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Deep soil carbon accrual after land restoration in the Atlantic Forest, Brazil

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Abstract

Soils play a crucial role in the global carbon cycle, not only by storing most of terrestrial carbon but also by actively participating in carbon sequestration and emission processes. Reliable estimates of soil organic carbon stocks changes after land use change are essential for developing effective climate change mitigation strategies. However, traditional estimates often focus on topsoil, overlooking the substantial contribution of deeper soil layers, particularly in the tropics. This thesis addresses this gap by providing global estimates of deep soil carbon stocks and examining the impact of various land restoration approaches on these stocks within the Atlantic Forest of Brazil, in addition to assessing deep soil carbon stability and temperature sensitivity.

I compiled a comprehensive database of over 12,000 soil profiles, ranging in depth from 200 to 500 cm, to update global estimates of deep soil carbon stocks in relation to climate, soil type, and land use. Additionally, I studied the response of deep soil carbon to different land restoration approaches, including reforestation, natural regeneration, and agroforestry systems, using a paired site design in triplicates for each method. Samples were taken from rural sites in the Atlantic Forest, with adjacent arable land and a secondary forest serving as references. I also evaluated soil carbon stability and temperature sensitivity at various depths along the soil profile to understand the factors influencing temperature sensitivity in restored lands.

The soil organic carbon stock for the 0-200 cm depth interval was 19% larger than previously thought, adding an extra 336 Pg of carbon stored in soils compared to previous global estimates. Soils in tropical climate have the highest stock in the 0-300 cm layer, with an average of 314 Mg ha^{-1}. Forests significantly contribute to this deep carbon pool, with 69% of the soil organic carbon stock in the 0-300 cm layer located below 40 cm. The land restoration case study in the Atlantic Forest showed no significant differences in total ecosystem carbon sequestration among the three restoration methods, although reforestation sites promoted higher plant aboveground carbon stocks than natural regeneration and agroforestry systems. There was an inverse relationship between aboveground carbon and deep soil carbon sequestration, and reforestation sites with fast aboveground growth caused a loss of 27 Mg ha^{-1} of soil organic carbon in the 40-300 cm layer. Moreover, reforestation sites had the lowest deep soil carbon stability, whereas agroforestry system presented the highest. Further, deep soil temperature sensitivity in restored lands was mostly influenced by soil fertility, particularly phosphorus content in the subsoil and nitrogen content in the deep soil.

The results showed that agroforestry systems and natural regeneration, which sequestered more carbon in deep soil in addition to increasing deep soil carbon stability, can enhance climate change mitigation benefits more effectively than reforestation, which is the most commonly used restoration approach in the Atlantic Forest. Transitioning from reforestation to these alternative approaches, where feasible, can bolster carbon stability and maximize the climate mitigation potential of land restoration efforts in the Atlantic Forest. In conclusion, this thesis demonstrated the significant contribution of deep soil carbon to total soil carbon dynamics, emphasizing the need to include deep soil layers in assessments of carbon sequestration following land restoration.

Zusammenfassung

Böden beeinflussen signifikant den globalen Kohlenstoffkreislauf als C-Speicher, -Quelle und -Senke. Doch zuverlässige Schätzungen der Boden-C-Vorräte und wie diese sich durch Landnutzung ändern, konzentrieren sich in der Regel nur auf den Oberboden und vernachlässigen den tieferen Unterboden. Diese Arbeit schließt diese Forschungslücke, indem sie weltweit die C-Vorräte im tieferen Unterboden schätzt sowie als Fallstudie im Atlantischen Regenwald Brasiliens untersucht, wie sich unterschiedlichen Landnutzungen auf die C-Vorräte und die Stabilität des tiefen Bodenkohlenstoffs auswirken.

Ich habe eine Datenbank von über 12.000 Bodenprofilen erstellt, die Tiefen bis zu mindestens 200 cm und mehr erfassen. Damit wurden früher publizierte Schätzungen der globalen Kohlenstoffvorräte im tiefen Boden in Abhängigkeit von Klima, Bodentyp und Landnutzung aktualisiert. Weiterhin habe ich im Atlantischen Regenwald unterschiedliche Ansätze zur Bodenrestaurierung, wie Aufforstung, natürlicher Regeneration und Agroforstsysteme, unter Anwendung eines gepaarten Standortdesigns mit dreifachen Wiederholungen untersucht, um die C-Anreicherung im Unterboden zu ermitteln. Proben aus angrenzenden Ackerböden und aus Sekundärwaldflächen dienten als Referenz. Mittels Inkubationen im Labor wurde die Abbaubarkeit des Unterbodenkohlenstoffs bei unterschiedlichen Temperaturen ermittelt.

Meine Schätzungen ergaben, dass der Vorrat an organischem Bodenkohlenstoff für das Tiefenintervall von 0-200 cm um 19% größer ist als bisher angenommen, was weltweit 336 Pg zusätzlichen Kohlenstoff in Böden entspricht. Die Böden in tropischen Klimazonen weisen den höchsten Vorrat in 0-300 cm-Bodentiefe auf, im Schnitt 314 Mg C ha^{-1}. Wälder tragen signifikant zu diesem tiefen Kohlenstoffpool bei, wobei 69% des Vorrats an organischem Bodenkohlenstoff in den oberen 300 cm Bodentiefe unterhalb von 40 cm liegen. Die Fallstudie zur Bodenrestaurierung im Atlantischen Regenwald zeigte keine signifikanten Unterschiede zwischen den drei Restaurierungsansätzen hinsichtlich Gesamtkohlenstoffvorräte im Ökosystem. Zwar zeigten Aufforstungsflächen höhere Kohlenstoffvorräte im Boden als natürliche Regenerations- und Agroforstsysteme, allerdings bestand eine umgekehrte Beziehung zwischen oberirdischem Kohlenstoff und den Kohlenstoffvorräten im tiefen Boden. Aufforstungsflächen mit schnellem oberirdischem Biomassezuwachs führten zu einem C-Verlust von 27 Mg ha^{-1} im Unterboden > 40 cm Tiefe. Darüber hinaus zeigte der tiefe Humus unter den Aufforstungsflächen die niedrigste Stabilität gegenüber mikrobiellem Abbau, wohingegen derjenige im Agroforstsystem die höchste Stabilität aufwies. Die Bodenfruchtbarkeit, insbesondere die Gehalte an P und N im Unterboden nehmen hierfür eine Schlüsselstellung ein.

Die Ergebnisse zeigen, dass Agroforstsysteme und natürliche Regenerationsflächen mehr zur Kohlenstoffspeicherung und damit zur Abschwächung des Klimawandels beitragen als Aufforstung, der am weitesten verbreitete Restaurierungsansatz im Atlantischen Regenwald. Zugleich erhöhen die Alternativen zur Aufforstung die Stabilität des Unterboden-C gegenüber mikrobiellem Abbau. Insgesamt lässt sich mit dieser Arbeit ein bedeutender Beitrag des tiefen Bodenkohlenstoffs für die C-Speicherung in Böden belegen, die nicht immer mit einem Anstieg der oberirdischen Biomasse korreliert und daher gesondert erfasst werden sollte.

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List of Abbreviations

AG	Agriculture
AGB	Aboveground biomass
AGC	Aboveground carbon
AFSa	Agroforestry system in advanced stage
AFSi	Agroforestry system in intermediate stage
C	Carbon
CO_2e	CO_2 equivalent
DBH	Diameter at breast height
ha	hectare
Mg	Megagrams
Mha	Million hectares
NR	Natural regeneration
Pg	Petagrams
Qr	Basal-to-substrate induced respiration ratio
Q10	Temperature sensitivity coefficient
RE	Reforestation with mixed native species
SF	Secondary forest
SIR	Substrate induced respiration
SOC	Soil organic carbon

1. CHAPTER 1: GENERAL INTRODUCTION

1.1. Rationale

In response to the growing concern over climate change, there is a pressing need for effective mitigation measures (Halonen et al., 2021; Zurek et al., 2022). One such strategy that holds promise is land restoration (Lewis et al., 2019). By rehabilitating degraded environments, land restoration offers significant opportunity for carbon sequestration (Griscom et al., 2020). Key to this process is the restoration of vegetation cover, which acts as a natural carbon sink, absorbs atmospheric carbon dioxide (CO_2) and sequesters it within biomass and soil substrates (Lewis et al., 2019).

Brazil exhibits significant potential for land restoration, with particular emphasis on the restoration of the Atlantic Forest (Brancalion et al., 2019; Crouzeilles et al., 2019; Rodrigues et al., 2011), a region of paramount importance due to extensive historical deforestation, (Carlucci et al., 2021; Strassburg et al., 2020). The significance of this ecosystem is underscored by initiatives such as the Restoration Pact, a collaborative effort involving multiple stakeholders aimed at promoting land restoration in the Atlantic Forest region (Guedes Pinto & Voivodic, 2021; Pinto et al., 2014).

The evaluation of potential carbon sequestration through land restoration has predominantly focused on aboveground biomass assessments (Domke et al., 2020; Strassburg et al., 2020). Integrating soil carbon pools into these assessments can significantly enhance the estimated sequestration potential of land restoration initiatives (Lewis et al., 2019; Zanini et al., 2021). Moreover, deeper soil layers are often overlooked in restoration evaluations (Mendes et al., 2019) despite their susceptibility to alterations (Lan et al., 2021; Mobley et al., 2015).

Deep soil carbon is a critical component of soil carbon dynamics (Kögel-Knabner & Amelung, 2021). Although surface soils typically exhibit higher carbon concentrations, the stocks present in deep soil layers play a significant role, comprising more than half of the total soil carbon pool in tropical environments (Borchard et al., 2019; Strey et al., 2017). Yet, these deeper layers are susceptible to changes, with the potential for both increases and decreases in stored carbon following land-use change (Hong et al., 2020; Jackson et al., 2002). Consequently, incorporating assessments of deep soil layers into land restoration evaluations is imperative to better understand the efficacy of restoration practices in mitigating carbon emissions.

1.2. State-of-the-art

1.2.1. Land restoration and climate change mitigation potential

To adhere to the critical objective of constraining global warming to 1.5 °C, the world must strive for net-zero carbon emissions by mid-century (IPCC, 2022). This ambitious goal requires not only a reduction in the emissions but also the removal of CO_2 from the atmosphere (Roe et al., 2021). Among the most prominent strategies for carbon removal is land restoration, which holds significant promise in mitigating climate change by sequestering carbon in plant biomass and soil (Busch et al., 2019; Griscom et al., 2017).

Griscom et al. (2017) concluded that land restoration is the natural climate solution - land-based management practices that reduce emissions and sequester carbon - with highest sequestration potential estimated to range from 2.7 to 17.9 Pg carbon dioxide equivalent (CO_2e) per year by 2030, depending on the assumption of land availability. Similarly, Roe et al. (2021) pointed out that afforestation and reforestation hold the highest potential among land-based measures (natural processes on land to absorb and store carbon), reaching 8.5 Pg CO_2e y^{-1}. Consequently, land restoration emerges as one of the most promising alternatives for removing carbon from the atmosphere, underscoring its pivotal role in global efforts to tackle climate change (Lewis et al., 2019).

The United Nations designated 2021-2030 as the Decade on Ecosystem Restoration, significantly elevating global awareness and commitments towards ecosystem restoration initiatives (Fischer et al., 2021). International multi-sectoral commitments have been set up to unite different stakeholders to facilitate the implementation of restoration projects, as for example the Bonn Challenge and the New York Declaration on Forests (Sewell et al., 2020). Global pledges are made to restore 350 million hectares land by 2030 (Sewell et al., 2020), with a notable focus on tropical regions (Griscom et al., 2020). In Brazil, the Atlantic Forest Restoration Pact was created in 2009 with the goal of restoring 15 million hectares of degraded and deforested land by 2050 (Crouzeilles et al., 2019).

The Atlantic Forest is a biome renowned for its rich ecological diversity and unique species (Joly et al., 2014). The Atlantic Forest once covered approximately 1.5 million km^2 along the eastern coast of Brazil, extending into parts of Paraguay and Argentina. However, due to deforestation and urbanization, it has been reduced to less than 12% of its original size (Ribeiro et al., 2009), making one of the world's most threatened and fragmented tropical forests (Lima et al., 2015, 2024).

The carbon density within the Atlantic Forest Biome parallels that of the Amazon, which has larger territorial coverage, resulting in a comparatively higher carbon stock (Longo et al., 2016; Vieira et al., 2011). Notably, the Atlantic Forest Biome allocates a larger area for restoration initiatives than the Amazon Biome due to historically greater levels of deforestation than in the Amazon, resulting in an original cover of approximately 12% (Ribeiro et al., 2011), rendering it a focal point for restoration endeavours (Leite et al., 2012; Soares-Filho et al., 2014). Moreover, the significant biodiversity in this region further accentuates the status of the Atlantic Forest as a restoration hotspot on a global scale (Melo et al., 2013; Rezende et al., 2018).

Different approaches can be used to restore degraded lands, ranging from active restoration with intensive use of external inputs and energy to passive restoration with little or no input (Meli et al., 2017). The debate over which method promotes the highest aboveground growth is ongoing in tropical forests (Crouzeilles et al., 2017; Díaz-García et al., 2020). In the Atlantic Forest, reforestation with mixed native species - an active restoration approach - promotes the highest growth when assessing aboveground biomass and topsoil (Brancalion et al., 2021; Zanini et al., 2021). However, an assessment that integrates deep soil carbon pools is still missing. Hence, incorporating deep soil into the assessment framework of land restoration in the Atlantic Forest could help to understand the full climate change mitigation potential of these efforts.

1.2.2. The importance of deep soil layers

Studies evaluating carbon stocks in ecosystems often prioritize the assessment of aboveground pools, overlooking substantial carbon reservoir within the soil pool (Mendes et al., 2019). Consequently, the exclusion of soil carbon leads to an underestimation of the ecosystem carbon stock. Furthermore, when soil is considered, studies commonly focus on topsoil layers (Yost & Hartemink, 2020), neglecting a significant portion of soil carbon stock located in deeper layers (Borchard et al., 2019; Kögel-Knabner & Amelung, 2021). Hence, deep soil layers constitute a crucial component of ecosystem carbon dynamics and should be included in comprehensive carbon assessments.

Global studies pointing at the importance of deep soil layers are already decades old. Richter and Markewitz (1995) showed a rapid decrease in carbon (C) concentration in the first 200 cm of the soil, but significant amounts were still found down to 600 cm in a soil profile in the USA. Mikhailova et al. (2000) demonstrated the importance of roots to the C cycle extending to 200 cm in Russia. In Malawi, the introduction of leguminous tree *Gliricidia* spp. (Fabaceae) to maize crops increased the soil organic carbon (SOC) stock by 200 cm (Makumba et al., 2007). More recently, land use was found to statistically influence the SOC concentration at depths of 500 cm in China (Yu et al., 2019).

In Brazil, the contribution of deep layers to the overall SOC stock has been demonstrated. Cerri and Volkoff (1987) found that 78% of the soil C was stored below 40 cm in a 500-cm deep soil profile in the Amazon forest. Later, in a primary forest in the Amazon, Sommer et al. (2000) found that 70% of the C was stored below 40 cm in a 600-cm deep profile, whereas in a nearby palm plantation, it increased to 78%. More recently, Strey et al. (2017) found that in a 1000-cm deep soil profile, 80% of the C is stored below 30 cm in primary Amazon forests. Overall, these studies show that deep soil layers are an important component of the carbon cycle because they hold a higher share of the SOC stock.

