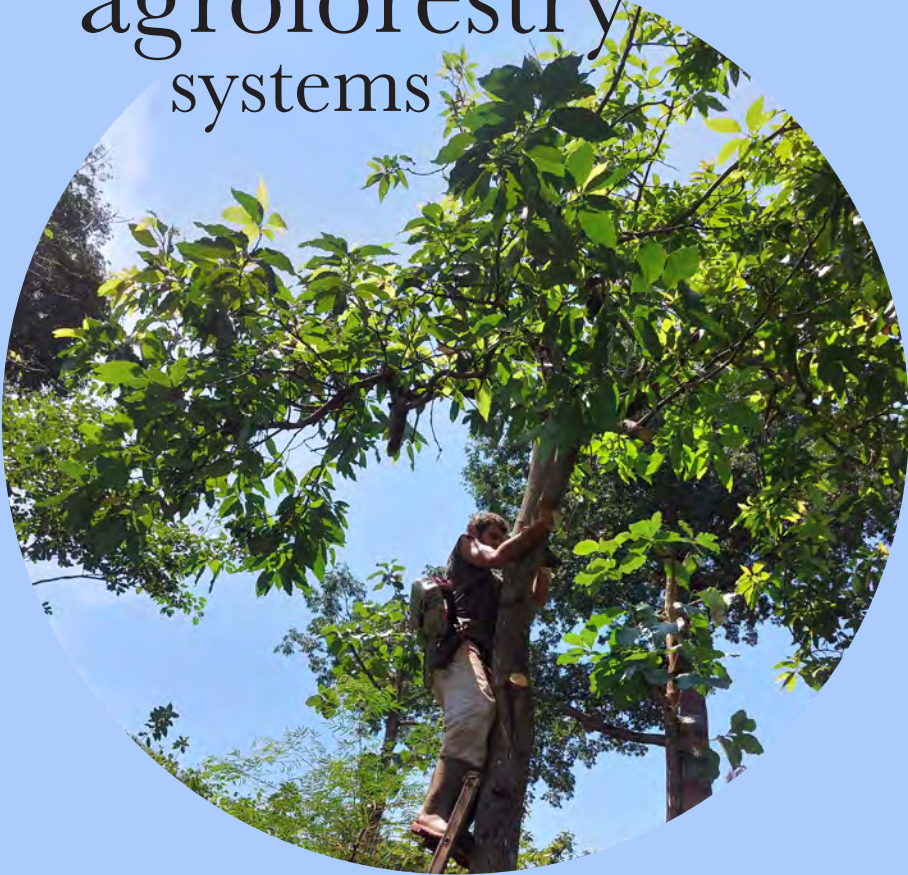


On the  
complexity of  
agroforestry  
systems



Jonas P. Steinfeld

## Propositions

1. Complexity is a fundamental but often overlooked driver of ecosystem service delivery. (this thesis)
2. Pruning and mulching is a powerful yet understudied practice to enhance ecosystem services in agroforestry systems. (this thesis)
3. The long-term trend of simplifying agricultural systems needs to be reversed to achieve food security within planetary boundaries.
4. Blaming eucalypts and other invasive species for environmental degradation equates to shooting the messenger.
5. Practicing farming should be an integral part of an agricultural scientist's daily life.
6. Rather than paying farmers for ecosystem services, polluters should pay for ecosystem dis-services.
7. There are not too many people on this planet but just too few in the countryside.

Propositions belonging to the thesis, entitled  
On the complexity of agroforestry systems  
Jonas Peter Steinfeld  
Wageningen, 20 March 2024

# On the complexity of agroforestry systems

Jonas P. Steinfeld

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Production Ecology & Resource Conservation.

# On the complexity of agroforestry systems

Jonas P. Steinfeld

## **Thesis**

submitted in fulfilment of the requirements for the degree of doctor  
at Wageningen University  
by the authority of the Rector Magnificus,  
Prof. Dr C. Kroeze,  
in the presence of the  
Thesis Committee appointed by the Academic Board  
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**Jonas Peter Steinfeld**

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*To all future agroforestry farmers*

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**Agroforestry system on Fazenda São Luiz in the municipality of São Joaquim da Barra, São Paulo, Brazil.**

Coffee (*Coffea arabica*) is grown under the shade of diverse fruit, timber and biomass trees. The farmer prunes the upper canopy trees drastically once a year.





01





CHAPTER 1

# General Introduction

01

## 1.1 Challenges for farming

**W**hat is the future of farming? There is much uncertainty around this question, but some things are known: farmers will have to produce food with less dependence on finite mineral fertilizers (Barbieri et al., 2021), while storing more soil organic carbon (SOC) in their soils to mitigate the worst of climate change and also adapt to it by improving water regulation (IPCC, 2022; Paustian et al., 2016). How to achieve this depends on who is asked, as farming will have to become more circular (De Boer and van Ittersum, 2018; Liaros, 2021), regenerative (Schreefel et al., 2020), nature-based (Lafortezza et al., 2018), climate-smart (Descheemaeker et al., 2020) or agroecological (Altieri and Nicholls, 2020). What all of these approaches have in common is that soils play a crucial role in them and that agricultural systems will have to be designed to increase soils' capacity to provide multiple ecosystem services. Designing such future-proof systems often involves adding components (e.g. diversifying crops) with the aim of strengthening their internal capacity to cycle nutrients, store SOC and water (Furey and Tilman, 2021; Jones et al., 2023).

When diverse components interact in a system and produce new properties, this system can be called “complex” (Filotas et al., 2014). Forests are often used as prime examples of complex ecosystems where the numerous interactions between plants, minerals, microbes and many other organisms result in high ecosystem functioning (Parrott, 2010), which for human societies means high provision of ecosystem services (Costanza et al., 1997; Manning et al., 2018). In forests, “undisturbed complexifying development” results in the emergence of these ecosystem services (Müller, 2005), but what about disturbed “un-natural” agricultural systems? Most agricultural management is aimed at reducing complexity, as simplified, homogenous systems can be managed with fewer people and higher economic returns to labour inputs. Would it make sense for future farmers to “complexify” their systems to enhance ecosystem services?

## 1.2 Agroforestry and its ecological benefits

Agroforestry is an umbrella term for a wide variety of traditional and modern agricultural systems that integrate trees and crops (Fig. 1.1) (Nair, 1985), and as such they are by definition more complex than monocultures (Nair et al., 2021). In ancient times, this integration of trees and crops used to be common in places as diverse as Brazil (Maezumi et al., 2018), India (Nogué et al., 2017), China (Hsiung et al., 1995) or Roman Italy (Lelle and

Gold, 1994). Since the 1970's, scientific interest in such systems has picked up due to agroforestry's potential to combine food production and the provision of ecosystem services and new, modern versions of these ancient systems have been developed by scientists and pioneering farmers (Nair et al., 2021).

The ecosystem service provision of agroforestry compared to monocultures has been extensively studied, particularly in Brazil and other tropical countries (Wolz and DeLucia, 2018). Meta-analyses have focussed on comparing SOC stocks under agroforestry and monocultures, and report positive effects ranging from 19% in a global study (Shi et al., 2018), and 21% (Kuyah et al., 2019; Muchane et al., 2020) to 26% (Chatterjee et al., 2018; Ma et al., 2020) in the tropics. Also nutrient availability of phosphorus (P) has been found to increase by 11% (Muchane et al., 2020) to 20% (Kuyah et al., 2019), as well as increases in water regulation by 20% (*idem*). Meta-analyses also attest to the positive outcomes on yields that can be achieved in agroforestry, generating win-win situations (Jezeer et al., 2017; Kuyah et al., 2019).



**Figure 1.1** | Examples of agroforestry systems from this study, representing a complexity gradient.

### 1.3 Knowledge gaps

Despite the considerable amount of research on agroforestry systems, knowledge gaps remain, e.g. on how their complexity influences the provision of ecosystem services (Schwarz et al., 2021). Many different types of agroforestry can be defined, such as silvopastures or multistrata agroforestry systems (Fig. 1.1) (Atangana et al., 2013; Schroth and do Socorro Souza da Mota, 2014). Some meta-analyses report differences in ecosystem service provision between these types (Feliciano et al., 2018; Santos et al., 2019; Shi et al., 2018), whereas another one does not (Kuyah et al., 2019). Some meta-analyses also report inconsistent findings for the same types of agroforestry systems (e.g. Feliciano et al., 2018; and Shi et al., 2018). Agroforestry types are mostly qualitatively defined (Toledo and Moguel, 2012) which makes it methodologically more difficult to establish their degree of complexity and compare it to their provision of ecosystem services. For instance, it is often not clear how agroforestry types differ in terms of taxonomic and functional diversity, structural complexity and management.

The complexity of agroforestry systems likely influences their nutrient cycling capacity which is important for productivity (Nesper et al., 2018). Nutrient dynamics in soils also play a key role in determining the permanence of SOC stocks, as e.g. N, P and Ca are

critical components of biochemical processes that lead to SOC stabilization (Rowley et al., 2017; Spohn, 2020; Tang et al., 2023). From a climate change mitigation perspective, it is important to know whether agricultural soils store SOC in physically stable forms, such as mineral-associated organic carbon (MAOC), or as more labile particulate organic carbon (POC) (Lugato et al., 2021). For farmers, it is important to know whether trade-offs or synergies exist between the two ecosystem services of nutrient cycling and long-term SOC storage (Moinet et al., 2023). However, as methodological consensus on how to assess the stability of SOC in the soil scientific community has only been reached in recent years (Cotrufo and Lavelle, 2022; Just et al., 2021), still little is known about the permanence of SOC stocks in agroforestry systems and how this links to nutrient cycling and agroforestry complexity.

Furthermore, knowledge gaps persist on the context-dependency of the benefits generated by agroforestry systems (Cardinael et al., 2018). The provision of soil-based ecosystem services is strongly related to inherent soil properties such as soil texture, e.g. soils with higher clay and silt contents have a natural advantage in storing SOC (Georgiou et al., 2022), or to storing water. The relative effect of complexity on specific ecosystem services may therefore be enhanced or diminished, depending on soil texture (Muchane et al., 2020). The effect may also vary with soil depth, or age of a system (Ma et al., 2020; Shi et al., 2018).

Next to the ecological benefits, it is crucial for farmers to know how increasing complexity impacts labour demand (Kansanga et al., 2020). It has been shown that for some agroforestry farmers keeping labour demand moderate is even more important than maximising productivity (Fujisawa et al., 2020; Scudder et al., 2022; Tilden et al., 2023) and in the future, labour availability is projected to be a major constraint for farmers (Ryan, 2023). It is often postulated that adopting more complex agroforestry systems increases labour demand (Brodt et al., 2019; Esche et al., 2022; Scudder et al., 2022; Smith et al., 2022), but labour inputs required to perform specific activities, such as weeding, might also decrease (Armengot et al., 2016). It is therefore relevant to assess the impact of agroforestry complexity on total labour demand, and also on specific labour activities.

Addressing these knowledge gaps requires data collection in agroforestry systems of varying complexity, from simple to highly complex. However, 74% of agroforestry plots on experimental farms have highly simplified designs and contain only a single tree species (Wolz and DeLucia, 2018). While more long-term research trials on more complex agroforestry systems are needed (Lovell et al., 2017), pioneering farmers who have already implemented more complex agroforestry systems represent real-life opportunities to study the effect of innovative designs and practices following an approach called 'learning from the future' (Valencia et al., 2022).

## 1.4 Challenges and agroforestry in Brazil

The challenges for farmers to produce food while increasing soil-based ecosystem services are particularly evident in Brazil. Here, farmers on sandy soils are already experiencing yield losses due to increased frequency of droughts (Rattis et al., 2021). Deeply weathered

tropical soils in Brazil also induce nutrient fixation when fertilized and farmers have responded to this by applying fertilizers such as inorganic P at unsustainable rates (Withers et al., 2018). Ferralsols, the most common soil type in Brazil (Camargo et al., 1986), have high SOC storage capacity (Fujisaki et al., 2018) but have nevertheless experienced large SOC losses in the last 200 years (Sanderman et al., 2017). These losses affect farmers in multiple ways, as SOC has also been shown to be important to achieve high yields in Ferralsols (Carvalho Mendes et al., 2021).

Brazil is also a hotspot of agroforestry development (Fig. 1.2) (Wolz and DeLucia, 2018) and most agroforestry research has focussed on the country's third largest biome, the Atlantic Forest (Schuler et al., 2022). It was shown that the provision of ecosystem services in this biome differs between simple and biodiverse agroforestry systems (Santos et al., 2019). However, particularly for Brazil's second largest biome, the *Cerrado*, Schuler et al. (2022) point out the need for more research. The state of São Paulo, in south-eastern Brazil, hosts both the Atlantic Forest and *Cerrado* biomes, and has seen an increase in new agroforestry implementation on former pastures in recent years (Agroicone, 2022) which made it an ideal region for this study.



**Figure 1.2** | Map showing agroforestry sites (bubble size refers to number of documented sites) in Brazil as registered by the *MapSaf* project during 2017 - 2022 and published on <https://mapeamentosaf.eco.br/projeto>.



## 1.5 Objectives

The overarching objectives of this thesis were to assess the potential of innovative Brazilian agroforestry systems to provide multiple soil-based ecosystem services across different contexts and to assess how their uptake can affect farm labour demand. The underlying hypothesis was that the degree of complexity of agroforestry systems is a key factor in determining their level of ecosystem services provision. Furthermore, I hypothesized that increasing complexity will also lead to increased labour demands.

## 1.6 Research questions

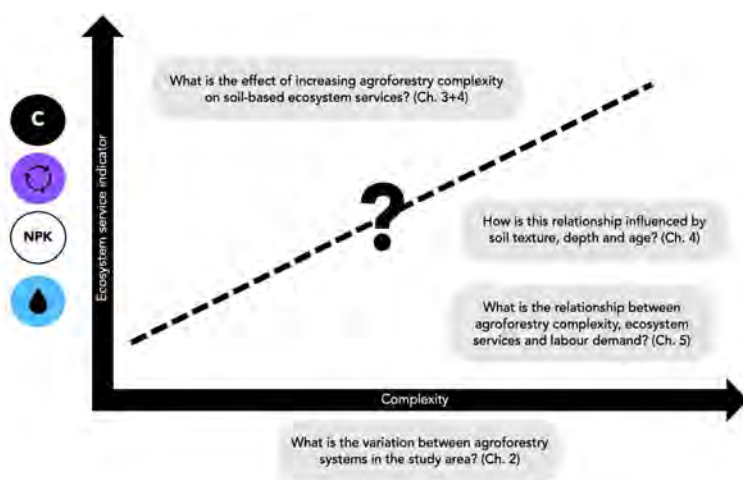
In order to address the overarching objectives, four research chapters were pursued in this thesis with their individual research questions (Fig. 1.3):

*Ch. 2) How do agroforestry systems in the study region differ in terms of taxonomic and functional diversity, spatial structure and management? How do these characteristics relate to the potential for nutrient cycling in agroforestry systems?*

*Ch. 3) How does agroforestry complexity affect nutrient cycling and SOC storage in MAOC and POC fractions? What is the relationship between nutrient cycling and SOC storage?*

*Ch. 4) What is the relationship between agroforestry complexity and the simultaneous provision of three important soil-based ecosystem services (SOC storage, nutrient provision and water regulation)? How is this relationship influenced by soil texture, depth and age of the agroforestry system?*

*Ch. 5) What is the relationship between agroforestry complexity, ecosystem services and labour demand? What is the effect of complexity on specific labour tasks?*



**Figure 1.3** | Conceptual overview of the research questions addressed in this thesis. Symbols next to the y-axis refer to soil-based ecosystem services, namely (top to bottom) soil organic carbon storage and cycling, nutrient cycling, nutrient provision and water regulation.

## 1.7 Study region

### *Climate and soils in central São Paulo state, Brazil*

The climate in the study region is tropical moist with dry winters (Aw) according to Köppen's classification. Mean annual precipitation ranges from 1,350 to 1,550 mm. The rainy season occurs during October-March, coinciding with a higher temperature (austral summer) and the dry season starts in April and ends in October (Alvares et al., 2013; Rodríguez-Lado et al., 2007). The present climate and vegetation in central São Paulo state emerged between 3000 and 3500 years ago (Scheel-Ybert et al., 2003).

Dominant soil types in the study region are Ferralsols and Acrisols (Rossi, 2017). Ferralsols (*Latossolos* in Brazilian soil classification) cover >60% of Brazil's land area (Camargo et al., 1988), are highly weathered and dominated by low-activity 1:1 clay minerals (generally, kaolinite) and Fe and Al oxyhydroxides in the fine fraction (Schaefer et al., 2008). The coarse fraction is dominated by quartz (Schaefer et al., 2004). The diagnostic feature of Ferralsols is their ferralitic B horizon, which can be sandy loam or finer (Klamt and van Reeuwijk, 1993), whereas topsoil texture can vary further. In the sampled sites, soil texture (0-30 cm) ranged from very sandy to very clayey (clay content range 25 – 620 g kg<sup>-1</sup>).

### *Agricultural and socio-economic background*

In order to understand labour dynamics in the studied agroforestry systems, it is important to consider the socio-economic conditions of the study region. São Paulo is classified as a 'very highly developed' state (according to the United Nation's Human Development Index, based on education, income and longevity indices) and also an economic powerhouse, generating 34% of Brazilian gross domestic product (GDP). To put this in perspective, the state's economic output amounts to more than that of Argentina, Bolivia, Paraguay and Uruguay combined (Tavares, 2016). This is mainly generated through industrial and service activities and only 2% of São Paulo's GDP is attributed to agriculture (Martinelli et al., 2011). Therefore, the labour market offers a broad range of options next to farming. Historically, however, the production of agricultural products, such as coffee and sugar, has been of crucial socioeconomic importance. Nowadays, highly mechanized sugarcane production for sugar and ethanol is the dominating agricultural activity in the state (Monteiro et al., 2015). Brazilian agricultural policy tools are mainly subsidized credit, and direct payments to farmers are very low compared to OECD countries and estimated at only 3% of farm incomes (Martinelli et al., 2011).

## 1.8 Methodological approach

### *Gradients*

Multiple gradients had to be defined in order to assess the relationship between the complexity of agroforestry systems and the provision of ecosystem services, and whether this effect depends on soil texture, depth and age. In order to test the effect of gradual increases, preference was given to continuous variables (e.g. clay content) over categorical ones (e.g. soil texture class) where possible. That is not to say that categorical variables were not used, or are not useful, but that continuous variables served the purpose better. This approach has been used in other studies and showed important interactions between diversity and management intensity (Cerda et al., 2017), as well as shade cover and yields (Blaser et al., 2018; Jezeer et al., 2018).

### *Measuring complexity*

Defining the complexity of a system is challenging (Parrott, 2010), and in this thesis the objective was not to define the best measure of complexity itself, but rather to define a measure of complexity that can best explain the provision of ecosystem services. Therefore, the first step was to identify a broad range of continuous variables (**Chapter 2**) that can approximate two main elements of complex systems: heterogeneity and interactions (Filotas et al., 2014). Heterogeneity of an agroforestry system can be expressed as its taxonomic or functional diversity (e.g. species richness or leaf traits) (Bullock et al., 2022; Naeem, 2013), or the heterogeneity of biomass distribution in a 3D space (Seidel et al., 2019). Aboveground interactions between components in agroforestry systems are shaped by farmer management, e.g. pruning or mowing frequency (Tschardt et al., 2011). In a second step, the relationships between these variables and proxies of nutrient cycling were assessed (**Chapter 2**). In a third step, a composite complexity index was constructed from these variables and used to test its relationship with a range of ecosystem service indicators (**Chapters 3 and 4**).

### *Indicators of ecosystem services*

The assessment of ecosystem service provision requires the measurement of proxy indicators (Bünemann et al., 2018). In this thesis, commonly used proxies were applied to quantify the provision of nutrient cycling, SOC storage and cycling, nutrient provision and water regulation. Nutrient cycling capacity was assessed by collecting litter samples (Fig. 1.4) and determining their dry weight and nutrient (N, P, K, Ca, Mg) contents (Froufe et al., 2019; Hartemink, 2005; Santos et al., 2017). SOC storage and cycling was assessed by collecting soil samples and physically fractionating them to determine stabilized MAOC and more labile POC stocks (Cambardella and Elliott, 1992; Cotrufo et al., 2019), as well as microbial biomass C (Alfaro-Flores et al., 2015; Lori et al., 2017; Vance et al., 1987). Plant-available nutrient stocks of macronutrients (N, P, K, Ca, Mg) and pH were used as proxies of nutrient provision (Cherubin et al., 2016; Matos et al., 2022). Indicators of soil

structure, such as macro- and microporosity (Bouma, 1991; Locatelli et al., 2022a), and available water capacity (Tomasella et al., 2000) were used as proxies for water regulation.



**Figure 1.4** | Impressions from the data collection process. Determining species diversity (top left), collecting litter samples (top right), taking undisturbed soil samples (bottom left), physical fractionation of MAOC and POC using a 53 $\mu$ m sieve (bottom centre) and drying of soil samples (bottom right).

### *Participatory research*

In this research project, I sought to follow an agroecological approach to science (*sensu* HLPE, 2019). That means that my goal was to solve a real-world problem, the lack of ecosystem service provision from agricultural systems, by combining different scientific disciplines (such as soil science, functional ecology, agronomy) and working closely with farmers (*idem*). The collaboration with farmers happened in a reflective and iterative way, where their observations informed my hypotheses, and my findings were shared in workshops and each farmer received an individual report (Fig. 1.5). This collaboration culminated in a joint paper (**Chapter 5**) to which a core group of pioneering farmers contributed substantially.



**Figure 1.5** | Throughout the thesis project, many interactions with farmers took place. Farmers were interviewed about labour demands in their agroforestry systems (top left and bottom left), received individual reports with soil data from their plots (top right) and participated in workshops about main challenges to scaling complex agroforestry (bottom right).

## 1.9 Thesis outline

In the following, a brief overview of the four research chapters (Fig. 1.6) and the general discussion is given:

In **Chapter 2**, 30 agroforestry systems are comprehensively described in terms of taxonomic and functional diversity, spatial structure and management and classified as silvopastures, multistrata or successional agroforestry systems. A LiDAR-derived structural complexity index is used to compare these three different types of agroforestry systems. Nutrient (N, P, K, Ca, Mg) stocks in litter are used as proxies for nutrient cycling and a model selection procedure is applied to determine those agroforestry characteristics which are most related to the provision of this ecosystem service.

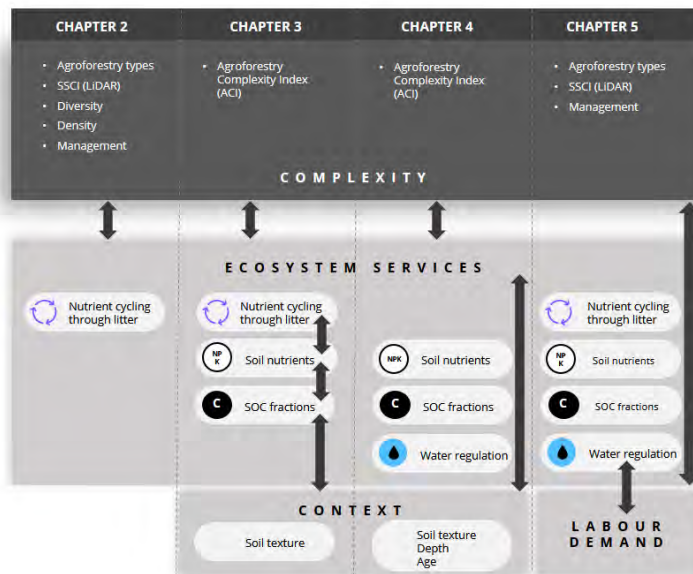
In **Chapter 3**, the Agroforestry Complexity Index (ACI) is defined. It quantifies **1**) how taxonomically diverse the trees in a system are (species richness), **2**) how close trees are to each other (stem density) and **3**) how often the farmers prune these trees and deposit the cut biomass on the soil right next to the trees (pruning & mulching frequency). Using the ACI, the cascading effects of agroforestry complexity on nutrient cycling (litter to soil), and in soil between nutrients and SOC fractions are tested using structural equation models.



In **Chapter 4**, the ACI is employed to assess the relationship between agroforestry complexity (ACI) and the simultaneous provision of SOC storage, nutrient provision and water regulation, and whether this relationship is influenced by soil texture, soil depth and age. Indicators for SOC storage are mineral-associated organic C, particulate organic C and microbial biomass C. For nutrient provision, P, CEC and pH are used as indicators. For water regulation, macro- and microporosity as well as available water capacity are used. Data from 201 samples which were collected across the 38 sites are used in linear mixed models to test for significance and assess effect sizes of the ACI and interactions with the co-variables (soil texture, depth and age).

In **Chapter 5**, the relationship between agroforestry complexity, ecosystem services and labour demand is explored using a subsample of 10 agroforestry systems. Detailed data on labour time spent on fertilization, pest control, weeding, crop management, biomass management and harvesting are compared to corresponding monocultures (secondary data). Furthermore, the main challenges to scaling agroforestry systems as perceived by farmers are discussed in workshops and farmers were asked to quantify the severity of each challenge for their own context in a survey on Likert-scales.

In **Chapter 6**, the results from this thesis are put in the context of recent studies on diversification, and the ACI is compared to other measures which were employed to measure complexity. Diversification and complexification are conceptually discussed and knowledge gaps for future research outlined. Lastly, challenges for scaling complex agroforestry systems for high ecosystem service provision are discussed.




**Figure 1.6** | Overview of research chapters. Arrows refer to relationships that were assessed.

## **Acknowledgements**

I thank my promotor Rachel Creamer and daily supervisor Felix Bianchi for their feedback on this chapter.







**Data collection in successional horticulture system on Fazenda da Toca, Itirapina, São Paulo, Brazil.**

Vegetable consortia are grown with *in situ* mulch, generated from nearby biomass trees and grasses. The LiDAR-derived Stand Structural Complexity Index (SSCI) of this system is 3.6, tree species richness 10 and stem density 1.100 ha<sup>-1</sup>.





02

# Identifying agroforestry characteristics for enhanced nutrient cycling potential in Brazil

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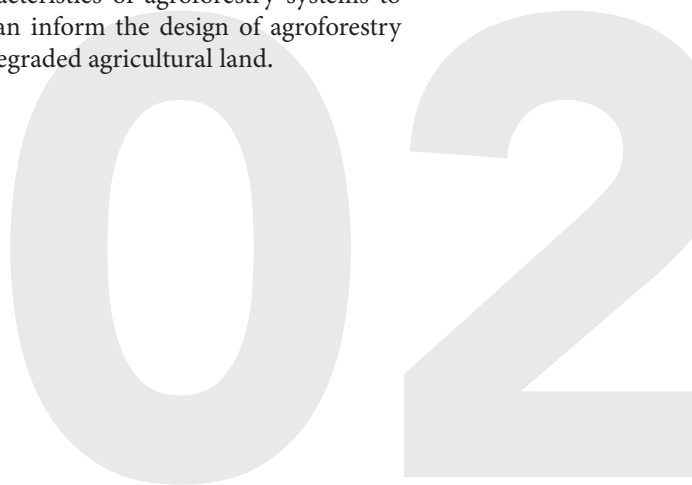
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## ABSTRACT

Tropical soils are prone to rapid degradation if not managed well, and agroforestry systems have the potential to restore degraded soils and support agricultural production together with other ecosystem services. In Brazil, an increasing number of pioneering farmers are establishing agroforestry systems on previously cleared farmland. However, while there are a wide range of agroforestry systems, this diversity has hardly been quantified, and it is not clear how these systems differ in their capacity for nutrient cycling to reverse soil degradation. The objectives of the study were to assess innovative agroforestry systems in terms of taxonomic and functional diversity, spatial structure and management, and to assess how these systems differ in terms of structural complexity and their potential for nutrient cycling. We assessed a LiDAR-derived stand structural complexity index (SSCI), interrow spacing, stem density, tree species richness and diversity, community weighted means (CWM) of foliar nitrogen and wood density, livestock density, pruning and mowing regimes in 30 agroforestry systems in the state of São Paulo, Brazil. We used N, P, K, Ca and Mg stocks in litter as a proxy for nutrient cycling. The agroforestry systems could be broadly categorized into silvopastures, multistrata and successional agroforestry systems. These types spanned a gradient of structural complexity, and this complexity was positively associated with tree species richness and planting density. Litter nutrient stocks were positively associated with pruning and mulching, and negatively associated with CWM of wood density, indicating the importance of pioneer trees. Overall, our results suggest that densely planted, pruned agroforestry systems that contain high species richness, including pioneer trees, contain relatively high amounts of N, P, K, Ca and Mg in their litter. These findings provide insight in the key characteristics of agroforestry systems to support nutrient cycling, and can inform the design of agroforestry systems for the regeneration of degraded agricultural land.





## 2.1 Introduction

Agroforestry systems are ancient production systems which are traditionally developed by integrating desired plant species into forests (Maezumi et al., 2018; Nogué et al., 2017). However, agroforestry systems may also help to restore degraded land, which requires the design of systems suitable for cleared farmland without existing tree cover (FAO, 2017; Wolz et al., 2018). The restoration of agricultural land is particularly urgent in the tropics, as tropical soils are often susceptible to rapid degradation if not managed appropriately (Lal, 2015; Lehmann et al., 2012), and agroforestry systems are well-suited for these environments (Muchane et al., 2020). Agroforestry is an umbrella term for the deliberate integration of crops and woody perennials in the same management unit, and encompasses a wide variety of systems (Nair, 1985). This integration of plant species can enhance biomass production and increase nutrient flows via litter (Fonte and Six, 2010). However, the capacity of agroforestry systems to enhance *in situ* nutrient cycling can be variable and context dependent (Barrios et al., 2017; Sauvadet et al., 2019; Veldkamp et al., 2023). This is particularly the case for phosphorus (P) (Muchane et al., 2020; Nesper et al., 2018), the most critical nutrient in tropical agriculture (Roy et al., 2016). Countries such as Brazil face immense economic and environmental costs to restore degraded and P deficient agricultural soils (Withers et al., 2018). Therefore, there is a need for a better understanding of the key characteristics of agroforestry systems that moderate their capacity to provide *in situ* nutrient inputs via litter (Veldkamp et al., 2023).

Agroforestry systems are managed according to farmers' needs and preferences, and therefore vary in species composition and tree density among others (Valencia et al., 2015). This variation can be captured by classifying agroforestry systems into distinct types (Atangana et al., 2013) or by quantifying key properties, such as stand structural complexity (Seidel et al., 2021), which is defined as the heterogeneity of biomass distribution in a three-dimensional space (Ehbrecht et al., 2021). In forests, structural complexity can be quantified by LiDAR (Newnham et al., 2015), and is strongly related to net primary productivity (Ali et al., 2016; Gough et al., 2019; Stark et al., 2012), microclimate (Ehbrecht et al., 2019) and habitat for biodiversity (Ishii et al., 2004). In agroforestry systems, structural complexity has been associated with carbon storage (Ali and Mattsson, 2017; Cardozo et al., 2022), habitat for biodiversity (Ibarra et al., 2021) and productivity (Jagoret et al., 2017b). Due to the tight association of structural complexity and the provision of several ecosystem services, there is a need for a better understanding of what agroforestry design characteristics influence the structural complexity of agroforestry systems.

In Brazil, there are a wide variety of agroforestry systems, ranging from silvopastoral systems where timber trees are planted into pastures to increasingly biodiverse fruit and veg-

etable agroforestry systems (Schuler et al., 2022). While traditional agroforestry systems can be found in cocoa producing regions in north-east Brazil, there has recently been an increase in newly planted, innovative agroforestry systems in the south-eastern state of São Paulo (Agroicone, 2022; MapSAF, 2022). Here, farmers are combining a range of crops and tree species at varying densities (Guerreiro et al., 2013; Vinholis et al., 2020), and some farmers also apply management practices to generate *in situ* mulch by mowing cover crops and intensively pruning trees (Martinelli et al., 2017; Miccolis et al., 2017). Such practices can enrich soils with nutrients (Froufe et al., 2019) and improve the availability of beneficial soil microbial populations (Chaudhary et al., 2023). Most research on these agroforestry systems focusses on the Atlantic Forest biome, whereas other biomes, such as the *Cerrado*, have received less attention (Schuler et al., 2022). The meta-analysis by Santos et al. (2019) suggested a relationship between the type of agroforestry system and the provision of ecosystem services in the Atlantic Forest biome. While this relationship holds promise for designing and managing agroforestry systems for particular desired ecosystem services, this relationship still requires further exploration.

The objectives of this study were to explore and describe the types of innovative agroforestry systems in the *Cerrado* and Atlantic Forest/*Cerrado* ecotone of São Paulo state, and to assess the capacity of these systems to support nutrient cycling via litter. Specific objectives were to (i) describe agroforestry systems in terms of taxonomic and functional diversity, spatial structure and management, (ii) assess how these agroforestry characteristics influence their structural complexity, and (iii) assess the relationship between agroforestry characteristics and nutrient stocks in litter.

