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ABSTRACT

Leaf-cutting ants of the genus *Atta* are widely distributed throughout the American tropics and subtropics and rival other herbivores in the consumption of surrounding foliage. Although numerous studies have been conducted on the role these insects play in herbivory and organic matter dynamics, only a handful of studies have examined their impacts on soil greenhouse gas emissions. Our study investigated fluxes of carbon dioxide (CO_2) and methane (CH_4) from three nests of *Atta cephalotes* using a portable greenhouse gas analyzer, and measured CO_2 and CH_4 emissions from soils containing nest holes that ranged 5.2–152.1 g CO_2-C and −1.1 to 15,264.7 mg CH_4-C m^{-2} day^{-1}, respectively. Fluxes of CO_2 and CH_4 were positively correlated above nest holes, but not in patches of soil away from leaf-cutting ant nests. Nearby non-nest soil emissions were significantly lower, ranging from 0.6 to 6.0 g CO_2-C and −1.3 to 0.77 mg CH_4-C m^{-2} day^{-1}. Fluxes of both gases among nests and among holes within a single nest were highly variable. This preliminary dataset is small in scale both temporarily and geographically, but the discovery of substantial greenhouse gas fluxes from *Atta cephalotes* nests may have important implications for carbon budgets of tropical and subtropical American forests. Further work will be necessary to determine the mechanisms behind enhanced greenhouse gas emissions from leaf-cutting ant nests, and how this may alter ecosystem-scale CO_2 emissions and CH_4 sink strength in tropical forest soils.

1. Introduction

Tropical forests play a critically important role in the global carbon (C) cycle, storing an estimated 11–30 % of the world's soil C pool, and 46 % of the world's living C pool (Brown and Lugo, 1982; Jobbágy and Jackson, 2000). Tropical forests have long been considered sinks for the greenhouse gases (GHG) carbon dioxide (CO_2) and methane (CH_4) (Brown and Lugo, 2017; Luyssaert et al., 2008), but some recent studies suggests that they may emit more C than they absorb (i.e. they are net sources of GHG) due to deforestation and reduced biomass density (Baccini et al., 2017). CH_4 is a particularly important GHG because it has an atmospheric warming potential between 34–86 times greater than

that of CO_2 (on a per mass basis) (IPCC, 2013). Soil CH_4 fluxes, which are controlled by many different factors, are strongly influenced by the availability of oxygen (O_2) and a C source (e.g. labile organic matter or CO_2) (Teh et al., 2005). When O_2 is available, CH_4-producing archaea are outcompeted by other groups of soil microorganisms, but in the presence of strongly reducing conditions and readily available labile C, CH_4 emissions are enhanced (Megonigal et al., 2004). Tropical forest soils have generally been considered to be CH_4 sinks (Aronson et al., 2019; Potter et al., 1996), and tropical forest soils are estimated to contribute 28 % of the global annual soil consumption (6.2 Tg year^{-1}) of CH_4 (Dutaur and Verchot, 2007). However, recent research indicates that wet tropical forests may be a weaker CH_4 sink than previously

assumed and may even be a net source (Frankenberg et al., 2005; Nisbet et al., 2014; Teh et al., 2005). While there have been substantial advances in models that predict the drivers of global storage and fluxes of these gases in terrestrial ecosystems, much of this work is based on measurements in temperate ecosystems. Although these models are likely to play an important role in calculating soil C budgets, most fail to incorporate the effects of soil invertebrates, such as ants, on GHG flux (Filser et al., 2016). This is despite the fact that several species of ants – in addition to other soil invertebrates such as earthworms, dung beetles, and termites – can substantially alter soil GHG fluxes (Brümmer et al., 2009; Jílková et al., 2015, 2016; Lubbers et al., 2013; Slade et al., 2016; Wu et al., 2013).

Leaf-cutting ants (*Acromyrmex* and *Atta* spp., Formicidae) are a ubiquitous feature of tropical forests and are considered ecosystem engineers due to their substantial impacts on soil structure and nutrient content, plant biomass, and plant community structure around their nests (Haines, 1975, 1978). They are considered a dominant herbivore in the Neotropics, although their herbivory consists of harvesting plant material to provide food for the symbiotic nest fungus *Leucoagaricus gongylophorus* (Aylward et al., 2013; Moreira-Soto et al., 2017), which is subsequently used to nourish the colony's brood (Meyer et al., 2011). The majority of forest-dwelling leaf-cutting ant species dispose of exhausted leaf material and pathogen-infected fungus in underground refuse chambers (Farji-Brener et al., 2016). In doing so, leaf-cutting ants translocate large amounts of C and nutrients associated with plant material from the surrounding area to their nests, where this material is stored underground, decomposed and concentrated (Perfecto and Vandermeer, 1993; Sousa-Souto et al., 2012). *A. cephalotes* nests have been shown in recent studies to be sources of enhanced CO_2 flux (Fernandez-Bou et al., 2019). Because of their translocation of substantial amounts of labile C to oxygen-depleted soils where methanogenesis occurs, it is reasonable to assume that the nests of *A. cephalotes* could also be substantial sources of CH_4.

The objectives of our study were to measure GHG (CH_4, CO_2) fluxes from the nests of *Atta cephalotes* and nearby reference soils in a tropical lowland rainforest, and to determine if fluxes above nest holes were higher than those from nest soils without holes, and soils not associated with nests. We hypothesized that fluxes of CH_4 and CO_2 from patches of soil containing *A. cephalotes* nest holes would be significantly larger (positive values representing emissions) than fluxes from soils without nest holes.

2. Methods

2.1. Study area

This study was conducted in the La Selva Biological Research Station (hereafter "La Selva"), Sarapiquí, Costa Rica, northeastern Caribbean slope (10°25′51″N, 84°00′59″W). La Selva encompasses over 1,500 ha of lowland tropical forest in northeastern Costa Rica and covers several different land use types, including primary forest, secondary forest, former plantations, regenerating agricultural pastures, and swamps (Matlock and Hartshorn, 1999). Mean annual precipitation is 4.26 m (1986–2015) (Fernandez-Bou et al., 2019), and mean monthly temperature ranges from 24.7 °C in January to 27.1 °C in August (Sanford et al., 1994). Soils present in La Selva are andic dystropept, andic humitropept, inceptisols, and ultisols (Mata-Chávez and Sancho, 1987). While La Selva is home to over 400 species of ants (Longino et al., 2002), *Atta cephalotes* is one of the most prominent, and the most abundant leaf-cutting ant species in the area (Perfecto and Vandermeer, 1993).

