

Rapid recovery of soil respiration during tropical forest secondary succession on former pastures

Claire Beckstoffer^{a,b,*}, Jefferson S. Hall^b, Whendee L. Silver^a

^a Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA 94720, United States

^b SI ForestGEO, Smithsonian Tropical Research Institute, Roosevelt Avenue, Ancon, Balboa 401, Panama

ARTICLE INFO

Keywords:

Soil respiration
Tropical secondary forest
Forest regeneration
Carbon flux
Moist forest

ABSTRACT

Tropical forests are responsible for the highest rates of soil respiration globally. As the natural regeneration of secondary forest becomes increasingly widespread in the tropics, there is a need to understand how pasture-to-forest conversion and secondary forest succession impact patterns and drivers of soil carbon dioxide (CO₂) fluxes. This study examined changes in soil respiration rates and associated drivers (soil temperature, gravimetric moisture content, soil organic carbon (C), and forest floor mass) in replicate age classes across a tropical secondary forest chronosequence including pasture and forests ranging in age from 7 to over 80 years since establishment. Soil CO₂ fluxes were highest in the pasture plots ($10.0 \pm 0.85 \mu\text{mol m}^{-2} \text{s}^{-1}$) and declined by nearly two-fold during the early pasture-to-forest transition. Soil CO₂ fluxes remained fairly consistent across the forest successional sere. Soil respiration was primarily influenced by soil temperature across the chronosequence, while soil moisture and substrate availability mediated the impact of temperature on soil CO₂ fluxes. The rapid decline of soil CO₂ fluxes during pasture-to-forest conversion was surprising and has not been reported in previous studies, most of which have focused on fluxes in different stand ages after forest establishment. Our results suggest that soil temperature may be a key driver of soil CO₂ fluxes during secondary tropical forest regrowth on pasture lands, potentially due to significant shifts in microclimate conditions during the early stages of forest establishment.

1. Introduction

Soil respiration is a major component of carbon (C) fluxes in forest ecosystems. As the primary pathway through which C is emitted from the soil to the atmosphere, soil respiration plays an important role in the ecosystem C balance and in mediating atmospheric CO₂ concentrations (Ryan and Law, 2005). Soil respiration is a particularly important flux in tropical forests, which are responsible for the highest rates of soil carbon dioxide (CO₂) emissions globally (Bond-Lamberty and Thomson, 2010; Gutiérrez Del Arroyo and Wood, 2021). Gaining a better understanding of the patterns and drivers of soil respiration in tropical forests is critical, as even small relative changes in soil respiration can have large climate impacts.

Studying soil respiration dynamics in tropical secondary forests is especially important, as secondary forests have now become a dominant form of tropical forest ecosystem (Chazdon, 2014; Hall et al., 2022; Lugo and Brown, 1992). The increasing deforestation of primary forest and the growing number of global reforestation incentives are likely to drive

the continued expansion of secondary forests ("International database on REDD+ projects and programmes," 2022; Kyoto Protocol., 1998; Weisse and Goldman., 2022). For example, the government of Panama has identified 240,510 ha of pasture and shrubland for potential forest restoration and aims to reforest 51,000 ha by 2025 (Ministerio de Ambiente., 2022,2020). Secondary forest regrowth may be particularly prevalent in areas where landowners prefer to allow their agricultural lands to regenerate naturally as a less time and resource-intensive strategy for reforestation (Holl and Aide, 2011; Sinacore et al., 2023). Given the increasing importance of secondary forest regrowth in the tropics, it is critical to examine the controls on soil respiration during natural forest regeneration to understand how this land transition will impact the climate over the coming decades.

Soil respiration is the sum of root respiration, resulting from plant metabolism and belowground C allocation, and microbial respiration, which is derived from substrate quantity and quality (Chen et al., 2011; Metcalfe et al., 2011; Ryan and Law, 2005). Soil temperature and moisture content significantly impact rates of soil CO₂ flux, affecting

* Correspondence to: University of California, 355 Bryant St. San Francisco, Berkeley, CA 94107, United States.

E-mail address: cbeckstoffer@berkeley.edu (C. Beckstoffer).

both microbial and root respiration rates (Kotrocó et al., 2023; Schindlbacher et al., 2009; Wood et al., 2013; Zhou et al., 2013). Aboveground biomass growth and changing community composition during secondary succession can influence microclimate conditions and soil biogeochemical properties, impacting drivers of soil respiration (Epihov et al., 2021; Lebríja-Trejos et al., 2011; Li et al., 2013; Teixeira et al., 2020; Yesilonis et al., 2016). In particular, secondary forest succession has been shown to impact soil temperature, water availability, microbial community structure, and soil C and nutrient concentrations, all of which may have significant impacts on soil CO₂ fluxes (Lebríja-Trejos et al., 2011; Li et al., 2013; Ostertag et al., 2008; Silver et al., 2004; Teixeira et al., 2020; Wang et al., 2022; Yan et al., 2023; Yesilonis et al., 2016). Secondary forest community composition and successional rates are sensitive to regional and local factors such as climate, soil nutrient availability and previous land use (Anderson-Teixeira et al., 2013; Moran et al., 2000; van Breugel et al., 2019). Secondary forest succession rates and interactions with below-ground biogeochemical functions are likely to be important drivers of soil respiration at local scales.

