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# Soil pyrogenic carbon in southern Amazonia: Interaction between soil, climate, and above-ground biomass

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The Amazon forest represents one of the world's largest terrestrial carbon reservoirs. Here, we evaluated the role of soil texture, climate, vegetation, and distance to savanna on the distribution and stocks of soil pyrogenic carbon (PyC) in intact forests with no history of recent fire spanning the southern Amazonia forest-Cerrado Zone of Transition (ZOT). In 19 one hectare forest plots, including three Amazonian Dark Earth (ADE, terra preta) sites with high soil PyC, we measured all trees and lianas with diameter  $\geq 10$  cm and analyzed soil physicochemical properties, including texture and PyC stocks. We quantified PyC stocks as a proportion of total organic carbon using hydrogen pyrolysis. We used multiple linear regression and variance partitioning to determine which variables best explain soil PyC variation. For all forests combined, soil PyC stocks ranged between 0.9 and 6.8 Mg/ha to 30 cm depth (mean  $2.3 \pm 1.5$  Mg/ha) and PyC, on average, represented 4.3% of the total soil organic carbon (SOC). The most parsimonious model (based on AICc) included soil clay content and above-ground biomass (AGB) as the main predictors, explaining 71% of soil PyC variation. After removal of the ADE plots, PyC stocks ranged between 0.9 and 3.8 Mg/ha (mean  $1.9 \pm 0.8$  Mg/ha<sup>-1</sup>) and PyC continued to represent ~4% of the total SOC. The most parsimonious models without ADE included AGB and sand as the best predictors, with sand and PyC having an inverse relationship, and sand explaining 65% of the soil PyC variation. Partial regression analysis did not identify any of the components (climatic, environmental, and edaphic), pure or shared, as important in explaining soil PyC variation with or without ADE plots. We observed a substantial amount of soil PyC, even excluding ADE forests; however, contrary to

expectations, soil PyC stocks were not higher nearer to the fire-dependent Cerrado than more humid regions of Amazonia. Our findings that soil texture and AGB explain the distribution and amount of soil PyC in ZOT forests will help to improve model estimates of SOC change with further climatic warming.

#### KEYWORDS

fire, biomass, drought, Holocene, Zone of Transition, Cerrado, soil organic carbon, fire fuel

## Introduction

The Amazon rainforest is one of the world's largest terrestrial above- and below-ground carbon (C) pools (Feldpausch et al., 2012; Ometto et al., 2014). With intensifying land-use change and severe drought, C emissions due to fire are increasing (Aragão et al., 2018), thereby reversing a multi-decadal C sink (Phillips et al., 2009; Brienen et al., 2015; Feldpausch et al., 2016). The central and northwestern regions of Amazonia, with high annual precipitation and short or no dry season, are resistant to burning in non-drought years (Pontes-Lopes et al., 2021), but in the transitional forests of southern Amazonia, a combination of lower annual precipitation, a pronounced dry season, and proximity to flammable Cerrado vegetation, increase the probability of fire in the presence of ignition sources (Balch et al., 2008; Nogueira et al., 2019; Prestes et al., 2020). The conditions promoting fire and the proportion of closed-canopy forest cover have varied over time, with palaeo-records for south and southwestern Amazonia indicating drier conditions during the mid-Holocene (Burbridge et al., 2004; Kury et al., 2021), followed by an expansion of closed-canopy forest over the past 2000 years (Burbridge et al., 2004).

Across the southern Amazon forest-Cerrado zone of ecological tension (ZOT), therefore, fire has been a long-term driver of vegetation dynamics (Mayle et al., 2000, 2007; Kury et al., 2021) and has contributed to the transformation of a proportion of above-ground biomass (AGB) into charcoal (Koele et al., 2017). The development of soil pyrogenic carbon (PyC), a soil organic carbon (SOC) fraction formed by the incomplete combustion of plant biomass, represents a SOC fraction resistant to decomposition that may persist for millennia (Bird et al., 2015). Determining the drivers of variation in soil PyC stocks is important for understanding past fire history, the contribution of PyC to total SOC stocks, and determining SOC sensitivity to current and future fire regimes and climate change.

Global estimates suggest an annual PyC production of 50–270 Tg (Kuhlbusch and Crutzen, 1995; Wei et al., 2018), with vegetation burning being responsible for up to 28 Tg year<sup>-1</sup>. Estimated inputs of PyC by atmospheric deposition from fossil fuel and biomass burning aerosols in Amazonia

(~6 kg km<sup>-2</sup> yr<sup>-1</sup>) are low (Coppola et al., 2019), indicating many thousands of years would be required to develop the current Amazonian soil PyC stock, estimated to be 2.67 Pg over 0–100 cm soil depth (Koele et al., 2017). Therefore, the majority of soil PyC found in forests with no history of recent fire is likely due to *in situ* historical fires and/or local edaphic or topographic conditions favoring the long-term accumulation of soil PyC. Radiocarbon dating of macro-charcoal fragments from the soil indicates Amazon forests have periodically burned throughout the Holocene (Sanford et al., 1985; Goulart et al., 2017; McMichael and Bush, 2019; Feldpausch et al., 2022), with some old-growth forests having fire return intervals of hundreds of years and having experienced no fire for the last ~800 years (Feldpausch et al., 2022).

The PyC fraction of SOC is more resistant to degradation than other SOC forms due to its refractory polyaromatic composition, as well as potential interactions with the clay mineral matrix through the formation of organo-mineral associations (Czimczik and Masiello, 2007). However, there is still uncertainty about how long PyC persists in soils, or how global warming will affect soil PyC stocks. Some studies have shown that most of the larger PyC particles remain in the soil after fire (Suman et al., 1997; Gustafsson and Gschwend, 1998; Masiello, 2004). Depending on the temperature of formation and organic matter of origin of PyC, mineral phase, and soil texture, PyC can remain in the soil much longer than other forms of SOC, and in some cases for thousands of years (Singh et al., 2014; Bird et al., 2015). Dissolved and fine particulate PyC fractions may be translocated to deeper soil depths and lost to erosion over years to decades, especially with heavy rainfall (Major et al., 2010), and these effects may be greater in sandy soils and forests with steeper slopes (Rumpel et al., 2006).

