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Increasing complexity of agroforestry systems benefits nutrient cycling and mineral-associated organic carbon storage, in south-eastern Brazil

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ABSTRACT

Agroforestry systems are often promoted as solutions to address land degradation and climate change. However, agroforestry is an umbrella term for a large variety of systems and it is not clear how their degree of complexity influences their provision of soil-based ecosystem services, such as soil organic carbon (SOC) storage and nutrient cycling. Furthermore, a knowledge gap remains whether agroforestry systems perform equally well on all soil types. The objectives of this study were 1) to assess the links between agroforestry complexity, nutrient cycling and SOC fractions, and 2) to assess how soil texture influences these relationships in Brazilian agroforestry systems. We sampled 59 agroforestry plots across 30 sites in Sao Paulo state, Brazil, and 8 monocrop sites (6 pastures and 2 crop monocultures). The 38 sites represented a soil textural gradient, ranging from very sandy to very clayey (clay content range 25 – 620 g kg^{-1}). An Agroforestry Complexity Index (ACI) was defined based on tree species richness, stem density and pruning management. Nutrient (N, P, K, Ca, Mg) and C contents were determined in litter and soil (0–30 cm depth) samples, and mineral-associated organic C (MAOC) and particulate organic C (POC) in soil samples were assessed as well. ACI was positively associated with C, N, P, Ca and Mg stocks in litter, and these litter nutrients were in turn positively associated with the corresponding soil nutrient stocks. Associations between soil nutrients and MAOC were stronger on sandy soils than on clayey soils, particularly for P, Ca and Cation Exchange Capacity (CEC). For POC, robust relationships with nutrients were only found on sandy soils. Structural Equation Models indicated causal relationships between agroforestry complexity, P and Ca cycling, and MAOC and POC stocks in topsoils. Our results indicate that nutrients effectively cycle from *in situ* mulch into plant-available soil pools and highlight the synergies between nutrient cycling and stable C stocks that can be achieved in complex agroforestry systems. These synergies seem to be particularly strong on sandy soils (<15 % clay).

1. Introduction

Climate change and soil degradation are becoming increasingly urgent problems for tropical agriculture (UNCCD, 2022). Land use change from native forests to agriculture causes carbon (C) losses to the atmosphere, both from aboveground and soil organic C (SOC) stocks (Don et al., 2011; Shukla et al., 2019). Farmers and policy makers have committed to restore SOC stocks in soils to mitigate climate change and for this, scientific recommendations are needed on what agricultural practices have potential to do so (Paustian et al., 2016; Sanderman et al., 2017). However, also nutrient cycles in tropical soils quickly diminish with land use change to agricultural production (Metcalfe et al., 2014). To circumvent this problem, farmers apply large amounts of mineral fertilizers to overcome inherently low soil fertility associated with deeply weathered tropical soils, whose mineral composition often induces a high fixation capacity of nutrients such as soil phosphorus (P)

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(Roy et al., 2016). To overcome the high nutrient fixation capacity, in countries such as Brazil, farmers have been applying inorganic P at rates twice the demand of crops since the 1970's, leading Withers et al. (2018) to propose that a redesign of Brazilian farming systems is needed to make better use of secondary (e.g. organic) sources of P. Hence, a major challenge for tropical agriculture in countries like Brazil is to find solutions that can restore SOC stocks and simultaneously benefit soil fertility.

Agroforestry systems are promoted as solutions that address both climate change mitigation and nutrient cycling (Cardinael et al., 2021; FAO, 2017). Agroforestry is an umbrella term for systems that integrate crops and/or animals with trees, and as such embrace a great diversity of traditional and modern systems (Nair et al., 2021; Wolz and DeLucia, 2018). A growing number of *meta*-analyses attest to the C sequestration potential of agroforestry in general (Beillouin et al., 2021; De Stefano and Jacobson, 2017; Hübner et al., 2021; Ma et al., 2020; Muchane et al., 2020; Shi et al., 2018). However, when comparing different types of agroforestry, *meta*-analyses often report conflicting results, e.g. Feliciano et al. (2018) reported larger SOC increases in silvopastures compared to multistrata home gardens, while Shi et al. (2018) report the opposite. In the Brazilian Atlantic Forest biome, the *meta*-analysis of Santos et al. (2019) showed that the provision of supporting ecosystem services, such as nutrient cycling, increased from monocultures to simple agroforestry systems and were highest in biodiverse agroforests. However, the provision of regulating services, such as SOC storage, was lower in simple agroforestry systems than in monocultural systems, but again, was highest in more complex, biodiverse agroforestry. Soil texture has been hypothesized to be an important variable influencing SOC storage in tropical agroforestry systems, but conclusive evidence is still lacking (Muchane et al., 2020). Hence, knowledge gaps about SOC storage and nutrient cycling in agroforestry systems remain (Lorenz and Lal, 2014; Schwarz et al., 2021).

In order to effectively mitigate climate change, it is crucial to account for the permanence of SOC stocks (Kristensen et al., 2022; Lehmann et al., 2020). To assess the stability of SOC stocks, two functionally distinct SOC pools can be defined: SOC associated with clay and fine silt particles (<53 μm), known as mineral-associated organic C (MAOC), and particulate organic C (POC; 53–2000 μm) (Cotrufo and Lavallee, 2022). Conditions for the formation of the more stable MAOC are more favourable in soils with relatively high clay contents (Georgiou et al., 2022), but also increasing molecular diversity of plant inputs to soil can enhance SOC persistence, and hence sequestration potential (Lehmann et al., 2020). Increasing SOC stocks of soils with varying textures implies different trade-offs, because clayey underutilized pastures in the tropics might have the highest SOC sequestration potential (Mitchell et al., 2021), whereas on sandy soils SOC accrual might be lower and less permanent (Lugato et al., 2021). However, sandy soils might benefit most in terms of soil fertility from increasing C inputs, creating co-benefits for climate change mitigation and agricultural production and reducing trade-offs (Moinet et al., 2023). Furthermore, nutrient inputs also play a role for SOC management, as Spohn (2020) proposes that to facilitate SOC sequestration increased P inputs may also be required. It is therefore pertinent to gain more knowledge on how promising agricultural solutions, such as agroforestry, perform on varying soil textures in terms of SOC storage and nutrient cycling and to assess whether synergies or trade-offs exist between these two ecosystem services.

1.1. Objectives

Brazil is an agricultural producer of global importance and has committed to reducing external fertilizer dependency and stepping up climate change mitigation efforts (MAPA, 2021). The country is also home to a growing number of agroforestry systems, with an increase in the area under agroforestry of 4 million ha from 2012 to 2017 (Gori Maia et al., 2021). These agroforestry systems represent a complexity gradient, spanning from relatively simple silvopastoral systems to highly biodiverse agroforests (Schuler et al., 2022). Hence, in this observational study we aimed to better understand how the variation in agroforestry complexity relates to SOC storage and nutrient cycling in the topsoil and whether these two ecosystem services are linked. We hypothesized that nutrient inputs through litter would increase in more complex systems, and that this in turn would positively affect MAOC and POC stocks. A minor objective was to assess whether links between SOC storage and nutrient cycling in agroforestry systems are influenced by soil texture.

