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Research paper

# Conversion of grazed pastures to energy cane as a biofuel feedstock alters the emission of GHGs from soils in Southeastern United States

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ABSTRACT

The cultivation of energy cane throughout the Southeastern United States may displace grazed pastures on organic soil (Histosols) to meet growing demands for biofuels. We combined results from a field experiment with a biogeochemical model to improve our understanding of how the conversion of pasture to energy cane during early crop establishment affected soil GHG ($CO_2$, $CH_4$, and $N_2O$) exchange with the atmosphere. GHG fluxes were measured under both land uses during wet, hot and cool, dry times of year, and following a fertilization event. We also simulated the impact of changes in precipitation on GHG exchange. Higher fertilization of cane contributed to greater emission of $N_2O$ than pasture during warmer and wetter times of the year. The model predicted that energy cane emitted more nitrogen than pasture during simulated wetter than drier years. The modeled emission factor for $N_2O$ was 20 to 30-fold higher than the default value from IPCC (1%), suggesting that the default IPCC value could dramatically underestimate the consequences of this land conversion on the climate system. Predicted soil $CH_4$ and $CO_2$ fluxes were higher in pasture than energy cane, and this difference was not affected by increasing precipitation. Model simulations predicted that soils under first year cane emit more GHGs than pasture, particularly during wet years, but this difference disappeared two years after energy cane establishment. Our results suggest that management practices may be important in determining soil GHG emissions from energy cane on organic soils particularly during the first year of cane establishment.

## 1. Introduction

Land use change – transforming land cover or changing management practices – impacts climate by affecting the emission of greenhouse gases (GHGs; $N_2O$, $CH_4$ and $CO_2$) from ecosystems [1–3]. The need for alternative energies is accelerating the conversion of marginal land and managed ecosystems to biofuel crops [4], and these changes are likely to impact the exchange of GHGs with the atmosphere [5].

Currently, most renewable fuel in the US is derived from corn ethanol; however, the Renewable Fuel Standard mandates that 60.6 billion L of renewable fuels must be supplied by ligno-cellulosic sources or other advanced renewable fuels by 2022. Crops grown in the Southeastern USA will contribute to meet the demand for renewable fuels from ligno-cellulosic feedstocks [6,7]. Because of its high biomass yields [8,9], energy cane (*Saccharum* spp. L), a high-cellulose producing variety of sugarcane, is a promising perennial crop for ligno-cellulosic fuel production that can be grown in regions of the Southeastern US such as Florida [10–12].

In the subtropical and tropical regions of Florida, grazed pastures, which cover > 30% of the total land area (170405 km$^2$) [13], will potentially be replaced by energy cane plantations. Most of these grazed pastures are planted in the highly organic soil, Histosols [14–17]. The cultivation of Histosols is likely to emit substantial amounts of carbon and nitrogen to the atmosphere [18,19], although the impact of this conversion on climate is uncertain [20–22].

The changes in vegetation and management associated with converting grazed pasture to energy cane plantations could alter the emission of GHGs from soils, especially following cultivation after land conversion [11,23–26]. For example, soils are usually tilled during the establishment of new crops, which can accelerate soil organic matter mineralization increasing $CO_2$ and $N_2O$ losses from soils [26,27]. Once energy cane is established on land previously occupied by grazed

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pastures, it will likely have higher yields and litter compared with pastures where grazers forage on plant biomass [23,28]. However, long-established grazed pastures will likely have higher root biomass than energy cane particularly during early establishment. Maximizing the productivity of energy cane will also require fertilization and irrigation [21], and the removal of grazers in energy cane plantations will eliminate dung and urine deposition [29].

Precipitation is a main driver of GHG emissions and will interact with land use change to modulate emission [30–32]. Most of Florida is sub-tropical (Cfa Köppen-Geiger climate, humid subtropical climate [33]) with distinct wet and dry seasons, and as in many subtropical and tropical regions, it has large interannual variation of precipitation which is predicted to become even larger during this century [34,35]. The influence of this land conversion on climate might be greater during wetter than drier times of the year as well as during wetter than drier years as increased precipitation enhances soil GHG emissions.

Here, we investigated how the conversion of pasture to energy cane affects the emission of GHGs from highly organic Histosols in Florida during early establishment by combining results from a field experiment and a mechanistic biogeochemical model. We hypothesize that the conversion of pasture to energy cane will increase the emission of $N_2O$, $CH_4$, and $CO_2$ from soils, particularly during warmer and wetter times of year. To test this hypothesis, we measured GHG fluxes from soils under pasture and energy cane during wet, hot and during cool, dry times of year and following a fertilization event. The fertilization event occurred during the dry season as typical for the cultivation of energy cane. We also examined whether changes in precipitation affect the magnitude of impact of land conversion on soil GHG emissions by simulating fluxes during wetter and drier years using the process-based biogeochemical model DayCent (v.4.5) [36,37].

## 2. Material and methods

### 2.1. Study site

Measurements were made in 2011, 2012 and 2013 on private land in Highlands County, FL (27°21′49″ N, 81°14′56″ W). This area has a subtropical climate with two distinct seasons, a wet, hot season from June through September, with relatively dry and cool conditions during the rest of the year. Mean annual precipitation (1980–2012) was 1310 mm, with two-thirds of total annual precipitation falling from June to September [38]. Mean annual temperature (1980–2012) was 22 °C [38].

To investigate how the conversion of pasture to energy cane influenced point soil GHG fluxes we established experimental plots (300 $m^2$) in commercial energy cane plantations and nearby pastures (< 1 km). The experiment consisted of 12 plots, providing replicates of each of the following three land uses: 1) grazed pasture (GP); 2) energy cane planted in 2010 and harvested in Nov 2011 (EC-2010); and, 3) energy cane planted in 2011 (EC-2011) (Table 1). Plots within each of the four replicates of each land use were 250 m apart.

The dominant vegetation at the GP sites was bahiagrass (*Paspalum notatum* Flueggé) that served as forage for cattle (*Bos taurus* L.) (Table 1). Bahiagrass is a $C_4$ perennial grass that was first introduced in Florida in 1913 and covers approximately 8094 $km^2$ of the state [39]. The GP sites were drained during 1960–1980, and were established in 1981. Since their establishment, the sites have been grazed by cattle at stocking rates of 0.01 $km^{-2}$ (or 1 $ha^{-1}$ [23]). The GP sites have not been fertilized in the last 10 years. Prior to land conversion, the energy cane sites were managed identically to the GP sites. Soils in energy cane plantations and pastures were hyperthermic Terric Haplosaprists that belong to the Histosol order. Soils have a bulk density of 0.5 g $cm^{-3}$, and a carbon and nitrogen content in the top 0.3 meters of 12.35 ± 3.0% and 0.96 ± 0.3%, respectively.

Energy cane plantations were established according to typical agronomic practices for this region (Fig. 1 [15]). Six months before planting energy cane, soils were tilled to a depth of 0.25 m to 0.30 m. After tillage, beds were kept weed free until planting by using an herbicide (Atrazine 50 FW; applied 5 and 1 months before planting at a rate of 0.2 g $m^{-2}$). Three-budded cane stalk cuttings were hand planted at a row spacing of 1.5 m and with 4 cm to 6 cm distance between stalk cuttings.

