

RESEARCH ARTICLE

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Key Points:

- Leaf-cutter ant nests change the CO₂ dynamics, reducing surrounding soil CO₂ concentration and increasing total emissions
- For same precipitation amounts, nest soils accumulate less CO₂ than nonnest soils; effects remain more than two years in abandoned nests
- Nest vents emitted up to 100,000 times more CO₂ than soil surface, and increased soil CO₂ emissions at the ecosystem level by 0.2 to 0.7%

Supporting Information:

- Supporting Information S1
- Spanish Translation of Supporting Information S2

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The Role of the Ecosystem Engineer, the Leaf-Cutter Ant *Atta cephalotes*, on Soil CO₂ Dynamics in a Wet Tropical Rainforest

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Abstract Leaf-cutter ants are dominant herbivores that disturb the soil and create biogeochemical hot spots. We studied how leaf-cutter ant *Atta cephalotes* impacts soil CO₂ dynamics in a wet Neotropical forest. We measured soil CO₂ concentration monthly over 2.5 years at multiple depths in nonnest and nest soils (some of which were abandoned during the study) and assessed CO₂ production. We also measured nest and nonnest soil efflux, nest vent efflux, and vent concentration. Nest soils exhibited lower CO₂ accumulation than nonnest soils for the same precipitation amounts. During wet periods, soil CO₂ concentrations increased across all depths, but were significantly less in nest than in nonnest soils. Differences were nonsignificant during drier periods. Surface efflux was equal across nest and nonnest plots (5 μmol CO₂ m⁻² s⁻¹), while vent efflux was substantially (10³ to 10⁵ times) greater, a finding attributed to free convection and sporadic forced convection. Vent CO₂ concentrations were less than in soil, suggesting CO₂ efflux from the soil matrix into the nest. Legacy effects in abandoned nests were still observable after more than two years. These findings indicate that leaf-cutter ant nests provide alternative transport pathways to soil CO₂ that increase total emissions and decrease soil CO₂ concentrations, and have a lasting impact. Estimated total nest-soil CO₂ emissions were 15 to 60% more than in nonnest soils, contributing 0.2 to 0.7% to ecosystem-scale soil emissions. The observed CO₂ dynamics illuminate the significant carbon footprint of ecosystem engineer *Atta cephalotes* and have biogeochemical implications for rainforest ecosystems.

Plain Language Summary Leaf-cutter ants modify their habitat to the extent that they are called ecosystem engineers. Living throughout the Americas, they construct massive nests to which they import the vegetation they harvest to feed a fungus they cultivate as their main food source. We studied the most common leaf-cutter ant in Costa Rica to assess the impact of its nests on carbon dioxide (CO₂) levels in surrounding soils and on soil CO₂ emissions. In the Costa Rican rainforest, heavy rains easily clog the clayey soils, accumulating CO₂ from microbial and root respiration. During wet periods, we observed lower CO₂ concentrations in nest soils relative to nonnest soils. We attribute this difference to the nest structure, which provides ventilation for both nest CO₂ and the CO₂ originated in the surrounding soil. We also found that soil CO₂ emissions were the same in nest and nonnest soils, but nest openings had emissions 100,000 times greater. Consequently, nests and their surrounding soils emit 15 to 60% more CO₂ than the equivalent nonnest soil areas. This difference, together with the expanding range of leaf-cutter ants, favored by human activities and warmer climate, has implications with respect to the global carbon cycle.

1. Introduction

Biogeochemical hot spots influence and can control the carbon balance and nutrient dynamics of whole ecosystems (Harms & Grimm, 2008; Leon et al., 2014; McClain et al., 2003). Soils are one of the largest global pools of carbon and the most heterogeneous, yet Earth system models currently rely on assumptions about soil carbon dynamics that add inaccuracy to global carbon estimates and climate change projections (Conant et al., 2011; Luo et al., 2016; Todd-Brown et al., 2013). Autotrophic and heterotrophic soil respiration, the former from roots and symbiotic microbes and the latter by saprotrophic prokaryotes and fungi, are an integral part of soil carbon dynamics that is most commonly assessed by measuring carbon dioxide (CO₂) efflux from the soil surface. This efflux exhibits spatiotemporal variability due to soil heterogeneity and environmental factors such as soil water content, temperature, and oxygen availability (Davidson, Janssens, et al., 2006). Environmental factors are dynamic and under influence from a changing climate. Soil fauna change soil structure and biogeochemical processes in ways that can stimulate or inhibit soil CO₂ efflux (Gutiérrez & Jones, 2006), further complicating estimates of soil carbon pools and fluxes. In this work, we focus on understanding the impact of a dominant member of the soil fauna, leaf-cutter ant *Atta cephalotes*, on soil CO₂ dynamics in tropical rainforest ecosystems.

Leaf-cutter ants are major herbivores in the Americas, and their large underground nests (Figure 1) are home to huge colonies that harvest hundreds of kilograms of fresh vegetation per year (Hughes & Goulson, 2002; Wirth et al., 2003). They are ecosystem engineers in tropical forest ecosystems (Blanton & Ewel, 1985; Urbas et al., 2007), that is, organisms that create and modify habitats by changing the surrounding biotic or abiotic components, regulating the availability of resources for other species (e.g., Jones et al., 1994). They change the habitat by creating canopy gaps (Corrêa et al., 2010), by transferring organic matter underground, by enhancing soil aeration and turnover rates (Perfecto & Vandermeer, 1993), and by increasing soil nutrient availability and nitrogen fixation (Pinto-Tomás et al., 2009). Nests appear as mounds of excavated soil marked by numerous entrances and gas vents that lead to an intricate network of tunnels and chambers. This structure extends 2 to 7 m belowground, depending on species (Jonkman, 1980a; Moreira et al., 2004). Leaf-cutter ants do not consume the vegetation itself; instead, they cultivate an obligate symbiotic fungus (*Leucoagaricus gongylophorus* (A. Møller) Heim) on the harvested vegetation in a network of subterranean chambers (Aylward et al., 2013; Hölldobler & Wilson, 2010; Suen et al., 2011). As the fungus decomposes the leaf fragments, it produces hyphal nodules (gongyliidia) that serve as food for the colony. Given the large vegetation input combined with fungal and ant activity, it is not surprising that leaf-cutter ant nests are hot spots for biogeochemical cycling (Costa et al., 2008; Pinto-Tomás et al., 2009). As forests become increasingly fragmented across the Neotropics (as for agriculture and grazing), leaf-cutter ants are becoming more abundant (Corrêa et al., 2010; da Silva et al., 2017; Siqueira et al., 2017) and their impact on soil carbon dynamics is expected to increase. Hence, understanding soil carbon dynamics requires accounting for their impacts.