2. Methods

2.1. Agroforestry in Sao Paulo state, Brazil

The Brazilian state of São Paulo, home to both the Atlantic Forest and Cerrado biomes, has one of the highest concentrations of recently established agroforestry systems (Agroicone, 2022; MapSAF, 2022). Innovative farmers have been experimenting with silvopastoral systems, often by planting widely-spaced rows of eucalypt trees into pastures (de Souza Filho et al., 2021). Other farmers have integrated cover crops, service and timber trees with fruit-bearing trees such as lime or coffee (Toca, 2019), resulting in multistrata agroforestry systems. A growing number of farmers are implementing even higher levels of species diversity attempting to mimic natural successional patterns observed in secondary forests, while managing service trees with intensive pruning to generate *in situ* mulch. This has become known as syntropic or successional agroforestry (Andrade et al., 2020).

2.2. Study region and sites

The study region is a transition zone between Atlantic Forest and Cerrado biomes. Fig. 1 shows the distribution of the 38 sites in relation to the soil types found in the State of Sao Paulo, and most of the systems were located on highly weathered Ferralsols (*latossolos* in Brazilian soil classification, Rossi (2017)). The climate is classified as Cwa according to Köppen criteria with humid summers and dry winters and average annual precipitation of 1600 mm (Alvares et al., 2014).

Thirty-eight sites were purposefully selected in the central-East of São Paulo state, of which thirty were agroforestry and eight monocrop sites, consisting of pastures (6) and organically managed monocrops (2, soy-maize rotations), to represent a complexity gradient (Fig. 2). Selection criteria for agroforestry sites were based on tree species diversity, spatial structure, management and age. Detailed information on the sites (species, management, soil texture) can be found in supplementary Table 1. Mean age of the agroforestry sites at the time of sampling was 5.2 (±0.66, SE) years reflecting the relatively recent increase in adoption of innovative agroforestry systems in the State of Sao Paulo. In the *meta*-analysis of Ma et al. (2020) it was shown that tropical agroforestry systems can reach a new SOC equilibrium 5 years after land use conversion to agricultural systems. We do not assume that such equilibria have been reached in all systems, but that enough time had passed at sampling for agroforestry management effects to dominate over previous land uses. The sampled agroforestry systems are comprehensively described in Steinfeld et al. (2023).

2.3. Complexity assessment

2.3.1. Agroforestry cBackspaceComplexity Index (ACI)

To assess the complexity gradient we used three metrics that represent this complexity and are relevant for SOC storage and nutrient cycling: 1) tree species richness, 2) tree stem density and 3) pruning & mulching frequency (Table 1). Tree species richness is one of the main drivers of C accumulation in agroforestry systems (Ma et al., 2020), and is a good indicator of taxonomic diversity. Tree stem density influences SOC storage in agroforestry systems (Cardinael et al., 2018; Saha et al., 2010), and is a good indicator the of spatial structure of agroforestry

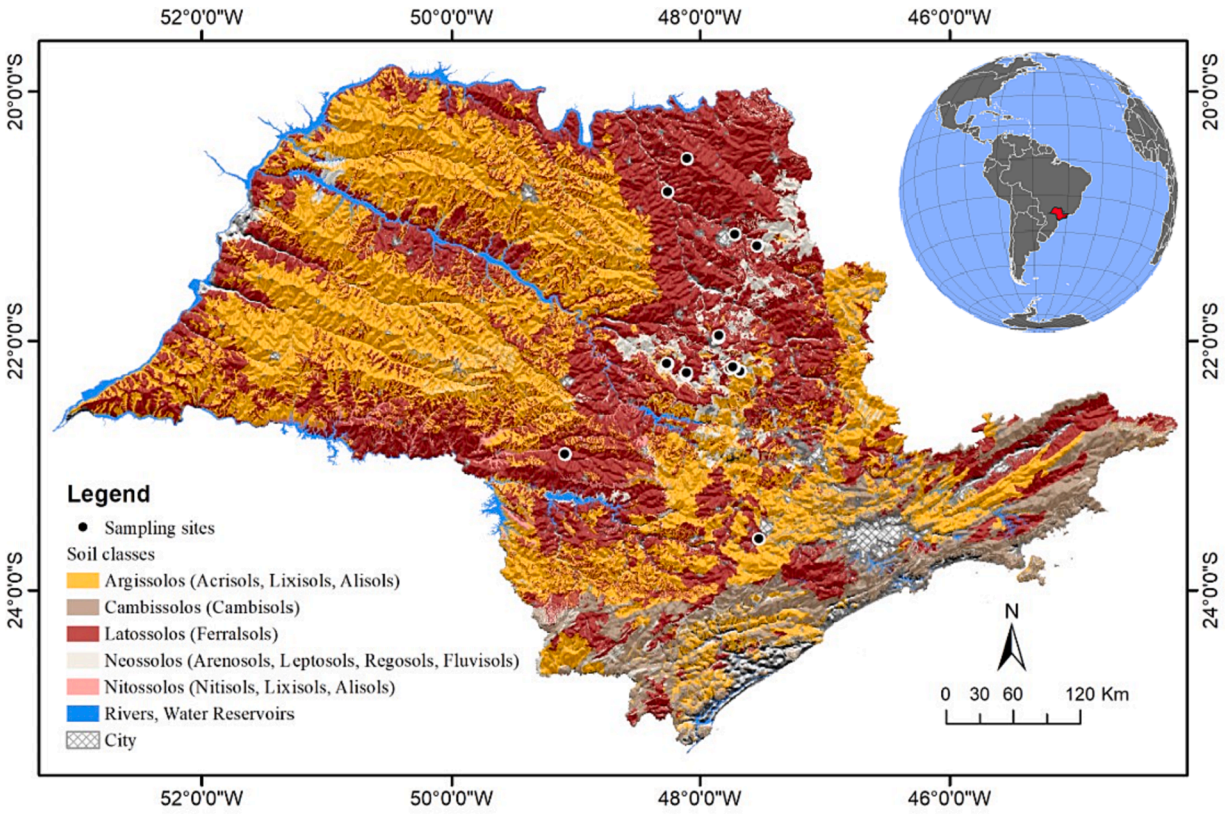

Fig. 1. Map of São Paulo state with soil types indicated according to the Brazilian classification system (WRB in brackets) and sampling sites indicated with white circled black dots. Note that due to the scale of the map one dot might represent several sites when they are relatively close to each other.

Fig. 2. Complexity gradient among sampled agroforestry systems as defined by the Agroforestry Complexity Index: A: 0, B: 0.12, C: 0.28, D: 1.52, E: 1.63, F: 2.14. See text for the explanation of the index.

Table 1
Diversity, density and management metrics used to compose the agroforestry complexity index of 38 systems, of which thirty were agroforestry systems, six were pastures and two organically managed monocrops (maize - soy rotation).

