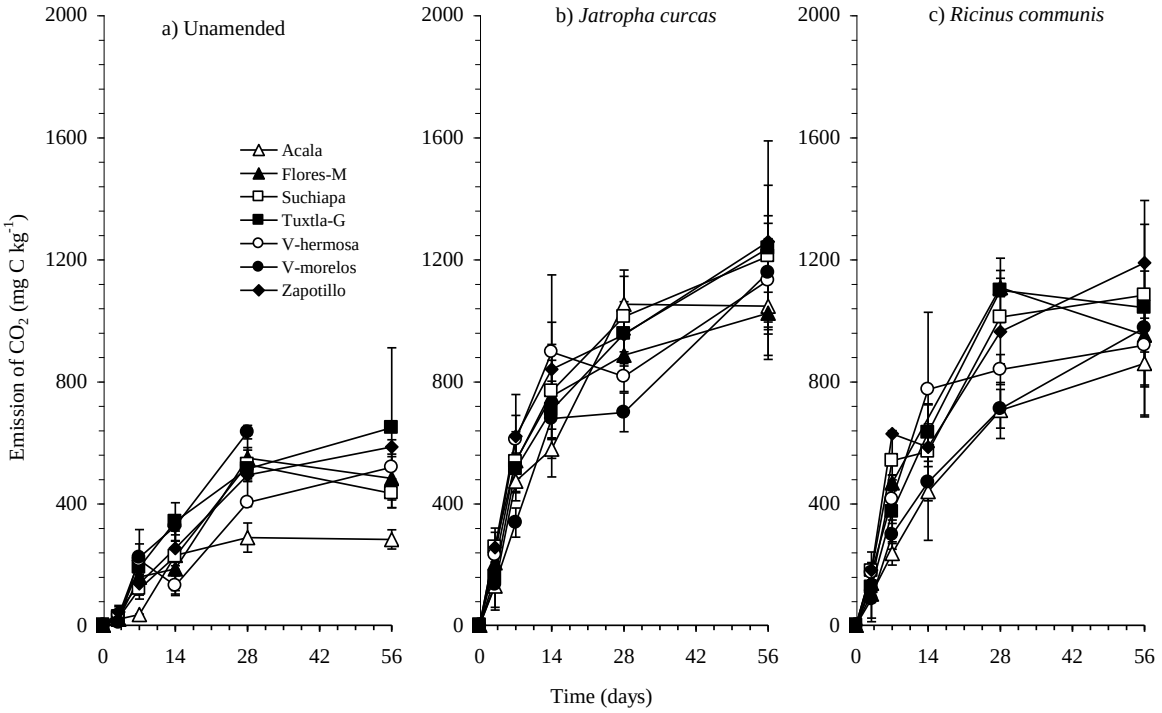

a) Unamended
b) *Jatropha curcas*
c) *Ricinus communis*
Acala
Flores-M
Suchiapa
Tuxtla-G
V-hermosa
V-morelos
Zapotillo
Emission of CO_2 (mg C kg^{-1})
Time (days)
0
400
800
1200
1600
2000
0
14
28
42
56