**Table 1**
DayCent simulation site characteristics and model parameters that vary or do not vary by land use (GP and energy cane).

| Parameters that do not vary by land use | |
|---|---|
| Latitude, Longitude | 27°21′49″ N, 81°14′56″ W |
| Soil type | Histosol |
| Soil carbon, nitrogen (%) | 12.35 ± 3.0%; 0.96 ± 0.3% |
| Soil bulk density | 0.5 g cm-3 |
| Site history | Before 1982, mix of perennial grasses species<br>From 1982 to 2010, warm season pasture with grazing.<br>After Nov, 2010 wetter and drier years simulations were run on GP and energy cane for two full years |
| Historical climate record | Daily climate from 1982-2010 with mean annual precipitation of 1270 mm, and mean annual temperature of 22 °C. |
| Simulated wetter year | 1145 mm of water during wet season, 296 mm of water during dry season<br>Average annual temperature of 22 °C |
| Simulated drier year | 824 mm of water during wet season, 296 mm of water during dry season<br>Average annual temperature of 22 °C |

| Parameters that vary by land use | GP | Energy cane |
|---|---|---|
| Symbiotic N fixation | Yes | No |
| Non-symbiotic N fixation | Yes | Yes |
| Grazing | Yes | No |
| Maximum C:N | 120 | 120 |
| Minimum C:N | 70 | 70 |
| Maximum belowground allocation (%) | 30 | 30 |
| Minimum belowground allocation (%) | 20 | 5 |

Energy cane typically is fertilized annually near the end of the dry season [39]. The timing of fertilization varied between different energy cane stands (Fig. 1). At each fertilization event, 2.8 g $m^{-2}$ of nitrogen as ammonium sulfate was applied. Dolomite ($CaMg(CO_3)_2$) was applied to energy cane plantations before the establishment of the crops at a rate of 9.1 g $km^{-2}$. In addition to natural rainfall, energy cane sites were irrigated with a linear move sprinkler system equivalent to 305 mm of additional precipitation applied over the canopy from January to June.

The EC-2010 crops were harvested by hand between 5 cm and 10 cm above the ground level, one year after planting using machetes in Nov 2011 (Fig. 1). Only aboveground biomass was harvested, leaving belowground biomass intact. These crops were not tilled after harvest, and post-harvest residues were left on the field.

### 2.2. Soil GHG measurements

To capture the effect of climate variation, soil $N_2O$, $CH_4$, and $CO_2$ fluxes were measured three times during the wet-hot season (July 11, 2011, September 4, 2011, and June 14, 2012), and twice during the dry-cool season (December 7, 2011 and March 1, 2011) (Fig. 1). At each sampling period, measurements were made between 11:00 and 15:00 (UTC - 5) over 3 days to 4 days. Measurements at each plot for each land use were sampled randomly at each sampling period to minimize confounding effects on soil GHG fluxes resulting from daily variability.

An experiment was also conducted to determine soil GHG emissions following fertilization. Fluxes of $N_2O$, $CH_4$, and $CO_2$ were measured in a total of 16 unfertilized plots and 16 fertilized plots at both GP and EC-

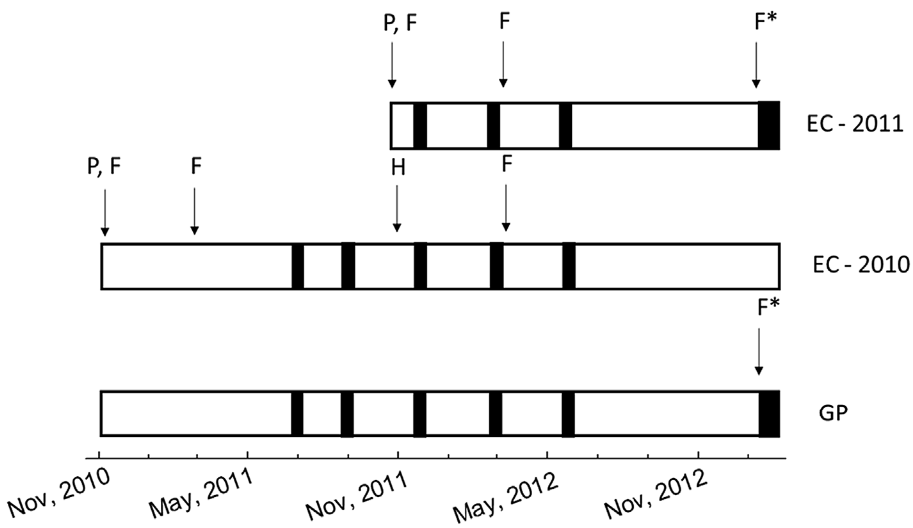


**Fig. 1.** Experimental plot characteristics and management practices for GP, EC-2010 (cane planted in 2010 and harvested in Nov 2011), and EC-2011 (cane planted in 2011) crops. P, F and H refer to plantation, fertilization and harvest. Shaded areas refer to times when soil GHG measurements were taken over 3 days to 4 days in July 11, September 4 and December 7, 2011, and in March 1 and June 14, 2012, and one day before and for 12 days immediately after a fertilization event in January 15, 2013 in EC-2011. Asterisks refer to the 12 days fertilization experiment when soil GHG fluxes were measured in fertilized and unfertilized EC-2011 and GP.

2011 experimental plots (Fig. 1). Plots were fertilized during the dry season on January 15, 2013 with 2.8 g $m^{-2}$ of nitrogen as ammonium sulfate (Fig. 1). The fertilized and unfertilized plots were 10 meters apart to reduce the potential for lateral movement of fertilizer into the unfertilized ones. Soil GHG fluxes were measured one day before and for 12 days following the fertilization event (January 15, 2013; Fig. 1).

Soil $N_2O$ and $CH_4$ fluxes were measured with a static chamber [40,41]. The polyvinyl chloride (PVC) static chamber (3.6 $dm^{-3}$) was placed on a 20-cm diameter PVC collars inserted 5 cm into the soil. Four collars were permanently installed in each experimental plot giving a total of 16 measurements for each land use type. For energy cane, soil $N_2O$ and $CH_4$ fluxes were measured within and between the rows. Gas samples were collected at 0, 10, 20, and 30 min after the chamber was sealed on the collar in 12 $cm^{-3}$ evacuated glass vials sealed with a butyl rubber, Teflon-coated septa (Sun SRI, Rockwood, TN). Immediately after collection, gas samples were analyzed by gas chromatography (Shimadzu 2014 Greenhouse Gas Analyzer, Shimadzu Scientific Instruments, Columbia, MD). Fluxes were calculated using linear and quadratic regression methods from the change in gas concentration in the chambers over time (as recommended in Refs. [42,43]). Both the linear and non-linear quadratic methods yielded similar soil $N_2O$ and $CH_4$ fluxes at each sampling point, suggesting that the assumption that fluxes were linear in this study was accurate [43]. In this study, fluxes shown were obtained from linear regressions with $R^2$ higher than 70% [42,44].