Complexity dimension	Agroforestry complexity metric	Unit	Mean	Min	Max
Taxonomic diversity	Tree species richness	Number of species ($plot^{-1}$)	5.6	0	16
Spatial structure	Stem density	Woody stems (ha^{-1})	1394	0	8356
Management	Pruning & mulching	Frequency ($year^{-1}$)	0.6	0	2

systems. Lastly, shade tree management influences SOC storage and nutrient cycling (Cardinael et al., 2021; Tscharntke et al., 2011) and a large share of the farmers participating in our study managed trees by intensive pruning & mulching (also known as chop & drop, Young, 2017).This practice has been shown to positively influence C cycling in long-term experiments (Schneidewind et al., 2019) as well as other agroforestry systems in the state of São Paulo, Brazil (Cezar et al., 2015; Froufe et al., 2020). Therefore, the frequency of pruning & mulching was included in our complexity assessment to represent its management dimension.

Data collection to quantify the complexity of the agroforestry sites is described in detail in Steinfeld et al. (2023). In short, a tree species inventory was conducted in three tree rows on a total of at least 75 individual trees per site (Fig. 3). Stem density was determined by counting the tree stems in each of the sampling plots and applying appropriate expansion factors to calculate to a per hectare basis. Data on the frequency of pruning & mulching per year was collected in a questionnaire from the managing farmers.

The Agroforestry Complexity Index (ACI) was derived by min–max transforming the values of tree species richness, stem density and pruning & mulching frequency into a value between 0 and 1, and adding

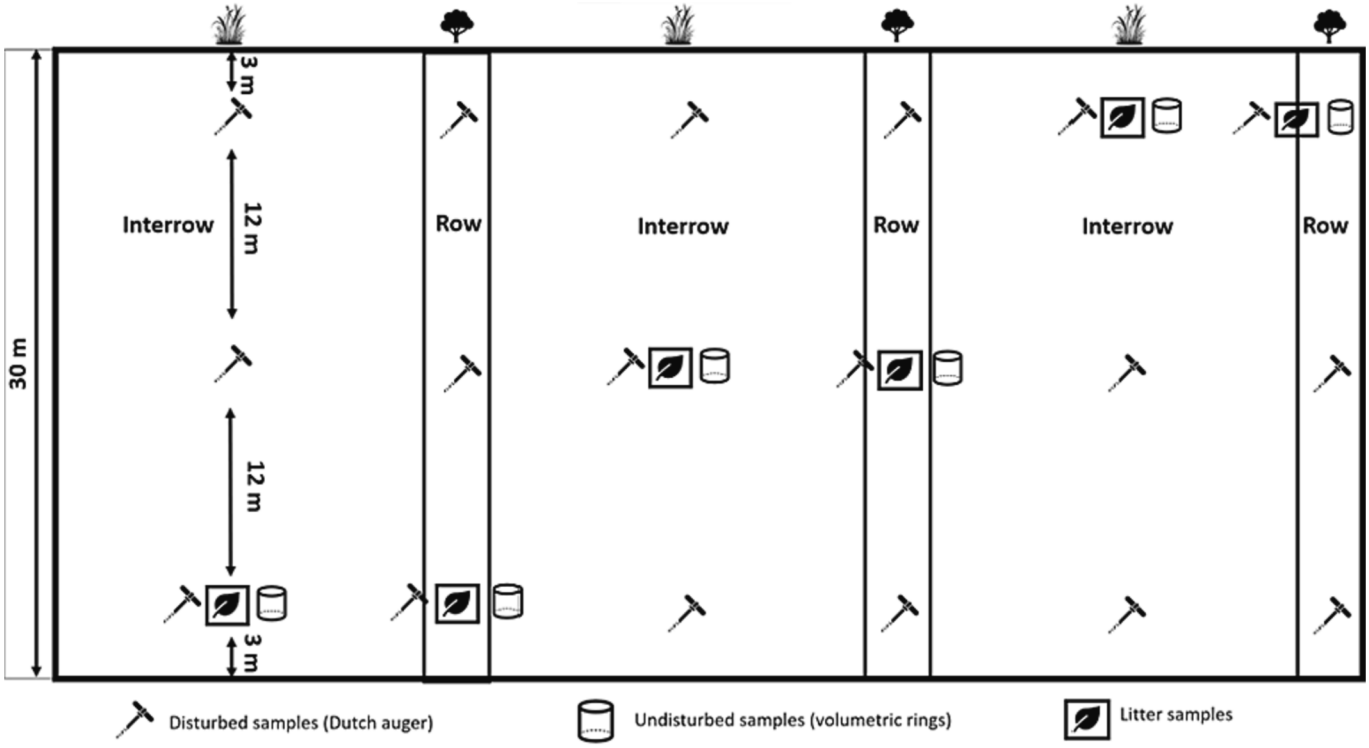

Fig. 3. Schematic representation of sampling design applied in the agroforestry plots. Tree rows (rows) and spaces between tree rows (interrows) constituted subplots. Disturbed soil samples (0–10, 10–20, 20–30 cm depth) were collected at 18 points (9 from rows, 9 from interrows) and agglomerated into one composite sample per depth increment and subplot. Undisturbed samples were collected in volumetric rings at three corresponding depth intervals in three rows and three adjacent interrows. Litter samples were collected using a 0.5 x 05 m frame on the same locations as undisturbed samples prior to opening the soil pit.

these up. The ACI values therefore range between 0 and 3. This approach has been applied in several other similar indices (Blüthgen et al., 2012; Bondi et al., 2021; Cerda et al., 2017; Mas and Dietsch, 2003) to ensure that the component scores received an equal weight.

2.4. Litter sampling and nutrient analysis

Litter was collected from the surface of the mineral soil layer using a 0.5 x 0.5 m quadrant, in both tree rows and interrows in each of the transects in the plot. This resulted in three row and three interrow samples per system (Fig. 3). Leaf litter and woody branches < 2 cm in diameter were collected, dried at 70° C for 48 h, weighted and finely ground in the laboratory for further chemical analysis. Subsamples from this ground material were then used to determine fine litter C content by combustion in a muffle oven, nitrogen (N) concentration via the Kjeldahl method and P concentrations using the Vanadomolybdate method with determination via spectroscopy. Potassium (K) was analysed via flame photometry and calcium (Ca) and magnesium (Mg) were extracted with HCl and determined via atomic absorption spectroscopy. All litter nutrient analyses were carried out at the commercial lab of the University of São Paulo/ESALQ campus and procedures are detailed in MAPA (2017). Nutrient concentrations were multiplied by the dry weight of the sample and converted to kg ha^{-1}. The reported stocks per system are averages of three row and three interrow samples, which were weighted based on the area they covered in the sampling plots.

Deadwood C sampling was conducted in the same sampling points following Pearson et al. (2005) by measuring diameter and length of deadwood logs. Samples were not taken to the laboratory and an intermediate density class of 0.349 g cm^{-3} was applied for deadwood biomass estimation (Clark et al., 2002). A deadwood C fraction of 0.47 g cm^{-3} was applied according to Martin et al. (2021) to estimate deadwood C stocks. These estimated deadwood C stocks were combined with C stocks sampled from fine litter.