Fig. 2

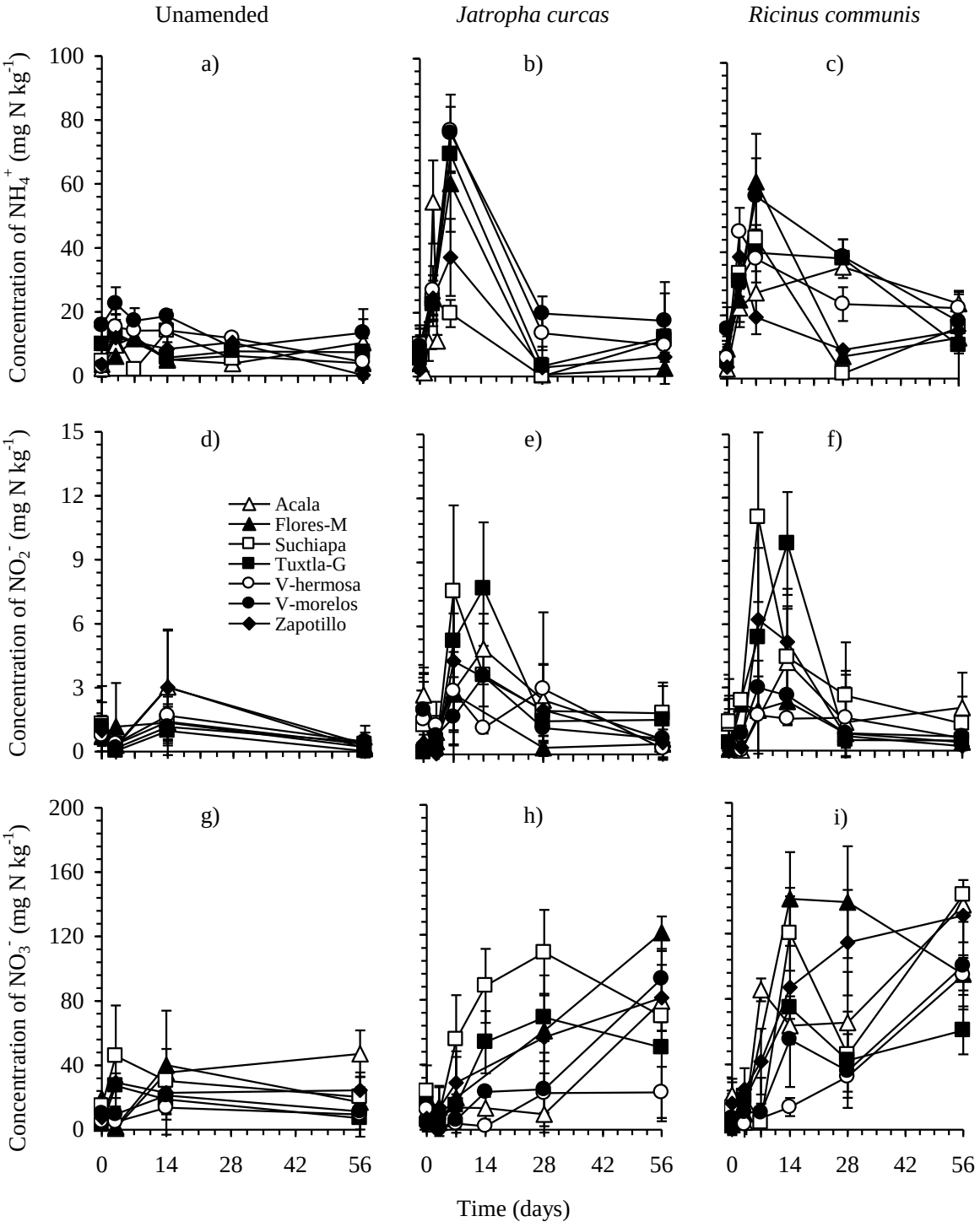

Unamended
Jatropha curcas
Ricinus communis
a)
b)
c)
d)
e)
f)
g)
h)
i)
Concentration of NH_4^+ (mg N kg^{-1})
Concentration of NO_2^- (mg N kg^{-1})
Concentration of NO_3^- (mg N kg^{-1})
Acala
Flores-M
Suchiapa
Tuxtla-G
V-hermosa
V-morelos
Zapotillo
Time (days)

Fig. 3

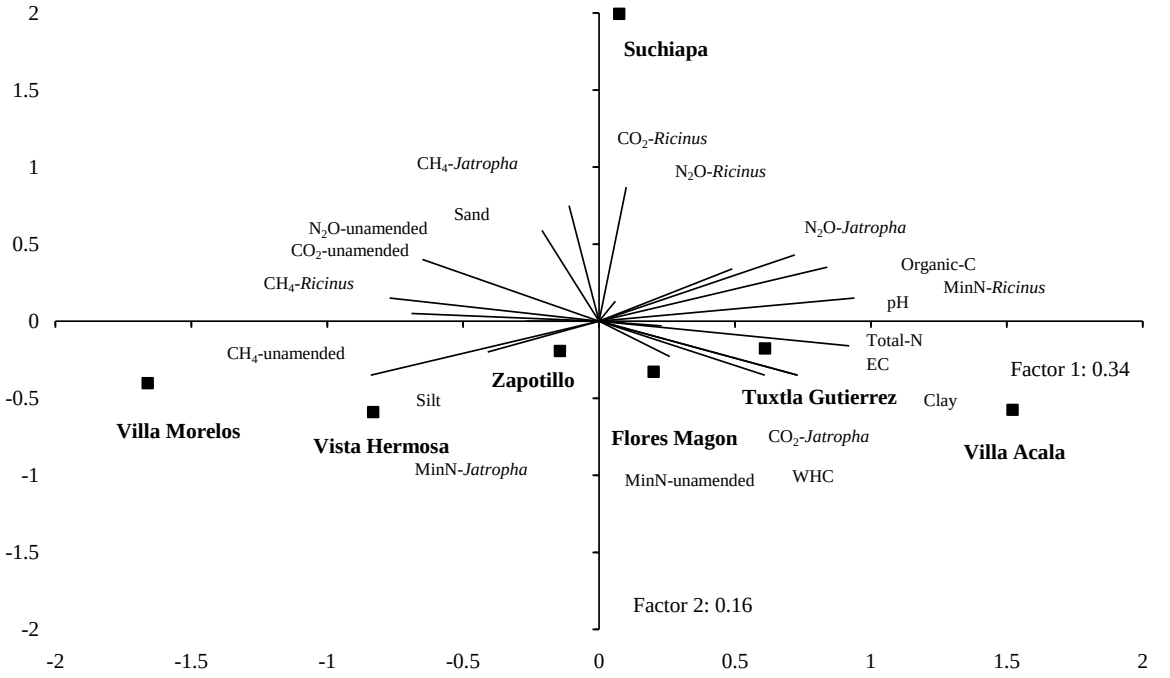
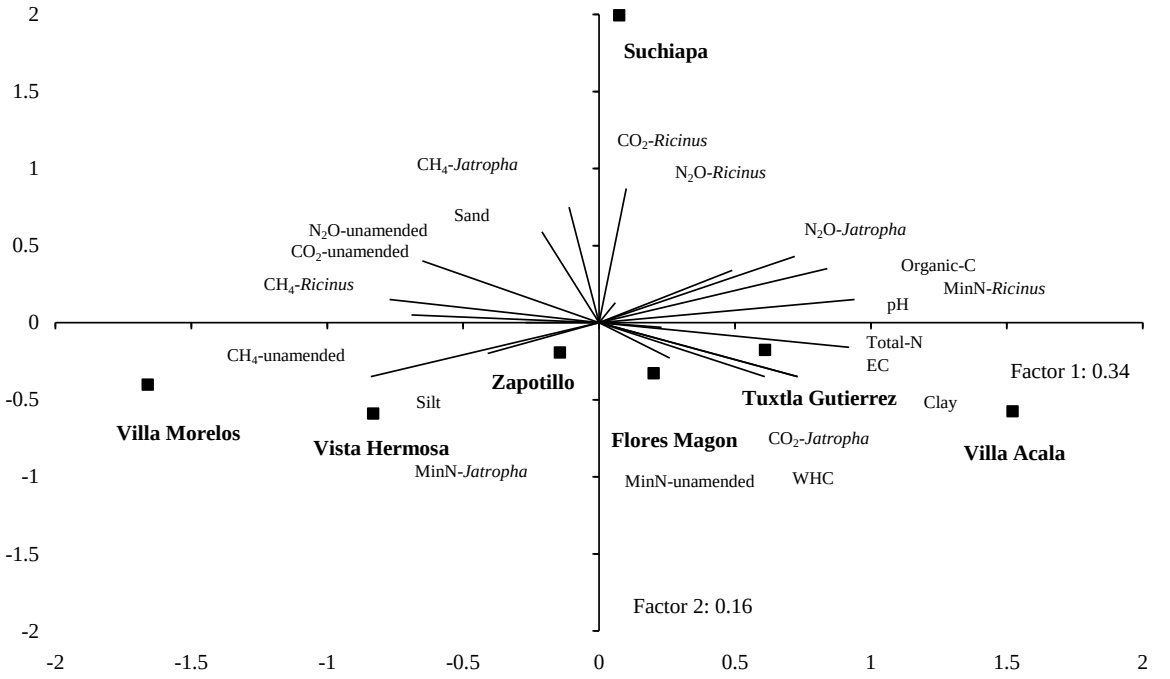