Soil $CO_2$ fluxes were measured with an infrared gas analyzer (LI-6400, LI-COR, Inc., Lincoln, NE, USA) connected to a soil respiration chamber (LI-6400-09 LICOR). In each experimental plot and two months prior to this experiment, 4 PVC (10-cm diameter) collars were inserted to a depth of 3 cm, providing a total of 16 soil respiration measurements for each land use. For the energy cane site, soil respiration ($R_{soil}$) was measured within and between rows.

### 2.3. Soil temperature, moisture, and aboveground and belowground biomass measurements

Soil temperature to a depth of 20 cm was measured near the PVC collars with a Type E temperature probe (Omega Engineering, Inc., CO) attached to a thermocouple adapter on the LI-6400-09 soil respiration chamber. Three soil temperature measurements were collected at each experimental plot. For the energy cane, soil temperature was an average of a between and within row crop measurement. Soil moisture content to a depth of 20 cm was determined gravimetrically. Two soil cores (3-cm diameter) were collected with a soil corer (JMC Backsaver, Clements Associates, Inc., IA) near each PVC collar and combined to create a single sample. Each soil sample for energy cane was a composite of soil collected between and within rows.

Aboveground standing biomass and litter biomass were collected at each sampling period and plot from 0.5 m × 0.5 m quadrats for pastures and 0.75 m × 0.75 m quadrats for energy cane. Aboveground standing biomass and litter biomass were dried at 60 °C until reaching constant mass.

Root biomass was measured at each sampling period. Four 5-cm diameter soil cores (one within each plot) to a depth of 20-cm were collected using a soil corer (AMS split core sampler; AMS, Inc., ID). Each sample was a composite of two soil cores, collected between and within rows for energy cane. Samples were frozen to −20 °C until processing. Roots were separated from soil, rinsed with deionized water and oven dried at 60 °C until reaching constant mass.

### 2.4. Statistical analysis of field data

Differences ($p < 0.05$) in GHG fluxes, soil temperature and moisture, biomass, and litter stocks between sampling periods were tested using a complete block repeated measures ANOVA with land use and sampling period as fixed factors. Differences ($p < 0.05$) in these variables within each sampling period were analyzed with analysis of variance (ANOVA) with land use as the fixed factor. For the fertilization experiment, differences ($p < 0.05$) in soil GHG fluxes within each sampling day were tested using a complete block repeated measures ANOVA with land use and fertilization treatment and their interaction as factors. A simple regression analysis (SRA) was used to investigate the influence of soil temperature and moisture on soil GHG fluxes, after transforming the data to ensure normality and homogeneity of variances. The combined effect of soil temperature and moisture on these fluxes was evaluated using a general linear model (GLM). All statistical tests were conducted with Statgraphics Centurion XVI (Statistical Graphics Corporation, Rockville, MD, USA).

### 2.5. Model description and parameterization

We used the biogeochemical model DayCent (v.4.5), which has been extensively used to simulate fluxes in crops and grasslands including sugar cane [36,45,46] (Appendix A), to predict how differences in precipitation affected soil GHG emissions following land conversion. Model inputs included vegetation cover, daily precipitation and temperature, soil texture, current nitrogen deposition [47], and current and historical land use practices for each specific land use type (Table 1). In this study, the potential plant productivity value was adjusted to match above- and belowground productivity measurements from our field

**Table 2**
Above- and belowground biomass, and litter biomass in GP (grazed pasture), EC-2010 (planted in 2010 and harvested in Nov 2011) and EC-2011 (cane planted in 2011) crops. Values are means ± standard error at each sampling period. Sampling periods refer to measurements taken over 3 days to 4 days in July 11, September 4 and December 7, 2011, and in March 1 and June 14, 2012. Mean values within the same sampling period with different letter denote statistical differences ($p < 0.05$; ANOVA). †refers to times after EC-2010 was harvested. N/A refers to non-applicable.

| | | 2011 | | | 2012 | |
|---|---|---|---|---|---|---|
| | | Jul | Sep | Dec | Mar | Jun |
| Aboveground Biomass ($Mg\ m^{-2}$) | GP | 0.7 ± 0.1a | 0.5 ± 0.1a | 0.6 ± 0.1a | 0.2 ± 0.1a | 0.3 ± 0.1a |
| | EC-2010 | 4.24 ± 0.4b | 5.8 ± 0.8b | 0.2 ± 0.1b† | 1.8 ± 0.3b† | 4.4 ± 0.2b† |
| | EC-2011 | NA | NA | 0.1 ± 0.1b | 0.7 ± 0.1c | 2.9 ± 0.5c |
| Litter biomass ($10^{-6}\ Mg\ m^{-2}$) | GP | 207.2 ± 59.2a | 128.8 ± 11.5a | 226.3 ± 31.8a | 220.0 ± 27.1a | 259.0 ± 32.9a |
| | EC-2010 | 310.8 ± 61.9a | 574.0 ± 116.0b | 618.3 ± 128.7b† | 507.4 ± 89.9b† | 600.3 ± 158.2b† |
| | EC-2011 | NA | NA | 0.1 ± 0.5c | 145.1 ± 23.2c | 264.4 ± 49.2a |
| Belowground biomass ($10^{-6}\ Mg\ m^{-2}$) | GP | 614.8 ± 138.3a | 600.7 ± 21.7a | 562.6 ± 88.7a | 610.2 ± 134.5a | 634.2 ± 59.4a |
| | EC-2010 | 332.3 ± 62.6b | 391.4 ± 92.1b | 363.8 ± 29.5b† | 334.4 ± 46.1b† | 375.2 ± 23.8b† |
| | EC-2011 | NA | NA | 69.1 ± 1.6c | 176.7 ± 14.8c | 271.3 ± 19.4c |

experiment (Table 2), and we optimized predicted soil GHG fluxes based on observed GHG from our field experiment [37,45,48–52]. Predicted soil $CO_2$ and $CH_4$ fluxes were optimized by adjusting the fraction of $CO_2$ from soil respiration used to produce $CH_4$. Predicted soil $N_2O$ fluxes were optimized by adjusting the maximum proportion of nitrified nitrogen lost as $N_2O$ at field capacity [37,45,48–52].

For historical DayCent simulations (Table 1), daily climate from 1850-2010 was used [38]. Historical simulations included a mix of perennial grasses species, and symbiotic nitrogen gas fixing plants as the dominant historic vegetation type in south-central Florida was savanna [23], followed by land conversion in 1982 to GP until Oct 2010 (Table 1). For GP simulations, DayCent was parameterized for symbiotic and non-symbiotic fixation, carbon and nitrogen content, and C allocation to the belowground component, and to model soil organic carbon (SOC) dynamics to a depth of 30 cm (Table 1). Grazing was simulated by annually removing 10% of live shoot and 1.0% of standing dead shoots as in Ref. [23] (Table 1). For the simulation of energy cane, aboveground biomass was removed and soils were tilled to a depth of 30 cm in May, 2010. Six months later, the landscape was converted to energy cane plantations. Planting, harvesting and fertilizing of cane was the same as in our field experimental site (Table 1; Fig. 1) and was similar to typical agronomic practices for this region [15].