2.5. Soil sampling

Disturbed soil samples were taken at 18 (9 row, 9 interrow) points in each sampling plot at three depth intervals (0–10, 10–20, 20–30 cm) using a Dutch auger and agglomerated into composite row and interrow samples for each depth, resulting in 6 composite samples per system (3 row and 3 interrow; Fig. 3). Undisturbed soil samples were retrieved using volumetric rings at six (3 row, 3 interrow) points in each plot at the same depth intervals as the disturbed samples, resulting in 18 samples per site (Fig. 3). Disturbed samples were dried and ground (2 mm) for chemical analysis.

2.6. Soil texture

Soil texture was determined using the Buyocous (densimeter) method (Dane and Topp, 2020). Soil textural classes were defined based on clay content as sandy ($<$150 clay g kg^{-1}), loamy (150–320 clay g kg^{-1}) and clayey ($>$320 clay g kg^{-1}) (Muchane et al., 2020; Shirazi and Boersma, 1984).

2.7. Soil nutrients

Total N was determined using the Kjeldahl method. P was extracted using ion exchange resin and determined via colorimetry at 725 nm wavelength. K, Ca and Mg were extracted using respective ion exchange resins and determined via atomic absorption spectroscopy. Al was determined by titration with KCl 1 mol/L and potential toxic acidity (H + Al) using a SMP buffer solution. Cation Exchange Capacity (CEC) was determined by adding the sum of bases with H + Al. All analyses were carried out at the University of São Paulo/ESALQ campus' commercial lab and procedures are detailed in van Raij et al. (2001).

2.8. Soil C analysis and physical fractionation

Soil physical fractionation to obtain MAOC and POC fractions was carried out according to the method of Cotrufo et al. (2019) and adopted from Cambardella and Elliott (1992), where soil was fractionated by size (53 μm) after full dispersion using dilute sodium hexametaphospate (0.5 %) and glass beads in a horizontal shaker for 16 h (140 rpm). Soil was rinsed through a sieve (53 μm), where soil that remained on the surface of the sieve was collected as POC, and soil that passed through was collected as MAOC. Both fractions were dried at 60 °C and subsequently C and N were determined via dry combustion using a LECO TruSpec CN (LECO Corporation, St. Joseph, MI, USA). N of the POC fraction was close to or below detectable levels (80 ppm for N, 50 ppm for C) for a large share of samples, and is therefore not reported.

2.9. Conversion to equivalent soil Mass (ESM) for C stocks

In order to account for differences in bulk densities between sites, equivalent soil layers (0–10, 10–20, 20–30 cm) were calculated using the field measured bulk density (Ellert and Bettany, 1995; Locatelli et al., 2022). This Equivalent Soil Mass (ESM) method is highly recommended over the Fixed Depth (FD) method where differences in bulk densities are not accounted for (von Haden et al., 2020; Wendt and Hauser, 2013). As samples represent a very large textural gradient (clay content range: 25 – 620 g kg^{-1}) we did not apply a single bulk density reference value for all samples, but a reference per previously defined soil textural classes of sandy, loamy and clayey (Heuscher et al., 2005; Manrique and Jones, 1991). Mean bulk density per textural class was used as reference value to calculate equivalent soil layers. Finally, C stocks were derived by multiplying the measured bulk density with the calculated equivalent soil layer. Overall, ESM and FD C stocks did not differ statistically, and we used ESM for further data analysis.

2.10. Statistical analysis

Our dataset consisted of 38 sites which were aggregated from 67 subplots (8 mono, 29 rows, 30 interrows as one agroforestry site was not planted in tree rows). Where applicable, we used the 67 subplots for data analysis and accounted for their nested structure by adding 'site' as a random factor in mixed models (Zuur et al., 2009). This was the case for analysing the relationships between litter nutrients and soil nutrients, as these datapoints came from the same subplots. The analysis was conducted using the *lme4* (Bates et al., 2015) and *lmerTest* (Kuznetsova et al., 2017) packages in R. Marginal (R^2m) and conditional (R^2c) coefficients of determination for mixed models were calculated using the *r.squaredGLMM* function based on Nakagawa et al. (2013).

Where the nested structure could not be easily accounted for, e.g. in structural equation models, we used the aggregated site dataset where all samples per site were pooled. As the ACI was determined at site level, we also used the aggregated dataset in analyses where ACI was employed as an explanatory variable (e.g. litter nutrients ~ ACI) in linear multiple regression models.

The relationships between complexity, nutrient cycling and C stocks were tested using structural equation models (SEM) and the R package *lavaan* (Rosseel, 2012). Twelve different structural equation models were developed to assess the effect of ACI on litter nutrients, soil nutrients and the final response variables, POC or MAOC stocks (Fig. 4). Clay content was also included in all models. SEMs were deemed to have good fit if the following criteria were met: Comparative Fit index (CFI) $\geq$ 0.95 (Hu and Bentler, 1999), p value (χ2) > 0.05 and standardized root mean square residual (SRMR) < 0.08 (West et al., 2012). We removed one outlier from the dataset with exceptionally high litter nutrient values as we suspected that the large amount of banana residue in it had not adequately dried, leading to inflated litter nutrient values.

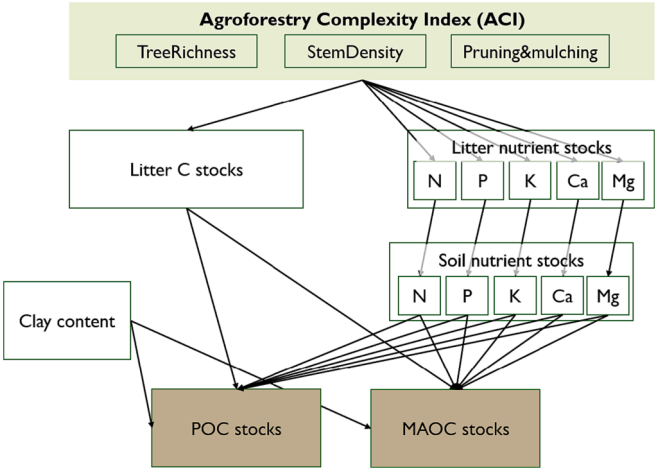

Fig. 4. Schematic representation of pathways that were tested using Structural Equation Models, linking the Agroforestry Complexity Index (ACI) with carbon stocks (MAOC and POC). Arrows indicate the 12 different path models that were tested.

3. Results

3.1. Relationships between ACI and litter nutrient stocks

The Agroforestry Complexity Index (ACI) was significantly positively associated to litter C (R^2: 0.36, $p < 0.0001$) and litter nutrient (P, N, Ca and Mg) stocks (Fig. 5). The strongest of the nutrient stock correlations was found for litter P stocks (R^2: 0.48, $p < 0.0001$), followed by litter N stocks (R^2: 0.37, $p < 0.0001$). Litter K stocks did not have a significant relationship with ACI.

3.2. Relationships between litter and soil nutrients

Linear mixed models indicated that all litter and soil nutrients (Total N, P, K, Ca, Mg) were significantly positively associated (Table 2). Clay content (g kg^{-1}) was also significantly associated to all soil nutrients, except for P.