The effect of leaf-cutter ant nests on soil CO₂ concentrations and emissions (Figure 1) is not well understood. As nutrient hot spots, it is reasonable to propose that nest soils emit more CO₂ than nonnest soils (Figure 1, pathways 1 and 2), especially given that CO₂ concentrations in nest tunnels are higher than background (atmospheric) levels and can exceed 5% (by volume) in vents connected to fungal and refuse chambers (Bollazzi et al., 2012; Harmon et al., 2015; Kleineidam & Roces, 2000). Leaf-cutter ant colonies constantly excavate their nest ventilation network to maintain adequate CO₂ and O₂ concentrations (Figure 1, pathway 2). For instance, grassland species *Atta vollenweideri* extend their vent openings by creating turrets above ground level to allow wind forced convection to drive ventilation (Halboth & Roces, 2017; Kleineidam & Roces, 2000). For most leaf-cutter ant species, vent CO₂ emission rates have not been well characterized, nor has the potential connection between the nest air and the surrounding nest soils (Figure 1, pathway 3). If the air in the nest has lower CO₂ concentration than the surrounding soil, given the large surface of nest walls and tunnels, the CO₂ emissions from the soil matrix to the nest air can be significant. If the opposite gradient occurs, it can be a relevant ventilation pathway for the nest. Accurate characterization of the nest and nest soil emissions will improve our understanding of the role of this ecosystem engineer in rainforest carbon cycling.

The complex behavioral and metabolic processes in leaf-cutter ant nests, coupled with their intricate architecture, lead to soil CO₂ efflux regimes that combine diffusive and convective gas transport and are challenging to quantify. For instance, forced convection (pressure-driven flux) is caused by windy conditions in *A. vollenweideri* nests (Kleineidam et al., 2001). Free convection, caused by significant gas density differences resulting from nonuniform temperature and vapor moisture content, has not been studied in

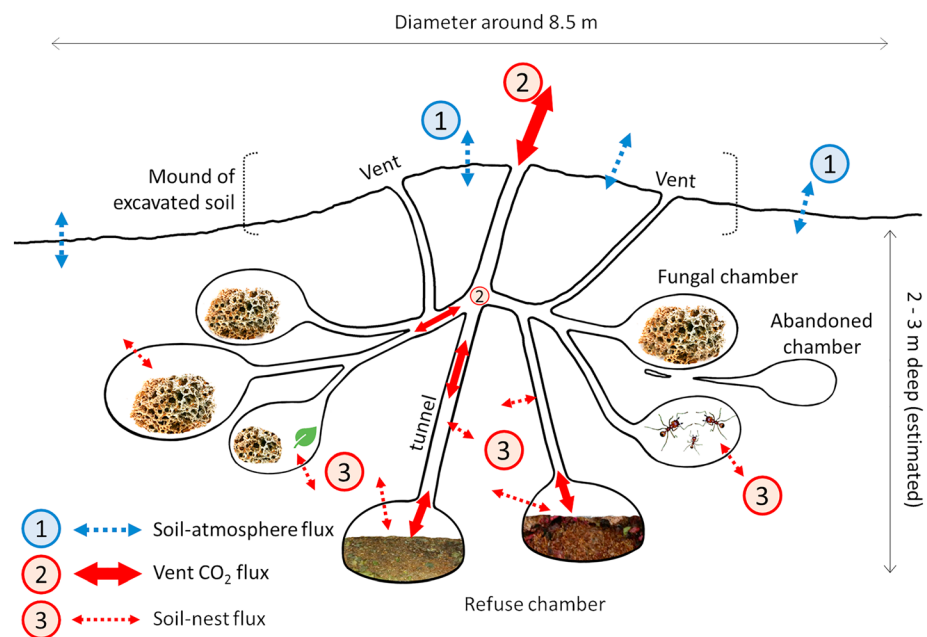


Figure 1. Conceptual diagram of CO₂ exchange and transport pathways in *Atta cephalotes* nests, including (1) soil surface CO₂ efflux; (2) nest vent CO₂ flux (convection and diffusion), where vent CO₂ stems from nest production (primarily fungal activity and refuse decay); and (3) soil-nest efflux. We estimate that the nests in our study area were around 2 to 3 m deep (based on excavations in the same region), and the average nest influence area was 67 m², with 32 vents.

leaf-cutter ant nests. It may play an important role in *A. cephalotes* nests built under dense canopies at times when the temperature and water vapor levels differ between the nest chambers and the atmosphere. Soil temperature and moisture content can significantly affect both soil gas diffusion and CO₂ production rates, and their relative contributions to soil CO₂ efflux can be difficult to separate. Within the soil matrix, diffusion from higher to lower concentrations is typically the dominant soil gas transport process, although instances of nondiffusive transport (convection) have been noted in soil respiration studies (Rey, 2015; Roland et al., 2015). Soil fauna can also affect soil properties pertinent to gas transport. For example, *Formica* ant nest excavation is known to reduce soil bulk density and decrease tortuosity (Drager et al., 2016), facilitating soil gas diffusion.

The goal of our study was to assess the impact of leaf-cutter ant *A. cephalotes* nests (both active and abandoned) on soil CO₂ dynamics (concentrations and emissions) in a lowland tropical rainforest. Our hypothesis was that leaf-cutter ant nest soils have greater CO₂ emissions than nonnest soils. To test it, we quantified soil CO₂ concentrations inside nest plots, CO₂ concentrations in nest vents, and CO₂ efflux from the soil surface and vent openings. We asked three related research questions: (1) What effect does the leaf-cutter ant nest structure have on CO₂ concentrations in the surrounding soil matrix under dry and wet weather conditions? (2) What is the influence of leaf-cutter ant soil excavation on surface CO₂ efflux? (3) What connection exists between nest vent CO₂ efflux and surrounding nest soil CO₂ concentration? By answering these questions, we aimed to assess the ecosystem-scale contribution of leaf-cutter ant nests to wet rainforest soil CO₂ emissions.