## 2.2 Methods

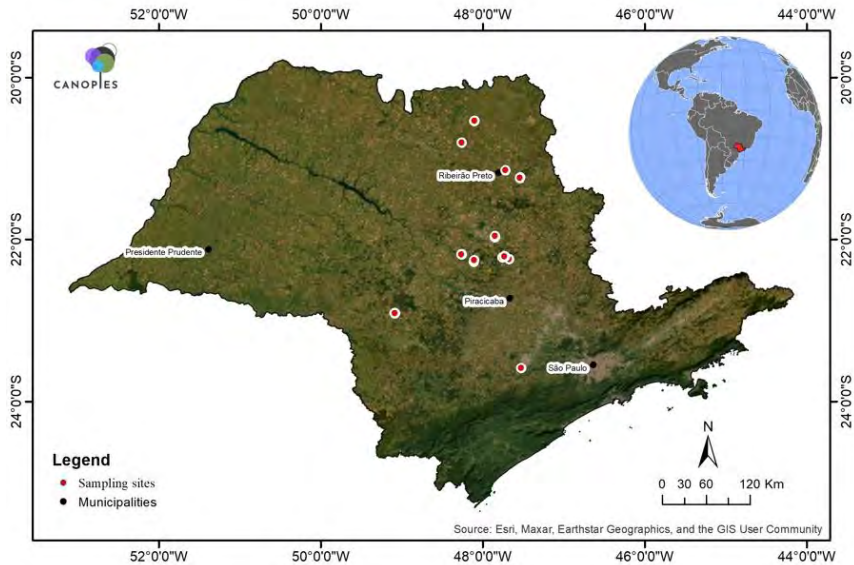
### 2.2.1 Study region

The study area was located in the central-east part of the São Paulo state, Brazil, in a transition zone between the Atlantic Forest and the *Cerrado* biomes. The predominant soil types in the region are highly weathered Ferralsols (*Latossolos* in Brazilian soil classification) and Acrisols, spanning a gradient in soil texture from very sandy to very clayey (Rossi, 2017). The climate is classified as Cwa according to Köppen criteria with humid summers and dry winters, and an average annual precipitation ranging from 1,350 to 1,550 mm. The dominant crops in the region include sugarcane, grains, citrus fruits, pastures and eucalypt plantations. Unlike other regions of Brazil, agroforestry systems are not part of the traditional agricultural landscape. However, in recent years a growing number of newly implemented agroforestry systems have been registered (Agroicone, 2022).

### 2.2.2 Selection of agroforestry systems

We selected agroforestry farmers in the *Cerrado*/Atlantic Forest ecotone in the state of São Paulo that have implemented innovative agroforestry on previously agricul-

tural land with the help of local experts, researchers and farmer networks (Fig. 2.1). Here, agroforestry systems are deemed innovative if they were purposefully and systematically designed to meet multiple objectives (Smith et al., 2012; Wolz et al., 2018). In practice, this entailed systems with cash crops, as well as trees or grasses that fulfil complementary functions, such as producing *in situ* green manure. This approach deviates from more traditional agroforestry where often remnants of secondary or primary vegetation are left to fulfil functions that are deemed important and only the crop of commercial interest is planted (e.g. leaving scattered trees in planted pastures or planting coffee under native forest canopy).



**Figure 2.1** | Location of the study area in the state of São Paulo, south-eastern Brazil. The distribution of sampling sites is indicated by red dots (some dots contain multiple sites).

Thirty agroforestry systems were selected on both small- and large-scale farms (farm size range 1 - >2000 ha). The mean age of the systems at the time of sampling was  $5.2 \pm 0.6$  years (range 3–14). The agroforestry systems hosted a diversity of crops such as fruits, e.g. coffee (*Coffea arabica*), lime (*Citrus latifolia*), and avocado (*Persea americana*), timber species, such as lemon eucalyptus (*Corymbia citriodora*) and mahogany (*Khaya senegalensis*), a range of vegetables and beef cattle (Fig. 2.2, Suppl. Table 2.1). Besides crops, in some systems ‘support species’ were planted, such as leguminous gliricidia trees (*Gliricidia sepium*) or fast-growing guinea grass (*Panicum maximum cv Mombaça*). In 28 out of 30 agroforestry systems, organic fertilizers (e.g., compost and rock meal) were used at generally low levels, and in two silvopastoral systems on an experimental farm chemical fertilizers were applied to maintain base saturation at recommended levels.

### 2.2.3 Classification of agroforestry systems into types

The selected agroforestry systems were classified into broad agroforestry system types and further distinguished into more specific types based on literature and the consultation of a local agroforestry expert (Table 2.1). In one case of doubt, we used expert judgement to make the final decision.

**Table 2.1** | Description of three agroforestry types and six agroforestry sub-types used for the classification of 30 agroforestry systems.

Agroforestry system type	Description	References	Sub-type	References
Silvopastures	Integration of trees and livestock, mainly in grazing systems	Jose et al. (2017)	Integrated Livestock-Forestry (ILF)	Guerreiro et al. (2013)
			Intensive Silvopastoral Systems (SSPi)	Calle et al. (2012)
Multistrata	Integration of two or more strata of trees or shrubs, no animals	Schroth and do Socorro Souza da Mota (2014)	Simple multistrata	Schroth and do Socorro Souza da Mota (2014)
			Complex multistrata	Schroth and do Socorro Souza da Mota (2014)
Successional	Integration of two or more strata of trees or shrubs, plant species selection based on ecological succession, and dynamic management	Andrade et al. (2020); Götsch (1994); Miccolis et al. (2016)	Successional horticulture	Miccolis et al. (2017); Padovan et al. (2021)
			Successional perennial	Young (2017)

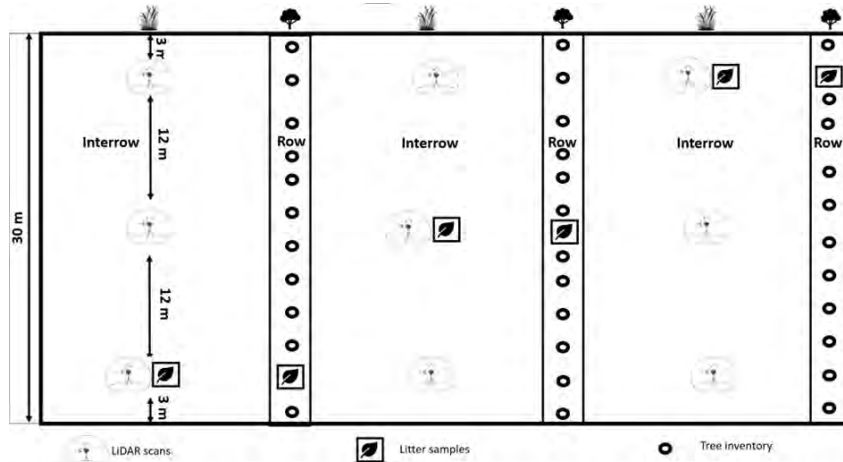
### 2.2.4 Sampling design and methodology

In each of the 30 agroforestry systems, a sampling plot was established at the centre of the field to minimize edge effects. Twenty-nine out of 30 systems were planted in linear rows and in these systems interrows were defined as the spaces between tree rows where no woody vegetation was present. Hence, sampling plots were divided into 'row' and 'interrow' areas. Sampling plots were 30 m long and three adjacent rows and interrows wide. The plot width was variable and ranged from 15 to 64 m. In the only system where trees were not planted in linear rows, a representative 30 x 30 m sampling plot was established at the centre of the field. Areas with steep slopes were avoided, and sampling plots were visually assessed to ensure that these were representative of the system. Farmers were consulted to verify that sampling plots had a representative management history of the agroforestry system.

We assessed a selection of metrics to characterize the agroforestry systems in terms of spatial structure, taxonomic diversity, functional diversity and management (Table 2.1). These metrics were chosen to allow for a holistic assessment of the complexity of the agroforestry systems. Data were collected by conducting field mea-



surements between January-February 2021, by conducting interviews with farmers, and using the TRY database, which contains a large set of plant functional trait data (Kattge et al., 2020; Mariano et al., 2021).



**Figure 2.2** | Sampling design used in the agroforestry systems. Plots were 30 m long and three adjacent rows and interrows wide. LiDAR scans were performed at nine locations within each plot (LiDAR symbol); litter samples (leaf symbol) were taken from each row and interrow following a transect at the centre of the row/interrrow. Per row, 25 trees (circles) were identified and their diameter at breast height (DBH) measured.

### 2.2.5 Spatial structure: interrow width, stem density and Stand Structural Complexity Index (SSCI)

Distances between rows were measured to determine interrow width (m) and trees in rows were counted and converted to stem density ( $\text{ha}^{-1}$ ). Stand structural complexity was assessed by single terrestrial laser scans at nine locations in each system according to the sampling design (Fig. 2.2) using a Faro Focus 3D laser scanner (Faro Technologies Inc., Lake Marry, Florida, USA) mounted on a tripod at 1.3 m height. Scans were converted into xyz files using Faro Scene software and the resulting point cloud was processed into the Stand Structural Complexity Index (SSCI) (R-code available here: <https://github.com/ehbrechtetal/Stand-structural-complexity-index---SSCI>). The SSCI is based on the fractal dimension of cross-sectional polygons derived from the 3D point cloud. The fractal dimension is a scale-invariant, mathematical measure of shape complexity (Mandelbrot, 1975), that does not take the stand's vertical structure into account. Therefore, fractal dimension values for each scan are scaled by using the effective number of layers (ENL, Ehbrecht et al., 2016). ENL quantifies the number of canopy layers that are effectively occupied by foliage and woody components. Based on these two components of fractal dimension and ENL, the SSCI quantifies the heterogeneity of biomass distribution in a 3D space. SSCI values increase with increasing stand density and verti-

cal stratification (for further details see Ehbrecht et al., 2017; Ehbrecht et al., 2021). The index has been used in numerous studies to investigate impacts of management and species composition on 3D vegetation structure (e.g. Asbeck and Frey, 2021; Juchheim et al., 2019; Willim et al., 2022) and to study effects of 3D forest structure on ecosystem functions and services (e.g. Donfack et al., 2021; Röhl et al., 2019).

**Table 2.2** | Overview of the metrics used for the characterization of agroforestry systems in terms of spatial structure, taxonomic diversity, functional diversity and management. For each metric the unit and collection method is indicated.

	Metrics	Unit	Data collection method
Spatial structure	Stand Structural Complexity Index (SSCI)	Dimensionless	Single terrestrial laser scans
	Stem density	Woody stems ha <sup>-1</sup>	In-field measurements
	Interrow width	m	
Taxonomic diversity	Species richness in rows and interrows	Number of species plot <sup>-1</sup>	Vegetation survey
	Exponential Shannon-Wiener index (H')	dimensionless	
Functional diversity	Leaf N community weighted mean (CWM)	N g kg <sup>-1</sup>	TRY database and Mariano et al. (2021)
	Wood density community weighted mean (CWM)	g cm <sup>-3</sup>	TRY database
	Successional groups	Number of successional groups plot <sup>-1</sup>	Literature
Management	Livestock density	Heads ha <sup>-1</sup>	Farmer interview
	Pruning & mulching	Frequency year <sup>-1</sup>	Farmer interview
	Mowing & mulching	Frequency year <sup>-1</sup>	Farmer interview

### 2.2.6 Taxonomic diversity: species richness and exponential Shannon-Wiener index

Tree species within plots (25 per row, total 75 per plot; Fig. 2.2) were identified to species level and the number of trees per species counted. Herbaceous crop plants, including cover crops, were identified to species level and their soil cover (as percentage) in the interrow area was visually estimated. The density of spontaneous herbaceous vegetation in interrows was generally low and was therefore not assessed. Species data was used to calculate richness and the exponential Shannon-Wiener index (exp H') (Hill, 1973; Jost, 2006).

### 2.2.7 Functional traits: Leaf N concentration, wood density and successional groups

Leaf N concentration ( $\text{g kg}^{-1}$ ) and wood density ( $\text{g cm}^{-3}$ ) data per tree species were sourced from the TRY database (Kattge et al., 2020), and community weighted means (CWM) per system were derived by weighting trait values by the relative abundance of each species (De Bello et al., 2021). Foliar N trait data were obtained from the Brazilian database of Mariano et al. (2021) and TRY. As these databases contained multiple values per species, a stepwise strategy was used to select data-points that were most representative for the study region (supplementary method). The recorded tree species were allocated to four successional groups (pioneer, early secondary, late secondary or climax species) and herbaceous plants in five successional groups (based on time until harvest: <45 days, <90 days, <120 days, <12 months, perennial) (Chacel, 2018). The validity and consistency of the grouping was checked by triangulating the data with a local agroforestry expert and with (grey) literature (Götsch, 1994; Miccolis et al., 2016; Yana and Weinert, 2001). We used the number of successional groups in plots for further analyses.

### 2.2.8 Management

Information on management, age and previous land use of the agroforestry plots was obtained by interviews with farmers. A large share of farmers applied *in situ* mulching, e.g. by mowing interrows and applying the cut material as mulch and/or by intensively pruning service trees and applying the residue as mulch. Here, the frequencies (number of times) of mowing & mulching and pruning & mulching per year are reported. Silvopastoral systems were the only systems containing livestock, and also had generally low taxonomic and functional diversity of plants. Due to the high collinearity between livestock density and diversity indices we used the diversity indices for further analysis and did not further explore relationships of livestock density and litter nutrient stocks.

### 2.2.9 Nutrient cycling: Litter nutrient stocks

Litter was sampled within a 0.5 x 0.5 m quadrant in each of the three tree rows and each of the three interrows along a transect in the plot (Fig. 2.2). Leaf litter and woody branches <2 cm in diameter were collected, dried at 70° C for 48h, weighed and finely ground in the laboratory for further chemical analysis. Subsamples from this ground material were then used to determine N concentration via the Kjeldahl method and P concentrations using the Vanadomolybdate method with determination via spectroscopy. K was analysed via flame photometry and Ca and Mg were extracted with HCl and determined via atomic absorption spectroscopy. All nutrient analyses were carried out at the University of São Paulo and followed standard procedures (MAPA, 2017). Nutrient concentrations were multiplied by the dry weight of the sample and converted to  $\text{kg ha}^{-1}$ . The reported nutrient stocks are averages of rows and interrows, which were weighted based on their area proportions in the sampling plots.

### 2.2.10 Statistical analyses

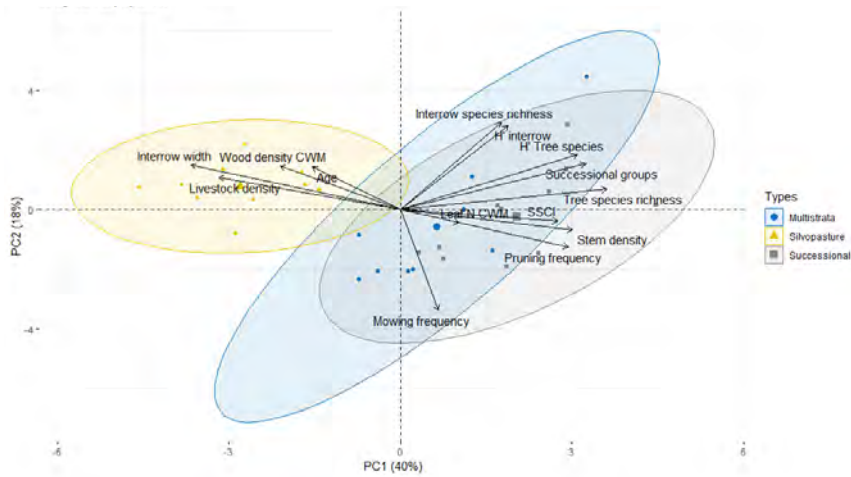
Principal component analysis (PCA) was used to explore relationships between indicators for spatial structure, taxonomic diversity, functional diversity and management (Table 2.2). Data were centered around their mean and the PCA was performed using the *FactoMineR* package, and extracted and visualized using the *factoextra* R package (Kassambara A, 2020). Metrics between agroforestry types were tested using non-parametric Kruskal-Wallis and Dunn post-hoc tests. Relationships between litter nutrient stocks (response variables) and SSCI, stem density (log10-transformed), interrow width, tree species richness and Shannon diversity in rows and interrows, leaf N, wood density, pruning & mulching, and mowing & mulching (explanatory variables) were explored using simple and multiple linear regression models. Full models contained all explanatory variables and the *dredge* function of the *MuMIn* package (Barton, 2022) was used for model selection based on the AICc criterion. Models within  $\Delta\text{AICc} < 2$  were considered and interactions were tested between variables in the most parsimonious models. Variance Inflation Factors (VIF) were checked for all models to assess collinearity and all reported models had VIF values  $< 2.5$  (Witten and James, 2013). All analyses were carried out in R (R Core Team, 2022).

## 2.3 Results

### 2.3.1 Spatial structure, taxonomic and functional diversity and management of agroforestry system types

PCA indicated a separation between silvopastoral systems on the one hand and multistrata and successional systems on the other along PC1 (40% of variation; Fig. 2.3). Silvopastoral systems were associated with larger interrow widths and higher wood density CWM, while multistrata and successional systems were associated with tree species richness, stem density and pruning frequency. PC2 (18% of variation) was largely defined by the mowing regime of the interrows and to a lesser extent by the taxonomic plant species richness of the interrows (Fig. 2.3).

The 30 agroforestry systems spanned a gradient in spatial structure and taxonomic diversity whereby silvopastures were the simplest, followed by multistrata agroforestry systems, and successional agroforestry systems were generally the most complex (Table 2.3). Silvopastoral systems had a significantly lower stem density and tree species diversity (exp H') than successional systems. Differences in interrow width, tree species richness and wood density CWM between multistrata and successional systems were not statistically significant (Table 2.3). Wood density CWM was significantly higher in silvopastoral than in the other two agroforestry types, while Leaf N concentrations CWM were not statistically different between the three types. Silvopastoral systems were the only systems with livestock, but applied almost no mowing & mulching or pruning & mulching management. Mowing & mulching was most frequently applied in multistrata systems, and pruning & mulching was most frequently applied in successional systems.



**Figure 2.3** | Principal component analysis of the data collected in silvopastures (yellow), multistrata agroforestry systems (blue), and successional agroforestry systems (grey). The proportion of explained variance is indicated on the axes.

When focussing on sub-types of agroforestry systems, there was a general pattern for tree species richness and functional diversity (successional group richness) of integrated Livestock-Forestry (iLF) < intensive Silvopastoral Systems (SSPi) < simple multistrata < complex multistrata < successional horticulture < successional perennial systems (Table 2.3). Stem density and SSCI also tended to increase in that order, whereas interrow width decreased in that order. SSPi tended to have a higher tree species richness and diversity than iLF, both in rows and interrows. Complex multistrata systems tended to have a higher species diversity and tree density than simple multistrata systems. Successional horticulture systems had a higher interrow species diversity (mostly vegetables) and similar tree species richness and diversity than successional perennial systems. Pruning & mulching frequency was slightly higher in horticultural than perennial successional systems.

**Table 2.3** | Mean values ( $\pm$  standard errors) of the variables reflecting spatial structure, taxonomic diversity, functional diversity and management of the agroforestry types that the 30 sampled systems were assigned to. Differences between silvopastures, multistrata and successional agroforestry systems were tested using the Kruskal-Wallis and Dunn post-hoc test ( $p_{\text{Holm-adj.}} < 0.05$ ) and are indicated by letters. Differences between sub-types were not statistically tested due to low levels of replication.

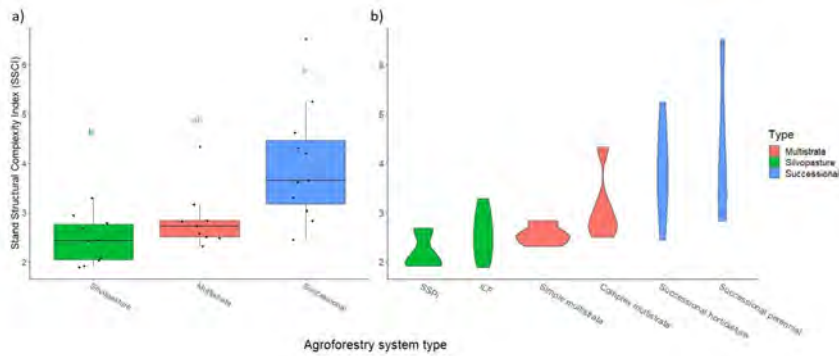
		Silvopastures (n=10)		Multistrata (n=9)		Successional (n=11)	
		Integrated Livestock-Forestry (n=7)	Intensive silvopastoral systems (n=3)	Simple multistrata (n=4)	Complex multistrata (n=5)	Successional horticulture (n=7)	Successional perennial (n=4)
Spatial structure	Stand Structural Complexity Index (SSCI)	2.49 $\pm$ 0.15 b		2.87 $\pm$ 0.20 ab		3.98 $\pm$ 0.35 a	
		2.64 $\pm$ 0.19	2.19 $\pm$ 0.24	2.53 $\pm$ 0.11	3.12 $\pm$ 0.32	3.83 $\pm$ 0.36	4.24 $\pm$ 0.82
	Stem density (ha <sup>-1</sup> )	283 $\pm$ 45 b		1255 $\pm$ 247 ab		3533 $\pm$ 710 a	
		240 $\pm$ 55	381 $\pm$ 50	1550 $\pm$ 540	1019 $\pm$ 118	3694 $\pm$ 969	3251 $\pm$ 1141
Interrow width (m)		23.10 $\pm$ 1.89 a		5.39 $\pm$ 0.33 b		4.32 $\pm$ 0.39 b	
		24.43 $\pm$ 2.58	20 $\pm$ 0	5.25 $\pm$ 0.75	5.5 $\pm$ 0.22	4.5 $\pm$ 0.54	4 $\pm$ 0.57
Taxonomic diversity	Tree species richness	2.80 $\pm$ 0.75 a		7.78 $\pm$ 1.80 b		10.45 $\pm$ 0.86 b	
		2 $\pm$ 0.84	4.67 $\pm$ 0.88	4.5 $\pm$ 1.55	10.4 $\pm$ 2.50	10.14 $\pm$ 1.01	11 $\pm$ 1.73
	H' tree species	2.04 $\pm$ 0.42 b		3.79 $\pm$ 1.12 ab		4.87 $\pm$ 0.38 a	
		1.62 $\pm$ 0.52	3.01 $\pm$ 0.54	2.19 $\pm$ 0.25	5.07 $\pm$ 1.88	5.51 $\pm$ 0.39	3.75 $\pm$ 0.40
	Interrow species richness	1.70 $\pm$ 0.39 a		3.56 $\pm$ 1.95 a		2.64 $\pm$ 0.59 a	
	1.71 $\pm$ 0.56	1.67 $\pm$ 0.33	2 $\pm$ 0.71	4.8 $\pm$ 3.55	3.29 $\pm$ 0.81	4 $\pm$ 0.50	
H' interrow species		1.38 $\pm$ 0.19 a		1.71 $\pm$ 0.34 a		2.10 $\pm$ 0.47 a	
		1.39 $\pm$ 0.26	1.35 $\pm$ 0.22	1.75 $\pm$ 0.47	1.68 $\pm$ 0.53	2.52 $\pm$ 0.67	1.36 $\pm$ 0.36
Functional diversity	Successional group richness	2.70 $\pm$ 0.42 b		3.89 $\pm$ 0.51 ab		4.91 $\pm$ 0.44 a	
		2.29 $\pm$ 0.52	3.67 $\pm$ 0.33	3.75 $\pm$ 1.1	4 $\pm$ 0.45	5.29 $\pm$ 0.56	4.25 $\pm$ 0.63
	Leaf N (g kg <sup>-1</sup> ) CWM	25.67 $\pm$ 2.60 a		29.86 $\pm$ 0.83 a		26.55 $\pm$ 1.90 a	
		22.22 $\pm$ 2.77	33.72 $\pm$ 0.77	29.94 $\pm$ 1.62	29.79 $\pm$ 0.95	27.42 $\pm$ 1.52	25.04 $\pm$ 4.84
Wood density (g cm <sup>-3</sup> ) CWM		0.69 $\pm$ 0.04 a		0.44 $\pm$ 0.07 b		0.44 $\pm$ 0.05 b	
		0.72 $\pm$ 0.04	0.63 $\pm$ 0.13	0.35 $\pm$ 0.11	0.51 $\pm$ 0.10	0.46 $\pm$ 0.06	0.39 $\pm$ 0.11
Management	Livestock density (ha <sup>-1</sup> )	1.52 $\pm$ 0.24 a		0 b		0 b	
		1.49 (0.31)	1.6 (0.38)	0	0	0	0
	Mowing & mulching frequency (year <sup>-1</sup> )	0.30 $\pm$ 0.30 b		3.94 $\pm$ 0.97 a		2.64 $\pm$ 0.88 ab	
		0.43 $\pm$ 0.43	0	3.75 $\pm$ 1.64	4.1 $\pm$ 1.33	2 $\pm$ 1.15	3.75 $\pm$ 1.31
	Pruning & mulching frequency (year <sup>-1</sup> )	0 b		0.81 $\pm$ 0.27 a		1.58 $\pm$ 0.18 a	
	0	0	0.5 $\pm$ 0.29	1.05 $\pm$ 0.42	1.86 $\pm$ 0.14	1.1 $\pm$ 0.33	



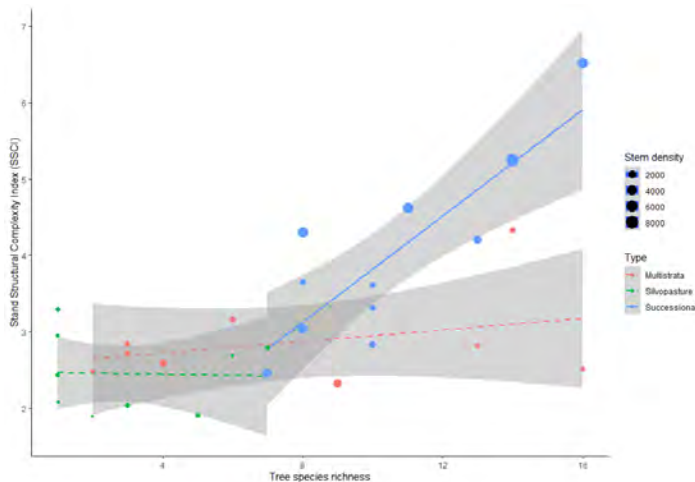
### 2.3.2 Explaining variation in stand structural complexity

Successional agroforestry systems had significantly higher SSCI values than silvopastures (Fig. 2.4a). There was substantial variation in SSCI in the 30 agroforestry systems, with the highest SSCI ( $6.5 \pm 0.3$ ) in a successional perennial system and the lowest in an iLF with few scattered trees ( $1.9 \pm 0.5$ ).

The most parsimonious model for SSCI indicated that SSCI was positively associated with tree species richness, stem density and their interaction ( $R^2=0.71$ ,  $p<0.001$ ; Suppl. Table 2.2). The combination of high tree species richness and stem density



**Figure 2.4** | Stand structural complexity index of silvopastures, multistrata and successional agroforestry systems. Letters indicate significant differences ( $p<0.05$ ; Kruskal-Wallis and Dunn's posthoc test) (a). Differences between the six sub-types were not tested statistically due to low levels of replication and their distribution is visualized in a violin plot (b). SSPi: intensive silvopastoral systems (Spanish acronym), iLF: integrated Livestock-Forestry.

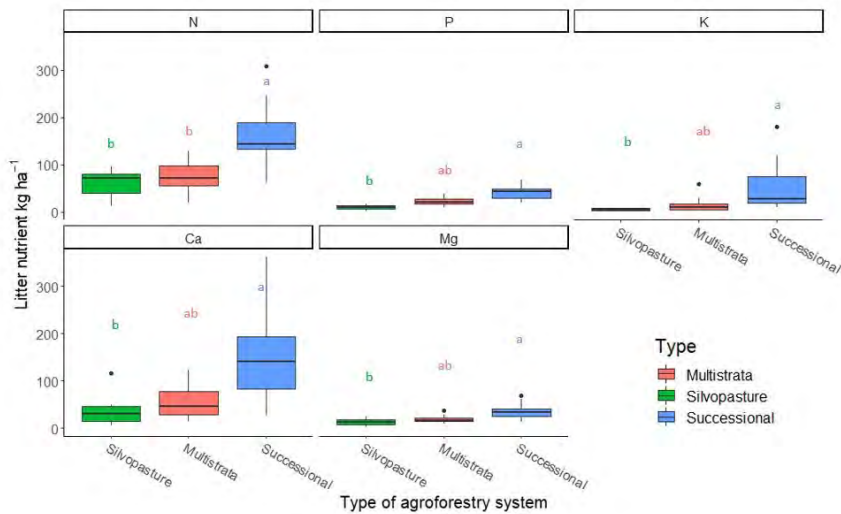


**Figure 2.5** | Relationship between stand structural complexity index (SSCI), tree species richness and stem density. Successional systems are designed to combine high species diversity with high stem density (bubble size), which results in a relatively high structural complexity. In silvopastoral and multistrata systems, increasing tree species richness does not increase SSCI as they tend to be planted at lower densities.

was most prevalent in successional systems, and less so in multistrata and silvopastoral systems (Fig. 2.5).

### 2.3.3 Variation in litter nutrient stocks and best explanatory metrics

Successional systems had consistently higher N, P, Ca and Mg nutrient stocks in litter than silvopastoral and multistrata systems (Fig. 2.6). For P, mean values show a ranking of silvopastures < multistrata < successional, but differences were only significant between successional and silvopasture systems (Fig. 2.6). Also, for N, Ca and Mg, successional systems had higher litter nutrient stocks than silvopastures, but for Ca and Mg these differences were not statistically significant between multistrata and successional systems. For N, however, successional systems had significantly higher litter stocks than multistrata systems.



**Figure 2.6** | Litter nutrient stocks of N, P, K, Ca and Mg (kg ha<sup>-1</sup>) in 30 agroforestry systems. Differences between silvopastures, multistrata and successional systems were tested using the Dunn posthoc test ( $p < 0.05$ ) and significant differences are indicated by different letters.

When shifting from comparing agroforestry types to employing the full range of metrics as explanatory variables, the model selection procedure indicated that pruning & mulching frequency was contained in the selection of most parsimonious models ( $\Delta AIC < 2$ ) and was positively associated with all litter nutrient stocks (Table 2.4). However, the most parsimonious model for P stocks in litter indicated that there was a significant interaction between pruning & mulching and stem density (log) ( $R^2 = 0.46$ ,  $p < 0.01$ ). Wood density CWM was negatively associated with N, K, Ca and Mg litter stocks. Tree species richness was positively associated with P, K and Ca stocks in litter, while SSCI was positively associated with litter Ca stocks.



**Table 2.4** | Results of model selection procedure to determine the most parsimonious models for N, P, K, Ca and Mg stocks in litter based on full models that contained all explanatory variables listed in Table 2.1. Models with  $\Delta AIC < 2$  are reported, and all models were tested for collinearity using the Variance Inflation Factor (VIF). All reported variables had VIF of below 2.5. Asterisks indicate significance levels: \*  $p < 0.05$ ; \*\*  $p < 0.01$ .

Response variable	Explanatory variable	Estimate	R <sup>2</sup>	p	$\Delta AICc$
Litter N stock	Pruning frequency +	4.233e-01 *	0.33	0.001	0
	Wood density CWM	-3.836e-01 *			
Litter P stock	Pruning frequency +	3.1397 *	0.46	0.006	0
	Stem density (log) +	0.2208			
	Pruning: Stem density	-0.3997 *			
	Pruning frequency +	4.225e-01 *	0.38	<0.001	1.90
	Tree species richness	3.534e-01 *			
Litter K stock	Wood density CWM	-4.212e-01 *	0.18	0.02	0
	Wood density CWM +	-3.845e-01 *	0.16	0.03	1.0
	Pruning frequency	2.197e-01			
	Tree species richness	3.780e-01 *	0.14	0.04	1.19
Litter Ca stock	SSCI +	3.935e-01 *	0.29	0.005	0
	Wood density CWM	-3.099e-01			
	SSCI	5.025e-01 **	0.25	0.005	0.76
	Pruning frequency +	3.460e-01 *	0.26	0.007	0.83
	Wood density CWM	-3.906e-01 *			
	Tree species richness	4.940e-01 **	0.24	0.006	1.09
Litter Mg stock	Pruning frequency +	3.408e-01	0.21	0.01	0
	Wood density CWM	-3.345e-01			
	Stem density (log)	0.3480 *	0.19	0.01	0
	Pruning frequency	3.967e-01 *	0.16	0.03	1.30

## 2.4 Discussion

### 2.4.1 General findings

Soil degradation is estimated to affect 40% of agricultural land and 50% of people globally (UNCCD, 2022). Agroforestry systems can contribute to soil regeneration through *in situ* nutrient cycling, but this potential may be system specific (Barrios et al., 2017). Based on the analysis of 30 innovative agroforestry systems which were established on previously agricultural land we report three key findings. First, we found that silvopastoral systems represented a clearly different agroecological context than multistrata and successional agroforestry systems. Silvopastoral systems were associated with relatively large interrow widths and higher wood density, reflecting management recommendations for interrow spacing (Vieira Junior et al., 2022) and economically motivated choices of planting high value timber trees. Multistrata and successional systems were associated with relatively high tree species richness, stem density, successional groups and pruning & mulching frequency. Secondly, variation in the LiDAR-derived SSCI was best explained by the combination of tree species richness and stem density. This explains the highest structural complexity in successional systems, as these systems typically had both high species richness and tree density. Third, stocks of N, P, K, Ca and Mg in litter were the lowest in silvopastures, followed by multistrata, and the highest in successional agroforestry systems. Variations in litter stocks of N, K, and Mg were explained by the frequency of pruning & mulching and wood density CWM. P litter stocks were associated with the interaction between pruning frequency and stem density, while Ca litter stocks were associated with wood density CWM and SSCI.