3.3. Relationships between soil nutrients and SOC fractions

On sandy soils, MAOC stocks were strongly positively associated with total N, P, K, Ca, Mg and CEC (Fig. 6). These relationships were particularly strong for CEC (R^2: 0.94, $p < 0.0001$), Ca (R^2: 0.90, $p < 0.0001$) and P (R^2: 0.84, $p < 0.0001$), and weakest for K (R^2: 0.23, $p = 0.04$). In general, the relationships of MAOC stocks with soil nutrients and CEC were less pronounced on loamy and clayey soils (Fig. 6). MAOC was significantly related to Total N and Mg on loamy soils, and to K (R^2: 0.65, $p = 0.02$) and Mg on clayey soils.

POC stocks showed a similar pattern as MAOC, with strong positive associations with soil nutrients on sandy soils, and much less so on loamy and clayey soils (Fig. 7). On sandy soils, POC was most strongly related to P (R^2: 0.80, $p < 0.0001$), Mg (R^2: 0.67, $p < 0.0001$) and CEC (R^2: 0.63, $p < 0.0001$).

3.4. Testing links between ACI, litter nutrients, soil nutrients and C fractions using structural equation models

A series of SEMs tested the effect of ACI on litter C stocks and, in turn, on POC and MAOC stocks (Fig. 8), and the effect of ACI on the five nutrients under consideration (N, P, K, Ca, Mg) and POC and MAOC stocks (Fig. 9). The models linking ACI, litter (fine + deadwood) C stocks and POC and MAOC stocks, respectively, were highly consistent with the data and both had a CFI of 1.0 (Fig. 8). The ACI also linked litter P stocks,

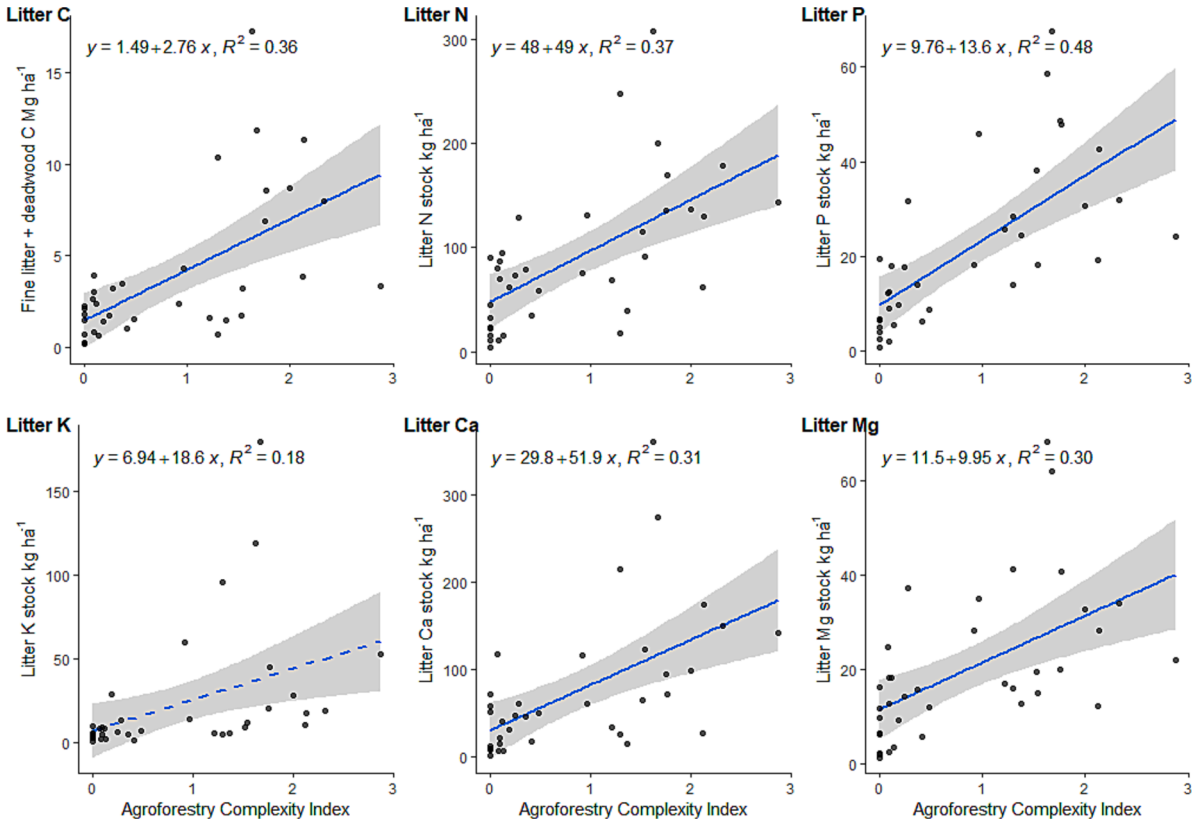

Fig. 5. Linear relationships between litter C and nutrients stocks (kg ha^{-1}) and the Agroforestry Complexity Index (ACI). Solid and dashed lines indicate significant ($p < 0.05$ level) and non-significant relationships ($p > 0.05$).

Table 2
Output of linear mixed models testing the relationships between soil nutrients (response variables) and litter nutrients, clay content and their interaction (explanatory variables).

Response variable	Explanatory variables	Estimate	Pr(>|t|)	Sig	R^2 m	R^2 c
	Litter N stock	1.45E-01	0.0268	*	0.60	0.86
	Clay	7.46E-01	1.06E-08	***		
Total N	Litter N stock * Clay	−3.46E-02	0.7271			
	Litter P stock	4.25E-01	1.56E-05	***	0.21	0.69
	Clay	5.20E-02	0.689			
P	Litter P stock * Clay	5.95E-03	0.939			
	Litter K stock	3.28E-01	0.00359	**	0.53	0.87
	Clay	5.50E-01	6.29E-06	***		
K	Litter K stock * Clay	−2.68E-02	−0.446			
	Litter Ca stock	3.44E-01	0.00263	**	0.28	0.59
	Clay	2.90E-01	0.0267	*		
Ca	Litter Ca stock * Clay	6.23E-02	0.50981			
	Litter Mg stock	3.34E-01	3.49E-06	***	0.45	0.88
	Clay	5.01E-01	8.91E-05	***		
Mg	Litter Mg stock * Clay	−2.83E-02	0.592			

Data were normalized and p-values were obtained using Satterthwaite approximations. Estimates, P-values, R^2m (marginal coefficient of determination) and R^2c (conditional coefficient of determination) are shown. R^2m describes the proportion of variance explained by the fixed factors, conditional R^2c describes the variance explained by fixed and random factors combined.

soil P stocks and, in turn, MAOC and POC stocks (CFI of both 1.0; Fig. 9). Similar effects were found for the SEMs containing ACI, litter Ca, soil Ca and MAOC (CFI 0.99) and POC stocks (CFI 0.97). The SEMs tested for total N, K and Mg were not sufficiently consistent with the data to support the hypothesis that these path models reflect dominant mechanisms of SOC dynamics in the sampled systems (Fig. 9).