Energy cane, as a perennial grass species, is typically separated into different growth cycles according to harvest and regrowth. The first growth cycle, termed 'plant cane' begins with establishment and continues until the first harvest. After this initial harvest, the period of regrowth until the next harvest is referred to as a 'ratoon' and is usually names based on order with the initial ratoon referred to as the 'first ratoon'. For the simulation of energy cane, the cycle of energy cane included plant cane (from planting to harvesting) and energy cane after first ratoon (after harvesting).

The effect of wet and dry years on GHG exchange from GP and energy cane was simulated with DayCent over a two-year period (from Nov 2010 to Nov 2012; Appendix A). Changes in precipitation were imposed during the wet season only, when soil GHG fluxes are predicted to change more rapidly [23,53–55]. To define wet and dry years for the model, we created a frequency distribution for precipitation during the wet season at our site from 1982-2010 (Appendix A; from 1982 to 2010 [38]). Precipitation during the wet season varied from 520 mm to 1222 mm. Because the distribution was highly non-normal, we selected most common wet year (1145 mm) and the most common dry year (824 mm) for our simulations. Wet and dry years in our simulations shared the same frequency of rainfall events, the amount of precipitation during the dry season (296 mm), and the same temperature record.

### 2.6. Model validation

The model was validated against aboveground productivity data from our experimental plots (Table 2), and from studies in tropical and subtropical sugar cane plantations (8 observations) and GP (6 observations) grown in Histosols (Appendix A). The model was also evaluated against data obtained from studies that reported annual GHG fluxes in tropical and subtropical sugar cane plantations (38 observations) and GP (14 observations) (Appendix A). We did not use point soil GHG flux measurements to validate DayCent because the correlation between observed and modeled hourly or daily GHG fluxes is usually poor [50,56–58]. Simple linear regression and a t-student test were used to compare observed and predicted data using Matlab® v. 7.8.0 (Appendix A).

In this study, we defined the soil GHG emission as the sum of $N_2O$, $CH_4$ and $CO_2$ fluxes from soil in $CO_2$-equivalents as in Refs. [59–61]. The relative contribution of each gas flux to soil GHG emissions was calculated assuming global warming potentials of 298 for $N_2O$ and 28 for $CH_4$ (according to [62]; on a time horizon of 100 years). Because we wanted to calculate the GHG emission from soils, and soil $CO_2$ emissions derive from the aerobic respiration of roots and soil microbes, emissions of $CO_2$ from soils used for these calculations included both $CO_2$ sources (as in Refs. [59–61]).

The emission factor (EF) for $N_2O$ from nitrogen fertilizer, the proportion of fertilizer emitted as $N_2O$ after application, for energy cane plantations was estimated from predicted annual $N_2O$ fluxes during wetter and drier years using DayCent.

EF was estimated as in Ref. [63] using Equation (1):

$$EF = \frac{net\ N\ flux}{N\ applied} \tag{1}$$

Net N flux was estimated as follows:

$$net\ N\ flux = (N_2O\ flux - N_2O\ flux\ (ambient)) \times \frac{14.007}{44.013} \tag{2}$$

where $N_2O$ flux refers to annual $N_2O$ fluxes from energy cane crops when nitrogen was applied, $N_2O$ flux (ambient) refers to annual $N_2O$ fluxes from crops when no nitrogen was applied. Numbers refer to the ratio of the atomic mass of nitrogen and molecular weight of $N_2O$.

### 2.7. Uncertainty in model predictions

Uncertainty in NPP was calculated by comparing observed and model NPP. The error in $R_{soil}$ was calculated by error propagation of root and soil microbial respiration uncertainties, and by assuming that uncertainty in root respiration equaled uncertainty in belowground NPP and that uncertainty in soil microbial respiration was 1.5x larger than in total NPP [52,64]. In our study, uncertainty in $N_2O$ and $CH_4$

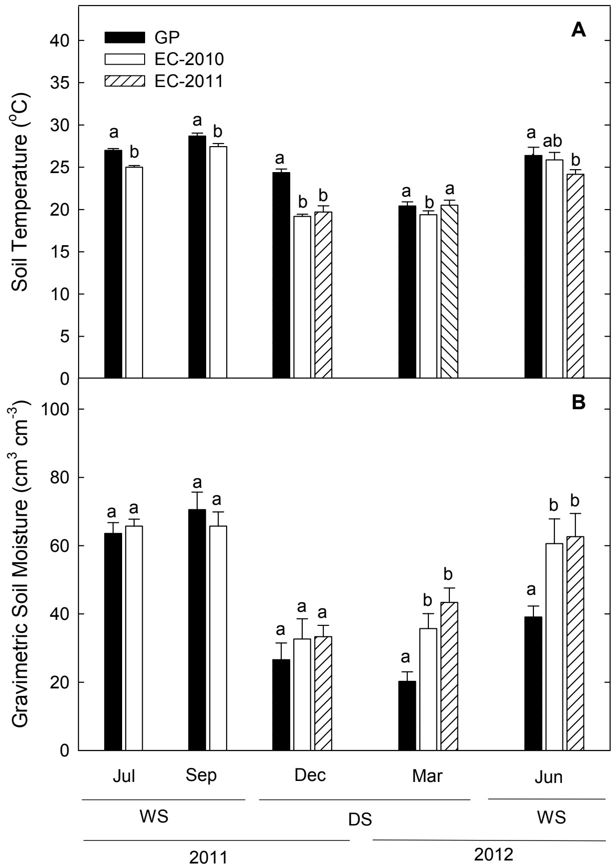


**Fig. 2.** Seasonal variation in soil temperature (A) and moisture (B) in GP (grazed pasture), EC-2010 (cane planted in 2010 and harvested in Nov 2011) and EC-2011 (cane planted in 2011). Values are means ± standard error at each sampling period. Sampling periods refer to measurements taken over 3 days to 4 days in July 11, September 4 and December 7, 2011, and in March 1 and June 14, 2012. WS refers to wet season, and DS to dry season. Mean values within the same sampling period with different letter denote statistical differences ($p < 0.05$; ANOVA).

estimates derived from the model was assumed to be constant for all land use types as in Refs. [64,69], and to be equal to the upper uncertainty estimate derived from studies that evaluated modeled vs. observed fluxes from grasslands, pastures, and agricultural crops [37,45,49–51,65–68].

## 3. Results

Over a complete wet-dry cycle, conversion from grazed pasture (GP) to energy cane consistently reduced soil temperature (Fig. 2A). Soil temperature for both land uses was higher in July, September, and June (wet season), than in December and March (dry season; Fig. 2A). Soil moisture in energy cane crops was similar to GP except in March and June when energy cane was irrigated and GP was not (Fig. 2B). For both vegetation types, soil moisture was higher in July, September, and June, than in December and March (Fig. 2B).

Aboveground standing biomass was higher in EC-2010 (planted in Nov 2010, and harvested one year later) and EC-2011 (planted in Nov, 2011) than in GP, with the exception of GP having higher biomass than EC-2010 in December, one month after cane was harvested (Table 2), and higher than EC-2011 in December, one month after the crop was planted (Table 2). Litter biomass consistently was higher in EC-2010 crops than in GP (Table 2). However, it was lower in EC-2011 crops than in GP or even similar ($p = 0.12$; Table 2). Root biomass was higher in GP than in EC-2010 crops, and higher in GP than in EC-2011 (Table 2). Root biomass in EC-2011 crops was consistently lower than in EC-2010 (Table 2).