### 2.4.2 Agroforestry types

Our study highlights the diversity of agroforestry systems established on previous agricultural land (mostly pastures) in south-eastern Brazil, which range from relatively simple to highly complex systems. Integrated livestock-forestry systems were developed by Brazilian research institute EMBRAPA (EMBRAPA, 2022) and their adoption in São Paulo state depended on the innovative capacity of farmers (de Souza Filho et al., 2020). The concepts behind intensive silvopastoral systems with high densities of fodder shrubs originate from Colombia and central America where their adoption has been fostered by innovation networks between farmers and researchers (Calle et al., 2013). Simple multistrata systems, which typically consist of combinations of two to three tree crops, are representative of experimental alley cropping systems in the global tropics, whereas the complex multistrata systems in this study had considerably higher species diversity than average tropical alley cropping systems (Wolz and DeLucia, 2018). Both horticultural and perennial successional systems are unique to Brazil as they were developed by local farmers, most notably by Ernst Götsch (Andrade et al., 2020; Götsch, 1994). While successional systems partly resemble tropical homegardens (Kumar and Nair, 2006), their commercial orientation and size, linear design in rows and intensive pruning and mulching regime set them apart from typical homegardens (Miccolis et al., 2017).

The three types and six sub-types described here showcase pioneering examples of agroforestry systems which can serve both further research as well as real examples for farmers interested in transitioning towards agroforestry (Valencia et al., 2022).

### 2.4.3 Stand structural complexity

The combination of high species diversity and high planting density resulted in high structural complexity, as evidenced by the LiDAR-derived SSCI. This finding offers farmers practical guidelines for the design of systems with high structural complexity, and also explains why successional systems were structurally more complex than silvopastoral systems. SSCI values of the studied successional agroforestry systems were similar to those from a biodiversity enrichment experiment in Indonesia where up to six native species were added to oil palm monocultures (Zemp et al., 2019). The highest SSCI value in this study ( $6.5 \pm 0.3$ ) was a 15 year old successional agroforest with SSCI values comparable to native woodlands in the Neotropics (Ehbrecht et al., 2021). SSCI values of silvopastoral and multistrata systems were in a similar range as in German silvopastoral systems which were older and had a lower tree density than most of the agroforestry systems presented here (Seidel et al., 2021). However, in this study, the association between SSCI and age was not significant. SSCI can be used as an indicator for microclimate or habitat suitability of a wide range of biota. However, further research is needed to underpin the relationship of the SSCI and productivity as well as other ecosystem services in agroforestry systems.

### 2.4.4 Litter nutrient stocks

High litter nutrient stocks were associated with the practice of pruning & mulching, which is in line with previous studies from southern São Paulo state (Froufe et al., 2019) and experimental agroforestry systems in Bolivia (Schneidewind et al., 2018) and Costa Rica (Russo and Budowski, 1986). Particularly for litter P stocks, the recycling of this critical nutrient will become ever more important for farmers as global P fertilizer stocks are finite (Withers et al., 2018). While the bioavailability of P for crops was not tested in this study, other studies suggest that organic P inputs can stimulate the formation of long-term slow release P sources for plants (Malik et al., 2012) through soil microbial processing (Gao et al., 2019; Maranguit and Kuzyakov, 2019; Tang et al., 2014). Soil organic P stocks were also the main source of plant extractable P when no chemical fertilizers were used (Soltangheisi et al., 2018), and in native tropical forests, P was suggested to cycle directly from litter to plants (Sayer and Tanner, 2010). Litter nutrient stocks in our successional agroforestry systems were in the same range as those in a canopy pruning experiment in a tropical forest where pruning also lead to significant increases in litter N and P concentrations (Silver et al., 2014). Pruned leaf and twig material has not gone through a process of senescence and associated nutrient withdrawal, and has therefore relatively high nutrient concentrations (Noodén and Leopold, 1988).

Besides pruning, wood density CWM, tree species richness and tree stem density were positively associated with litter nutrient stocks. Wood density CWM was

negatively associated with litter N, K, Ca and Mg stocks, indicating that agroforestry systems that are dominated by trees with low wood density benefitted from relatively high litter nutrient enrichment. Wood density CWM was associated with the proportion of pioneer species ( $R^2=0.47$ ,  $p<0.001$ , Suppl. Fig. 2.1), suggesting a relationship between the growth strategy of trees and litter nutrient stocks. Elevated levels of N, P and Ca in litterfall from pioneer trees have been reported in tropical forests and attributed to the ability of pioneers to mobilize nutrients from degraded soil (Aidar et al., 2003; Santiago, 2010; Vasconcelos and Luizão, 2004). The positive association between tree species richness and nutrient cycling is also in line with studies which showed the interactive effects of the resulting litter diversity and soil biodiversity on the provision of multiple ecosystem services (Gaitanis et al., 2023; Liu et al., 2023). The association between stem density and litter nutrient input is likely related to higher aboveground biomass in dense stands, as has also been shown in Brazilian secondary forests (Teixeira et al., 2020).

#### 2.4.5 Insights for agroforestry system design

Globally, 74% of scientific agroforestry experiments only included a single tree species (Wolz and DeLucia, 2018), while the farmers managing successional agroforests presented here included on average 10 tree species and two herbaceous species in the interrows. Based on our metrics of functional diversity, results indicate that a substantial share of pioneer trees can enhance nutrient cycling. Pioneer trees are fast-growing and, if managed well (e.g. by periodic pruning), can also provide adequate shade for slower-growing, late successional fruit or high value timber trees (Brancalion et al., 2019). Integration of species with different life cycles also allows for denser plantations, as fast-growing species can be harvested or thinned out over time. Systems with the highest litter nutrient stocks in this study had about 3500 trees  $\text{ha}^{-1}$ , which is substantially more than in most agroforestry studies (Ma et al., 2020). While coffee and cocoa are the most prominent agroforestry crops globally (Jezeer et al., 2017), our observations suggest that a wide range of crops (including limes, avocados and vegetables) can be grown under shade, especially if pruning management of service trees is used to regulate light interception and to support *in situ* nutrient cycling (Tschardt et al., 2011). Pruning and mulching of functionally diverse and dense agroforests also enhanced tree crop productivity in Bolivia, but requires substantial labour input (Armengot et al., 2016; Esche et al., 2022). Agroforestry design should therefore also take into account implications for labour demand.



## 2.5 Conclusions

Our findings indicate that more complex agroforestry systems have higher nutrient cycling potential, and that the design of such systems should take into account the taxonomic and functional diversity, spatial structure and management of trees. The establishment of species-enriched agroforestry systems with a high tree density and a high proportion of pioneer tree species, in combination with intense pruning & mulching, can increase litter nutrient stocks, and potentially stimulate nutrient cycling to reverse soil degradation. As all systems in this study were established on previous agricultural land without tree cover, the results show promise for the restoration of degraded farmland. Moving forward, agroforestry research should focus on how to complexify agroforestry systems to increase the provision ecosystem services, while keeping the required labour input manageable (Lovell et al., 2017; Wolz et al., 2018).

## Acknowledgements

We gratefully acknowledge the help and support of all participating farmers, and would like to give thanks for the fruitful discussions about agroforestry management we had with Ana-Clara Rocha, Maridélia Arriba, Rodrigo Junqueira, Denise Amador, Hemes Lopes, Maria-Fernanda Guerreiro and Gustavo Fernandes Gonçalves. Special thanks go to Luiz Octavio Ramos Filho (EMBRAPA Environment) for sharing his expertise and support. We would also like to thank Rodney Rizzo, Guilherme Fernando Muller and Marcelo Gomes Barroca Xavier for their support during field work.

## Supplementary material

### Supplementary Method: Functional traits secondary data selection

Firstly, duplicated entries were deleted, as well as all values with an error risk greater than three units of standard deviation from the species mean of each trait (Wang et al., 2020). Minimum and maximum trait values per site were also discarded to obtain a more representative dataset. Secondly, the geographical origin of the trait measurement was adopted as a selection criterion (Monnet et al., 2021). Specifically, the dataset was split into four different units, containing trait values based on their geographies: 1) traits measured in the Brazilian Atlantic Rainforest or Cerrado biomes (where studied agroforestry systems were located), 2) measured in Brazil (at a country-level), 3) in countries located at a 10-30 degrees latitude (also countries with only a part of their territory within this latitude were considered eligible), or 4) globally. The same division was made both at a species-specific and gender-specific level, resulting in eight sub-datasets. Thirdly, for each of these datasets, the mean functional trait value was calculated (Adler et al., 2014). Finally, per each species, the “first available” trait value was selected, giving preference to the most local sub-dataset over the others (in order: same biome > Brazil > lat. 10-30 > global). If no spe-

cies-level data was present, genus-level information was used instead, with the same selection logic. When genus-level information was not present, other literature sources were consulted. If also genus-level information was not retrievable in the literature (9 cases out of 306), the value of the closest phylogenetically-related species at a family level present in the dataset was instead used (e.g. *Zingiber officinale* for *Curcuma longa*) (Flo et al., 2019).

**Supplementary Table 2.1** | Detailed information on sampling sites, including agroforestry types, main crops, timber species, support species, management and age.

System ID	Type (broad)	Type (detailed)	Main crop	Timber species	Support species	Management	Age (years)
AFS1	Multistrata	Complex multistrata	Hibiscus, beans, medicinals	-	Natives	No pruning, little mowing; low organic fertilization	4
AFS2	Silvopasture	iLF	Rotation of feed crops and beans	Lemon eucalypt ( <i>Corymbia citriodora</i> )	-	Tillage, low organic fertilization	11
AFS3	Silvopasture	iLF	Grass ( <i>Urochloa brizantha</i> cv Miranda)	Lemon eucalypt ( <i>Corymbia citriodora</i> )	-	Grazing, no inputs in last 10 years	11
AFS4	Silvopasture	iLF	Grass ( <i>Urochloa brizantha</i> cv Miranda)	Lemon eucalypt ( <i>Corymbia citriodora</i> )	-	Grazing, no inputs in last 9 years	10
AFS5	Successional	Successional horticulture	Vegetables, coffee, diverse fruits, vanilla	Mahogany ( <i>Khaya senegalensis</i> ), Jequitiba ( <i>Cariniana legalis</i> )	Eucalypt ( <i>Eucalyptus urograndis</i> ), Gliricidia ( <i>Gliricidia sepium</i> ), Mombaça ( <i>Panicum maximum</i> )	Frequent pruning, mowing and mulching; organic fertilization for vegetables, none for trees	3.5
AFS6	Successional	Successional horticulture	Vegetables, lemon, diverse fruits	Louro Pardo ( <i>Cordia trichotoma</i> )	Eucalypt ( <i>Eucalyptus urograndis</i> ), Gliricidia ( <i>Gliricidia sepium</i> ), Mombaça ( <i>Panicum maximum</i> )	Frequent pruning, mowing and mulching; organic fertilization for vegetables, none for trees	3
AFS7	Successional	Complex multistrata	Lime	Mahogany ( <i>Khaya senegalensis</i> ), Red cedar ( <i>Toona ciliata</i> )	Eucalypt ( <i>Eucalyptus urograndis</i> ), Gliricidia ( <i>Gliricidia sepium</i> ), Inga ( <i>Inga vera</i> ), Guapuruvu ( <i>Schizolobium parahyba</i> ), Mombaça ( <i>Panicum maximum</i> )	Pruning, mowing and mulching; organic fertilization for lime trees	3

System ID	Type (broad)	Type (detailed)	Main crop	Timber species	Support species	Management	Age (years)
AFS8	Multistrata	Complex multistrata	Lime	Mahogany ( <i>Khaya senegalensis</i> )	Eucalypt ( <i>Eucalyptus urograndis</i> ), Mombaça ( <i>Panicum maximum</i> ), Banana (not present anymore at time of sampling)	Pruning, mowing and mulching;	4
AFS9	Successional	Successional horticulture	Vegetables, banana	-	Eucalypt ( <i>Eucalyptus urograndis</i> ), Mulberry ( <i>Morus nigra</i> ), natives	Frequent pruning, mowing and mulching, organic inputs for vegetables, none for trees	3
AFS10	Multistrata	Simple multistrata	Avocado, macadamia	-	Signal grass ( <i>Urochloa decumbens</i> )	Mowing	3
AFS11	Silvopasture	iLF	Palisade grass ( <i>Urochloa ruziensis</i> )	Eucalypt ( <i>Eucalyptus urograndis</i> )	-	Grazing	5
AFS12	Successional	Successional perennial	Diverse fruits	-	Natives	Pruning, mowing	4.5
AFS13	Multistrata	Simple multistrata	Avocado, papaya, maize	-	Signal grass ( <i>Urochloa decumbens</i> )	Mowing, tillage	4
AFS14	Multistrata	Simple multistrata	Banana	Mahogany ( <i>Khaya senegalensis</i> )	Signal grass ( <i>Urochloa decumbens</i> )	Mowing	5
AFS15	Multistrata	Complex multistrata	Citrus fruits, curcuma	Mahogany ( <i>Khaya senegalensis</i> ), Jequitiba ( <i>Cariniana legalis</i> ), Louro Pardo, ( <i>Cordia trichotoma</i> )	Signal grass ( <i>Urochloa decumbens</i> )	Mowing	4
AFS16	Silvopasture	iSPS	Switch grass ( <i>Panicum maximum</i> cv Mombaça)	-	Eucalypt ( <i>Eucalyptus urograndis</i> ), Acacia ( <i>Acacia mangium</i> ), Gliricidia ( <i>Gliricidia sepium</i> )	Grazing	3
AFS17	Silvopasture	iSPS	Switch grass ( <i>Panicum maximum</i> cv Mombaça), Leucena ( <i>Leucaena leucocephala</i> )	Mahogany ( <i>Khaya senegalensis</i> )	Eucalypt ( <i>Eucalyptus urograndis</i> ), Acacia ( <i>Acacia mangium</i> ), Gliricidia ( <i>Gliricidia sepium</i> )	Grazing	3

## AGROFORESTRY CHARACTERISTICS

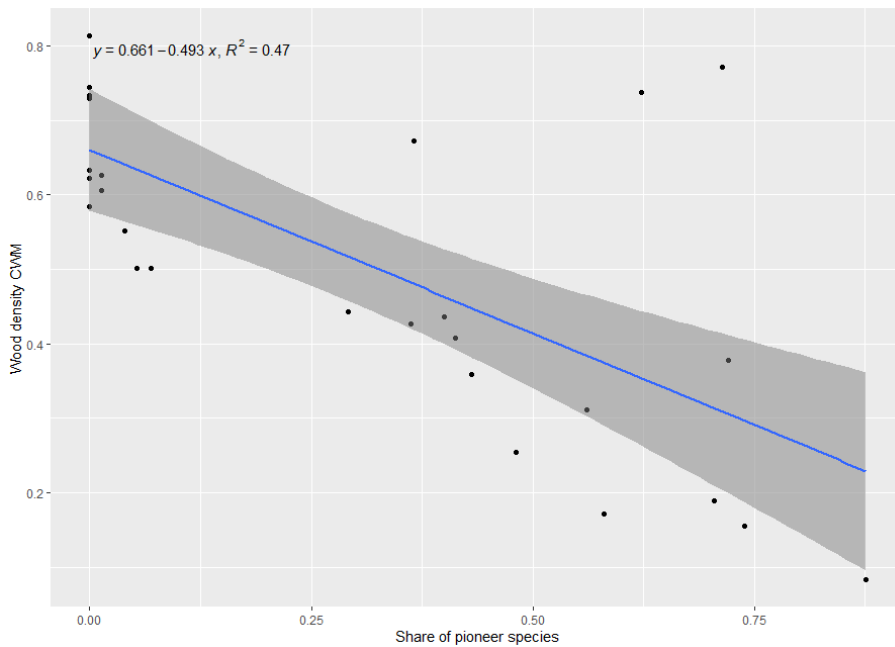
System ID	Type (broad)	Type (detailed)	Main crop	Timber species	Support species	Management	Age (years)
AFS18	Silvopasture	iSPS	Switch grass ( <i>Panicum maximum</i> cv Mombaça), Mexican sunflower ( <i>Tithonia diversifolia</i> )	Mahogany ( <i>Khaya senegalensis</i> )	Eucalypt ( <i>Eucalyptus urograndis</i> ), Acacia ( <i>Acacia mangium</i> ), Gliricidia ( <i>Gliricidia sepium</i> )	Grazing	3
AFS19	Successional	Successional horticulture	Vegetables, coffee, diverse fruits	-	Gliricidia ( <i>Gliricidia sepium</i> ), natives	Pruning	3.5
AFS20	Successional	Successional perennial	Banana, diverse fruits	-	Gliricidia ( <i>Gliricidia sepium</i> ), natives, Mombaça ( <i>Panicum maximum</i> )	Pruning, mowing	3.5
AFS21	Successional	Successional horticulture	Curcuma, banana, vegetables, medicinals	-	Natives	Pruning	3.5
AFS22	Successional	Complex multistrata	Citrus fruits, banana, coffee	-	Natives	Rare pruning	3
AFS23	Silvopasture	iLF	Signal grass ( <i>Urochloa decumbens</i> )	Natives	-	Grazing	12
AFS24	Silvopasture	iLF	Palisade grass ( <i>Urochloa ruziensis</i> )	Eucalypt ( <i>Eucalyptus urograndis</i> )	-	Grazing	9
AFS25	Successional	Successional horticulture	Vegetables, medicinals, diverse fruits	-	Gliricidia ( <i>Gliricidia sepium</i> ), Mulberry ( <i>Morus nigra</i> )	Pruning	3
AFS26	Successional	Successional perennial	Coffee	Teak ( <i>Tectona grandis</i> )	Natives	Pruning, mowing	15
AFS27	Multistrata	Simple multistrata	Rotation of field intercrops	-	Gliricidia ( <i>Gliricidia sepium</i> ), natives	Pruning, tillage	3
AFS28	Successional	Successional horticulture	Vegetables, banana	-	Gliricidia ( <i>Gliricidia sepium</i> ), natives	Pruning	8
AFS29	Silvopasture	iLF	Switch grass ( <i>Panicum maximum</i> cv Mombaça)	-	Amendoim bravo ( <i>Pterogyne nitens</i> )	Grazing	8
AFS30	Successional	Successional perennial	Coffee, diverse fruits	Mahogany ( <i>Khaya senegalensis</i> )	Gliricidia ( <i>Gliricidia sepium</i> ), natives	Pruning, mowing	3.5



**Supplementary Table 2.2** | Model output relating to Fig. 2.5 showing how the stand structural complexity index is associated to tree species richness, stem density (log) and their interaction.

Response variable	SSCI			
Residuals: Min	1Q	Median	3Q	Max
-1.18866	-0.41012	0.00318	0.41528	0.87522
Coefficients	Estimate	SE	Sig	
(Intercept)	-3.51281	0.77565	<0.001	***
Stem density (log)	0.46468	0.10994	<0.001	***
Tree species richness	-2.54590	0.65407	<0.001	***
Stem density: Tree species richness	0.41554	0.09206	<0.001	***
Adj. R <sup>2</sup> : 0.71			p-value: <0.001	


**Supplementary Figure 2.1** | Relationship between wood density (g cm<sup>-3</sup> community weighted mean) and the share of pioneer species in the sampled systems (p<0.001).



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**Litter sampling in successional agroforestry system on Fazenda da Toca, Itirapina, São Paulo, Brazil.**

A 0.5 x 0.5 m wooden frame was used to sample litter at six locations in each agroforestry system. Fine litter was then dried, weighted and analysed for N, P, K, Ca and Mg contents in the laboratory.





03



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

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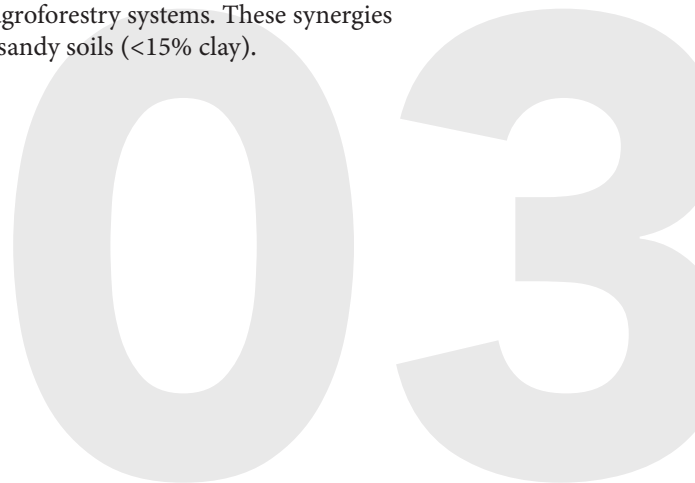
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## ABSTRACT

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 São 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<sup>-1</sup>). 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).



## 3.1 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 (Metcalf 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) (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 1970s, 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  $\mu\text{m}$ ), known as mineral-associated organic C (MAOC), and particulate organic C (POC; 53-2000  $\mu\text{m}$ ) (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 the 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.

### 3.1.2 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 – 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.

## 3.2 Methods

### 3.2.1 Agroforestry in São 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 experiment-



ing with silvopastoral systems, often by planting widely-spaced rows of eucalypt trees into pastures (de Souza Filho et al., 2020). 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).

### 3.2.2 Study region and sites

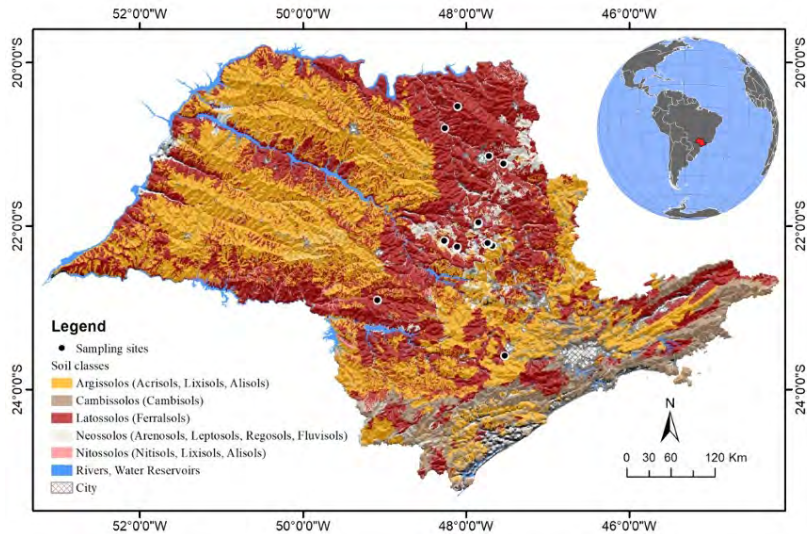
The study region is a transition zone between Atlantic Forest and *Cerrado* biomes. Figure 3.1 shows the distribution of the 38 sites in relation to the soil types found in the State of São Paulo, and most of the systems were located on highly weathered Ferralsols (*Latosolos* in Brazilian soil classification, Rossi (2017)). The climate is classified as Cwa according to Köppen criteria with humid summers and dry winters and average annual precipitation of 1,350 - 1,550 mm (Alvares et al., 2013).

Thirty-eight sites were purposefully selected in the central-East of São Paulo state, of which 30 were agroforestry and eight monocrop sites, consisting of pastures (6) and organically managed monocrops (2, soy-maize rotations), to represent a complexity gradient (Fig. 3.2 and Chapter 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 3.1. Mean age of the agroforestry sites at the time of sampling was 5.2 ( $\pm 0.66$ , SE) years, reflecting the relatively recent increase in adoption of innovative agroforestry systems in the State of São 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. (2024).

### 3.2.3 Agroforestry Complexity 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. 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 (stems ha<sup>-1</sup>) influences SOC storage in agroforestry systems (Cardinael et al., 2018; Saha et al., 2009), and is a good indicator of the spatial structure of agroforestry systems. Lastly, shade tree management influences SOC storage and nutrient cycling (Cardinael et al., 2021; Tscharrntke 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., 2018) as well as other agroforestry systems in the state of São Paulo, Brazil (Cezar et al., 2015; Froufe et al., 2019). Therefore, the frequency of pruning & mulching was included in our complexity assessment to represent its management dimension.



**Figure 3.1** | 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.



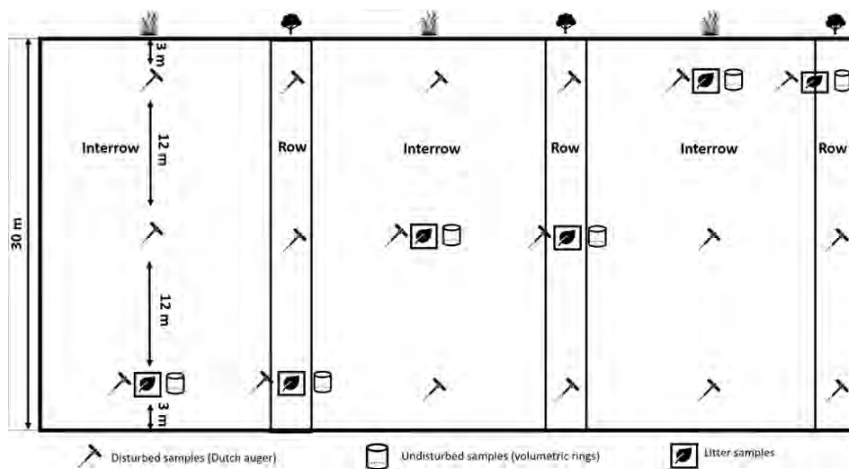
**Figure 3.2** | Complexity gradient among sampled agroforestry systems and pasture as defined by the Agroforestry Complexity Index: A: 0, B: 0.12, C: 0.28, D: 1.52, E: 1.63, F: 2.14. See text for the explanation of the index.

Data collection to quantify the complexity of the agroforestry sites is described in detail in Chapter 2. In short, a tree species inventory was conducted in three tree rows on a total of at least 75 individual trees per site (Fig. 3.3). 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 (Table 3.1) into a value between 0 and 1, and adding 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., 2020; Cerda et al., 2017; Mas and Dietsch, 2003) to ensure that the component scores received an equal weight.

**Table 3.1** | 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 – soybean rotation).

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



**Figure 3.3** | Schematic representation of sampling design applied in the agroforestry plots. Tree rows (rows) and spaces between tree rows (interrows) constituted sub-plots. 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 0.5 m frame on the same locations as undisturbed samples prior to opening the soil pit.

### 3.2.4 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. 3.3). Leaf litter and woody branches <2 cm in diameter were collected, dried at 70° C for 48h, 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<sup>-1</sup>. 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<sup>-3</sup> was applied for deadwood biomass estimation (Clark et al., 2002). A deadwood C fraction of 0.47 g cm<sup>-3</sup> 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.

### 3.2.5 Soil sampling

Disturbed soil samples were taken at 18 (nine row, nine 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 six composite samples per site (three row and three interrow; Fig. 3.3). Undisturbed soil samples were retrieved using volumetric rings at six (three row, three interrow) points in each plot at the same depth intervals as the disturbed samples, resulting in 18 samples per system (Fig. 3.3). Disturbed samples were dried and ground (2 mm) for chemical analysis.

### 3.2.6 Soil texture

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



### 3.2.7 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<sup>-1</sup> 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).

### 3.2.8 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) as adopted from Cambardella and Elliott (1992), where soil was fractionated by size (53 µm) after full dispersion using dilute sodium hexametaphosphate (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.

### 3.2.9 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., 2022b). 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<sup>-1</sup>) 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.

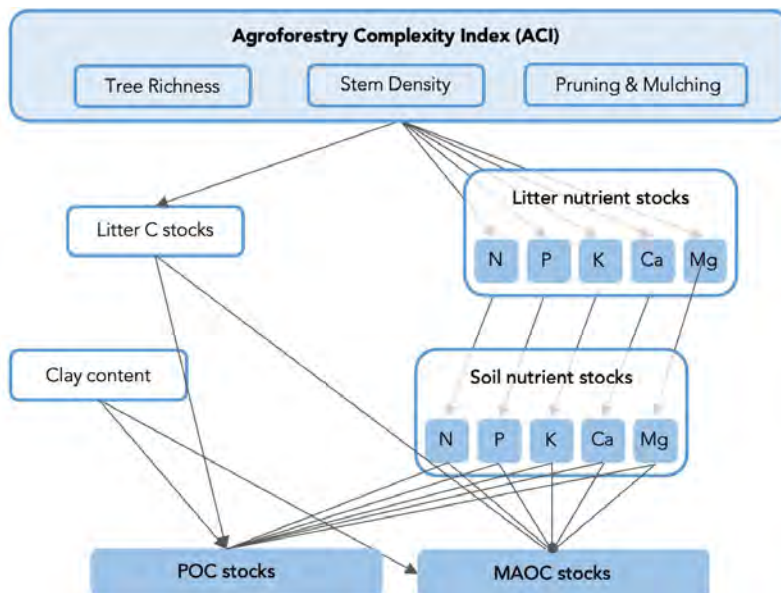
### 3.2.10 Statistical analysis

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 and Schielzeth (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  $\sim$  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. 3.4). 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 ( $\chi^2$ )  $> 0.05$  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.

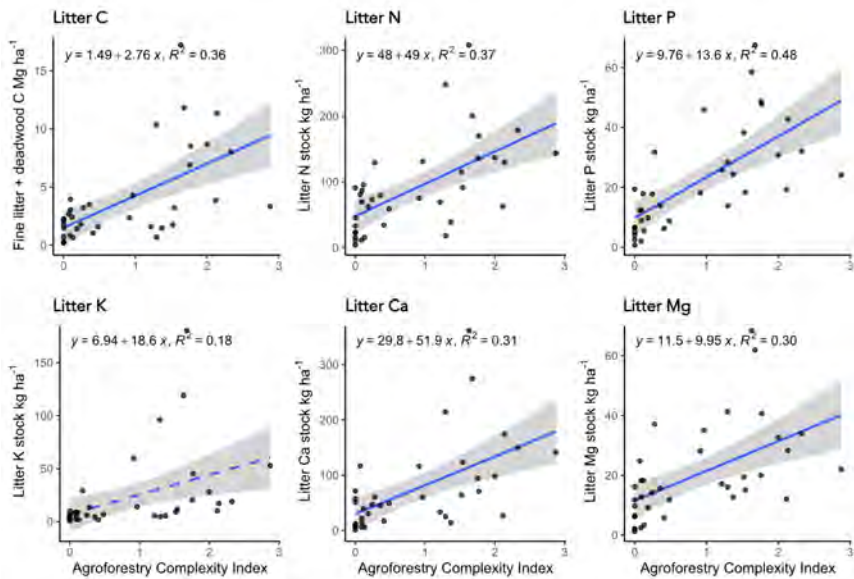


**Figure 3.4** | Schematic representation of pathways that were tested using Structural Equation Models, linking the Agroforestry Complexity Index (ACI) with carbon stocks (MAOC and POC). Arrows indicate the 12 different path models that were tested.

### 3.3 Results

#### 3.3.1 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. 3.5). 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.



**Figure 3.5** | Linear relationships between litter C and nutrients stocks (kg ha<sup>-1</sup>) and the Agroforestry Complexity Index (ACI). Solid and dashed lines indicate significant ( $p < 0.05$  level) and non-significant relationships ( $p > 0.05$ ).

#### 3.3.2 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 3.2). Clay content (g kg<sup>-1</sup>) was also significantly associated to all soil nutrients, except for P.

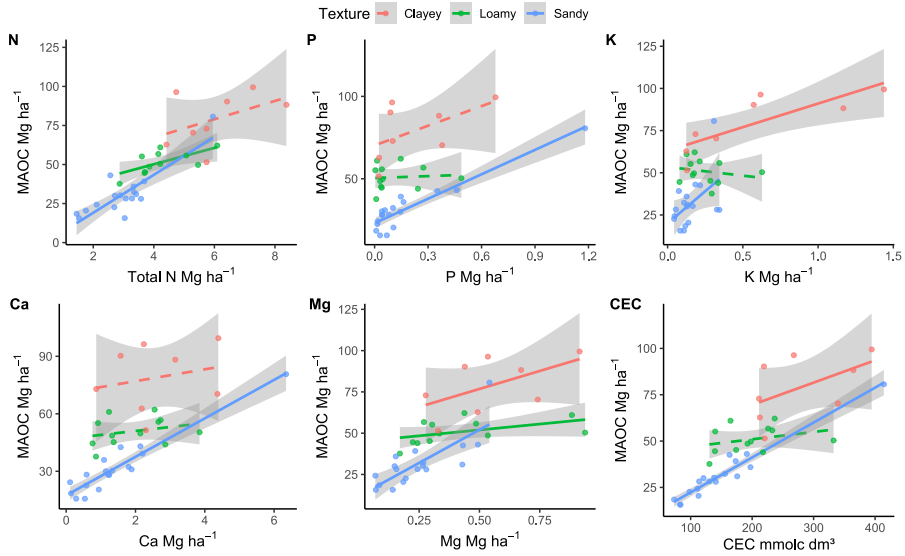
**Table 3.2** | Output of linear mixed models testing the relationships between soil nutrients (response variables) and litter nutrients, clay content and their interaction (explanatory variables). Data were normalized and p-values were obtained using Satterthwaite approximations. Estimates, p-values,  $R^2m$  (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.