2. Study Site and Methods

2.1. Location and Site Selection

This study was conducted at La Selva Biological Station, in the Atlantic lowlands of northeastern Costa Rica (Figure 2; 10°25'19"N, 84°00'54"W; 37 to 135 m above sea level). The forest there is categorized as premontane tropical moist forest (Sanford et al., 1994), with an average annual rainfall of 4.26 m (1986 to 2015). Soils are volcanically derived oxisols (Kleber et al., 2007) and are relatively fertile for wet Neotropical

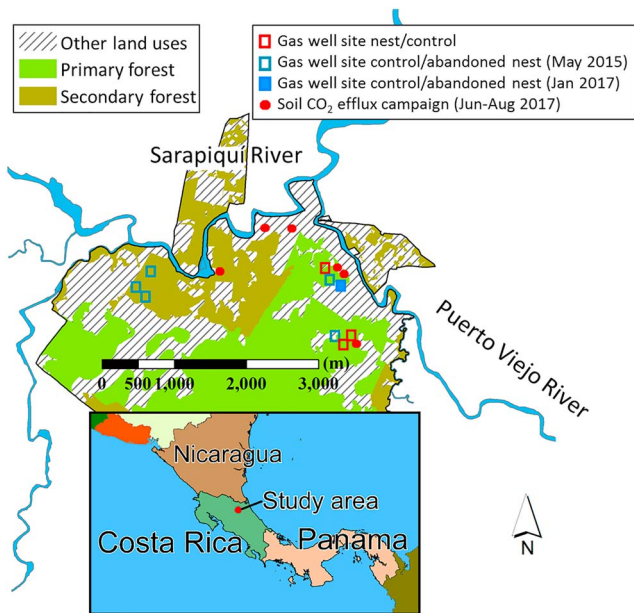


Figure 2. Map illustrating the study locations at La Selva Biological Station: open red squares depict active-nest/nonnest soil sites (3), where the nests remained active throughout the study (March 2015 to December 2017); open blue squares depict active-nest/nonnest soil sites (5), where the nests were abandoned early in the study (May to August 2015); and solid blue square depicts the active-nest/nonnest soil site, where the nest was abandoned late in the study (January 2017). After the nest abandonments, we performed soil surface efflux campaigns on all the abandoned-nest/nonnest soil sites and on six additional active-nest/nonnest soil sites (solid red circles). We also measured nest vent CO_2 flux and vent CO_2 concentration in the latter six active-nest plots.

forests (Powers et al., 2005). The site consists of old growth and secondary growth forest on recent alluvial terraces and weathered residual plateaus and slopes.

In early 2015, we selected nine long-term study sites, each consisting of a leaf-cutter ant nest plot paired with its nonnest plot as control (Figure 3). Nonnest plots were selected at least 20 m apart from their corresponding nest, on a similar slope and with the same vegetation cover. The nine nest/nonnest sites included locations on alluvial soil within primary forest and on residual (volcanic) soil within primary and secondary forest. Six nest plots were abandoned by their colonies during periods of heavy rains and flooding. We continued to observe the abandoned nests to assess legacy effects. Hence, our long-term study sites included nonnest, active-nest, and abandoned-nest soils. In addition to the long-term study, we also executed intensive short-term sampling campaigns aimed at characterizing soil surface CO_2 efflux and vent efflux. Due to the nest abandonments, we identified six additional active-nest and nonnest soil sites (Figure 2) for the efflux campaigns.

2.2. Instrument Installation and Sampling

All plots were set up on a 5×6 -m grid to provide orientation for the gas and efflux sampling. For the long-term plots, we also designated a 1-m wide passage to allow researcher and instrument access on the plot while avoiding soil compaction (which could bias efflux measurements) on the majority of the plot (Figure 3). We installed three gas wells at 20-, 60-, and 100-cm depths near the center of the plot. The gas wells consisted of stainless steel tubes (9.5-mm diameter), each with a perforated lower end wrapped in a fine metal mesh to prevent particle intrusion into the well (Schwendenmann et al., 2003). The gas samples were collected approximately monthly using a gastight polypropylene syringe with a one-way stopcock and sideport needle. The probe and syringe were flushed by drawing and discarding 30 mL of soil gas from the probes at 20- and 60-cm depths, and 60 mL for the probe at 100 cm. A 50-mL sample was then collected from each depth and analyzed for CO_2 concentration within a few hours of collection

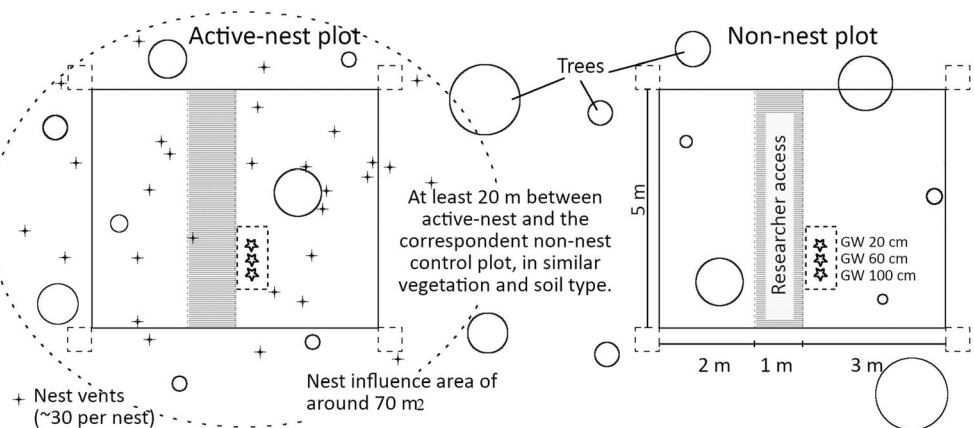


Figure 3. Schematic of a site with gas sampling ports. Each site included (left) a pair of active-nest and (right) a nonnest control soil plots. Each plot consisted on an area of 6×5 m (black, solid rectangles), with a 1-m wide passage (shaded rectangle) to access the three gas wells at 20-, 60-, and 100-cm depths (shown as three stars) near the center of the plot. At each corner of the plot, we placed 0.5×0.5 -m litter and coarse wood traps (dashed-line squares; see section 2.2 in the supporting information). Nest vents are represented with “cross” (average number surveyed was 32 vents per nest), and approximate tree circumferences are shown for scale.

using a custom bench top system built around a Li820 infrared gas analyzer (LI-COR, Lincoln, NE, USA) with a manual injection port. Each sample was measured 3 times and the average CO₂ peak concentrations were converted to absolute sample CO₂ concentrations using a standard curve. Because many gas samples had concentrations above the highest standard used and outside the Li820 specification, those samples were diluted 10-fold with CO₂-free air.

Soil gas sampling began in March 2015. Between May and August 2015, we observed that five nests were abandoned. We attributed the abandonments to the intense rainfall and flooding at La Selva during May and June 2015 (Figure 4). One additional nest was abandoned in January 2017, again after heavy rainfall. Over the duration of the study (about 2.5 years), this represents a turnover rate for *A. cephalotes* nests of about 18% yr⁻¹, which is consistent with the 23% yr⁻¹ turnover rate estimated by Perfecto and Vandermeer (1993) also at La Selva. Nests that persisted for the duration of the study included one in alluvial soil and two in residual soil, all in primary forest. In sum, by the end of the study, our long-term soil CO₂ concentration monitoring sites encompassed three active-nest soil plots, five plots with nests abandoned early in our study, one plot which nest was abandoned late in the study, and nine paired nonnest soil plots.