The ZOT between the two largest Brazilian biomes, the Amazon forests and the Cerrado, is located in a seasonal climate, with a dry period of up to 6 months, average temperature above 25°C, and annual precipitation predominantly between 1400 and 2000 mm (Silva et al., 2008). While an increase in temperatures with global warming (IPCC, 2018) is likely to increase the rate of respiration of SOC to the atmosphere (Davidson et al., 2006), for recalcitrant PyC fractions losses could be much lower since PyC stocks are more resistant to degradation in response to warming (Lehmann et al., 2008). The

more open vegetation and the low stratum that dries during the dry season, that are typical of the Cerrado (Ribeiro and Walter, 2008), favor the entry of fires in transition forests at higher frequency than moist forests (Balch et al., 2008) and therefore, likely the production of PyC. Although these seasonal evergreen forests show relative fire resistance, this resistance significantly changes with an increase in fire frequency, and intensity, exacerbated by extreme weather events and land-use change (Balch et al., 2008; Brando et al., 2020). Thus, these factors, combined with the precipitation gradient and differences in vegetation structure such as height (Barbier et al., 2010; Feldpausch et al., 2011), biomass (Fauset et al., 2015), and composition (Morandi et al., 2016) across the transition may interact to affect soil PyC stocks.

Some factors could also act ambiguously to affect SOC and PyC stocks across the ZOT. For example, AGB stocks are generally greater with higher precipitation, providing more fuel for fire and potentially more charcoal during burning (Fearnside et al., 2001). However, higher AGB and higher precipitation can maintain higher sub-canopy moisture, preventing combustion of dead leaves and small branches that act as fine fuel and thereby reduce the capacity of these components to carry a fire (Ray et al., 2005). Likewise, soils with more clay can protect PyC from decomposition through the formation of organo-mineral compounds (Glaser et al., 2003); however, the lower moisture content (Metcalf et al., 2007) of sandier soils may slow the decomposition of SOC and PyC. Therefore, a combination of edaphic, climatic, and vegetation factors may interact to affect soil PyC. Thus, we hypothesize that forests located in regions with lower annual precipitation, combined with more clayey soils, will have higher PyC than sandy soils located in regions with higher precipitation. Furthermore, we hypothesize that PyC stocks will vary with AGB, as AGB directly reflects the amount of material that can be converted to PyC during burning (Fearnside et al., 2001; Preston and Schmidt, 2006). Our study aims to evaluate the interaction of multiple drivers in controlling the distribution and stocks of forest PyC across the Amazon-Cerrado transition. We address the following questions: (1) How much soil PyC is stored in forests across the Amazon-Cerrado transition? (2) How do PyC stocks relate to soil texture, forest AGB, and climate? (3) How does the distance of each sampled location to the current Cerrado biome border influence forest soil PyC?

## Materials and methods

### Study area

We conducted this study in 19 permanent forest plots (forests of the Amazon-Cerrado transition), representing structurally intact forests with no history of recent fire (at least since the availability of satellite data) in the southeastern

and southwestern border zone of the Amazon Basin, with sixteen plots located in Brazil and three in Bolivia (Table 1 and Figure 1). Plots in Brazil's forests, in southeastern Amazonia, are distributed over two states: Mato Grosso, covering the municipalities of Nova Xavantina, Ribeirão Cascalheira, Querência, São Félix do Araguaia, Porto Alegre do Norte, Santa Terezinha, Gaúcha do Norte and Alta Floresta; and in Pará, in the municipality of Santana do Araguaia. The plots in Bolivia in southwestern Amazonia, are located in the Department of Santa Cruz, bordering Mato Grosso/Brazil.

We collected vegetation and soil data following the standard RAINFOR protocol (Phillips et al., 2010); all plot data are archived and managed through ForestPlots.net (Lopez-Gonzalez et al., 2009, 2011) and contribute to regional and international plot networks (ForestPlots.net et al., 2021). The average annual precipitation of the region varies between 1,451 and 2,353 mm and the average temperature from 23.9 to 26.9°C (Worldclim 1.4, Hijmans et al., 2005; Table 1).

The forests of the Amazon-Cerrado transition grow on deep, highly weathered soils, mainly represented by Ultisols and Oxisols with low pH and cation exchange capacity (CEC), as well as largely occluded P due to the high levels of Fe and Al oxide minerals (Quesada et al., 2011). As a result of extensive sandstone and granitic rocks (Sombroek, 1966), the Brazilian soils are quite sandy and have low fertility (Reatto et al., 2008). The Ultisols comprise soils consisting of mineral material, with the presence of a textural B horizon of clay of low or high activity combined with low base saturation (EMBRAPA, 2006). Their striking feature is an increase in clay from the surface horizon A to the subsurface B. The depth ranges from shallow to deep (IBGE, 2015).

Oxisols are very weathered, deep and well-drained soils. In general, they are strongly acidic, with low base saturation, dystrophic or aluminum-rich soils (EMBRAPA, 2006). Together with the Ultisols, they are the most extensive soils in Brazil (IBGE, 2015). Many different vegetation types are found on this soil type, ranging from more open formations, such as *campo cerrado*, to forest formations such as *cerradão* (Marimon-Junior and Haridasan, 2005), to seasonal evergreen and ever wet forests (Ivanauskas et al., 2008). Pre-Columbian land-use also modified vegetation and soils through the production of Anthrosols (Amazon Dark Earths, ADE, *terra preta*), small ancient agricultural patches of up to several hectares of soil highly enriched in PyC that are now covered by mature forests (Oliveira et al., 2020). While ADEs are found throughout Amazonia (Palace et al., 2017), estimates suggest they only represent a small fraction (~3%) of Amazonian soils (McMichael et al., 2014). Pre-Columbian land-use and fire management appears to have declined sharply across Amazonia following the Great Dying after 1492 (Goulart et al., 2017; McMichael and Bush, 2019; Feldpausch et al., 2022) due to the introduction of Old-World diseases during post-Columbian European colonization (Dull et al., 2010).

**TABLE 1** Forest plot codes (RAINFOR codes), states (MT, Mato Grosso; PA, Pará; SC, Santa Cruz province), Country (BR, Brazil and BO, Bolivia), geographic location, annual precipitation ( $P_A$ ), average annual temperature ( $T_A$ ), soil types (Soil Survey Staff, 1999), and soil PyC/Total Organic Carbon (TOC) of areas sampled along the Amazon-Cerrado transition.