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

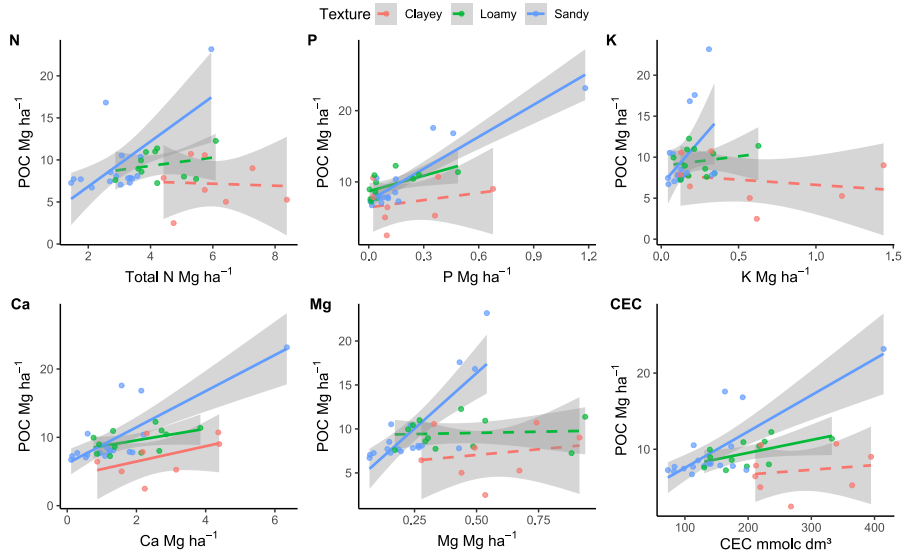
### 3.3.3 Relationships between soil nutrients and SOC fractions

On sandy soils, MAOC stocks were strongly positively associated with total N, P, K, Ca, Mg and CEC (Fig. 3.6). 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. 3.6). 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. 3.7). 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,  $p < 0.0001$ ).



**Figure 3.6** | 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 ( $p > 0.05$ ).

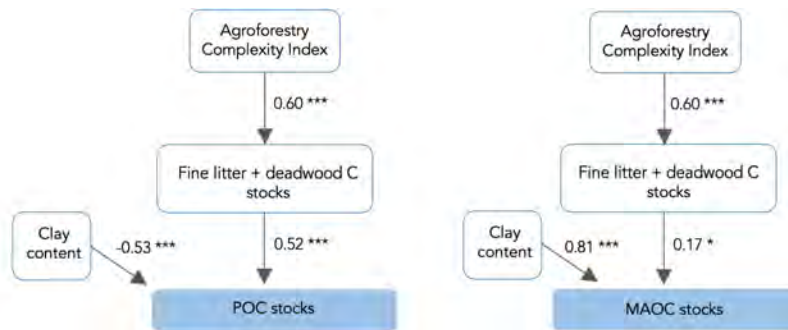


**Figure 3.7** | Relationships between soil nutrients (Total N, P, K, Ca, Mg), Cation Exchange Capacity (CEC) and particulate organic C (POC) stocks in the 0-30 cm soil layer. Significant ( $p < 0.05$  level) and non-significant relationships ( $p > 0.05$ ).



### 3.3.4 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. 3.8), and the effect of ACI on the five nutrients under consideration (N, P, K, Ca, Mg) and POC and MAOC stocks (Fig. 3.9). 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. 3.8). The ACI also linked litter P stocks, soil P stocks and, in turn, MAOC and POC stocks (CFI of both 1.0; Fig. 3.9). 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. 3.9).

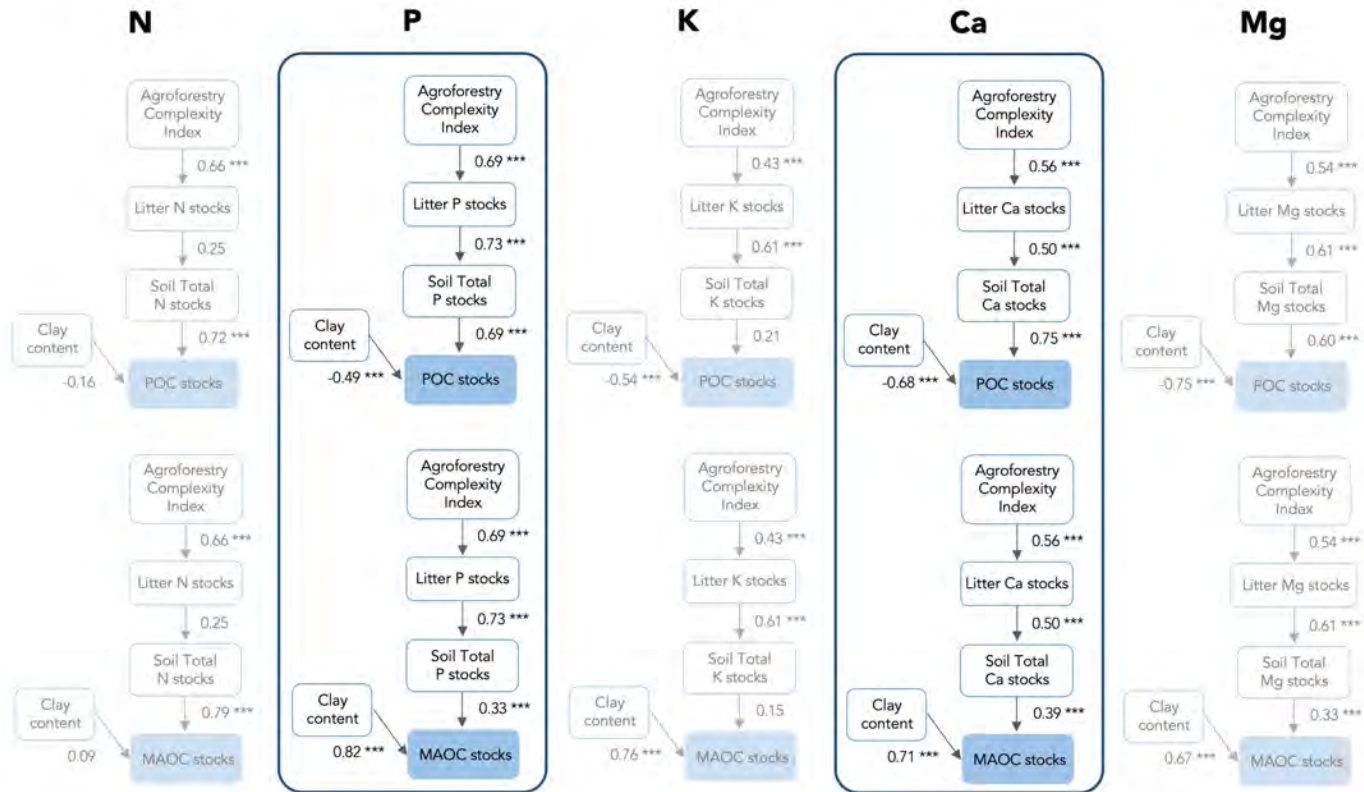


**Figure 3.8** | Outcomes of structural equation models testing the links between agroforestry complexity, C stocks of fine litter + deadwood and POC and MAOC stocks in soil. Both models receive high support from the data (both CFI: 1.0). The coefficients from the structural equation models are displayed next to arrows and their significance levels indicated.

## 3.4 Discussion

### 3.4.1 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



**Figure 3.9** | 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.

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.

### 3.4.2 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; Marangui 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 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 and Leopold, 1988). Steinfeld et al. (2024) showed that pruning & mulching frequency had high explanatory power for litter nutrient stocks in the studied sites. Froufe et al. (2019) and Matos et al. (2020) also described the benefits of pruning & 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.

### 3.4.3 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., 2017). Humic acids have also been shown to adsorb to calcium phosphate precipitates (Alvarez et al., 2004; Cao et al., 2007; Grossl and Inskip, 1991) potentially stabilising C through organo-organic interactions at molecular interfaces (Rowley et al., 2017). 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 and biological interactions in soils with contrasting clay contents (Bacq-Labreuil et al., 2018).

### 3.4.4 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., 2019; Schneidewind et al., 2018) 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., 2022).

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., 2020). Therefore, better incentives to implement medium-highly complex agroforestry systems in these areas are recommended. Such incentives need to include farm-

er 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 the 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).

### 3.4.5 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., 2024), and the links reported here with SOC suggest that it is an important metric to take into account (Tschardt 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.

## 3.5 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.



## Supplementary material

**Supplementary Table 3.1** | Characteristics of sampled agroforestry systems (AFS), pasture and monocrop sites.

System ID	Type	Main production	Timber species	Support species	Management	Age (years)	Soil texture (g kg <sup>-1</sup> )		
							Clay	Silt	Sand
P1	Pasture	Brachiaria grass	-	-	Grazing	10	42	18	940
AFS1	Multistrata	Hibiscus, beans, medicinals	-	Natives	No pruning, little mowing; low organic fertilization	4	59	21	920
AFS2	Silvopasture	Rotation of feed crops and beans	Lemon eucalypt ( <i>Corymbia citriodora</i> )	-	Tillage, low organic fertilization	11	25	21	954
AFS3	Silvopasture	Grass ( <i>Urochloa brizantha</i> cv Miranda)	Lemon eucalypt ( <i>Corymbia citriodora</i> )	-	Grazing, no inputs in last 10 years	11	92	20	888
AFS4	Silvopasture	Grass ( <i>Urochloa brizantha</i> cv Miranda)	Lemon eucalypt ( <i>Corymbia citriodora</i> )	-	Grazing, no inputs in last 9 years	10	40	19	941
AFS5	Successional	Vegetables, coffee, diverse fruits, vanilla	Mahogany ( <i>Khaya senegalensis</i> ), Jequitiba ( <i>Cariniana legalis</i> )	Eucalypt ( <i>Euclayptus urograndis</i> ), Gliricidia ( <i>Gliricidia sepium</i> ), Mombaça ( <i>Panicum maximum</i> )	Frequent pruning, mowing and mulching; organic fertilization for vegetables, none for trees	3.5	102	29	869
AFS6	Successional	Vegetables, lemon, diverse fruits	Louro Pardo ( <i>Cordia trichotoma</i> )	Eucalypt ( <i>Euclayptus urograndis</i> ), Gliricidia ( <i>Gliricidia sepium</i> ), Mombaça ( <i>Panicum maximum</i> )	Frequent pruning, mowing and mulching; organic fertilization for vegetables, none for trees	3	90	22	889
AFS7	Successional	Lime	Mahogany ( <i>Khaya senegalensis</i> ), Red cedar ( <i>Toona ciliata</i> )	Eucalypt ( <i>Euclayptus urograndis</i> ), Gliricidia ( <i>Gliricidia sepium</i> ), Inga ( <i>Inga vera</i> ), Guapuruvu ( <i>Schizolobium parahyba</i> ), Mombaça ( <i>Panicum maximum</i> )	Pruning, mowing and mulching; organic fertilization for lime trees	3	85	22	893

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System ID	Type	Main production	Timber species	Support species	Management	Age (years)	Soil texture (g kg <sup>-1</sup> )		
							Clay	Silt	Sand
AFS8	Multistrata	Lime	Mahogany ( <i>Khaya senegalensis</i> )	Eucalypt ( <i>Eucalyptus urograndis</i> ), Mombaça ( <i>Panicum maximum</i> ), Banana (not present anymore at time of sampling)	Pruning, mowing and mulching;	4	97	26	877
Mono1	Monocrop	Maize	-	-	Organic fertilization and pest control	5	226	19	755
AFS9	Successional	Vegetables, banana	-	Eucalypt ( <i>Eucalyptus urograndis</i> ), Mulberry ( <i>Morus nigra</i> ), natives	Frequent pruning, mowing and mulching, organic inputs for vegetables, none for trees	3	134	24	842
AFS10	Multistrata	Avocado, macademia	-	Signal grass ( <i>Urochloa decumbens</i> )	Mowing	3	188	22	790
P2	Pasture	Brachiaria grass	-	-	Grazing	5	95	33	872
AFS11	Silvopasture	Palisade grass ( <i>Urochloa ruziensis</i> )	Eucalypt ( <i>Eucalyptus urograndis</i> )	-	Grazing	5	62	9	930
AFS12	Successional	Diverse fruits	-	Natives	Pruning, mowing	4.5	170	28	802
AFS13	Multistrata	Avocado, papaya, maize	-	Signal grass ( <i>Urochloa decumbens</i> )	Mowing, tillage	4	231	179	590
AFS14	Multistrata	Banana	Mahogany ( <i>Khaya senegalensis</i> )	Signal grass ( <i>Urochloa decumbens</i> )	Mowing	5	243	164	594
AFS15	Multistrata	Citrus fruits, curcuma	Mahogany ( <i>Khaya senegalensis</i> ), Jequitiba ( <i>Cariniana legalis</i> ), Louro Pardo, ( <i>Cordia trichotoma</i> )	Signal grass ( <i>Urochloa decumbens</i> )	Mowing	4	278	124	598
P3	Pasture	Brachiaria grass	-	-	Grazing	10	319	127	554

System ID	Type	Main production	Timber species	Support species	Management	Age (years)	Soil texture (g kg <sup>-1</sup> )		
							Clay	Silt	Sand
M2	Monocrop	Soy	-	-	Organic fertilization and pest control	4	151	17	832
AFS16	Silvopasture	Switch grass ( <i>Panicum maximum</i> cv Mombaça)	-	Eucalypt ( <i>Eucalyptus urograndis</i> ), Acacia ( <i>Acacia mangium</i> ), Gliricidia ( <i>Gliricidia sepium</i> )	Grazing	3	156	19	825
AFS17	Silvopasture	Switch grass ( <i>Panicum maximum</i> cv Mombaça), Leucena ( <i>Leucaena leucocephala</i> )	Mahogany ( <i>Khaya senegalensis</i> )	Eucalypt ( <i>Eucalyptus urograndis</i> ), Acacia ( <i>Acacia mangium</i> ), Gliricidia ( <i>Gliricidia sepium</i> )	Grazing	3	125	25	850
AFS18	Silvopasture	Switch grass ( <i>Panicum maximum</i> cv Mombaça), Mexican sunflower ( <i>Tithonia diversifolia</i> )	Mahogany ( <i>Khaya senegalensis</i> )	Eucalypt ( <i>Eucalyptus urograndis</i> ), Acacia ( <i>Acacia mangium</i> ), Gliricidia ( <i>Gliricidia sepium</i> )	Grazing	3	157	18	826
P4	Pasture	Switch grass ( <i>Panicum maximum</i> cv Mombaça)	-	-	Grazing	3	141	23	836
AFS19	Successional	Vegetables, coffee, diverse fruits	-	Gliricidia ( <i>Gliricidia sepium</i> ), natives	Pruning	3.5	236	57	707
AFS20	Successional	Banana, diverse fruits	-	Gliricidia ( <i>Gliricidia sepium</i> ), natives, Mombaça ( <i>Panicum maximum</i> )	Pruning, mowing	3.5	265	28	707
P5	Pasture	Brachiaria grass	-	-	Extensive	5	293	19	688
AFS21	Successional	Curcuma, banana, vegetables, medicinals	-	Natives	Pruning	3.5	240	23	737
AFS22	Successional	Citrus fruits, banana, coffee	-	Natives	Rare pruning	3	94	21	885

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System ID	Type	Main production	Timber species	Support species	Management	Age (years)	Soil texture (g kg <sup>-1</sup> )		
							Clay	Silt	Sand
AFS23	Silvopasture	Signal grass ( <i>Urochloa decumbens</i> )	Natives	-	Grazing	12	274	24	702
AFS24	Silvopasture	Palisade grass ( <i>Urochloa ruziensis</i> )	Eucalypt ( <i>Eucalyptus urograndis</i> )	-	Grazing	9	344	49	607
P6	Pasture	Brachiaria grass	-	-	Grazing	12	274	15	711
AFS25	Successional	Vegetables, medicinals, diverse fruits	-	Gliricidia ( <i>Gliricidia sepium</i> ), Mulberry ( <i>Morus nigra</i> )	Pruning	3	483	118	399
AFS26	Successional	Coffee	Teak ( <i>Tectona grandis</i> )	Natives	Pruning, mowing	15	537	294	169
AFS27	Multistrata	Rotation of field intercrops	-	Gliricidia ( <i>Gliricidia sepium</i> ), natives	Pruning, tillage	3	580	204	216
AFS28	Successional	Vegetables, banana	-	Gliricidia ( <i>G. sepium</i> ), natives	Pruning	8	567	274	159
AFS29	Silvopasture	Switch grass ( <i>Panicum maximum</i> cv Mombaça)	-	Amendoim bravo ( <i>Pterogyne nitens</i> )	Grazing	8	559	349	92
AFS30	Successional	Coffee, diverse fruits	Mahogany ( <i>Khaya senegalensis</i> )	Gliricidia ( <i>Gliricidia sepium</i> ), natives	Pruning, mowing	3.5	578	307	116





**Soil sampling using volumetric rings in a multistrata agroforestry system (left) and a silvopasture (right).**

These samples were used to determine soil bulk density, total porosity, as well as macro- and microporosity. These soil structural properties are highly relevant for water regulation in soils.

ROAD\_BANANA  
0-10  
E3

ROAD\_BANANA  
10-20  
E3

ROAD\_BANANA  
20-30  
E3





EMBRAPA  
0-10  
E1

EMBRAPA  
10-20  
E1

EMBRAPA  
20-30  
E1

04



# Soil organic carbon storage, nutrient provision and water regulation boosted by increasing complexity of agroforestry systems in south-eastern Brazil

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## ABSTRACT

Diversified systems such as agroforestry are often promoted based on the assumption that they can increase the simultaneous provision of multiple soil-based ecosystem services and constitute more resilient systems in the face of environmental change. However, such systems are also more complex and transitioning towards higher complexity is a challenge for most farmers whose starting points are highly simplified monocultural systems. Therefore, it is relevant to investigate what the benefits of gradual increases in complexity are and also whether increasing complexity yields the same response in different contexts. The objective of this study was to empirically test the effect of agroforestry complexity on the simultaneous provision of three important soil-based ecosystem services (SOC storage and cycling, nutrient provision, water regulation) and determine whether age, and inherent soil properties, such as texture and soil depth, mediate this relationship. We analysed a total of 201 soil samples, taken at three depth intervals (0-10, 10-20, 20-30) in 67 plots across 30 agroforestry and 8 monocultural sites that represent a complexity gradient. This complexity was defined by the Agroforestry Complexity Index (ACI), which is based on tree species richness, stem density and pruning & mulching management. Sites were spread across a soil textural gradient ranging from very sandy to very clayey (clay content range 25 – 620 g kg<sup>-1</sup>) in the centre-east of São Paulo State, Brazil and ranged in age from 3 – 14 years. Indicators of ecosystem service provision were measured and used in linear mixed models as response variables, being mineral-associated organic C (MAOC), particulate organic C (POC) and microbial biomass C (MBC) to reflect C storage and cycling, soil P (resin), Cation Exchange Capacity (CEC) and pH to reflect nutrient provision and macroporosity, microporosity and estimated available water capacity (AWC) to reflect water regulation. The ACI was positively associated with indicators of SOC storage and cycling (MAOC, POC), nutrient provision (P and CEC) and water regulation (macroporosity and AWC). However, for MAOC, POC, pH and AWC these effects were mediated by soil texture, indicating less strong effects on clayey soils. For POC and CEC, depth was a significant mediator of ACI, indicating a decrease in effect size from 0-10 to 20-30 cm. Although the effect of complexity is weaker on clayey soils, significant three-way interactions between ACI, clay and depth for MAOC, POC, P, CEC and AWC indicate that in these soils the effects reach deeper into the soil profile. The tested age gradient did not reveal significant effects, except for pH where ACI and age showed a combined positive effect. MBC and microporosity were positively associated with clay content, but not with ACI. This study clearly demonstrates the ecological benefits of increasing the complexity of agroforestry systems. However, it also shows the context dependency of these effects and can therefore inform farmers and other stakeholders on where to most efficiently invest in complexifying agroforestry systems.

## CHAPTER 4

## 4.1 Introduction

In order to remain resilient in the face of environmental change, farmers need to manage their soils in ways that increase their capacity to simultaneously provide vital ecosystem services, such as soil organic carbon (SOC) storage and cycling, the provision of nutrients and water regulation (IPCC, 2022). Diversification of agricultural systems has been proven to be effective in increasing the provision of these ecosystem services (Beillouin et al., 2021; Tamburini et al., 2020). However, large variation exists in the range and extent to which these ecosystem services are delivered between different types of diversified systems and it is not clear why (Beillouin et al., 2021; Lamichhane, 2023). Diversified production systems are composed of heterogeneous, interacting components, and therefore neatly fit the definition of complex systems (Filotas et al., 2014). An often implicit, but rarely explicitly tested hypothesis is that the capacity of an agricultural system to provide a range of ecosystem services is defined by its level of complexity (Malézieux, 2011; Parrott, 2010). In order to provide farmers and policy makers with advice on how to design systems that provide more SOC storage and cycling, nutrient provision and water regulation, the relationship between complexity and the simultaneous provision of these ecosystem services should therefore be further explored.

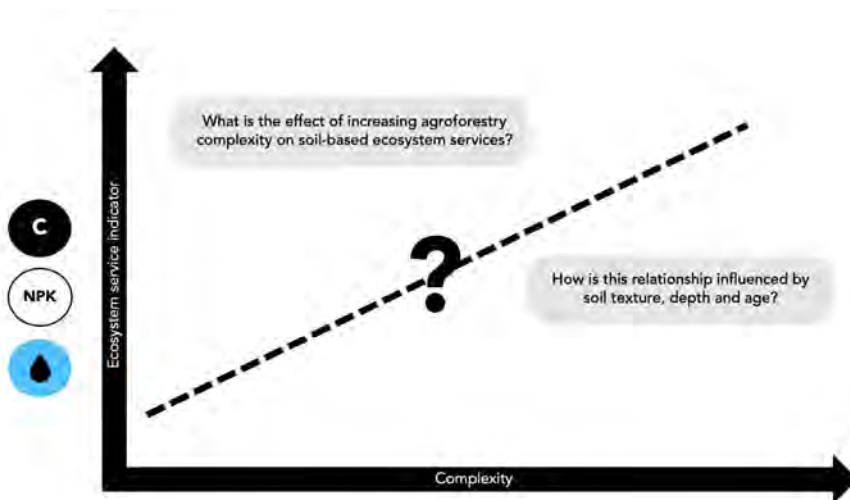
Agroforestry, the integration of crops and trees (Nair et al., 2021), has been shown to be the most effective diversification strategy in providing multiple ecosystem services (Beillouin et al., 2021). More than 100 types of agroforestry systems have been defined in the literature (Atangana et al., 2013) from very simple systems through to highly complex systems which aim to mimic natural forest structures. When assessing the benefits of agroforestry systems on the delivery of soil-based ecosystem services, most studies have only focused on the comparison to strongly contrasting systems such as monocultures (De Stefano and Jacobson, 2017; Feliciano et al., 2018; Hübner et al., 2021; Kuyah et al., 2019; Ma et al., 2020; Muchane et al., 2020; Santos et al., 2019; Shi et al., 2018; Torralba et al., 2016). Some studies compare between the different types of agroforestry systems, such as silvopastures and multistrata systems but this often results in conflicting outcomes (see Feliciano et al., 2018; and Shi et al., 2018). Therefore, this paper will move away from the categorisation of agroforestry systems and use a continuous complexity gradient to assess why some agroforests provide more ecosystem services than others.

Quantifying the complexity of a system can be challenging (Parrott, 2010). Nevertheless, a continuous measure of an agroforestry systems' degree of complexity can be approximated by assessing the heterogeneity of its components, e.g. by quantifying species diversity (Naeem, 2013) or spatial structure (Seidel et al., 2019; Seidel et al., 2021). Interactions between these components abound, and are also influenced by farmers' management, e.g.



by the frequency of pruning and *in situ* mulching (Schneidewind et al., 2018; Steinfeld et al., 2024; Tschardt et al., 2011). To account for this multidimensionality of complexity, composite indices can be constructed by adding relevant and standardized metrics (Paoli et al., 2016; Rebout et al., 2021). The Agroforestry Complexity Index (ACI) is such a composite index, based on tree species richness, tree stem density and pruning & mulching frequency (Steinfeld et al., 2023).

More complex, often referred to as regenerative, systems are being promoted to enhance the capacity of the soil to deliver multiple ecosystem services (Schreefel et al., 2020), but it is often not clear to what extent they perform across different contexts (Giller et al., 2021). E.g. in a previous study on the effect of increasing agroforestry complexity, the synergies between nutrient cycling and SOC storage were shown to be more likely on sandy soils than on clayey soils (Steinfeld et al., 2023). It is not clear, however, whether this also holds for properties relevant for water regulation, which will become increasingly important for climate change adaptation (UNCCD, 2022). Likewise, other factors such as depth might interact with complexity, e.g. that some soil properties such as nutrient availability only significantly increase in more complex systems in the most shallow layers (0-10 cm) but not in deeper layers (e.g. 20-30 cm). This has been observed in naturally regenerating forests in south-eastern Brazil (Bieluczyk et al., 2023b). While global studies show that C sequestration rates ( $\text{Mg ha}^{-1} \text{ year}^{-1}$ ) are highest in the early years of agroforestry implementation (Feliciano et al., 2018; Ma et al., 2020), it could still be that complexity and age positively interact, meaning that older and more complex systems would have stronger effects on ecosystem service indicators than younger ones.



**Figure 4.1** | Graphic representation of the research questions addressed in this study. Symbols on the left represent three soil-based ecosystem services SOC storage and cycling (black), nutrient provision (white) and water regulation (blue). Specific ecosystem service indicators as well as the Agroforestry Complexity Index are described in the methods section.

### 4.1.2 Objectives

The objective of this study was, therefore, to empirically test the effect of agroforestry complexity on the simultaneous provision of three important soil-based ecosystem services (SOC storage and cycling, nutrient provision, and water regulation) and to determine whether age, and inherent soil properties such as texture and topsoil depth mediate this relationship (Fig. 4.1).

## 4.2. Methods

### 4.2.1 Climate and soils in the study region

The study took place in the central region of the state of São Paulo, south-eastern Brazil. The climate in this region is tropical moist with dry winters (Aw) according to Köppen's classification. Mean annual precipitation ranges from 1,350 to 1,550 mm. The rainy season occurs during October-March, coinciding with a higher temperature (austral summer) and the dry season starts in April and ends in October (Alvares et al., 2013; Rodríguez-Lado et al., 2007). The study region harbours two biomes, the Atlantic Forest and the *Cerrado* (Brazilian savannah), both considered threatened biodiversity hotspots (Garcia and Ballester, 2016; Myers et al., 2000). Southeast Brazil is also estimated to have been a historic SOC hotspot with accelerated SOC losses in the last 200 years (Sanderman et al., 2017). Soils in the region are typically highly weathered, with Ferralsols (*Latossolos* in Brazilian soil classification) being the most frequent soil type (Rossi, 2017). Topsoil texture can vary in these soils, but generally, the fine fraction is dominated by low activity 1:1 clays and the coarse fraction by quartz (Schaefer et al., 2008). The clay content of our sites in the 0-30 cm layer varied from 25 – 620 g kg<sup>-1</sup>, representing a large textural gradient (Fig. 4.2).



**Figure 4.2** | Predominant soil texture map of São Paulo State, Brazil according to Rossi (2017) and sampled sites ( $n=38$ ) (left). On the right, soil texture (mean of three depth intervals) as determined via laboratory analysis of the 67 plots (circled dots) that were sampled across the 38 sites.

### 4.2.2 Agroforestry in the study region

São Paulo State has been a hotspot of agroforestry projects in recent years (Agricone, 2022; MapSAF, 2022) and innovative farmers have been integrating trees in a variety of ways, resulting in a complexity gradient of systems (Steinfeld et al., 2024). Relatively simple silvopastures (Fig. 4.3, B), such as integrated-Crop-Livestock-Forestry systems (EMBRAPA, 2022; Guerreiro et al., 2013) combine pastures with fast-growing timber production (mostly eucalypt cultivars). More complex ‘multi-strata’ systems (Fig. 4.3, C and D) typically integrate several tree and crop species forming multi-layered agroforests for fruit production. Most complex ‘successional’ systems (Fig. 4.3, E and F) combine high species diversity and density levels with intensive pruning & mulching management to promote *in situ* organic matter cycling (Andrade et al., 2020; Götsch, 1994). We identified 30 agroforestry sites that represent these three types of increasing complexity, which are comprehensively described in Steinfeld et al. (2024).



**Figure 4.3** | Complexity gradient among sampled sites (monocultures and agroforestry) 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.

### 4.2.3 Sites and Agroforestry Complexity Index

The 30 agroforestry sites were complemented with 8 monocultural sites (6 pastures and 2 maize/soybean rotations, Fig. 4.3 A), totalling 38 sites representing a complexity gradient. This gradient was quantified using our Agroforestry Complexity Index (ACI), which is introduced in Steinfeld et al. (2023). Briefly, it is based on three components that reflect taxonomic diversity, spatial structure and management. Tree species richness, stem density and pruning & mulching frequency were assessed in the 38 sites, standardized to values between 0-1 and added to calculate the ACI for each site, making this index in structure similar to other agroforestry indices (e.g. Cerda et al., 2017; Mas and Dietsch, 2003) or a complexity index devised for marine ecological research (Paoli et al., 2016). The ACI has previously been shown to be positively associated with nutrient cycling (N, P, Ca, Mg) through litter at the same sites that were analysed in the present study (Steinfeld et al., 2023). The time since implementation of the sites, referred to as age, ranged from 3 – 14 years.

#### *Sampling plots*

All but one of the 30 agroforestry systems were planted in linear rows and at each site an area of 30 m length was demarcated. The width of this area was variable but always three tree rows wide, including the space in between rows (interrows). In

this area, rows and interrows were sampled separately, hence yielding two plots per agroforestry site (suppl. Fig. 4.1). In the monocultural fields and the one agroforestry site that was not planted in rows, an area of 30 x 30 m was used for sampling, meaning these sites had only one plot. Representativeness of the sampled areas was assessed visually and confirmed with farmers, and steep slopes were avoided.

#### 4.2.4. Soil sampling

Between January and February 2021, disturbed soil samples were collected using a Dutch auger at nine points per plot (suppl. Fig. 4.1) and pooled to create a composite sample. Sampling was done for three depth intervals (0-10 cm, 10-20 cm, 20-30 cm) resulting in a total of 201 disturbed soil samples (collected across the 67 agroforestry plots). Samples were stored in cool boxes and transported to the lab for further analysis within a maximum of 4 days. Undisturbed soil samples were collected using volumetric rings at three points per plot (suppl. Fig. 4.1) and in the centre of the corresponding depth intervals (0-10 cm, 10-20 cm, 20-30 cm), resulting in a total of 603 samples which were transported to the laboratory for further analysis.

#### 4.2.5. Soil analysis

##### *Soil texture*

Soil texture was determined for the 201 disturbed soil samples using the Buycocou (densimeter) method (Dane and Topp, 2020) and five sand fractions (very coarse, coarse, medium, fine, very fine) were determined according to USDA size classes.

##### *Ecosystem service indicators*

We used commonly applied indicators as proxies for the provision of soil-based ecosystem services (Bünemann et al., 2018). For SOC storage and cycling, we used mineral-associated organic C (MAOC), particulate organic C (POC) and microbial biomass C (MBC). MAOC constitutes a stabilised pool of SOC as it is physically and chemically more protected than other fractions, whereas POC tends to cycle faster and therefore, constitutes an essential resource for soil biota (Cotrufo and Lavelle, 2022; Kögel-Knabner et al., 2022). MBC is a measure of the quantity of soil biota and commonly applied in Brazilian soil biological monitoring (Alves De Castro Lopes et al., 2013).

For nutrient provision, we used available P (resin), Cation Exchange Capacity (CEC) and pH. P constitutes the most critical nutrient in Brazilian agriculture (Withers et al., 2018), CEC represents the availability of  $K^+$ ,  $Ca^{2+}$  and  $Mg^{2+}$  in relation to  $Al^{3+}$ , and pH directly influences the availability of macro- and micronutrients.

For water regulation, we used macro- and microporosity and available water capacity (AWC). Macroporosity measures how well water can infiltrate and percolate through the soil (Bouma, 1991), whereas microporosity is related to soil water retention (de Jonge et al., 2000). AWC is a composite measure which reflects plant-available water storage (Tomasella et al., 2000).



### *SOC storage and cycling*

Soil physical fractionation of the disturbed soil samples to obtain MAOC and POC fractions was carried out according to the method of Cotrufo et al. (2019) and adapted from Cambardella and Elliott (1992). Soil was fractionated by size after full dispersion using dilute sodium hexametaphosphate (0.5%) and glass beads in a horizontal shaker for 16 h (140 rpm). Soil was rinsed through a sieve (53  $\mu\text{m}$ ), 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 concentrations were determined via dry combustion using a LECO TruSpec CN (LECO Corporation, St. Joseph, MI, USA). Stocks were corrected for differences in soil bulk densities between sites by using the equivalent soil mass (ESM) approach (Ellert and Bettany, 1995) and taking reference values specific to each soil textural class (clayey, loamy, sandy, see Chapter 3 for further details).

MBC (mg C g<sup>-1</sup> dry soil) was determined using the fumigation extraction method (Vance et al., 1987).

### *Nutrient provision*

P was extracted using ion exchange resin and determined via colorimetry at 725 nm wavelength. Cations (K, Ca and Mg) were extracted using respective ion exchange resins and determined via atomic absorption spectroscopy, and pH was measured using CaCl<sub>2</sub> 0,01 mol L<sup>-1</sup>. Aluminium (Al) was determined by titration with KCl 1 mol L<sup>-1</sup> 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' lab, and procedures are detailed in van Raij et al. (2001).

### *Water regulation*

Macro- and microporosity were derived following the method of EMBRAPA (2017) by estimating microporosity (m<sup>3</sup> m<sup>-3</sup>, pores < 50  $\mu\text{m}$ ) as the volumetric water (VW) at -6 kPa water potential and macroporosity (m<sup>3</sup> m<sup>-3</sup>, pores > 50  $\mu\text{m}$ ) as the difference between total porosity and microporosity. Total porosity was derived according to the following formula:

$$\text{Total soil porosity (cm}^3 \text{ cm}^{-3}\text{)} = 1 - ((\text{bulk density g cm}^{-3})/(\text{particle density g cm}^{-3})).$$

For total porosity and bulk density, all 603 undisturbed soil samples were analysed and the mean of each plot's and depth's triplicate (n=201) was used for statistical analysis. For macro- and microporosity, samples from 13 plots (117 of the 603 samples) were not measured due to an administrative error, and so for statistical analysis only data from the reduced number of 54 plots (n=162) were used. The pedotransfer function (PTF) of Tomasella et al. (2000) for Brazilian soils was used to estimate available water capacity (AWC), using bulk density, SOC, coarse sand, fine sand, silt and clay.