The impacts of secondary forest succession on soil respiration have received less attention in the tropics relative to temperate and boreal ecosystems (Das Gupta and Mackenzie, 2016; Duan et al., 2022; Kim et al., 2014; Susyan et al., 2011; Wang et al., 2010). The majority of studies in the tropics have focused on tropical dry forests, while the effects of secondary forest regrowth on soil respiration in tropical moist forests have been less thoroughly explored (Aryal et al., 2017; Calvo-Rodriguez et al., 2020; Huang et al., 2016; Peixoto et al., 2017; Rodtassana et al., 2021; Vargas-Terminel et al., 2022; Wang et al., 2017). Previous studies in the tropics have also focused primarily on changes in soil CO₂ flux between different stand ages after forest establishment, while few have examined soil CO₂ flux dynamics during the earliest stages of forest regeneration, specifically the pasture-to-forest transition (Calvo-Rodriguez et al., 2020; Salimon et al., 2004). The existing literature reports a wide variety of soil respiration responses to secondary forest succession, as well as a lack of consensus on the dependence of soil respiration on specific drivers such as forest floor mass, soil temperature, net primary productivity, root biomass, and soil organic C concentrations (Aryal et al., 2017; Calvo-Rodriguez et al., 2020; Duan et al., 2022; Gao et al., 2019; Huang et al., 2016; Rodtassana et al., 2021; Sigau and Abdul-Hamid, 2018; Susyan et al., 2011; Wang et al., 2017). In order to capture a more holistic picture of the impacts of natural forest regeneration on soil respiration in the tropics, there is a need to further constrain the drivers of soil CO₂ flux during both pasture conversion and secondary succession on a landscape scale.

In this study we explored changes in soil CO₂ fluxes together with a suite of potential drivers across a secondary forest chronosequence in Agua Salud, Panama. We measured soil CO₂ fluxes, soil temperature, gravimetric moisture content, forest floor mass and soil organic C concentrations in pasture and three secondary forest age classes. Our study aimed to address the following questions: i) how do soil CO₂ fluxes vary with secondary forest age? ii) how do soil temperature, soil gravimetric moisture content, forest floor mass and soil organic C vary across secondary forest succession and how are these variables related to soil CO₂ fluxes?

2. Methods

2.1. Study region

Our study was conducted within the Agua Salud Project, located adjacent to Soberanía National Park in the central part of the Panama Canal Watershed (9°13' N, 79°47' W, 330 masl) (van Breugel et al., 2013). The study area is characterized by a strongly dissected pre-tertiary basalt plateau with narrow interfluvies, linear slopes averaging 42 % and narrow or no valley floors (Hassler et al., 2011;

Neumann-Cosel et al., 2011). Soils in the study area are strongly weathered, infertile and well-drained Oxisols (Inceptic Hapludox) and Inceptisols (Oxic and Typic Dystrudepts), with pH values (in water) ranging from 4.4 to 5.8 (van Breugel et al., 2019).

The forest in the study area is classified as a tropical moist forest based on the Holdridge life zone system. There is a distinct wet season beginning in early May and ending in mid-December (Ogden et al., 2013). The average annual rainfall in Agua Salud between 2015 and 2022 was 2506 mm while the mean daily temperature was 24.5 °C and varied only little throughout the year (Paton, 2023).

Land use in the area consisted of a mosaic of cattle pastures and cultivated fields, fallows, secondary forests, plantations and fragments of mature forest (van Breugel et al., 2013).

The study area was dominated by different ages of secondary forest, with approximately 8.3 % of the area under active pasture (van Breugel et al., 2019). The stocking density was 0.87 animals per hectare in pasture sites (Chavarria et al., 2021). Adjacent Soberanía National Park contained mature forest (Hassler et al., 2011; Neumann-Cosel et al., 2011).

2.2. Site selection and chronosequence approach

The effects of secondary forest regrowth on soil CO₂ respiration were investigated using a “space-for-time” chronosequence approach. Twenty sites were selected across the Agua Salud watershed and grouped into four different age classes: sites under active pasture, 7-year-old secondary forest (the period with canopy closure but with high stem density), secondary forest ranging between 24 and 32 years old (representing a range of mid-successional environments typical of the region), and mature forest over 80 years old (which represented over 80 % of the aboveground biomass of 300-year-old forest on nearby Barro Colorado Island (BCI) and was the oldest forest in or near the study site) (see Table 1, Supplementary Materials) (Hall et al., 2022). For simplicity, we will refer to these age classes as pasture (P), early succession (ES), mid-succession (MS), and late succession (LS), respectively. We chose to measure pasture and 7-year-old secondary forest due to the significant changes in ecosystem structure and species composition during early succession, which could have significant impacts on soil CO₂ fluxes. Soil C storage in Agua Salud did not vary significantly among secondary forests between 7 and 15 years old in a previous study (Neumann-Cosel et al., 2011) but showed a significant linear increase over the first 40 years of succession (Püspök, 2019), thus we chose to measure 24–32-year old forests in order to capture changes in the soil C cycle that might occur on a longer timescale. Finally, we chose to measure mature forests over 80 years old to compare soil respiration dynamics between early and mid-successional sites to sites that more closely resembled an old growth forest system.

Sites were similar with respect to soil order, climate and previous land use, allowing for an effective comparison among secondary forest age classes within our study area. Beginning in the 1950s, land use in the study area was dominated by extensive cattle ranching and small-scale shifting cultivation (van Breugel et al., 2013). The early and mid-successional plots in this study were all active pasture for over 2 decades prior to reforestation. Aboveground vegetation varied between pasture, ES (basal area = 11.5 ± 1.23 m² per ha, aboveground biomass = 41.9 ± 5.92 Mg per ha, stem density = 7588 ± 838 individuals per ha), MS (basal area = 24.0 ± 1.23 m² per ha, aboveground biomass = 109 ± 7.20 Mg per ha, stem density = 6500 ± 489 individuals per ha) and LS plots (basal area = 24.1 ± 0.824 m² per ha, aboveground biomass = 136 ± 6.11 Mg per ha, stem density = 4350 ± 534 individuals per ha) (Hall et al., 2022; Lai et al., 2018, 2017; van Breugel et al., 2011). Biomass growth was determined by using locally derived allometric equations from van Breugel et al. (2011) according to Lai et al. (2017), (2018) and extrapolating to older forest based on Hall et al. (2022); stems > 1 cm DBH were included.