Plot code	Municipalities/ province	State/ Country	Lat	Lon	$P_A$ (mm)	$T_A$ (°C)	Soil types	PyC/TOC
ALF-02	Alta Floresta	MT/BR	-9.58	-55.92	2,353	25.6	Entisols	0.065
FLO-01	Ribeirão Cascalheira	MT/BR	-12.81	-51.85	1,613	25.5	Oxisols	0.042
FLO-02	Ribeirão Cascalheira	MT/BR	-12.76	-51.88	1,621	25.6	Oxisols	0.046
FRP-01	São Félix do Araguaia	MT/BR	-11.48	-51.52	1,634	26.9	Oxisols	0.029
GAU-02	Gaúcha do Norte	MT/BR	-13.43	-53.31	1,701	24.1	Oxisols	0.045
GAU-04	Gaúcha do Norte	MT/BR	-13.1	-53.35	1,795	24.7	Oxisols	0.030
GAU-05	Gaúcha do Norte	MT/BR	-12.98	-52.92	1,757	24.9	Oxisols	0.063
GAU-06	Gaúcha do Norte	MT/BR	-13.31	-53.41	1,729	24.7	Oxisols	0.059
HCC-21	José M. de Velasco	SC/BO	-14.53	-60.74	1,513	21.4	Ultisols	0.039
LFB-01	José M. de Velasco	SC/BO	-14.58	-60.83	1,451	23.9	Ultisols	0.058
LFB-02	José M. de Velasco	SC/BO	-14.58	-60.83	1,451	23.9	Ultisols	0.046
POA-01	Porto Alegre do Norte	MT/BR	-10.96	-52.17	1,774	26.1	Ultisols	0.030
SAA-01	Santana do Araguaia	PA/BR	-9.79	-50.43	1,815	26.8	Ultisols	0.064
SAA-02	Santana do Araguaia	PA/BR	-9.64	-50.45	1,778	26.6	Ultisols	0.060
SAT-01	Santa Terezinha	MT/BR	-9.84	-50.46	1,821	26.7	Ultisols	0.025
TAN-02	Querência	MT/BR	-13.08	-52.38	1,625	24.9	Oxisols	0.025
TAN-03	Querência	MT/BR	-12.83	-52.35	1,679	25.1	Oxisols	0.046
TAN-04	Querência	MT/BR	-12.92	-52.37	1,662	25.0	Oxisols	0.044
VCR-02	Nova Xavantina	MT/BR	-14.83	-52.17	1,512	25.2	Oxisols	0.028

RAINFOR = Amazon Forest Inventory Network ([ForestPlots.net](https://ForestPlots.net)).

Soils were classified according to the RADAMBRASIL soil map ([RADAMBRASIL, 1982](https://radambrasil.gov.br/)).

## Plot selection

We selected 19 one hectare forest plots that were within the forest-cerrado ZOT, with plots in intact forest at maximum and minimum distances of 52.2 and 0.2 km from cerrado. The Alta Floresta plot (ALF-02) is geographically the farthest into the Amazon forest biome and the Nova Xavantina plot (VCR-02) closest to the Cerrado biome. The plots span a 1540 km length of the ZOT representing large-scale variations in vegetation and environment.

## Data collection

### Data collection and processing

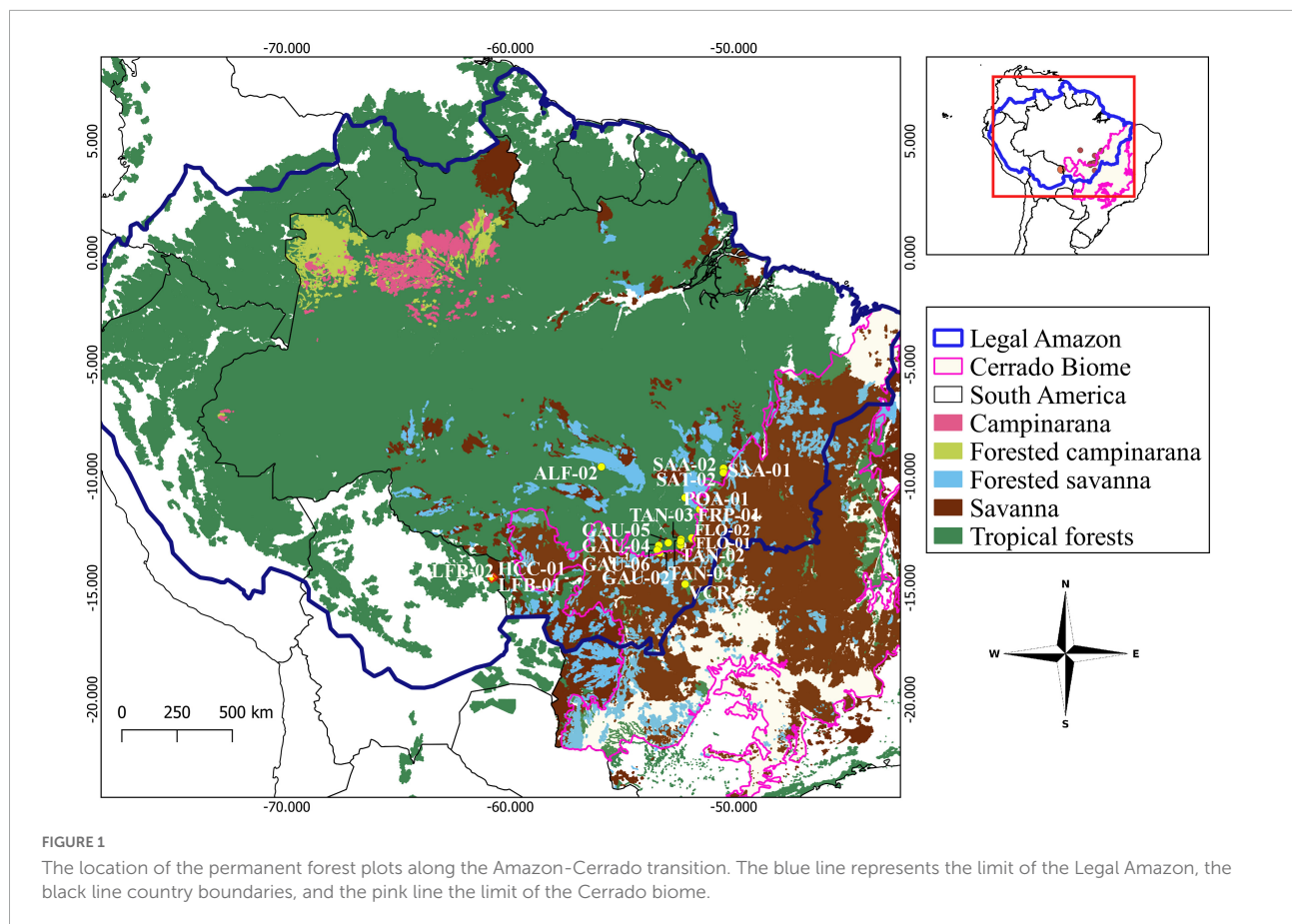
For the soil analysis, we collected soil samples at 0–5, 5–10, 10–20, and 20–30 cm depth distributed over five random points in each plot using a Edelman soil auger. We stored samples in plastic bags and dried them in the laboratory. After drying, we analyzed the samples for textural characteristics (granulometry), including sand, silt, and clay, using the analysis protocol for assessing soil of *Empresa Brasileira de Pesquisa Agropecuária* (Silva et al., 1998; EMBRAPA, 2011). To quantify soil PyC abundance, we prepared soil for analysis by grinding 10 g of soil with a ball mill. Using hydrogen pyrolysis (Meredith et al., 2012), we quantified the polycyclic aromatic component of PyC

as a proportion of total organic carbon (in  $\text{g}/\text{dm}^3$ ). Initially,  $\sim 1$  g of ground soil was catalyst loaded, subsequently pressurized to 150 bar of hydrogen gas and heated from 250 to 300°C over 1 min, then ramped at 8°C per minute to a final temperature of 550°C which was maintained for 2 min (Koele et al., 2017). This technique is assumed to remove all non-PyC from the sample and elemental analysis of the original and treated samples provide an accurate estimate of the abundances of SOC and PyC. PyC results from this technique are highly reproducible, with determinations from triplicate analyses within  $\pm 2\%$  across all samples (Meredith et al., 2012).