Suchiapa
CO2-Ricinus
N2O-Ricinus
CH4-Jatropha
Sand
N2O-unamended
CO2-unamended
CH4-Ricinus
N2O-Jatropha
Organic-C
MinN-Ricinus
pH
Total-N
EC
CH4-unamended
Zapotillo
Silt
Tuxtla Gutierrez
Clay
Villa Morelos
Vista Hermosa
MinN-Jatropha
Flores Magon
CO2-Jatropha
Villa Acala
MinN-unamended
WHC
Factor 1: 0.34
Factor 2: 0.16
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2

Fig. 3


Suchiapa
CO2-Ricinus
N2O-Ricinus
CH4-Jatropha
Sand
N2O-unamended
CO2-unamended
CH4-Ricinus
N2O-Jatropha
Organic-C
MinN-Ricinus
pH
Total-N
EC
CH4-unamended
Zapotillo
Silt
Tuxtla Gutierrez
Clay
Villa Morelos
Vista Hermosa
MinN-Jatropha
Flores Magon
CO2-Jatropha
Villa Acala
MinN-unamended
WHC
Factor 1: 0.34
Factor 2: 0.16
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2

Artículo ya enviado a la revista

Letters in Applied Microbiology

A method to produce ^{13}C-enriched plant material and to study its incorporation into microbial DNA

Running head: **^{13}C-marked material incorporated in microbial DNA**

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Significance and impact of the study

New molecular biology techniques have increased our knowledge of the soil microbial community structures in soil. The functional importance of these microorganisms in soil, e.g. decomposition of organic material, is still largely unknowm. Maize was ^{13}C labelled and amended to soil. The soil was incubated, the DNA extracted after 1, 3, 7, 14 and 28 days and the separation of ^{13}C labelled DNA from the unlabelled DNA optimized. The DNA was of sufficient quality to identify bacteria involved in the

mineralization of the maize residue. The proposed technique allows to study the functionality of microbial populations in different ecosystems.

Abstract

Crop residues are important to maintain soil fertility as they improve soil structure and provide nutrients to cultivated plants, but little is known about the microorganisms are involved in their degradation. Maize was cultivated in a $^{13}CO_2$ enriched environment, added to soil and incubated aerobically. The total DNA was extracted from soil and the light (unlabelled) and heavy DNA fraction (^{13}C-labelled) separated by ultracentrifugation. The heavy and light DNA was cloned and characterized. The ^{13}C enrichment in the plant shoot was 35 atom% and 14 atom% in the roots. The buoyant density of the light DNA fraction in CsCl was 1.72 g ml^{-1} and the heavy fraction 1.75 g ml^{-1} when centrifuged at 45,000 rpm and 20 °C for 40 h. The 16S rDNA of the extracted ^{13}C-labelled DNA was amplified by PCR, cloned and identified. It was found that application of ^{13}C pulse labelled maize plants to soil allow to identify the microorganisms involved in the degradation of crop residues.

Keywords 16S rRNA, Pulse labelling with $^{13}CO_2$, neutral detergent fraction (NDF), growth chamber, stable isotope probing (SIP).

Introduction

$^{13}CO_2$ pulse labelling and stable isotope probing (SIP) methods offer the potential to track ^{13}C-labelled plant photosynthate into phylogenetic groups of microbial taxa in the rhizosphere, elucidate nutrient cycles and organism interactions within ecosystems

(Vandenkoornhuyse et al. 2007). Isotopic techniques have been widely used to study C cycling in soil-plant systems (Jones et al. 2009). Until recently, such studies used ^{14}C for its availability and ease of determination and sensitivity. The advances in the analysis of stable isotopes have prompted interest in the use of ^{13}C as a tracer, and methods have been developed for labelling plants with $^{13}CO_2$. The main advantage of ^{13}C over ^{14}C is that it is not radioactive, so that labelling chambers do not need extensive precautions against leaks and analysis can be conducted routinely and safely. These labelling experiments have been used to produce labelled plant material or microorganisms to investigate C dynamics in these organisms or in soil. Bromand et al. (2001), studied the partition of ^{13}C in different plant components, such as grain, chaff, stem and roots of plants cultivated wheat (*Triticum aestivum* L.) by exposing them to an atmosphere enriched with $^{13}CO_2$. They observed a similar enrichment of the different plant components: grain with 3.41%, chaff 3.41%, stem 3.65% and root 3.50% of the ^{13}C atoms. As the abundance of ^{13}C remained homogeneous in the different plant components, ^{13}C-labelled plant biomass can thus be used to study dynamics of C and microbial population in soil.