2.2. Field measurements

All GHG flux measurements were performed between January 3–5, 2017. Three nests of *A. cephalotes* were measured in three different habitat types: a secondary forest and former cacao plantation

("secondary"), a section of primary forest with a nest under full canopy cover but not far from a grassy cleared area ("primary"), and a clear-cut area near a guardhouse on the forest edge, with low tree cover ("disturbed"). Disturbed and secondary forest sites were dominated by alluvial soils and primary forest soils were categorized as residual soils. At each nest, fluxes of CH_4 and CO_2 were measured at the largest hole, at three smaller nest holes of varying distances from the largest hole, and on one patch of nest soil cleared by ants but containing no nest holes. Fluxes above twelve nest holes were measured in total, and in each case only one vent hole was included in the flux chamber at each time. For reference, Soil fluxes of CH_4 and CO_2 were also measured at eight sampling points in a patch of secondary forest, and eight sampling points in a patch of primary forest near nests but not heavily influenced by leaf-cutting ants (no visible trails, no cleared patches of soil). GHG flux sampling points in each patch of forest were each 10 m away from a central point and arranged in a regular octagon. In cases where a sampling location was obstructed by a tree or the ground was too uneven to take a flux reading, a new point no more than 1 m away was used.

GHG fluxes were measured using a Los Gatos Ultraportable GHG flux analyzer and a 21 cm diameter ×30 cm tall cylindrical transparent static flux chamber, similar to previously described methods (Brannon et al., 2016; Martin and Moseman-Valtierra, 2015). All GHG flux measurements were performed between 9:00 and 16:00 to avoid confounding effects of diurnal variability, and were conducted for 4−6 min per plot, based on observed periods for linear rates of change. Positive values represent emissions and negative values represent uptake (flux of gas from the air into soil). Hobo® data loggers (Onset, Bourne, MA) were suspended within flux chambers during all flux measurements to record air temperature at 30 s intervals, and the Ideal Gas Law was used to calculate changes in gas concentrations over time using within-chamber air temperatures and ambient atmospheric pressure and accounting for chamber volume and footprint area.

At each gas sampling point, a polyvinyl chloride (PVC) collar with a sharpened edge was inserted into the upper cm of soil. Collars were left in place for 30 min prior to flux measurements to reduce the effects of soil disturbance, and a gas flux chamber was subsequently placed over the collar. The gap between the chamber and collar was sealed with water, and after placement of the chamber, the first thirty seconds of data were omitted to allow mixing of transient gases that remained in the chamber from prior to sealing. All fluxes were expressed per m^2 of soil by dividing by the cross-sectional area of the cylindrical flux chamber (0.032 m^2). Each point was measured twice, once in the morning, and once in the afternoon to account for changes in flux throughout the day. Temperature was recorded for each point using a temperature probe at a depth of 5 cm. During flux measurement temperature sensors were placed inside and outside the gas measuring chamber and readings were compared to identify if the flux measuring equipment was generating a microhabitat not representative of the soil. Salinity of surface soil in parts per thousand was recorded using a handheld refractometer, and pH of topsoil was recorded using a handheld pH meter. We obtained daily average soil water volumetric content (% WVC) from the La Selva weather station (https://anetium.ots.ac.cr/meteoro/default.php?pestacion=201). Average conditions during the measurement of soil gas fluxes are shown in Table 1.

Table 1

Average environmental conditions and characteristics of surface soils during the study period. Confidence intervals (95 %) are provided in parentheses after each average value. VWC = volumetric water content.

air temperature (°C)	soil pH	soil salinity (ppt)	soil temp °C	soil moisture (% VWC)
26.80 (0.26)	5.23 (0.19)	3.86 (0.72)	23.95 (0.15)	50.73 (1.19)
n = 32	n = 32	n = 32	n = 32	n = 3

The area of each leaf cutter ant nest was calculated by measuring the total surface area of soil that had been cleared of vegetation by ants, using previously published methods (Hernández et al., 1999; Varón et al., 2011). Briefly, the area was estimated as an ellipse ($\pi \times l/2 \times w/2$), where l is the greatest length along the cleared area and w is the greatest dimension across the nest and perpendicular to l. The density of holes in each nest was quantified using a grid system of 0.25×0.25-m quadrats covering the entire nest area. The area of each hole was calculated using the same formula as above, with the diameter of each hole being measured along two perpendicular axes and the area being calculated as an ellipse. Summary measurements for each nest are provided in Table 2.

Total CO_2 and CH_4 fluxes for each nest were estimated as follows. Flux of GHG above each nest hole was corrected for the amount of gas consumed by bare soil surrounding the hole within the flux chamber, according to the following equation: $[(F_{hole} \times SA_{chamber}) - (F_{non-hole} \times SA_{non-hole})]/SA_{hole}$, where F_{hole} equals the flux of CO_2 or CH_4 (mg C m^{-2} d^{-1}) in a given patch of soil containing an *A. cephalotes* nest hole; $SA_{chamber}$ equals the total ground surface area (m^2) enclosed in the flux chamber; SA_{hole} equals the area of the nest hole within the flux chamber; $F_{non-hole}$ equals the median flux of CO_2 or CH_4 (mg C m^{-2} d^{-1}) from all soils without *A. cephalotes* nest holes (including non-nest soils) in the current study; and $SA_{non-hole}$ equals the total solid soil surface area (minus the area of the nest hole) enclosed within the flux chamber (m^2). The median of the resulting values for GHG flux per area of hole within a single nest was then multiplied by the total nest area covered by holes (m^2) within the same nest. The median value for GHG flux from bare soil across all measurement locations was multiplied by the total area of bare soil within a single nest. These two values were added together to estimate the total flux of CO_2 or CH_4 from each nest (mg C nest^{-1} day^{-1}). Note that using only nest soils without holes when calculating $F_{non-hole}$ would have provided average CH_4 and CO_2 flux values of -0.11 and 3137 mg CH_4-C or CO_2-C m^{-2} d^{-1}, respectively, while using all soils without holes (including non-nest soils) produced average CH_4 and CO_2 flux values of -0.25 and 3477, respectively. Because fluxes from holes are so much larger than fluxes from soils without holes, these differences had a negligible effect on final estimates. Estimated CH_4-C and CO_2-C fluxes per nest calculated with the two flux values for soils without holes differed by less than 0.5 and 5200 mg CH_4-C or CO_2-C nest^{-1} d^{-1} (average = 0.22 and 1854).