### Pyrogenic carbon stock and above-ground biomass of vegetation

We quantified the PyC stocks (0–30 cm) using mean bulk density by FAO major soil grouping (Batjes, 1996), and converted this to tons per hectare ( $\text{Mg}/\text{ha}$ ) for each forest. We determined the biomass for each plot using the pantropical allometric model of Chave et al. (2005),  $\text{AGB} = 0.0509 \times \rho D^2 H$ ; where the coefficient F (0.0509) is dependent on the diameter ( $D$ ): height ( $H$ ) ratio,  $\rho$  is the wood density ( $\text{g}/\text{cm}^3$ ), compiled from the DRYAD global database (Chave et al., 2009; Zanne et al., 2009),  $D$  is the individual diameter of each tree (cm) at breast height or above deformities or buttresses and  $H$  (m) is the total tree height. We estimated the height for each tree





from a pantropical allometric equation  $H = a(1 - \exp(-bDc))$  (Feldpausch et al., 2012), where the coefficients  $a$ ,  $b$ , and  $c$  were determined for each region. Together, these equations minimize the error in biomass estimation, as they consider geographic location, forest environment, and structure (see Feldpausch et al., 2011, 2012).

### Climate variables

Data for temperature (maximum and minimum), average annual precipitation, coefficient of precipitation variation (seasonality), and soil water deficit in the dry season were compiled from meteorological stations between 1950 and 2000, with a spatial resolution of approximately 1 km (30 s) using WorldClim (Hijmans et al., 2005). We extracted climate data using the *raster* (Hijmans, 2016) and *rgdal* (Bivand et al., 2016) packages in R (R Core Team, 2020). We calculated potential evapotranspiration for all plots from the Tropical Rainfall Monitoring Mission (TRMM) data, version 3B42, V7, mainly derived from satellite data (NASA, 2011) with a range of 50° North to 50° South, using the same packages mentioned above and also *vegan* (Oksanen et al., 2016).

The Maximum Climatological Water Deficit (MCWD) (Aragão et al., 2007) was estimated for each plot as the value of the cumulative water deficit in all months of the year,

starting in the rainy season and ending in the dry season, using monthly data from TRMM (NASA, 2011). This index is based on the approximate monthly transpiration of tropical forests (100 mm/month). When this value is less than 100 mm, the forest is assumed to be in water deficit, which is then accumulated monthly. We generated these data using the *raster* and *rgdal* packages (Bivand et al., 2016; Hijmans, 2016).

We measured the shortest distance from each forest plot to the Cerrado biome (savanna environment) using Google Earth Pro. To reduce uncertainties associated with anthropogenically altered areas between the biomes, we used the transition band delimited by Marques (2016) as a kmz extension and superimposed this layer on the Google Earth image as boundaries for the two biomes. We used this distance in the present study as a predictor of fires, as the closer to the Cerrado biome, which has characteristics that favor the entry of fire (Balch et al., 2008; Ribeiro and Walter, 2008), the greater the chances of exposure to fire in prehistory and in modern times, mainly due to land-use change.

### Data analysis

To test which predictor variables best explain soil PyC variation, we applied Multiple Linear Regression (OLS) models for (1) the distance from the forests (plots) to the Cerrado

Biome; (2) the climate (MCWD, evapotranspiration of the warmest months,  $T_A$ ,  $P_A$ , coefficient of variation of precipitation and average temperature of the warmest months); (3) soil texture (silt, sand, and clay) and (4) current AGB of each plot.

To identify collinearity and remove predictor variables with low correlations to the response variable ( $r < 0.1$ ), we applied Pearson's correlation (Supplementary Table 3). We removed the collinearity by removing from our models the variables with  $r > 0.8$  (Supplementary Tables 1, 2). With the remaining variables, we applied planned models (ICtab function) of corrected Akaike and corrected Akaike Information Criterion corrected (AICc) (Anderson and Burnham, 2002). This argument creates a table by fitting the models in ascending order, with the best model (most parsimonious), with the smallest delta ( $\Delta\text{AICc} = 0$ ), and the greatest weight (WEIGHT, measures the weight in favor of each model) at the top.

We also tested the multi-collinearity of the complete models selected by AICc from the Variance Inflation Factor (VIF), where the variables should present values less than 10 (Quinn and Keough, 2002). As we did not observe VIF greater than 10, we continued with the remaining variables in the models. We also evaluated the impact of the possible presence of very discrepant data in the model by the diagnosis of Bonferroni outliers. As outliers were detected, we applied the *tsclean* function and replaced the outliers with linear interpolated values (Box-Cox) (Hyndman, 2016).

To confirm the independence of the data ( $p > 0.05$ ), we tested the Spatial Autocorrelation (SAC) of the residuals of the models, complete, selected (obtained by selecting the AICc models), the response variable, and the Geographically Weighted Regression-GWR by the Moran index (Legendre and Legendre, 1998; Diniz-Filho et al., 2003). We also tested the assumptions of multiple linear regression by evaluating the normality of the residuals (Shapiro-Wilk) and by graphically displaying the values predicted by the model vs. residues for homoscedasticity and linearity (Legendre and Legendre, 1998; Supplementary Figures 1, 2). As we did not observe significant SAC and the assumptions of the analysis were not violated, we continued using the OLS multiple linear regression model.

We previously tested for possible local effects on the response variable by comparing the OLS and GWR models (Fotheringham et al., 2002), with the Gaussian spatial function. As there was no increase in the GWR regression model on the OLS ( $F_{1,4}$ , 6.4-effective parameter number = 0.54,  $R^2 = 0.19$ ,  $\text{AICc} = -42.6$ ,  $p = 0.63$  and  $F_{1,5}$ , 5-effective parameter number = 0.43,  $R^2 = 0.11$ ,  $\text{AICc} = -52.5$ ,  $p = 0.78$ ), we chose to continue the analyses using only the OLS and assumed an absence of local effects.

To assess which sets of variables best explain the variation in the soil PyC response, we divided the variables into pure climatic, edaphic, and environmental fractions (biomass and distance), fractions shared between them, and the residue.