To assess the role of CO₂ production on soil CO₂ concentration, we conducted a literature survey on leaf-cutter ant nests and soil biomass production. We also collected fine litter and coarse woody debris from our plots every other week, approximately. To collect fine litter, we placed two litter traps (50 × 50 cm) at 1 m above the ground on the NE and SW corners of each plot (Figure 3). To collect coarse woody debris, we delimited two equal-sized traps on the ground surface on the NW and SE corners of each plot. The fine litter fall consisted of leaves (<50 cm) and woody parts (<1 cm), while the coarse woody debris consisted of leaves (>50 cm) and woody parts (>1 and <10 cm). We trimmed plant materials along the edge of the traps. The samples were placed in paper bags, dried at 70 °C for 72 hr, and weighted to the nearest 0.1 g.

2.3. Soil and Vent CO₂ Efflux Sampling

Between June and August 2017, we conducted sampling campaigns to measure soil moisture, soil surface and nest vent CO₂ efflux, and vent CO₂ concentration at the six added sites. The plots were set up with the same sampling grid (Figure 3), but without the gas sampling wells. At each plot, we measured soil surface efflux and soil moisture at four random points in three to five sampling events over the two-month period. To measure surface efflux, we installed 10-cm-diameter PVC collars on the ground surface 1 hr prior to sampling for 5 min using low-cost CO₂ flux chambers (modified from Harmon et al., 2015) equipped with NDIR CO₂ sensors (model MH-Z16, Winsen Technology Co., Henan, China) and relative humidity and temperature sensors (model HTU21D, TE Connectivity). In addition, we measured the soil moisture and temperature at a 5-cm depth within the collar after each soil efflux measurement using a Decagon sensor connected to a data-logging meter (sensor model GS3 and meter model ProCheck, Decagon Devices, Pullman, WA, USA). We validated our sensor measurements using standard equipment (Li820 infrared gas analyzer, LI-COR, Lincoln, NE, USA). Detailed specifications for the CO₂ efflux detection chambers are available in section 2.3 in the supporting information.

During each vent sampling event, we measured the CO₂ efflux from six vents in each of the six active-nest plots. In preliminary tests, we observed that vent CO₂ efflux was several orders of magnitude larger than soil surface efflux, that is, greater than would be possible by gas diffusion only. This led us to conclude that free and/or forced convection were likely occurring (Hölldobler & Wilson, 2010; Kleineidam et al., 2001). While the chambers were vented with respect to soil CO₂ diffusive efflux measurements, they obstructed convective vapor flow from the nest vents. We opened an 18-mm-diameter hole in the side of each chamber to minimize this obstruction and its potential biasing of vent CO₂ measurements. In addition, we used the vent cross-sectional area as the CO₂-emitting area to estimate the CO₂ efflux, instead of considering the chamber area. To test for wind as a possible forced convection driver, we installed anemometers in two of our sites for two months during the efflux sampling campaign. Wind over mounded soil formations can cause sufficient pressure drops over vent openings to drive forced convection (Jackson & Hunt, 1975; Kleineidam et al., 2001; Vogel et al., 1973).

We observed a temperature rise in our CO₂ chamber during sampling that we attributed to free convection (that is, warmer air rising out of the nest vents). We dismissed solar radiation on the chamber as a potential heat source because all measurements were conducted under dense canopy. Since the rate of free convection is related to the air density and vent geometry (which impact how gas expands), any extension or

obstruction of the vent during measurements (as with our flux chamber) may bias the measurements. To minimize the potential for bias, we considered only the early portion of the efflux time series data for which chamber temperature changes were less than 0.2 °C and exhibited a linear response in CO₂ concentrations. Other studies focused on measuring CO₂ advection from soil prescribe use of the linear segment of the time series (Lewicki et al., 2005). From the linear response, and using the vent cross-sectional area as effective CO₂-emitting area, we estimated the vent CO₂ efflux in the same manner as is done for soil surface efflux measurements. At present, there is no standard method to measure convection coming from these vents, and we acknowledge these estimations are approximate and likely lower than the true values. Besides the effect the chamber may have on convection from the vents, a significant source of underestimation of the flux is derived from the response time of the CO₂ sensor. Since the sensor delays a few seconds to read the current CO₂ concentration of the well-mixed chamber, the slope used to calculate the efflux is less steep. In our calibration analyses, the Li820 gas analyzer reacted to changes in 10 s, while the MH-Z16 response time was slower (about 30 s).

To identify the direction of the CO₂ concentration gradient between the soil matrix and the nest air, we measured CO₂ concentrations inside the nest vents and compared these values with the soil CO₂ concentrations. Using a small air pump and plastic tubing, we directed air from 10 to 20 cm inside the vent through a cell equipped with an NDIR CO₂ sensor. We obstructed the vent opening to ensure the extracted gas samples originated in the tunnel and not from nearby surface air. We measured four vents in three nests for periods ranging from 3 to 20 min. For each measurement cycle, we assumed that the maximum concentration observed was the internal vent concentration.

2.4. Assessing the Role of Precipitation

Precipitation drives soil moisture, which has a significant impact on soil CO₂ dynamics. To assess the potential effect of precipitation on soil CO₂ concentration, we computed the daily moving average for periods ranging from 1 to 365 days prior to each gas well sampling event, that is, the daily averages considering data segments of one day, two days, etc., through 365 days. Using precipitation data from La Selva meteorological station, we classified each sampling event as “wet” or “dry” relative to the calculated historical (1986–2015) daily mean precipitation (11.7 mm day⁻¹). The La Selva meteorological station is located in an open area, while our sites were located under dense forest canopy. Therefore, timing of the precipitation on our plots was likely accurate, but the amount was likely less than reported values due to canopy interception which varies with event intensity (Loescher et al., 2002).

2.5. Data Analysis

To study the effect of LCA nest structure on soil CO₂ concentration under dry and wet periods (our first research question), we looked for differences in observed soil CO₂ concentrations between the three plot types (active-nest versus abandoned-nest versus nonnest soils), for the two soil types and two canopy covers, at various depths (20, 60, and 100 cm) and considering the aforementioned range of dry and wet periods (moving daily average from 1 to 365 days). We tested the entire soil CO₂ concentration data set using a generalized linear mixed model from the MASS package (Venables & Ripley, 2002) programmed in R (R Core Team, 2017). We used penalized quasi-likelihood to fit the model to the data, since this technique has been shown to perform well in comparison with other more complex procedures (Bolker et al., 2009; Breslow, 2004). The fixed factors were the plot type, depth, precipitation class (dry or wet period), soil type, and canopy cover, and we considered all possible interactions among them. We defined one random factor, the unique gas well sampling port identifier, which is a proxy for sampling location. The variables soil type and canopy cover and their interactions with the other factors and the interactions between depth and plot type were not significant (see section 2.5 in the supporting information). We removed these interactions from the model but retained the other factors. Thus, the resulting generalized linear mixed model tested for differences among plot types during dry and wet periods ranging in length from 1 to 365 days.