4. Discussion

4.1. General findings

Our findings highlight the synergies between nutrient cycling and SOC storage that can be achieved by increasing the complexity of agroforestry systems, and the importance of soil texture for these dynamics in agroforestry systems in south-eastern Brazil. Litter to soil cycling of P and Ca was important for more labile POC, as well as stabilised MAOC stocks. Fine litter + deadwood C stocks were positively related with higher stocks of POC and MAOC, however the relationship with POC was much stronger than that for MAOC. Not all agroforestry systems performed the same because their complexity (defined as the sum of the standardised species richness, stem density and pruning management) influenced the strength of the synergies between nutrient cycling and C storage. Furthermore, these systems did not perform equally well on all soil types, as sandy soils were shown to be particularly suited to achieve co-benefits of complexifying agroforestry.

4.2. Nutrient cycling

Relationships between litter nutrients and soil nutrients were strongest for P, indicating that this critical nutrient is effectively being cycled via *in situ* biomass inputs into plant-available soil pools. Effective P cycling from organic sources has been shown in previous studies (Gao et al., 2019; Malik et al., 2012; Maranguit and Kuzyakov, 2019; Richards et al., 2021; Tang et al., 2014) and has even been suggested to cycle directly from litter to forest trees (Sayer and Tanner, 2010). Soil organic

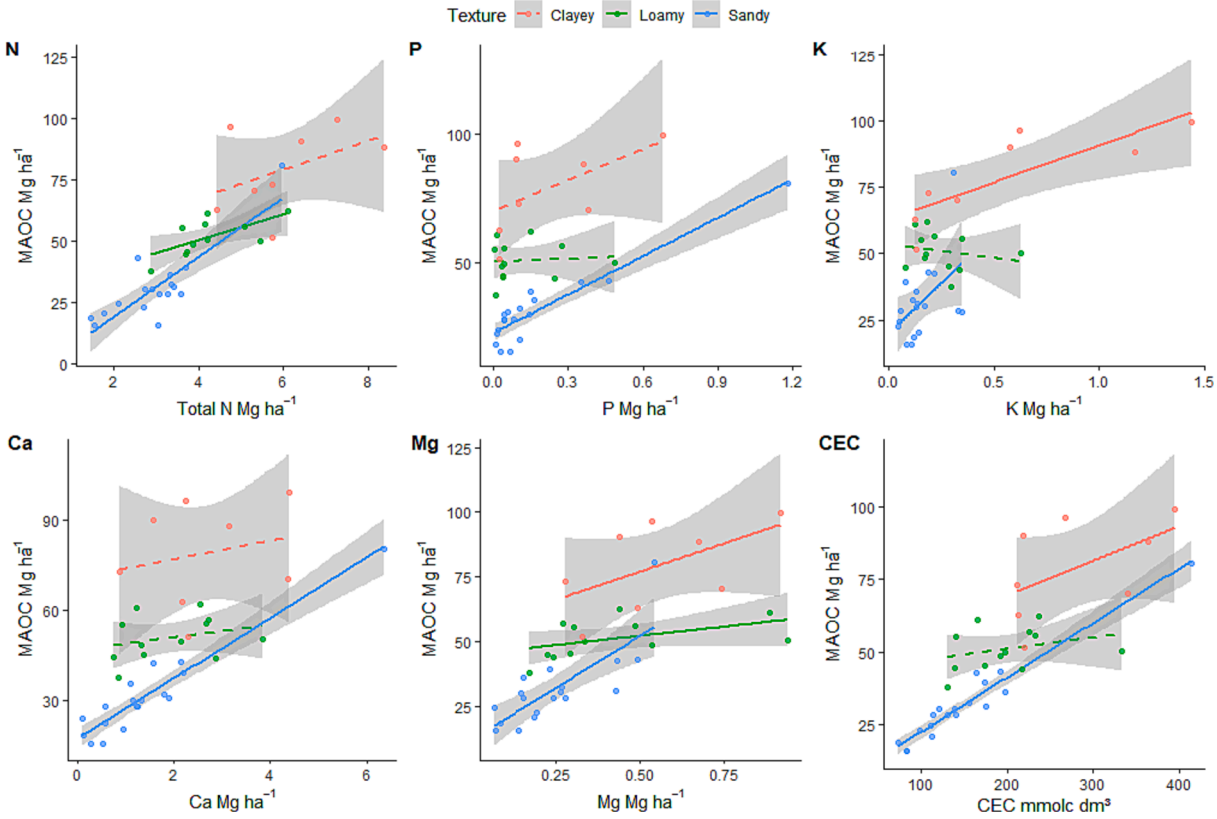

Fig. 6. Relationships between soil nutrients (Total N, P, K, Ca, Mg), Cation Exchange Capacity (CEC) and mineral-associated organic C (MAOC) stocks in the 0–30 cm soil layer. Clayey, loamy and sandy soils are indicated in blue, green and red, respectively. Solid and dashed lines indicate significant ($p < 0.05$ level) and non-significant relationships ($p > 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

P stocks were also found to be the main source of plant extractable P when chemical fertilizers were not used (Liu et al., 2018; Richards et al., 2021; Soltangheisi et al., 2018), which is the case in almost all of the systems studied here. As our dataset contains some sites ($n = 8$) on heavy clay Ferralsols known for their high P fixation capacity (Roy et al., 2016), it is somewhat unexpected that soil P was not influenced by clay content. For this subset of clayey soils, the correlation between litter P and soil P was very strong (R^2: 0.90, not reported), but the low number of sites merits caution in the interpretation of this result. Nonetheless, overall our results highlight the importance of *in situ* cycling of P from mulch biomass in tropical systems.

Soil stocks of total N, K, Ca and Mg were positively associated with litter stocks of these nutrients, as well as clay content. These findings provide evidence for the effective cycling of the full range of macronutrients from *in situ* biomass. The material collected was in varying degrees of decomposition and we did not collect freshly cut biomass, so we cannot determine how commonly applied metrics such as C/N or C/P ratios of fresh material influenced their decomposition. However, we postulate that particularly pruned residues have favourable C/nutrient ratios as these have not gone through senescence and the associated nutrient withdrawal (Noodén et al., 1988). Steinfeld et al. (2023) showed that pruning & mulching frequency had high explanatory power for litter nutrient stocks in the studied sites. Froufe et al. (2020) and Matos et al. (2020) also showed evidence of the benefits of pruning and mulching for nutrient cycling in south-eastern Brazil. We therefore propose that pruning & mulching is an effective management practice to reduce the reliance on mineral fertilizers alone for farmers in the region.

4.3. Nutrient cycling and C fractions

The structural equation models showed that P and Ca cycling from litter to soil was strongly linked to MAOC and POC in the 38 sites. The availability of Ca^{2+} has been shown to be linked with MAOC (Bai et al., 2020; Pu et al., 2021; Yu et al., 2022) because as a divalent cation it can bridge negatively charged sites in SOC and on clay mineral surfaces (Rowley et al., 2018). Humic acids have also been shown to adsorb to calcium phosphate precipitates (Alvarez et al., 2004; Cao et al., 2007; Grossl and Inskeep, 1991) potentially stabilising C through organo-organic interactions at molecular interfaces (Rowley et al., 2018). Positive interactions between SOC fractions and nutrient stocks are also likely, as the strong correlation between P and POC on sandy soils (R^2: 0.80) in this study may indicate that particulate organic matter is a relevant source of P, as suggested in previous studies in tropical soils (Damian et al., 2020; Salas et al., 2003). However, Spohn et al. (2022) suggest a trade-off between P availability and SOC storage in clayey Ferralsols similar to the clayey sites in this study, as they report desorption of SOC from mineral surfaces after adding phosphate in a lab experiment.