2.3. Statistical analyses

Flux data were log-transformed to meet the assumptions of normality, heteroskedasticity, and linearity, and a small positive constant (2) was added to all CH_4 flux values before applying a natural log transformation. Linear regression was used to test for a correlation between CO_2 and CH_4 fluxes from leaf-cutting ant nest holes, and between CO_2 and CH_4 flux and hole area. Differences in GHG flux between soils containing nest holes and non-nest-associated soils were tested with two-tailed t-tests, with the assumption of heterogeneous variances in the case of CH_4 fluxes. All analyses were conducted in R Studio version 1.2.1335 (RStudio Team, 2020).

Table 2
Nest surface area (S.A.), number of holes, and median and range of hole surface areas (S.A., in cm^2) for each *A. cephalotes* nest included in the study.

nest site	nest S.A. (m^2)	nest holes	hole S.A. median, range (cm^2)	summed hole S.A. (cm^2 nest^{-1})
primary forest	45.24	41	3.14 (0.76−120.95)	235.62
disturbed area	148.39	46	0.98 (0.20−122.52)	249.56
secondary forest	90.28	12	3.14 (0.79−235.62)	426.86

3. Results

3.1. Soil and hole fluxes of CO_2 and CH_4

Nest hole-associated fluxes of GHG were significantly higher than fluxes from non-nest-associated soils (CO_2 $t_{1,14.04} = 2.93$, $p < 0.05$; CH_4 $t_{1,14.29} = 2.90$, $p < 0.05$, Fig. 1, Table 3). Fluxes of CO_2 and CH_4 were positively correlated in patches of soil containing holes from *A. cephalotes* nests ($R^2 = 0.59$, $F_{1,10} = 14.41$, $p < 0.01$), but not in non-nest soils. The magnitude of GHG flux from holes was highly variable among nests and among holes within a single nest (Fig. 1), but there was no correlation between fluxes of CO_2 or CH_4 and the size (area) of nest holes. Estimated total GHG fluxes from whole nests were also highly variable (ranging 0.24 to 0.58 kg CO_2-C, and -13 to 5,880 mg CH_4-C nest^{-1} d^{-1}, Fig. 2), with all nests potentially acting as sources for CO_2, but one nest possibly taking up more CH_4 than it emitted and acting as a net sink (see primary forest, Fig. 2b).

4. Discussion

4.1. Implications for sink or source status of tropical forest soils

We measured substantially elevated emissions of CO_2 and CH_4 from *A. cephalotes* nest holes relative to nest and non-nest soil without holes (Fig. 1), and although there was considerable variability in emission rates from each hole and nest, our measurements suggest that CO_2 and CH_4 fluxes from holes are high enough that *A. cephalotes* nests may be net sources of CH_4 (Fig. 2). This is in contrast to general perceptions of tropical forest soils, which are usually regarded as fairly strong CH_4 sinks (Dutaur and Verchot, 2007) based on the results of on-the-ground measurements that exclude ant nests. Our highest estimated CH_4 emission rates per nest (5,880 mg CH_4-C d^{-1}), if sustained for an entire year (2.15 kg yr^{-1}) could offset nearly 2/3 of the average CH_4 sink of an entire hectare of tropical forest soils (-3.33 kg CH_4 ha^{-1} yr^{-1}, see Dutaur and Verchot (2007)). However, CH_4 soil fluxes vary seasonally with temperature and soil moisture levels, and this temporal variability is not reflected in our study. the scaling of *Atta* nest emissions to larger areas is further complicated by the high variability of nest density estimates. Some studies provide estimates for single *Atta* species, while others lump several taxa together, making it difficult to estimate both the combined densities of all species and the density of a single species (such as *A. cephalotes*) alone. Existing estimates of *A. cephalotes* nest densities (excluding other *Atta* species or multiple species in combination) range from 0 to 3.5 nests ha^{-1} (average = 1.06 ± 0.62 [mean ± 95 % CI]) in mature or primary forests, 0.5–16 nests ha^{-1} (average = 4.68 ± 5.62) in secondary forests, and 2.6–102 nests ha^{-1} (average = 37.77 ± 45.07) in agricultural sites and other disturbed areas (Bianchi, 1998; Cherrett, 1968; Fernandez-Bou et al., 2019; Jaffe and Vilela, 1989; Rockwood, 1973; Sendoya et al., 2014; Soper et al., 2019). Depending upon land use and leaf-cutting ant nest density, *Atta* nest emissions have the potential to alter the CH_4 sink status of tropical forests. However, given the limited temporal and spatial replication in our study and variability in observed emissions, this needs further investigation.