### 4.2.6. Statistical analysis

Linear mixed effect models (Zuur et al., 2009) were used to explore the relationships between ACI, clay, depth and age (fixed effects) and ecosystem service indicators (response variables). As the dataset has a nested structure, the random effect 'site' was used to account for the fact that of the 201 samples, six samples per depth always came from the same site and were therefore more likely to be correlated (same microclimate, history, etc). Out of those six, however, there is a further differentiation as those from the same plot are even more likely to be correlated than those from the other plot, hence the additional random effect 'plot'. We report marginal  $R^2$  and conditional  $R^2$  of these mixed models (Nakagawa and Schielzeth, 2013).

For the analysis, data were scaled and in the case of P log-transformed to conform to assumptions of normality. Correlation between explanatory variables (ACI, clay, depth, age) was low or non-existent (suppl. Fig. 4.2 and 4.3). Full models with four fixed effects were tested and as age was only significant for pH, it was dropped and for the other ecosystem service indicators, models with ACI \* clay \* depth as fixed effects were used to facilitate interpretation. Significant effects were used to predict ecosystem service indicators in linear models and visualized using the predict function of the *sjPlot* package in R (Brooks et al., 2017; Johnson, 2014).

## 4.3 Results

Nine ecosystem service indicators were tested using separate mixed models (Table 4.1) and the detailed output can be found in the supplementary material (suppl. Fig. 4.4-4.6, suppl. Tables). In general, soil depth was the most significant determinant for eight out of nine indicators (MAOC, POC, MBC, P, CEC, pH, macroporosity, AWC) with negative effects, meaning that these indicator values were highest in the 0-10 cm layer and decreased to 20-30 cm depth. This decrease was strongest for POC and P (log). Clay was positively associated with MAOC, MBC, CEC, microporosity and AWC, and negatively with POC. The two variables where clay content had the strongest effect on were MAOC and microporosity. In contrast to depth and clay, age did not significantly correlate with any of the ecosystem service indicators.

### 4.3.1. ACI and SOC storage and cycling

MAOC and POC stocks were significantly and positively associated with ACI with a larger standardized effect size for POC (0.44), than for MAOC (0.25). However, for both MAOC and POC this relationship was mediated by clay content, as the interaction ACI \* clay was significant and negative (Table 4.1). This interaction can also be observed in Fig. 4.4, where the slope of the predicted line for sandy sites was steeper compared to the line for clayey sites. For POC, the interaction term ACI \* depth was also significant, indicating a decreasing effect of complexity on POC with depth, but this was not the case for MAOC. However, both MAOC and POC demonstrated significant three-way interactions between ACI \* clay \* depth, with

positive estimates. This indicates that the decrease with depth is less pronounced in more complex systems on clayey soils which can also be observed in Fig. 4.4, e.g. in the small decrease in predicted MAOC stocks between 0-10, 10-20 and 20-30 for high ACI sites on clayey soils. MBC did not have a significant relationship with ACI.

#### 4.3.2. ACI and nutrient provision

For P and CEC, the correlation with ACI was significant (standardised effect sizes of 0.55 and 0.42, respectively) (Table 4.1). The interaction of clay content and ACI was not significant for P and CEC, but for CEC, the interaction between ACI and depth was significant and negative (-0.16). That means that soil texture did not influence the positive effect of ACI on the available nutrient contents of P and CEC (see also almost parallel lines in Fig. 4.4), but that for CEC this effect diminished significantly in deeper soil layers (20-30 cm). Both P and CEC had a significant three-way interaction between ACI \* clay \* depth, meaning that in more complex systems on clayey soils, P and CEC did not decrease as much with depth as in less complex systems. For pH, we did not find a general positive effect of ACI, but both interaction terms ACI \* clay and ACI \* age (standardized effect sizes -0.32 and 0.45, respectively) were significant. This suggests that the effect of ACI on pH only becomes relevant in soils with low clay content and with increasing age, and can also be observed in the flat slope of the line predicted for clayey soils, in contrast to the relatively steep positive slope predicted for pH on sandy soils (Fig. 4.4).

#### 4.3.3. ACI and water regulation

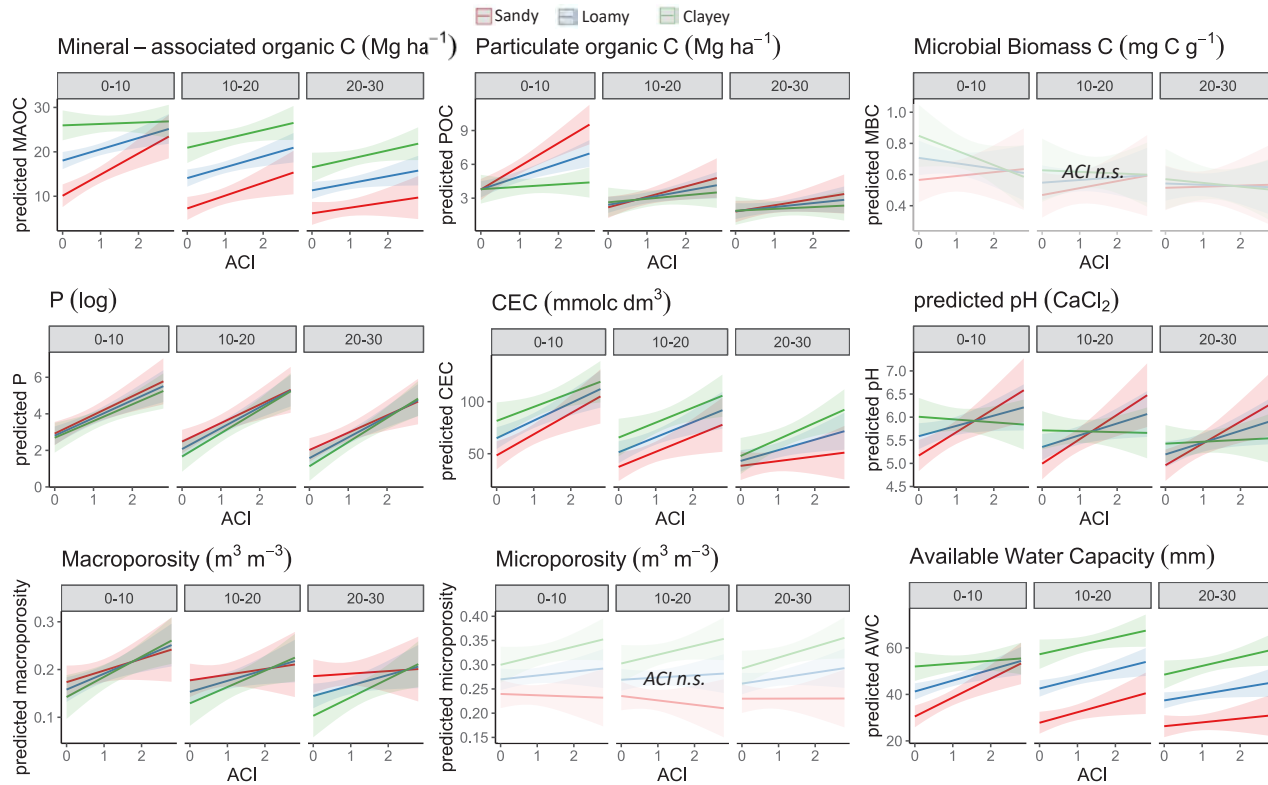
The ACI was positively associated with AWC and macroporosity, but not microporosity. For macroporosity, this positive relationship was stronger than for AWC (estimated standardized effect sizes 0.46 and 0.25, respectively) and not mediated by any other variable. For AWC, the positive effect diminished significantly with increasing clay content (ACI \* clay estimate size: -0.18), which can also be observed in the steeper slope of the predicted line for sandy soils for the 0-10 cm layer (Fig. 4.4). The three-way interaction ACI \* clay \* depth was significant for AWC (0.24), which just as for indicators of SOC storage and nutrient provision means that the decrease with depth is less pronounced in more complex systems on clayey soils.

**Table 4.1** | Standardised effect sizes and significance levels from the tested mixed models. In a first round, fixed effects were ACI, clay, depth, age and their interactions. In these models, age did not have significant effects for any ecosystem service indicator except for pH, and so was removed from the remaining models which were rerun as: Response variable ~ ACI \* Clay \* Depth.

\*PTF: Pedotransfer function

Ecosystem service	Response variable	Intercept	Clay	Depth	Age	Agroforestry Complexity Index (ACI)	Interactions				R <sup>2</sup> marginal / R <sup>2</sup> conditional
							ACI*Clay	ACI* Depth	ACI*Clay*Depth	ACI* Age	
C storage and cycling	MAOC	0.48***	0.68***	-0.89***	/	0.25**	-0.22 **	-0.09	0.25***	/	0.72/0.88
	POC	0.76***	-0.40***	-1.24***	/	0.44***	-0.35 **	-0.30*	0.28*	/	0.39/0.60
	Microbial Biomass C	0.32*	0.30*	-0.49***	/	-0.10	-0.17	0.07	0.13	/	0.07/0.69
Nutrient provision	P (log)	3.73 ***	-0.16	-1.07***	/	0.55***	-0.04	0.14	0.20*	/	0.40/0.90
	CEC	0.43***	0.40***	-0.84***	/	0.42***	-0.08	-0.16*	0.09**	/	0.46/0.86
	pH	0.52**	0.18	-0.49***	-0.21	0.24	-0.32**	0.06	0.04	0.45*	0.50/0.93
Water regulation	Macroporosity	0.21	-0.13	-0.35**	/	0.46**	0.14	-0.19	0.12	/	0.19/0.68
	Microporosity	-0.13	0.56***	-0.08	/	0.06	0.12	0.04	0.01	/	0.57/0.90
	Available Water Capacity (PTF*)	0.12	0.48***	-0.37***	/	0.25**	-0.18*	-0.11	0.24**	/	0.72/0.86





**Figure 4.4** | Modelled relationships between explanatory variables clay, depth and ACI on nine soil-based ecosystem service indicators. *n.s.*: non-significant

## 4.4 Discussion

### 4.4.1 What is the effect of increasing agroforestry complexity on the simultaneous provision of multiple ecosystem services?

In this paper, we present a generally positive effect of increasing complexity on the provision of multiple soil-based ecosystem services (Fig. 4.4). This effect was shown to depend on several interactions, but after accounting for these, a positive effect remained overall significant for MAOC, POC, P, CEC, macroporosity and AWC across all sites (Table 4.1, suppl. Fig. 4.4-4.6). This expands on the findings of Santos et al. (2019) who showed higher provision of supporting ecosystem services (such as nutrient provision) and regulating services (such as C storage or water regulation) from more biodiverse systems when compared to simple agroforestry systems, in the Brazilian Atlantic Forest biome. Similar results were found in Indonesia, where treatments of increasingly complex rice systems have shown a corresponding increase in the provision of ecosystem services such as productivity, pest regulation and nutrient cycling (Khumairoh et al., 2021; Khumairoh et al., 2018). Also, in Beillouin et al. (2021)'s global meta-analysis of the effects of crop diversification, agroforestry, as arguably the most complex of the studied practices, showed the strongest positive effects on associated biodiversity, agricultural production and soil quality. However, the ecological benefits from increased complexity are likely to come with increased labour demand (Esche et al., 2022; Gosling et al., 2021; Scudder et al., 2022). That poses a challenge for many farmers and calls for more research into how to complexify systems while keeping them manageable (Wolz et al., 2018).

While our study underlines the ecological importance of agroforestry complexity, we did not explore the mechanisms that drive these outcomes. However, based on previous studies, we postulate that in these diversified systems the practice of pruning 'biomass' trees and using the generated residues as mulch is of crucial importance to enhance multiple ecosystem services. While such *in situ* mulching can provide C inputs of 4 Mg C ha<sup>-1</sup> year<sup>-1</sup> (Schneidewind et al., 2018), in our sites it was also shown that the nutrients in this mulch litter, particularly Ca<sup>2+</sup> and P, facilitate synergies between ecosystem services (Steinfeld et al., 2024). E.g. Ca<sup>2+</sup> inputs to soils support the formation of MAOC (Rowley et al., 2017), positively impact soil structure in soils with low activity clays (Wuddivira and Camps-Roach, 2007) and are also known to elevate pH. Higher pH is associated with better habitat for many microorganisms (Aciego Pietri and Brookes, 2008), particularly fungi (Labouyrie et al., 2023; Riedo et al., 2021). The mulch layer of pruned agroforests in south-eastern Brazil, as well as Honduras, was also found to provide habitat to a high diversity of soil macrofauna (Matos et al., 2020; Pauli et al., 2011). The improved conditions for macro- and microorganisms in increasingly diverse, dense and mulched agroforests are likely to result in positive feedback loops which further improve the soil properties important for ecosystem service delivery (Philippot et al., 2023). A positive relationship between our soil biological indicator MBC and the ACI would be coherent with these hypothesized pathways. However, the fact that we did not find a significant relationship suggests that this indicator is too insensitive (Ritz et

al., 2009), or that it is not the total amount of microbial biomass that increases with complexity, but that changes in taxonomic community composition are more important (Labouyrie et al., 2023).

#### 4.4.2 How does soil texture influence the effect of complexity?

The positive effect of complexity diminished with increasing clay content for MAOC, POC, pH and AWC. Thus, in agroforestry systems situated on sandier soils, the effects found with increasing complexity were stronger than those found in systems with higher clay contents (Table 4.1, Fig. 4.3). Muchane et al. (2020) also found that agroforestry has larger effects on sandy soils for SOC stocks, but, contrary to our results, could not find sufficient evidence for greater pH increases on sandy soils. Clay provides strong physical protection of SOC and abundant binding sites for cations (Georgiou et al., 2022; Mitchell et al., 2021). Clayey soils also provide distinct microbial habitat (Labouyrie et al., 2023), which confers higher resilience to disturbance and buffers potential negative effects of agricultural management (Crowther et al., 2014; Neal et al., 2020). Sandy soils, on the other hand, have weaker structure, offer less protection to C and nutrients and rapidly deteriorate under mismanagement (Yost and Hartemink, 2019). As such, the effects of more complex systems (e.g. with higher and more diverse plant inputs, high root density and shaded soil surface) are more expressive on these naturally more vulnerable soils.

Sandy soils cover 8% of the Brazilian territory, and 15% of the *Cerrado* biome (Donagemma et al., 2016), which due to their lower productive potential are often used as extensive pastures. While these soils are already more challenging to manage, the effects of climate change are predicted to put their provision of ecosystem services (e.g. SOC storage) at even greater risk (Lugato et al., 2021). In fact, Brazilian farmers on sandy soils are already experiencing higher yield losses due to changing climate patterns (Rattis et al., 2021). However, research has also shown that Brazilian farmers perceive sandy soils as particularly suitable for integrated systems, such as silvopastures (de Souza Filho et al., 2020; Gil et al., 2016). Our results indicate that targeting these farmers to implement medium – highly complex agroforestry would benefit ecosystem services such as SOC storage (both POC and MAOC) and water regulation significantly and support both climate change mitigation and adaptation. In São Paulo State, subsidised credit lines for such investments already exist but de Souza Filho et al. (2020) showed that for cattle ranchers improved extension service would be even more critical.

#### 4.4.3 How does soil depth (0-30 cm) influence the effect of complexity?

All ecosystem service indicators (except for microporosity) significantly decreased with depth, and for POC and CEC the positive effect of ACI also significantly diminished from 0-10 cm to 20-30 cm, albeit the relationship with ACI remained positive also at 20-30 cm. As POC results from fragmentation of plant residues (Cotrufo et al., 2015) it requires bioturbation to be incorporated in the soil (Frouz, 2018) and usually accumulates in the upper layers. With regards to CEC, we hypothesize that the strong effect of complexity in the 0-10 cm layer is related to the enhanced

cycling of nutrients such as  $\text{Ca}^{2+}$  from *in situ* mulch which was demonstrated in Steinfeld et al. (2023). Nevertheless, our findings suggest that the positive effect of increasing complexity on MAOC, P, macroporosity and AWC is not constrained to the most shallow topsoil (0-10 cm) layer, but impacts (at least) the top 30 cm evenly. That is in contrast to studies on forest restoration conducted in the same region, which showed that even after 15 years positive effects on multiple soil-based ecosystem services were concentrated in the 0-10 cm layer (Bieluczyk et al., 2023a; Bieluczyk et al., 2023b).

However, the natural decrease with depth was less pronounced in more complex systems on clayey soils, partially offsetting weakening effect of ACI on clayey soils. This is indicated by the positive three-way interaction between ACI, clay and depth for MAOC, POC, P, CEC and AWC. Microporosity was only significantly related to clay (not to ACI), but we hypothesize that it is the favourable microstructure of our clayey soils that is conducive for higher C and litter nutrient inputs in complex systems to 'leach' deeper. As SOC is also crucial for improving AWC, it can also explain why AWC improved at depth in these clayey soils. Globally, soil surface layers (0-30 cm) are estimated to contain 53% of the SOC stock down to one metre (Balesdent et al., 2018). If that ratio is applied to our data, some of the clayey sites might contain SOC stocks in the order of  $200 \text{ Mg ha}^{-1}$  at one metre depth, in line with other studies of Brazilian clayey Ferralsols under agroforestry, where SOC stocks of well above  $200 \text{ Mg ha}^{-1}$  have been reported (Araujo et al., 2013; Gama-Rodrigues et al., 2010). Clayey Ferralsols have a highly uniform and deep soil profile and developed during millions of years under the heavy influence of soil biota (Schaefer, 2001). However, it remains unclear whether subsoils are as sensitive as topsoils to the effects of gradual increases in agroforestry complexity.

#### 4.4.4 Does age influence the effect of complexity?

The age gradient tested in this study was not a relevant factor for any ecosystem service indicator, and the interaction ACI \* age was only significant for pH. In their meta-analysis, Shi et al. (2018) show significant differences in SOC stocks amongst a range of age classes of agroforestry systems only after 50 years and Li et al. (2022) conversely report that agroforestry effects diminish over time. Another meta-analysis reported that SOC stocks change fastest during the first 10 years and then start plateauing in tropical agroforestry systems (Ma et al., 2020). The effect of age on other soil-based ecosystem services is less well studied but we assume that the dynamics are generally similar, due to the central role that SOC plays in most processes in soil (Hoffland et al., 2020). We conclude therefore that the benefits of complexity in our tropical sites came into effect relatively fast and were already detectable in systems as young as 3 years. It must be noted though, that our age gradient was relatively small (3-14 years) and further effects could unfold as agroforestry systems mature further.

The notable exception to the insignificance of age in our study is the combined positive effect of ACI and age on pH. Also, the interaction between ACI and clay is significant for pH, meaning that particularly on sandy soils, pH tends to increase in more complex systems over time which could reflect successional changes in micro-

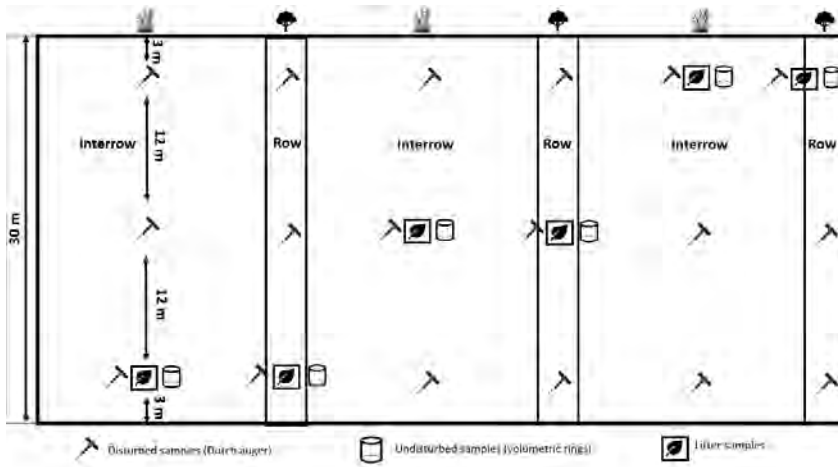


bial communities in highly complex systems. It should be noted that farmers of older, highly complex systems in our study maintained frequent pruning & mulching regimes over the years which is probably necessary to sustain resilient microbiomes (Lehmann et al., 2020; Leite et al., 2023). Hence, while our results overall suggest that complexification can increase ecosystem service provision relatively fast, the associated practices such as maintaining high diversity, density and *in situ* biomass cycling, should be maintained in the long-term.

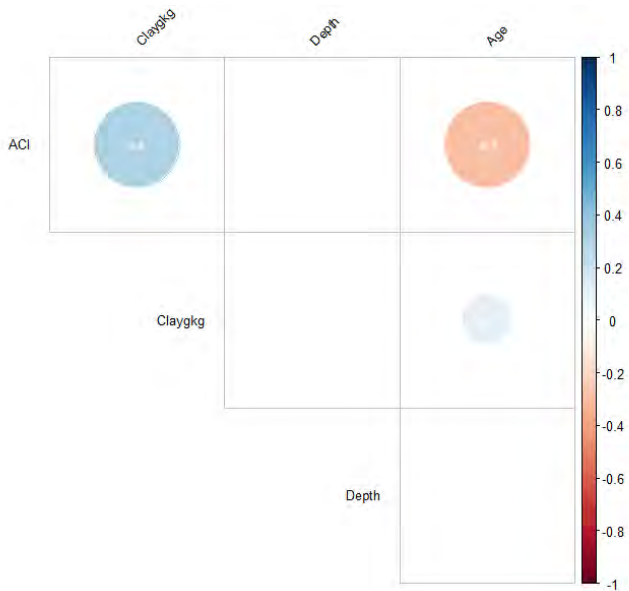
## 4.5 Conclusions

More complex systems such as agroforestry are promoted to enhance the provision of ecosystem services, but most studies only compare contrasting levels of complexity. Knowledge gaps about the effects of gradual increases in agroforestry complexity on soil-based ecosystem services remain, and whether such effects depend on soil texture, soil depth or age. Our study of a complexity gradient supports a generally positive effect of increasing agroforestry complexity on SOC storage, nutrient provision and water regulation. However, this effect was mediated by soil texture, being stronger on sandy soils. For POC and CEC the effect was also primarily concentrated in the 0-10 cm layer of the soil, while for other indicators the effect was evenly relevant over the 0 – 30 cm profile. Age, however, was not a relevant factor for all but one ecosystem service indicator, indicating that the positive effects of complexity were achieved relatively fast. We therefore conclude that complexifying agroforestry systems, e.g. by increasing their species diversity, density and intensifying pruning & mulching management, benefits ecosystem services especially on sandy soils.

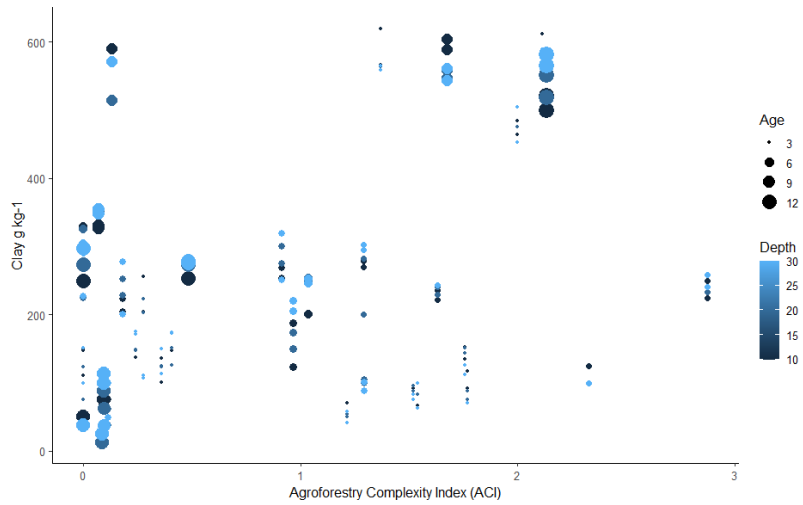
## Supplementary material



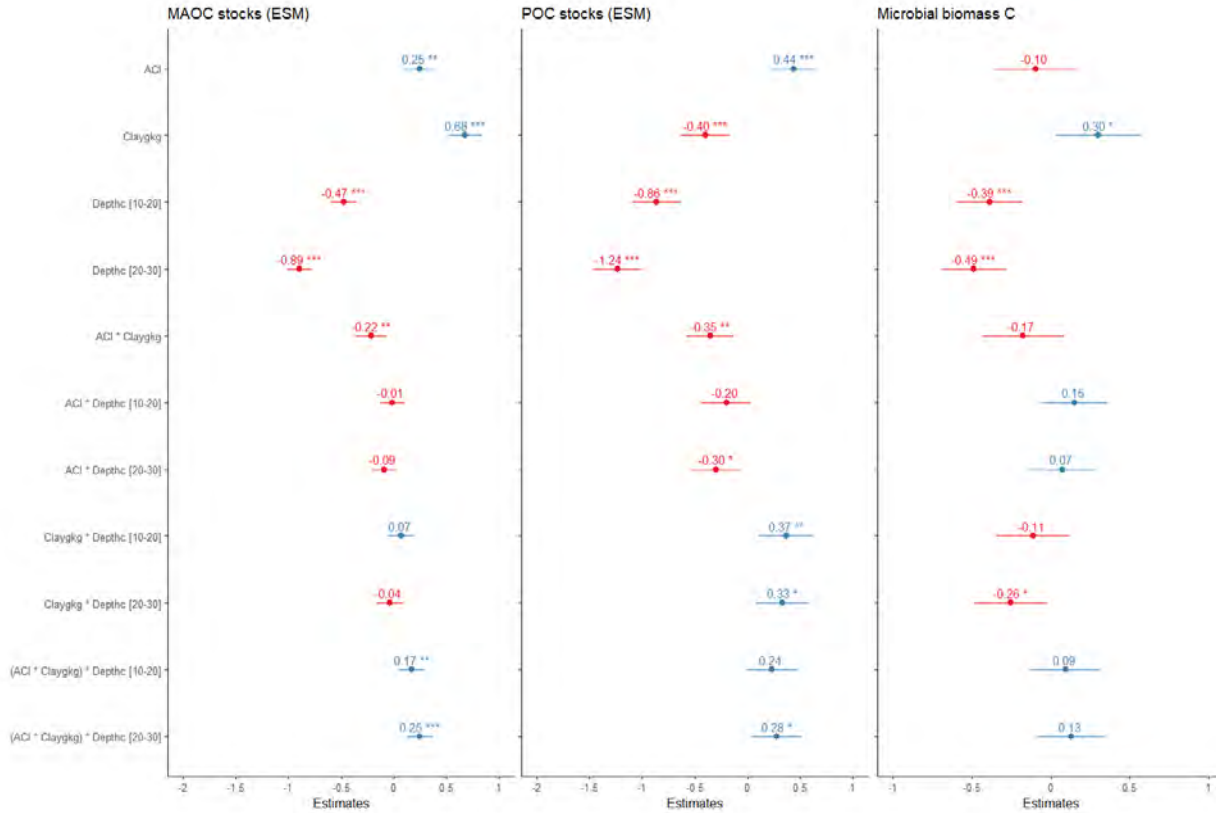
**Supplementary Figure 4.1** | Sampling design used in data collection (see Chapter 3 for explanation).



**Supplementary Figure 4.2** | Correlation graph between fixed effects ACI, Clay, Age and Depth. Pearson correlation coefficients are reported.

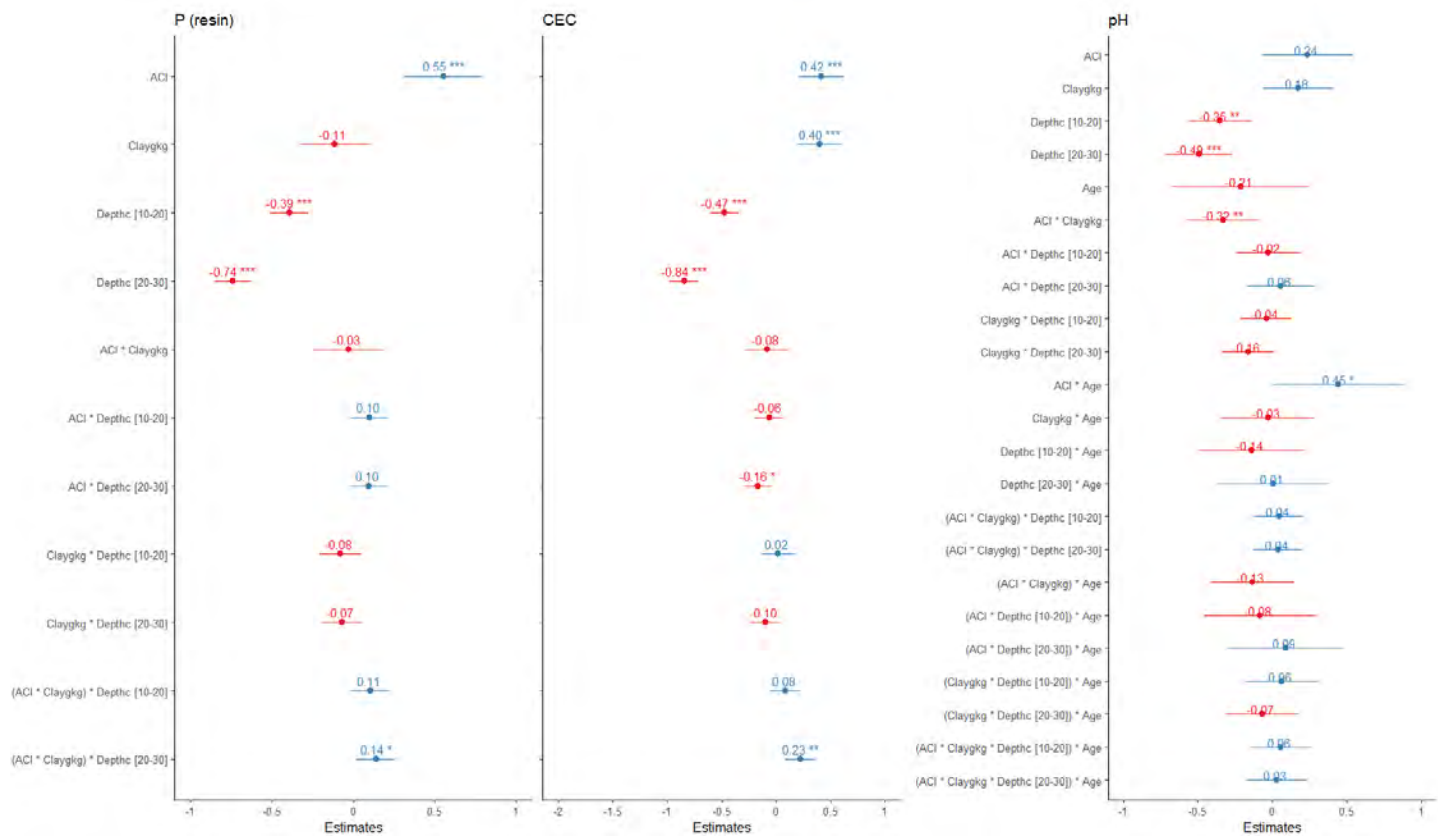


**Supplementary Figure 4.3** | Distribution of soil samples (n=201) in relation to soil texture (clay content), complexity (ACI), age (bubble size) and depth (colour).

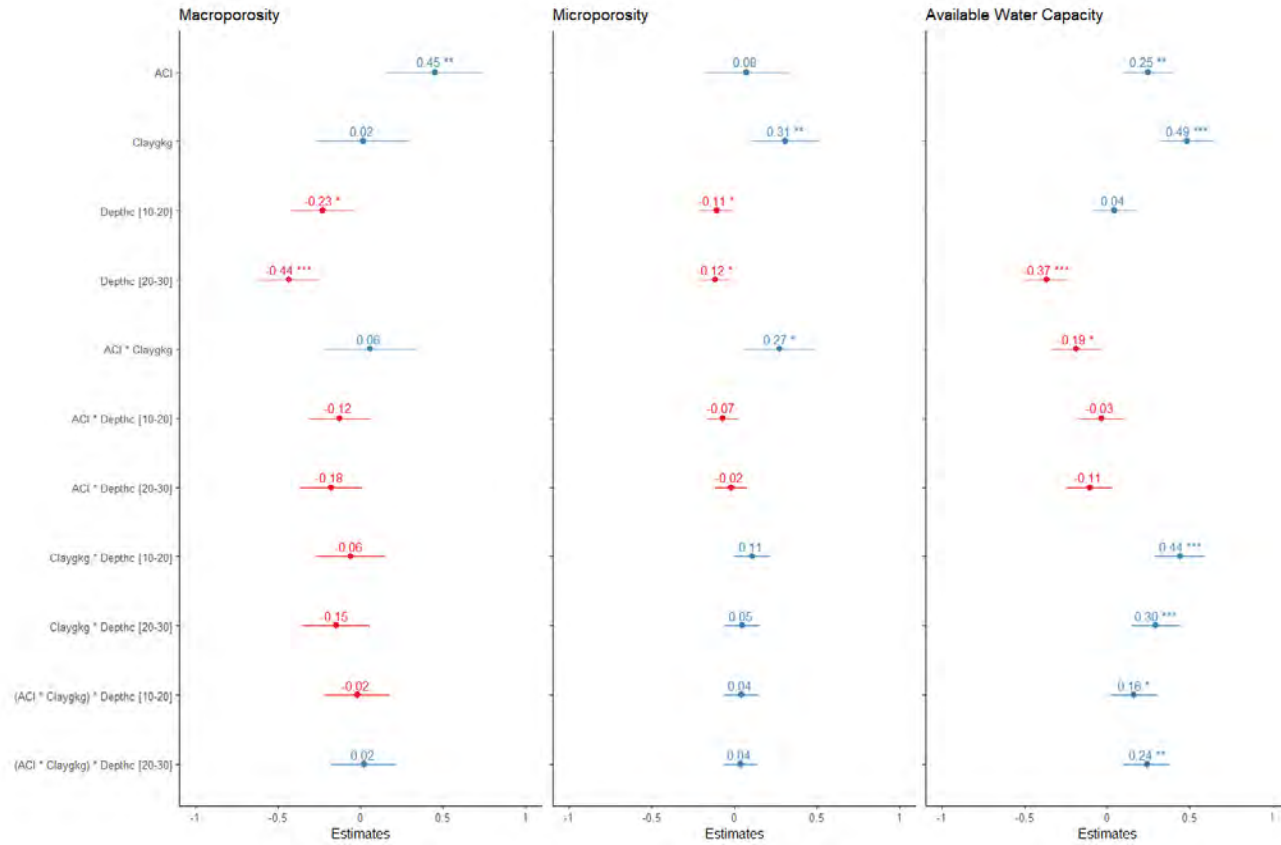


**Supplementary Figure 4.4** | Model output for ecosystem service indicators of SOC storage and cycling. Estimates are standardised effect sizes. ESM refers to equivalent soil mass (Wendt and Hauser, 2013).