Since the response variable is a single vector, we applied a partial regression model based on linear regression. This analysis divides the variation in the response variable into components explained in two or more data sets and the fractions combined between them (Legendre and Legendre, 1998). The significance of pure fractions A, B, and C were tested by Redundancy analysis – RDA, using 999 permutations with a significance level of 0.05.

All analyses were done using the R program (R Core Team, 2020). We performed all the procedures for the GWR analysis with the *spgwr* package (Bivand and Yu, 2017), OLS and partial linear regression with the *vegan* package (Oksanen et al., 2016), Spatial Autocorrelation-ACE (Moran Index) with the *spacemaker* package (Dray, 2013), AICc model selection with the *bbmle* package (Bolker and Team, 2016), VIF and data discrepancy test (Bonferroni test) with the *car* package (Fox and Weisberg, 2011), and the correction of outliers with the *forecast* package (Hyndman, 2016). All of the above packages were used together with the *vegan* package (Oksanen et al., 2016). We standardized all data using the normalized argument (make margin sum of squares equal to one) of the *decostand* function (Oksanen et al., 2016) and used a significance level of 5% for all analyses. Maps and figures were produced with the packages *Rdoby*, *lattice*, *plyr*, *reshape*, *boot*, *zoo*, *ggplot2*, *maps*, *GISTools*, *mapdata*, *shape*, and *maptools* (Davison and Hinkley, 1997; Zeileis and Grothendieck, 2005; Wickham, 2007, 2009, 2011; Sarkar, 2008; Brunson and Chen, 2014; Soetaert, 2014; Becker et al., 2016; Bivand and Lewin-Koh, 2016; Cauty and Ripley, 2016; Richard et al., 2016).

## Results

The PyC stocks in the soils of the forests spanning the ZOT ranged between 0.9 and 6.8  $\text{Mg ha}^{-1}$  to 0–30 cm depth (with an average of  $2.3 \pm 1.5$ ) (Figure 2), with PyC representing 4.3%

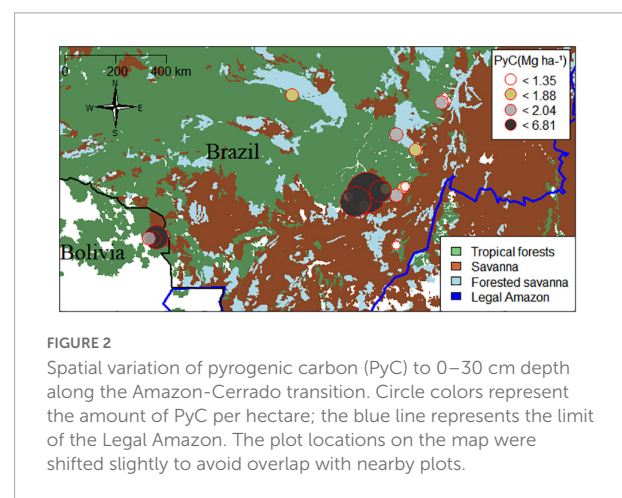


TABLE 2 Models with and without ADE plots to estimate soil PyC.

Models with ADE	Variables	$\Delta$ AICc	df	Weight
Model 1	biomass + clay	0	4	0.93
Model 2	biomass + sand	5.8	4	0.05
Model 3	biomass + distance + sand	8.6	5	0.01
Model 4	distance + sand + mcwd + biomass	11.4	6	0.003
Model 5	distance + mcwd + clay	14.3	5	< 0.001
Model 6	biomass + distance	18.5	4	< 0.001
Model 7	mcwd + distance	23.6	4	< 0.001

Summary model 1 with ADE	Estimates/intercept	t/F-statistic	P	df	R <sup>2</sup>
Biomass	0.01	−2.84	= 0.01	1	
Clay	0.007	3.91	< 0.001	1	
Result model	−2.01	20.00	< 0.001	2/16	0.71

Models without ADE	Variables	$\Delta$ AICc	df	Weight
Model 1	biomass + sand	0	4	0.64
Model 2	distance + sand + biomass	3.1	5	0.14
Model 3	distance + mcwd + clay	3.7	5	0.10
Model 4	biomass + clay	3.8	4	0.02
Model 5	biomass + mcwd + sand + distance	6.5	6	0.02
Model 6	biomass + distance	18.2	4	0.00
Model 7	mcwd + distance	18.3	4	0.00

Summary model 1 without ADE	Estimates/intercept	t/F-statistic	P	df	R <sup>2</sup>
Biomass	0.26	1.76	0.10	1	
Sand	−0.81	−5.51	< 0.001	1	
Result model	> −0.001	16.56	< 0.001	2/13	0.72

Significant value ( $\leq 0.05$ ) in bold.

Selection of models was determined by the corrected Akaike Information Criterion (AICc) for different models designed using environmental variables from the Amazon-Cerrado transition.

$\Delta$  AICc, indicates the best model in ascending order; df, degree of freedom of the model; Weight, measures the force in favor of each model; Clay, clay content; Biomass, aboveground biomass.

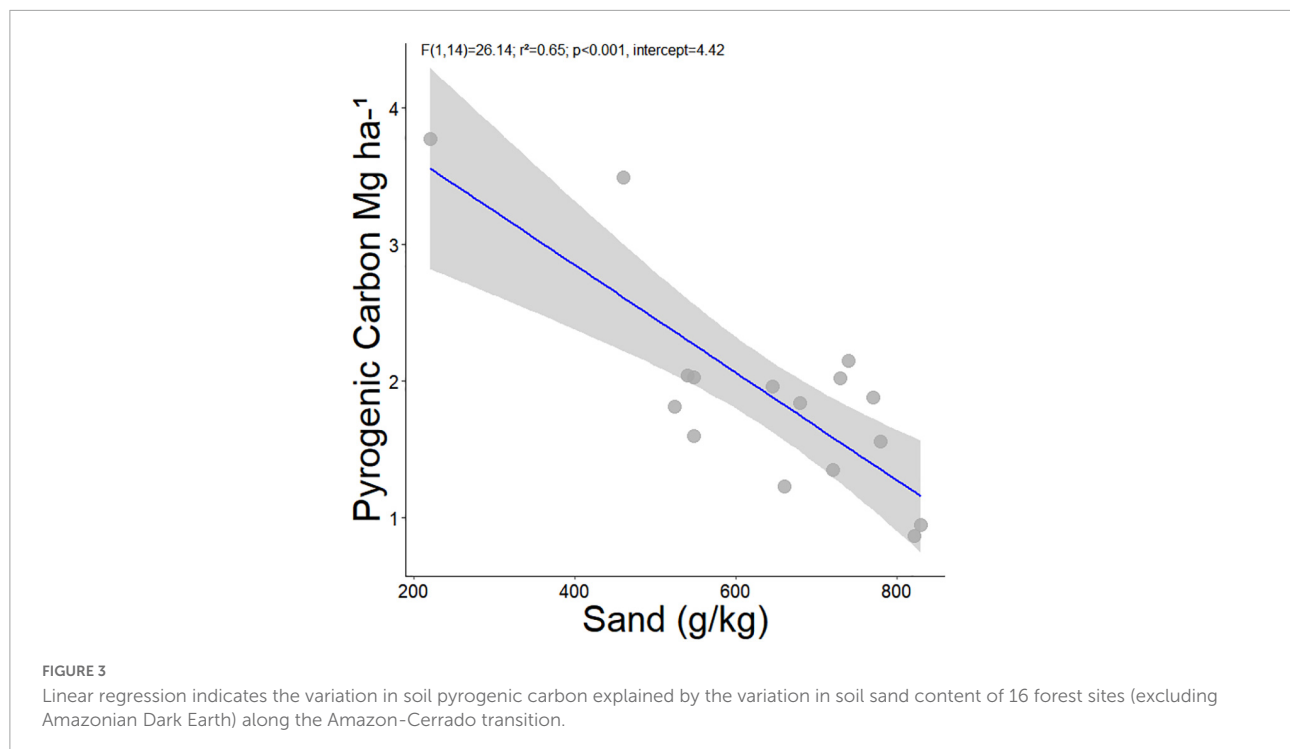
Collinearity values can be found in the [Supplementary Table 2](#).