In the Atlantic Forest, some studies assessed layers below topsoil, but rarely below 100 cm. Vicente et al. (2016) found different SOC stocks among natural forests, planted forests, and pasture in a 100-cm soil profile and in individual layers up to 100 cm in the Atlantic Forest. In the same biome, Monroe et al. (2016) found different SOC stocks in a 100-cm profile among agroforest systems and pasture, although the stock in layers below 40 cm did not differ. Interestingly, Gama-Rodrigues et al. (2010) found that a traditional cocoa agroforestry system had higher SOC stock in topsoil than native Atlantic Forest, although the stock in the 60-100 cm layer was higher in the latter than in the former. Therefore, the layers below topsoil appear to be influenced by land use in the Atlantic Forest Biome.

When deep soil layers are included in ecosystem C assessments, soil tends to be the dominant pool. Borchard et al. (2019) demonstrated that the SOC stock in the 0-300 cm layer represented about 80% of the total C in an agroforestry system in Indonesia. In a secondary forest in Singapore, Ngo et al. (2013) found that the SOC stock in the 0-300 cm soil layer was 52% of the total ecosystem C, whereas aboveground C accounted for 38%. In a preserved Atlantic Forest remnant, Vieira et al. (2011) noted that the SOC stock in the 0-100 cm layer was twice that of the aboveground C stock. Moreover, when deeper soil layers are included in the assessment of management practices, the overall conclusions can change. For example, the interpretation of no tillage on SOC stocks changed from a 9% increase when topsoil was considered to a 13% decrease when the assessment was extended up to 75 cm deep (Olson & Al-Kaisi, 2015). In a study of land use change from grazed meadows to shrubland, Chen et al. (2022) found a 35% increase in SOC stock after including the 40-120 cm soil layer in the analysis. Thus, including layers below the topsoil highlights the importance of the deep soil carbon pool to the ecosystem and can alter the overall interpretation of the results.

In summary, deep soil layers serve as substantial reservoirs of SOC, often comprising a significant portion of the total SOC stock. Consequently, when accounting for deep soil layers, the cumulative SOC stock typically exceeds that of aboveground carbon stocks. Also, it is noteworthy that subsoil layers can be influenced by land use practices. Nevertheless, research investigating soil depths exceeding 100 cm in the Atlantic Forest is limited. Hence, it is essential to integrate deep soil layers into carbon stock assessments within this biome to capture a more comprehensive understanding of the total carbon dynamics.

1.2.3. Soil respiration and temperature sensitivity

Soil respiration is a fundamental process in terrestrial ecosystems, in which microorganisms metabolize organic carbon compounds present within the soil matrix, releasing CO_2 as a by-product (Ryan & Law, 2005; Xu & Shang, 2016). Soil respiration can be categorized into autotrophic respiration, which originates from plant roots, and heterotrophic respiration, which is driven by soil microorganisms decomposing organic matter (Cheng & Kuzyakov, 2005; Feng & Zhu, 2019). Heterotrophic respiration accounts for the majority of soil respiration and thus plays a crucial role in the global carbon cycle (Lei et al., 2021; Schlesinger & Andrews, 2000; Xu & Shang, 2016). Understanding soil carbon respiration is of paramount importance for predicting feedback between terrestrial ecosystems and climate change, as it regulates the net exchange of carbon between soil and the atmosphere (Bond-Lamberty et al., 2018; Nissan et al., 2023). Thus, assessing soil carbon respiration is essential for understanding ecosystem carbon dynamics and elucidating its implications for climate change mitigation strategies.

The rate of soil heterotrophic respiration is an indicator of soil carbon stability because it reflects the relative decomposability or stability of soil organic matter (SOM) to microbial decay. Soil incubation experiments are often used to assess soil carbon stability, which measures the CO_2 emitted under controlled conditions (Schädel et al., 2020). Since carbon stability indicates the resistance and inaccessibility of organic matter to biological decomposition, soil incubation experiments are the only biological test that directly assesses carbon stability (Schädel et al., 2020). Although other methods exist to partition SOM into pools of varying stability (von Lützow et al., 2007), they do not provide a direct biological assessment.

Soil respiration is influenced by a combination of intrinsic soil characteristics and environmental factors (Davidson & Janssens, 2006; Xu & Shang, 2016). The influence of temperature on soil respiration is referred to soil temperature sensitivity and represented by the parameter Q10, which is defined as the factor by which soil respiration increases when the soil temperature increases by 10°C. The higher the Q10 value, the more soil carbon is respired per 10°C increase in temperature (Meyer et al., 2018; Qi et al., 2002). Soil temperature sensitivity is an important characteristic of soils and organic matter, as it plays a key role in the global carbon cycle. Slight changes in soil temperature can significantly impact the amount of CO_2 released from soils, which is a major source of atmospheric CO_2 (Bond-Lamberty et al., 2018; Schlesinger & Andrews, 2000; Xu & Shang, 2016). As temperatures are projected to rise due to climate change, soils are anticipated to respond with heightened respiration rates, thereby establishing a negative feedback mechanism in response to climate change (Haaf et al., 2021; Nissan et al., 2023).

Contrasting findings regarding soil temperature sensitivity across different depths of the soil profile were observed, along with the identification of various factors that influence this sensitivity. Fierer et al. (2003) found that temperature sensitivity in deeper soil layers was 3.9, higher than that topsoil (3.0) under grasslands and attributed this to a decrease in carbon quality and changes in the microbial community. In forest ecosystems in China, Li et al. (2020) also found higher temperature sensitivity in deeper layers due to differences in carbon quality. Higher Q10 with depth was also found by Soong et al. (2021) in a conifer forest, although they attributed it to different organic matter protection mechanisms along the soil profile. In permafrost regions, Mu et al. (2016) found higher Q10 in deeper layers, raising awareness of the negative impact of thawing on the climate cycle. In contrast to these studies that found higher Q10 with depth, Gillabel et al. (2010) showed that physical SOM protection in deeper layers in an agricultural area promoted a decrease in temperature sensitivity with depth: it ranged from 1.9 to 3.8 depending on the fraction assessed in the topsoil to 0.9 to 1.4 in the subsoil. Similar results of mineral protection were also attributed to negatively influenced deep soil Q10 in a tropical Andisol in a forest ecosystem (McGrath et al., 2022). Yet, Wordell-Dietrich et al. (2017) associated the decrease in Q10 with depth to a combination of physical protection, OM quality, and microbial community in a beech forest. Lastly, and contrasting to all previously cited studies, Pries et al. (2017) found a constant Q10 along the soil profile in a two-year whole-soil warming experiment conducted in a coniferous forest.

These diverse findings on deep soil temperature sensitivity highlight its multifaceted nature. The interplay between factors such as OM quality and protection and microbial community dynamics underscores the complexity of this phenomenon. Considering the spatial variability of these factors along soil profiles, it is plausible to expect corresponding variations in temperature sensitivity. Therefore, a comprehensive analysis that considers the combined influence of these factors can shed light on their relative importance in shaping soil temperature sensitivity patterns along soil profiles.

1.2.4. Land restoration approaches

Several approaches can be used to restore degraded lands. In the case of forest land restoration, the most common approaches are natural regeneration, planting seedlings, direct seeding, soil transposition, and nucleation (Fowler et al., 2015; Nunes et al., 2020; Raupp et al., 2020; Schlawin & Zahawi, 2008). In general, approaches can be grouped into active and passive methods (Meli et al., 2017), although in practice there is a continuum of intervention possibilities, ranging from unassisted to intensively assisted recovery (Chazdon et al., 2021). Ecosystem resilience, past land use history, and the surrounding environment should be used to define which restoration strategy should be implemented; however the higher the degradation level, the higher the need to intervene and apply more active approaches (Holl & Aide, 2011; Stanturf et al., 2014).

Passive land restoration involced natural processes tha restore degraded ecosystems without direct human interventions (Díaz-García et al., 2020). The resilience of ecosystems is required for natural regeneration to occur (Chazdon et al., 2021; Holl & Aide, 2011). Passive restoration is frequently regarded as a cost-effective approach, particularly when nearby forest fragments serve as seed sources (Lawson & Michler, 2014; Shono et al., 2007). A review of 113 studies found that passive restoration promoted better restoration success for biodiversity and forest structure parameters than active restoration (Crouzeilles et al., 2017). On the other hand, Reid et al. (2018) argued that the greater success of natural regeneration may be due to selection bias. They argued that evaluations of natural regeneration occurred in areas where restoration had succeeded, whereas evaluations of active restoration were carried out under a broad range of conditions, thus undermining general comparisons. Hence, paired site studies are critical for comparing the success of different restoration approaches.

Active land restoration involves direct human intervention to restore degraded ecosystems, such as by reintroducing vegetation through planting and seeding (Dimson & Gillespie, 2020). Active restoration aims to accelerate the recovery process or change the ecological succession of a site that has passed a threshold of degradation and cannot recover naturally (Chazdon et al., 2021). In contrast, passive restoration requires minimal to no human interventions and relies on natural regeneration processes (Bechara et al., 2016).

In the Atlantic Forest, most restoration interventions are performed actively via seedling planting, followed by passive restoration (Brancalion et al., 2016; Guerra et al., 2020). The history of land restoration in the Atlantic Forest can be divided into five phases according to Rodrigues et al. (2009), and can be summarized as: 1) implemented before 1920, forest restoration projects aimed primarily at soil and water resource protection. Species selection was based on economic value rather than ecological principles, leading to the use of exotic species without careful analysis, resulting in problems of alien species invasion; 2) from the 1970s to the late 1980s, reforestation projects shifted towards forest succession, prioritizing native species, although a limited number were typically planted. These species were often fast-growing but not necessarily local, leading to high mortality rates due to poor seedling adaptation; 3) focus on replicating the floristic, structural, and successional processes of regional forest remnants. Knowledge of gap dynamics was applied with the increased use of regional species alongside nonregional and exotic ones. Reforestation projects adopted a modular approach, planting a diverse mix of pioneers, secondary, and climatic species; 4) around the year 2000, efforts shifted towards restoring ecological processes rather than replicating nearby forest remnants. The emphasis was placed on the trajectory of forest development rather than its final structure. Additionally, traditional planting methodologies came under scrutiny, leading to the emergence of alternative approaches; 5) currently, there is an emphasis on income generation through reforestation, incorporating both timber and non-timber products, and payment for ecosystem services. The planning of reforestation projects now includes techniques aimed at reducing restoration costs.

An attractive restoration strategy for small-scale farmers involves the adoption of successional agroforestry systems, which offer the dual benefit of generating income while concurrently rehabilitating the land (Blinn et al., 2013; Padovan et al., 2022; Shennan-Farpón et al., 2022). Successional agroforestry systems combine the principles of agroecology and natural regeneration to produce secondary forests, while ensuring food security for small-scale landholders in tropical regions (Miccolis et al., 2019). This approach mimics the process of forest development and builds a multi-layer forest structure in the final stages (Cardozo et al., 2022). Initially, a mix of native and exotic trees is planted in rows. In the initial stages (up to 5-8 years depending on the system characteristics), agricultural crops are planted between rows. During this period, trees are pruned to produce organic material and provide nutrients to the soil (Young, 2017). Also, fruits are harvested from the planted trees, like avocado, mango, cacao, guava, and banana. After canopy closure, sowing crops is not possible anymore, but the system continues to produce fruits until later stages, although the amount decreases over time (Gama-Rodrigues et al., 2021; Padovan et al., 2022). In the final stages, the area will resemble a multi strata secondary forest (Vieira et al., 2009).

Studies comparing active and passive restoration approaches in the Atlantic Forest show a more pronounced response of active restoration to aboveground carbon accumulation, although it has no effect on topsoil organic carbon (OC). Zanini et al. (2021) found that active restoration promoted higher tree growth after five years, although no differences were found in the SOC stock in the 0-20 cm layer. Brancalion et al. (2021) evaluated passive and active restoration across a chronosequence of 60 years and noted that active restoration accumulated 50% more aboveground C stock than passive restoration, whereas the SOC stock in the 0-20 cm layer did not differ between the approaches. Ferez et al. (2015) evaluated the effect of a high-input silvicultural system for reforestation in the Atlantic Forest and found that the aboveground stock was more than 3-fold higher than that of a low-input system after 6 years of planting, although there was no difference in the SOC stock in the 0-30 cm layer.

Studies on agroforestry systems also denote their capacity for carbon sequestration (Froufe et al., 2011; Monroe et al., 2016; Thomazini et al., 2015), although comparisons of agroforestry with other methods are more scarce. Pontes et al. (2019) did not find differences in the aboveground biomass stock of agroforestry systems and mixed species plantations 20 years after establishment. Similarly, Badari et al. (2020) did not find any differences between the aboveground biomasses of the two systems after 15 years. In both studies, soil was not included in the analysis, thus precluding a comparison of the effects of each approach on this pool.

A comparative analysis of various restoration approaches is essential for determining the most effective strategy for carbon sequestration. However, there is a notable scarcity of studies comparing active restoration with mixed-species plantation, passive restoration via natural regeneration, and agroforestry systems, which are the predominant methods utilized in the Atlantic Forest region. Furthermore, to the best of my knowledge, no previous study has comprehensively compared these approaches while incorporating both aboveground biomass and deep soil carbon pools into the analysis. Given that the inclusion of deeper soil layers can significantly influence the interpretation of the results, it is imperative to incorporate this information to offer more robust recommendations for land restoration aimed at maximizing carbon sequestration potential.

1.3. Objectives

This study aims at assessing the potential of land restoration to tackle climate change by sequestering carbon, with a particular focus on deep soil carbon. I hypothesize that including deep soil layers in land restoration assessments will alter our understanding of the climate change mitigation potential of restoration efforts, because deep soil layers contain a significant portion of ecosystem carbon and are susceptible to changes following the introduction of trees. I will, on the one hand, examine deep soil carbon changes after land restoration. On the other hand, I will examine soil carbon stability in a future warmer climate. To address this objective, the study is divided into three chapters. In the first chapter, I analysed global patterns of deep soil carbon stocks, demonstrating how climate and land use influence deep soil carbon stocks. I created a large database, updating results that are decades old. In the second chapter, I assessed how different land restoration approaches influence deep soil carbon stocks in a rural settlement in the Atlantic Forest of Brazil. Here, I collected soil up to 300 cm, determined C content in the laboratory, and calculated carbon stocks, in addition to measuring and calculating aboveground carbon stock. In the third chapter, I determine potential soil carbon losses by measuring soil respiration at increasing temperatures. Specifically, I address the following questions:

1. How do climate, land use, and soil type influence deep soil carbon stocks?

   Information on global carbon stocks is important for guiding management strategies to tackle climate change. Yet, information on deep soil carbon stocks globally is particularly scarce, and most relevant studies that estimated deep soil carbon stocks in relation to land use and soil type are decades old. Since their publication, a great amount of new data have been made available, although no comprehensive study has analysed them. Moreover, there does not seem to be any comprehensive study that demonstrates variations in deep soil carbon in different climatic regions. I hypothesized that i) colder climates present larger topsoil organic carbon stock and lower contribution from deep soil to the overall carbon pool due to slower carbon decomposition rates, and ii) forest ecosystems have larger total SOC stocks than croplands and grasslands due to larger litter inputs, but a smaller proportion of organic carbon comes from deep soil layers. Thus, I created a unique deep soil organic carbon stock database by uniting different databases and literature data and assessed global patterns of deep soil carbon stock.