4.2. Potential mechanisms behind enhanced CH_4 flux from Atta nests

Soils not containing *Atta* nests at the La Selva Biological Station are generally CH_4 sinks, even when close to saturation (Aronson et al., 2019). However, prior to the year 2019 studies of soil CH_4 fluxes throughout the tropics have generally excluded ant colonies. Enhanced microbial activity has been noted in previous studies of leaf-cutting ant nests (Fernandez-Bou et al., 2020, 2019; Fernandez et al., 2014; Majeed et al., 2018; Soper et al., 2019; Sousa-Souto et al., 2012), but substantial CH_4 emissions had only been observed in aboveground refuse piles of *Atta colombica* (Soper et al., 2019). Several factors may contribute to the elevated CO_2 and CH_4 emissions from *A. cephalotes* nests observed in the
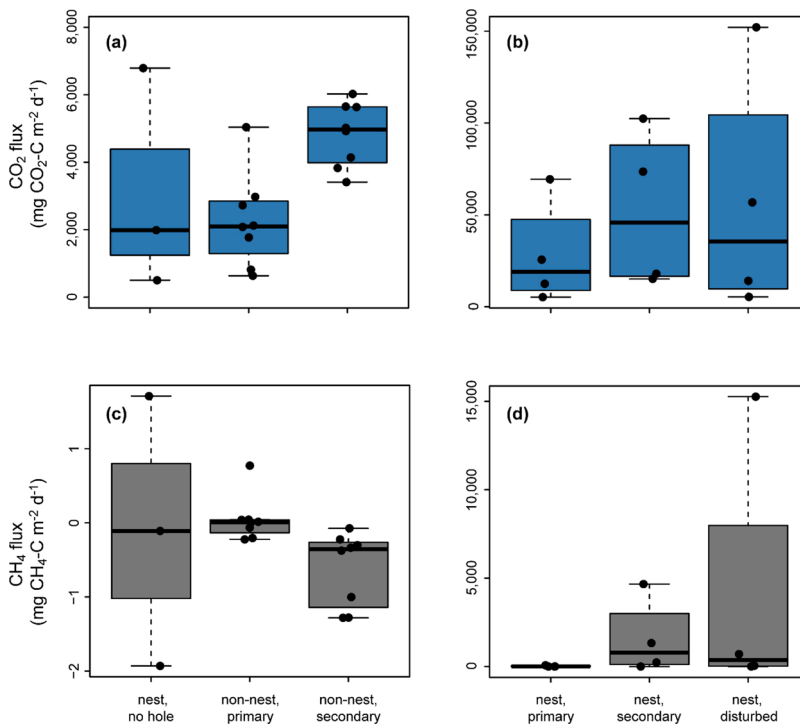
Fig. 1. Box plots of soil greenhouse gas fluxes including CO_2 emissions in patches of soil without (a) and with (b) a single *A. cephalotes* nest hole, and CH_4 fluxes in patches of soil without (c) and with (d) a single *A. cephalotes* nest hole. The two panels on the left (a, c) include fluxes of CO_2 and CH_4 (respectively) from patches of soil above nests but without holes (nest soil, no hole), and non-nest-associated soils in primary (non-nest, primary) and secondary (non-nest, secondary) forests. The two panels on the right (b, d) include fluxes of CO_2 and CH_4 (respectively) from patches of nest soil containing a single hole, in a primary forest (nest, primary), in a secondary forest / former plantation (nest, secondary), and in a cleared area (nest, disturbed). Note that the scales of vertical axes differ in all panels.

Table 3

Average (mean) soil fluxes of CO_2 and CH_4, from *A. cephalotes* nest areas and non-nest soils. Confidence intervals (95 %) and ranges are provided to the right of each average value. The column labeled "% sinks" provides the percentage of flux measurements that were negative (uptake of CO_2 or CH_4 by soil) within a given category. Raw data are available in the supplementary material (Table S1).

	CO_2 flux (mg C m^{-2} day^{-1})			CH_4 flux (mg C m^{-2} day^{-1})		
	mean ± 95 % C.I.	range (min – max)	% sinks (soil uptake)	mean ± 95 % C.I.	range (min – max)	% sinks (soil uptake)
non-nest soil, secondary forest (n = 8)	4,828 ± 657	(3,409–6,024)	0	−0.61 ± 0.34	(−1.28– −0.07)	100 %
non-nest soil, primary forest (n = 8)	2,269 ± 961	(633–5,038)	0	0.05 ± 0.22	(-0.22–0.77)	37.5 %
A. cephalotes nest soil without holes (n = 3 total, 1 per nest)	3,092 ± 3,720	(500–6,790)	0	−0.11 ± 2.06	(−1.93–1.71)	66.7 %
A. cephalotes nest soil, primary forest, one nest hole (n = 4)	28,177 ± 28,161	(5,228–69,383)	0	18 ± 34	(−1.08–65)	50 %
A. cephalotes nest soil, secondary forest, one nest hole (n = 4)	52,268 ± 42,029	(15,159–102,392)	0	1,556 ± 2,109	(−1.10–4,665)	25 %
A. cephalotes nest soil, disturbed area, one nest hole (n = 4)	57,080 ± 65,870	(5,332–152,091)	0	4,003 ± 7,364	(2.14–15,265)	0

current study. Fresh organic matter (cut plant material) is continually translocated from above- to below-ground in *A. cephalotes* nests, and the large amounts of decomposing leaf litter within underground waste management chambers of *A. cephalotes* nests are likely to be a major source of emissions. Similar to the enhancement of CH_4 and N_2O emissions by *A. colombica* waste above ground (Soper et al., 2019), *A. cephalotes* refuse piles may provide the labile C necessary to enhance CH_4 fluxes below ground. During an excavation of two *A. cephalotes* nests in Costa Rica, Fernandez-Bou et al. (2020) noted that refuse chambers ranged from 20−100 L in volume but were only about 5% full of refuse despite the fact that they were receiving a steady supply of organic matter, indicating rapid rates of decomposition. The fact that the refuse chambers were partially flooded while the other chambers and tunnels of the nest were dry suggested that they may serve other functional roles such as draining floodwaters away from other parts of the nest. High organic matter loads and saturation with water are the two factors most frequently cited as driving elevated CH_4 fluxes in tropical soils, and the combination of the two in refuse chambers increases the likelihood that refuse chambers may contribute more than other parts of the nest to methane emissions (Verchot et al., 2000). The existence of

subterranean tunnels alone may enhance CH_4 exchange with the atmosphere. *Atta* spp. nest tunnels extend to depths up to 7 m (Swanson et al., 2019), providing a direct connection between soil sites of methanogenesis and the atmosphere. This could allow elevated concentrations of CH_4 in anoxic layers of moist tropical soil (Teh et al., 2005) to rapidly bypass oxic, methanotrophic soils via nest tunnels and holes, rather than slowly diffusing upward through soil layers and being oxidized before reaching the atmosphere.