Sites in each age class were representative of the total geographic

area of the Agua Salud watershed covering approximately 54 km² (Fig. 1). A site was defined as a single slope “segment” in both pasture and secondary forest (van Breugel et al., 2013). Each site contained an upslope and downslope transect in order to capture topographic variation. All sites in the MS age class and 2 sites from the LS age class contained two 2 m x 50 m transects, one upslope and one downslope, separated by > 30 m. Sites in the ES age class spanned 30 m x 30 m, with an upslope and downslope transect separated by approximately 20 m. All pasture sites and three LS sites were new to this study and did not contain preexisting site boundaries, thus an upslope and downslope transect were constructed at the time of measurement, along the same slope segment and separated by > 30 m. Samples for CO₂ fluxes, gravimetric moisture content, temperature, forest floor mass, and soil organic C concentrations were taken in each transect, each at 5 m intervals, except when thick roots or ant hills prevented placement of a soil collar, in which case the sampling location was moved an additional 5 m (total n = 5 samples per transect, 2 transects per site).

Sites were sampled during the 2022 wet season from September 2 to December 2. As with Nottingham et al. (2022), we chose to sample soils during the wet season to ensure that soil moisture was not a limiting factor for soil microbial activity and soil CO₂ fluxes (Nottingham et al., 2022). Soils in Agua Salud also experience large cracks during the dry season, making it difficult to obtain reliable flux values using a survey chamber method. Furthermore, several studies have shown that soil CO₂

fluxes in the wet season account for a large majority of annual emissions in tropical forests (Davidson et al., 2000; Huang et al., 2016; Rodtassana et al., 2021). We investigated the drivers of wet season soil respiration in order to understand dynamics that may dominate trends in total annual CO₂ respiration from tropical soils. The order of sampling was randomized across age classes to avoid bias from weekly or monthly weather trends.

2.3. Soil CO₂ flux measurements

Soil CO₂ flux measurements were performed using an infra-red gas analyzer and a portable, automated opaque survey chamber (LI-8100 IRGA, LI-8100–103 survey chamber, LI-COR, Inc., Lincoln, Nebraska, USA). To measure soil CO₂ fluxes, collars (PVC, 20 cm inner diameter, 13 cm depth) were first installed 3–5 cm deep along each transect (5 per transect). Collars were inserted 30–45 minutes prior to flux measurements in order to minimize the impacts of soil disturbance and root severance on fluxes (Keller et al., 2005; van Haren et al., 2010). Four measurements were taken per collar (from the top of the collar to the soil surface, along the inner diameter) and then averaged to find the approximate volume within the collar. Flux measurements were collected for a duration of 90 seconds and the chamber was flushed with ambient air for 30 seconds in between measurements. One flux measurement was taken per soil collar. In three (of 200 flux measurements)