2. What is the carbon sequestration potential of different land restoration approaches in the Atlantic Forest?

   Different approaches have been implemented to restore degraded lands in the Atlantic Forest. They differ in carbon sequestration when the aboveground carbon pool is assessed, although only small differences are found in topsoil carbon. However, carbon sequestration potentials seem to be unknown when deep soil carbon pool is included in the analysis. Because root growth and turnover follow aboveground living biomass, I hypothesized that soil organic carbon stock changes after land restoration follow aboveground biomass pattern. Here, I assessed the carbon sequestration potential of the most commonly sed approaches of land restoration in the Atlantic Forest by including deep soil carbon pools in the analysis.

3. What is the effect of land restoration on the stability of soil carbon along the soil profile in a future warmer climate?

The long-term mitigation potential of land restoration interventions depends on the stability of the sequestered carbon. Soil carbon stability and temperature sensitivity is known to change with depth, although previous studies have shown contrasting results. Since land restoration may alter soil profiles' traits, soil respiration and temperature sensitivity are likely to be affected. I hypothesized that i) the respiration responses to warming decreases with soil depth, but that ii) the degree of this effect declines with restoration progress since other factors other than molecular structure play a role in temperature sensitivity in deep soil. Here, I measured soil respiration and its sensitivity to temperature increases in the topsoil, subsoil, and deep soil and compared it with paired agriculture and reference forests.

2. CHAPTER 2: GLOBAL PATTERNS OF DEEP SOIL ORGANIC CARBON STOCKS

2.1. Introduction

Soils constitute an important component of the global carbon budget, accounting for more than three times the plant aboveground biomass and more than the plant aboveground biomass and atmospheric carbon pools combined (Friedlingstein et al., 2022; Jackson et al., 2017; Lal, 2018). However, these estimates are considered conservative as they do not encompass deeper soil layers, since the carbon stock located below the topsoil can represent more than 50% of total SOC stock (Borchard et al., 2019; Kögel-Knabner & Amelung, 2021; Strey et al., 2017). Consequently, including deep soil is key to reliable estimates of the global carbon budget.

Deep SOC stock is a function of edaphoclimatic conditions and land use (Gross & Harrison, 2019; Jackson et al., 2017; Kögel-Knabner & Amelung, 2021; Lorenz & Lal, 2005). In an analysis of 313 profiles around the world, Mathieu et al. (2015) found that the reference soil group is the main influencing factor of deep soil C age, denoting the importance of clay content and mineralogy to deep soil C dynamics. Indeed, Batjes (1996) found great variations in the SOC depth distribution among the reference soil groups to 200 cm. Beside geology, also climate affected the depth distribution of SOC. Precipitation and temperature have been shown to be key drivers of net primary productivity and biomass decomposition, and both processes affect deep soil C stock (Han et al., 2018; Hobley et al., 2015; Tan et al., 2020; Van Der Voort et al., 2019; Wiesmeier et al., 2019). Jobbagy & Jackson (2000), in a global review of the depth distribution of SOC, conclude that the contribution of deep soil to the overall SOC storage decreases with increasing precipitation. However, estimates of deep soil carbon stock according to major climatic types are still lacking. Land use modulates the depth distribution of SOC, primarily via its influence on SOC dynamics and the root system, although these effects are largely restricted to topsoil and upper subsoil layers (Duarte-Guardia et al., 2020; Gmach et al., 2020; Gross & Harrison, 2019; Poeplau & Don, 2013; van den Berg et al., 2012). Globally, Jobbagy & Jackson (2000) found that the share of deep soil C stock is larger in forests compared to grasslands, probably due to deeper root systems (Jackson et al., 1996; Lorenz & Lal, 2005).

The review papers and meta-analyses are useful for synthesizing data and creating general conclusions (Torraco, 2016); yet, only few papers consider deep SOC. Lorenz & Lal (2005) present a narrative review depicting the importance of the depth distribution of SOC and its contribution to the overall carbon budget. Different metanalysis studied the impact of land use change on the SOC stock in the 0-100 cm layer. Guo & Gifford (2002) showed that the transition from forest to plantation decreased SOC stocks. The results of Shi et al. (2013) demonstrated that the afforestation of grasslands reduced SOC, although with decreasing magnitude of change with depth. Chatterjee et al. (2018) showed an increase in SOC stock after the transition from cropland or pasture to agroforestry systems. A meta-analysis by Duarte-Guardia et al. (2020) revealed that a transition from grassland to cropland decreases SOC stock in the 0-100 cm layer, but a transition from grassland to forest increases. Yet, Marín-Spiotta & Sharma (2013) showed that SOC stock in the 0-100 cm layer differs among the different forest types in the tropics, being slightly higher under N-fixer species. Overall, these meta-analyses reveal a continuum of SOC stocks in the 0-100 cm layer, progressing from forests to agroforests, grasslands, plantations, and finally croplands.

In relation to the reference soil group, Batjes (1996) presented estimates of SOC down to 200 cm using data from 4,353 soil profiles from WISE database. Lastly, Jobbagy & Jackson (2000) estimated SOC stock to 300 cm of different land uses and found higher contribution of deep SOC stock in forests than grasslands. Their results are based on approximately 2,700 soil profiles from three databases, with extrapolation to deeper layers based on topsoil properties. Thereby, estimates of deep SOC stock (down to 200 cm of soil and beyond) are more than two decades old. Moreover, recent estimates do not include layers below 100 cm. Thus, estimates of deep SOC should be carried out taking into account the findings of the last 20 years in order to review and, if necessary, update the contribution of deep SOC to the global carbon budget.

We compiled data from three different databases and the literature to create a database of SOC stocks containing more than 12,000 soil profiles with a minimum depth of 200 cm. When available, we also included data to 500 cm depth. The objective of this study is to provide an updated estimate of deep SOC stocks and to relate it to the reference soil groups, climates and land uses. We hypothesize that 1) updated soil carbon information might revise our understanding of overall ecosystem C storage, particularly for tropical forests and cropland, where several new data evolved in the last two decades. In addition, we hypothesize that 2) soils in colder climates present lower contribution of deep soil to the overall C pool, due to the larger topsoil SOC stocks, while the opposite is true for soils in arid climates, and that 3) forest ecosystems comprise larger SOC stocks in the whole profile than croplands and grasslands, although with smaller share of deep layers due to larger contributions from the topsoil.

2.2. Material and methods

2.2.1. Data source and extraction

The data used in this work is a compilation from four different sources: 1) NCSS database; 2) WoSIS database; 3) WISE database and; 4) data extracted from articles published in peer reviewed journals. The National Cooperative Soil Survey (NCSS) is the Soil Characterization Database provided by the United States Department of Agriculture (NCSS, n.d.). The World Soil Information Service (WoSIS) database is provided by the International Soil Reference and Information Centre - ISRIC (Batjes et al., 2020). The World Inventory of Soil Emission Potentials (WISE) database is a global environmental research dataset also developed by ISRIC (Batjes, 2009).

For the three databases, we extracted data of soil profiles that contained information of soil organic carbon content (% or g kg^{-1}) up to a minimum depth of 200 cm. We also extracted other soil parameters like bulk density, rock fragment content, and clay content, in addition to profile information like coordinates, country, year, climate, and soil classification. Furthermore, information of land use was extracted from the WISE database.

For the data extracted from the literature, we used Google Scholar and Web of Science to search for articles published in peer reviewed journals. We used a set of keywords in different combinations among them: soil, carbon, organic, organic carbon, organic matter, deep, deep soil, subsoil, soil carbon stock, carbon storage. After the search, we scanned the abstract and the "Material and Methods" chapter to check if the article reports information of soil carbon (stock or content) to a minimum depth of 200 cm. In addition to that, we also found articles in the reference list of previous reviews of deep soil (Chatterjee et al., 2018; Feliciano et al., 2018; Guo & Gifford, 2002; Harrison et al., 2011; Jobbagy & Jackson, 2000; Lorenz & Lal, 2005; Marín-Spiotta & Sharma, 2013; Shi et al., 2013). Furthermore, we found some articles cited in the already selected deep soil papers. Different from the databases, we extracted either the information of soil carbon content (% or g kg^{-1}) or SOC stock (kg m^{-2} or Mg ha^{-1}). We also extracted information of land use, in addition to soil (pH, clay, bulk density, and rock fragments) and profile information (coordinates, country, year, climate and reference soil group) when available.

For the soil profiles that did not report the climate classification, we extracted this information from Kottek et al. (2006). We then combined the climates in four groups: tropical, dry, temperate, and cold, representing the Köppen climates A, B, C and D, respectively. The main characteristics of each climate are: A) Tropical = average temperature of the coldest month is higher than 18°C; B) Dry = accumulated annual precipitation at least half of the potential evapotranspiration; C) Temperate = average temperature of the coldest month is between -3 and 18°C; D) Cold = average temperature of the coldest month is lower than -3°C (for detailed criteria of each climate type please check Kottek et al. (2006). We did not include polar climate due to the small number of soil profiles in the respective regions. In addition to the climate classification, we also extracted the reference soil group from FAO & IIASA (2023) for the profiles that did not report this information.

Information on land use was extracted from the literature and WISE database, and grouped into three categories: croplands, grasslands, and forestlands. We further divided the forestlands into four types: primary and secondary forests, woodlands, agroforest, and plantation. Cropland comprises areas of annual or perennial agricultural production, regardless of the tillage used, fertilization and irrigation systems. Paddy fields are also included in this category. Grassland comprises either native or planted areas covered with grasses, managed or not. Forestlands comprise all areas dominated by trees, encompassing a broad range of tree density. For the different forestland types, we grouped primary and secondary forests due to the small number of primary forests identified, and because the literature did not always provide clear information allowing to distinguish between them. Woodland is a transition from cropland and grassland to forest. In addition to native shrublands and wooded grasslands such as savannas, this forest type includes abandoned croplands and grasslands that show some level of tree regeneration, but not enough to categorize them as forest. Planted forests and agroforestry with less than five years were included in this forest type. Agroforestry includes areas of agricultural production in combination with trees, such as successional agroforestry systems, alley cropping, shaded coffee systems, improved fallows, and rubber gardens. Plantations are usually planted single-species forests, irrespective of management activities such as thinning or pruning.

2.2.2. Soil organic carbon stock estimate

Soil profiles with less than three reported layers were excluded from our database. We also excluded soil profiles without the topsoil layers or missing an intermediate layer thicker than 50 cm. When an intermediate layer less thick than 50 cm was missing, data were interpolated from adjacent layers.

The soil organic carbon stock was calculated for each layer according to equation 1:

$$SOC = C\ x\ Th\ x\ BD\ x\ (1 - \left(\frac{RF}{100}\right)$$

where SOC is the soil organic carbon stock (Mg ha^{-1}), C is the soil organic carbon content (%), Th is the thickness of the soil layer (cm), BD is the soil bulk density (g cm^{-3}), and RF is the rock fragment (%).

When the information of rock fragment was absent, we regarded it as zero. When BD was not reported, we estimated it based on equation 2 proposed by Adams (1973) and used in previous reviews and meta-analyses of SOC (Chatterjee et al., 2018; Guo & Gifford, 2002; Marín-Spiotta & Sharma, 2013; Post & Kwon, 2000):

$$BD = \frac{100}{\frac{\mathrm{OM}}{0.24} + (\frac{100 - OM}{1.64})}$$

where BD is bulk density (g cm^{-3}) and OM is soil organic matter content (%). Organic matter was calculated by dividing soil organic carbon content by 0.58 (Guo & Gifford, 2002).

Since the reported depths differed among the soil profiles, we determined the SOC stock in harmonized depths to allow comparisons. First, we calculated the cumulative SOC stock in the soil profile and then we calculated the SOC in the following depths by interpolation: 0-40, 40-100, 100-200, 200-300, and 300-500 cm.

As we used different databases, we checked for duplicate soil profiles. When two soil profiles had the same coordinate, year, and stock, we excluded one of them. When the soil profiles reported the same coordinate, but different stock or year, we decided case by case checking the original soil profile data (depth of each sampled layer, carbon concentration, bulk density, and rock fragments). For instance, in case of minor differences in bulk density that resulted in different stocks, one of the profiles was excluded. On the other hand, when there were major differences in the soil profile, we regarded them as different profiles, but sampled on the same area and registered with the same coordinate.

The estimate of SOC stock for the reference soil group was based on the average stock in each depth to that specific soil group. We then estimated the global soil carbon stocks by multiplying the average carbon stock of each reference soil group by its global area extracted from FAO & IIASA (2023). We carried out an estimation with organic soils (>15% C) and one without, but in both estimates we excluded Cryosols because the sampling number was small in comparison to the share of global distribution represented by this reference soil group.

2.2.3. Limitations of the dataset

The intention of this study is to provide estimates of deep soil carbon stocks, which are underrepresented in the literature, based on an updated dataset covering the period from 1905 to 2023. Carbon stocks in the topsoil are not our primary focus, since good regional and global estimates are provided by other authors, such as Gomes et al. (2019), Liu et al. (2013), Meersmans et al. (2012), and Stockmann et al. (2015).

Due to the different data sources, the geographical origin of the soil samples, the variety of soil sampling and laboratory analyses and other factors, we expected that our dataset would have a number of limitations. Some of these limitations are discussed below:

- We calculated C stocks using the share of rock fragments provided with the information about the soil profiles. However, only 16% of the data contains this information. If there was no information about rock fragments, we regard it as zero. Nonetheless, the reported average content of rock fragments is below 15% at all soil depths (Table 7-3, Appendix A), thus unlikely to be a relevant control factor for deep soil C stocks.

- Thirty-nine percent of the data contains information on bulk density, and missing values were estimated based on Adams (1973), which is the preferred method in previous review papers that estimate SOC stock (Chatterjee et al., 2018; Guo & Gifford, 2002; Marín-Spiotta & Sharma, 2013). Although the overall correlation between reported and estimated bulk densities is high (r=0.69), the errors of using this pedotransfer function may be large for Anthrosols, Arenosols, Solonetz, Lixisols and Gypsisols, which present low correlation (Figure 7-5, Appendix A). Moreover, Adams' method (Adams, 1973) underestimates bulk density higher than 1.6 g cm^3, usually found in layers with low organic C concentration (De Vos et al., 2005; Figure 7-5, Appendix A). Therefore, there is a particular risk that bulk density is underestimated for deep soil layers, which in turn results in an underestimation of SOC stock. Hence, the contribution of deep soil C to overall ecosystem C stocks is possibly even slightly larger than reported here.

- We acknowledge that the concentration of profiles in a few regions (Figure 2-1) may lead to biased estimates. However, the profiles in the USA encompass all climates, land-use categories, and reference soil groups (exception for Anthrosols). In Australia, profiles are found in tropical, arid, and temperate climates and in most soil groups. Therefore, although the profile distribution is concentrated in a few countries, they present major soil groups, climates, and land uses, thus reducing a potential geographic bias on deep soil C comparisons.

- Our database contains soil profiles sampled over several decades. In the meantime, laboratory techniques have evolved, and the determination of C concentration has become more precise (Chatterjee et al., 2009). Yet, we deem it unlikely that variations in analytical methodologies undermine the overall interpretation of our results. Usually, the Walkley and Black method (Walkley, 1947) using $K_2Cr_20_7$ for wet combustion has been applied in earlier studies and in many laboratories of the tropics and subtropics (Campos, 2010; Bernoux et al., 1998; Miyazawa et al., 2000), while automated C/N analyses after dry combustion dominates recent SOC studies (Fuentes et al., 2022;

Stockmann et al., 2013; Tautges et al., 2019). Both methods usually compare well, and if not, conversion factors have been developed for the different Walkley and Black approaches (Chatterjee et al., 2009; Meersmans et al., 2009; Pansu & Gautheyrou, 2006; Sato et al., 2014). In particular, the Walkley and Black methodology is not sensitive to inorganic C, which is common in dry, arid climates, and where the average SOC stocks of our study are high.