Supplementary Figure 4.5 | Model output for ecosystem service indicators of nutrient provision. Estimates are standardised effect sizes.



**Supplementary Figure 4.6** | Model output for ecosystem service indicators of water regulation. Estimates are standardised effect sizes.

## Supplementary tables

MAOC STOCKS (ESM)			
Predictors	Estimates	CI	p
(Intercept)	0.48	0.32 – 0.63	<0.001
ACI	0.25	0.09 – 0.40	0.002
Claygkg	0.68	0.52 – 0.84	<0.001
Depthc10-20	-0.47	-0.59 – -0.35	<0.001
Depthc20-30	-0.89	-1.01 – -0.77	<0.001
ACI * Claygkg	-0.22	-0.37 – -0.06	0.006
ACI:Depthc10-20	-0.01	-0.13 – 0.11	0.873
ACI:Depthc20-30	-0.09	-0.21 – 0.03	0.127
Claygkg:Depthc10-20	0.07	-0.06 – 0.20	0.293
Claygkg:Depthc20-30	-0.04	-0.17 – 0.09	0.562
ACI:Claygkg:Depthc10-20	0.17	0.05 – 0.30	0.007
ACI:Claygkg:Depthc20-30	0.25	0.12 – 0.37	<0.001
Random Effects			
$\sigma^2$	0.11		
$\tau_{00}$ Subplot	0.03		
$\tau_{00}$ System	0.13		
ICC	0.60		
N <sub>System</sub>	38		
N <sub>Subplot</sub>	67		
Observations	201		
Marginal R <sup>2</sup> / Conditional R <sup>2</sup>	0.718 / 0.888		

POC STOCKS (ESM)			
Predictors	Estimates	CI	p
(Intercept)	0.76	0.54 – 0.98	<0.001
ACI	0.44	0.22 – 0.66	<0.001
Claygkg	-0.40	-0.63 – -0.17	0.001
Depthc10-20	-0.86	-1.10 – -0.63	<0.001
Depthc20-30	-1.24	-1.47 – -1.00	<0.001
ACI * Claygkg	-0.35	-0.58 – -0.13	0.002
ACI:Depthc10-20	-0.20	-0.43 – 0.04	0.095
ACI:Depthc20-30	-0.30	-0.53 – -0.07	0.012
Claygkg:Depthc10-20	0.37	0.11 – 0.62	0.005
Claygkg:Depthc20-30	0.33	0.08 – 0.58	0.011
ACI:Claygkg:Depthc10-20	0.24	-0.01 – 0.48	0.059
ACI:Claygkg:Depthc20-30	0.28	0.04 – 0.52	0.023
Random Effects			
$\sigma^2$	0.42		
$\tau_{00}$ Subplot	0.04		
$\tau_{00}$ System	0.16		
ICC	0.33		
$N_{\text{System}}$	38		
$N_{\text{Subplot}}$	67		
Observations	201		
Marginal $R^2$ / Conditional $R^2$	0.395 / 0.597		



MICROBIAL BIOMASS C			
Predictors	Estimates	CI	p
(Intercept)	0.32	0.06 – 0.58	0.015
ACI	-0.10	-0.35 – 0.16	0.459
Claygkg	0.30	0.03 – 0.57	0.028
Depthc10-20	-0.39	-0.60 – -0.18	<0.001
Depthc20-30	-0.49	-0.70 – -0.28	<0.001
ACI * Claygkg	-0.17	-0.44 – 0.09	0.188
ACI:Depthc10-20	0.15	-0.06 – 0.36	0.160
ACI:Depthc20-30	0.07	-0.14 – 0.28	0.492
Claygkg:Depthc10-20	-0.11	-0.34 – 0.12	0.345
Claygkg:Depthc20-30	-0.26	-0.48 – -0.03	0.029
ACI:Claygkg:Depthc10-20	0.09	-0.13 – 0.32	0.402
ACI:Claygkg:Depthc20-30	0.13	-0.09 – 0.35	0.242
Random Effects			
$\sigma^2$	0.33		
$\tau_{00}$ Subplot	0.64		
$\tau_{00}$ System	0.01		
ICC	0.67		
$N_{System}$	38		
$N_{Subplot}$	65		
Observations	195		
Marginal $R^2$ / Conditional $R^2$	0.071 / 0.690		

Soil P (log )			
Predictors	Estimates	CI	p
(Intercept)	0.36	0.12 – 0.60	0.004
ACI	0.55	0.31 – 0.79	<0.001
Claygkg	-0.11	-0.33 – 0.10	0.307
Depthc10-20	-0.39	-0.51 – -0.27	<0.001
Depthc20-30	-0.74	-0.85 – -0.62	<0.001
ACI * Claygkg	-0.03	-0.25 – 0.19	0.783
ACI:Depthc10-20	0.10	-0.02 – 0.22	0.092
ACI:Depthc20-30	0.10	-0.02 – 0.21	0.109
Claygkg:Depthc10-20	-0.08	-0.21 – 0.05	0.220
Claygkg:Depthc20-30	-0.07	-0.20 – 0.06	0.282
ACI:Claygkg:Depthc10-20	0.11	-0.02 – 0.23	0.094
ACI:Claygkg:Depthc20-30	0.14	0.02 – 0.26	0.026
Random Effects			
$\sigma^2$	0.10		
$\tau_{00}$ Subplot	0.22		
$\tau_{00}$ System	0.32		
ICC	0.84		
$N_{\text{System}}$	38		
$N_{\text{Subplot}}$	67		
Observations	201		
Marginal $R^2$ / Conditional $R^2$	0.400 / 0.903		

CEC			
Predictors	Estimates	CI	p
(Intercept)	0.43	0.22 – 0.65	<0.001
ACI	0.42	0.21 – 0.63	<0.001
Claygkg	0.40	0.19 – 0.61	<0.001
Depthc10-20	-0.47	-0.61 – -0.34	<0.001
Depthc20-30	-0.84	-0.98 – -0.71	<0.001
ACI * Claygkg	-0.08	-0.29 – 0.12	0.416
ACI:Depthc10-20	-0.06	-0.19 – 0.07	0.372
ACI:Depthc20-30	-0.16	-0.30 – -0.03	0.016
Claygkg:Depthc10-20	0.02	-0.13 – 0.17	0.779
Claygkg:Depthc20-30	-0.10	-0.24 – 0.05	0.194
ACI:Claygkg:Depthc10-20	0.08	-0.06 – 0.22	0.243
ACI:Claygkg:Depthc20-30	0.23	0.09 – 0.36	0.001
Random Effects			
$\sigma^2$	0.14		
$\tau_{00}$ Subplot	0.18		
$\tau_{00}$ System	0.22		
ICC	0.75		
$N_{System}$	38		
$N_{Subplot}$	67		
Observations	201		
Marginal R <sup>2</sup> / Conditional R <sup>2</sup>	0.464 / 0.864		

pH			
Predictors	Estimates	CI	p
(Intercept)	0.52	0.21 – 0.83	0.001
ACI	0.24	-0.07 – 0.54	0.126
Claygkg	0.18	-0.06 – 0.41	0.138
Depthc10-20	-0.35	-0.56 – -0.13	0.002
Depthc20-30	-0.49	-0.71 – -0.26	<0.001
Age	-0.21	-0.67 – 0.25	0.362
ACI * Claygkg	-0.32	-0.57 – -0.08	0.008
ACI:Depthc10-20	-0.02	-0.24 – 0.19	0.825
ACI:Depthc20-30	0.06	-0.17 – 0.28	0.603
Claygkg:Depthc10-20	-0.04	-0.21 – 0.13	0.662
Claygkg:Depthc20-30	-0.16	-0.33 – 0.01	0.070
ACI * Age	0.45	0.00 – 0.89	0.050
Claygkg * Age	-0.03	-0.34 – 0.29	0.869
Depthc10-20:Age	-0.14	-0.50 – 0.22	0.447
Depthc20-30:Age	0.01	-0.37 – 0.38	0.971
ACI:Claygkg:Depthc10-20	0.04	-0.12 – 0.21	0.589
ACI:Claygkg:Depthc20-30	0.04	-0.12 – 0.20	0.632
(ACI * Claygkg) * Age	-0.13	-0.41 – 0.15	0.351
ACI:Depthc10-20:Age	-0.08	-0.45 – 0.29	0.667
ACI:Depthc20-30:Age	0.09	-0.30 – 0.48	0.648
Claygkg:Depthc10-20:Age	0.06	-0.18 – 0.31	0.609
Claygkg:Depthc20-30:Age	-0.07	-0.31 – 0.18	0.598
ACI:Claygkg:Depthc10-20:Age	0.06	-0.15 – 0.27	0.569
ACI:Claygkg:Depthc20-30:Age	0.03	-0.17 – 0.24	0.749
<b>Random Effects</b>			
$\sigma^2$	0.07		
$\tau_{00}$ Subplot	0.18		
$\tau_{00}$ System	0.29		
ICC	0.87		
$N_{\text{System}}$	38		
$N_{\text{Subplot}}$	67		
Observations	201		
Marginal R <sup>2</sup> / Conditional R <sup>2</sup>	0.496 / 0.934		


MACROPOROSITY			
Predictors	Estimates	CI	p
(Intercept)	0.21	-0.11 – 0.52	0.201
ACI	0.46	0.13 – 0.79	0.007
Claygkg	-0.13	-0.45 – 0.19	0.431
Depthc10-20	-0.23	-0.47 – 0.01	0.057
Depthc20-30	-0.35	-0.59 – -0.11	0.004
ACI * Claygkg	0.14	-0.19 – 0.46	0.418
ACI:Depthc10-20	-0.16	-0.41 – 0.09	0.217
ACI:Depthc20-30	-0.19	-0.45 – 0.06	0.135
Claygkg:Depthc10-20	-0.12	-0.39 – 0.16	0.408
Claygkg:Depthc20-30	-0.34	-0.61 – -0.06	0.016
ACI:Claygkg:Depthc10-20	0.03	-0.24 – 0.31	0.812
ACI:Claygkg:Depthc20-30	0.12	-0.16 – 0.39	0.399
Random Effects			
$\sigma^2$	0.34		
$\tau_{00}$ Subplot	0.08		
$\tau_{00}$ System	0.44		
ICC	0.60		
$N_{System}$	31		
$N_{Subplot}$	54		
Observations	162		
Marginal $R^2$ / Conditional $R^2$	0.192 / 0.679		



MICROPOROSITY			
Predictors	Estimates	CI	p
(Intercept)	-0.13	-0.34 – 0.09	0.250
ACI	0.06	-0.16 – 0.29	0.590
Claykg	0.56	0.36 – 0.76	<0.001
Depthc10-20	-0.08	-0.19 – 0.04	0.210
Depthc20-30	-0.08	-0.20 – 0.04	0.186
ACI * Claykg	0.12	-0.08 – 0.33	0.240
ACI:Depthc10-20	-0.05	-0.17 – 0.08	0.468
ACI:Depthc20-30	0.04	-0.09 – 0.16	0.567
Claykg:Depthc10-20	0.09	-0.05 – 0.22	0.219
Claykg:Depthc20-30	0.02	-0.11 – 0.16	0.753
ACI:Claykg:Depthc10-20	0.03	-0.10 – 0.17	0.635
ACI:Claykg:Depthc20-30	0.01	-0.13 – 0.14	0.932
Random Effects			
$\sigma^2$	0.08		
$\tau_{00}$ Subplot	0.00		
$\tau_{00}$ System	0.27		
$N_{System}$	31		
$N_{Subplot}$	54		
Observations	162		
Marginal R <sup>2</sup> / Conditional R <sup>2</sup>	0.851 / NA		

AVAILABLE WATER CAPACITY			
Predictors	Estimates	CI	p
(Intercept)	0.12	-0.03 – 0.28	0.128
ACI	0.25	0.10 – 0.40	0.002
Claygkg	0.48	0.32 – 0.64	<0.001
Depthc10-20	0.04	-0.09 – 0.18	0.505
Depthc20-30	-0.37	-0.50 – -0.24	<0.001
ACI * Claygkg	-0.18	-0.34 – -0.03	0.019
ACI:Depthc10-20	-0.03	-0.16 – 0.10	0.622
ACI:Depthc20-30	-0.11	-0.24 – 0.03	0.113
Claygkg:Depthc10-20	0.44	0.29 – 0.58	<0.001
Claygkg:Depthc20-30	0.30	0.15 – 0.44	<0.001
ACI:Claygkg:Depthc10-20	0.16	0.02 – 0.30	0.023
ACI:Claygkg:Depthc20-30	0.24	0.10 – 0.38	0.001
Random Effects			
$\sigma^2$	0.13		
$\tau_{00}$ Subplot	0.02		
$\tau_{00}$ System	0.12		
ICC	0.52		
$N_{System}$	38		
$N_{Subplot}$	67		
Observations	201		
Marginal $R^2$ / Conditional $R^2$	0.719 / 0.865		





**Pruning of 'biomass trees' in an agroforestry system on a small farm in Mairinque, São Paulo, Brazil.**

The pruned branches and leaves in this agroforestry system are chipped with a machine and applied as mulch in the same tree row. This practice is common amongst successional agroforestry farmers in the study region.





05



# Scaling complex agroforestry: constrained by labour demand?

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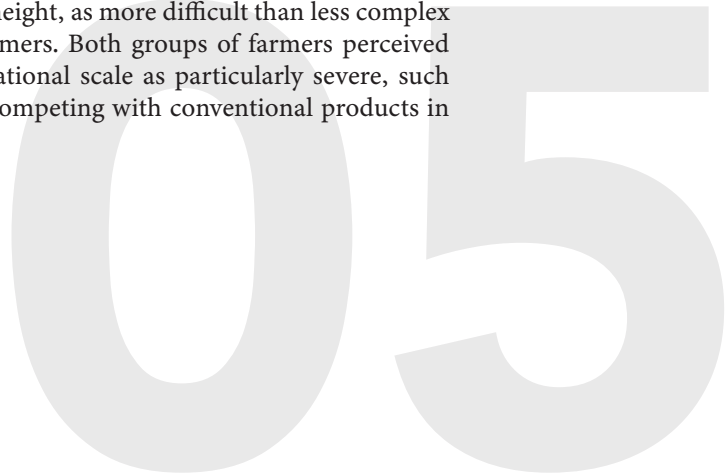
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## ABSTRACT

More complex agricultural systems such as agroforestry can increase much needed soil-based ecosystem services, but it is often postulated that agroforestry requires higher labour demand. As farmers are experiencing increasing labour shortages, it is important to assess whether agroforestry complexity indeed increases labour demand. We used ten case-study systems that represent a complexity gradient in south-eastern Brazil to assess total labour demand, as well as labour inputs for fertilization, weeding, pest control, crop management, biomass management and harvesting. We also compared labour requirements to reference monocultures and surveyed farmers on challenges regarding the scaling of their agroforestry systems. Our results confirm that in general, higher agroforestry complexity indeed correlates to higher labour demands and to higher ecosystem service provision. However, our results also show more intricate relationships: the trade-off between complexity and labour requirements was particularly evident in successional agroforestry systems, which had highest structural complexity levels; in less complex multistrata agroforestry systems, which are still more complex than monocultures, total labour demand was found to be lower than in reference monocultures. In successional systems, substantially more time was invested on *in situ* biomass management and less time was spent on pest control and weeding than in monocultural systems. These findings suggest that increasing complexity in itself does not by definition lead to increased labour demands (e.g. multistrata), but rather that it is the purposeful maximisation of ecosystem service delivery that requires labour intensive management (e.g. pruning & mulching). Furthermore, farmers of more complex successional agroforestry perceived challenges at field and farm scale, such as access to adapted machinery and pruning at height, as more difficult than less complex multistrata agroforestry farmers. Both groups of farmers perceived challenges at regional to national scale as particularly severe, such as access to subsidies and competing with conventional products in the market.



## 5.1 Introduction

Farmers worldwide need to respond to land degradation and climate change by managing their soils to produce food while simultaneously increasing the provision of other ecosystem services (Schulte et al., 2015). A significant challenge to such multifunctional land management is the higher labour inputs that have been associated with management practices that increase the provision of ecosystems services (Brodt et al., 2019; Wolz et al., 2018), against a contextual backdrop of labour shortages in many rural communities around the world (Ryan, 2023).

The challenge of managing labour requirements is paramount in one of the exemplar multifunctional systems: agroforestry. Agroforestry involves the integration of trees and crops, which makes these systems more complex than monocultures (Nair et al., 2021). There are many types of agroforestry systems, that differ in terms of species diversity and density, and their resulting structural complexity (Jagoret et al., 2017a; Seidel et al., 2021; Steinfeld et al., 2024). This ‘complexification’ has been shown to enhance the provision of ecosystem services (Santos et al., 2019; Steinfeld et al., 2023), e.g. by facilitating ecological interactions between trees and crops (Isaac and Borden, 2019). These interactions occur via soils (Sauvadet et al., 2020), but can also be actively managed and augmented by management practices such as pruning ‘biomass’ trees and using the residues as mulch for crops (Esche et al., 2022). Across South America, this practice has been shown to significantly enhance *in situ* nutrient cycling (Froufe et al., 2019; Russo and Budowski, 1986; Schneidewind et al., 2018). Particularly in Brazil, this practice is being taken up by an increasing number of agroforestry farmers (Andrade et al., 2020; Miccolis et al., 2017). In a preceding case study of 30 agroforestry farms in the Brazilian state of São Paulo, the nutrients in mulch generated from pruning in complex agroforestry systems have been shown to enhance also other soil-based ecosystem services such as SOC storage (Steinfeld et al., 2023).

The integration of trees and crops in agroforestry can impact labour requirements in numerous ways (Nair et al., 2021): the exact relationships between complexity and labour demands are yet ambiguous. This uncertainty constrains the rapid scaling of these systems to larger areas or supply chains, since labour demand is a key decision variable for the adoption of agricultural practices (Adimassu et al., 2015; Fujisawa et al., 2020). For agroforestry systems, this ambiguity may partly be explained by the variation in labour demand for specific crops. For instance, it is well known that vegetable production is labour intensive due to manual harvesting, weeding and other crop management tasks. In comparison, perennial fruit crops are often less labour demanding (Godoy, 1992). In France, a modelling exercise of labour dynamics suggested that integrating fruit trees into

vegetable market garden systems can reduce total labour demand (De Lapparent et al., 2023). At the same time, in coffee agroforestry, shade from canopy trees can induce less synchronised ripening and therefore increase harvesting time (Vaast et al., 2006). This may not be the case for crops that occupy higher canopy layers themselves, such as avocados. Labour requirements in agroforestry systems may also be affected by the management of ‘biomass’ trees (Armengot et al., 2016; Esche et al., 2022; Scudder et al., 2022), the density of trees (Nyberg et al., 2020) or management of secondary crops (Huang et al., 2022). In addition, the relationship between complexity and labour requirements is confounded by interactions between specific types of labour activities. For example: more time spent mulching could potentially reduce labour demand for other activities such as weeding (Armengot et al., 2016). This may even reduce total labour demand; however, this needs further exploration (Ferguson and Lovell, 2017).

Unpacking the triangular relationships between labour demand, agroforestry complexity and the provision of ecosystem services is an important step in making agroforestry systems attainable to a wider range of farmers and geographies. Therefore, this study aims to address this knowledge gap by disentangling these relationships for ten innovative agroforestry systems in south-eastern Brazil that were selected along a gradient of complexity. Specifically, in this paper we assess (i) the relationships between agroforestry complexity, labour demand and soil-based ecosystem services and (ii) the implications, as perceived by farmers themselves, for scaling agroforestry systems.

## 5.2 Methods

### 5.2.1 Selection of agroforestry sites

This study was part of a larger project (CANOPIES, Nabuurs et al., 2022) that studied the design and multifunctionality of 30 pioneering farms that have successfully implemented innovative agroforestry systems and as such present unique opportunities to learn from (Valencia et al., 2022). Previous work in this project found positive relationships between agroforestry complexity and the provision of soil-based ecosystem services, namely soil organic carbon (SOC) storage, nutrient provision and water regulation (Steinfeld et al., 2023, Chapter 4).

For this current study, a subsample of 10 agroforestry sites was selected from this larger sample. Selection criteria for the 10 sites were 1) to be representative of the established complexity gradient in terms of the Stand Structural Complexity Index (SSCI) and Agroforestry Complexity Index (Steinfeld et al., 2023; Steinfeld et al., 2024, suppl. Fig. 1), and 2) to be able to provide high quality data on labour inputs. While silvopastures (also referred to as integrated Livestock-Forestry systems), were included in the overall CANOPIES project, these were excluded from this current study, as extensive literature on labour inputs and economic performance in these systems already exists (Bendahan et al., 2018; Costa et al., 2018; Gil et al., 2015; Magalhães et al., 2018).

### 5.2.2 Study region: socio-economic and bio-physical characteristics

All agroforestry sites were located in the central region of the state of São Paulo, in south-eastern Brazil. In São Paulo state, agriculture represents a relatively small share of the economy, and service and industrial sectors compete with farms in labour markets (IBGE, 2019). The agricultural sector is dominated by highly mechanized large-scale production of conventional monocultures (e.g. sugarcane), but in the study region perennial crops, especially citrus, are also an important commodity. Brazilian agricultural policy tools consist mainly of subsidized credit, and direct payments to farmers are low compared to OECD countries at only 3% of farm income (Martinelli et al., 2011).

The study region is located in the ecotone between the Atlantic Forest and *Cerrado* (Brazilian savannah) biomes, which are both considered biodiversity hotspots (Garcia and Ballester, 2016; Myers et al., 2000). The climate is tropical moist with dry winters (Aw) according to Köppen's classification. Mean annual precipitation ranges from 1,350 to 1,550 mm with a dry season between April and October (Alvares et al., 2013; Rodríguez-Lado et al., 2007). The most common soil types in the region are Ferralsols and Acrisols (Rossi, 2017); due to intense weathering over long periods, these soils are often nutrient deficient, particularly in terms of phosphorus (P) (Roy et al., 2016). In addition, South-eastern Brazil has been a hotspot of accelerated SOC losses over the last 200 years (Sanderman et al., 2017).

### 5.2.3 Agroforestry types

All agroforestry sites have previously been classified as either multistrata or successional agroforestry systems, which can be further differentiated into simple multistrata, complex multistrata, successional horticulture and perennial horticulture (Steinfeld et al., 2024). While both multistrata and successional agroforestry systems are characterized by relatively high species diversity, successional systems are planted at higher densities and contain a mix of pioneer and climax species to mimic ecological succession. Additionally, management of successional agroforestry systems centres around pruning 'biomass trees' that serve the purpose of generating *in situ* mulch (Andrade et al., 2020; Götsch, 1994; Miccolis et al., 2017). For more detailed information on the agroforestry types, we refer to Chapter 2.

### 5.2.4 Labour demand: primary data collection

Data on labour hours was collected in structured surveys with the managers of the agroforestry plots. Participants of the survey were asked to refer to a representative full agricultural year and provide information on labour hours spent on fertilization, pest control, weeding, crop management, biomass management (includes mowing, pruning & mulching) and harvesting (see Table 5.2 for examples). Initial planting time is not reported as the focus of this study was on the management rather than the establishment of agroforestry. Eight labour hours were assumed to constitute one labour day, and data was transformed to a per ha basis. Time spent on initial planting and maintenance of farm infrastructure are not reported, and all data refer to years where production was already at a commercially viable level, e.g.



**Table 5.1** | Overview of agroforestry systems used in this study and key characteristics. \*Types refer to classifications and Stand Structural Complexity Index (SSCI) measurements reported in Chapter 2.

Identifier	Type*	SSCI*	Plot size (ha)	Age (year)	Tree species richness (exponential Shannon diversity index)	Tree stem density (ha <sup>-1</sup> )	Cash crop(s)
AFS10	Multistrata (simple multistrata)	1.9	0.5	3.0	3.0 (2.7)	749	Fruits (Banana, avocado)
AFS13	Multistrata (simple multistrata)	2.5	0.3	4.0	2.0 (2.0)	495	Fruits (Avocado)
AFS14	Multistrata (simple multistrata)	2.6	0.8	5.0	4.0 (1.6)	2394	Fruits (Banana)
AFS1	Multistrata (complex multistrata)	2.5	0.5	3.0	16.0 (12.1)	746	Diverse medicinal herbs and vegetables
AFS7	Multistrata (complex multistrata)	2.7	6.4	4.0	3.0 (1.9)	899	Fruits (Lime)
AFS8	Multistrata (complex multistrata)	3.2	21.0	3.0	6.0 (2.3)	1209	Fruits (Lime)
AFS9	Successional (successional horticulture)	3.6	0.5	3.0	10.0 (7.0)	1100	Diverse vegetables
AFS28	Successional (successional horticulture)	4.2	0.2	8.0	13.0 (5.8)	3038	Diverse vegetables
AFS5	Successional (successional horticulture)	4.6	0.2	3.5	11.0 (6.1)	5334	Diverse vegetables
AFS30	Successional (successional perennial)	6.5	0.8	15.0	16.0 (4.8)	5308	Coffee

in the case of citrus agroforestry at least four years after establishment, when labour demand for management activities can be assumed to remain relatively stable over the productive lifespan of these systems. In 8 out of 10 sites, the use of a tractor was very low or non-existent. For the two systems that had significant tractor hours (AFS7 and AFS8) labour hours of the tractor driver were used. In some cases, the surveys were supported by the availability of pre-existing detailed labour records shared by the farmers. All data inputs was processed and standardised to a per ha basis and iteratively evaluated with the farmers to ensure good representability (Arco-Verde and Amaro, 2015).

### 5.2.5 Reference values monocultures

We compare our agroforestry sites with monocultures of their respective cash crops to 1) assess whether they require more or less labour than commonplace monocultural commodity production systems, and 2) be able to cross-compare agroforestry systems that cultivate different crops. Reference data on the labour requirements of the respective crops in monocultures were sourced from published articles and databases from Brazilian extension services, and selected based on comparability (Table 5.3). In those agroforestry systems with a productive focus on a single cash crop (e.g. coffee), a single reference was used that was most comparable in terms of variety, planting density but also harvesting method (e.g. manually-harvested *arabica* coffee instead of machine-harvested *canephora* varieties). For agroforestry systems consisting of a diversity of cash crops (e.g. vegetable consortia), reference values for each individual crop were sourced and weighted based on the share of time they occupy in a given rotation, to construct a virtual mixed reference crop. Finally, the differential labour requirements of the various agroforestry systems were established by subtracting the reference values from the primary labour data collected (reported as ‘difference to monoculture reference ha<sup>-1</sup> year<sup>-1</sup>’).

**Table 5.2** | Examples of the six labour activities which were recorded in the study.

Activity	Examples
<b>Fertilization</b>	Application of manure, compost or rockmeal, mostly manually
<b>Pest control</b>	Spraying of (organic) pesticides
<b>Weeding</b>	Manual weeding (by hand or uprooting with hoe)
<b>Crop management</b>	Planting of annuals, pruning of tomato plants, removal of dried leaves from bananas
<b>Biomass management</b>	Pruning of service trees, mowing (manually or mechanically) of green manure crops, application of generated mulch around the base of trees
<b>Harvesting</b>	Manual harvesting of ripe coffee berries, limes or vegetables

**Table 5.3** | Reference monocultures and their sources. \*A virtual reference crop was created for vegetable consortia, based on the weighted average of each individual monocrop.

Reference monoculture		Total labour days ha <sup>-1</sup> year <sup>-1</sup>	Used for sites	Region	Reference
<b>Lime (<i>Citrus x latifolia</i>)</b>	4 <sup>th</sup> year	50	AFS8	Brasília, DF, Brazil	EMATER-DF (2021)
	5 <sup>th</sup> year	62	AFS7		
<b>Banana (<i>Musa sapientum</i>)</b>		107	AFS10, AFS14	Brasília, DF, Brazil	EMATER-DF (2021)
<b>Avocado (<i>Persea americana</i>)</b>	4 <sup>th</sup> year	41	AFS10, AFS13	Espírito Santo, Brazil	Partichelli et al. (2018)
<b>Medicinal crop (<i>Cyperus articulatus</i>)</b>		74	AFS1	Pará, Brazil	Castellani et al. (2011)
<b>Coffee (<i>Coffea arabica</i>)</b>		81	AFS30	Espírito Santo, Brazil	Krohling (2013)
<b>Consortium based on eight monoculture vegetables*</b>	Radish ( <i>Raphanus sativus</i> ), 5%	106	AFS5, AFS9, AFS28	Brasília DF, Brazil	EMATER-DF (2021)
	Rocket ( <i>Eruca sativa</i> ), 3%	90			
	Lettuce ( <i>Lactuca sativa</i> ), 6%	163			
	Broccoli ( <i>Brassica oleracea</i> var <i>italica</i> ), 13%	85			
	Eggplant ( <i>Solanum melongena</i> ), 19%	175			
	Carrot ( <i>Daucus carota</i> subsp. <i>sativus</i> ), 13%	132			
	Onion ( <i>Allium cepa</i> ), 13%	116			
	Tomato ( <i>Solanum lycopersicum</i> ), 22%	281			
		164			

### 5.2.6 Structural complexity: LiDAR-derived Stand Structural Complexity Index (SSCI)

In an earlier study, Steinfeld et al. (2024) assessed the structural complexity by performing single terrestrial laser scans at nine locations in each system using a Faro Focus 3D laser scanner (Faro Technologies Inc., Lake Marry, Florida, USA) mounted on a tripod at 1.3 m height. Scans were converted into xyz files using Faro Scene software and the resulting point cloud was processed into the Stand Structural Complexity Index (SSCI) (R-code available here: <https://github.com/ehbrechtetal/Stand-structural-complexity-index---SSCI>). The SSCI quantifies the heterogeneity of biomass distribution in a 3D space and SSCI values increase with increasing stand density and vertical stratification (for further details see Ehbrecht et al., 2017; Ehbrecht et al., 2021). The previous study showed that the SSCI is related to the combination of tree species richness and stem density, and therefore reflects both the taxonomic diversity and spatial structure of these particular agroforestry systems (Steinfeld et al., 2024).

### 5.2.7 Indicators for ecosystem services: soil and litter sampling

As described in Chapter 4, we chose indicators that relate to three important soil-based ecosystem services in the study region: SOC storage, nutrient cycling and provision and water regulation (Cherubin et al., 2016). Indicators were soil organic carbon (SOC) stocks to reflect SOC storage, soil available P (resin) and Cation Exchange Capacity (CEC) to reflect nutrient provision, and available water capacity (AWC) to reflect water regulation (Bünemann et al., 2018; Cherubin et al., 2016).

At each site, soil samples were collected separately from tree rows and interrows. Procedures and sampling design are detailed in Steinfeld et al. (2023). Composite soil samples as well as undisturbed soil samples in volumetric rings were collected at three depth intervals (0-10, 10-20, 20-30 cm). Composite samples were analysed for P (resin) contents, Cation Exchange Capacity (CEC) and soil texture (van Raij et al., 2001). They were also physically fractionated to determine mineral-associated organic carbon and particulate organic carbon contents (Cambardella and Elliott, 1992; Cotrufo et al., 2019), and in this study we report the sum of these two fractions as total SOC. Undisturbed samples were used to determine bulk density, and this data, together with soil texture and SOC content was used to estimate available water capacity (AWC) according to Tomasella et al. (2000).

### 5.2.8 Statistical analysis

Indicators of ecosystem services, namely soil fertility (P, CEC), water regulation (AWC) and SOC storage (SOC), were transformed into z-scores. Statistical differences between multistrata (n=6) and successional systems (n=4) were tested using the nonparametric Wilcoxon rank-sum test with labour demand as response variable using the R package *ggstatsplot* (Patil, 2021).