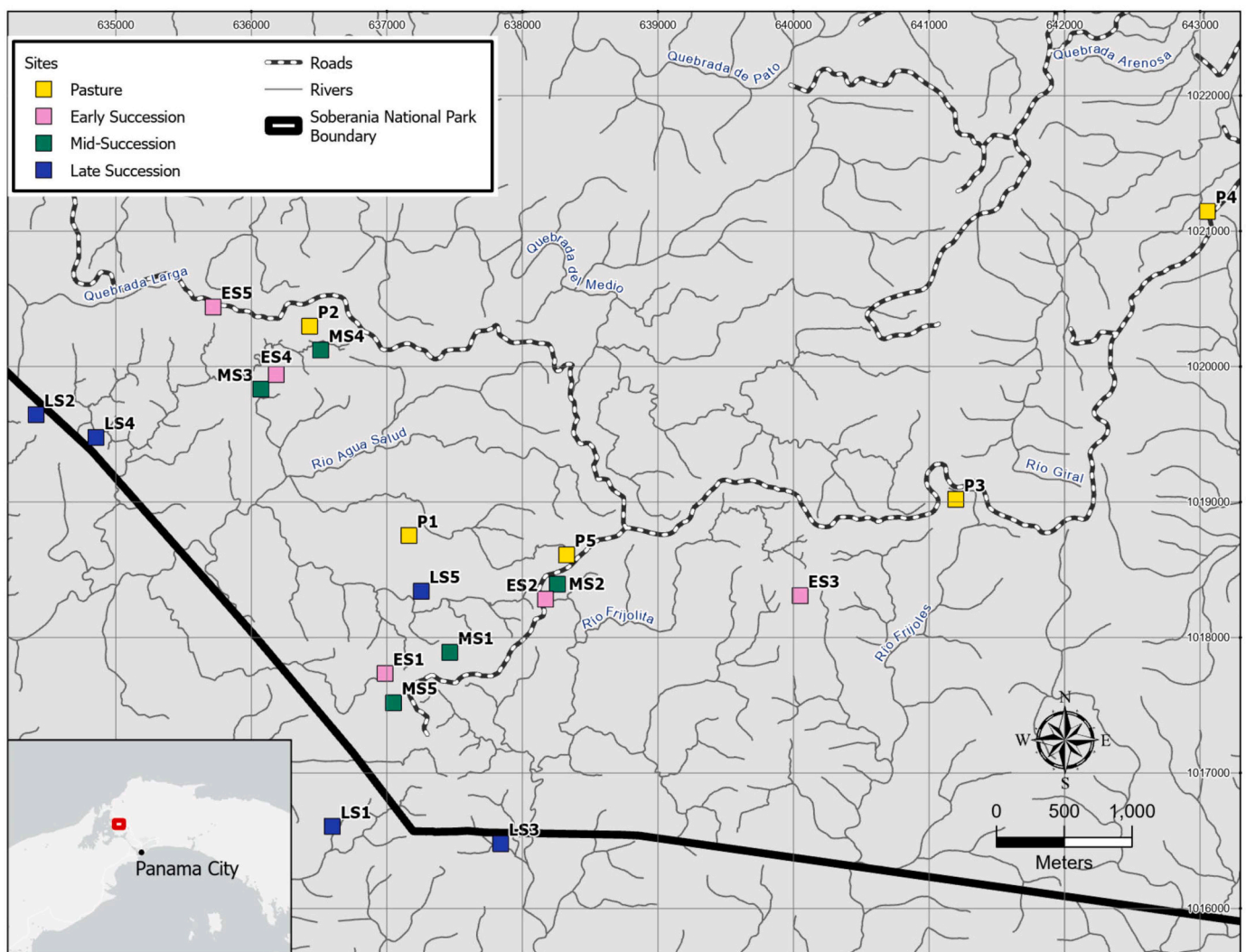


Fig. 1. Sampling Plots in the Agua Salud Study Area. P1-P5 are pasture sites, ES1-ES5 were early successional sites, MS1-MS5 are mid-successional sites, and LS1-LS5 were late successional sites.

we immediately repeated the measurement two more times when we got an anomalously high CO₂ emission rate (>4 standard deviations above the mean for the respective age class), likely due to accidental pressure to the chamber or a large insect or organism in the chamber base. In these cases, the value was lower during the subsequent two measurements which were within 1 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of each other, so the lower value was used. To further avoid interference from large insects, spiders or worms, soil collars were visually screened before each flux measurement was taken (but not physically searched, to prevent disturbances to soil or soil microbes). After the flux measurement was completed, a physical search of each collar was performed. If any large insects, spiders or worms were discovered in either the initial screen or physical search, the collar was removed and replaced nearby, and a new flux measurement was taken after 30 minutes.

Dense vegetation was not present along the forest floor in our ES, MS, and LS forest sites, however, thick grass was present in our pasture sites. We conducted a clipping test in 3 pasture sites to determine whether foliar respiration from the grass interfered with our soil respiration measurements. Soil CO₂ fluxes were measured at each collar ($n = 10$ per site) prior to clipping all aboveground vegetation, and then after clipping. No significant differences in fluxes were observed between clipped and unclipped collars during the sampling period (Table 2, [Supplementary Materials](#)).

2.4. Soil sampling

Soils were sampled from 0 to 5 cm depth at a random location within 2 m of each soil collar using a 2.2 cm diameter soil probe (#77453 33" soil probe, Forestry Suppliers, Inc., Jackson, Mississippi, USA). Each sampling location was cleared of surface litter and debris prior to sample collection. Three soil cores were collected per collar and then homogenized to form a single sample. All 10 soil samples per site were analyzed for gravimetric moisture content while 5 samples per site were analyzed for soil organic carbon concentrations. Soils were stored at room temperature until analysis.

To measure soil moisture, approximately 10 g of wet soil per sample were separated and weighed. Each 10-g sample was placed in an oven at 105 °C for 72 hours, when it reached a constant weight. Soils were then removed from the oven and re-weighed to calculate gravimetric moisture content. Soil temperature was measured immediately following flux measurements. A soil thermometer probe was inserted into the soil in the center of the soil collar to approximately 10 cm depth (OMEGA HH501BJK Thermometer, OMEGA Engineering, Norwalk CT).

Forest floor mass was sampled by installing a 25 × 25 cm (inside area) frame flat against the soil with long nails. Frames were placed at a random location within 2 m of each soil collar and one sample was collected per soil collar. Forest floor material (defined as all recognizable dead plant material, such as leaf litter, twigs and debris) was collected until only bare soil and live roots and plants were contained within the frame ([Silver et al., 1994](#)). Forest floor samples were oven-dried for 48 hours at 55 °C and then weighed to determine mass.

Soil samples (5 per site) were air-dried in the laboratory to measure soil organic C. Subsamples ranging from approximately 20 to 40 g were air-dried in open tins in Panama for 12–30 days prior to sieving and grinding. Subsamples were then sieved and hand-sorted to remove rocks, fine roots and leaf litter. Approximately half of the subsamples were ground in Panama to a fine powder using a mortar and pestle ([Ostertag et al., 2008](#)). The rest of the subsamples were resealed in ziploc bags and subsequently ground in Berkeley using a ball-grinder (SPEX Sample Prep Mixer Mill 8000D, Metuchen, New Jersey, USA). Total soil C concentrations were measured using an elemental analyzer at UC Berkeley (CE Elantech, Lakewood NJ) ([Ryals et al., 2014](#)). Soils in Agua Salud do not contain any carbonates, therefore total C values reflect soil organic C content ([Ogden et al., 2013](#)).

2.5. Statistical analysis

All analyses were performed using R Statistical Software (v2.1.461; [R Core Team., 2022](#)). Differences in soil CO₂ flux, soil temperature, gravimetric moisture content, forest floor mass, and soil organic C concentrations across forest age classes were analyzed using a one-way analysis of variance (ANOVA) and Tukey's HSD post-hoc test ($p \leq 0.1$) ([Fox and Weisberg, 2019](#)). Data were tested for normality and if not normally distributed, a Kruskal-Wallis test was performed instead, followed by Dunn's test for multiple comparisons ([Ogle et al., 2023](#)). The ANOVAs were performed using site average values for CO₂ flux, soil temperature, gravimetric moisture content, forest floor mass, and soil organic C ($n = 5$ per age class).

We used a linear mixed effects model (lmer command from the lme4 package in R) to analyze the effect of each soil parameter on soil CO₂ flux ([Bates et al., 2015](#)). Although we took 200 total measurements for CO₂ flux, soil temperature, gravimetric moisture content, and forest floor mass, our input data set was constrained to 98 points as the lmer model removes rows with missing soil organic C values. Our full model contained soil temperature, gravimetric moisture content, forest floor mass and soil organic C, as well as 2-way interactions between all variables, as fixed effects with site as a random intercept. Models were fitted using the restricted maximum likelihood method (REML). A type III ANOVA and summary table were generated for our full model with p-values calculated via Satterthwaite's degrees of freedom method ([Kuznetsova et al., 2017](#); [Luke, 2017](#)). Mean sums of squares, number of parameters, and F-values are extracted from the ANOVA function applied to the full model in R, while parameter estimates, standard errors and p-values for parameter estimates were extracted from the summary of the full model. To assess the fit of our model, we calculated a conditional R² value using Nakagawa's method for generalized linear mixed-effects models, using the performance package in R ([Nakagawa and Schielzeth, 2013](#); [Nakagawa et al., 2017](#)).