- When the reference soil group information was not available, we retrieved it from FAO & IIASA (2023), which has a resolution of approximately 1 km. This means that the coordinated provided in the database might not represent the exact retrieved reference soil group due to the natural variation of soils within 1 km. Moreover, some profiles were located on the border of two contrasting soils. This might cause variations in the SOC estimate of reference soil groups with small sample size, such as Solonchaks that presented surprisingly high SOC.
- Lastly, our dataset only takes into account information based on real carbon concentration measurements, instead of using functions to extrapolate to deeper layers. The results of functions that derive deep SOC contents based on topsoil measurements are sensitive to soil type and the model applied (James et al., 2014; Wade et al., 2019), and their use in global datasets like the one used in this study might lead to large errors. Also, our data present low correlation between top- and deep soil (Figure 7-4, Appendix A). Thus, the results shown here are not affected by this uncertainty.

2.2.4. Statistical analysis

For the analysis of climate and land use, we first checked for differences in the clay content: all land uses do not differ statistically (Figure 7-1, Appendix A), whereas the climates show only small variations in the clay content, although with statistically significant difference due to the large sample size (Figure 7-3, Appendix A). We then assessed the SOC data distribution with the Shapiro-Wilk test as well as with skewness and kurtosis tests. The results show that the SOC data are not normally distributed, in addition to being asymmetric and heavy-tailed. Moreover, the visual analysis with a histogram shows that the data have a Poisson-like distribution. We then performed a Poisson regression (Wooldridge, 2010), using SOC as the dependent variable. We ran a regression for each independent variable (land use and climate), and also included depth as a second independent variable in each model, as well as the interaction between depth and the independent variables. For climate, we included only mineral soils in the analysis in order to avoid bias due to unbalanced distribution of organic profiles among the climates. Statistical differences among the levels of the independent variables were checked with LSD test ($p<0.05$). All analyses were performed using Stata 16.1 (StataCorp LLC, College Station, USA).

2.3. Results

2.3.1. Database

The combination of four different sources resulted in a database of 12,796 soil profiles after the exclusion of 3,246 duplicate ones, distributed as follows: 10,262 with a maximum depth of 200 cm, 2,006 reaching 300 cm, and 528 up to 500 cm. Of these, 12,751 profiles contained reference soil group information and 12,351 climate information.

The literature search resulted in 53 publications (see Appendix A for the list of studies), summing 248 profiles. Together with these profiles, 413 profiles from the WISE database also contained information of land use, totaling 661 profiles with this information.

The profiles were distributed unevenly across the five continents, covering 115 different countries (Figure 2-1). The United States were by far the most frequently studied region with 7,850 profiles (61%), followed by Australia with 2,016 profiles (16%). Another five countries had more than 100 profiles: Ethiopia (359), Czech Republic (256), Brazil (240), Canada (164) and Cameroon (128). The remaining 14% of the profiles were distributed across 108 countries, with little information from China and West and Southwest Europe, and hardly any information from Russia, Northern Africa, the Middle East, and the Argentinean Pampa.

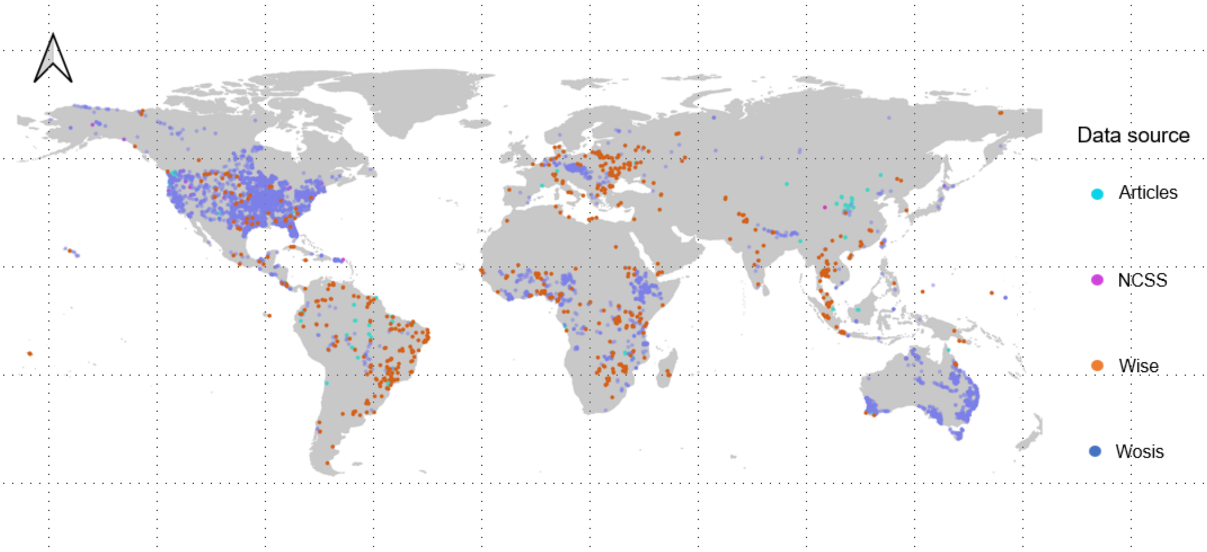

Figure 2-1. Global distribution of the soil profiles according to the data source. Articles = literature; NCSS = National Cooperative Soil Survey (NCSS); Wise = World Inventory of Soil Emission Potentials; Wosis = World Soil Information Service (WoSIS). Map courtesy: Dr. Luiz Felipe Galizia

2.3.2. Soil organic carbon stock distribution according to the reference soil group

For the reference soil groups that presented profile data to a depth of 500 cm, the SOC stock ranged from 99 Mg ha^{-1} (coefficient of variation CV=0.2%) in Gypsisols to 5,502 Mg ha^{-1} (CV=11%) in Histosols, with an average stock of 468 Mg ha^{-1} (CV=235%). Considering the upper 200 cm of the soil profile, the average SOC stock was 212 Mg ha^{-1} (CV=175%), with a minimum of 66 Mg ha^{-1} (CV=131%) in Stagnosols and a maximum of 2,280 Mg ha^{-1} (CV=36%) in Histosols (Table 2-1).

The depth distribution of SOC stocks varied more than twofold among the different soils. In the 0-200 cm layer, the share of the 100-200 cm soil layer ranged from 21% in Chernozem to 47% in Histosol. On average, 35% of the SOC was in the 100-200 cm soil layer (Figure 2-2).

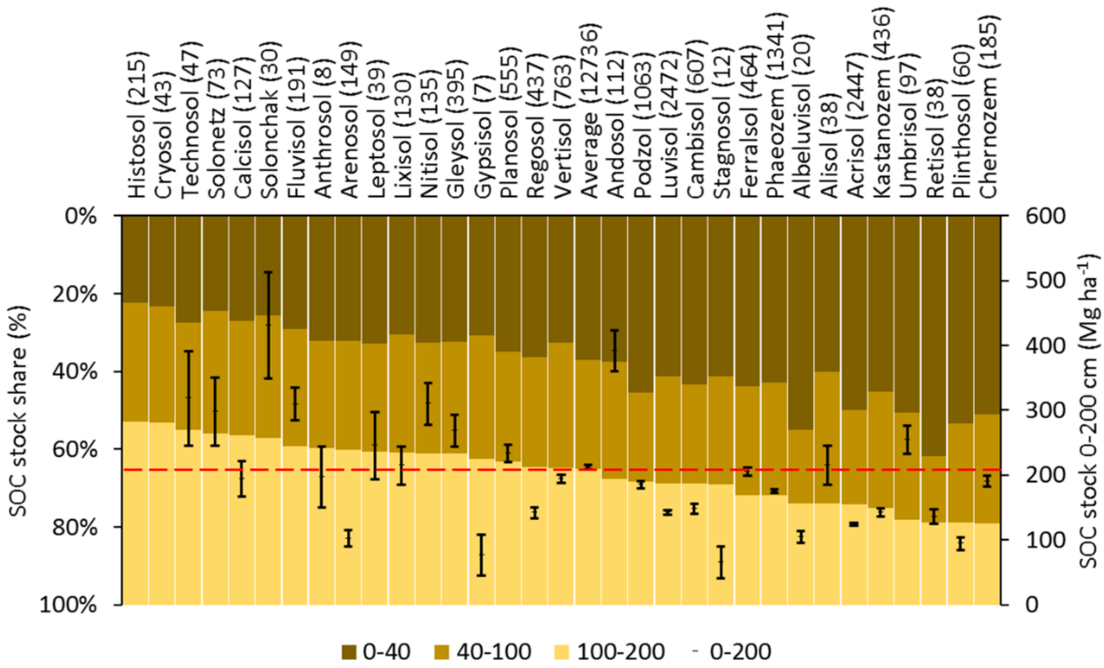

Figure 2-2. Depth distribution of soil organic carbon in the reference soil groups, sorted by decreasing share of SOC stock in 100-200 cm. 0-40, 40-100 and 100-200 refer to the SOC share (%) in its respective layer. The red dashed line shows the average share of SOC stock in the 100-200 cm layer in relation to the 0-200 cm layer. The bars show the average soil organic carbon stock (Mg ha^{-1}) in the 0-200 cm depth interval of each reference soil group (error bars are ± 1 standard error). SOC stock of Histosol (2,280 ± 56 Mg ha^{-1}) and Cryosol (1,803 ± 145 Mg ha^{-1}) are not shown to preserve scale. Numbers in parentheses after the reference soil group are the sample size.

The sequence of soils in Figure 2-2 generally showed low SOC stocks in soil with root restricting layers due to anoxic conditions when wet and possibly physically barrier when dry (Planosols, Stagnosols), and little weathered or sandy subsoil (Regosol, Arenosol, Gypsisol), though with exceptions (Gleysols, Calcisols). Highest values occurred in Histosols and

Cryosols, while elevated SOC stocks were found in Andosols, and surprisingly the Solonchaks. In general, soils with elevated fertility such as Chernozems, Umbrisols, Alisols, Phaeozems, Nitisol, Anthrosols and Fluvisols had larger SOC stocks than the remaining less fertile soil groups, with exception of the Ferralsols (Table 2-1; Figure 2-2), that usually occur in areas with high potential productivity, largely unaffected by limitations in precipitation and temperature. Yet, there was small overall correlation between the absolute amount of SOC stored and the share of that in deep soil ($R^2 = 0.11$, Figure 7-4, Appendix A).

Table 2-1. Soil organic carbon stock (SOC, Mg ha^{-1}) in the reference soil groups in selected depth intervals. CV = coefficient of variation (%); n = sample size.

	0-40 cm			0-100 cm			0-200 cm			0-300 cm			0-500 cm		
Reference soil group	SOC	CV	n	SOC	CV	N	SOC	CV	n	SOC	CV	n	SOC	CV	n
Acrisol	62.0	64	2447	92.0	72	2447	124.2	87	2447	146.0	89	211	196.2	105	23
Albeluvisol	57.3	54	20	77.0	43	20	104.2	39	20	135.1	35	3	215.1	32	2
Alisol	86.0	73	38	159.1	89	38	215.1	86	38	244.8	51	4	-	-	0
Andosol	147.2	73	112	264.6	77	112	391.7	84	112	597.1	104	5	-	-	0
Anthrosol	63.7	120	8	117.9	91	8	197.6	67	8	239.1	73	2	-	-	0
Arenosol	32.7	112	149	61.5	124	149	102.1	151	149	193.7	170	22	268.7	58	6
Calcisol	52.3	113	127	109.3	143	127	193.9	160	127	381.6	141	9	-	-	0
Cambisol	64.4	93	607	102.0	108	607	148.2	133	607	195.1	159	75	315.8	223	34
Chernozem	97.3	57	185	150.7	58	185	190.3	59	185	221.2	48	41	255.2	48	13
Cryosol	419.2	48	43	960.6	49	43	1803	53	43	2657	58	16	3929	82	4
Ferralsol	90.2	57	464	147.2	59	464	205.2	65	464	238.7	40	128	299.5	38	15
Fluvisol	90.4	89	191	183.0	101	191	309.4	111	191	499.0	83	48	536.7	25	2
Gleysol	86.9	141	395	163.5	162	395	267.8	181	395	584.9	134	43	990.9	179	22
Gypsisol	23.6	99	7	47.9	101	7	76.6	109	7	81.6	72	3	98.8	0.2	2
Histosol	509.7	46	215	1208	38	215	2280	36	215	3313	37	71	5502	11	6
Kastanozem	64.1	55	436	106.8	66	436	142.1	85	436	170.0	115	78	195.7	31	31
Leptosol	80.7	87	39	148.9	109	39	245.5	131	39	278.5	47	8	306.4	-	1
Lixisol	65.5	99	130	130.6	131	130	214.6	156	130	284.6	178	24	321.3	22	13
Luvisol	58.6	74	2472	97.6	91	2472	141.9	116	2472	187.4	151	347	245.2	191	103
Nitisol	101.1	68	135	189.1	91	135	309.9	120	135	542.2	138	23	1815	67	4
Phaeozem	74.9	53	1341	125.6	56	1341	174.8	60	1341	209.2	54	113	272.3	50	40
Planosol	81.9	74	555	147.7	96	555	233.5	130	555	329.0	155	108	609.5	173	24
Plinthosol	50.4	90	60	74.6	87	60	94.5	82	60	105.3	77	5	115.2	-	1
Podzol	84.1	93	1063	126.7	95	1063	185.3	102	1063	272.9	104	338	410.0	97	124
Regosol	51.4	83	437	91.6	99	437	141.6	120	437	177.2	100	52	233.5	79	12
Retisol	83.8	50	38	107.0	45	38	135.7	51	38	160.2	11	6	-	-	0
Solonchak	110.2	93	30	246.1	103	30	431.2	105	30	571.4	100	12	843.6		1

Solonetz	73.0	115	73	166.9	139	73	297.9	152	73	469.7	140	3	-	-	0
Stagnosol	27.1	131	12	45.3	130	12	65.6	131	12	-	-	0	-	-	0
Technosol	87.2	109	47	175.1	137	47	318.2	156	47	454.0	177	14	783.2	199	9
Umbrisol	128.9	63	97	198.5	78	97	254.3	83	97	284.0	74	26	303.3	-	1
Vertisol	63.4	67	763	125.8	76	763	193.9	96	763	245.0	125	148	278.7	43	30
Average	78.4	113	12736	138.1	143	12736	212.1	175	12736	318.8	196	1986	468.1	235	523

2.3.3. Soil organic carbon stock distribution according to climate

When grouping SOC stocks according to climate region, we did not include the 300-500 cm layer due to the limited sample size (3-7% of topsoil samples). Tropical climates had the largest SOC stock per hectare, averaging to 314 Mg ha^{-1} for the top 300 cm soil (Table 2-2); yet, tropical soils were also richer in clay than other sites (Figure 7-3, Appendix A), though likely also different in clay mineralogy (data usually not reported). For the same depth interval, there were small differences between arid (194 Mg ha^{-1}), temperate (206 Mg ha^{-1}) and cold (195 Mg ha^{-1}) climates. The distribution of SOC along the soil profile, however, varied considerably between the different climates. In the arid climate, 54% of SOC was found below 100 cm, whereas it was 48% in temperate and 34% in cold climates (located mostly at northern latitudes), thus inversely reflecting SOC stocks in the topsoil (0-40cm). Soil organic carbon stocks in the topsoil were largest in cold climates, but the lowest in all layers below 40 cm depth. In contrast, tropical soils stored the largest amounts in all layers below 40 cm (Table 2-2). Overall, tropical and temperate climates showed similar distribution pattern of SOC in the profile, although stocks are higher in tropical climate. Soils of dry climates (Köppen climate A) presented the most homogenous distribution, whereas cold climates (Köppen climate D) the biggest contrast between topsoil and deep soil layers (Figure 2-3).