The stable isotope probing (SIP) methodology is a powerful technique. It can reveal the functional importance of microorganisms in a soil and has the capacity to reveal the uptake of labelled carbon substrates in complex bacterial communities (Dumont and Murrell, 2005). The SIP methodology typically involves the application of a stable isotope-labelled substrate to the environment of interest and then assessing the assimilation of the isotope into microbial biomarkers, such as nucleic acids, phospholipid fatty acids and ergosterol. Of these biomarkers, nucleic acids might be the most useful as they contain the most diverse taxonomic information and have a fast

turnover rate. Typically, following the addition of a ^{13}C labelled substrate, the nucleic acids are extracted and the ^{13}C-labelled portion is separated from the rest using density gradient centrifugation for subsequent molecular analysis (Radajewski et al. 2000).

Crop residues left on the field are important to maintain the soil fertility, serve as a C substrate for soil microorganisms, and provide nutrients for plants, such as nitrogen. Their degradation and the microorganisms involved are thus a key factor in soil fertility. Maize plants were cultivated in an ^{13}C-enriched atmosphere and the labelled plant material was added to an agricultural soil. The soil was incubated aerobically, the DNA extracted and the light and heavy DNA separated by ultracentrifugation. The objective of this study was to enrich maize with ^{13}C in a growth chamber, apply it to a soil and to show that it was possible to separate the light and heavy DNA fraction thereby allowing to identify the microorganisms involved in the degradation of crop residues.

Results and Discussion

^{13}C labelled maize

The total N content was 34 g kg^{-1} and the C content 422 g kg^{-1}. The size of the soluble fraction was 599 g kg^{-1}, the lignin content 99 g kg^{-1} and the (hemi)cellulose content 211 g kg^{-1}. The amount of polyphenols was 2.4 g kg^{-1} and the ash content 62 g kg^{-1}. The ^{13}C enrichment in plant shoot was 35 atom% and 14 atom% in the roots. The 35 atom% ^{13}C enrichment was higher than the 13.8 and 16.8 atom% ^{13}C reported for leaves of field pea (*Pisum sativum* L.) and canola (*Brassica napus* L.) (Sangster et al. 2010). However, the 14 atom% ^{13}C enrichment in roots of maize was lower than the 37 atom% ^{13}C reported for a genetically starch-modified potato (*Solanum tuberosum* L.) cultivar

(Hannula et al. 2012). The enrichment percentage of the plant residues depends on the time of applied pulse, $^{13}CO_2$ concentration, plant maturity, plant parts and different type chambers (Moore-Kucera and Dick 2008).

Decomposition of dried maize and SOM during incubation

The $^{12}CO_2$ production in the maize-amended soil was 10-times larger than in the unamended soil (Fig. 1). The decomposition of the maize residue was high at the beginning of the experiment and decreased thereafter. This larger emission of CO_2 at the onset of the experiment has also been observed by Abro et al. (2011). It is attributed to the rapid decomposition of the extractable polysaccharides in the maize residues. The decomposition of the more resistant fraction of the organic residue, i.e. hemicellulose and lignin, was lower so that its effect on the emission of CO_2 was less noticeable at the onset. However, other factors such as C:N and lignin:N ratio are also known to affect organic material decomposition.

Incorporation of ^{13}C in bacterial DNA

A highly effective separation of ^{12}C- and ^{13}C-DNA was obtained with the ultracentrifugation conditions applied. The optimum conditions to separate the ^{13}C labelled DNA from *E. coli* from the unlabelled DNA was centrifugation at 45,000 rpm and 20 °C for 40 h (Table 1). The bands were clearly separated by 10 mm (Fig. 2a). The width between the upper part of the visible smear (unlabelled DNA) and the bottom of the visible band (^{13}C-labelled DNA) was similar to values reported by Padmanabhan et al. (2003). They applied ^{13}C- labelled compounds (glucose, phenol, caffeine, and

naphthalene) to soil and conditions for separation of ^{12}C- and ^{13}C-labelled were 41,900 rpm at 20ºC for 66 h.

The buoyant density (ND) of the unlabelled DNA or the "light" fraction from *E. coli* in CsCl was 1.72 and 1.75 for the ^{13}C labelled DNA or "heavy" DNA (Table 1). Lueders et al. (2004a) found that the BD of the ^{13}C-labelled DNA of *Methylobacterium extorquens* grown on 99% ^{13}C-methanol increased with 0.04 g ml^{-1} compared to that of unlabelled DNA.

The same conditions (45,000 rpm at 20 °C for 40 h) were applied to separate ^{13}C-labelled and unlabelled DNA extracted from soil amended with ^{13}C-labelled maize plantlets after 1, 3, 7, 14 and 28 days. Under UV light, the extracted DNA showed a clearly visible smear of unlabelled DNA and a separate smear of ^{13}C-labelled DNA after 1, 3, 7 and 14 days (Fig. 2b), but at day 28 no clear separation of unlabelled and ^{13}C-labelled occurred (Fig. 2c). At day 1, 3, 7 and 14, the smear of the ^{13}C-labelled DNA was positioned below that of ^{12}C-DNA in the density gradient indicating a higher BD. The repartition of DNA among gradient fractions of increasing density is illustrated in Fig. 3. Similar profiles were obtained with DNA extracted from soil at day 1, 3, 7 and 14. The BD of 1.75 DNA g ml^{-1} for ^{13}C-labelled DNA extracted from soil amended with maize residue was similar to values reported by Lueders et al. 2004b. They also reported the distribution of the total DNA in the different gradient fractions and it was similar as found in this study.