Given precipitation-related differences between nest and nonnest soil CO₂ concentrations (discussed in section 3.1), we explored the effect of precipitation intensity on soil CO₂ concentrations at different depths and for different plot types. To do so, we calculated the correlation between soil CO₂ concentrations and the moving average of daily precipitation from 1 to 365 days for each depth and plot type. We calculated the correlation coefficient (Pearson's *r*) as an indicator of negligible ($r < 0.3$), weak ($0.3 < r < 0.5$),

moderate ($0.5 < r < 0.7$), and strong ($r > 0.7$) correlation between soil CO₂ concentrations and the running average precipitation for a given period.

To investigate what impact (if any) LCA nest excavation has on soil surface CO₂ efflux (our second research question), we looked for differences between efflux measurements and soil moisture content for the three plot types. We again used a generalized linear mixed model approach, with plot type and canopy cover as fixed factors, and plot ID (sampling grid number) nested within efflux sampling event as random factors (associated, respectively, with variability in location and timing of the measurements). We explored potential differences in the soil gas diffusion pathway in the three plot types by estimating soil tortuosity factors for the top 20 cm of the soil profile (Jury et al., 1991; see section 2.5 in the supporting information).

To explore the question of connectivity between nest vents and the surrounding soil CO₂ concentrations (our third research question), we compared measured soil and vent CO₂ concentrations to determine whether the concentration gradient supported fluxes from the soil to the nest air (chambers and tunnels) or vice versa (details in section 3.3). We then incorporated this outcome into the overall estimate of CO₂ emissions from LCA nests.

3. Results and Discussion

3.1. Soil CO₂ Concentrations

Soil CO₂ concentrations were elevated during wet periods relative to dry periods (Figure 4), with a major change evident during the transition from the extremely wet to drier periods (September 2015 to May 2016). In response to our first research question (regarding the effect of the LCA nest structure on soil CO₂ concentrations under dry and wet weather conditions), soil CO₂ concentrations were significantly lower in active-nest soils than in nonnest soils during wetter-than-average periods, while for drier-than-average conditions differences were not significant (e.g., Tables 1 and S1). This pattern occurred for all dry/wet averaging periods greater than 30 days, which is consistent with our monthly sampling interval. In general, soil CO₂ concentrations exhibited high variability. The highest soil CO₂ concentration for nonnest, active-nest, and abandoned-nest plots (6.8, 3.5, and 3.8%, respectively; Table S1) and greatest differences among plot type mean values occurred near the end of the unusually wet period in September 2015. The lowest soil CO₂ concentrations were measured near the end of a dry period in May 2016 (1, 0.7, and 0.6%, respectively; Table S1), which was associated with an El Niño–Southern Oscillation event.

Other studies have observed wet and dry seasonal differences in soil CO₂ concentrations in tropical (including La Selva) and temperate forests (Hashimoto et al., 2004, 2007; Schwendenmann et al., 2003; Sotta et al., 2007). For example, Sotta et al. (2007) observed soil CO₂ concentrations ranging from less than 1% during the dry season to greater than 6% in the wet season, which is comparable to our range of observations for nonnest soils (Figure 4). These large differences are attributed to inhibited gas diffusion during wet periods, when the soil becomes more saturated (Schwendenmann & Veldkamp, 2006; Solomon & Cerling, 1987). In nest soils, the ventilation network may provide potentially entrapped gas an alternative transport pathway that is insensitive to seasonal precipitation.

We observed that the same precipitation amount leads to greater accumulation of CO₂ in nonnest soils than in nest soils (consistent with our previous findings; Figure 5). The correlation between soil CO₂ concentration and precipitation calculated for each of the three depths (20, 60, and 100 cm) revealed differences between nest and nonnest soils when averaging periods were greater than about 30 days (Figure 5; see section 3.1 in the supporting information for discussion about shorter averaging periods). Observed soil CO₂ concentrations tended to increase with soil depth for all three plot types as is expected in most soils (Davidson, Savage, et al., 2006; Tang et al., 2003), and particularly in nonnest tropical soils (Hashimoto et al., 2004, 2007; Schwendenmann et al., 2003; Sotta et al., 2007). This is potentially due to longer gas exchange pathways between the deeper soil matrix and the atmosphere. Soil CO₂ concentration in nonnest plots exhibited a strong correlation with precipitation that increased with depth, being the strongest at 100 cm ($r = 0.75$), while in active-nest soils correlation was weaker at 20 and 60 cm, and negligible at 100 cm ($r = 0.28$). In abandoned-nest soils, correlation was relatively weak across all depths, similar to active-nest soils for 20 and 60 cm, but greater at the 100-cm depth. These results support the idea that the nest ventilation network may act as an alternative transport pathway to reduce soil CO₂ concentrations, particularly in deeper soil layers.