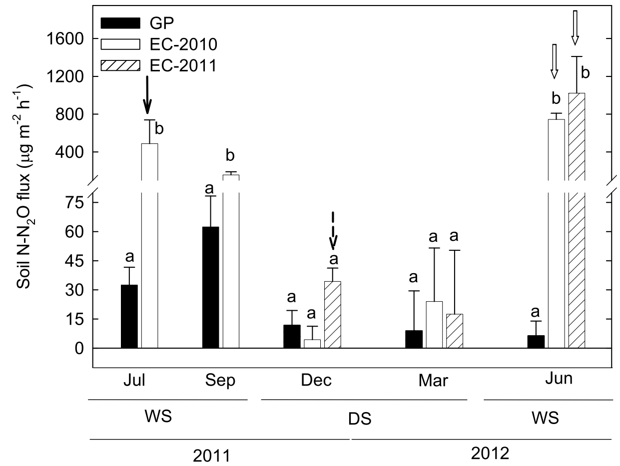


**Fig. 3.** Seasonal variation of soil $N_2O$ fluxes in GP (grazed pasture), EC-2010 (cane planted in 2010 and harvested in Nov 2011) and EC-2011 (cane planted in 2011). Positive soil $N_2O$ fluxes indicate that the soil is a net source of $N_2O$ to the atmosphere. Values are means ± standard error at each sampling period. Sampling periods refer to measurements taken over 3 days to 4 days in July 11, September 4 and December 7, 2011, and in March 1 and June 14, 2012. WS refers to wet season, and DS to dry season. Arrows indicate fertilizer application on energy cane crops (Fig. 1). Mean values within the same sampling period with different letter denote statistical differences ($p < 0.05$; ANOVA).

The conversion of pasture to energy cane crops increased the emission of point $N_2O$ fluxes from soils, particularly during the wet season (e.g. EC-2010 in July and September, and EC-2010 and EC-2011 in June; Fig. 3). Under drier and cooler soil conditions, soil $N_2O$ fluxes were similar in energy cane crops and in GP, even in December one month after EC-2011 had been fertilized (Fig. 3). Differences in soil moisture explained much of the variation in soil $N_2O$ fluxes in GP and energy cane crops ($R^2 = 76\%$ in GP, and $R^2 = 78\%$ in energy cane crops; Appendix B). Differences in soil temperature explained more variation in fluxes in GP than in energy cane crops ($R^2 = 43\%$ in GP, and $R^2 = 68\%$ in energy cane crops; Appendix B).

Nitrogen fertilization caused a transient increase in soil $N_2O$ fluxes in both EC-2011 and GP, and fluxes were larger in fertilized than unfertilized plots for both land uses (Fig. 4). Following fertilization, soil $N_2O$ fluxes in EC-2011 reached a maximum of 104 µg $m^{-2}h^{-1}$ returning to pre-fertilization levels after 9 days (Fig. 4). This maximum

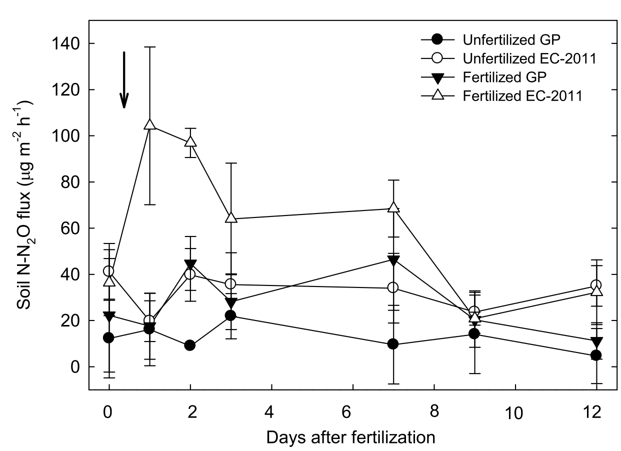


**Fig. 4.** Soil $N_2O$ fluxes from fertilized and unfertilized plots of GP (grazed pasture) and EC-2011 (cane planted in 2011). The fertilization event (arrow) on the fertilized plots occurred in January 15, 2013 (dry season) after measuring soil $N_2O$ fluxes. Positive or negative soil $N_2O$ fluxes reflect that the soil is a net source or a sink of $N_2O$ relative to the atmosphere, respectively. Values are means ± standard error for each sampling period. Soil temperature and volumetric soil moisture values were as in Table 2. Mean values within the same sampling period with different letter denote statistical differences ($p < 0.05$; ANOVA).

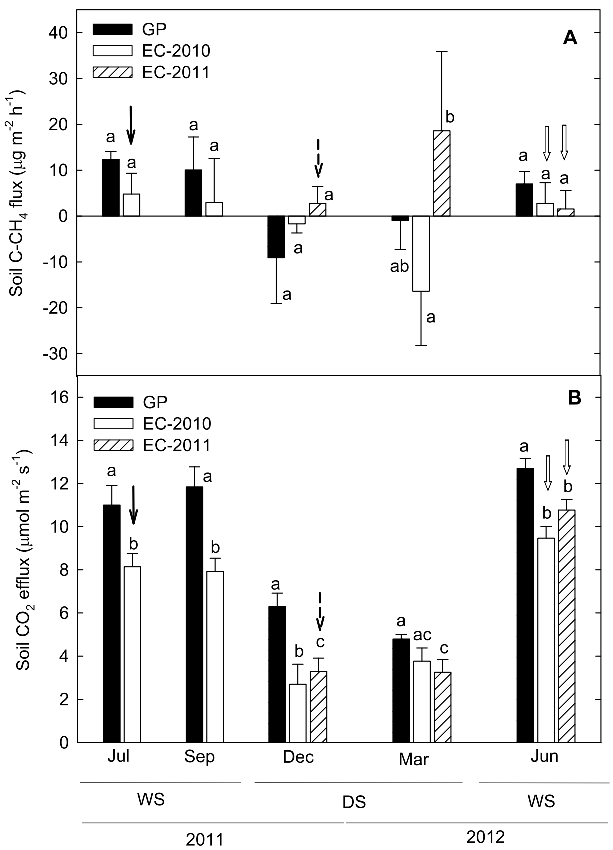


**Fig. 5.** Seasonal variation of soil $CH_4$ fluxes (A) and total soil $CO_2$ efflux (B) in GP (grazed pasture), EC-2010 (cane planted in 2010 and harvested in Nov 2011) and EC-2011 (cane planted in 2011). Positive or negative soil $CH_4$ fluxes reflect that the soil is a net source or a sink of $CH_4$ relative to the atmosphere, respectively. Values are means ± standard error at each sampling period. Sampling periods refer to measurements taken over 3 days to 4 days in July 11, September 4 and December 7, 2011, and on March 1 and June 14, 2012. Arrows indicate fertilizer application on energy cane crops in March 10 (solid) and November 14, 2011 (dashed), and March 10, 2012 (empty). WS refers to wet season, and DS to dry season. Mean values within the same sampling period with different letter denote statistical differences ($p < 0.05$; ANOVA).

was lower than soil $N_2O$ emissions in EC-2011 crops in June when soil was wetter and warmer (Figs. 2 and 4). Fluxes 9 days after the fertilization event were similar to fluxes measured one year earlier during the dry season (e.g. EC-2010 in December and March; Figs. 3 and 4).