The positive correlation between CO_2 and CH_4 fluxes from *A. cephalotes* nest holes could indicate that aerophilic and methanogenic microbes are highly active in coupled aerobic and anaerobic microsites, possibly near to one another in specific regions of the nest. In previous studies of tropical soil GHG fluxes, it has been suggested that high levels of aerobic microbial respiration deplete oxygen concentrations in soils, thereby creating anaerobic microsites within soils which in turn enhance methanogenesis (Verchot et al., 2000). High densities of plant roots and resulting root respiration and release of labile root exudates may create anoxic microsites and enhance methanogenesis in soils (Philippot et al., 2009; Sørensen, 1997; Waldo et al., 2019), but while the nest soils of some *Atta* species contain significantly higher densities and biomass of
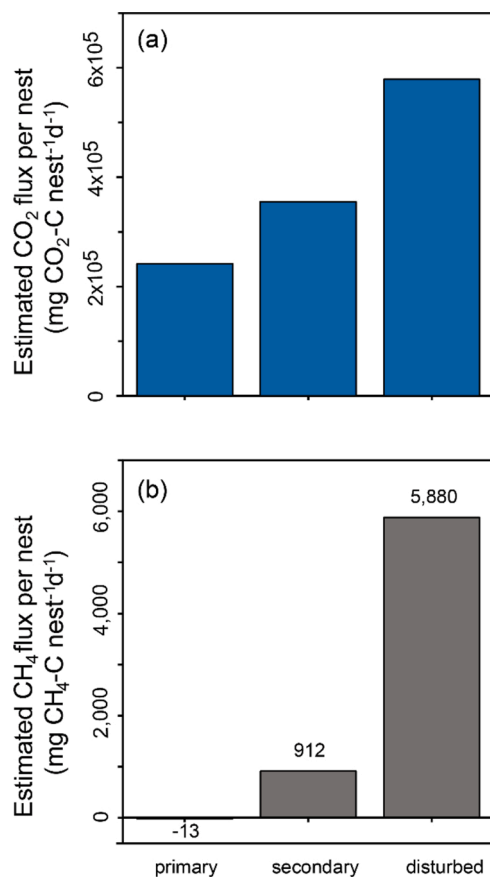
Fig. 2. Estimated single-day total nest flux of (a) CO_2 and (b) CH_4 per each *A. cephalotes* nest in primary and secondary forest ("primary", "secondary", respectively) and in a cleared area (disturbed) on the day of measurement. Note that the scales of the vertical axes differ in the two panels. Calculations are described in the Methods section.

roots, this effect may differ among species depending on whether refuse is disposed of outside of the nest or in underground refuse chambers (Farji-Brener and Medina, 2000; Moutinho et al., 2003). A portion of the elevated CO_2 fluxes may also be due in part to the oxidation of CH_4 as it travels upward from the deeper soils of the nest. Conversely, previous studies have shown CO_2 to be an important source of C for methanogenesis in tropical forest soils (Teh et al., 2005), and it is possible that air within leaf-cutting ant nest chambers and tunnels, enriched in CO_2 and low in O_2 (Bollazzi et al., 2012; Fernandez-Bou et al., 2019), may provide ideal conditions for methanogenesis. Finally, the observed correlation between CO_2 and CH_4 fluxes may reflect that different nest holes play different roles as part of a complex ventilated nest. At any given time some nest holes will serve as entrance and others as an exit, and gas fluxes from *A. cephalotes* nests are controlled by temperature gradients within the nest that drive free convection, and sporadic wind-forced convection (Fernandez-Bou et al., 2020). Future studies of ant nest CO_2 and CH_4 fluxes should take into account the interplay between altered physical characteristics brought about by ants. For example, increased canopy openness above *A. cephalotes* nests allow substantially more light to reach soils compared to non-nest sites, consequently increasing soil temperature amplitudes and reducing moisture in the soil surrounding nests (Corrêa et al., 2010; Meyer et al., 2011). All these factors may alter nest soil fluxes of GHGs.

4.3. Land use change and the role of Atta nests in tropical CH4 flux

Land use change within the tropics may increase the potential for leaf-cutting ants to alter C emissions in tropical soils. Primary forest land

area is decreasing (Hansen et al., 2013; Watson et al., 2016), having been altered by humans for agriculture and other uses. *A. cephalotes* and other species of leaf-cutting ants are often regarded as pests, as they prefer the leaves of several economically valuable plants such as cocoa, citrus, coffee, cotton, maize, and cassava, as well as displaying a preference for fertilized crops (Montoya-Lerma et al., 2012). Higher densities of leaf-cutting ants are observed in secondary forests and disturbed areas relative to primary forests (Farji-Brener, 2001), and very high nest densities have been observed in several different agricultural settings (>100 colonies ha^{-1} in some coffee farms) (Bianchi, 1998; Hernandez and Jaffe, 1995; Varón et al., 2011; Zanetti et al., 2000). The magnitude of fluxes potentially arising from leaf-cutting ant nests in disturbed areas may solidify the roles of degraded tropical forests as sources, at least for CH_4, and further clearing of land for agriculture may further enhance leaf-cutting ant nest contributions to total GHG emissions.

4.4. Suggestions for future research

Given their high densities and potentially large effects on C exchange between soils and the atmosphere, the activity leaf-cutting ants may prove to be an important component of C budgets in the ecosystems where they occur. If further research confirms leaf-cutting ant nests to be net sources of CH_4 and CO_2 over annual time scales, then tropical forest C cycling models could incorporate an accelerated living biomass to CO_2 and CH_4 emission pathway that is mediated by leaf-cutting ants. However, our findings should be interpreted with caution. Measurements were collected within a small area of Costa Rican lowland tropical forest, from three nests, within a short window of 3 days. Our results clearly show that GHG fluxes were highly variable from one nest to another as well as among holes within individual nests. Brief, geographically limited sampling periods such as ours are not sufficient to make definitive conclusions about the biogeochemistry of an entire ecosystem or region, as these fluxes are likely to vary with colony age and size, season, elevation, weather conditions, soil types, soil moisture, time of day (Fernandez-Bou et al., 2020), and among broader regions where leaf-cutting ants occur. For example, average soil moisture at the time of our measurements (50.73 % VWC) was within the range that appeared to enhance soil CO_2 and CH_4 efflux in previous studies at the La Selva Biological Station (Aronson et al., 2019; Schwendenmann et al., 2003), and therefore the nest emissions of CO_2 and CH_4 measured in the current study may be higher than if they had been measured during drier conditions. Future studies should target larger sample sizes with repeated measurements of multiple leaf-cutting ant species' nests, broader spatial and temporal coverage to include multiple regions and seasons, and the measurement of fluxes immediately before and after rain events that may cause pulsed releases of CO_2 and CH_4. As the activities of *Atta* spp. change with colony age and over the course of each day and year, and as environmental conditions change between wet and dry seasons, so may the fluxes of GHG from *Atta* nests.