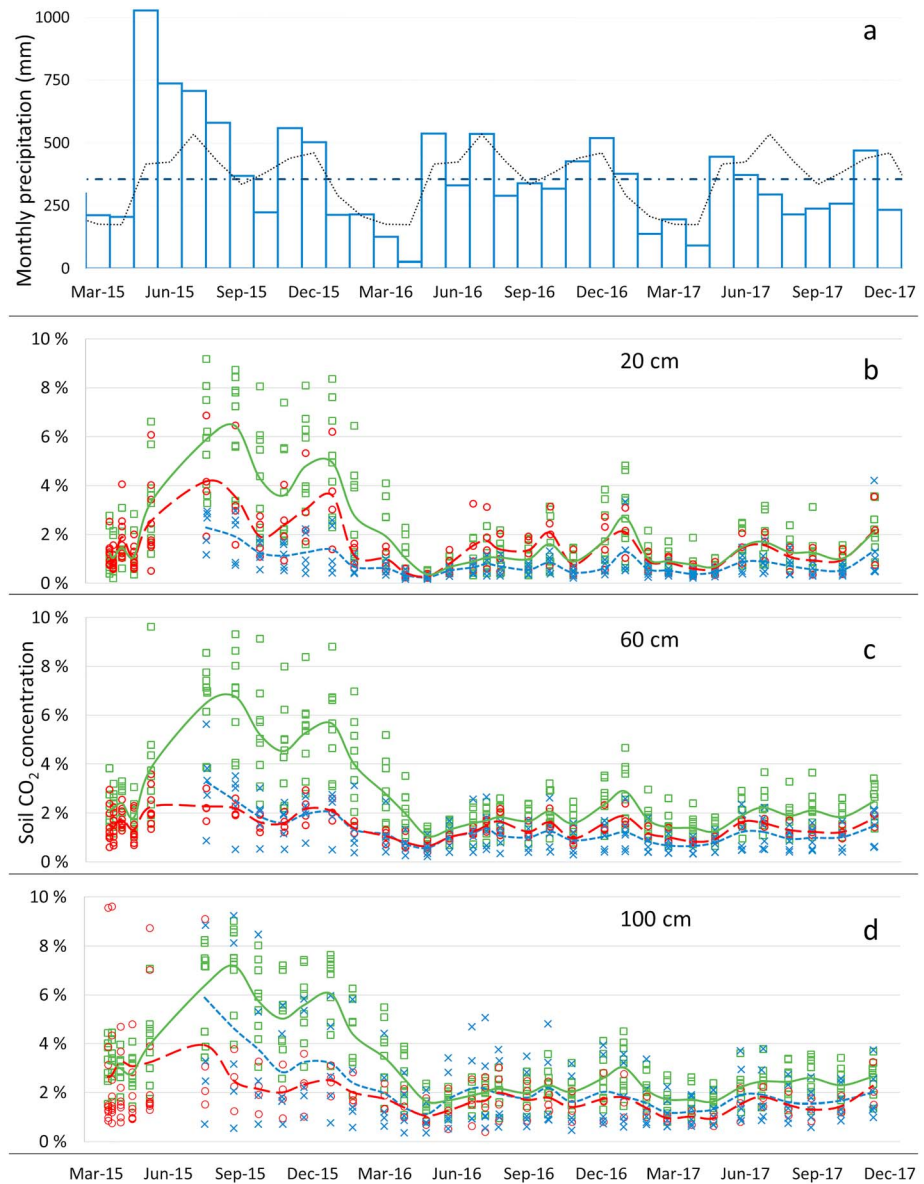


Figure 4. (a) Precipitation is presented by monthly total (bars), historical monthly average (1986 to 2015; dotted line), and mean monthly average (dot-dashed line). Soil CO₂ concentrations at (b) 20-cm, (c) 60-cm, and (d) 100-cm depths for nonnest control soils (green squares), abandoned-nest soils (blue crosses), and active-nests soils (red circles), and their respective mean values (solid green, short-dashed blue, and long-dashed red lines, respectively). Soil CO₂ concentration was higher in nonnest soils than in nest soils (active and abandoned), and increased with increasing depth.

The lower CO₂ concentration we observed in nest soils is likely due to the ventilation network that provides an alternative pathway for CO₂. Nest excavation studies have shown that leaf-cutter ant nests present complex and large geometry (Gonçalves, 1942; Jonkman, 1980b; Mariconi et al., 1961; Moreira et al., 2004; Moser, 2006), representing internal surface areas for soil-nest gas exchange up to hundreds of square meters (Table S2). We also observed lower soil CO₂ concentrations in abandoned-nest soils during wet periods, supporting the explanation of alternative pathways for CO₂ diffusion.

3.2. Soil CO₂ Production

Examination of the carbon sources present in nest soils provides further evidence that ventilation in nest soils is the main mechanism that reduces nest soil CO₂ concentrations. Kuzyakov (2006) suggested five biogenic sources of CO₂: (1) decomposition of plant matter, (2) priming effect of root exudation or of plant residue

Table 1

Soil CO₂ Concentrations as Described by the Observed Mean, Standard Deviation, Median, and Generalized Linear Mixed Model-Based Expected Mean and Its Relative Error With Respect to the Observed Mean for Nonnest, Active-Nest, and Abandoned-Nest Soils During Dry and Wet Periods, Considering the Dry and Wet Classification Defined by the 90-Day Average Precipitation Prior to Each Sampling Event

P90d	Depth	Nonnest Control (%)					Active Nest (%)					Abandoned Nest (%)				
		μ	σ	M	E	MAPE	μ	σ	M	E	MAPE	μ	σ	M	E	MAPE
Dry	20	1.3	1.1	0.9	1.3	2	1.2	0.9	0.9	1.2	1	0.6	0.4	0.5	0.5	-12
	60	2.0	1.1	1.6	1.9	-3	1.3	0.5	1.2	1.8	41	1.0	0.6	0.8	0.8	-20
	100	2.4	1.1	2.2	2.6	8	1.9	2.0	1.4	2.5	30	1.7	1.2	1.3	1.1	-36
Wet	20	2.9	2.4	2.0	2.9	1	2.0	1.5	1.6	1.8	-9	1.1	0.8	0.8	1.3	14
	60	3.6	2.2	2.8	3.5	-3	1.7	0.6	1.7	2.1	24	1.6	1.1	1.4	1.5	-7
	100	4.0	2.1	3.4	4.2	7	2.4	2.0	1.9	2.6	8	2.8	2.6	2.0	1.8	-36

Note. μ , σ , and M are the mean, standard deviation, and median values of the observed soil CO₂ concentrations (%); E is the expected mean (from the GLMM); and MAPE is the minimum average percentage error of the model expected mean with respect to the mean value. Dry and wet classification based on precipitation record 90 days prior to each sampling event.

addition, (3) microbial decomposition of soil organic matter, (4) root respiration, and (5) rhizomicrobial respiration (or decomposition of fine roots). In our site, fine leaf litter mass was similar across plot types, although slightly greater at nest (active and abandoned) plots compared to nonnest plots (average \pm standard deviation values of $6,148 \pm 2,858$, $6,203 \pm 2,551$, and $5,404 \pm 2,614$ kg ha⁻¹ yr⁻¹, respectively; Table S3). In tropical, nutrient-limited soils, addition of nitrogen enhances microbial respiration and decomposition of organic matter (Cleveland et al., 2006; Cleveland & Townsend, 2006). Since *Atta* nests fix nitrogen (Pinto-Tomás et al., 2009), microbial activity in nests is expected to be similar or greater than in nonnest soils. Moreover, in active-nest soils, hyphal and root production (47.3 and 31.1 kg C m⁻³ yr⁻¹, respectively) were substantially higher than in nonnest soils (14.8 and 5.6 kg C m⁻³ yr⁻¹, respectively) due to higher turnover rates (Swanson, 2017). Together, these values suggest that decomposition of plant matter, priming effects, and microbial decomposition are similar or greater in nest soils relative to nonnest soils, and that root and rhizomicrobial respiration is greater in nest soils. Despite higher soil CO₂ production, nest soils presented lower CO₂ concentration than nonnest soils, implying that CO₂ production is not the driver of this difference.