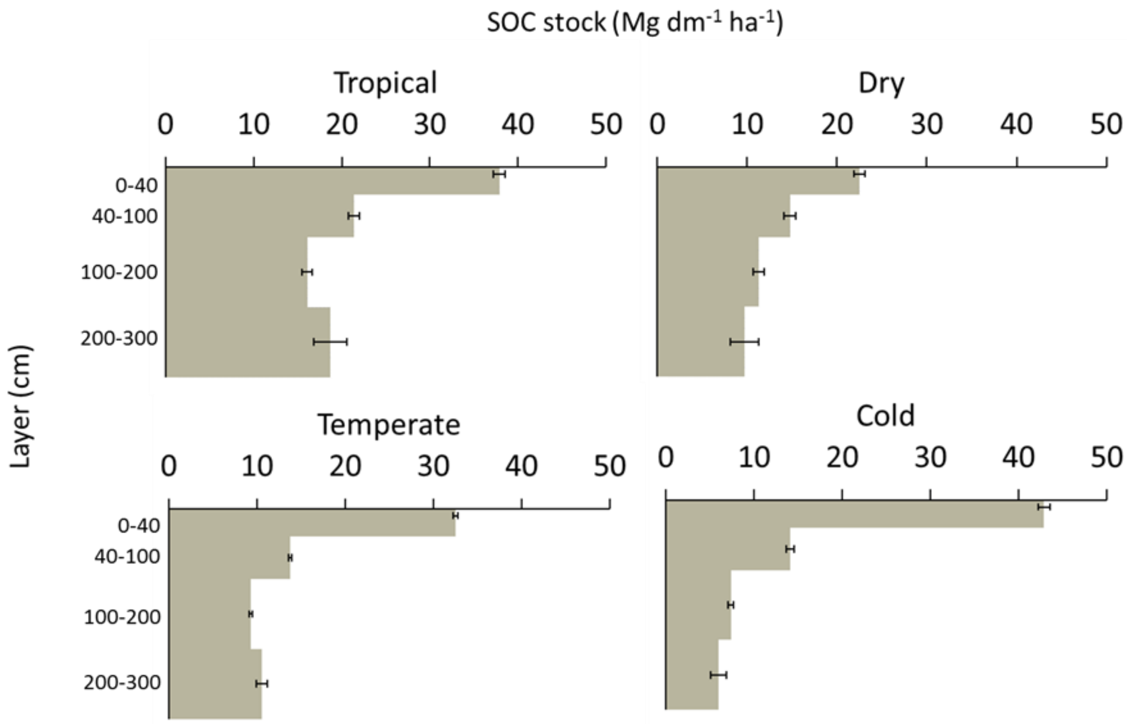

Figure 2-3. Distribution of soil organic carbon stock per decimetre of layer thickness (SOC, Mg dm^{-1} ha^{-1}) along the soil profile to a depth of 300 cm, in the studied climates. Error bars indicate the standard error.

2.3.4. Soil organic carbon stock distribution according to land use

Forestlands presented the largest average SOC stock in the 0-300 cm soil layer with 264 Mg ha^{-1} (300-500 cm layer not evaluated in this regard due to limited data – approximately 10% of topsoil data). Grasslands showed larger SOC stocks than croplands in the 0-100 cm layer. However, SOC stocks in deeper soil layers, i.e., in 100-200 cm and 200-300 cm, were larger in croplands than in grasslands. Moreover, the total SOC stock per hectare (0-300 cm) was larger in croplands than in grasslands, summing 242 Mg ha^{-1} and 228 Mg ha^{-1}, respectively (Table 2-2). Soil texture did not vary significantly across land-use types (Figure 7-1, Appendix A).

The different land-use categories did not have much influence on the distribution pattern of SOC, with all land uses showing a decreasing SOC density along the soil profile (Figure 2-4). Although the distribution pattern was similar, layer specific differences in the SOC stock led to a range of share below topsoil from 49% in croplands to 32% in grasslands.

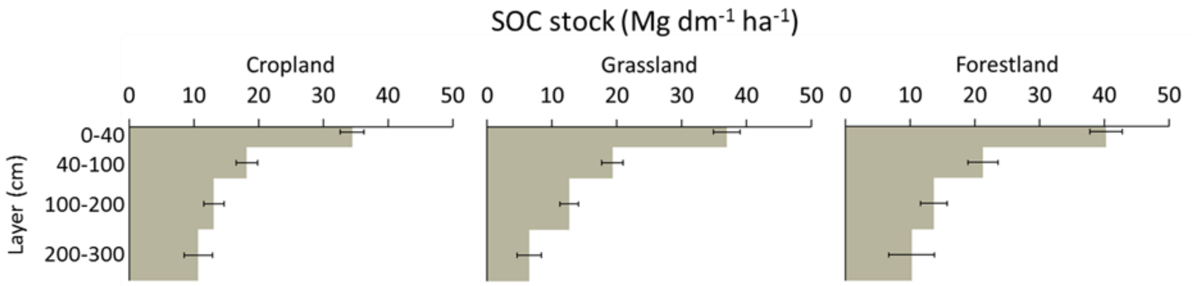

Figure 2-4. Distribution of soil organic carbon stock per decimetre of layer thickness (SOC, Mg dm^{-1} ha^{-1}) along the soil profile to a depth of 300 cm, in the studied land uses. Error bars indicate the standard error.

The SOC distribution varied greatly among different forest types, though (Figure 2-5): primary and secondary forest and agroforests presented similar total (0-300 cm) SOC stock, with 327 and 320 Mg ha^{-1}, respectively. Similarly, the total SOC stock in woodlands and plantations did not differ significantly, summing to 129 and 124 Mg ha^{-1}. Yet, the SOC in woodlands and plantations was significantly lower than in primary and secondary forests or agroforests. The primary and secondary forests stored almost half of the SOC below 100 cm, whereas this contribution was least in plantations, with deep soil (100-300 cm) comprising only 35% of total SOC (Table 2-2).

Intriguingly, plantations and woodlands presented even lower SOC stocks than croplands in all layers. Moreover, the SOC stock in the 200-300 cm layer of croplands was larger than in woodland, agroforest and plantation. This explains the lower SOC stock in this layer of overall forestlands compared to croplands (Table 2-2).

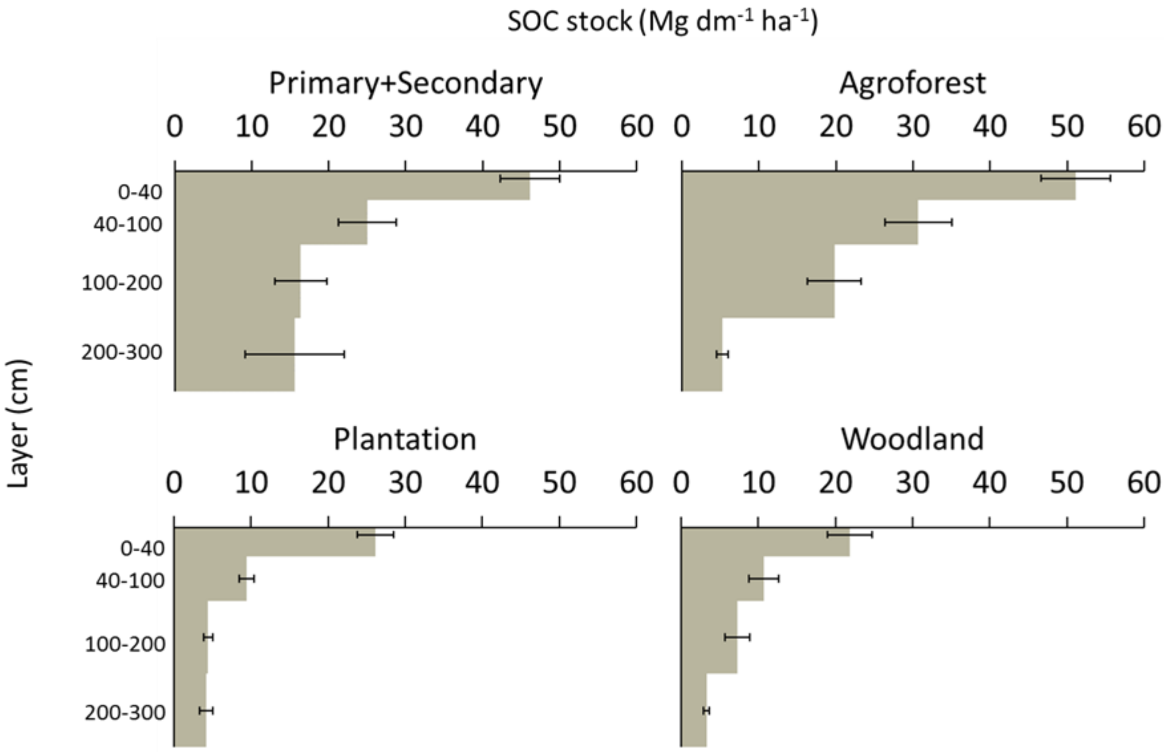

Figure 2-5. Distribution of soil organic carbon stock per decimetre of layer thickness (SOC, Mg dm^{-1} ha^{-1}) along the soil profile to a depth of 300 cm, in the studied forest types. Error bars indicate the standard error.

Table 2-2. Soil organic carbon stock (Mg ha^{-1}), soil organic carbon stock share (%) and cumulative soil organic carbon stock (Mg ha^{-1}) in the studied layers (cm) according to the climate (upper part), land use (middle) and forest type (lower part). TRO = Tropical, ARI = Arid, TEM = Temperate and COL = Cold. CRO = cropland, GRA = grassland and FOR = forestland. P+S = primary + secondary forests, AGR = agroforests, PLA = plantation forests and WOO = woodlands. Different letters in the SOC stock indicate statistical difference in the same layer (LSD, $p<0.05$).

	Sample size (n)				SOC stock (Mg ha^{-1})				SOC share (%)				Cumulative SOC stock[1] (Mg ha^{-1})			
Layer (cm)	TRO	ARI	TEM	COL	TRO	ARI	TEM	COL	TRO	ARI	TEM	COL	TRO	ARI	TEM	COL
0-40	1315	1218	8108	1191	75.7 b	45.0 d	65.0 c	85.8 a	24.1	23.2	31.6	44.0	75.7	45.0	65.0	85.8
40-100	1315	1218	8108	1191	64.2 a	44.3 b	41.2 b	42.3 b	20.4	22.8	20.0	21.7	139.9	89.3	106.2	128.1
100-200	1315	1218	8108	1191	80.5 a	56.4 b	46.5 c	37.0 d	25.6	29.1	22.6	19.0	220.3	145.7	152.6	165.0
200-300	225	213	1156	141	93.4 a	48.4 b	52.9 b	29.9 c	29.8	25.0	25.7	15.3	313.8	194.1	205.6	194.9

Layer (cm)	CRO	GRA	FOR	CRO	GRA	FOR	CRO	GRA	FOR	CRO	GRA	FOR
0-40	284	123	241	69.0 c	73.9 b	80.5 a	28.4	32.5	30.5	69.0	73.9	80.5
40-100	284	123	241	54.6 c	58.0 b	63.8 a	22.5	25.5	24.2	123.5	131.8	144.3
100-200	284	123	241	65.5 b	63.3 c	68.3 a	27.0	27.8	25.9	189.1	195.1	212.6
200-300	70	35	99	53.4 a	32.4 c	51.1 b	22.0	14.3	19.4	242.5	227.6	263.7

Layer (cm)	P+S	AGR	PLA	WOO	P+S	AGR	PLA	WOO	P+S	AGR	PLA	WOO	P+S	AGR	PLA	WOO
0-40	141	29	37	34	92.3 b	102.2 a	52.3 c	43.7 d	28.2	32.0	42.3	33.9	92.3	102.2	52.3	43.7
40-100	141	29	37	34	75.0 b	92.1 a	28.2 d	32.1 c	22.9	28.8	22.8	24.9	167.2	194.3	80.5	75.8
100-200	141	29	37	34	81.8 b	99.0 a	22.1 d	36.5 c	25.0	31.0	17.9	28.3	249.1	293.2	102.6	112.4
200-300	53	10	18	18	77.8 a	26.3 b	21.0 c	16.5 d	23.8	8.2	17.0	12.8	326.9	319.5	123.6	128.9

1) Cumulative here means that SOC stocks are summed from the topsoil to the respective depth.

2.3.5. Global deep soil organic carbon stock

The global terrestrial SOC stock up to 200 cm summed 2,036 Pg (2,554 Pg if organic soils were included), with 35% of the stock in the 100-200 cm layer when the estimate is based on the profile data. Permafrost soils are not included in this estimate due to poor data. Considering the 0-500 cm soil profile, 62% of the global stock was found in the 100-500 cm layer (Table 2-3).

Table 2-3. Global estimates of SOC stock to specific depths, in Petagram (Pg). Mineral = soil profiles with C content larger than 15% and Cryosols were excluded. Mineral + organic = soils with C content larger than 15% were included, but Cryosols excluded. Sample size is the number of soil profiles used to calculate the stock in the respective depth.

Depth (cm)	Sample size n	Mineral (Pg)	Mineral + organic (Pg)
0-40	12,736	764	901
0-100	12,736	1,331	1,623
0-200	12,736	2,036	2,554
0-300	1,986	2,738	3,603
0-500	523	3,461	4,113

2.4. Discussion

2.4.1. Relation between soil organic carbon stock and reference soil group

We present updated estimates of deep SOC stocks for the major reference soil groups. An earlier analysis of them had been provided by Batjes (1996) for the depth of 0-200 cm. Since Batjes (1996) used the FAO-UNESCO (1974) soil classification system (FAO-UNESCO, 1974), comparison of every soil group is not possible, due to the inclusion and exclusion of soil groups during updates in the classification system, in addition to modifications in the diagnostic criteria of some soils (Schad, 2023). Nevertheless, we selected specific soil groups to give an overview of depth distribution of SOC.

For the Acrisols, a typical soil in subtropical and tropical climates, our results reveal a SOC stock that is 19% larger than that reported by Batjes (1996) for the upper 200 cm of soil. This difference in SOC stock is largely due to a larger share of the 100-200 cm layer, which has to be changed from formerly 10% to 26% of total SOC based on the newly created database. For Chernozems, a typical soil of temperate climates, we found slightly smaller total SOC stocks than reported earlier, but a much smaller share of SOC in the 100-200 cm layer: it has to be changed from 36% to 21%. Critical estimates for our global C budget are still the organic soils: for Histosols, our results revealed that the averaged SOC stock per hectare is 5% larger than previously estimated, though with less contribution from deep soil (from 64% to 47%) (Table 2-1). Still accurate assessment of SOC stocks in organic soils remain challenging, also due to difficulties in correct assessments of soil bulk density in bogs and fens, as well as in organic layer thickness of, e.g., folic Histosols in the Tundra (Schimmel & Amelung, 2023).