Additional suggestions for future studies include a better characterization of changing air flow conditions during flux measurements. In this study, most CO_2 and CH_4 fluxes from *A. cephalotes* nest holes were substantially larger than fluxes from soils not colonized by *Atta* nests, but at times fluxes from nest holes were somewhat curvilinear or showed variability that potentially indicated changes in air flow to the flux chamber (Fig. S1). These occasional non-linear fluxes may have been due to changing air movement within the underground tunnel network. Closed chamber measurements on a complex nest structure have inherent limitations and static chamber flux measurements on nest holes should therefore be interpreted with caution. The complex nest architecture likely contributes to the large among-nest and within-nest variability in GHG fluxes observed in the current study (Fig. 1, Table S1), as different nest holes may function as air entrances or exits for GHGs (Bollazzi et al., 2012). Pressure sensors within flux chambers could help to detect, if they occur, any changes in air flow during a chamber incubation that pushes or pulls CO_2 and CH_4 to/from the flux chamber.

The use of multiple chambers coupled with a multiplexer may allow a single GHG analyzer to simultaneously measure gas fluxes from multiple nest holes. Additionally, open path gas analyzer systems could capture concentration and mass flow of any gas of interest, without interfering with air flow patterns in nest holes.

At equivalent levels of CH_4 flux as those measured in this study, and at published densities of *A. cephalotes*, it is possible that leaf-cutting ants may collectively reduce CH_4 sink strength in lowland tropical forests, potentially even converting some areas into net sources of CH_4 to the atmosphere. The range of estimates for densities of *Atta* spp. nests are highly variable within the same region, and in order to understand their effects on tropical forests' roles as sources or sinks of CO_2 and CH_4 at larger scales, better estimates of nest density are crucial. Furthermore, future studies attempting to determine leaf-cutting ant effects on forest C budgets should clearly delineate study systems both spatially and temporally. For example, considering only the nest site, nests are likely a net CO_2 source. However, if the foraging area and associated translocation of leaf C is included, conclusions about ant effects on C budgets may be different. Leaf-cutting ants may simply concentrate CO_2 emissions in a single location (otherwise occurring as more spatially diffuse emissions during organic matter decomposition across the landscape), rather than enhance them. Finally, a clearer determination of the factors influencing variability in emissions among nests (and among holes within an individual nest) are needed, and the elucidation of the factors driving these differences provides an exciting avenue for new research.

Author contribution statement

Conceptualization, A.S.M. and R.M.M; Methodology, A.S.M. and R.M.M.; Formal analysis, A.S.M., R.M.M., C.S.D., E.B.J., J.J.Q. and D.Y.; Investigation, A.S.M., R.M.M., C.S.D., E.B.J., J.J.Q. and D.Y.; Resources, R.M.M.; Data curation, A.S.M. and R.M.M.; Writing—original draft preparation, A.S.M. and R.M.M.; writing—review and editing, A.S.M., C.S.D., and D.Y.; visualization, A.S.M.; supervision, A.S.M. and R.M.M.; project administration, A.S.M. and R.M.M.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.pedobi.2021.150754.

References

Aronson, E.L., Dierick, D., Botthoff, J.K., Oberbauer, S., Zelikova, T.J., Harmon, T.C., Rundel, P., Johnson, R.F., Swanson, A.C., Pinto-Tomás, A.A., Artavia-León, A.,
Matarrita-Carranza, B., Allen, M.F., 2019. ENSO-influenced drought drives methane flux dynamics in a tropical wet forest soil. J. Geophys. Res. Biogeosci. 124, 2267–2276.

Aylward, F.O., Burnum-Johnson, K.E., Tringe, S.G., Teiling, C., Tremmel, D.M., Moeller, J.A., Scott, J.J., Barry, K.W., Piehowski, P.D., Nicora, C.D., Malfatti, S.A., Monroe, M.E., Purvine, S.O., Goodwin, L.A., Smith, R.D., Weinstock, G.M., Gerardo, N.M., Suen, G., Lipton, M.S., Currie, C.R., 2013. *Leucoagaricus gongylophorus* produces diverse enzymes for the degradation of recalcitrant plant polymers in leaf-cutter ant fungus gardens. Appl. Environ. Microbiol. 79, 3770–3778.

Baccini, A., Walker, W., Carvalho, L., Farina, M., Sulla-Menashe, D., Houghton, R.A., 2017. Tropical forests are a net carbon source based on aboveground measurements of gain and loss. Science 358, 230–234.

Bianchi, G., 1998. Densidad poblacional de hormigas corta-doras (*Atta cephalotes*) en la reserva biologica La Selva, Costa Rica. In: Farji-Brener, A.G., Chinchilla, F. (Eds.), Ecologia tropical y conservacion 98-2. O.T.S. Course Book, San Jose, Costa Rica, pp. 289–291.

Bollazzi, M., Forti, L.C., Roces, F., 2012. Ventilation of the giant nests of *Atta* leaf-cutting ants: does underground circulating air enter the fungus chambers? Insectes Soc. 59, 487–498.

Brannon, E.Q., Moseman-Valtierra, S.M., Rella, C.W., Martin, R.M., Chen, X., Tang, J., 2016. Evaluation of laser-based spectrometers for greenhouse gas flux measurements in coastal marshes. Limnol. Oceanogr. Methods 14, 466–476.

Brown, S., Lugo, A.E., 1982. The storage and production of organic matter in tropical forests and their role in the global carbon cycle. Biotropica 14, 161–187.

Brown, S., Lugo, A.E., 2017. Trailblazing the carbon cycle of tropical forests from Puerto Rico. Forests 8, 101.

Brümmer, C., Papen, H., Wassmann, R., Brüggemann, N., 2009. Fluxes of CH_4 and CO_2 from soil and termite mounds in south Sudanian savanna of Burkina Faso (West Africa). Global Biogeochem. Cycles 23. GB1001.

Cherrett, J.M., 1968. Some aspects of the distribution of pest species of leafcutting ants in the Caribbean. Proc. Am. Soc. Hortic. Sci. 12, 295–310.

Corrêa, M.M., Silva, P.S.D., Wirth, R., Tabarelli, M., Leal, I.R., 2010. How leaf-cutting ants impact forests: drastic nest effects on light environment and plant assemblages. Oecologia 162, 103–115.

Dutaur, L., Verchot, L.V., 2007. A global inventory of the soil CH_4 sink. Global Biogeochem. Cycles 21. GB4013.