3.3. Soil CO₂ Efflux

Soil surface CO₂ efflux measurements (Figure 6a) exhibited relatively high variability and no significant differences between nonnest, active-nest, and abandoned-nest plots (regarding our second question: what

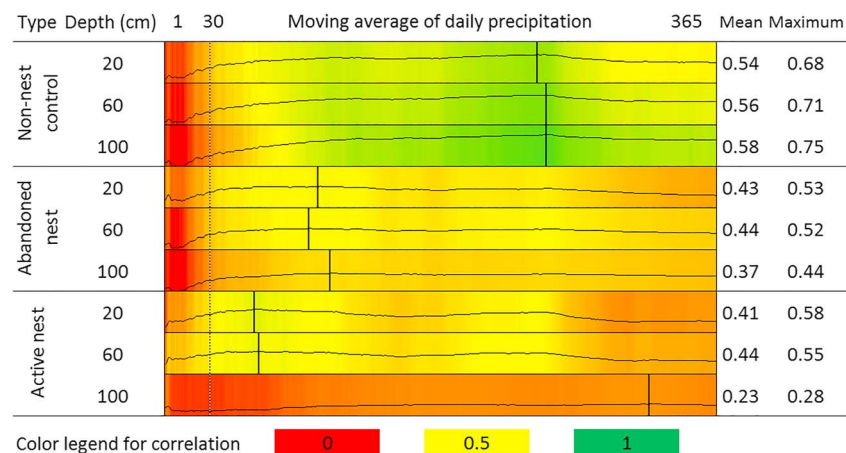


Figure 5. Heat maps for each plot type and depth (365 columns and 3 rows for each of the three plot types), where each cell color depicts the correlation coefficient (r) between soil CO₂ concentration and the daily precipitation moving average (1 to 365 days; columns) for nonnest, abandoned-nest, and active-nest soils at 20-, 60-, and 100-cm depths (rows), and the horizontal black lines represent the correlation from 1 to 365 days, and the vertical black segments indicate when the maximum correlation occurred.

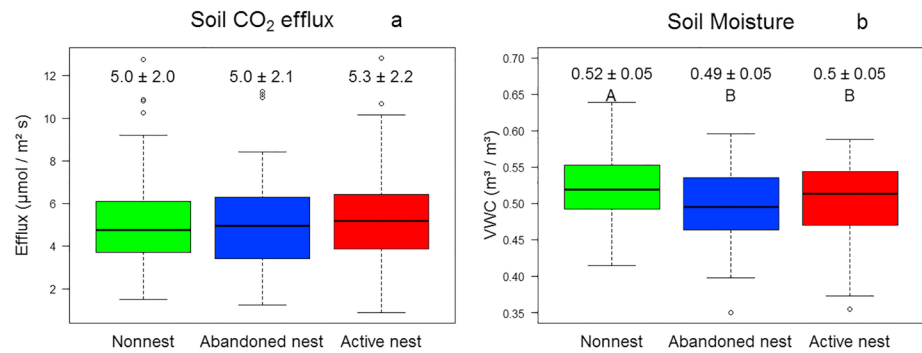


Figure 6. (a) Soil surface CO₂ efflux and (b) soil moisture from the nonnest control (green), abandoned-nest (blue), and active-nest (red) soils. Different letters denote significant differences ($p < 0.05$).

is the influence of LCA soil excavation on surface CO₂ efflux?). Efflux values were similar to other reported values for La Selva soils (Schwendenmann & Veldkamp, 2006). Based on these findings, it is not likely that observed lower soil CO₂ concentrations in nest soils were due to increased soil surface CO₂ efflux (section 3.1). We observed a small but significant difference in surface soil moisture content in nonnest soils (0.52; Figure 6b) compared to abandoned-nest (0.49) and active-nest soils (0.50), which is consistent with the previous finding that soil moisture content decreases slightly near *A. cephalotes* nests (Meyer et al., 2011). These measurements fall within the range of volumetric water content in these clayey soils (0.40–0.65 and 0.30–0.55 for shallow alluvial and residual soils, respectively), and surface CO₂ efflux varies with moisture content at this site (Schwendenmann & Veldkamp, 2006). However, the difference in soil moisture content we observed did not affect the soil CO₂ efflux values observed across different plot types.

We measured a small difference in the soil CO₂ concentration gradients between the soil surface and 20-cm depth, which implies a 15% greater soil tortuosity factor (less tortuous path) in nest soils relative to nonnest soils (Table S4). Such a difference in tortuosity, if real, would be consistent with minor differences in soil moisture content and pore structure, and could result from changes in soil structure via LCA nest excavation and maintenance. However, given the uncertainty in these observations, this explanation is largely speculative, and our second research question requires further investigation.

3.4. Vent CO₂ Efflux

Vent CO₂ efflux calculated from our observations were 3 to 5 orders of magnitude greater than surface efflux rates (average 15,450 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, standard deviation 45,274 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, maximum 434,000 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). Vent CO₂ efflux varied substantially in strength and duration of signal (Figure 7), presumably due to differences in vent connectivity to nest fungal and refuse chambers. Efflux values of this magnitude are too large to be attributed to gas diffusion, and are probably caused by free (density-driven) and/or forced (pressure-driven) convection. Given the numerous connections between the nest interior and the atmosphere, the pressure differential needed for forced convection is unlikely to occur. An exception may be wind-triggered forced convection, which is a ventilation driver for *A. vollenweideri* nests (Kleineidam et al., 2001). We considered this possibility, but the measurements from our anemometers revealed that wind events were rare at our plots, since the nests were located within the dense forest understory. However, the detection limit for our anemometer was about 0.5 m s^{-1} , and therefore, we cannot rule out the effect of wind. Given the near quiescent conditions in the dense forest canopy, we believe that free convection is also likely to help drive nest ventilation. In this case, higher local nest temperatures and relative humidity enable less dense air, rich in CO₂, to rise out of the vents, and colder, dryer, and therefore denser air that is relatively poor in CO₂ to drain into the vents. The issue of CO₂ fluid dynamics in nest vents warrants additional detailed investigation.

Our third research question is related to the potential sources of vent CO₂ (what is the connection between CO₂ nest vent emissions and surrounding nest soil?). Observed internal vent CO₂ concentration varied substantially among vents, as would be expected from the vent efflux results. For the three nests tested, mean vent concentrations were 0.31% (standard deviation 0.16%), 0.61% (0.41%), and 1.02% (0.44%). These vent

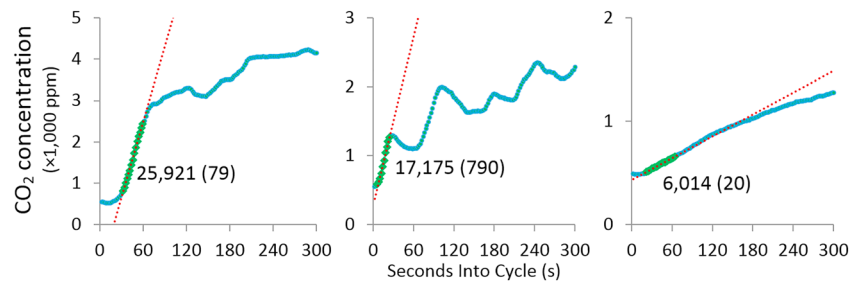


Figure 7. Observed transient vent CO₂ concentrations (blue circles) illustrating typical vent responses which varied in magnitude (slope) and direction (positive and negative slope segments interpreted as periods of CO₂ efflux and air influx, respectively). Values shown are the vent efflux rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) calculated from the linear regression (dotted red line) using the green highlighted data and the vent area shown in parentheses (10^{-6} m^2).