### 5.2.9 Identification of main challenges to scaling complexity: workshops and survey

In March and September 2024, two workshops were held with seven agroforestry farmers and a local agroforestry expert to define the main challenges that farmers experience related to their systems. In the first workshop, an open discussion was held to include a variety of topics, ranging from specific management issues such as weeding or pruning, to more contextual topics such as access to land. In the second workshop, these challenges were revisited and ordered according to the scale (field, farm, regional, national) at which they are most relevant. Subsequently, the managers of 10 case study systems plus an additional agroforestry manager of a highly complex system for which it was not possible to get high quality labour data, were individually surveyed to rate how they experience the severity of each challenge on a Likert scale (1=no difficulty – 5=very difficult) (Kvakkestad et al., 2015).

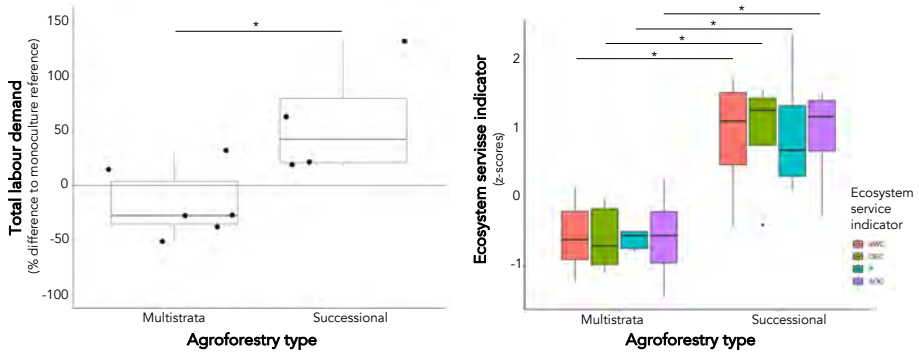
## 5.3 Results

Labour demand varied substantially between the 10 sampled agroforestry systems and ranged from 30 labour days ha<sup>-1</sup> year<sup>-1</sup> in a simple multistrata system growing avocados to 379 labour days ha<sup>-1</sup> year<sup>-1</sup> in a successional horticulture system growing a diverse range of vegetables, with an overall mean of 123 ± 35 total labour days ha<sup>-1</sup> year<sup>-1</sup> (suppl. Table 5.1). In the following sections, we report differentials to monoculture references, to enable better comparison between agroforestry systems growing different crops.

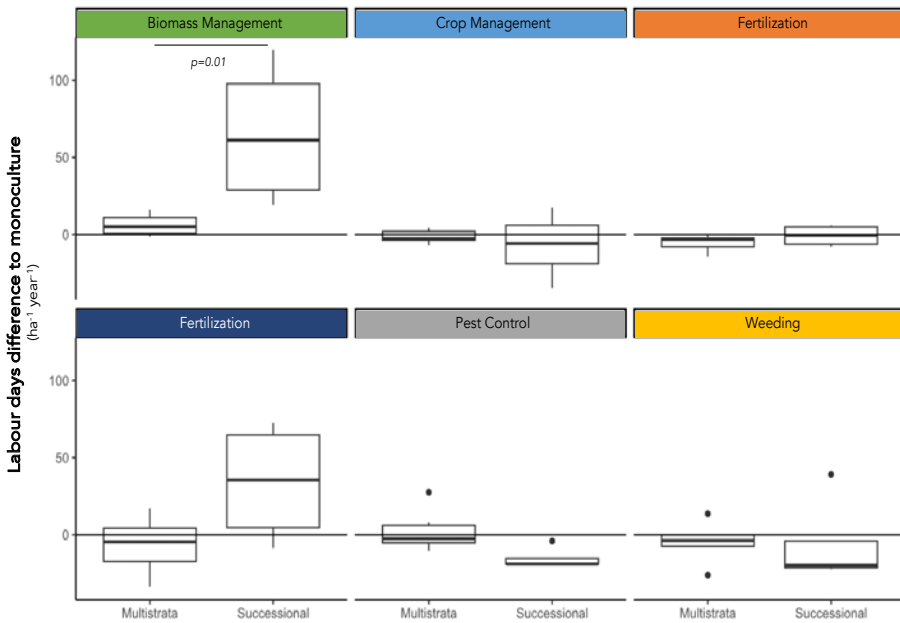
### 5.3.1 Agroforestry complexity and labour demand

The SSCI by itself did not exhibit a significant relationship with differential labour demands ( $R^2$ : 0.15,  $p=0.27$ ) but significant patterns were observed when assessing differences between two types of agroforestry: successional agroforestry systems required higher labour inputs than multistrata agroforestry systems ( $p<0.05$ ), and also had significantly higher levels of AWC ( $p<0.05$ ), CEC ( $p<0.05$ ), P ( $p<0.01$ ) and SOC ( $p<0.05$ ). All successional agroforestry systems required higher labour inputs than monocultural references, whereas four out of six multistrata systems required less labour than monocultures (Fig. 5.1).

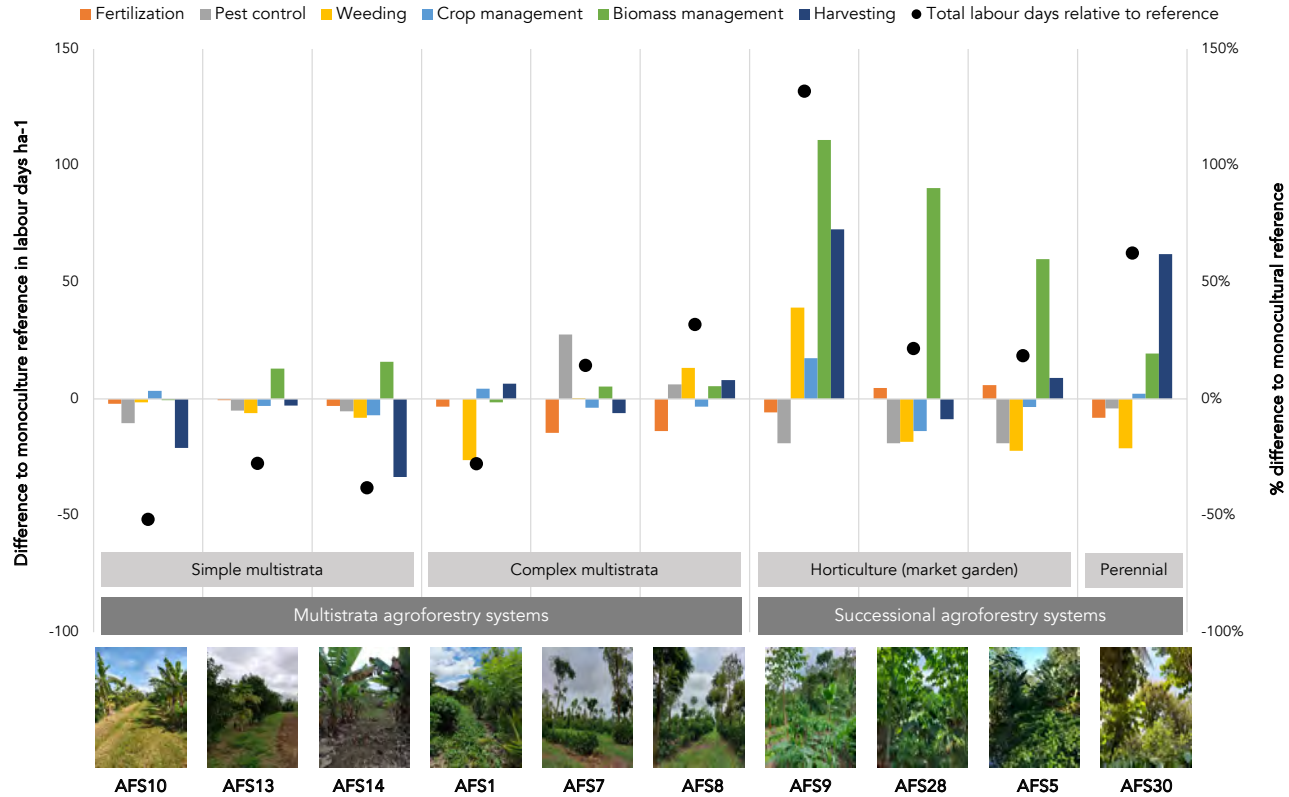
Further sub-classification of agroforestry types showed that simple multistrata systems spent on average 39% less labour days ha<sup>-1</sup> year<sup>-1</sup> than reference monocultures, complex multistrata 6% more, successional horticulture systems 57% more and the successional perennial system had 63% higher labour demand than reference monocultures (Fig. 5.2). One successional horticulture system (AFS9) had substantially higher labour demand than the two other successional horticulture systems, particularly for harvesting and weeding. This farmer reported a persistent nut grass (*Cyperus rotundus*) infestation, which in a conventional context would have required little extra time to control. Farmers of successional horticulture systems spent highest amounts of labour time on biomass management.



**Figure 5.1** | Differences in total labour demand (relative to monocultural reference) between multistrata and successional agroforestry systems (left side). On the right side, differences in soil based-ecosystem service provision as indicated by available water capacity (AWC), Cation Exchange Capacity (CEC), available P, and soil organic carbon (SOC) stocks and transformed to z-scores. All differences are statistically significant ( $p < 0.05$ ).



**Figure 5.2** | Differences in labour demand per labour activity compared to monocultural reference (horizontal line) and between multistrata and successional agroforestry systems. Significant differences between agroforestry types are indicated.



**Figure 5.3** | Systems are ordered according to their structural complexity (SSCI) from lowest to highest (left to right). The left y-axis shows differentials to the systems respective monoculture references in labour days ha<sup>-1</sup> year<sup>-1</sup> for specific labour tasks (bars) and on the right y-axis differentials are given in % for total labour demand (points). Agroforestry types and sub-types are based on Chapter 2.

When assessing specific labour activities (Fig. 5.3), successional and multistrata agroforestry systems differed significantly in time spent on biomass management. When comparing successional systems to monocultural references, all sampled systems spent less time on pest control and all but one (AFS9) spent less time on weeding. Three out of four successional systems spent more time on harvesting than monocultural references. When comparing multistrata systems to monocultural references, similar amounts of labour time were spent on activities such as crop management and pest control. Multistrata farmers had most notable reductions as compared to monocultural references in time spent on harvesting, and to a lesser extent on weeding and fertilization.

### 5.3.2 Challenges as perceived by pioneering farmers

The two workshops conducted to define the main challenges faced by agroforestry farmers in this study resulted in 12 challenges which were ordered according to the spatial scale at which they are most relevant (Fig. 5.4). For example, the challenges “weeding without removing mulch cover” or “pruning at height” were classified to be most relevant at the field scale, while “managing the diversity of tasks that arise in increasingly complex agroforestry systems” were deemed most relevant at the farm scale. At field scale, conducting pruning at height (also known as pollarding) had the highest mean difficulty score (3.3), and at farm scale it was the increased diversity of tasks (mean 3.1) which was considered most challenging. “Finding qualified and motivated farm workers” was a major challenge for all farmers (mean score 4.1) and was considered as an example of a challenge beyond farm scale, as it depends on and reflects regional socioeconomic conditions. Access to extension services, financial subsidies and land was a major challenge especially for farmers of successional agroforestry systems; one which can only be addressed at regional to national level through policymaking. Noteworthy, the two systems located on a large-scale farm (AFS7 and AFS8) reported low difficulty levels with accessing extension services, finance or land. The highest average difficulty score across all scales was attributed to ‘competing with conventional products in the market’ (4.4), followed by accessing financial subsidies (4.1). The lowest average score (2.0) was attributed to “knowledge about species response to management”, which shows the pioneering farmers’ confidence in the practical knowledge on agroforestry management that they have already acquired. Overall, challenges related to regional and national scales were perceived as more difficult to overcome (mean 3.3 by multistrata farmers and 4.5 by successional farmers) than field and farm scale challenges (mean 2.2 by multistrata farmers and 3.3 by successional farmers).



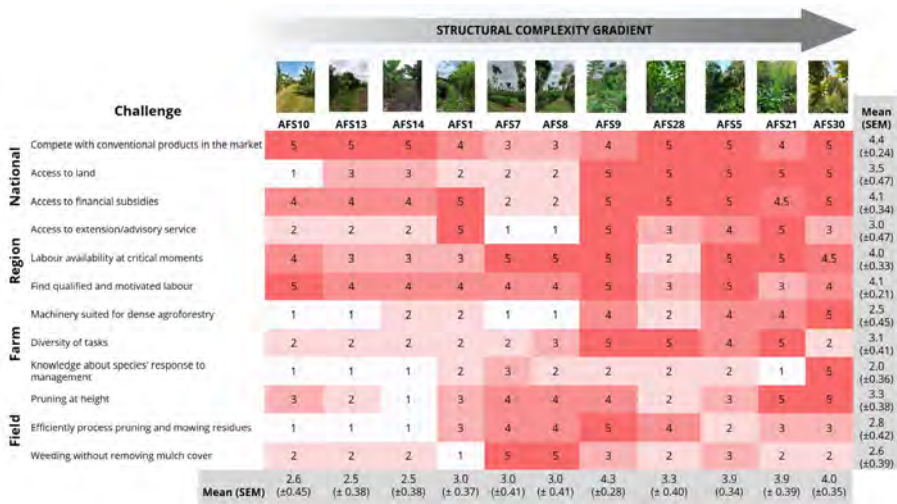


Figure 5.4 | Challenges were defined in two workshops with pioneering farmers, and consequently managers indicated how difficult (1=not difficult – 5=very difficult) the respective challenges were experienced in their contexts. Left column indicates at which scale (field – national) these challenges are relevant.

## 5.4 Discussion

### 5.4.1 Key findings

In this paper, we unpacked the triangular relationships between complexity, multi-functionality and labour requirements. Our results confirm that in general, higher agroforestry complexity indeed correlates to higher labour demands and to higher ecosystem service provision. However, our results also show more intricate relationships: the trade-off between complexity and labour requirements was particularly evident in successional agroforestry systems, which had highest structural complexity levels. In less complex multistrata agroforestry systems, which are still more complex than monocultures, total labour demand was found to be lower than in reference monocultures. In successional systems, substantially more time was invested on *in situ* biomass management and less time was spent on pest control and weeding than in monocultural systems. These findings suggest that increasing complexity in itself does not by definition lead to increased labour demands (e.g. multistrata), but rather that it is the purposeful maximisation of ecosystem service delivery that requires labour intensive management (e.g. pruning & mulching).

### 5.4.2 Agroforestry complexity and labour demand

A majority of the sampled multistrata agroforestry systems required relatively low labour inputs, particularly for activities such as harvesting and weeding. All managers of agroforestry systems with lower labour demand than monoculture references

stated the explicit objective to keep labour demand moderate to low, which is a common strategy amongst many agroforestry farmers around the world, even if it is associated with low productivity (Adimassu et al., 2015; Fujisawa et al., 2020; Scudder et al., 2022; Tilden et al., 2023). From a profitability point of view, this can be beneficial as labour costs are often the largest expense and incremental productivity gains in response to increased labour inputs do not always pay off (Davidova et al., 2022; Esche et al., 2022; Scudder et al., 2022). As multistrata systems can nevertheless be expected to provide more ecosystem services than simplified monocultures (Santos et al., 2019; Steinfeld et al., 2023), these low-labour multistrata agroforestry systems are showcasing a viable approach to multifunctional land management.

### 5.4.3 Specific labour activities

Ecosystem service provision is further augmented in highly complex successional agroforestry systems (Fig. 5.2b, Santos et al., 2019; Steinfeld et al., 2023). Our study shows that managing this complexity requires higher labour inputs than less complex agroforestry systems and monocultures (Fig. 5.1a). Our results suggest that this is not primarily related to the management requirements associated with the higher structural complexity itself but rather to the *in situ* biomass management that is practised in these systems. Particularly successional horticulture systems had high labour demand, as maintaining adequate shade levels and providing mulch for vegetables requires more frequent tree pruning. This is in line with another study that postulates that the integration of trees *per se* does not increase labour demand, but that the impact on labour results from interactions between trees and crops (Ferguson and Lovell, 2017).

Our study shows that the higher pruning & mulching frequencies in successional agroforestry systems benefit soil-based ecosystem services, which is in line with previous studies using a larger dataset (Steinfeld et al., 2023; Steinfeld et al., 2024). However, the additional time spent on *in situ* biomass management not only increased ecosystem services, but it also reduced the labour requirements for other tasks, such as pest control and weeding; an explicit example of synergies between labour activities. The weed-suppressive effect of mulching has been well-established (Mudare et al., 2023), as are the higher natural enemy populations in organic mulches which can benefit biological pest control (Mudare et al., 2023; Pauli et al., 2011). This replaces potentially hazardous chemical pest control measures and eliminates the risk of pesticide contamination (Dhananjayan et al., 2020).

More research is needed to assess whether these synergies between labour activities and the increased provision of ecosystem services from *in situ* biomass management pays off in economic terms. In Bolivian cocoa agroforestry systems, pruning & mulching was indeed shown to increase productivity, but at current farm gate prices this did not always offset the increased costs for the labour (Esche et al., 2022). A complete economic assessment should also account for savings in fertilizer usage, as well as savings in pesticide costs but this would require more detailed quantification of yield responses in agroforestry systems (Andreotti et al., 2018; Cerda et al., 2020). At this stage, our results highlight the importance of gains in operational efficiency in biomass management as key to the potential for scaling successional agroforestry (De Morais et al., 2023): this is the area where further research would be of imme-

diate benefit to farmers.

#### 5.4.4 Challenges for scaling complex agroforestry

##### *Challenges at field to farm scale*

In line with our results (Fig. 5.4), the lack of appropriately customised equipment has been put forward as a major challenge to scaling agroforestry in places as diverse as California in the USA (Brodt et al., 2019), Kenya (Nyberg et al., 2020) India (Upendranadh and Subbaiah, 2016) and Northern Europe (Graves et al., 2009). Most agricultural machinery has been developed to deliver maximum efficiency in homogeneous row crops (Ditzler and Driessen, 2022), and little resources have thus far been invested in developing technical solutions specifically for agroforestry. In our study sites, further efficiency gains require investments into the development of more suitable equipment, such as walk-behind reapers instead of brush cutters for mowing & mulching (De Morais et al., 2023) or even robots adapted to multi-layered agroforests (Chowdhary et al., 2019). There is ample scope for the development of mechanization that supports those tasks that generate ecosystem services (e.g. pruning at height), attract new generations of farmers (Nyberg et al., 2020) and adapt to the individual levels of physical engagement that farmers desire (Ditzler and Driessen, 2022). Finally, the most difficult challenge reported at farm scale, i.e. managing the diversity of tasks (Fig. 5.4), could be facilitated with technologies such as specific farm management support tools or apps.

##### *Challenges at regional to national scale*

In principle, higher labour demands at farm scale could translate into the creation of additional job opportunities at the regional scale which can positively support rural economies (Smith et al., 2022). However, the pioneering farmers reported significant difficulties in finding qualified and motivated people in their regions (Fig. 5.4), turning the lack of labour availability into a major impediment to scaling agroforestry systems. In addition, farmers reported that despite the availability of subsidised credit lines for agroforestry, the banks which are intermediating these credits are often unwilling to underwrite the risks associated with these innovative and therefore lesser known systems. Furthermore, creditors are less likely to finance workers' salaries than large machinery. Some scope for investment exists in the form of private market initiatives such as carbon credits. However, for small or mid-sized farms, such schemes are associated with prohibitive costs for monitoring, reporting and verification (Tamba et al., 2021). Finally, the limited availability of specialised extension service was highlighted as an impediment by farmers, in line with other studies in the region (de Souza Filho et al., 2020; Vinholis et al., 2020). In short: agricultural equipment, financial incentives and extension services have all evolved to support monocultural commodities and all require significant development to also support the scaling of multifunctional agroforestry systems.

## 5.5 Conclusions

Agroforestry systems have been proposed as exemplar farming systems that deliver not only on food, but also on the contemporary societal requirements for multifunctionality (Beillouin et al., 2021; Cardinael et al., 2021; FAO, 2017). However, agroforestry systems are complex and more difficult to manage. This study assessed the triangular relationships between the complexity of agroforestry systems, labour requirements and the delivery of ecosystem services using ten case-study agroforestry systems along a gradient of complexity. Specifically, we tested the hypothesis that increased complexity results in higher labour requirements. Our results show that these relationships are more nuanced: we found that multistrata agroforestry systems in fact require proportionally less labour than their equivalent monocultural crops, while delivering more ecosystem services. For successional agroforestry, our results are less straight: while these systems deliver ecosystem services at much higher rates than multistrata agroforestry systems, this is predicated on very large time investments in *in situ* biomass management. While this activity enhances nutrient cycling and reduces the labour requirements for weed and pest control, it contributes to an overall increase in labour requirements.

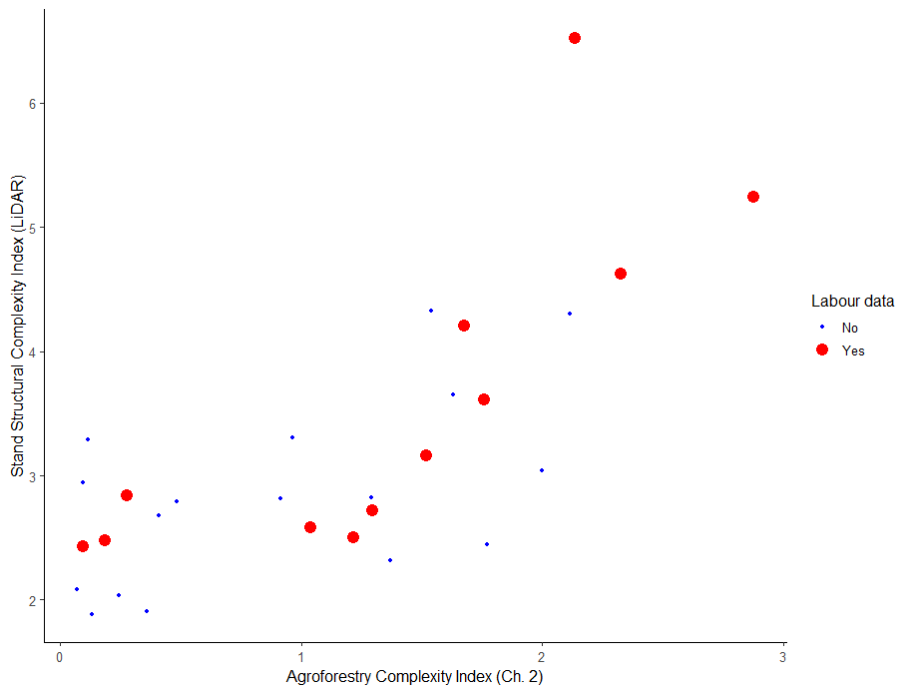
While the creation of job opportunities may be considered positive in the context of rural development and mitigating unsustainable urbanisation, farmers cited the lack of suitable labour as a main constraint to scaling agroforestry. In addition, our study underscores the need for enabling technological, financial and knowledge environments to facilitate larger-scale transitions to agroforestry, as current technologies, financial mechanisms and extension services have evolved to serve the requirements of monocultural commodity production. This implies that the point of initiative for transitions to agroforestry may indeed be within the realm of regional or national actors such as research institutes, extension services, financial institutes and government departments, rather than individual farmers.

## Acknowledgements

We thank Marcelo Francia Arco-Verde for initial discussions on our study design. Our deep gratitude goes to all farm managers who dedicated their time to participate in this study.



## Supplementary material




**Supplementary Figure 5.1** | The subsample (10) of agroforestry systems was representative of the larger sample of agroforestry systems (30), as can be seen by their distribution along the Stand Structural Complexity Index and Agroforestry Complexity Index (Chapters 2 and 3).

**Supplementary Table 5.1** | Total labour days ha<sup>-1</sup> year<sup>-1</sup> and labour days spent on specific labour tasks in the sampled agroforestry systems.

Identifier	Type	Crop	Total labour days ha <sup>-1</sup> year <sup>-1</sup>	Labour days ha <sup>-1</sup> year <sup>-1</sup>					
				Fertilization	Pest control	Weeding	Crop management	Biomass management	Harvesting
AFS10	Multistrata	Banana, avocado	39	3	1	6	7	3	20
AFS13	Multistrata	Avocado	30	2	6	0	0	12	9
AFS14	Multistrata	Banana	67	3	7	0	4	16	36.5
AFS1	Multistrata	Diverse medicinal herbs and vegetables	53	1	0	9	6	5	32
AFS7	Multistrata	Lime	71	8	35	4	0	6	19
AFS8	Multistrata	Lime	66	3	13	18	1	5	26
AFS9	Successional	Diverse vegetables	379	14	0	69	56	111	130
AFS28	Successional	Diverse vegetables	199	15	0	12	34	90	48
AFS5	Successional	Diverse vegetables	194	16	0	8	44	60	66
AFS30	Successional	Coffee	132	0	0	4	3	25	99







**Agroforestry system in smallholder settlement Mario Lago near Ribeirão Preto, São Paulo, Brazil**

The Agroforestry Complexity Index of this system is 1.63. The farmer produces a variety of crops (coffee and pineapple amongst others) and sells these in local markets where premium prices for his ecologically beneficial management are often hard to attain.





06





CHAPTER 6

# General discussion

06

## 6.1 Relevance and general findings

The future for farmers has not become any easier in the last four years during which this thesis was produced. In 2019, it was still widely believed feasible to limit climate change to 1.5°C above pre-industrial levels (IPCC, 2019). Now, in 2023, scientific consensus is that farmers have to prepare for a future that passes this threshold significantly as policy and societal action has largely stalled (IPCC, 2023). Farmers were distracted by highly fluctuating fertilizer prices and messages to prioritize short-term yields over long-term actions to respond to environmental change (Neik et al., 2023). The need to enhance soil-based ecosystem services in agricultural systems, such as nutrient cycling, SOC storage and water regulation, has become even more urgent (UNCCD, 2022).

On the other hand, the scientific progress about ways for farmers to address these challenges has not stalled during these four years. A plethora of meta-analyses were published in high impact journals between 2019 and 2023, and their message is clear: diversifying agricultural systems benefits ecosystem services (Beillouin et al., 2021; Dainese et al., 2019; He et al., 2023; Ricciardi et al., 2021; Tamburini et al., 2020; Teixeira et al., 2022), without necessarily compromising yields (Dainese et al., 2019; Tamburini et al., 2020) or potentially even increasing yields (Beillouin et al., 2021). Out of several diversification strategies, agroforestry was shown to provide the strongest positive effects on ecosystem service provision (Beillouin et al., 2021). Nevertheless, even after assessing thousands of studies these meta-analyses still point out great variability, and the factors causing it remain unclear (Beillouin et al., 2021; Lamichhane, 2023).

Exploring why some agroforestry systems provide more ecosystem services than others was a main motivation of this study, and I hypothesized that the underlying complexity of agroforestry systems is one of the main drivers of their ecological benefits. This complexity was quantified in the Agroforestry Complexity Index (ACI). Using this index, the general findings from this thesis are broadly in line with the benefits reported in recent meta-analyses: increasing agroforestry complexity was clearly associated with increased nutrient cycling and provision, SOC storage and water regulation. The findings also point out an important caveat: agroforestry complexity does not work equally well across all contexts, as it was shown that its relative effects on delivering ecosystem services, such as SOC storage and water regulation, were stronger on sandy soils than on clayey soils.

Another important caveat was found in relation to farm labour demand: while increasing agroforestry complexity can decrease labour demand for some activities, such as weeding and pest control, those practices that are most important to enhance ecosystem service provision, such as pruning & mulching, increase total labour demand. That means that

those agroforestry systems with highest ecosystem service provision also required higher labour inputs than monocultural references and simpler agroforestry systems.

## 6.2 Employing a complexity gradient to gain deeper insight into ecosystem service delivery

Throughout this thesis, a distinctive approach was employed to assess the importance of agroforestry complexity. The general importance of agroforestry complexity for their ecological performance is an often-implicit assumption in the literature (Chatterjee et al., 2018; Schroth and do Socorro Souza da Mota, 2014; Shi et al., 2018), but it is rarely explicitly tested. In a review on Brazilian agroforestry systems it was shown that valuable insights on their ecosystem service provision could be generated by distinguishing between agroforestry systems based on species diversity, spatial structure and application of ecological design principals (Santos et al., 2019). This promising approach was refined in this thesis, and a quantitative complexity gradient was developed to provide more nuanced information about agroforestry's potential for farmers and other stakeholders (Teixeira et al., 2022). In the following, I will discuss this process and evaluate this approach.

### 6.2.1 First step: quantifying the many dimensions of complexity

First, I had to take stock and understand what actually makes an agroforest complex. Agroecosystems are complex in multiple ways (Naeem, 2013; Vandermeer and Perfecto, 2017) and a wide range of methods were employed throughout this thesis to describe the various dimensions of complexity (**Chapter 1**). A literature review was conducted to define the most relevant complexity dimensions: taxonomic diversity (Malézieux, 2011; Malézieux et al., 2009; Santos et al., 2019), functional diversity (Naeem, 2013; Santos et al., 2021; Wood et al., 2015), spatial structure (Jagoret et al., 2017a; Seidel et al., 2021; Zemp et al., 2019), time (Malézieux, 2011; Notaro et al., 2022; Young, 2017) and management (Cerda et al., 2017; Jezeer et al., 2018). Based on these dimensions, (mostly continuous) metrics were defined that capture variation between agroforestry systems, such as species richness or the exponential Shannon diversity index (taxonomic diversity), stem density or a LiDAR-derived structural complexity index (spatial structure), community weighted means of wood density and foliar N (functional diversity) or the frequency of management interactions. The temporal dimension was most challenging to capture as the agroforestry systems had productive lifespans of 20 years or more, and it was assumed that at the point of sampling they had reached a relatively stable state in their evolution. This first step provided a wealth of data to do justice to the multidimensionality of agroforestry complexity.

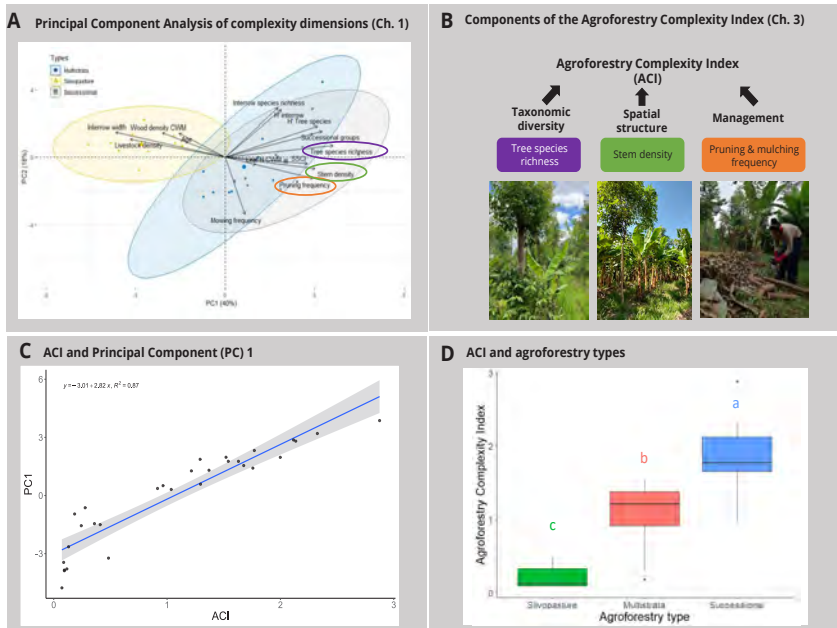
This first step also involved defining those metrics most relevant in the context of the innovative Brazilian agroforestry farmers who were the subject of this study. For instance, the management dimension of agroforestry is often quantified as the frequency of chemical input applications (De Beenhouwer et al., 2014; Jezeer et al.,



2018) or weeding intensity (Cerda et al., 2017). In this study, none of the agroforestry farmers applied non-organic pesticides and only in two silvopastoral systems were chemical fertilizers applied at all. Much more relevant, however, was how farmers managed biomass within their systems: all multistrata and successional farmers either mowed and/or pruned grasses and trees to leave this cut biomass as a mulch cover on the soil (**Chapter 1**). The farmers also designed their systems based on functional traits, such as successional group, shade tolerance and regrowth capacity after pruning (Andrade et al., 2020; Miccolis et al., 2016; Young, 2017), and so taxonomic and functional diversity indices co-correlated strongly in the dataset (not reported). Therefore, the metrics to represent complexity in this study might not apply in other regions but were tailored to the study's specific context.

### 6.2.2 Second step: composing a multidimensional complexity index

In a second step, the Agroforestry Complexity Index (ACI) was devised as a multidimensional, composite index (**Chapter 3**). The metrics chosen to compose the ACI were species richness, stem density and the frequency of pruning & mulching (Fig. 6.1B). This selection was based on 1) their representativeness of the systems overall complexity (Fig. 6.1A and C), 2) ease of measurement and 3) relevance for nutrient cycling (**Chapter 2**). Metrics of taxonomic diversity (species richness) and spatial structure (stem density) are commonly applied in combination to describe agroforestry complexity (Cerda et al., 2017; Jagoret et al., 2017a; Jezeer et al., 2018; Sonwa et al., 2018) and particularly pruning & mulching showed high relevance for nutrient stocks in litter (**Chapter 2**). The metrics representing the different complexity dimensions were standardized and added, just as in composite complexity indices from the realms of theoretical biology (Rebout et al., 2021) or marine ecology (Paoli et al., 2016). The resulting ACI was representative of the variation across multiple complexity dimensions and well suited to distinguish between silvopastures, multistrata and successional agroforestry systems (Fig. 6.1D).



**Figure 6.1** | The Agroforestry Complexity Index (ACI) was based on three components representative of the overall variation between the 30 agroforestry systems as can be seen from the Principal Component Analysis (A), composition of the ACI (B), high correlation between the ACI and Principal Component 1 (C), differences in ACI between silvopastures, multistrata and successional agroforestry types are significant ( $p < 0.05$ ) (D).