Farji-Brener, A.G., Elizalde, L., Fernández-Marín, H., Amador-Vargas, S., 2016. Social life and sanitary risks: evolutionary and current ecological conditions determine waste management in leaf-cutting ants. Proc. R. Soc. B 283, 20160625.

Farji-Brener, A.G., 2001. Why are leaf-cutting ants more common in early secondary forests than in old-growth tropical forests? An evaluation of the palatable forage hypothesis. Oikos 92, 169–177.

Farji-Brener, A.G., Medina, C.A., 2000. The importance of where to dump the refuse: seed banks and fine roots in nests of the leaf-cutting ants *Atta cephalotes* and *A. colombica*. Biotropica 32, 120–126.

Fernandez, A., Farji-Brener, A.G., Satti, P., 2014. Moisture enhances the positive effect of leaf-cutting ant refuse dumps on soil biota activity. Austral Ecol. 39, 198–203.

Fernandez-Bou, A.S., Dierick, D., Swanson, A.C., Allen, M.F., Alvarado, A.G.F., Artavia-León, A., Carrasquillo-Quintana, O., Lachman, D.A., Oberbauer, S., Pinto-Tomás, A.A., Rodríguez-Reyes, Y., Rundel, P., Schwendenmann, L., Zelikova, T.J., Harmon, T.C., 2019. The role of the ecosystem engineer, the leaf-cutter ant *Atta cephalotes*, on soil CO_2 dynamics in a wet tropical rainforest. J. Geophys. Res. Biogeosci. 124, 260–273.

Fernandez-Bou, A.S., Dierick, D., Harmon, T.C., 2020. Diel pattern driven by free convection controls leaf-cutter ant nest ventilation and greenhouse gas emissions in a Neotropical rain forest. Oecologia 192, 591–601.

Filser, J., Faber, J.H., Tiunov, A.V., Brussaard, L., Frouz, J., Deyn, G.D., Uvarov, A.V., Berg, M.P., Lavelle, P., Loreau, M., Wall, D.H., Querner, P., Eijsackers, H., Jiménez, J.J., 2016. Soil fauna: key to new carbon models. SOIL 2, 565–582.

Frankenberg, C., Meirink, J.F., Weele, M.v., Platt, U., Wagner, T., 2005. Assessing methane emissions from global space-borne observations. Science 308, 1010–1014.

Haines, B., 1975. Impact of leaf-cutting ants on the vegetation development at Barro Colorado Island. In: Golley, F.B., Medina, E. (Eds.), Tropical Ecological Systems: Trends in Terrestrial and Aquatic Research. Springer-Verlag New York Inc., New York, New York, pp. 99–111.

Haines, B., 1978. Element and energy flows through colonies of the leaf-cutting ant, *Atta colombica*, in Panama. Biotropica 10, 270–277.

Hansen, M.C., Potapov, P.V., Moore, R., Hancher, M., Turubanova, S.A., Tyukavina, A., Thau, D., Stehman, S.V., Goetz, S.J., Loveland, T.R., Kommareddy, A., Egorov, A., Chini, L., Justice, C.O., Townshend, J.R.G., 2013. High-resolution global maps of 21st-century forest cover change. Science 342, 850–853.

Hernandez, J.V., Jaffe, K., 1995. Dani economico causado por populacoes de formigas *Atta laevigata* (F. Smith) em plantacoes de *Pinus caribea* Mor. e elementos para o manejo de paraga. Anais da Sociedade Entomologica do Brasil 24, 287–297.

Hernández, J.V., Ramos, C., Borjas, M., Jaffe, K., 1999. Growth of *Atta laevigata* (Hymenoptera: Formicidae) nests in pine plantations. Florida Entomol. 82, 97–103.

IPCC, 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jaffe, K., Vilela, E., 1989. On nest densities of the leaf-cutting ant *Atta cephalotes* in tropical primary forest. Biotropica 21, 234–236.

Jílková, V., Picek, T., Frouz, J., 2015. Seasonal changes in methane and carbon dioxide flux in wood ant (*Formica aquilonia*) nests and the surrounding forest soil. Pedobiologia 58, 7–12.

Jílková, V., Picek, T., Šestauberová, M., Krištůfek, V., Cajthaml, T., Frouz, J., 2016. Methane and carbon dioxide flux in the profile of wood ant (Formica aquilonia) nests and the surrounding forest floor during a laboratory incubation. FEMS Microbiol. Ecol. 92, fiw141.

Jobbágy, E.G., Jackson, R.B., 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecol. Appl. 10, 423–436.

Longino, J.T., Coddington, J., Colwell, R.K., 2002. The ant fauna of a tropical rain forest: estimating species richness three different ways. Ecology 8, 689–702.

Lubbers, I.M., Groenigen, K.Jv., Fonte, S.J., Six, J., Brussaard, L., Groenigen, J.Wv., 2013. Greenhouse-gas emissions from soils increased by earthworms. Nat. Clim. Change 3, 187–194.

Luyssaert, S., Schulze, E.-D., Börner, A., Knohl, A., Hessenmöller, D., Law, B.E., Ciais, P., Grace, J., 2008. Old-growth forests as global carbon sinks. Nature 455, 213–215.

Majeed, M.Z., Miambi, E., Barois, I., Bernoux, M., Brauman, A., 2018. Characterization of N_2O emissions and associated microbial communities from the ant mounds in soils of a humid tropical rainforest. Folia Microbiol. 63, 381–389.

Martin, R.M., Moseman-Valtierra, S., 2015. Greenhouse gas fluxes vary between *Phragmites australis* and native vegetation zones in coastal wetlands along a salinity gradient. Wetlands 35, 1021–1031.

Mata-Chávez, R., Sancho, F., 1987. Mapa de estudio detallado de suelos. Organización para Estudios Tropicales, Estación Biológica La Selva.

Matlock, R.B., Hartshorn, G.S., 1999. La Selva Biological Station (OTS). Bull. Ecol. Soc. Am. 80, 188–193.

Megonigal, J.P., E., H.M, Visscher, P.T., 2004. Anaerobic metabolism: linkages to trace gases and aerobic processes. In: Schlesinger, W.H. (Ed.), Biogeochemistry. Elsevier-Pergamon, Oxford, pp. 317–324.