In our field experiment, the conversion of pasture to energy cane did not affect point $CH_4$ fluxes from soils ($p = 0.3$; Fig. 5A). In July, September and June when conditions were relatively warm and wet, GP and cane crops were small net sources of $CH_4$ to the atmosphere, but at times became net sinks for $CH_4$ as soils dried in December and March (Fig. 5A). Soil $CH_4$ fluxes in GP were correlated with soil moisture but not with temperature (Appendix B). In energy cane crops, no significant correlation between fluxes and moisture or temperature was found ($p = 0.15$ for soil moisture, $p = 0.3$ for soil temperature; Appendix B).

On average, fertilized plots were a stronger net sink of $CH_4$ than unfertilized plots for both land uses. During the fertilization event in EC-2011 and GP during the dry season, average net $CH_4$ emissions were $-0.06 \pm 0.02$ μmol $m^{-2}$ $h^{-1}$ for unfertilized cane and $-1.1 \pm 0.4$ μmol $m^{-2}$ $h^{-1}$ for fertilized cane, and they were $-1.2 \pm 0.5$ μmol $m^{-2}$ $h^{-1}$ for unfertilized GP and $-5.3 \pm 2$ μmol $m^{-2}$ $h^{-1}$ for fertilized GP.

Measurements of soil $CO_2$ fluxes were consistently higher from GP than from energy cane crops (Fig. 5B). Soil $CO_2$ efflux in GP and cane sites was the lowest in December and March when soil moisture and temperature were low (Fig. 1A and B), and highest in July, September and June (Fig. 4B). Soil $CO_2$ efflux was highly correlated with both soil temperature and moisture for both GP and energy cane crops (Appendix B). There was no statistically significant effect of fertilization on $CO_2$ flux in either land use type (data not shown).

To determine whether changes in precipitation affect soil GHG emissions from GP and EC, we simulated fluxes during wet and dry years using DayCent (v.4.5). We found good agreement between modeled aboveground biomass and data from our experimental plots as well with literature values for GP and sugar cane grown on Histosols ($R^2 = 75\%$ for energy cane, and $R^2 = 87\%$ for GP; Appendix A), indicating that our predictions provided a good representation of the productivity that drives the biogeochemical dynamics of DayCent.

To further evaluate predicted GHG fluxes from soil, we compared predicted soil GHG values against reported GHG data from published studies (Appendix A). Modeled annual $N_2O$, $CH_4$ and $CO_2$ fluxes from soil were within the range reported for annual fluxes from tropical and subtropical pastures and sugar cane (Appendix A).

Predicted annual soil $N_2O$ fluxes were higher in plant and first ratoon cane than in GP for simulated wet and dry years, and the difference in fluxes between land uses was greater in wetter than drier years (Fig. 6A &B). Soils under plant cane emitted 0.6 g $m^{-2}$ $yr^{-1}$ more nitrogen than under GP during wet than dry years (Fig. 6A), while soils under first ratoon cane emitted 0.3 g $m^{-2}$ $yr^{-1}$ more nitrogen than under GP during wet than dry years (Fig. 6B). Predicted annual $N_2O$ fluxes were higher for both energy cane crops and GP during wetter than drier years.

The emission factor (EF) for $N_2O$ from nitrogen fertilizers in plant and first ratoon cane plantations was higher during wetter than drier years, but similar for plant and first ratoon cane. During drier years, EF was 21% for both plant and first ratoon cane. During wet years, EF was 28% for plant cane, and 33% for first ratoon cane. On average, unfertilized plant and first ratoon cane emitted $0.5 \pm 1.3$ g $m^{-2}$ $yr^{-1}$ of nitrogen to the atmosphere, and it was within the range for annual fluxes from unfertilized sugar cane (Appendix A).

Predicted annual soil $CH_4$ emissions were higher in GP than in plant and first ratoon cane, and the difference in emissions between crops was similar during wet and dry years (Fig. 6C and D). Predicted annual soil $CH_4$ emissions for both GP, and plant and first ratoon cane crops were higher during wet than dry years.

Predicted annual soil $CO_2$ fluxes were higher in GP than in plant and first ratoon cane (Fig. 6E and F). Differences between land use type were similar during wet and dry years (Fig. 6E and F). Predicted annual soil $CO_2$ fluxes within each land use type were similar during wet and dry years.

To compare the emission of GHGs from soil between land use during wet and dry years we converted soil emissions of $N_2O$, $CH_4$, and $CO_2$ to $CO_2$ equivalents (Fig. 7). The difference in soil GHG emissions under energy cane and GP depended on the establishment phase of energy cane (Fig. 7). Under plant cane, soils emitted more GHGs than under GP, and this difference was larger during wet than dry years. However, soil GHG emissions from soils under GP and first ratoon cane were similar regardless of precipitation. Under plant cane, the contribution of $N_2O$ to overall GHG emissions from soil was larger than the contribution of $CO_2$. Under first ratoon cane, however, the contribution of $CO_2$ to overall GHG emissions from soils was similar or even larger than the contribution of $N_2O$. The contribution of $CH_4$ fluxes to soil GHG emissions was negligible, and they did not contribute to differences in soil GHG emissions between land uses. On average, the uncertainty in $CO_2$ fluxes explained most of the uncertainty in GHG emissions from soil under GP (average of 315 g $yr^{-1}$ of $CO_2$ equivalents for both wet and dry years), and the uncertainty in $N_2O$ fluxes explained most of the uncertainty in GHG emissions from soil under plant and first ratoon cane crops (average of 183 g $yr^{-1}$ of $CO_2$ equivalents for both wet and dry years and all cane crops).