### 6.2.3 Third step: multidimensional complexity and ecosystem services

In a third step, the ACI was used in regression models as a fixed effect to explain ecosystem service indicators (**Chapters 3 and 4**). To showcase the multidimensional ACI's suitability as a predictor of ecosystem service delivery, it can be compared to some 'less multidimensional' metrics that were also employed in this thesis (Table 6.1). For instance, the LiDAR-derived SSCI was shown to correlate strongly with species richness and stem density (**Chapter 1**) and can therefore be used as a proxy of two complexity dimensions. Stem density or tree richness as well as Shannon diversity on their own only capture single complexity dimensions. Substituting these metrics (SSCI, stem density, tree species richness and Shannon diversity) for the ACI in the same models that were employed in **Chapter 4** (Ecosystem service indicator  $\sim$  ACI \* Clay \* Depth \* Age) and evaluating these models based on the corrected Akaike Information Criterion (AICc) (Cavanaugh and Neath, 2019), shows that the ACI consistently constitutes the best ( $\Delta$ AICc: 0) or equally good ( $\Delta$ AICc > 2) model compared to models containing 'less multidimensional' metrics. Particularly for available water capacity (AWC), mineral-associated organic carbon (MAOC), particulate organic carbon (POC) and P, model performance was substantially better ( $\Delta$ AICc to second best model: 11.70, 10.95, 10.68 and 7.46, respectively). Based on this comparison and **Chapters 2, 3 and 4**, I conclude that the ACI was able to

explain more variation in ecosystem service provision than other metrics because it captured more (multidimensional) information on the agroforestry systems' complexity.

The approach of defining agroforestry gradients using multiple complexity dimensions simultaneously has also been successfully employed to explain ecosystem service provision in other studies (Santos et al., 2019; Teixeira et al., 2022). For instance, several studies on coffee agroforestry have defined gradients based on genetic diversity and spatial structure (Cerdeira et al., 2017; Cerdeira et al., 2020; De Beenhouwer et al., 2016; De Beenhouwer et al., 2014; Jezeer et al., 2018; Mas and Dietsch, 2003) and reported positive relationships with biodiversity habitat quality (De Beenhouwer et al., 2016; De Beenhouwer et al., 2014; Mas and Dietsch, 2003), and important interactions with management intensity for C storage and productivity (Cerdeira et al., 2017; Jezeer et al., 2018). An innovative approach to quantifying the spatial structure of cocoa agroforests using drone imagery was employed by Blaser et al. (2018) and the resulting 'one dimensional' spatial structure gradient was used to assess differences between agroforestry plots and monocultural plots. The authors found significant associations of this gradient with aboveground C storage and biodiversity habitat, but not with SOC storage or soil fertility (Blaser et al., 2018). Overall, these studies confirm that taking multiple complexity dimensions, including management, into account shows important ecosystem service patterns.

The importance of context (e.g. altitude) has been shown in studies employing agroforestry complexity gradients to explain ecosystem service delivery (Cerdeira et al., 2017). In this thesis, it was shown that soil texture is a major determinant of the strength of complexity effects on multiple ecosystem services (**Chapter 4**), as well as synergies between them (**Chapter 3**). This dependency on soil texture was indicated by significant interaction terms with clay content (**Chapter 4**) and in Table 6.1 it can be observed that the ACI was more 'sensitive' than other metrics to these interactions. A meta-analysis comparing agroforestry to monocultural references has also shown that agroforestry provides higher benefits for SOC storage on tropical sandy soils (Muchane et al., 2020). Also in Germany, sandy soils have been shown to be more responsive to management practices aimed at increasing SOC than clayey soils (Gocke et al., 2023). Using the ACI, this pattern was confirmed but also more detail provided: the combined effects of species diversity, density and pruning & mulching management are likely responsible for higher relative ecosystem service delivery on sandy soils.

Distinguishing between agroforestry systems based on their complexity reveals more nuanced information on the ecological benefits of agroforestry for stakeholders (Teixeira et al., 2022), and in Brazil it was shown that biodiverse agroforestry systems provide more ecosystem services than simplified ones (Santos et al., 2019). By developing the ACI, this thesis added further depth to this insight (**Chapters 3, 4**). This can be attributed to the multidimensional nature of the ACI, and therefore a similar, context-specific approach to quantifying agroecosystem complexity can be recommended to study the provision of ecosystem services in other places.

**Table 6.1** | Five different models, containing either the Agroforestry Complexity Index, Stand Structural Complexity Index, exponential Shannon diversity, tree species richness or stem density as fixed effects (together with clay, depth and age) to explain response variables related to ecosystem service indicators are compared. The corrected Akaike Information Criterion (AICc) is used to compare model performance, and best (lowest AICc) or equally good ( $\Delta AICc > 2$ ) models are highlighted in **bold**.

Ecosystem services	Response variable	Corrected Akaike Information Criterion (AICc)					Interactions			
		Models: response variable ~ ACI or SSCI or Sh or SD or TR * Clay * Depth					Only significant ( $p < 0.05$ ) interaction terms are reported			
		Agroforestry Complexity Index (ACI)	Stand Structural Complexity Index (SSCI)	Exponential Shannon Diversity Index (Sh)	Stem density (SD)	Tree species richness (TR)	Interaction with clay	Interaction with depth	Interaction with clay * depth	Interaction with age
C storage and cycling	MAOC	<b>290.16</b>	304.59	303.81	301.11	306.09	ACI**, SD*		ACI***, SD***, TR*, SSCI*	
	POC	<b>501.55</b>	518.20	515.34	512.24	521.66	ACI**, SD**, SSCI*	ACI*	ACI*	
	Microbial Biomass C	<b>515.41</b>	519.02	517.16	<b>514.48</b>	516.93				
Nutrient provision	P (log)	<b>498.34</b>	508.61	517.07	512.55	505.80			ACI*, TR**	
	CEC	<b>375.54</b>	392.97	<b>377.42</b>	395.52	385.99		ACI*, Sh*	ACI**, TR*, Sh*	
	pH	<b>351.74</b>	367.19	353.75	<b>353.28</b>	<b>352.42</b>	ACI**, SD*, TR*			ACI*
Water regulation	Macroporosity	<b>413.40</b>	418.62	416.02	<b>411.47</b>	415.93				
	Microporosity	<b>211.06</b>	215.08	<b>209.24</b>	<b>211.62</b>	213.78				
	Available Water Capacity (AWC)	<b>309.30</b>	321.10	323.93	321.01	325.71	ACI**		ACI**, SD**	



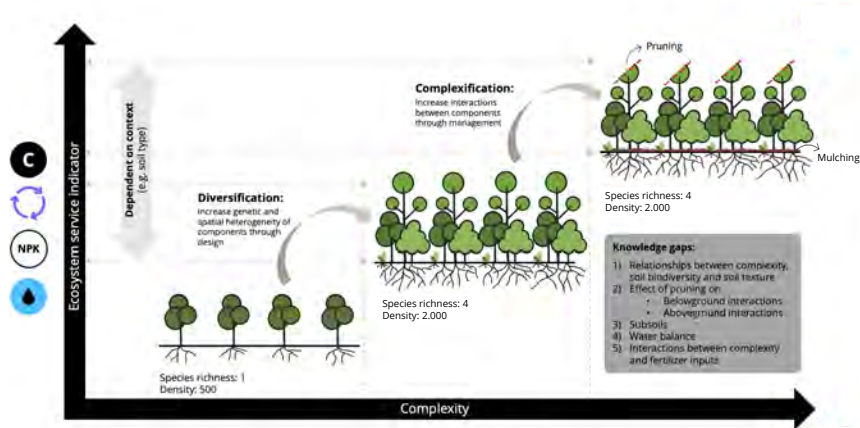
### 6.3 Increasing interactions through *in situ* biomass management

In all research chapters of this thesis, the importance of *in situ* biomass management (e.g. pruning & mulching) for enhancing soil-based ecosystem services was highlighted. Farmers applied this management in taxonomically and functionally diverse agroforestry systems, and pruning was mostly applied to pioneer trees which were intercropped for the specific purpose of generating *in situ* mulch (**Chapter 2**). However, the effects of pruning & mulching are likely more systemic than just the direct nutrient flows it generates. For instance, drastically reducing the crowns of tall canopy trees increases light interception for lower strata plants. This could trigger increased plant growth (Esche et al., 2022) and root exudation of these sub-canopy trees and grasses (Beer et al., 1998). Pruning & mulching also affects water dynamics: pruned trees temporarily transpire less (Buyinza et al., 2023), and the generated mulch reduces soil evaporation and increases water infiltration (Kearney et al., 2019; McIntyre et al., 2000; Nawaz et al., 2016). Modified moisture regimes greatly affect soil microbiomes, SOC decomposition and nutrient transfers in soils (Li and Schaeffer, 2020; Lützow et al., 2006). Therefore, *in situ* biomass management directly influences the interactions between components of the agroforestry systems, both above- and belowground.

### 6.4 Designing for diversity and managing for complexity

Diversifying agricultural systems results in increased heterogeneity, for instance by adding species or modifying the spatial pattern of crop fields (Ditzler et al., 2021). Many examples of diversification in agroforestry systems showcase designs based on scientific insights, such as adding trees for wind protection (Jacobs et al., 2022) or to provide shade for animals (Giro et al., 2019). More diverse components will almost inevitably interact, making these systems not only diverse but also complex (Bullock et al., 2022). The literature shows that this ‘diversifying by design’ results in variable but positive effects on the provision of multiple ecosystem services (Beilouin et al., 2021).

All of the agroforestry systems in this study are prime examples of diversification, as farmers designed them based on their knowledge of functional traits (**Chapter 2**). The most complex systems (e.g. successional agroforestry systems), however, went one step further and continuously applied management practices that were targeted at increasing the interactions between the diverse components of their systems. The results from this thesis (**Chapters 3, 4 and 5**) suggest that this targeted management (e.g. pruning & mulching) caused an additional ‘boost’ to ecosystem service provision. I propose that this combination of ‘diversifying through design’ and ‘increasing interactions through management’ could be referred to as ‘complexification’ (Fig. 6.2).



**Figure 6.2** | Conceptual overview of diversification and complexification. Species richness and density data are examples. Knowledge gaps are further outlined in the text below.

## 6.5 Outlook: Knowledge gaps

In **Chapter 3**, synergies between nutrient cycling and MAOC and POC stocks were shown, and also in **Chapter 4** there were no indications of trade-offs between SOC storage, nutrient provision and water regulation. Synergies between multiple ecosystem services, or soil ‘multifunctionality’, is an outcome often reported for agroforestry systems (Cerdeira et al., 2017; Li et al., 2022; Tschardt et al., 2011; Veldkamp et al., 2023). In Fig. 6.2, I propose that this increase in ecosystem service provision is caused by farmers’ ‘complexification’ but substantial knowledge gaps remain, particularly about mechanisms in soils that lead to these outcomes. Soils are incredibly diverse systems, teeming with life and a myriad of interactions between soil biological actors and the mineral matrix (Creamer et al., 2022) and even on fundamental processes such as SOC formation and persistence, our understanding is still evolving without a clear scientific consensus (Hoffland et al., 2020; Lehmann et al., 2020). It was shown that the soil microbiome is key in the delivery of soil-based ecosystem services (Neal et al., 2020) and highly responsive to management (Labouyrie et al., 2023), and yet, there is still much that needs to be uncovered. This is particularly the case for highly biologically active agricultural systems such as agroforestry (Schwarz et al., 2021).

Some key knowledge gaps that future research should address are the following (Fig. 6.2):

### 1) Relationships between complexity – soil biodiversity – soil texture

More and more evidence points to the importance of soil texture in shaping microbial communities (Crowther et al., 2014; Labouyrie et al., 2023), and this likely also influences the provision of ecosystem services. Witzgall et al. (2021) showed in an experimental setup that fungi play a key role in transforming

POC to MAOC, and that the rate of this activity was higher in sandy soils. Also in field trials in the study region, fungi were shown to be associated with changes between SOC pools over time (Bossolani et al., 2021). It is likely that agroforestry complexity has strong effects on microbial communities, even though the soil biological indicators tested in this thesis (microbial biomass C, betaglucosidase and acid phosphatase) could not confirm this. More research is needed on elucidating the role of soil biodiversity in the provision of ecosystem services (Creamer et al., 2022), and how agroforestry complexity and soil texture jointly influence biological processes in soil.

## 2) Effect of pruning on:

### a) Belowground interactions (root C inputs, effect on microbial community)

The effects of pruning on C, nutrient and biological dynamics in soils are largely unknown (Riedel et al., 2019). However, it was shown that drastic pruning induces root dieback (McIvor et al., 2018) and can therefore provide high quality organic matter inputs to soils (Clemmensen KE et al., 2013; McCormack et al., 2015). Dead roots might also turn into microbial hotspots, and pruned plants might induce microbial communities to increase nutrient solubilization (Spohn and Kuzyakov, 2014). Farmers also hypothesize that the release of chemical compounds from pruned trees in soil stimulates growth responses in neighbouring plants. All of these potential dynamics might have substantially contributed to the effects observed in pruned agroforests in this study, but need to be rigorously tested.

### b) Aboveground interactions (light interception and productivity)

Pruning of tall 'biomass' trees temporarily reduces tree crowns and increases light interception at lower strata. Positive effects of this practice on productivity have been shown for cocoa (Esche et al., 2022) and coffee (Schnabel et al., 2017), but remain underexplored for most other crops. Observations from farmers in this study suggest that citrus (particularly lime), banana and a wide range of vegetables could respond well to this dynamic shade management. A common practice amongst farmers in this study is to pile up pruning residues as mulch underneath tree rows, and the CO<sub>2</sub> released during the decomposition of this material might have additional fertilization effects on crops but that remains to be tested.

## 3) Subsoils

Results from this thesis show that effects of complexity on the provision of several ecosystem services are higher at 20-30 cm than at 0-10 cm in clayey soils. It is likely that these effects can be detected further down in soil profiles but this requires more studies. In tropical clayey soils, significant microbial activity has been shown at 3 m depth (Veldkamp et al., 2003) which is well within the range of tree roots commonly planted in the agroforestry systems. In sandy subsoils, SOC stocks might be substantially higher than expected due to increased tree root density at depth (Silver et al., 2000). Also nutrient uptake at depth (using deep rooted trees as nutrient 'pumps') should be further explored (Isaac and Borden, 2019).

#### 4) Water balance

Disentangling the effects of trees on water dynamics in agroforestry systems is challenging (Jacobs et al., 2022). Higher water uptake from increased root density in topsoil might be counteracted in more complex agroforestry by more even root distribution at deeper layers (Niether et al., 2017). Mulching is known to increase water retention, and lower temperatures in structurally more complex systems induce less evapotranspiration (Ehbrecht et al., 2019; Lin, 2010). Periodic pruning reduces total transpiration and has even been shown to reverse sap flows (Buyinza et al., 2023), but the sum of these effects, and especially the interactions between all of the aforementioned variables are unknown (Sarmiento-Soler et al., 2019).

#### 5) Interactions with fertilizer inputs

Fertilizer use amongst farmers in this study was generally low, but during initial planting it is common to use animal manures and natural soil amendments such as rock meal. The latter are often rich in Si, Ca and Al (Garbowski et al., 2023) and have been shown to stimulate microbially mediated nutrient solubilization when applied in combination with organic matter in Brazilian sandy soils (Lopes et al., 2021). The effectiveness of these less soluble inputs might depend on soil texture and agroforestry complexity due to specific microbial groups. More research is needed how rock meal and other fertilizers can be used to enhance or ‘kickstart’ nutrient cycling in agroforestry systems.

## 6.6 Outlook: Relevance for farmers

The ‘complexification’ which was shown to relate to increased ecosystem service provision (Fig. 6.2), also increased farm labour demand (**Chapter 5**). The importance of this finding was underlined by the participating farmers themselves, as they evaluated it to be one of the most severe challenges to scaling highly complex agroforestry systems. Successional agroforestry farmers also highlighted other pertinent challenges, such as the lack of available technology and policy support. Managers of less complex, multistrata agroforestry struggled less with challenges at the field and farm scale, such as availability of customized agricultural technology. Less complex silvopastures were not part of the subsample used in **Chapter 5**, but literature suggests that for these systems technology is largely available (Gil et al., 2015; Gil et al., 2016). The participating farmers in this study reported knowledge requirements to be less of an issue, but this is unlikely to be true for the average conventional farmer (Bendahan et al., 2018). For instance, even for relatively simple silvopastures access to knowledge was shown to be a significant constraint (Vinholis et al., 2020). These challenges make it more difficult for the large majority of conventional farmers to adopt more complex systems, increase ecosystem service provision and ultimately, become more resilient to environmental change (Anghinoni and Vezzani, 2021).

Nevertheless, I argue that within the current socio-economic conditions, silvopastures and multistrata agroforestry systems can be considered (relatively) low-hanging fruits for



farmers to achieve improved ecological performance. From a technological point of view, the tools to transition to integrated crop-livestock-forestry systems are available and actively promoted by Brazilian state funded agencies such as Embrapa (Gori Maia et al., 2021) or extension services such as Epagri (Vianna, 2022). For multistrata agroforestry systems, labour demand was in most cases lower than in corresponding monocultures (**Chapter 5**), making these systems attractive alternatives for small- to medium sized fruit producing farms with labour shortages. Next to knowledge diffusion about these systems, also good market access for secondary products such as timber or diverse fruits are key to enhance uptake amongst farmers (Huang et al., 2022).

For highly complex, successional agroforestry systems, I argue that more profound socio-economic changes are needed to enable wide-spread uptake. These would have to be supported by policies, but would also likely require an influx of new, skilled farmers to work in complexified systems (Nyberg et al., 2020) and a reversal of the trend of rural depopulation (Ryan, 2023). Making successional farming ‘easier’ through technology would certainly help, as long as it enhances farmers’ efficiency, and not completely eliminates their need to exist (Ditzler and Driessen, 2022). Adapted machinery for more efficient biomass management are being actively developed and tested by farmers and researchers in Brazil (De Morais et al., 2023). However, as long as degrading agricultural practices are still directly and indirectly supported by subsidies (Heyl et al., 2022), it will remain difficult for farmers of successional agroforestry to become sufficiently competitive in the market.

## 6.7 Final reflection

Gloomy, and yet scientific projections on climate change show that the future for farmers and society as a whole will be marked by strong perturbations (IPCC, 2022). And yet, the pioneering farmers in this study gave us a glimpse of how a more resilient future could look like (Valencia et al., 2022). The complexity gradient in this study showed that some of these agroforestry systems represent a rather small step from the current paradigm of simplification, and others can be seen as a giant leap. For some it only takes a few trees to be planted in a pasture, while the multi-layered, forest-like canopies of more complex systems must be reminiscent of how ancient Brazilian agroforests looked like in pre-Colombian times (Maezumi et al., 2018). Nevertheless, all of these systems were conceived and managed in a modern way, planted in linear rows and potentially mechanisable (Wolz et al., 2018). The public discourse often suggests that in order to respond to environmental challenges we have to choose between sparing or sharing, technified or natural (Loconto et al., 2020). The farmers in this study gave me the incredible opportunity to see that there might be a middle way, or maybe even a whole gradient of middle ways.

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# Summary

Agroforestry systems are often promoted to address the urgent challenges of land degradation and climate change by enhancing soil-based ecosystem services, such as nutrient cycling, water regulation and soil organic carbon (SOC) storage. However, agroforestry is an umbrella term for a large variety of systems which integrate trees and crops, and agroforestry system types may differ in the extent to which ecosystem services are provided. The complexity of agroforestry systems may be an important characteristic to explain ecosystem service provision levels, but this has not yet been assessed. It is also not clear how the level of ecosystem services provision by agroforestry systems are influenced by soil texture and depth, or age of the agroforestry system. Furthermore, knowledge gaps remain on how agroforestry complexity impacts farm labour demand. This lack of information hinders the implementation of multifunctional agroforestry systems that combine food production with enhanced ecosystem service provision.

Pioneering farmers have implemented a diversity of agroforestry systems in Brazil, ranging from relatively simple silvopastures where widely spaced timber trees are planted into pastures, to highly diverse and dense agroforests that mimic natural forests. The aims of this thesis were (i) to assess the relationship between agroforestry complexity and the provision of soil-based ecosystem services, (ii) to assess whether this relationship is context-dependent, and (iii) to assess the relationship between the complexity of agroforestry systems and labour demand, and identify other potential challenges for implementing agroforestry systems.

In **Chapter 2**, 30 agroforestry systems from the state of São Paulo, Brazil, are comprehensively described in terms of taxonomic and functional diversity, spatial structure and management, and classified as silvopastures, multistrata and successional agroforestry systems. Terrestrial LiDAR scans were performed to assess their structural complexity, and nutrient (N, P, K, Ca, Mg) stocks in litter were assessed as proxies for nutrient cycling. The LiDAR-derived Stand Structural Complexity Index (SSCI) was explained by tree species diversity and stem density in the agroforestry systems. Model selection indicated that the practice of pruning & mulching, tree species richness and stem density are important to enhance in situ nutrient cycling.

In **Chapter 3**, the Agroforestry Complexity Index (ACI) is proposed as a metric to explain variation in nutrient cycling and SOC storage. The ACI was derived from tree species richness, stem density and pruning & mulching frequency. Structural equation models indicated that ACI had direct effects on nutrient stocks in litter, and cascading effects on nutrient stocks and SOC fractions in soils. These models also indicated that higher cycling of Ca and P in more complex agroforestry systems had synergistic effects on SOC storage, particularly on sandy soils.

In **Chapter 4**, the relationships between the ecosystem services of SOC storage, nutrient provision and water regulation and ACI, soil texture, soil depth and the age of the agroforestry system are explored. Seven out of nine ecosystem service indicators were significantly positively associated with ACI. For nutrient provision (P and CEC) and macroporosity this effect was equally strong across soil types. However, for mineral associated organic carbon (MAOC), particulate organic carbon (POC), pH and available



water capacity (AWC) the effect size of the ACI was higher on sandy than on clayey soils. On clayey soils, however, three-way interactions indicated that positive effects of complexity on MAOC, POC, P, CEC and AWC were more pronounced at 20-30 cm depth than at 0-10 cm. This shows that agroforestry complexity might have more pronounced effects in deeper soil layers of clayey Ferralsols. The tested age gradient was not associated with ecosystem service provision, except for pH on sandy soils.

In **Chapter 5**, the relationship between agroforestry complexity, ecosystem services provision and labour demand is explored using a subsample of ten agroforestry systems. Detailed data on labour time spent on fertilization, pest control, weeding, crop management, biomass management and harvesting in agroforestry systems were compared to secondary reference data for corresponding monocultures. When comparing highly complex successional and less complex multistrata agroforestry systems, a trade-off was observed as higher labour demand in successional agroforestry was also associated with higher ecosystem service provision. The main contributor to both higher total labour demand and higher ecosystem service provisions was biomass management, which was in turn negatively associated with labour time for fertilization, pest control and weeding. Furthermore, important challenges to scaling agroforestry systems as perceived by farmers were, among others, lack of machinery for dense agroforestry systems and access to subsidies.

In **Chapter 6**, the development and usefulness of the ACI is evaluated in the context of agroforestry management and soil-based ecosystem services. Diversification and complexification are conceptually discussed and knowledge gaps for future research outlined. Lastly, challenges for the upscaling efforts to establish complex agroforestry systems for high ecosystem service provision are discussed.

## Resumo

Os sistemas agroflorestais (SAFs) são frequentemente promovidos para enfrentar desafios urgentes como a degradação da terra e das mudanças climáticas, porque esses sistemas aumentam a provisão de serviços ecossistêmicos, como a ciclagem de nutrientes, a regulação da água no solo e o armazenamento de carbono orgânico no solo (COS). No entanto, o termo SAF abrange uma grande variedade de sistemas que integram culturas florestais e agrícolas, desde os mais simples até sistemas de alta complexidade, e podem existir diferenças no nível de provisão de serviços ecossistêmicos. A complexidade dos SAFs pode ser uma característica importante para explicar os níveis de fornecimento de serviços ecossistêmicos, mas isso ainda não foi avaliado. Também não está claro como a textura, a profundidade do solo e a idade do SAF influenciam o nível de provisão de serviços ecossistêmicos. Além disso, ainda há lacunas de conhecimento sobre como a complexidade dos SAFs afeta a demanda de mão de obra agrícola. Essa falta de informações dificulta a implementação de SAFs multifuncionais que combinam a produção de alimentos com o aumento do fornecimento de serviços ecossistêmicos.

Agricultores pioneiros implementaram uma ampla diversidade de sistemas agroflorestais no Brasil, desde SAFs silvipastoris relativamente simples em que árvores madeiráveis são plantadas em pastagens, até SAFs altamente diversificados e densos que imitam florestas naturais. Os objetivos desta tese foram (i) avaliar a relação entre a complexidade agroflorestal e o fornecimento de serviços ecossistêmicos do solo, (ii) avaliar se essa relação depende do contexto e (iii) avaliar a relação entre a complexidade dos SAFs e a demanda de mão de obra e identificar outros possíveis desafios para a implementação de SAFs.

No **Capítulo 2**, 30 SAFs localizados no estado de São Paulo, Brasil, são descritos de forma abrangente em termos de diversidade taxonômica e funcional, estrutura espacial e manejo, e classificados como silvipastoris, multiestratos e sistemas agroflorestais sucessionais. Foram realizadas varreduras utilizando um LiDAR terrestre para avaliar sua complexidade estrutural, e os estoques de nutrientes (N, P, K, Ca, Mg) na serapilheira foram avaliados como indicadores da ciclagem de nutrientes. O *Stand Structural Complexity Index* (SSCI), derivado do LiDAR, foi determinado pela diversidade de espécies de árvores e pela densidade de plantio nos SAFs. A seleção de modelos estatísticos indicou que a prática de poda e aplicação de cobertura morta, a riqueza de espécies arbóreas e a densidade de árvores são importantes para melhorar a ciclagem de nutrientes *in situ*.

No **Capítulo 3**, o Índice de Complexidade Agroflorestal (ACI) é proposto como uma métrica para explicar a variação na ciclagem de nutrientes e no armazenamento de COS. O ACI foi derivado da riqueza de espécies arbóreas, da densidade de árvores e da frequência de poda e cobertura morta. A modelagem de equações estruturais indicou que o ACI teve efeitos diretos sobre os estoques de nutrientes na serapilheira e efeitos em cascata sobre os estoques de nutrientes e frações de COS nos solos. Esses modelos também indicaram que a maior ciclagem de Ca e P em SAFs mais complexos teve efeitos sinérgicos no armazenamento de SOC, especialmente em solos arenosos.

No **Capítulo 4**, são investigadas as relações entre os serviços ecossistêmicos de armazenamento de SOC, fornecimento de nutrientes e regulação da água e o ACI, a textura do solo, a profundidade do solo e a idade dos SAFs. Sete dos nove indicadores de serviços ecossistêmicos foram significativamente associados de forma positiva ao ACI. Para o fornecimento de nutrientes (P e CTC) e a macroporosidade, esse efeito foi igualmente forte em todos os tipos de solo. Entretanto, para o carbono orgânico associado a minerais (MAOC), carbono orgânico particulado (POC), pH e capacidade de água disponível (CAD), o tamanho do efeito do ACI foi maior em solos arenosos do que em solos argilosos. Em solos argilosos, no entanto, interações de três vias indicaram que os efeitos positivos da complexidade sobre MAOC, POC, P, CEC e CAD foram mais pronunciados a 20-30 cm de profundidade do que a 0-10 cm. Isso mostra que a complexidade agroflorestal pode ter efeitos mais pronunciados nas camadas mais profundas do solo de Latossolos argilosos. O gradiente de idade testado não foi associado à efeitos significativos, com exceção do pH em solos arenosos.

No **Capítulo 5**, a relação entre a complexidade agroflorestal, a provisão de serviços ecossistêmicos e a demanda de mão de obra é investigada usando uma subamostra de dez SAFs. Dados detalhados sobre o tempo de mão de obra gasto em fertilização, controle de pragas, capina, manejo cultural, manejo da biomassa e colheita nos SAFs foram comparados com dados de referência secundários para monoculturas correspondentes. Ao comparar sistemas agroflorestais sucessionais altamente complexos e sistemas agroflorestais multiestratos menos complexos, observou-se uma compensação, pois a maior demanda de mão de obra em sistemas agroflorestais sucessionais também foi associada a uma maior provisão de serviços ecossistêmicos. O principal contribuinte tanto para a maior demanda total de mão de obra quanto para a maior oferta de serviços ecossistêmicos foi o manejo da biomassa, que, por sua vez, foi negativamente associado ao tempo de mão de obra para fertilização, controle de pragas e capina. Além disso, desafios importantes para a ampliação dos SAFs, conforme percebido pelos agricultores, foram, entre outros, a falta de maquinário para SAFs densos e o acesso a subsídios.

No **Capítulo 6**, o desenvolvimento e a utilidade do ACI são avaliados no contexto do manejo agroflorestal e dos serviços ecossistêmicos do solo. A diversificação e a complexificação são discutidas conceitualmente e as lacunas de conhecimento para pesquisas futuras são delineadas. Por fim, são discutidos os desafios para os esforços de aumento de escala para estabelecer sistemas agroflorestais complexos para uma alta provisão de serviços ecossistêmicos.

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## About the author



Jonas was born on October 4th 1991 and grew up in the small town of Leichlingen, Germany, as the fourth child of his Swedish mother Eva and German father Ralph. During his high school period Jonas worked part-time as a gardener. Upon finishing high school, he walked from France to Spanish Santiago de Compostela and then went on to do his BSc in International Business at Maastricht University in the Netherlands. During an exchange semester at the University of São Paulo in Brazil, he fell in love with the local culture, and even more in love with his future wife Erica. After completing his BSc studies in Maastricht in 2014, Jonas moved to Brazil and started to work for the urban agriculture NGO Cidades Sem Fome. After two years of working with urban vegetable farmers in São Paulo's periphery, he was introduced to agroforestry and spent several months as an intern on agroforestry farms across Brazil in 2016. In 2017, Jonas started his MSc in Organic Agriculture at Wageningen University and graduated (*cum laude*) in 2019. In the same year, he started his PhD on Brazilian agroforestry systems at the Farming System Ecology and Soil Biology groups of Wageningen University. Erica and Jonas live together with their daughter Naomi and her soon-to-be born sister on a small homestead about 60 km from the city of São Paulo in Brazil.



# List of publications

## *Peer-reviewed articles*

**Steinfeld, J.P.**, Bianchi, F.J.J.A., Locatelli, J.L., Rizzo, R., de Resende, M.E.B., Ballester, M.V.R., Cerri, C.E., Bernardi, A.C. and Creamer, R.E., 2023. Increasing complexity of agroforestry systems benefits nutrient cycling and mineral-associated organic carbon storage, in south-eastern Brazil. *Geoderma* **440**, 116726.

**Steinfeld, J.P.**, Miatton, M., Creamer, R.E., Ehbrecht, M., Valencia, V., Ballester, M.V.R. and Bianchi, F.J.J.A., 2024. Identifying agroforestry characteristics for enhanced nutrient cycling potential in Brazil. *Agriculture, Ecosystems & Environment* **362**, 108828.

# PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities).



## Review/project proposal (9 ECTS)

- Agroforestry and ecosystem services in the Atlantic forest biome, Brazil
- CANOPIES proposal

## Post-graduate courses (4.1 ECTS)

- Workshop; Global Network of Lighthouse Farms (2019)
- Basic statistics; PE&RC and WIMEK (2019)
- Spring school world soils and their assessment; ISRIC (2021)

## Laboratory training and working visits (4.5 ECTS)

- Physical fractionation of soil organic matter; University of Sao Paulo/ESALQ (2021)

## Invited review of journal manuscripts (1 ECTS)

- Geoderma Regional: empirical approach for developing production environment soil health goals, New York, USA (2023)

## Deficiency, refresh, brush-up courses (0.3 ECTS)

- Forest inventory in R; Florestal Package (2021)

## Competence, skills and career-oriented activities (4.15)

- Searching and organising literature; WUR Library (2019)
- Research data management; WGS (2020)
- Project and time management; WGS (2020)
- Scientific integrity; WGS (2020)
- Creating and pitching virtual posters; In'to Languages (2022)

## PE&RC Annual meetings, seminars and PE&RC weekend/retreat (2.1 ECTS)

- PE&RC Weekend for first years (2019)
- PE&RC Biodiversity symposium (2019)
- PE&RC Last year retreat (2023)



### **Discussion groups/local seminars or scientific meetings (4.5 ECTS)**

- CENA/USP Literature review meetings (2020)
- reNature science team meetings (2020-2022)

### **International symposia, workshops and conferences (11.3 ECTS)**

- SIMA conference on agroforestry complexity; oral presentation; virtual (2021)
- UFSCar workshop on agroforestry; oral presentation; Sorocaba, Brazil (2021)
- BonaRes soils conference; poster presentation; Berlin, Germany (2023)

### **Societally relevant exposure (2.5 ECTS)**

- Presentations to farmers (2020-2023)
- Textbox on CANOPIES project in IPCC report (2022)

### **Lecturing/supervision of practicals/tutorials (6 ECTS)**

- Methodologies for reading sustainable foodscapes (2020-2022)
- Agroecology (2020-2023)

### **BSc/MSc thesis supervision (3 ECTS)**

- Metrics for defining complexity in agroforestry systems
- Labour demand in innovative agroforestry systems
- Soil organic carbon assessment in regenerative agriculture
- Al and Fe oxides and their relationship with SOC storage in sandy tropical soils under agroforestry

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