When taking all soils together, on average, the 0-200 cm SOC stock presented in our study (excluding Cryosols) was 33 Mg ha^{-1} and thus 19% larger than the weighted average of all soils reported by Batjes (1996), whereas in the 100-200 cm layer, our estimate was 5.8 Mg ha^{-1} or 9% higher. The overall share of SOC in the 100-200 cm layer in relation to 0-200 cm soil depth remains similar to that reported by Batjes (1996): it was 33% on average estimated earlier, whereas our results indicated that it is 35%. If we also include the SOC stock for the 200-300 and 300-500 cm soil layers, in addition to the 0-200 cm of Batjes (1996), we find that on average 108 and 149 Mg SOC are stored per hectare, respectively (Table 2-1).

The IPCC (Canadell et al., 2022) and the most recent global carbon budget (Friedlingstein et al., 2023) refer to a stock of 1,700 Pg in mineral soils in the 0-200 cm layer. Our estimate based on the SOC stock of each reference soil group shows that this SOC stock is 19% (336 Pg) larger, summing to 2,036 Pg SOC. Moreover, Jobbagy &

Jackson (2000) estimate the global SOC stock in the 200-300 cm layer to be 351 Pg, whereas our figure shows a twofold larger stock in this layer, with 702 Pg. Our estimate also adds an additional 723 Pg of SOC to the global estimate by including the 300-500 cm layer (Table 2-3); yet, this number is more uncertain than for the upper two meters of the soil profile due to the limited sample size. Nevertheless, the stock of organic carbon in mineral soils globally used by IPCC seems to be underestimated.

Our estimates do not represent the SOC stock in a specific period of time, since the data span over several decades, and changes in stock due to land use change and management might have occurred (Beillouin et al., 2022; Don et al., 2011; Gocke et al., 2023; Maia et al., 2010; Skadell et al., 2023). However, temporal changes in deep SOC stocks are likely slow, taking the high radiocarbon ages commonly found in such soils depths (Krull & Skjemstad, 2003; Mathieu et al., 2015; Paul et al., 1997). Clearly, our analyses show that if not including deep SOC, the terrestrial reservoir of organic C will be considerably underestimated.

The vertical distribution of SOC across reference soil groups is influenced by distinct mineralogical properties and related SOC stabilization processes, such as illuviation and pedoturbation mechanisms (Doetterl et al., 2015; Kögel-Knabner & Amelung, 2021; Mathieu et al., 2015; Schneider et al., 2021), as well as likely due to confounding site variables (Duarte-Guardia et al., 2020). Varied inputs of SOC into soils, influenced by factors such as fertility levels and restrictions on root penetration, further contribute to the observed disparities (Amelung et al., 2020; Poeplau et al., 2021). Nevertheless, using all data, our results do not show a clear separation of deep SOC stock and share according to distinct reference soil groups, e.g., deep SOC stocks in soils typically occurring in tropical environments (Acrisols, Ferralsols, Plinthosols) did not differ much from temperate soils (Podzols, Luvisols, Phaeozems), although different processes are responsible for the distribution of organic carbon in the soil profile. This indicates that at a global scale additional factors other than soil properties (expressed as a reference soil group) influence deep soil carbon stocks, such as climate, land use, and geology (Duarte-Guardia et al., 2020; Yu et al., 2019), which result in different pedological processes, e.g., larger C input in the tropics versus slower SOC decomposition rates in temperate climates.

2.4.2. Relation between soil organic carbon stock and climate

Our hypothesis that cold climates present a smaller contribution of deep soil to total SOC stock than warmer climates was supported. Also, we could support our hypothesis that in regions with dry climates larger parts of SOC are stored in deep soil layers, although the observation that the total SOC stock in dry climates exceeds that in temperate and cold soils contradicts our assumptions. Yet, SOC stocks in dry regions may be slightly overestimated due to large amounts of inorganic C (Apesteguia et al., 2018; A. Schmidt et al., 2012); yet, this cannot be controlled in a meta-analysis since detailed information on the laboratory techniques applied is not available. Noteworthy, tropical climates exhibited the largest SOC stock below 100 cm soil depth (Table 2-2), likely reflecting the deep weathering and thus horizon development in these soils, with potentially also deep root penetration, in addition to larger root density (Canadell et al., 1996; Jackson et al., 1996; Schenk & Jackson, 2002). Moreover, the oxidic mineral signature in tropical environments favor SOC stabilization (Chevallier et al., 2019; Kögel-Knabner & Amelung, 2021), in addition to the lower pH values that increase the surface charge of the oxides and reduce organic matter decomposition (Basile-Doelsch et al., 2020; O'Brien et al., 2015). Managing deep soil C is thus particularly important for the tropics and less for cold climates.

Temperature and precipitation are the main influencing factors of organic matter input and decomposition (Kirschbaum, 1995; O'Rourke et al., 2015; Ogle et al., 2005; Wiesmeier et al., 2019). Topsoil organic C stocks can decrease with increasing temperatures due to faster organic matter decomposition (Koven et al., 2017; Smith et al., 2005; Tan et al., 2020). On the other hand, there is an increase in topsoil organic C stocks with increasing precipitation due to larger net primary productivity and, in turn, C input (Amelung et al., 1998; Han et al., 2019; Hobley et al., 2015; Nichols, 1984; Saiz et al., 2012). Wiesmeier et al. (2019) pointed out that SOC storage does not always follow this pattern. In arid and semi-arid regions (dry climate), the SOC stock is limited by C input due to low water availability, whereas in regions with sufficient precipitation but cold temperatures, microbial activity is the limiting factor rather than input. Thus, on average, SOC stocks are larger in cold and moist areas, intermediate in warm and moist climates, and small in dry and hot regions. This pattern was also observed in our topsoil dataset. Cold climates show the largest SOC stocks in the topsoil, tropical climates show intermediate stocks, and dry climates show the smallest SOC stocks in the topsoil (Table 2-2).

Climatic effects on deep SOC are less clear, and results do not always point into the same direction. Temperature had either a negative (Gray et al., 2015; Jobbagy & Jackson, 2000; Marín-Spiotta & Sharma, 2013) or a positive (Han et al., 2018; Tuo et al., 2018) correlation with SOC in deep soil. Also, precipitation correlates either positively with deep SOC stocks (Gray et al., 2015; Han et al., 2018; Marín-Spiotta & Sharma,

2013) or negatively (Jobbagy & Jackson, 2000; Tuo et al., 2018). Marín-Spiotta & Sharma (2013) suggest that the reason for these contradictory findings is probably due to the study of different ranges of climatic gradients. For example, Tuo et al. (2018) found a positive correlation of SOC with mean annual temperatures ranging from 8.6 to 12.3°C, likely because rising temperatures in this range promote net primary productivity and thus C return into soil in the form of litter. Jobbagy & Jackson (2000), in turn, considered mean annual temperatures in the range of 0 to 30°C, i.e., there carbon dynamics were more affected by higher decomposition at elevated temperatures (Walker et al., 2018). Eventually, our results of deep SOC related to climate do not follow the general topsoil pattern pointed out by Wiesmeier et al. (2019). We found the largest deep SOC stocks in the warm areas of the tropics, intermediate ones in dry, and smallest ones in cold climates (Figure 2-3).

2.4.3. Relation between soil organic carbon stock and land use

Several field studies have already demonstrated that the influence of land use reaches deep soil layers (Borchard et al., 2019; Cardinael et al., 2015; Jackson et al., 2002; Kaonga & Bayliss-Smith, 2009; Mikhailova et al., 2000; Quartucci et al., 2023; Sommer et al., 2000; Tautges et al., 2019). Also at global scale, subsoil organic C stocks vary significantly with land use (Jobbagy & Jackson, 2000; Duarte-Guardia et al., 2020). Our results confirm these findings, although our estimates show an overall larger contribution of deep soil to the overall SOC stock than previously demonstrated by Jobbagy & Jackson (2000). The 100-300 cm layer represents 73% of the 0-100 cm stock in our study for grasslands, whereas in Jobbagy & Jackson (2000) it represents 43%. For forestlands, it is 83% in our study, with 56% for Jobbagy & Jackson (2000). According to their estimate for croplands, the 100-300 cm layer stores 65 Mg ha^{-1}, whereas in our estimates it sums 119 Mg ha^{-1} (Table 2-2). Approximately 35% of the soil profiles used in our study were assessed after Jobbagy & Jackson (2000), suggesting that SOC estimate with the inclusion of more recent publications discovered larger amounts of deep SOC.

We have to reject our hypothesis that forestlands have, on average, larger SOC stocks than croplands on a whole profile basis, since in the 200-300 cm layer the SOC stock in croplands is slightly larger. Yet, the results present great variation among forest types (Table 2-2). When comparing croplands with primary and secondary forests only, our hypothesis holds true. In our database, most sites with forest plantation were under agriculture production before plantation establishment. In principle, this could be a selection bias, where sites with lower organic carbon contents were converted to plantations. If this is true, we can assume that SOC content did not increase after the plantation was established, since these sites have been long converted to plantation (recently converted sites were classified as woodlands – see land use categories definition in Material and Methods). In addition, farmers may have preferred to select the better soils for deforestation and arable soil use, so that parts of the differences between these land uses can also be a selection bias.

Soil clay content differences among land uses are small, and thus cannot account for the differences in SOC stocks (Figure 7-1 and Figure 7-2, Appendix A). An uneven sampling distribution of forest types may contribute to the difference in SOC stock, since most of plantations are in regions with temperate climates (Table 7-1, Appendix A), which present small deep soil carbon stocks (Table 2-2). Other factors contributing to different SOC accrual are amount, quality and frequency of the C added, substrate C/N ratio, nutrient availability and composition of microbial communities (Bailey et al., 2019; Kuzyakov et al., 2000; Lehmann & Kleber, 2015; Meyer et al., 2018; Qiao et al., 2016; Sollins et al., 2007; Zhou et al., 2021) as well as potential priming effects on SOC, with larger susceptibility of deep soil layers (Bailey et al., 2019; Bernal et al., 2016; De Graaff et al., 2014; Gaudel et al., 2022; Meyer et al., 2018). In addition, afforestation has been shown to change the moisture regime and the composition of the bacterial community in deep soil (Kong et al., 2022), and ultimately, the response of deep soil C addition to decomposition depends on tree species composition (Angst et al., 2019; Gao et al., 2023; Voigtlaender et al., 2012; Yin et al., 2018), which are all not possible to evaluate here due to the lack of paired sites.

It has been well documented that organic C stocks in top and subsoils of grasslands exceed those in croplands (Deng et al., 2016; Don et al., 2011; Han et al., 2010; Reijneveld et al., 2009; Wiesmeier et al., 2013; Xie et al., 2007). However, our results contradict this common assumption, showing that croplands have larger SOC stocks in deep soil than grasslands. The higher SOC stock in top- and subsoil in grasslands is due to denser rooting (DuPont et al., 2010; Phillips et al., 2015) and more efficient accrual of litter-derived C (Hu et al., 2016; Rasse et al., 2005). However, 95% of the grass root biomass is concentrated in the first 60 cm of soil (Jackson et al., 1996; Lorenz & Lal, 2005), and only very few studies assessed SOC stocks deeper than 100 cm. Studies on SOC stocks deeper than 100 cm report that there is either no difference in deep SOC stocks of grasslands and croplands (Han et al., 2018; Mikhailova et al., 2000; Wang et al., 2016) or they also indicate larger SOC stocks in croplands (Yu et al., 2019). Our findings that croplands have larger deep SOC stock than grasslands do not seem to be influenced by climate, since there is a homogeneous distribution of land uses in all climates (Table 7-1, Appendix A), but could again be the result of different preferences in site selection, as in the case of some profiles in Fluvisols with high C concentration in deep soil of croplands (Table 7-2, Appendix A).

2.5. Conclusion

We created a deep soil organic carbon stock database of more than 12,000 soil profiles, with maximum depths ranging from 200 to 500 cm, and performed a global assessment with updates of main reference soil groups developed by Batjes (1996), of land uses developed by Jobbagy & Jackson (2000), and added estimates for the major climate types. Our results show a larger 0-200 cm stock for the average of the soil groups than previously reported, with larger contribution of deep soil to the overall SOC stocks, particularly in forestlands and grasslands, and an overall larger SOC stock below 100 cm in croplands than in grasslands. Deep soil C stocks also differ in different climates, but unlike previous reports for the topsoils, we find the largest stocks of deep SOC in the tropical areas, followed by arid lands. At the global scale, our estimates for mineral soils in the 0-200 cm layer are 19% larger than the current estimates used for global C modelling (Canadell et al., 2022; Friedlingstein et al., 2023), in addition to the need of adding 1,425 Pg from the 200-500 cm layer to the global estimates.

3. CHAPTER 3: DEEP SOIL CARBON LOSS OFFSETS RAPID ABOVEGROUND CARBON ACCUMULATION AFTER REFORESTATION

Modified on the basis of:

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3.1. Introduction

Carbon accrual in restored lands has been promoted as a natural solution for climate change mitigation (Griscom et al., 2017; Lewis et al., 2019). As a result of increased primary productivity through restoration interventions, carbon is accumulated in all aboveground plant biomass as well as in roots, litter, and soil (IPCC, 2006; Lewis et al., 2019). To account for increased SOC storage, usually the 0-30 cm (IPCC, 2019) or up to 30 to 40 cm soil depth ("4 per 1000" Initiative) are considered (Minasny et al., 2017), as reported in many studies assessing soil carbon sequestration after land restoration (Brancalion et al., 2021; Ferez et al., 2015; Zanini et al., 2021; Zhang et al., 2019). However, half or even more of soil C is stored in the subsoil (Duarte-Guardia et al., 2020; Jobbagy & Jackson, 2000; Schneider & Don, 2019). Particularly when land restoration is performed by planting trees, subsoil C and even deeper soil layers can contribute significantly to overall ecosystem carbon storage (Borchard et al., 2019; Lorenz & Lal, 2005; Rumpel & Kögel-knabner, 2011). This is especially important in tropical environments, where soils (mainly Ferralsols and Acrisols) reach several meters deep and accumulate a significant share of carbon below topsoil (Batjes, 2014; Kögel-Knabner & Amelung, 2021; Strey et al., 2017). It was observed, however, that C accrual upon land-use change in the topsoil was offset by losses of C in the subsoil (Kalbitz et al., 2013; Mobley et al., 2015; Steinmann et al., 2016). Thus, assessment of deeper soil layers in land restoration areas is needed to draw more reliable conclusions about the real potential of land restoration for C sequestration.

The impact of land use change on deep soil C stocks can vary depending on site conditions. In a semi-arid region in China, Zhao et al. (2014) found a SOC stock increase of 5-7 Mg ha^{-1} in the 1-2m layer after 30 years of land restoration. On the other hand, Mobley et al. (2015) show that soil carbon gain in the 0-7.5 cm layer was counterbalanced by losses from the 35-60 cm layer in in a 50-year-old loblolly pine forest planted on old cotton field in the USA. In a global meta-analysis, Shi et al. (2013) found that the land use change from cropland to shrubland promoted a SOC stock increase of around 0.11 Mg ha^{-1} yr^{-1} in the 40-100 cm layer. In tropical regions, land use change was found to have a positive effect on subsoil C (Ahirwal & Maiti, 2018; Vicente et al., 2016; Wang et al., 2017). However, our knowledge on deep soil C response to land-use change is limited.