of the total soil organic carbon (SOC) to 30 cm depth. The most parsimonious model, based on AICc, included soil clay and above-ground biomass as the main predictors of PyC, and these together explained 71% of the variation [ $F_{(2,16)} = 20$ ;  $r^2 = 0.71$ ;  $p < 0.001$ , intercept =  $-2.01$ ] (Table 2). When evaluating these variables in isolation, only clay explained a significant part of the model variance, however, with a loss in the explanatory power [ $F_{(1,17)} = 13.46$ ;  $r^2 = 0.44$ ;  $p = 0.001$ , intercept = 0.30] and in the estimative value, of 0.07 to 0.06 Mg of PyC for each unit of clay in the model.

Next, we evaluated the effect of removing the ADE plots from the models. As these sites are known to have been altered by pre-Columbian peoples, the ADE plots represent outliers compared to the PyC stocks found in non-ADE soils, and non-ADE plots represent the vast majority of soils across the ZOT. After the removal of the ADE plots, soil PyC stocks ranged between 0.9 and 3.8 (mean  $1.9 \pm 0.8$  Mg/ha PyC) to 0–30 cm

depth. The PyC, on average, continued to represent  $\sim 4\%$  of the total soil carbon inventory to 30 cm depth (Supplementary material 1). The most parsimonious model included soil sand content and biomass as the main predictors of PyC and these explained 72% ( $r^2$ ) of the variation. When evaluating these variables in isolation, only sand explained a significant part of the model variance (Table 2), with a slight loss in the explanatory power and estimation ( $-0.81$  to  $-0.004$ ) of the model [ $F_{(1,14)} = 26.14$ ;  $r^2 = 0.65$ ;  $p < 0.001$ , intercept = 4.42, Table 2 and Figure 3]. That is, there is a gain in the predictive power of PyC when evaluating the sand in the presence of the variable biomass; however, the variable sand has a predictive capacity three times that of biomass to explain the amount of PyC.

None of the components (climatic, environmental, or edaphic), pure or shared, was important in explaining soil PyC variation with or without ADE plots based on the partial regression models (Figures 4A,B and Table 3). However, when



we removed the ADE plots, there was a small increase in explanatory power of the pure edaphic component (Figure 4B). The fractions not explained by the models (residuals) were relatively high (Figures 4A,B).

## Discussion

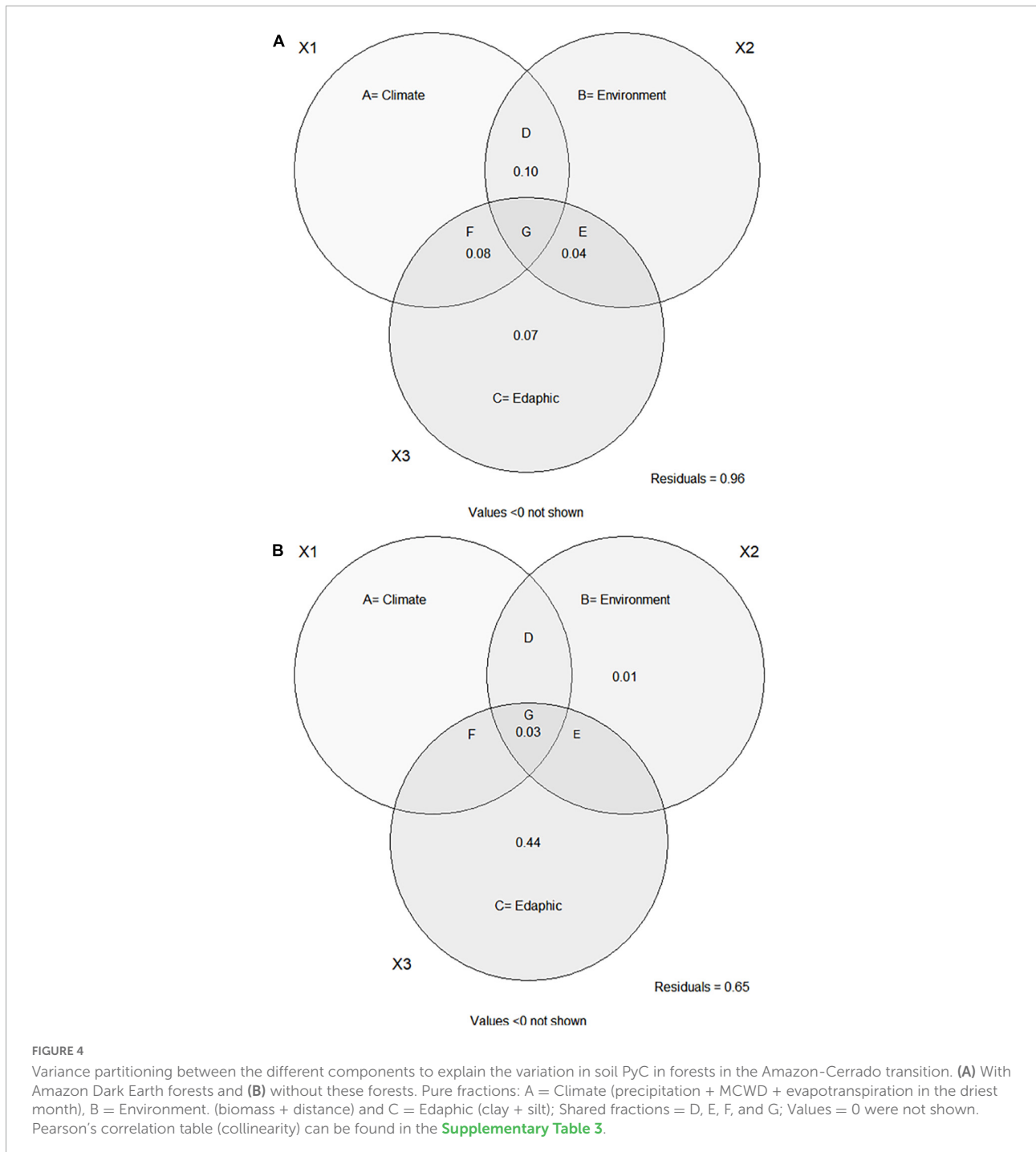
Studies over the past decade have begun to reveal the effects of historical fire on structurally intact forests with no history of recent fire throughout the Amazon Basin (Feldpausch et al., 2022), including higher than predicted PyC stocks (Bird et al., 2015; Koele et al., 2017). However, there remains large uncertainty about how soil PyC varies spatially (Carvalho et al., 2018), and the drivers of spatial variability, especially throughout the ZOT where fire risk is greater and fire has potentially affected vegetation and soils for millennia. Here, we show that soil PyC storage in the southern Amazon ZOT varies approximately eight fold (Figure 2), at local and regional scales of 1540 km, and these stocks are, on average, similar to humid regions of Amazonia where historical fire may have been less prevalent (Koele et al., 2017). Edaphic factors were one of the strongest predictors of soil PyC stocks, which follows expectations based on studies of biochar application and the capacity of clay to protect PyC through the formation of organo-mineral complexes (Czimczik and Masiello, 2007). Although there was substantial variation in  $T_A$  (4°C) and  $P_A$  (900 mm/yr) across the geographical focus on the ZOT, which are known to affect soil respiration rates (Davidson and Janssens, 2006;