concentrations are on average lower than the adjacent soil CO₂ concentrations (Figure 4 and Table 1). Thus, the nest structure may facilitate CO₂ diffusion from the soil matrix into the nest following the concentration gradient. Exceptions likely occur for highly active-nest zones (e.g., refuse chambers), where diffusion may occur in the opposite direction, particularly during dry periods when soil CO₂ concentrations are generally lower. Based on the relationship between nest ground surface area and nest internal surface area (Table S2), the range of plausible tortuosity values between the soil matrix and inside the nest, and the CO₂ concentration gradient between soil and the nest air, we estimate that the CO₂ efflux from the soil matrix into the nest is roughly 20% of the efflux from the soil directly to the atmosphere (see section 3.3 in the supporting information for calculation).

3.5. Ecosystem-Scale CO₂ Emissions

Active leaf-cutter ant nests continuously emit CO₂ originating from soil, root, fungus, and ant respiration, while intermittently receiving and accumulating large amounts of carbon as harvested vegetation. In this way, the nests act as hot spots of carbon transformation and CO₂ emissions and change the soil CO₂ dynamics in Neotropical rainforests. According to our research, the legacy effect in soil CO₂ dynamics after the nest abandonment can persist for more than two years.

We provide evidence that LCA nest structure reduces soil CO₂ concentrations compared to nonnest soils. This reduction does not appear to be driven by differences in CO₂ production or physical soil properties affecting surface CO₂ emissions. The major difference between active-nest and nonnest soils is the presence of the nest structure. While the vents emit CO₂ at much higher rates than the surrounding soil matrix, they occupy a much smaller area than the surrounding nest soil surface area. For example, our surveys indicated that the average nest surface area was 67 m², while the number of vents was 32 with openings averaging 0.00021 m², or around 0.007-m² total area of vent openings per nest (vent:nest area ratio of about 1:10,000). Thus, while vents emit substantially elevated CO₂ concentrations, their impact on soil CO₂ emissions is relatively small when scaled by area, a point detailed below.

To estimate nest-scale CO₂ emissions relative to nonnest soils, we integrated soil and vent emission observations using estimates of nest area, vent numbers, and vent opening size from our field observations (Table S5). A reasonable range of soil CO₂ efflux rates in this forest is 4 to 7 kg CO₂ m⁻² yr⁻¹ (as in this study and in Schwendenmann & Veldkamp, 2006), and nest surface area ranges from 30 to 70 m² (Wirth et al., 2003, based on Perfecto & Vandermeer, 1993; Tables 2 and S5). Given these values, soil ground surface of a nest emits 120 to 490 kg CO₂ annually. For vent efflux, based on an observed average vent efflux value of $2.1 \times 10^4 \text{ kg CO}_2 \cdot \text{m}^{-2} \text{ yr}^{-1}$, an average of 32 vents per mature nest, and average vent opening (0.00021 m²), the total vent CO₂ emissions is about 72 kg CO₂ per nest and year. These values suggest that an average *A. cephalotes* nest area emits around 200 to 600 kg CO₂ per nest and year, that is, 15 to 60% more than an equivalent area of soil in a lowland tropical forest. Considering the nest survey we conducted in 2015 (Tables 2 and S5), at least 1.2% of the La Selva surface of primary and secondary forest was occupied by *A. cephalotes* nests. That is equivalent to an additional 0.2 to 0.7% contribution of CO₂ from *A. cephalotes* in this Neotropical rainforest.

Table 2

Summary of the Nest Survey Conducted at La Selva in 2015

L.U.	Number of Nests	Number of Plots	Area (ha)	Total Area L.U. (ha)	% Surveyed	Nest Dens. (nest ha ⁻¹)	Nest Mean Area (m ²)	Percent Area Covered by Nests
POG	30	33	16.5	781	2.1	1.8	64	1.2
SF	12	15	7.5	347	2.2	1.6	76	1.2
Total	42	48	24	1128	2.1	1.8	67	1.2

Note. L.U.: land use type, POG: primary old forest, SF: secondary forest, area: area surveyed, total area L.U.: total area of that land use at La Selva, % surveyed: percentage of the total area surveyed, nest dens.: nest density. More details in Table S5.

4. Conclusions

Leaf-cutter ants are ecosystem engineers that continuously modify their nests to optimize environmental conditions for their colony. We studied the role of *A. cephalotes* in modifying soil CO₂ dynamics (concentrations and emissions) in a tropical wet forest in Costa Rica. During wet periods, clay-rich tropical soils tend to limit gas movement through the soil matrix and its exchange with the atmosphere, causing soil CO₂ concentrations to increase. While we found this to be true in nonnest soils, we found that soil CO₂ concentration increases were significantly attenuated in active and abandoned-nest soils relative to nonnest soils. Moreover, the influence of nest structure became more prominent with increasing depth, where gas exchange with the atmosphere requires longer dry periods.

Nest vent CO₂ efflux values were 10³ to 10⁵ times greater than soil CO₂ efflux, and we attributed them to free convection for our nest sites located in dense forest vegetation. Forced convection is likely playing a role in nest ventilation, but the common lack of wind suggests that it is not as relevant as for other *Atta* species. Vents had lower CO₂ concentrations than adjacent soil, pointing to diffusive transport of CO₂ from the soil matrix into the nest interior. The nest network (chambers and tunnels) has a surface area similar to the nest ground surface, and this structure facilitates gas exchange between the soil matrix and the nest air. Hence, nest vents play a major role in reducing soil CO₂ concentrations by emitting the CO₂ originating both from nest activities and microbial and root respiration in the soil matrix.

Nests and their surrounding soil areas emit 15 to 60% more CO₂ than the equivalent nonnest soils. This range translates to an enhancement in total CO₂ emissions of 0.2 to 0.7% in this Neotropical rainforest. While this estimated range of CO₂ emissions represents only a rough snapshot of active nests (involving assumptions about nest geometry and with vent contributions likely underestimated), it shows that leaf-cutter ants change the soil CO₂ dynamics and provides a reasonable starting point for assessing forest-scale carbon emissions catalyzed by this ecosystem engineer. Given that the range of leaf-cutter ants is expanding in response to land disturbances and warming climate, this difference illuminates the significant carbon footprint of ecosystem engineer *A. cephalotes* and has implications with respect to the global carbon cycle.

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