Active restoration through mixed native species planting and passive restoration via natural regeneration are the most common approaches of land restoration in tropical areas (Chazdon & Guariguata, 2016; Rodrigues et al., 2011). A third restoration approach is the implementation of successional agroforestry systems, an active restoration with a mix of native and exotic trees intercropped with agriculture, which have the capacity to mimic natural processes and thus to build forest-like structures in final stages (Miccolis et al., 2019; Young, 2017). In several tropical regions, such the Atlantic Forest of Brazil, active restoration was the most used approach (Brancalion et al., 2016). Indeed, greater aboveground biomass growth has been measured following active than following passive restoration (Brancalion et al., 2021; César et al., 2018), yet with little differences in topsoil C after 5 years of experiment duration (Ferez et al., 2015; Zanini et al., 2021). Most studies do not consider the deep soil C pool (Mendes et al., 2019), despite it is well known that many forest trees are able to develop deep-rooting systems (Canadell et al., 1996; Pierret et al., 2016). To be able to study such effects, paired site approaches are needed, which allow comparisons of different land-use practices under similar soil and climatic conditions.

The objective of this study is to quantify the C sequestration potential by different restoration approaches in the Atlantic Forest Biome in Brazil. We hypothesized that soil carbon stock changes follow aboveground biomass pattern. For this purpose, we determined C storage in aboveground biomass as well as roots, and soil down to a depth of 300 cm in native vegetation and agricultural areas (positive and negative references, respectively), and three different restoration approaches: natural regeneration (passive restoration), reforestation (active restoration) and agroforestry systems of different ages.

3.2. Material and methods

3.2.1. Site description

The study was conducted in the Ipanema rural settlement, belonging to the Landless Workers Movement (MST, in Portuguese) located in the municipality of Iperó, state of São Paulo, Brazil, about 120 km away from the capital São Paulo. The settlement was established in 1996 and covers an area of 1,744 ha, divided into 148 farms ranging in size from 8 to 20 ha (INCRA, 2017). It belongs to the Atlantic Forest Biome and is located inside and in the buffer zone of the Ipanema National Forest, which has a total area of 5,070 hectares (ICMBio, 2017). The climate is humid subtropical, oceanic without dry season and with hot summer (Cfa according to Köppen-Geiger climate classification) (Alvares et al., 2013). The mean annual temperature is 19.6 ^{o}C and the mean cumulated annual precipitation is 1,219 mm. The predominant soils in the area are Ferralsols and Acrisols (ICMBio, 2017; INCRA, 2020).

Large-scale deforestation took place for coffee plantations in the 19th century, which were then substituted gradually by sugarcane and pasture in the 20th century (Carlucci et al., 2021; Dean, 1997). From the mid-1970s to early 1990s, the area was managed by a public company that raised cattle in the flatter areas, in addition to implementing field trials of agricultural machinery (ICMBio, 2017; Oliveira & Carvalho, 2017).

3.2.2. Experimental design

We used a paired-site design to evaluate three different approaches of land restoration: reforestation (RE), natural regeneration (NR) and agroforestry system (AFS). For the agroforestry system, we evaluated an intermediate stage (AFSi; agroforestry with 5-6 years) and an advanced stage (AFSa; agroforestry with 18-20 years). We also evaluated areas of agriculture (AG) as a negative reference (control) and areas of secondary forest (SF) as a positive reference (target) (Figure 3-1). Each land cover was assigned and sampled in triplicate, therefore totalling 18 areas. Table 3-1 shows details about each land cover. For more information of forest structure indicators see Table 8-3.

All the area shared the same land-use history prior to the establishment of the rural settlement and all restoration areas were under agricultural use prior to land-use change. Therefore, we assume that there were no differences in the C stock between the agricultural and restored areas before land-use change. Hence, we used the adjacent agricultural sites as reference. This does not imply that its C stocks did not change in the last 18 years due to environmental effects, but the design assumes that if such effects occurred, they did so similarly at all sites. Minor changes in agricultural practices have taken place in the last years, but they were not profound technical changes such as no tillage, that might have influenced the C stocks.

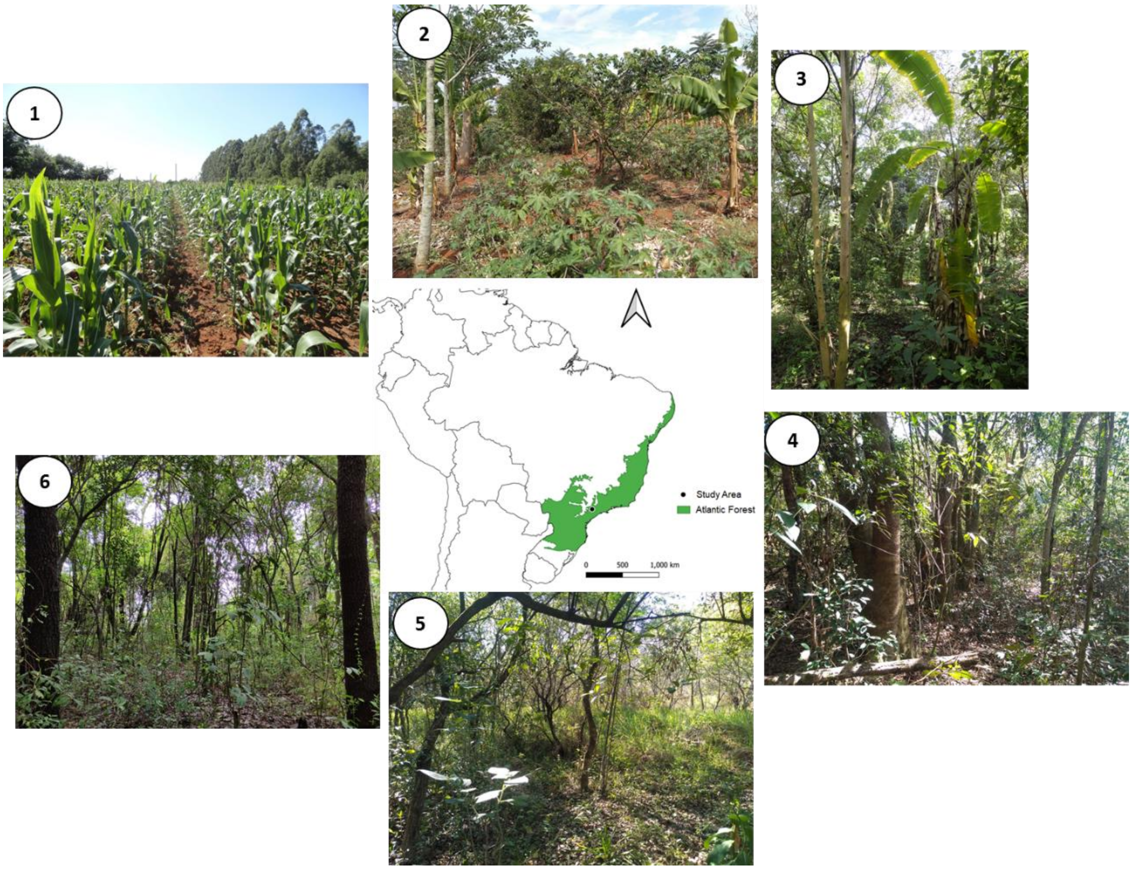

Figure 3-1. Pictures of the six land uses assessed: 1) agriculture (AG); 2) agroforestry system in intermediate stage (AFSi) – agriculture interspersed with trees (rows with native and fruit trees); 3) agroforestry system in advanced stage (AFSa) – the structure resembles a secondary forest - the banana plant is a remnant of intermediate stages; 4) reforestation (RE) – it is still possible to see the initial planting lines; 5) Natural regeneration (NR) – characterized by a great number of small individuals; 6) secondary forest (SF) – characterized by the presence of big trees. Centre: sampling location (black dot) within the Atlantic Forest Biome (the green area on the map represents the original biome cover). Map courtesy: Dr. Luiz Felipe Galizia.

Table 3-1. Description of the land cover under study, with averaged (± SE, n=3) information on age after land-use change from agriculture (in years), soil clay content (g kg^{-1}), and soil pH ($CaCl_2$).

Land cover	Age	Clay (g kg^{-1})	pH ($CaCl_2$)	Description
Agriculture (AG)	>25	0-40 cm – 285±78 40-100 cm – 347±88	0-40 cm - 4.9±0.17 40-100 cm - 4.4±0.23	Areas used for agriculture since settlement establishment (1996). Management usually consists of soil preparation with plow and harrow in the 0-20 cm layer. Liming and mineral fertilization are carried out at low rates (1-2 Mg ha^{-1} of lime, 40-80 kg ha^{-1} of N and 10-30 kg ha^{-1} of P) and at irregular intervals (2-4 years). Periods of 1-3 years fallow are implemented after 3-5 years of cropping. Most common crops are maize, common bean, cassava, okra, eggplant, and pepper.
Agroforestry system intermediate (AFSi)	5.7±0.3	0-40 cm – 314±63 40-100 cm – 397±86	0-40 cm - 5.0±0.17 40-100 cm - 4.7±0.19	Successional agroforestry system in an intermediate stage of succession, planted on former agricultural land. A mix of native and exotic trees were planted in spacing of 1.5-3.0 m between plants and 3.0-6.0 m between lines. Soil preparation consisted of plowing and harrowing and then manually prepared pits to plant the tree seedlings. In the first stages before canopy closure (0-4 years), management was targeted at crop production between the tree lines. After that, management is targeted at fruit production. Most common fruit-bearing plant species are banana, mango, guava, avocado, citrus, lychee, and black mulberry.
Agroforestry system advanced (AFSa)	19.0±1.0	0-40 cm – 414±71 40-100 cm – 504±73	0-40 cm - 5.1±0.05 40-100 cm - 4.6±0.25	Successional agroforestry system in an advanced stage of succession. These areas are the succession of AFSi and thus received similar management in early (crop production) and intermediate stages (fruit production). After that, management consisted of pruning bigger trees until they are approximately 5 m high. In advanced stages (>15 y), there are still some fruit trees producing under the canopy (guava, mango, avocado), but in fewer amount.
Reforestation (RE)	16.0±1.2	0-40 cm – 247±70 40-100 cm – 323±76	0-40 cm - 4.6±0.24 40-100 cm - 4.3±0.15	Active restoration by planting mixed native tree species in areas of former arable land. Soil preparation was first carried out with plowing and harrowing and then manually to prepare pits to plant the tree seedlings. Planting was performed in a spacing of 3.0 m between rows and 2.0 m between seedlings with 20-50 different species distributed randomly in the pits. Weeding took place in the initial stages in order to allow the forming canopy to surpass the average

				invasive grass height. After that, the area was fenced to promote physical isolation, and the natural regeneration process started to take place.
Natural regeneration (NR)	17.0±1.5	0-40 cm – 368±45 40-100 cm – 446±46	0-40 cm - 4.5±0.14 40-100 cm - 4.4±0.18	Passive restoration by a process of natural regeneration that occurred after the physical isolation of arable land by fencing. In the first stage (0-2 years), the area was invaded by grasses and shrubs, followed by small trees shading the grasses. As the regeneration advanced, larger trees established and multi-strata secondary forest was formed.
Secondary forest (SF)	>25	0-40 cm – 426±80 40-100 cm – 426±103	0-40 cm - 5.0±0.31 40-100 cm - 4.1±0.10	Areas covered with forest at least since the settlement establishment (1996). There has been no targeted management in the area (e.g., timber for energy or non-timber forest products). These are preservation areas set aside by farmers to comply with legal requirements.

3.2.3. Sampling and sample preparation

Field data were collected between November 2020 and March 2021. We assessed the carbon stocks of soil, living and dead plant biomass as recommended by IPCC (2006) in assessments of carbon sequestration in arable land converted to forest land. For living biomass, we assessed the aboveground biomass (AGB) and the root biomass. For the dead organic matter or necromass, we assessed the litter, standing woody necromass (SWN) and fallen woody necromass (FWN), which were separated into large woody necromass (LWN) and medium-sized woody necromass (MWN). The ecosystem (=total) C stock was then calculated as the sum of the amount of C stored in aboveground living and dead biomass, plus the amount of root C and of SOC.

3.2.3.1. Soil

We collected soil with a mechanized hand (gasoline-powered) soil corer of 10 cm diameter up to 300 cm in the following layers: 0-20, 20-40, 40-100, 100-150, 150-200, 200-300 cm. In each area we collected five samples in the 0-20 and 20-40 cm layers and three samples in the remaining layers. The soil was air dried and then 2 mm sieved. We also collected three undisturbed samples (100 cm^3 stainless steel ring) in each layer. The samples were dried at 105 ^oC in a ventilated oven, the dry weight was determined, and the bulk density was then calculated.

3.2.3.2. Roots

We sampled fine roots (diameter < 2.0 mm), and estimated coarse roots (diameter > 2.0 mm). For the fine roots, we used the same mechanized soil corer of soil samples to collect three samples of approximately 1 kg of soil in the same layers of the soil samples. Maximum rooting depth in the studied land uses varies depending on the species composition, but may range from 500 to 1,500 cm deep (Canadell et al., 1996). We washed the soil in a 250 µm mesh and placed the organic material in a glass bowl with water. Then, with a tweezer, we collected all the root pieces ≤ 2.0 mm diameter. Subsequently, the roots were dried at 65 ^oC for 72 hours and the weight determined. We first determined root density (g cm^{-3}) in each layer and root biomass per unit of soil dry weight and then converted to root biomass on a hectare basis using the calculated soil bulk density (Adriano et al., 2017). For the coarse roots, we first estimated total root biomass based on the aboveground living biomass (IPCC, 2006; Mokany et al., 2006). Then, we subtracted the fine roots to obtain coarse roots biomass.

3.2.3.3. *Aboveground living biomass*

We established three inventory plots of 200 m² (10 m x 20 m) in each area. Total height and diameter at breast height (DBH) were measured for all tree individuals with DBH ≥ 1.0 cm. We calculated tree biomass using the pantropical allometric model developed by Chave et al. (2014). Wood density was taken from the database developed by Chave et al. (2006). For the species that could not be identified in the field, we used a wood density value of 0.60 g cm^{-3}, which was the average wood density of all identified individuals in the reforestation, natural regeneration and secondary forest sites. The average wood density value used in this study is close to the mean wood density of 0.632 g cm^{-3} found in tropical South America (Chave et al., 2009) and the value of 0.603 g cm^{-3} used by Vieira et al. (2008) to estimate aboveground biomass in the Atlantic Forest. We then summed the biomass of all individuals inside the plot and extrapolated to a hectare basis.

3.2.3.4. *Aboveground necromass*

For the litter and woody necromass <2.0 cm, a square of 0.25 m² (0.5 m x 0.5 m) was deposited randomly at three different spots within each AGB sampling plot. The material inside the square was collected, dried, weighed and extrapolated to a hectare basis.

For the standing woody necromass (SWN), we followed the same methodology of AGB, and identified the dead individuals in the field inventory. The biomass per tree was calculated according to Chave et al. (2014) and a correction factor of 0.7609 was used to convert biomass to necromass (Maas et al., 2021).

For the fallen woody necromass (FWN), we used the intercept line method, arranging a 10m line on the ground and measured the diameter of all pieces of dead wood ≥ 2.0 cm diameter that touched the line. We defined as medium-sized woody necromass (MWN) pieces from 2.0 to 9.9 cm diameter and as large woody necromass (LWN) pieces ≥ 10.0 cm diameter. The decay class of each piece was also determined as follows: 1) solid; 2) intermediate; 3) highly decomposed (Maas et al., 2020). The volume was calculated according to van Wagner (1968) and then the biomass was calculated by multiplying the volume by the wood density of each class found in Maas et al. (2021). When the dead individual was a palm, we used the density reported by Tiepolo et al. (2002).