One fraction of the light DNA (BD 1.72 g ml^{-1} CsCl) and another of the heavy DNA (BD 1.75 g ml^{-1}) was sampled from the ultracentrifuge tubes. An 250-µl aliquot of the heavy DNA 1.75 g ml^{-1} CsCl was sampled from the ultracentrifuge tubes for PCR of the 16S rDNA, clonation and identification.

PCR, cloning and identification of the ^{13}C-labelled bacterial DNA

The ^{13}C labelled DNA extracted from the maize amended soil was separated by ultracentrifugation and the 16S rDNA was amplified successfully by PCR. It was possible to clone the obtained fragments and in a preliminary study some of them were identified.

The ^{13}C-DNA was of sufficient quality to clone it and identify the bacteria involved or directly benefitting from the degradation of the maize residue. Twelve clones were sequenced and identified (Table 2). The sequences belonged to Proteobacteria, Acidobacteria, Actinobacteria, Cloroflexi and Gemmatimonadetes. Six phylotypes were identified to the level of genus. The preliminary study identified five genera, i.e. *Blastococcus*, *Gemmatimonas*, *Skermella*, *Sphingosinicella* and *Sphingomonas*. A species belonging to the *Sphingosinicella,* e.g. *S. vermicomposti* was isolated from vermicompost and is thus presumably involved in degradation of organic material (Yasir et al. 2010). Different species belonging to the genus *Sphingomonas* have the capacity to degrade cellulose (Talia et al. 2012). Haichar et al. (2007) added ^{13}C-cellulose to soil and incubated it for 90 days. Sequencing and phylogenetic analysis indicated that most sequences were closely related to sequences from organisms known for their ability to degrade cellulose, such as *Dyella*, *Mesorhizobium* sp., *Sphingomonas* sp., and an uncultured Deltaproteobacterium. A phylotype belonging to the genus *Sphingomonas* was also found in this study.

This is the first study in which maize plants were pulse labelled with ^{13}C and added to soil to study the bacteria involved in the degradation of maize residues. The highly ^{13}C-enriched maize plants allowed to identify the organisms involved in its

decomposition by separating ^{13}C-labelled DNA from the other DNA by ultracentrifugation at 45,000 rpm and 20 °C for 40 h. The ^{13}C-labelled DNA was of good quality and the 16S rDNA was amplified by PCR. The amplified DNA could be cloned and a preliminary analysis of the 16S rDNA identified known degraders of cellulose, i.e. *Sphingomonas*. The proposed technique will allow to elucidate the microorganisms involved in the degradation of crop residues such as maize, so that the functionality of bacterial populations in soil can be determined.

Material and Methods

Growth chamber

An acrylic chamber of 105 l (surface 35 cm × 50 cm and 60 cm high) was used for pulse-labelling maize plants with $^{13}CO_2$. The chamber was fitted with a lid with two injection ports so that the growth chamber could be closed air-tight and flushed. The chamber contained a vial with an aqueous solution of $Ca(^{13}CO_3)_2$ (99 atom%) (Sigma, Aldrich, USA). The headspace was flushed with air bubbled through 1 M NaOH so that it was CO_2-free.

Cultivation of ^{13}C-enriched plants

Maize (*Zea mays* L.) seeds were surface-sterilized with 1.5 % (v/v) sodium hypochlorite for 12 min and thoroughly washed with sterile distilled water. Seeds were germinated on 0.8% agar-water plates to induce etiolation and incubated in the dark at 28 °C for 48 h. The maize seedlings with a root of approximately 2 cm were placed on sterilized and C-free vermiculite in the growth chamber and moistened with a nutritive Steiner

191 solution (Steiner 1961). The headspace was flushed with CO_2-free air. A pulse of $^{13}CO_2$
192 was released by injecting 50% p/v H_2SO_4 from a syringe through an injection port into
193 the Ca($^{13}CO_3$) solution. This process was repeated each day. After 25 days, the maize
194 plants were harvested, air-dried and characterized.
195 The isotopic enrichment of the ^{13}C in plant residue was determined at the University
196 of California-Davis stable isotope facility (USA) using a PDZ Europa ANCA-GSL
197 elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer
198 (Sercon Ltd., Cheshire, UK).
199
200 ^{13}C-labelled maize fractionation scheme
201 The maize residue was analyzed for total N, total C and ^{13}C, acid detergent fibre (ADF),
202 lignin, polyphenol and ash content in triplicate. Details of the fractionation technique
203 can be found in Ruíz-Valdiviezo et al. (2010).
204
205 Incubation and application of ^{13}C labelled maize residue
206 The sampling site is located in Otumba (Northern Latitude 19° 41' 36' Western
207 Longitude 98° 43' 24') in the state of Mexico, Mexico. Details of the sampling site and
208 climatical conditions can be found in Mendez-Bautista et al. (2010). The area is
209 cultivated with maize and common bean, receiving a minimum amount of inorganic
210 fertilizer without being irrigated.
211 Soil samples were collected on 8 January 2010, i.e. in the dry season. In an area of
212 10,000 m^2, three different 100 m^2 plots were outlined, sampled by augering 20 times the
213 0-20 cm soil layer, pooled so that three soil samples were obtained and taken to the
214 laboratory in black polyethylene bags. The samples were 5 mm sieved and

characterized. The soil is sandy loam with pH 7.6 and electrolytic conductivity (EC) 0.480 dS m^{-1}, an organic C content of 13.4 g C kg^{-1} soil, 0.7 g kg^{-1} of total N and WHC of 552 g kg^{-1} dry soil. The field based replication was maintained in the incubation study.