In the sandy sites, MAOC was positively associated with all nutrients, which was particularly evident for P (R^2: 0.84), Ca (R^2: 0.90) and CEC (R^2: 0.94). This suggests that the presence of P and/or cations is of critical importance for the stabilisation of SOC when clay content is low, e.g. through the formation of organo-mineral complexes (Kleber et al., 2015) and by enhancing the molecular diversity of substrate available to decomposers (Lehmann et al., 2020). A long-term field study in São Paulo state has shown that Ca amendments increased the relative importance of fungi in the microbial community (Bossolani et al., 2021) and particularly arbuscular mycorrhizal fungi (AMF) are known to positively associate with MAOC stocks (Averill et al., 2014; Craig et al., 2018), as well as favour nutrient cycling in agroforestry systems (Dierks et al., 2021; Dierks et al., 2022). Experimental evidence shows that on sandy soils fungi play a key role in the transformation of POC into MAOC (Witzgall et al., 2021). Thus, the synergies between nutrient cycling and SOC storage are mediated by soil texture, likely due to distinct chemical

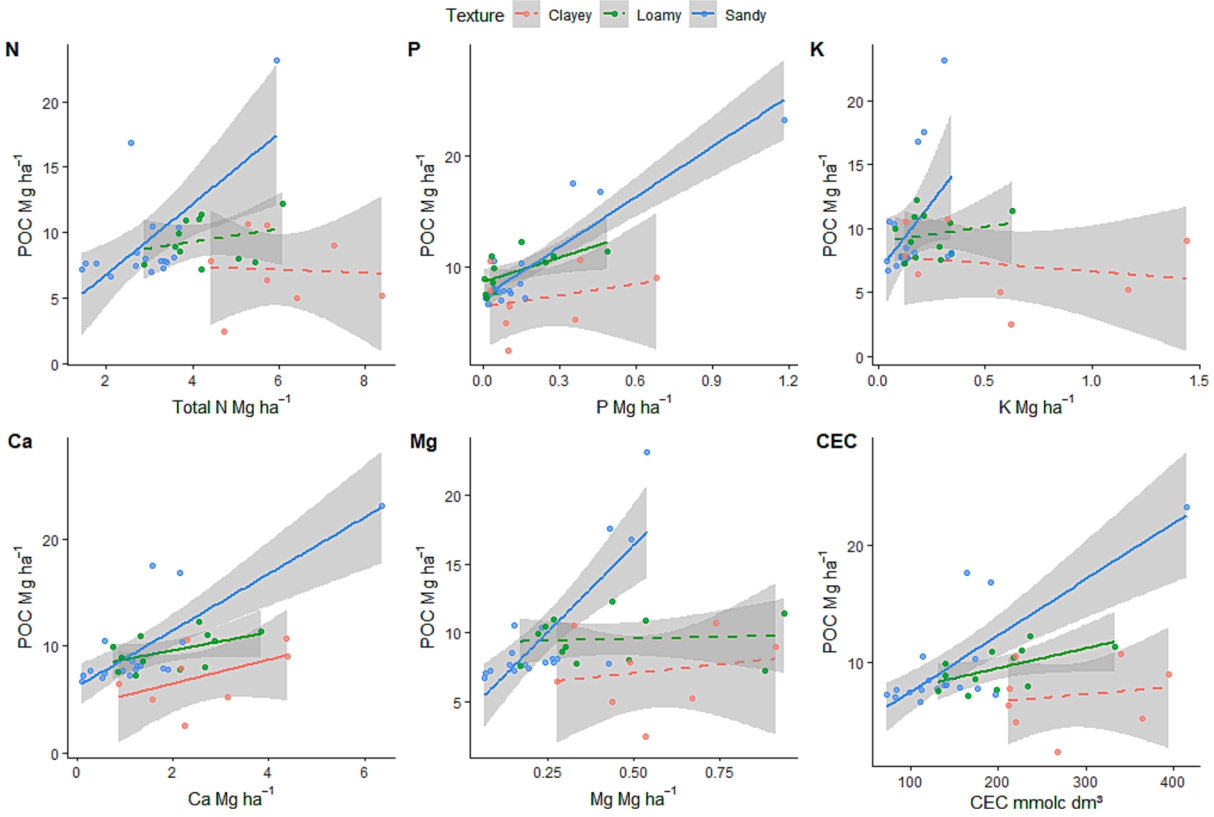

Fig. 7. Relationships between soil nutrients (Total N, P, K, Ca, Mg), Cation Exchange Capacity (CEC) and particulate organic C (POC) stocks in the 0–30 cm soil layer. Solid and dashed lines indicate significant ($p < 0.05$ level) and non-significant relationships ($p > 0.05$).

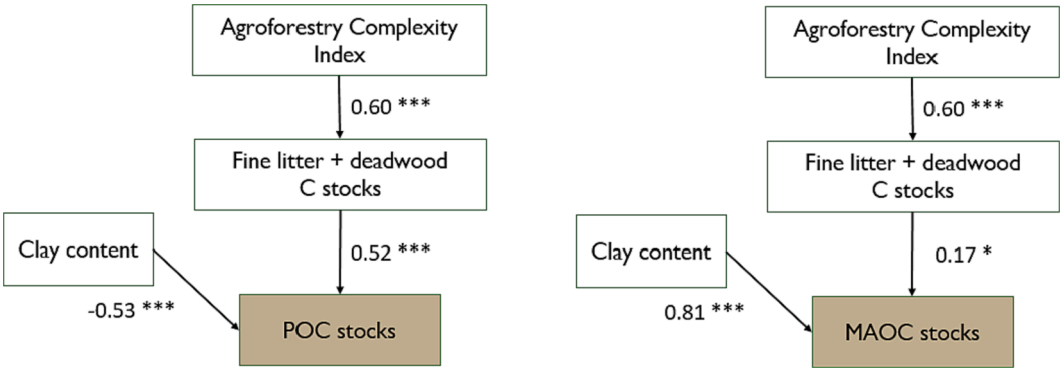

Fig. 8. Outcomes of structural equation models testing the link between agroforestry complexity, C stocks of fine litter + deadwood and POC and MAOC stocks in soil. Both models receive high support from the data (both CFI: 1.0). The coefficients from the structural equation models are displayed next to arrows and their significance levels indicated.

and biological interactions in soils with contrasting clay contents (Bacq-Labreuil et al., 2018).