To further examine the directions of the significant interaction terms in our full linear mixed effects model, we performed transformations of our parameter estimates (from our lmer model) using the deltaMethod function from the car package in R to generate point estimates and standard errors for our dependent variable, soil CO₂ flux ([Fox and Weisberg, 2019](#)). Each transformation was generated using the full range of values for one independent variable of interest, plus values representing the mean, one standard deviation below the mean, and one standard deviation above the mean for the covariate, while plugging in mean values for all other fixed effects.

Given the complexity of forest soils, we chose to include statistical significance up to the 90 % level (i.e., $p < 0.1$), acknowledging that this increases the probability of committing a Type I error ([Pretzsch et al., 2014](#); [Rastogi et al., 2023](#) in this journal). We also report the actual p-values throughout to allow for interpretation recognizing that the binary decision rule is somewhat arbitrary ([Murtaugh, 2014](#)).

3. Results

3.1. Soil CO₂ flux, soil properties and secondary forest age

Average soil CO₂ fluxes ranged from 5.3 to 10.0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ across the chronosequence. Soil CO₂ fluxes were significantly higher in the active pasture than in the ES, MS, and LS forests (p -values = <0.001, <0.001, and <0.001, respectively) ([Fig. 2](#)). There were no statistically significant differences in average CO₂ fluxes among the three older forested age classes. Soil respiration in pasture sites averaged $10.0 \pm 0.85 \mu\text{mol m}^{-2} \text{s}^{-1}$, almost double the average CO₂ flux in each of the older forest age classes.

Soil temperature ranged between 25.5 ± 0.29 °C in the LS forest and 29.1 ± 0.70 °C in the pasture. Mirroring trends in CO₂ fluxes, soil temperature was significantly higher in the pasture sites than in the ES, MS, and LS forests (p -values = <0.001, <0.001, <0.001, respectively).