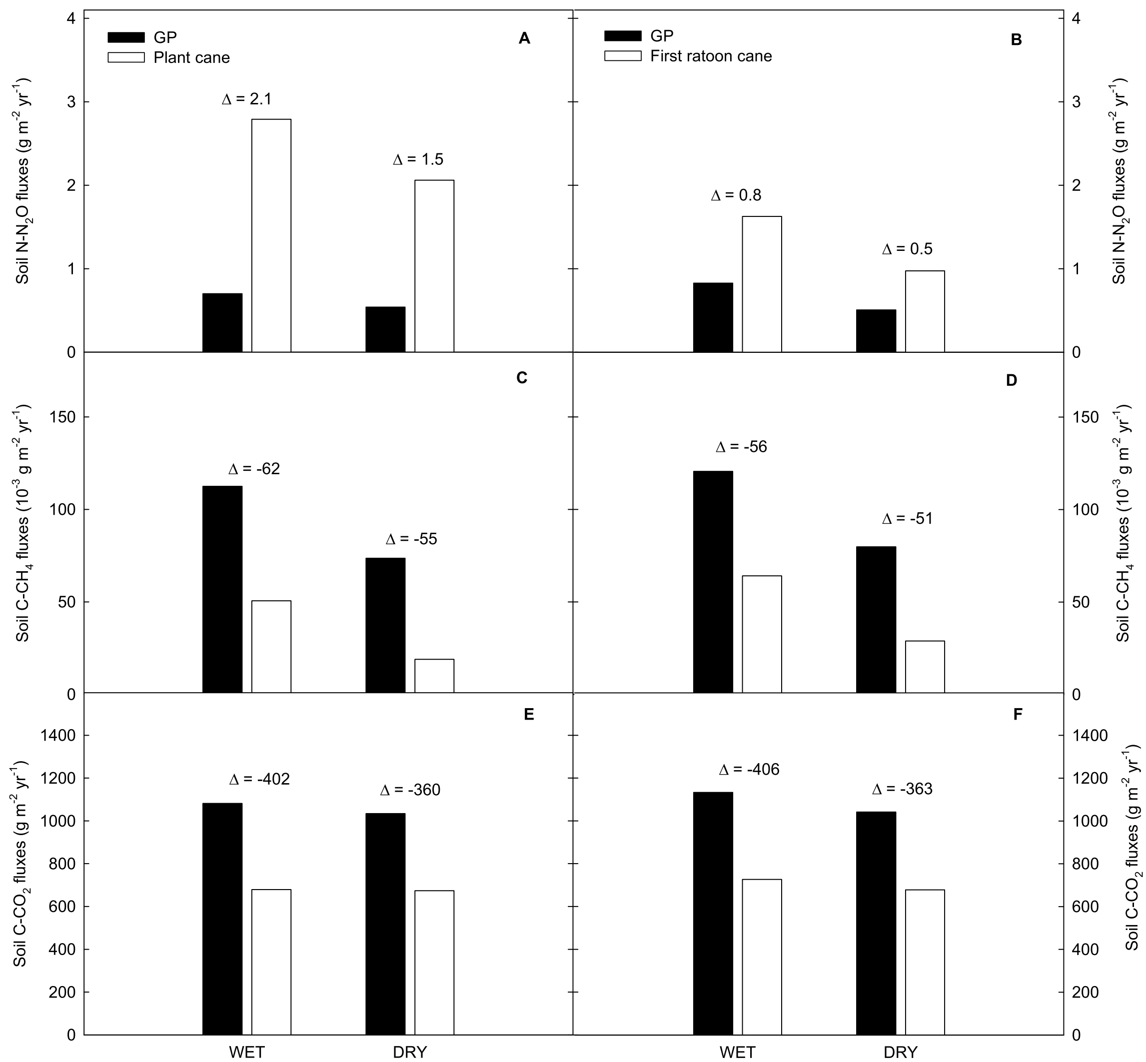


**Fig. 6.** Predicted soil $CO_2$, $N_2O$, and $CH_4$ emissions from GP (grazed pasture), plant (from planting to harvesting) and first ratoon cane (after harvesting) crops during wet and dry years. Positive soil $N_2O$ and $CH_4$ fluxes reflect that the soil is a net source of $N_2O$ relative to the atmosphere, respectively. Δ refers to difference in each GHG flux between plant or first ratoon cane and GP. Soil GHG fluxes were predicted using DayCent (v.4.5).

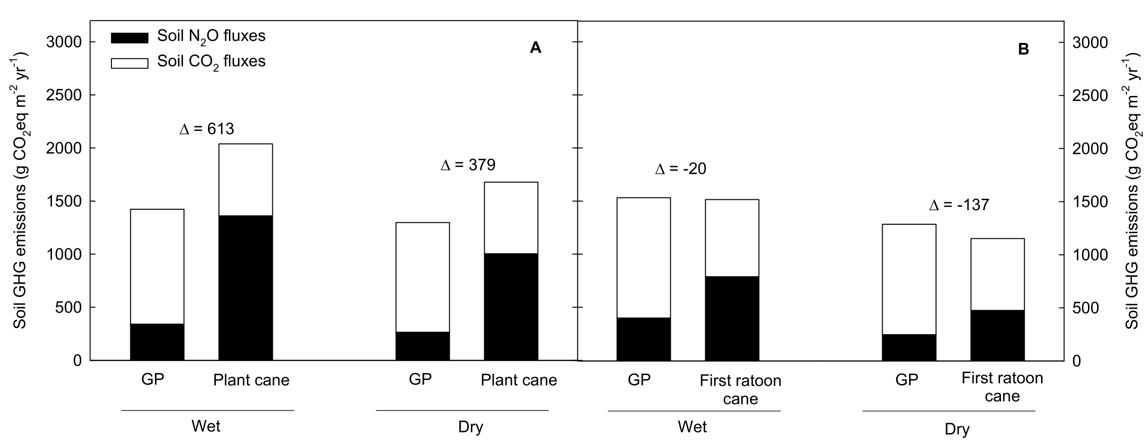


**Fig. 7.** Predicted net soil GHG emissions from GP (grazed pasture), plant (from planting to harvesting), and first ratoon cane (after harvesting) crops during wet and dry years. Net soil GHG emissions were calculated as the sum of predicted annual $CO_2$, $N_2O$ and $CH_4$ fluxes from soils as in Grover et al. [59], Braga do Carmo et al. [60], Chen et al. [61]. Δ refers to difference in soil GHG emissions between plant or first ratoon cane and GP. Soil GHG fluxes were predicted using DayCent (v.4.5). Global warming potentials of 298 for $N_2O$ and 28 for $CH_4$ were used (according to [62]; on a time horizon of 100 years). Because annual fluxes of $CH_4$ were smaller than fluxes of $CO_2$ and $N_2O$, they made a negligible contribution to total soil GHG fluxes and they are not shown in the Figure.

## 4. Discussion

Land conversion from pasture to energy cane plantations can increase the emission of GHGs from soils (i.e. $CO_2$ equivalents). Supporting our hypothesis, land conversion increased the emission of $N_2O$ during warmer and wetter times of the year, and precipitation modulated differences in predicted $N_2O$ fluxes between land uses. The difference in $N_2O$ emissions between land uses also depended on fertilization, because predicted $N_2O$ emissions were higher in plant than in first ratoon cane, and plant cane received twice as much fertilizer. Predicted emission factors (EFs) for $N_2O$ from nitrogen fertilizer in cane crops varied with precipitation, and were 20 to 30-fold higher than the default value from Intergovernmental Panel on Climate Change (IPCC; 1% [63]). Although point $CH_4$ measurements did not capture differences between land uses, predicted $CH_4$ emissions were higher in grazed pasture (GP) than in plant and first ratoon cane crops, and precipitation did not affect the impact of land conversion on $CH_4$ fluxes. Point and predicted soil $CO_2$ fluxes were consistently higher in GP than in energy cane crops. Contrary to our hypothesis, changes in precipitation did not affect the impact of land conversion on predicted soil $CO_2$ fluxes, and fluxes were similar in wet and dry years within each land use. During early establishment of energy cane, the impact of land conversion from GP to cane on the emission of GHGs from soils depended on the magnitude of soil $N_2O$ emissions under cane which varied with the amount of fertilizer and changes in precipitation.