The soil was adjusted to 40% water holding capacity (WHC) by adding distilled water and pre-incubated for 7 days in a drum containing a beaker with 100 ml to avoid desiccation and one beaker with 100 ml of 1 M NaOH solution to trap CO_2 evolved. Seven sub-samples of 5 g soil from each of the three soil samples were added to 60 ml glass flaks, amended with 23.7 mg ^{13}C-labelled maize residue (42.2% C) so that 2 g C kg^{-1} soil was added (considered the MAIZE treatment) or left unamended (considered the CONTROL treatment). One flask of each soil sample and each treatment was selected at random, the 5 g soil removed and extracted for DNA. This was considered the zero-time sample.

The remaining flasks were placed in 940 ml glass jars containing a vessel with 10 ml distilled water (H_2O) to avoid desiccation and a vessel with 20 ml 1 M sodium hydroxide (NaOH) to trap carbon dioxide (CO_2) evolved. The jars were sealed and incubated at 22 ± 2 °C for 56 days. An additional 18 jars without soil, but containing a vessel with 10 ml distilled H_2O and one with 20 ml 1 M NaOH, were sealed and served as controls to account for the CO_2 trapped from the atmosphere. After 1, 3, 7, 14, 28 and 56 days, one jar was selected at random from each treatment (n = 2) and soil (n = 3). Three flasks without soil were also selected at random. The flasks were opened, the vessel with 1 M NaOH removed, stoppered and the soil was treated as described for zero-time samples. All remaining jars were opened, aired for 10 min, resealed and further incubated.

239

240 Nucleic acid extraction

241 Total DNA was extracted directly from soil in triplicate by a method based on direct
242 cell lysis as described by Guo et al. (1997). Details of the modified technique can be
243 found in Valenzuela-Encinas et al. (2008). DNA samples were visualized and DNA was
244 quantified on a Nanodrop ND-1000 spectrophotometer (Thermo, Waltham, MA, USA).
245 Yield of the total DNA extracted from the microcosms was 2.1 µg g^{-1} soil. The DNA
246 was stored at -80 °C.

247

248 Separation of the ^{13}C-labelled DNA from the unlabelled DNA

249 The heavy and light DNA were separated by density gradient ultracentrifugation using
250 CsCl as described by Radajewski et al. (2000). The total extracted DNA was added to a
251 CsCl – SYBR Gold – TE buffer. The volume of the DNA extract was adjusted to 500 µl
252 with TE (10:1) buffer. SYBR® Gold DNA was used in the CsCl density gradients
253 instead of ethidium bromide as this simplifies the cleanup of fractions collected from
254 ultracentrifuge tubes. One gram of CsCl was dissolved in this solution and 25 µl
255 10,000× SYBR® Gold DNA stain (Invitrogen, Carlsbad, CA, USA) added. The
256 resulting mixture was added to 4.9 ml Optiseal tubes (Beckman Coulter, CA, USA).
257 The tubes were filled with 1 g ml^{-1} CsCl solution. The final density of the solution was
258 1.725 g ml^{-1}. The tubes were centrifuged at 45,000 rpm and 20 °C for 12, 24, 36 or 40
259 h in an Optima™ L-100 XP Ultracentrifuge (Beckman Coulter) equipped with an
260 ultracentrifuge rotor VTi 65.2 (Beckman Coulter, CA, USA). The DNA bands were
261 visualized by illumination with a portable UV lamp at 365 nm. Centrifuged gradients
262 were fractionated from bottom to top into 14 equal fractions (~150 µl). The fraction

densities were measured with a Reichert AR200 handheld digital refractometer. SYBR® Gold DNA stain was removed with isoamyl alcohol. The CsCl from the DNA solution was removed with Millipore`s Amicon® Ultra-0.5 centrifugal filters (Millipore, Carrigtwohill Co., CORK, Ireland) for concentration and purification of DNA solutions.

A tube with ^{13}C-labelled and unlabelled *Escherichia coli* was used as control. To obtain ^{13}C-DNA, *E. coli* JM 109 was grown on uniformly labelled ^{13}C-glucose (Sigma Aldrich, St. Louis MO, USA) in MM media which had the following composition (per litre): 10 g ^{13}C-Glucose; 5 g $(NH_4)_2SO_4$; 2 g $MgSO_4 \cdot 7H_2O$; 2 g K_2HPO_4; 1 g $FeSO_4 \cdot 7H_2O$ (pH 6.5-7.0). *Escherichia coli* DNA was extracted with a QIAcube automatic system and purified with the DNeasy Blood & Tissue Kit (Qiagen, USA) following the manufacturer's protocol. *Escherichia coli* grown on analytical grade glucose provided unlabelled DNA and was collected in the same way. Control tubes were prepared by mixing 5 µg ^{12}C-DNA and ^{13}C-DNA of *E. coli* and was loaded into a CsCl– SYBR Gold – TE buffer as described above.

Cloning, sequencing and nucleotide sequence accession numbers

The 16S rDNA fragments were obtained with the primers 46F and 1540R. Details of the cloning procedure and sequencing can be found in Valenzuela-Encinas et al. (2008). The sequence were deposited in the GenBank database and assigned the accession numbers JX163120 to JX163131.