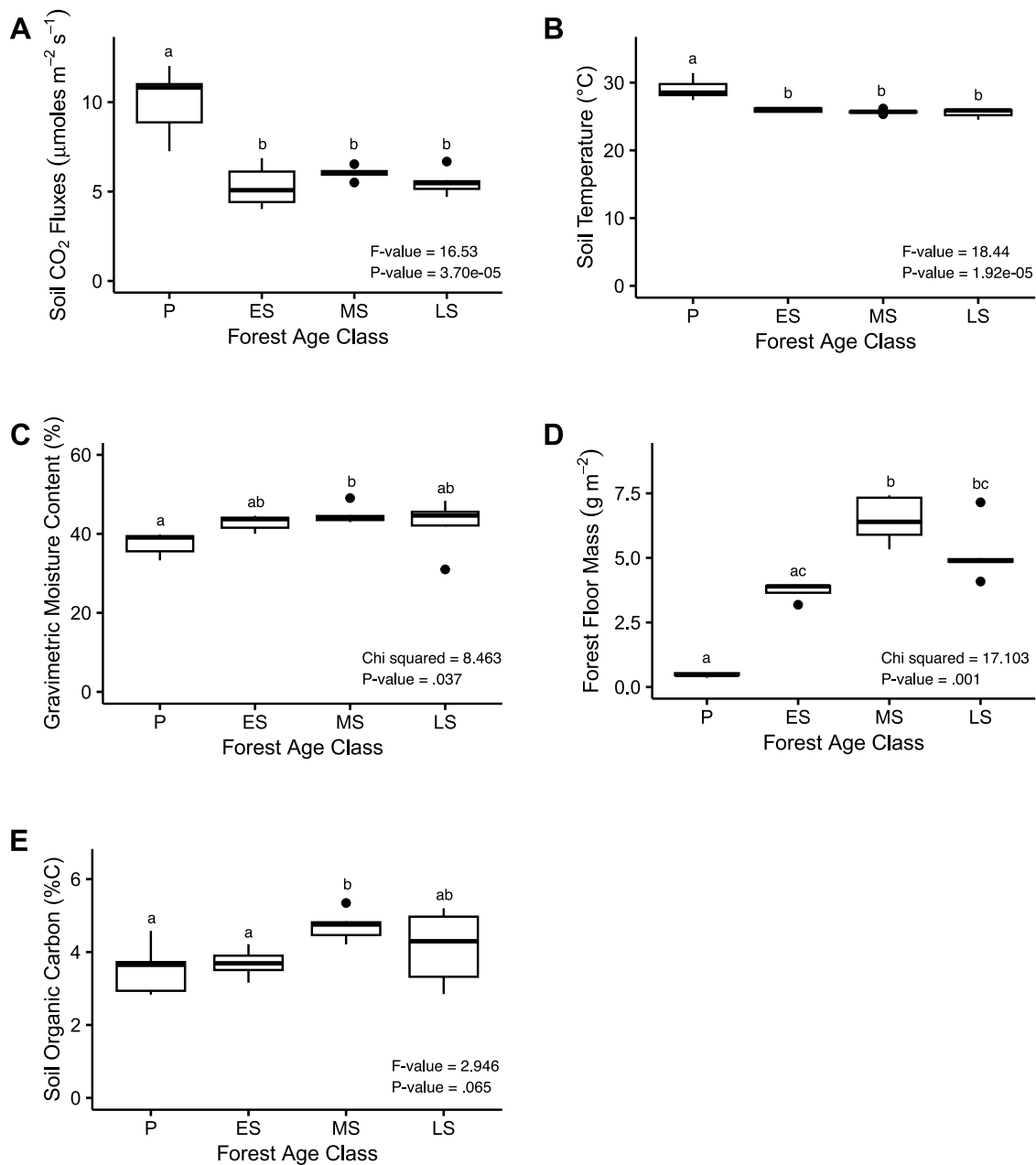


Fig. 2. Soil properties and secondary forest age. Soil properties (A, CO₂ fluxes; B, soil temperature; C, soil gravimetric moisture; D, forest floor mass; E, soil organic C concentration) are plotted in box plots with respect to forest age class. Significant differences between pairs of age classes are denoted by different letters (a,b,c) above the box plot. F-values and p-values are reported for the ANOVA while chi-squared values and p-values are reported for the Kruskal-Wallis test. Thick centerlines represent median values while lower and upper limits of the box represent the first and third quartiles, respectively. The ends of line extending from the edges of the box represent the minimum and maximum values. Dots represent outliers, which are defined as values greater than 1.5 x IQR above the third quartile, or 1.5 x IQR below the first quartile, where IQR = the interquartile range (third quartile - first quartile). Percent values for soil organic C and gravimetric moisture content indicate percent by mass.

(Fig. 2). There were no statistically significant differences in average soil temperature between the three older forest age classes.

Like soil CO₂ fluxes and soil temperature, soil moisture was not significantly different between the three older forest age classes. However, soil gravimetric moisture content in the active pasture was significantly lower than in the MS forest (p-value = 0.053). Soil gravimetric moisture content ranged from 37.1 % to 45.2 % across the chronosequence (Fig. 2).

Values for forest floor mass and soil organic C also generally increased across the chronosequence. Average forest floor mass spanned a large range from 0.47 g m⁻² to 6.48 g m⁻², with significantly lower values in the active pasture compared to the MS and LS forest (p-values

= 0.001 and 0.028, respectively) (Fig. 2). Forest floor mass was also significantly higher in the MS forest than in the ES forest (p-value = 0.072). Average soil organic C concentrations ranged from 3.5 % to 4.6 % across the chronosequence and were significantly higher in the MS forest compared to both the pasture and the ES forest (p-value = 0.093, 0.094, respectively) (Fig. 2).

3.2. Drivers of soil CO₂ fluxes

Based on our linear mixed effects model, soil temperature was the only independent main effect which significantly predicted changes in soil CO₂ fluxes (Table 1). Soil temperature was positively correlated

Table 1

Linear mixed effects model summary. Sums of squares, number of parameters, F-values, parameter estimates (coefficients), standard errors for parameter estimates, and p-values for parameter estimates for each fixed effect in our full model.

Fixed Effect	Mean Sum of Squares	Number of Parameters	F-value	Parameter Estimate	Std. Error	P-value
Soil Moisture	0.630	1	1.857	−0.140	0.103	0.177
Soil Temperature	2.029	1	5.98	0.493	0.202	0.017
Forest Floor Mass	0.065	1	0.191	0.049	0.112	0.664
Soil Organic C	0.242	1	0.713	0.080	0.094	0.401
Soil Moisture: Soil Temperature	1.202	1	3.542	−0.328	0.174	0.063
Soil Moisture: Forest Floor Mass	0.076	1	0.223	−0.061	0.130	0.638
Soil Temperature: Forest Floor Mass	0.018	1	0.054	−0.040	0.172	0.817
Soil Moisture: Soil Organic C	0.051	1	0.152	0.031	0.080	0.698
Soil Temperature: Soil Organic C	1.664	1	4.904	0.367	0.166	0.030
Forest Floor Mass: Soil Organic C	0.902	1	2.656	0.213	0.131	0.107

Model Equation: Soil CO₂ Flux ~ Soil Moisture + Soil Temperature + Forest Floor Mass + Soil Organic C + Soil Moisture: Soil Temperature + Soil Moisture: Forest Floor Mass + Soil Temperature: Forest Floor Mass + Soil Moisture: Soil Organic C + Soil Temperature: Soil Organic C + Forest Floor Mass: Soil Organic C + (1|Site)
Conditional R² = 0.647

with CO₂ flux (p-value = 0.017). Two-level interactions between soil temperature and soil organic C and soil temperature and soil moisture were also found to be significant predictors of soil CO₂ fluxes (p-values = 0.030 and 0.063, respectively) (Table 1).

The interaction between soil temperature and soil moisture was negatively correlated with soil CO₂ fluxes (Table 1). Higher moisture levels corresponded to a smaller positive effect of soil temperature on soil CO₂ fluxes (slope) (Fig. 3). On the other hand, the interaction between soil temperature and soil organic C was positively correlated with soil CO₂ fluxes, where higher soil organic C values corresponded to a greater positive effect of soil temperature on soil CO₂ fluxes (Fig. 3).

4. Discussion and conclusion

4.1. Changes in soil CO₂ flux and soil organic C concentrations during secondary succession

Soil CO₂ fluxes from pastures in Agua Salud were nearly two times higher than fluxes from naturally regenerating secondary forest. Our CO₂ flux values from pasture and forest sites fell within ranges previously reported in Panama (Nottingham et al., 2022; Schwendenmann et al., 2007; Wilsey et al., 2002). Previous studies have shown either an increase in soil respiration across a secondary forest chronosequence (Aryal et al., 2017; Gao et al., 2019; Huang et al., 2016; Sigau and Abdul-Hamid, 2018; Susyan et al., 2011), no change in soil CO₂ fluxes (Wang et al., 2010), or either higher or lower soil respiration rates in the intermediate successional stages, compared to early and late stages

(Calvo-Rodriguez et al., 2020; Duan et al., 2022; Wang et al., 2017). Notably, of the existing literature, only Calvo-Rodriguez et al. (2020) compared CO₂ fluxes during the early transition from pasture to secondary forest. Within our forested sites, the lack of change in soil respiration rates could be due to similar soil microclimate conditions (soil temperature and moisture) in our ES, MS and LS forests, while soil temperature increased significantly in pasture sites. Wang et al. (2010) also found no difference in soil respiration within secondary forests, but they did not report pasture fluxes. Our results suggest that measuring the early pasture-to-forest transition may be important to fully capture the impact of secondary forest regrowth on soil CO₂ fluxes.