Soils under sugar cane are typically fertilized at planting, as well as during the development of plant and first ratoon cane [70]. By fertilizing soils under cane, $N_2O$ emissions were higher compared to GP during warm and wet times of the year (Fig. 3), and at annual scales during simulated wetter and drier years (Fig. 6). The stimulating effect of nitrogen fertilization on $N_2O$ emissions was also evident in plant cane compared to first ratoon cane as the former was fertilized twice over the course of the year and had higher emissions than first ratoon cane (Fig. 6). In addition to N fertilization, both tillage activities and cultivation of soils could help explain increased soil $N_2O$ emissions in cane than in GP and in plant cane than in first ratoon cane (Fig. 6). Tillage and cultivation of no-till ecosystems can increase the emission of $N_2O$ from soils as these activities accelerate nitrogen mineralization and hence soil nitrogen availability and cane crops were tilled in this study [26,27,71].

Precipitation modulated the impact of land conversion on soil GHG emissions as differences in soil $N_2O$ emission between both energy cane crops and GP were larger during wet than dry years (Fig. 6). These modeling results reveal a synergistic interaction between both limiting factors as observed in other ecosystems [72–74]. Climatic models predict that subtropical regions will experience more frequent floods and extended drought periods in the future [35,75,76]. Thus, it is likely that future climatic conditions altering precipitation will modulate how this land conversion affects net $N_2O$ emissions from soil.

Although GP was not fertilized in this study, grazers likely increased the soil nitrogen available through dung and urine deposition. Higher $N_2O$ emissions in fertilized energy cane than in GP (Figs. 3 and 6) are consistent with the view that ammonium-based sulfate fertilizers, such as those applied to cane crops, have a stronger stimulating effect on $N_2O$ fluxes than urine [77–80], and that a substantial amount of nitrogen in urine is volatilized as $NH_3$ rather than produced as $N_2O$ [81].

The uneven distribution of urine and dung deposition could underestimate soil $N_2O$ fluxes from grazed grasslands [82,83]. In this study, although we measured soil $N_2O$ emissions from distributed randomly plots across GP sites to capture spatial variability, point soil $N_2O$ emissions could have missed large emissions from small areas due to urine and dung deposition patchiness (“hot spots”). However, the modeling experiment likely captured these hot spots as DayCent has been used to successfully simulate soil $N_2O$ emissions from grazed pastures and it includes the processes controlling $N_2O$ fluxes [36,45,84,85].

Because EFs for energy cane crops were higher than the default value from IPCC (EF1: 1% [63]), our results suggest that using the default value to estimate $N_2O$ fluxes during the establishment phase of energy cane could dramatically underestimate the consequences of land conversion on the climate system.

The difference between our predicted EFs and the default value from IPCC is likely explained by differences in climatic conditions between our study site and studies used to calculate the EF from IPCC [63]. In our study, greater precipitation increased EF as precipitation stimulates $N_2O$ fluxes from soils (Appendix B [86]), and the default value from IPCC was estimated from studies of crops around the world with limited representation from crops in subtropical and tropical climates with typically high precipitation [63].

The high organic content of soils at our site (Table 1) could also explain increased EFs compared to IPCC and other values reported for sugarcane on Brazilian soils (EF of 0.8% to 13%) [21,87]. The EF for fertilized crops grown on highly organic soils is likely above the default IPCC value as carbon stimulates microbial activity including soil denitrifiers that can accelerate $N_2O$ emissions [63,88–90].

The model predicted higher annual $CH_4$ emissions in GP than in plant and first ratoon cane, and increasingly higher $CH_4$ emissions from both land uses with more precipitation (Fig. 6). These results are explained by how DayCent represents the mechanisms that drive $CH_4$ dynamics that are based on our knowledge from global change experiments [36]. Greater deposits of dung and urine stimulate the production of $CH_4$ by anaerobic fermentation [91,92] and can inhibit methanotrophy [93], and in model simulations cattle were present in GP. Greater root biomass increases $CH_4$ production [94], and observed and predicted root biomass was larger in GP than in recently established cane (Table 2). In addition, greater simulated $CH_4$ emissions in pasture than energy cane crops could also be explained by how DayCent represents the effect of fertilizers on $CH_4$ fluxes. In our simulations and field experiment, we used ammonium sulfate fertilizers and this type of fertilizers can decrease the net $CH_4$ production and enhance net $CH_4$ consumption [77,93]. The application of this fertilizer can inhibit net $CH_4$ emissions as nitrogen limitation of methanotrophs is alleviated [95] and it inhibits methanogenic activity as sulfate serves as an alternative to $CO_2$ as electron acceptor for the anaerobic oxidation of organic matter [30,93,94]. Although we did not resolve differences in measured $CH_4$ fluxes from soils between land use type (Fig. 5A), the view that ammonium-based sulfate could increase net $CH_4$ uptake particularly during the dry season was evidenced by a stronger net sink of $CH_4$ in fertilized than unfertilized cane and GP in our 12 day experiment.

Measured and modeled soil $CO_2$ emissions were consistently higher from GP than cane crops according to our model and observations (Figs. 5B and 6). Factors controlling root and aerobic soil microbial respiration could help explain differences between land uses. Root respiration is often higher in ecosystems with greater root biomass [32,96]. Soil microbial respiration is stimulated by warm conditions, tillage, and increased C inputs from root and litter biomass [32,97,98]. Increased root and soil microbial respiration due to increased root biomass (Table 2) and warmer soils (Fig. 2A) in GP than in recently established cane might have compensated for increases in soil microbial decomposition in cane as a result of tillage activities and increased litter biomass (Table 2), explaining greater soil $CO_2$ emissions from soils under GP compared to energy cane crops (Figs. 5B and 6).

Although changes in soil moisture explained much of the variation in soil $CO_2$ emissions in our field experiment (Appendix B), changes in precipitation did not affect the difference in predicted soil $CO_2$ emissions between GP, and plant and first ratoon cane crops (Fig. 6). This does not necessarily mean that precipitation does not drive variations in fluxes, rather, this suggests that at the precipitation levels in our simulations, low soil wetness was likely not limiting root and soil microbe respiration.

The conversion of GP to energy cane increased the emission of GHGs from Histosols particularly during the first year of cane establishment (Fig. 7). This increase was in part explained by the stronger net $N_2O$ source of soils under cane than under GP (Fig. 7). However, as energy cane developed and received less fertilizer, differences in the emission of GHGs from soils between land uses were predicted to become smaller (Fig. 7). Our results are consistent with other studies showing large contribution of $N_2O$ fluxes from fertilized soils to the overall GHG source strength of soils during the establishment phase of bioenergy crops [25,26,99].

## 5. Conclusions

By combining a full factorial measurement campaign with a state-of-the-art biogeochemical model, our results suggest that during the establishment phase of energy cane using the default IPCC value to estimate $N_2O$ emissions could dramatically underestimate the consequences of this land conversion on the climate system. These findings strengthen the idea that the use of crop specific EF values should improve the accuracy of national and regional inventories for direct $N_2O$ emissions from fertilized cane crops. Although the cultivation of highly organic soil during the establishment phase of energy cane plantations can increase the GHG emissions from soils, the magnitude of impact of this land conversion on the climate system will depend on management practices (i.e. fertilization) and changes in precipitation.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biombioe.2017.11.020.

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