Lehmann et al., 2008), these variables were unimportant in predicting soil PyC. The inferred main drivers of soil PyC stocks differed when including the ADE plots rich in PyC, with biomass and soil clay (−88 correlation with sand, Supplementary material 3) content then being important drivers. This was due to biomass stocks on average being higher in ADE than the surrounding non-ADE forests in southern, but not eastern, Amazonia (Oliveira et al., 2020).

## Preservation of pyrogenic carbon, fire regime, and regional and global estimates

We hypothesized that soil PyC stocks would be higher in drier forests. On average, soil PyC content across the seasonally dry and fire-prone ZOT was similar to that found in more humid regions of Amazonia (Koele et al., 2017). This suggests that historical fire regimes may have not differed substantially from other regions of Amazonia. Alternatively, historical fire may have differed but the mechanisms that produced and preserved PyC may have differed, e.g., there could have been differences in the degree of recalcitrance of PyC formed during burning, which may have affected preservation potential, or differences in past climate may have affected either the incidence of fire or the preservation of PyC in the soil. For example, in biochar (purposefully carbonized biomass for agricultural use), the recalcitrance or resistance to





decomposition is related to the production temperature (Zhao et al., 2013).

The fraction of PyC in soils varies globally and with vegetation type and historic land-use. A study evaluating PyC in ADE and non-ADE forests in central and eastern Amazonia found that PyC represented 35% of the SOC in ADE soils and 14% in adjacent Oxisol (Glaser et al., 2001). A literature-based inventory and on a wider scale indicates that PyC may

represent up to 50% of total SOC (Reisser et al., 2016). High amounts of PyC (20%) as a fraction of total SOC were observed in Australian soils (Lehmann et al., 2008). As expected, we observed the highest values of soil PyC in ADE plots in our study, representing 5.07% (see [Supplementary Table 1](#)) of the total SOC. However, since ADE likely only covers a small fraction of the total area of Amazonia (~3%, McMichael et al., 2014), the higher PyC content of ADE soils would not

TABLE 3 Models with and without ADE to estimate soil PyC.

Models with ADE	Statistic ( <i>F</i> )	<i>P</i>	df	<i>R</i> <sup>2</sup> of the model
Pure climate	1.50	0.27	3	0.23
Pure environmental	0.16	0.85	2	0.05
Pure edaphic	1.22	0.32	2	0.14
Models without ADE				
Pure climate	0.99	0.46	3	0.17
Pure environmental	0.88	0.45	2	0.15
Pure edaphic	2.07	0.20	2	0.17

Partial regression models for different groups of explanatory variables sampled along with the Amazon-Cerrado transition. Pure climatic fraction (precipitation + MCWD + ATWQ = Average temperature of the warmest quarter); pure environmental fraction (AGBs + distance); pure edaphic fraction (sand + silt). Collinearity values can be found in the [Supplementary Table 3](#).

substantially increase SOC stocks across Amazonian soils, which are dominated by lower PyC soil contents. That soil PyC stocks were not substantially higher in the ZOT will be important to consider when modeling PyC and estimating the effects of climate change on SOC.

Increases in global temperature are significantly and positively correlated with changes in soil respiration (Bond-Lamberty and Thomson, 2010). There is a forecast for an increase in global temperature of up to 3°C (IPCC, 2018) and therefore also in soil carbon emissions, mainly caused by increased microbial activity, decomposition of organic matter, and root respiration (Davidson et al., 2006; Lehmann et al., 2008). There is evidence PyC is resistant to decomposition (Maestrini et al., 2015), with turnover times on the order of 1,300–2,600 years (Lehmann et al., 2008; Jones et al., 2019), and with models showing the presence of PyC in soils buffering losses of SOC under future climate warming. While PyC storage has been shown to decrease in warmer climates, no relationship was found between temperature and PyC mineralization across a climatic gradient of mean annual temperatures of 3.9–17.2, suggesting that warmer or cooler periods may have little impact on the stability of PyC currently in soils (Cheng et al., 2008). Egli et al. (2004) observed in a controlled laboratory experiment that increasing temperature reduces the amount of SOC storage. Despite this, the effects of increasing global temperature on the PyC stock in tropical forest soils remains uncertain.