Meyer, S.T., Leal, I.R., Tabarelli, M., Wirth, R., 2011. Ecosystem engineering by leaf-cutting ants: nests of Atta cephalotes drastically alter forest structure and microclimate. Ecol. Entomol. 36, 14–24.

Montoya-Lerma, J., Giraldo-Echeverri, C., Armbrecht, I., Farji-Brener, A., Calle, Z., 2012. Leaf-cutting ants revisited: towards rational management and control. Int. J. Pest Manag. 58, 225–247.

Moreira-Soto, R.D., Sanchez, E., Currie, C.R., Pinto-Tomás, A.A., 2017. Ultrastructural and microbial analyses of cellulose degradation in leaf-cutter ant colonies. Microbiology 163, 1578–1589.

Moutinho, P., Nepstad, D.C., Davidson, E.A., 2003. Influence of leaf-cutting ant nests on secondary forest growth and soil properties in Amazonia. Ecology 84, 1265–1276.

Nisbet, E.G., Dlugokencky, E.J., Bousquet, P., 2014. Methane on the rise-again. Science 343, 493–495.

Perfecto, I., Vandermeer, J., 1993. Distribution and turnover rate of a population of *Atta cephalotes* in a tropical rain forest in Costa Rica. Biotropica 25, 316–321.

Philippot, L., Hallin, S., Börjesson, G., Baggs, E.M., 2009. Biochemical cycling in the rhizosphere having an impact on global change. Plant Soil 321, 61–81.

Potter, C.S., Davidson, E.A., Verchot, L.V., 1996. Estimation of global biogeochemical controls and seasonality in soil methane consumption. Chemosphere 32, 2219–2246.

Rockwood, L.L., 1973. Distribution, density, and dispersion of two species of *Atta* (Hymenoptera: Formicidae) in Guanacaste Province, Costa Rica. J. Anim. Ecol. 42, 803–817.

RStudio Team, 2020. RStudio: Integrated Development for R. URL. RStudio, PBC, Boston, MA. http://www.rstudio.com/.

Sanford Jr, R.L., Paaby-Hansen, P., Luvall, J.C., Phillips-Rodríguez, E., 1994. Climate, geomorphology, and aquatic systems. In: McDade, L.A., Bawa, K.S., Hespenheide, H.A., Hartshorn, G.S. (Eds.), La Selva: Ecology and Natural History of a Neotropical Rain Forest. University of Chicago Press, Chicago, IL, p. 486.

Schwendenmann, L., Veldkamp, E., Brenes, T., O'Brien, J.J., Mackensen, J., 2003. Spatial and temporal variation in soil CO_2 efflux in an old-growth neotropical rain forest, La Selva, Costa Rica. Biogeochemistry 64, 111–128.

Sendoya, S.F., Silva, P.S.D., Farji-Brener, A.G., 2014. Does inundation risk affect leaf-cutting ant distribution? A study along a topographic gradient of a Costa Rican tropical wet forest. J. Trop. Ecol. 30, 89–92.

Slade, E.M., Riutta, T., Roslin, T., Tuomisto, H.L., 2016. The role of dung beetles in reducing greenhouse gas emissions from cattle farming. Sci. Rep. 6, 18140.

Sørensen, J., 1997. The rhizosphere as a habitat for soil microorganisms. In: van Elsas, J.D., Trevors, J.T., Wellington, E.M.H. (Eds.), Modern Soil Microbiology. Marcel Dekker Inc., New York, pp. 21–45.

Soper, F.M., Sullivan, B.W., Osborne, B.B., Shaw, A.N., Philippot, L., Cleveland, C.C., 2019. Leaf-cutter ants engineer large nitrous oxide hot spots in tropical forests. Proc. R. Soc. B 286, 20182504.

Sousa-Souto, L., Santos, D.C.d.J., Ambrogi, B.G., dos Santos, M.J.C., Guerra, M.B.B., Pereira-Filho, E.R., 2012. Increased CO_2 emission and organic matter decomposition by leaf-cutting ant nests in a coastal environment. Soil Biol. Biochem. 44, 21–25.

Swanson, A.C., Schwendenmann, L., Allen, M.F., Aronson, E.L., Artavia-León, A., Dierick, D., Fernandez-Bou, A.S., Harmon, T.C., Murillo-Cruz, C., Oberbauer, S.F., Pinto-Tomás, A.A., Rundel, P.W., Zelikova, T.J., 2019. Welcome to the *Atta* world: a framework for understanding the effects of leaf-cutter ants on ecosystem functions. Funct. Ecol. 33, 1386–1399.

Teh, Y.A., Silver, W.L., Conrad, M.E., 2005. Oxygen effects on methane production and oxidation in humid tropical forest soils. Glob. Change Biol. 11, 1283–1297.

Varón, E., Eigenbrode, S.D., Bosque-Pérez, N.A., Hilje, L., Jones, J., 2011. Coffee farm diversity and landscape features influence density of colonies of *Atta cephalotes* (Hymenoptera: Formicidae). J. Econ. Entomol. 104, 164–172.

Verchot, L.V., Davidson, E.A., Cattânio, J.H., Ackerman, I.L., 2000. Land-use change and biogeochemical controls of methane fluxes in soils of eastern Amazonia. Ecosystems 3, 41–56.

Waldo, N.B., Hunt, B.K., Fadely, E.C., Moran, J.J., Neumann, R.B., 2019. Plant root exudates increase methane emissions through direct and indirect pathways. Biogeochemistry 145, 213–234.

Watson, J.E.M., Shanahan, D.F., Marco, M.D., Allan, J., Laurance, W.F., Sanderson, E.W., Mackey, B., Venter, O., 2016. Catastrophic declines in wilderness areas undermine global environment targets. Curr. Biol. 26, 2929–2934.

Wu, H., Lu, X., Wu, D., Song, L., Yan, X., Liu, J., 2013. Ant mounds alter spatial and temporal patterns of CO_2, CH_4 and N_2O emissions from a marsh soil. Soil Biol. Biochem. 57, 884–891.

Zanetti, R., Vilela, E.F., Zanuncio, J.C., Leite, H.G., Freitas, G.D., 2000. Influência da espécie cultivada e da vegetação nativa circundante na densidade de sauveiros em Eucaliptais. Pesquisa Agropecuária Brasileira 35, 1911–1918.