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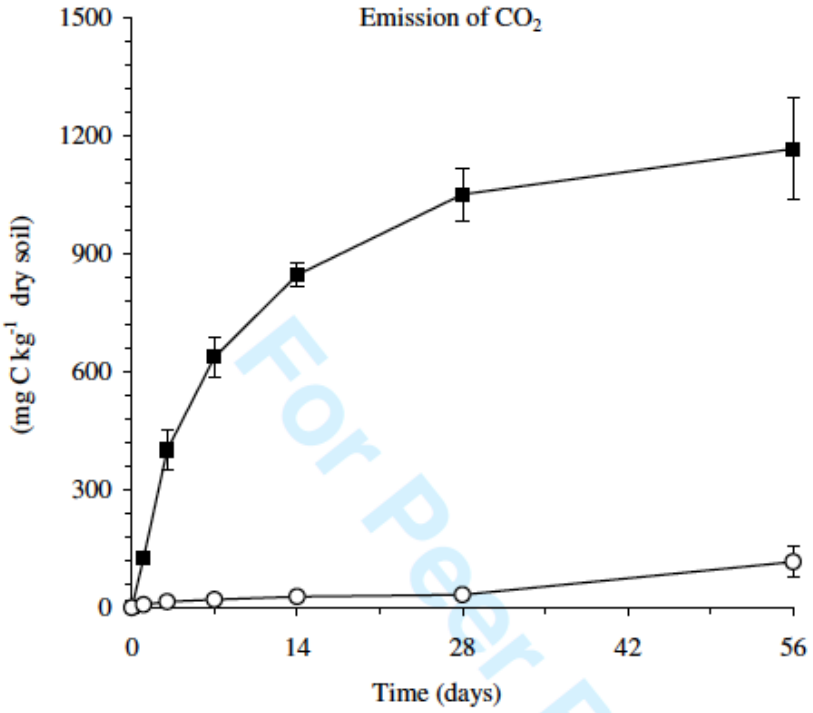
Legends to the figures

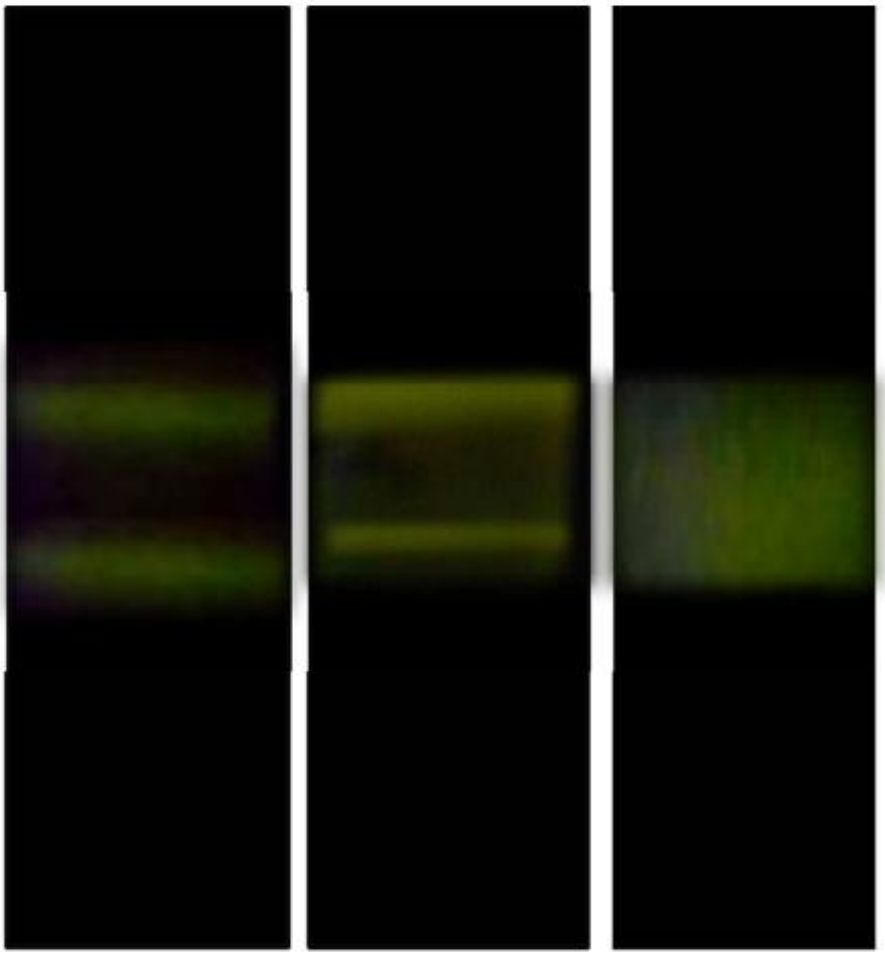
Fig. 1 Emission of CO_2 form a maize-amended and unamended soil incubated at 22 ±2°C aerobically for 56 days. Bars are ± std.

Fig. 2 Separation of light and heavy DNA from a) *E. coli* and b) extracted from soil amended with ^{13}C-labelled maize residue at day 3 and c) at day 28.

Fig. 3 Separation of light and heavy DNA extracted from a soil amended with ^{13}C-labelled maize and incubated for 3 days. The DNA content of each fraction was quantified fluorometrically.

Fig. 1





139x155mm (150 x 150 DPI)