The significant decline of soil CO₂ fluxes following pasture conversion demonstrates the potential for secondary forest regeneration to rapidly decrease soil CO₂ emissions. Furthermore, soil CO₂ fluxes remained relatively stable from 7 to 80+ years of secondary forest succession, suggesting that the reduced soil CO₂ flux rates may be maintained in the long-term by continued growth and development of secondary forest. Our observations of soil CO₂ flux dynamics were restricted to Panama's wet season. Several studies have shown that soil respiration rates in both pasture and secondary forest, as well as their associated drivers, may vary depending on season, although wet season fluxes generally account for the majority of emissions from moist forests (Davidson et al., 2000; Mukhopadhyay and Maiti, 2012; Rodtassana et al., 2021; Tanner et al., 2014; Wang et al., 2010; Wolf et al., 2011). Soil CO₂ fluxes across the Agua Salud chronosequence should also be measured during the dry season to capture potential annual patterns in CO₂ emissions following reforestation.

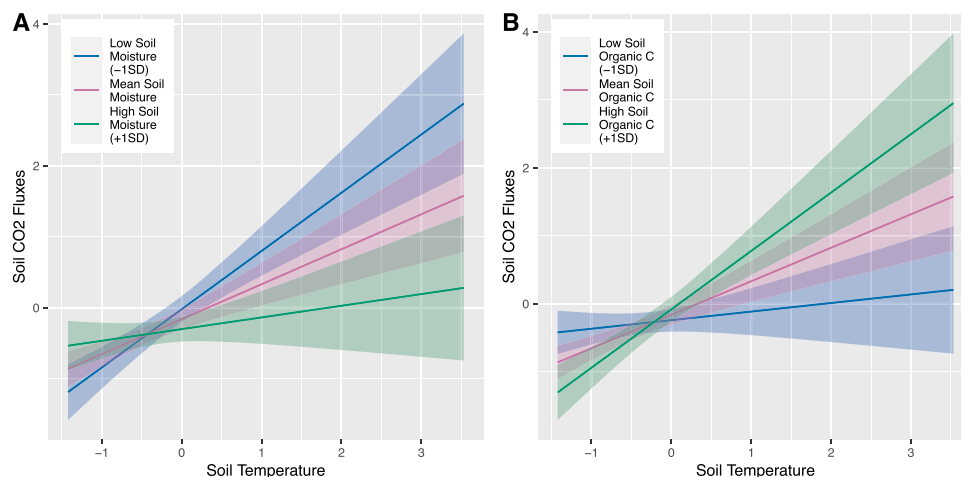


Fig. 3. Influence of interactions between soil properties on soil CO₂ fluxes. Relationships (± 1 standard error) between soil temperature and soil CO₂ fluxes for modifying values of the covariate (A, soil moisture; B, soil organic C), including the mean (0), +1 and −1 standard deviations of the covariate. Values for the dependent and independent variables are z-scored values.

In addition to reduced soil CO₂ emissions, increasing soil organic C concentrations were observed with secondary forest regrowth across our study area. Our soil organic C values in pasture and secondary forest were in agreement with ranges reported by a previous study that looked at soil organic C content down to 20 cm depth in the Agua Salud chronosequence (Neumann-Cosel et al., 2011). Our study showed significant increases in soil organic C concentrations from the pasture to MS secondary forests, but not between the pastures and the ES age class, suggesting that changes in soil organic C concentrations may occur on longer timescales and may not be significant within the first 0–15 years of secondary forest regrowth (Neumann-Cosel et al., 2011).

Temperature, moisture, and forest floor mass are likely to vary with secondary succession.

Observed changes in soil temperature, moisture, and forest floor mass during succession were consistent with changes in edaphic conditions reported by other studies in tropical secondary forests. Previous studies in tropical moist forests have also demonstrated a decline in soil temperature with secondary forest succession (Rottassana et al., 2021; Wang et al., 2017). This effect may be particularly relevant in systems that experience significant increases in leaf area and basal area during succession, minimizing the impact of direct solar radiation on the forest floor (Hashimoto and Suzuki, 2004; Lebrija-Trejos et al., 2011; Marthens et al., 2008; Schwartz et al., 2022). Indeed, in Agua Salud, light availability in the understorey has been shown to decrease with succession (van Breugel et al., 2013). This effect is likely responsible for the significant decrease in soil temperature in our sites by approximately 3 °C within only the first 7 years of secondary forest regrowth. Litterfall has also been shown to recover rapidly during the first few decades of tropical secondary forest succession, aligning with observed increases in forest floor mass between the pasture and MS secondary forests in Agua Salud (Ostertag et al., 2008; Powers and Marín-Spiotta, 2017). Changes in soil moisture during secondary forest succession are thought to arise due to a changing balance of evaporation and transpiration, where decreased evaporation rates due to early-successional shading may increase soil moisture, while greater transpiration rates from increased vegetation growth may decrease soil moisture in late-successional forests (Lebrija-Trejos et al., 2011). While increases in soil moisture with forest age have been observed, these trends tend to be more variable across studies (Lebrija-Trejos et al., 2011; Schwartz et al., 2022; Vargas-Terminel et al., 2022). However, other studies in tropical moist forests have demonstrated similar increases in soil moisture across a successional gradient and between young and intermediate successional forests (Wang et al., 2017; Rottassana et al., 2021). Changes in soil organic C stocks have been shown to follow less predictable trends during tropical forest succession, and may be significantly influenced by land use history and soil physical properties (Powers and Marín-Spiotta, 2017).