4.4. *Relevance for management and policies*

Our findings allow us to provide relevant insights for farmers and policy makers in the study region, e.g. on how to manage agroforestry systems for an increased provision of ecosystem services and where to incentivise what types of agroforestry. Regarding management, our results highlight the importance of generating *in situ* mulch containing both pruned leaves and woody material. This is in line with other studies that showed the importance of this practice for nutrient cycling from leaf litter (Froufe et al., 2020; Schneidewind et al., 2019) and woody logs for soil biodiversity (Leite et al., 2023). As our results show, the combination of nutrients and C inputs from this practice stimulates the formation of stabilised SOC stocks and, therefore, agroforestry farmers can enhance nutrient cycling and SOC storage, simultaneously. An important consideration, however, is the additional labour demand that the pruning of diverse agroforestry systems causes (Esche et al., 2023).

For policy makers and investors, our findings support the hypothesis of Muchane et al. (2020) that agroforestry systems on sandy soils have higher SOC accrual than on loamy or clayey soils in tropical and subtropical climates. These results further corroborate Brazilian legislation which already recognizes sandy soils as prime areas for integrated agroforestry systems in their low carbon agriculture plan (Brazilian Ministry of Agriculture, 2021). Currently, sandy soils in the state of São Paulo are predominantly used for extensive cattle ranching (de Souza Filho et al., 2021). Therefore, better incentives to implement medium-

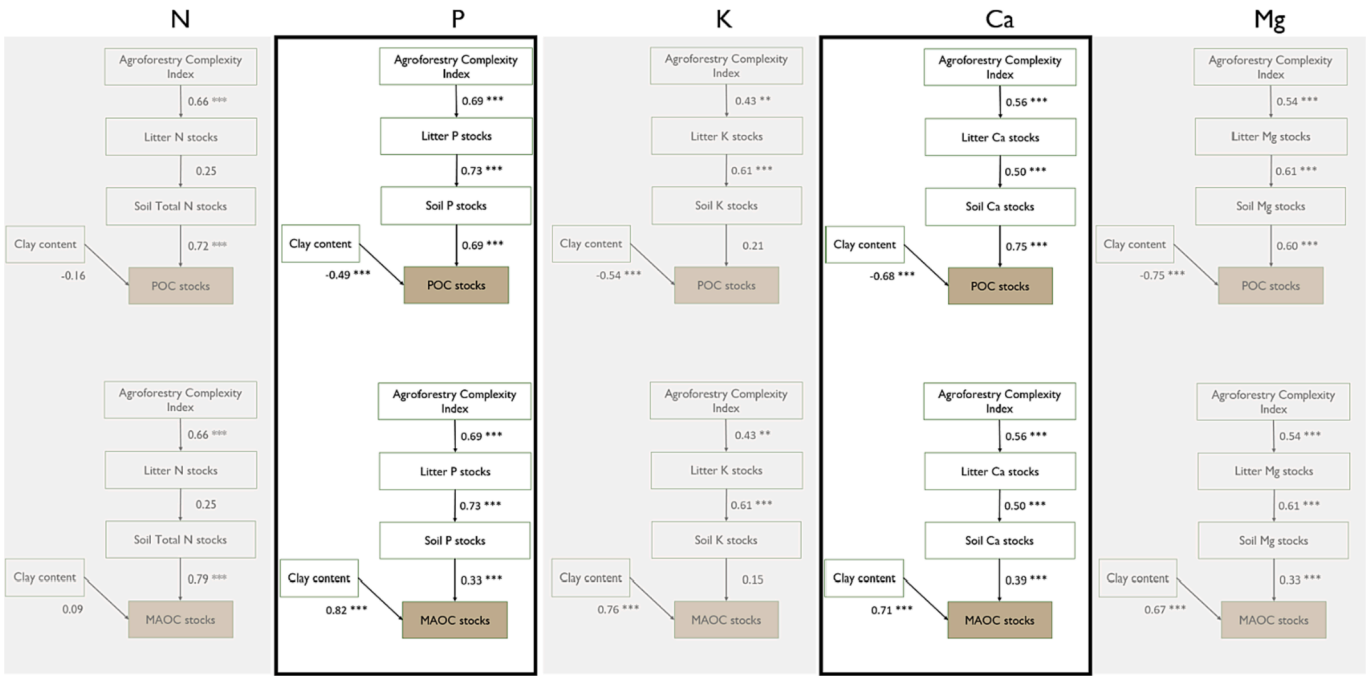

Fig. 9. Outcomes of structural equation models testing the links between agroforestry complexity, litter nutrient stocks, soil nutrients and POC and MAOC stocks. Models highlighted in black boxes receive high support from the data and non-highlighted models have goodness-of-fit measures below cut-off values. The coefficients from the structural equation models are displayed next to arrows and their significance levels indicated.

highly complex agroforestry systems in these areas are recommended. Such incentives need to include farmer training and extension, as converting pastures to complex agroforestry requires both knowledge and additional labour (Schroth et al., 2016). On clayey soils, however, the additional benefits of increasing complexity are less clear and agroforestry systems of lower complexity, such as integrated Crop-Livestock-Forestry systems, could already provide substantial ecological benefits (Bieluczyk et al., 2020; Carvalho Mendes et al., 2021) while having a less drastic, although still considerable, impact on farm reconfiguration (Gil et al., 2015). Our results can therefore be used to further refine the definition of priority areas for agroforestry implementation (de Mendonca et al., 2022).

4.5. Agroforestry complexity

It is common in studies to compare agroforestry as one generic category to contrasting land use types, such as monocultures. However, in this study we defined a continuous complexity gradient based on metrics that represent three key components: diversity, tree density and management. This approach of assessing gradients is in line with recommendations by Teixeira et al. (2022) and allowed us to reveal important nuances that would have otherwise remained hidden. Since we also included management (pruning & mulching frequency) in the definition of this gradient, we chose the term complexity instead of diversification (used e.g. in Beillouin et al., 2021; Hufnagel et al., 2020; Teixeira et al., 2022). Blaser et al. (2018) also assessed the provision of ecosystem services in relation to an agroforestry gradient which was based on shade tree cover, but did not find a positive relationship with neither soil fertility nor SOC storage. The high explanatory power that pruning & mulching frequency had on litter nutrient stocks in our sites (Steinfeld et al., 2023), and the links reported here with SOC suggest that it is an important metric to take into account (Tscharntke et al., 2011). Interactions between pruning and other attributes, such as tree species diversity, are likely but could not be thoroughly tested here as all systems that were pruned had at least moderate levels of tree diversity. Nevertheless, based on our results, we recommend study designs that incorporate gradients rather than contrasting categories, and encourage further research into the effects of using pruning residues for *in situ* mulching.

5. Conclusions

We sampled 38 sites that represent an agroforestry complexity gradient to test the relationship between complexity and the provision of nutrient cycling and SOC storage. An Agroforestry Complexity Index (ACI) was defined based on tree species richness, stem density and pruning management. Our findings highlight the synergies between nutrient cycling and SOC storage that can be achieved by increasing the complexity of agroforestry systems, and the importance of soil texture to moderate these dynamics. On sandy soils, relationships between soil nutrients and stable MAOC were strongest. Structural equation modelling indicated that P and Ca inputs from *in situ* mulching are particularly relevant for the formation of SOC stocks. Overall, our results show that complex agroforestry systems in south-eastern Brazil are suited to achieve co-benefits for soil fertility and SOC storage, especially on sandy soils.

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Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2023.116726.

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