## Pyrogenic carbon and ancient fire

In the ever wet forests of the Amazon, PyC in the soil usually results from ancient fires, both natural and those caused by people from pre-Columbian civilizations (Masiello, 2004; Lehmann et al., 2008; Goulart et al., 2017; McMichael and Bush, 2019; Feldpausch et al., 2022), as inputs from aerosols are relatively small (Coppola et al., 2019). There is evidence for ancient and frequent fires in closed canopy forest in

Amazonia (Hammond et al., 2007), especially during the Middle Holocene (Mayle and Power, 2008), with historical fire return intervals of ~450 years (Feldpausch et al., 2022). However, as there was an advance of the Amazon transitional forests over savanna formations in more recent times (Mayle et al., 2000, 2004; Marimon et al., 2006; Mayle and Power, 2008; Morandi et al., 2015), our plots that are currently forested could have previously supported savanna vegetation with lower biomass (Marimon et al., 2006). Therefore the rate of conversion of biomass to soil PyC may have been lower, resulting in only a moderate amount of PyC delivered to upper soil as observed in the present study. Furthermore, fire severity and fuel conversion efficiency likely varied greatly, as they do today. During modern fires, the conversion of forest AGB to PyC during deforestation varies between 3 and 10% (Fearnside et al., 1993; Preston and Schmidt, 2006).

## Predictors of pyrogenic carbon

Sand content (negative effect) was selected as the main predictors of PyC in non-ADE soils. It is possible that greater aeration of soils with a more sandy texture may have been responsible for the loss of PyC (Glaser et al., 2001) and resulting in lower soil PyC. More PyC may have also been illuviated downward through coarse-grained soils (Major et al., 2010). The model including the ADE soils indicated clay as one of the main predictors, which is inversely related to sand content (Supplementary material 3). We expect that the higher clay content explains part of the PyC variation in the soil due to the greater capacity of clay-rich soils to form organo-mineral compounds, together with organic matter, capable of protecting the charcoal from decomposition (Glaser et al., 2003). Another factor was the tendency of forests with higher AGB values (selected by the best model when including ADE sites) to explain part of this variation. It is predicted that increased aboveground biomass (Preston and Schmidt, 2006) leads to a larger amount of charcoal that can be formed during burning. Alternatively, greater biomass may be due to greater soil fertility in soils with high PyC, e.g., for forests growing on ADE soils in southern Amazonia, biomass is higher than the surrounding forests (Oliveira et al., 2020).

The selection of pure edaphic (texture) variables to explain the amount of soil PyC may have been due to the subtle edaphic differences along this extensive ecological zone of tension (Ab'Saber, 2002; Reatto et al., 2008; Silva et al., 2008; Quesada et al., 2011). However recent studies have shown that the extent and frequency of drought events in Amazonia have increased (Marengo et al., 2011), reducing tree growth and increasing mortality (Feldpausch et al., 2016), which may affect fire fuel loads. Models suggest that dry season stress will increase across the ZOT and that future climate may be more seasonal but still capable

of supporting forests rather than becoming savanna-like (Malhi et al., 2009), which may affect biomass storage, fire regimes, and PyC stocks.

## Pyrogenic carbon and distance to Cerrado

Contrary to expectations, we did not find higher soil PyC stocks in areas closer to the Cerrado biome. Evidence shows that when a fire reaches forests geographically close to savanna environments, many tree individuals die and allow light to enter, changing the microclimate (Balch et al., 2008) and favoring the entry of grasses (Silvério et al., 2013). Thus, the chance of new fires also increases, especially in warmer and drier years (Zarin et al., 2005), reinforcing a positive fire feedback and thereby potentially increasing the soil PyC stock over time. Whether more PyC is formed would also depend on fuel loads, fuel availability (moisture content), and fire behavior. A possible explanation for the absence of an effect of distance to savanna may lie in the soil texture, as, in the more sandy soils characteristic of the region closer to the savanna, PyC is more readily oxidized (Lima, 2014) or leached (Abney and Berhe, 2018) with losses from the surface through deeper translocation (Major et al., 2010). It may be that the modern transitional forest sites had Cerrado on them in the past and retain the PyC from those times (Marimon et al., 2006; Wright et al., 2020). Furthermore, biochar (PyC type) in the soil can also be transformed through the action of enzymes of microorganisms and environmental factors such as temperature (Madari et al., 2009). As a result, PyC may be chemically altered over time and thus not detected in the analytical window used by hydrogen pyrolysis.

## Limitations and future research

Geographically, our study sites cover an extensive part of the Forest-Cerrado ZOT, with a focus on closed-canopy, but transitional, forests; however, there are other forest types throughout the region (e.g., *cerradão* and patches of *cerrado* within a forest matrix; Marimon-Junior and Haridasan, 2005) that may have different PyC stocks and drivers of soil PyC distribution. Because we made use of existing plots, we were unable to use predefined points to systematically assess the effect of distance from the *cerrado* on soil PyC. The proportion of variance (residuals) unexplained by our models was relatively high, highlighting the importance of including new areas to fully explain the likely variation that exists across the expansive ZOT region. The ZOT forests, also known as the 'Arc of

Deforestation,' are threatened by the rapidly advancing agricultural frontier (Nogueira et al., 2007), placing the SOC stocks in these forests at risk. Continued research is needed to evaluate how SOC fractions, including PyC, will respond to climate change and to the landscape-scale changes in land-use.

## Conclusion

This study shows that there is only a moderate amount of PyC (~ 4% of total SOC) in the forest soils in the southern Amazonian ZOT. A much greater fraction of soil PyC in the ADE plots reinforces the importance of long-term conservation of ADE areas as a recalcitrant carbon stock. Soil PyC stocks in non-ADE forest plots are largely driven by edaphic properties. A sharp reduction in soil PyC in sandier soils and higher PyC in clayey soils highlights the physicochemical role of soil in the mineralization or the protection of soil PyC and its loss from the upper soil by illuviation to deeper soil levels. Biomass combined with edaphic variables explained part of the soil PyC variation, but only when including ADE forests. It will be important to include new forest types and soil samples at greater depths to more fully understand the variation observed in this study. Our surprising findings that soil PyC stocks are no greater in the ZOT than in the wetter regions of Amazonia, and that PyC stocks are not greater near the fire-dependent *cerrado*, require further investigation to understand variation in past fire history and factors that affect PyC formation and loss. This study improves understanding of the drivers of PyC across the ZOT, providing important new information to manage and conserve soils and to improve models to assess the impact of climate change on SOC.

## Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author/s.

## Author contributions

EO, TF, BM, and BM-J designed the study. EO analyzed the data. EO, AM, CAQ, LA, and PM analyzed the sampled soil. MB analyzed the soil PyC. CAQ analyzed the soil physicochemical properties. BM, PM, LA, OLP and AM contributed to the plot vegetation data. EO wrote the manuscript with TF, BM, and BM-J and with input from all co-authors. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/ffgc.2022.880963/full#supplementary-material>

### SUPPLEMENTARY FIGURE 1

Test of the assumptions of the multiple regression model for the variables with Amazon Dark Earth forests sampled along with the Amazon-Cerrado transition.

### SUPPLEMENTARY FIGURE 2

Test of the assumptions of the multiple regression model for the variables without Amazon Dark Earth forests sampled along with the Amazon-Cerrado transition.



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