4.2. Drivers of soil CO₂ flux

Soil temperature was a key predictor of soil CO₂ flux in our sites. Soil temperature has been widely reported as a significant driver of soil respiration as it can influence rates of both heterotrophic and autotrophic respiration (Duan et al., 2022; Nottingham et al., 2022; Schindlbacher et al., 2009; Wang et al., 2006, 2010). Specifically, higher soil temperatures may increase substrate availability and root enzyme activity, contributing to higher rates of root respiration (Atkin et al., 2000). Increasing soil temperatures may drive microbial respiration by boosting microbial growth and enzymatic activity, changing soil microbial community composition, and releasing labile substrates from stable mineral compounds (Nottingham et al., 2022). However, while soil temperature may be a strong driver of soil respiration seasonally, previous studies in tropical moist forests have suggested that soil temperature may not significantly impact soil CO₂ flux across a successional gradient due to a lack of variability in soil temperature between forest age classes (Rottassana et al., 2021; Wang et al., 2017). Our results

indicate that soil temperature may in fact be an important successional driver of soil respiration when considering the early pasture-to-forest transition. In particular, the significant increases in basal area and leaf area during the first years of pasture conversion may cause a large variation in soil temperature even in moist tropical climates, driving a decrease in soil CO₂ fluxes in early-successional forests compared to pasture sites.

The response of soil CO₂ fluxes to soil temperature was also dependent on values of soil gravimetric moisture content and soil organic C concentrations. We found that soil CO₂ fluxes were less sensitive to soil temperature under high soil moisture content, while the response of CO₂ fluxes to changes in soil temperature was greater under lower soil moisture content. Previous studies have observed parabolic responses of soil respiration to soil moisture, where soil moisture content above a specific threshold contributes to reduced rather than increased CO₂ fluxes (Wood et al., 2013). This effect could be a result of limited CO₂ diffusion or reduced microbial activity due to oxygen (O₂) depletion under high soil moisture (Wood et al., 2013). During the wet season in Panama, our soils had a high gravimetric moisture content (35–45 %), which likely placed our soils at or above this threshold where increasing moisture values would correspond to lower CO₂ flux rates. Thus, lower soil gravimetric moisture content may have reduced the limiting effects of low CO₂ diffusion and O₂ depletion on soil CO₂ fluxes, allowing for a greater response of soil respiration to increasing soil temperatures.

Unlike soil moisture, the interaction between soil temperature and soil organic C concentrations was positively correlated with soil CO₂ fluxes. Soil CO₂ fluxes were more sensitive to changes in soil temperature when soil organic C content was higher. Higher soil organic C content in soil may increase the sensitivity of soil respiration to soil temperature by providing more available substrates to support elevated rates of microbial growth (Duan et al., 2022; Gershenson et al., 2009).

4.3. Conclusion

Soil CO₂ fluxes declined by nearly two-fold during the early pasture-to-forest transition and remained relatively stable during the following 80+ years of secondary forest growth. Our results suggest that studying soil respiration dynamics during the full scope of secondary forest regrowth, including the initial pasture-to-forest conversion, is critically important to fully capture the magnitude of associated changes in soil CO₂ fluxes. Changes in soil CO₂ fluxes during the pasture-to-forest transition also shed light on important successional drivers of soil respiration that may not be captured in studies focusing solely on different stand ages well after forest establishment. In particular, our results demonstrate the importance of soil temperature as a driver of soil CO₂ flux during the regeneration of tropical moist forests, especially during early forest establishment when rapid changes in leaf and basal area may yield higher and more variable soil temperatures. The effect of soil temperature on soil CO₂ fluxes was also affected by soil moisture content and substrate availability. Overall, our results indicate that the conversion of pasture lands to naturally regenerating tropical forests may be an effective strategy to reduce soil CO₂ emissions within the first few years after forest establishment.

Funding source

This work was supported by the Smithsonian Tropical Research Institute through a short-term fellowship to Claire Beckstoffer and the Agua Salud Project and by partial funding from NSF Luquillo LTER DEB-1831952 to the University of Puerto Rico and UCB.

CRediT authorship contribution statement

Whendee L. Silver: Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Jefferson S. Hall:** Writing – review &

editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Claire Beckstoffer:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors have no competing interests to declare.

Data availability

I have shared the link to my data/code at the "Attach File" step.
[2022 Agua Salud Soil CO2 Fluxes - Raw Data \(Original data\)](#) (Mendeley Data)
[2022 Agua Salud LMER Code RStudio \(Original data\)](#) (Mendeley Data)

Acknowledgements

This work is a contribution of the Agua Salud Project, a collaboration between the Smithsonian Tropical Research Institute (STRI), the Panama Canal Authority (ACP) and the Ministry of the Environment of Panama (MiAmbiente). Agua Salud is part of the Smithsonian Institution Forest Global Earth Observatory (ForestGEO). This research was made possible thanks to funding from STRI and valuable support from the following STRI staff, and in kind support from the NSF-sponsored grant to the Luquillo Long-term Ecological Research Program. I would like to thank Mario Bailón, Guillermo Fernández, Johana Balbuena, and Victorino Montenegro for field work and data collection, Dayana Agudo for soil analyses, Adriana Tapia for project administration, Dr. Andy Nottingham for support on LICOR methodology, Dr. Michiel van Breugel for providing forest biomass data and calculations, and Milton Solano for preparing our site map in ArcGIS. I would like to thank Dr. Steve Beissinger for help with our linear mixed effects model analysis and data visualization. I would also like to acknowledge the resources and support provided by Dr. Whendee Silver's lab at my undergraduate institution and Gisela Gonzalez for help with elemental analysis.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.foreco.2024.122263](https://doi.org/10.1016/j.foreco.2024.122263).

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