Fig. 3

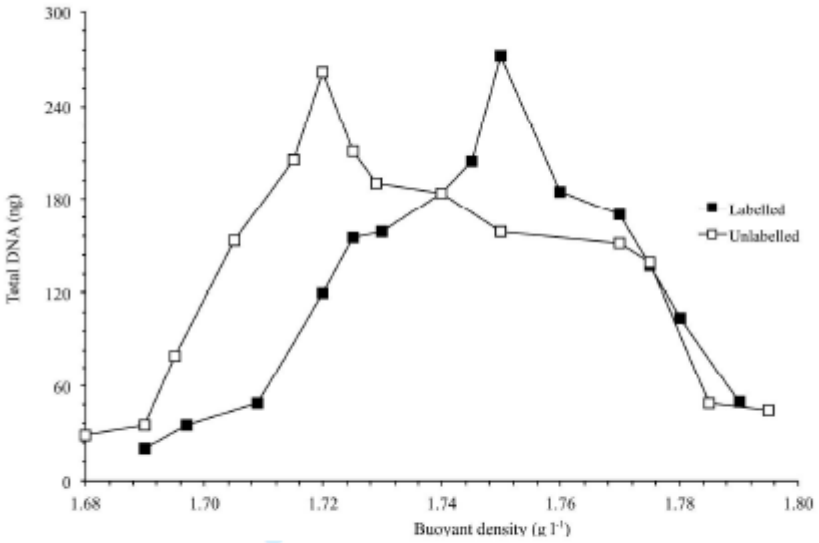


Table 1 Centrifugation conditions and buoyant density of ^{12}C-DNA and ^{13}C-labelled DNA in CsCl

Centrifugation condition	Separation (mm)	Density (g l^{-1})	
		^{12}C-DNA	^{13}C-DNA
20°C, 45, 000 rpm, 12 h	ND *	1.71	1.74
20°C, 45, 000 rpm, 24 h	8	1.70	1.74
20°C, 45, 000 rpm, 30 h	8	1.72	1.75
20°C, 45, 000 rpm, 40 h	10	1.72	1.75

*ND: No determined

Table 2 Classification of twelve clones involved or directly benefitting from the degradation of the maize residue when added to soil

Accession code	Phylum	Class	Subclass	Order	Suborder	Family	Genus
JX163120	Acidobacteria	Acidobacteria_Gp1	NC	NC	NC	NC	NC
JX163121	Proteobacteria	Alphaproteobacteria	NC	Rhodospirillales	NC	Rhodospirillaceae	NC
JX163122	Actinobacteria	Actinobacteria	Actinobacteridae	Actinomycetales	Frankineae	Geodermatophilaceae	Blastococcus
JX163123	Proteobacteria	Deltaproteobacteria	NC	Myxococcales	NC	NC	NC
JX163124	Actinobacteria	Actinobacteria	Rubrobacteridae	NC	NC	NC	NC
JX163125	Gemmatimonadetes	Gemmatimonadetes	NC	Gemmatimonadales	NC	Gemmatimonadaceae	Gemmatimonas
JX163126	Actinobacteria	Actinobacteria	NC	NC	NC	NC	NC
JX163127	Proteobacteria	Alphaproteobacteria	NC	Rhodospirillales	NC	Rhodospirillaceae	Skermanella
JX163128	Proteobacteria	Alphaproteobacteria	NC	Rhodospirillales	NC	Rhodospirillaceae	Skermanella
JX163129	Chloroflexi	Anaerolineae	NC	Anaerolineales	NC	Anaerolineaceae	NC
JX163130	Proteobacteria	Alphaproteobacteria	NC	Sphingomonadales	NC	Sphingomonadaceae	Sphingosinicella
JX163131	Proteobacteria	Alphaproteobacteria	NC	Sphingomonadales	NC	Sphingomonadaceae	Sphingomonas

NC: Not classified

Actividades extraordinarias

Como parte de los estudios de Doctorado en el grupo de trabajo del Dr. Luc Dendooven desarrollé otras investigaciones experimentales que han arrojado resultados con los que se escribieron otros artículos científicos publicados (3) y enviados (6) a revistas indizadas.

ARTÍCULOS PUBLICADOS EN REVISTAS INDIZADAS EN *ISI Web of Knowledge*sm

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Conclusiones

La cantidad de hojas de *Jatropha* descompuestas fue baja debido a que la emisión de CO_2 fue más baja que la producción de CO_2 de otros residuos derivados de plantas. Como tal, el cultivo del árbol aumentará la fertilidad mejorando el contenido carbono orgánico de los suelos degradados.

Así, también las hojas de *Jatropha* curcas no inhibieron la actividad de metanótrofos o indujeron sitios anaerobios incrementando la emisión de CH_4. Sin embargo, las emisiones de N_2O aumentaron en el suelo enmendado con hojas de *Jatropha* debido a un aumento en el ciclo de N, esto pudo ser posible debido a que las hojas de *Jatropha* curcas tuvieron una alta concentración de nitrógeno.

La adición de los residuos derivados de plantas a un suelo agrícola de Otumba, o bien, al suelo salino-alcalino del exlago de Texoco, permite la recuperación de suelos, mejorando su fertilidad y por ende, los redimientos de los cultivos; sin embargo, esto depende de la calidad de la materia orgánica que se adicione.

En condiciones de laboratorio, la aplicación de residuos de maíz marcados con ^{13}C a un suelo agrícola incrementa la producción de CO_2 significativamente durante el periodo de incubación.

La combinación de las técnicas isotópicas *'$^{13}CO_2$ pulse-labelling'* y ^{13}C-DNA SIP permitió identificar a los microorganismos involucrados en la biodegradación de residuos de plantas y su funcionalidad dentro del ecosistema.

Perspectivas

Con base en que los resultados obtenidos en esta investigación tienen gran utilidad para determinar la presencia de microorganismos activos en el proceso de biodegradacion de plantas, deben enriquecerse mediante la técnica isotópica *'$^{13}CO_2$ pulse-labelling'* otras especies de plantas y establecerse experimentos adicionales en los que se incorporen sus residuos marcados con ^{13}C a a suelos sujetos a diferentes perturbaciones. También, se pueden establecer otros experimentos donde se incorporen otros substratos marcados con ^{13}C y ^{15}N a los suelos que permitan identificar los microorganismos metabólicamente activos en diferentes procesos biológicos del suelo, utilizando la prueba DNA SIP establecida en este proyecto doctoral.

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