3.2.4. Determination of carbon stocks

3.2.4.1. Soil

The soil (air dried and 2 mm sieved) was milled in a ball mill and the carbon content determined in an elemental analyzer (Vario Micro Cube Elementar, Germany). Subsequently, we calculated soil carbon stock as follows:

$$SOC = C\% \ x \ BD \ x \ Th$$

Where: SOC = soil organic carbon stock, in Mg ha^{-1}; C% = soil carbon concentration, in %; BD = soil bulk density, in g cm^{-3}; Th = layer thickness, in cm. For detailed information of soil carbon concentration and bulk density see Table 8-1.

From the results of SOC in fixed depths layers, we calculated the SOC at equivalent soil mass (ESM), based on Wendt & Hauser (2013). The fine soil mass of the secondary forest (SF) was used as reference. Nevertheless, we report the results here as if they were in fixed depths to facilitate comparisons between land covers. See Table 8-2 (Appendix B) for information of soil masses.

3.2.4.2. Roots

Fine roots were milled in a ball mill and then carbon content was determined in an elemental analyzer (Vario Micro Cube Elementar, Germany). The fine root carbon stock was calculated by multiplying the root biomass by the root carbon content. For the coarse roots, we used the carbon content of woody biomass.

3.2.4.3. Aboveground living biomass

Carbon stock was calculated by multiplying the biomass stock by 0.457, which is the average C content in sub-montane Atlantic Forest in Vieira et al. (2011).

3.2.4.4. Aboveground necromass

For litter, we made a composite sample from each area and determined the carbon content in an Elementar Vario Micro Cube elemental analyzer. For the standing woody necromass, we used the carbon content of 0.43 reported by Maas et al. (2021). For the fallen woody necromass, we used the carbon content of its respective size class found in Maas et al. (2021).

3.2.5. *Data analysis*

We first performed Shapiro-Wilk and skewness and kurtosis tests to check for data normality. Standing woody necromass, SOC and ecosystem (total) did not show normal distribution. For the normally distributed data, we employed a general linear model with the carbon pool as the dependent variable and the land cover as the independent variable. For the others, we employed Poisson model with Huber-White robust estimates of the standard errors, since the data showed Poisson like distribution (Wooldridge, 2010). For the SOC, we also included the soil layer (and the interaction between land cover and soil layer) as an independent variable. Statistical differences of carbon stocks in each pool among the land covers were tested with LSD test ($p<0.05$).

We checked for differences in soil clay content among the land covers (LSD, $p<0.05$), and none of the restoration approaches showed statistically difference to the agriculture sites (Figure 8-1). Nonetheless, since a small variation in the clay content was observed among the land covers (Table 3-1; Figure 8-1, Appendix B), coupled with a moderate correlation between SOC and clay in deep soil (Figure 8-2, Appendix B), we also ran the SOC model including soil clay content as a continuous independent co-variable in order to adjust the results of SOC for this uncontrolled factor. We then checked for statistical differences of the SOC sequestration in each layer of each restoration approach (LSD, $p<0.05$), i.e., the SOC stock difference to agriculture (negative control).

When reporting the results of the carbon sequestration in all pools (section 3.3.3), the figures of SOC are not adjusted by soil clay content. When we present only the results of SOC sequestration (section 3.3.4), the results are presented as a range between the measured values (without clay as covariate) and estimated values (detrended with clay as covariate). All analyses were performed using Stata 16.1 (StataCorp LLC, College Station, USA).

3.3. Results

3.3.1. Carbon stocks and pools relationships

The reference secondary forest (SF) showed the highest average ecosystem (total) C stock (384±134 Mg ha^{-1}). The total C stock of the advanced (19-year-old) agroforestry system (311±27 Mg ha^{-1}), natural regeneration (310±38 Mg ha^{-1}) and reforestation systems (289±52 Mg ha^{-1}) did not differ at a p<0.05 significance level, but were significantly higher than at the site under agriculture (164±35 Mg ha^{-1}) (p<0.001, p<0.001 and p=0.032, respectively). With intermediate restoration time of only 5.7 years, the agroforestry system also exhibited an intermediate total carbon stock of 218±43 Mg ha^{-1}, which was lower than that of the advanced agroforestry system (p=0.029) (Figure 3-2 and Table 3-2).

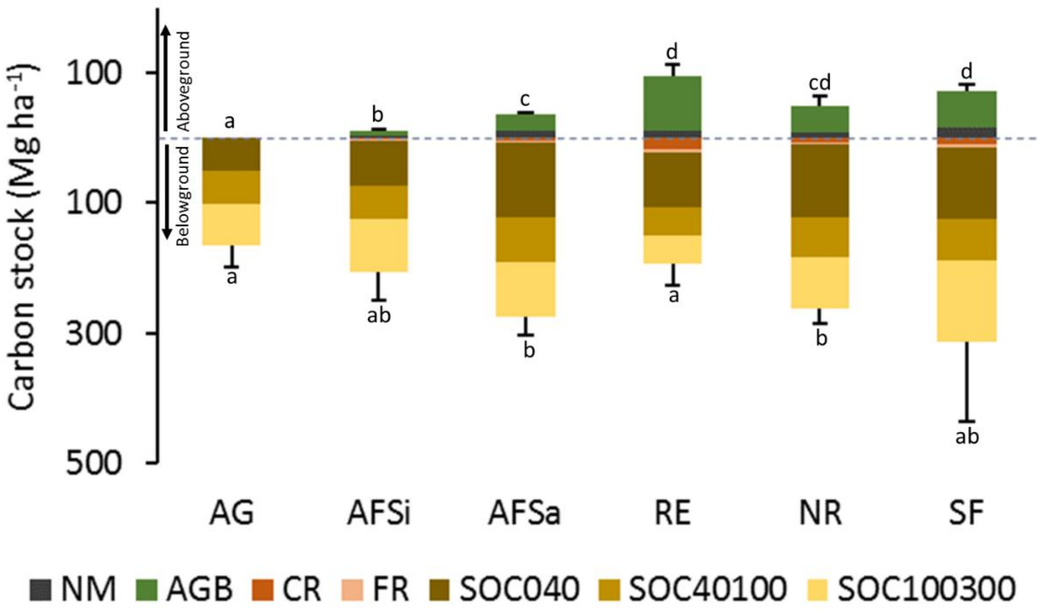

Figure 3-2. Carbon stocks (Mg ha^{-1}) in the measured pools: NM = aboveground necromass; AGB = aboveground living biomass; CR = coarse roots (diameter > 2.0 mm); FR = fine roots (diameter <2.0 mm) (sum of the whole soil profile = 0-300 cm); SOC040 = soil organic carbon in the 0 to 40 cm layer; SOC40100 = soil organic carbon in the 40 to 100cm layer; SOC100300 = soil organic carbon in the 100 to 300 cm layer. SOC pools based on an equivalent soil mass. Error bars represent standard error of the aboveground pools (NM + AGB) and belowground pools (CR + FR + SOC040 + SOC40100 + SOC100300). Different letters indicate statistical difference among the land covers for the aboveground and belowground pools (LSD, p<0.0.5) AG: Agriculture, AFSi: agroforestry system in intermediate stage, AFSa: agroforestry system in advanced stage, RE: reforestation, NR: natural regeneration, SF: secondary forest.

Table 3-2. Mean carbon stocks (Mg ha^{-1} with standard errors, n=3) of all carbon pools. Aboveground = aboveground living biomass with DBH ≥ 1.0 cm. Necromass = sum of litter + SWN + FWN. SWN = standing woody necromass. FWN = fallen woody necromass (sum of LWN + MWN). LWN = large woody necromass (diameter ≥ 10cm). MWN = medium-sized woody necromass (diameter between 2 and 10 cm). Coarse roots = diameter > 2.0 mm. Fine roots = diameter ≤ 2.0 mm in different soil layers. SOC = soil organic carbon in different soil layers (n=5 for the 0-20 and 20-40 cm layers and n=3 for the remaining). Ecosystem = sum of all pools (Aboveground + Necromass + Coarse roots + Fine roots + SOC 0-300 cm). AG: agriculture, AFSi: agroforestry system in intermediate stage, AFSa: agroforestry system in advanced stage, RE: reforestation, NR: natural regeneration, SF: secondary forest. Different letters in the same line (comparison of the same pool among the land covers) indicate statistical difference by LSD test (p<0,05).

Carbon pool	AG	AFSi	AFSa	RE	NR	SF
Aboveground		7.3 (0.5) a	25.3 (4.8) b	85.4 (14.9) d	39.8 (12.7) bc	56.3 (9.3) cd
Necromass		4.4 (0.9) a	10.3 (1.4) ab	10.5 (3.6) ab	8.3 (3.2) ab	14.8 (2.9) b
Litter		3.46 (0.94) a	6.13 (0.94) a	5.45 (1.64) a	5.83 (2.26) a	6.63 (0.96) a
SWN		0.00 (0.00) a	0.25 (0.24) ab	0.36 (0.10) b	0.43 (0.23) b	1.18 (0.60) b
FWN		0.92 (0.08) a	3.98 (0.70) b	4.70 (2.20) ab	2.03 (0.94) ab	7.02 (2.23) b
LWN		0.74 (0.04) a	3.42 (0.54) b	3.36 (2.39) ab	1.03 (0.57) a	5.48 (2.19) b
MWN		0.18 (0.10) a	0.56 (0.28) ab	1.33 (0.62) b	1.01 (0.38) ab	1.54 (0.19) b
Coarse roots		2.59 (0.61) a	3.42 (1.36) a	18.18 (4.94) b	6.79 (3.72) ab	10.60 (3.65) ab
Fine roots 0-300 cm	0.48 (0.36) a	1.38 (0.33) ab	3.30 (0.46) bc	5.07 (0.97) c	3.90 (1.28) bc	4.80 (1.20) c
Roots 0-20 cm	0.36 (0.30) a	0.75 (0.21) ab	1.63 (0.12) c	2.11 (0.81) c	1.46 (0.21) c	2.02 (0.86) bc
Roots 20-40 cm	0.05 (0.03) a	0.20 (0.04) b	0.44 (0.17) bcd	0.86 (0.14) e	0.38 (0.10) c	0.78 (0.14) de
Roots 40-100 cm	0.02 (0.01) a	0.15 (0.04) a	0.57 (0.06) b	1.07 (0.41) b	0.57 (0.20) b	1.04 (0.39) b
Roots 100-150 cm	0.01 (0.01) a	0.15 (0.09) ab	0.21 (0.07) b	0.30 (0.07) b	0.77 (0.44) b	0.28 (0.05) b
Roots 150-200 cm	0.01 (0.01) a	0.08 (0.04) b	0.23 (0.04) c	0.48 (0.33) abc	0.17 (0.07) bc	0.38 (0.17) c
Roots 200-300 cm	0.03 (0.01) a	0.06 (0.02) b	0.22 (0.08) c	0.25 (0.12) bc	0.55 (0.46) abc	0.30 (0.11) c
SOC 0-300 cm	163.8 (35.4) a	202.4 (42.1) ab	268.8 (28.1) b	169.9 (30.2) a	251.5 (20.2) b	297.7 (120.6) ab
SOC 0-20 cm	26.0 (6.0) a	39.0 (6.2) ab	77.2 (13.4) c	48.3 (4.9) b	62.0 (5.9) c	70.4 (7.9) c
SOC 20-40 cm	23.7 (4.7) a	29.6 (7.5) ab	36.7 (4.0) bc	34.2 (9.1) abc	48.1 (5.9) c	38.7 (5.3) bc
SOC 40-100 cm	52.1 (10.5) abc	50.9 (6.7) ab	70.0 (6.4) c	44.4 (7.4) a	62.0 (6.6) bc	63.6 (27.1) abc
SOC 100-150 cm	28.2 (6.3) ab	32.2 (3.9) b	33.9 (12.8) ab	18.1 (5.0) a	28.6 (8.0) ab	43.7 (22.2) ab
SOC 150-200 cm	16.4 (5.9) ab	21.4 (6.6) ab	24.2 (9.3) ab	10.9 (3.2) a	19.5 (3.9) b	31.0 (21.8) ab
SOC 200-300 cm	17.4 (7.4) ab	29.3 (11.8) ab	26.8 (9.8) ab	14.1 (4.8) a	31.4 (5.2) b	50.2 (40.2) ab
Ecosystem	164.3 (35.1) a	218.0 (42.6) ab	311.1 (26.5) c	289.1 (52.3) bc	310.3 (38.0) bc	384.3 (134.2) abc

The C stock in soil was the largest pool, accounting for between 59% (reforestation) and 93% (agroforestry system intermediate) of total ecosystem carbon stock (agriculture not considered). SOC stock in the first meter (0-1 m) was larger than the aboveground living biomass carbon in any land cover (agriculture not considered). Except for reforestation, the SOC stock in the 100-200 cm depth interval was larger than that of aboveground living biomass. Yet, in the case of the agroforestry systems (both intermediate and advanced), the SOC stock in deep subsoil (200-300 cm soil layer) was also larger than the C stock of the aboveground living biomass (Figure 3-2 and Table 3-2).

The reforestation sites had the largest aboveground living C stock (85±15 Mg ha^{-1}) among all sites, statistically larger than the advanced agroforestry system (25±5 Mg ha^{-1}; p=0.003) and the natural regeneration site (40±13 Mg ha^{-1}; p=0.042). Reforestation and natural regeneration did not differ from the reference secondary forest (56±9 Mg ha^{-1}), which implies that after 16 and 17 years these restoration approaches succeeded in recovering the aboveground living stock, respectively. The share of aboveground living biomass C pool relative to total ecosystem C, however, varied considerably among the restoration approaches, ranging from 3% in the intermediate agroforestry system to 30% in the reforestation sites.

Aboveground necromass stocks varied from 4.4±0.9 Mg ha^{-1} in intermediate agroforestry system to 14.8±2.9 Mg ha^{-1} in the reference secondary forest, with statistically significant difference between them (p=0.018). The aboveground necromass carbon stock represented a small share of ecosystem carbon, ranging from 2.0% (intermediate agroforestry system) to 3.9% (secondary forest) of total ecosystem carbon.

Reforestation showed the highest fine root carbon stock (5.1±1.0 Mg ha^{-1}), statistically different from agriculture (p=0.003) and intermediate agroforestry system (p=0.011). Overall, fine roots accounted for the lowest share among the studied pools, ranging from 0.3% in agriculture to 1.8% in reforestation of total ecosystem carbon. Coarse roots followed the aboveground stock, reaching 18.2 Mg ha^{-1} in the reforestation sites, statistically superior to agroforestry system with intermediate (2.6 Mg ha^{-1}, p=0.009) and advanced stage of development (3.4 Mg ha^{-1}, p=0.014).

3.3.2. Soil organic carbon stocks and depth distribution

The secondary forest had the highest total (0-300 cm) SOC stock of all sites studied, reaching 298±121 Mg ha^{-1}. The older agroforestry system (p=0.006) and the natural regeneration (p=0.010) site still exhibited a significant larger total SOC stock than the site under agriculture, which, however, stored similar amounts of SOC as did the reforestation site (Table 3-2).

The secondary forest had also the highest SOC stock below 40 cm, summing up to 189±111 Mg ha^{-1} in the 40-300 cm layer. The reforestation site stored less SOC below 40 cm than the agricultural site, 87±20 Mg ha^{-1} and 114±30 Mg ha^{-1}, respectively. In all land covers, more than half of the total SOC (0-300 cm) was found below 40 cm. Moreover, the share of SOC in topsoil declines by a factor of 1.5 when 300 cm instead of only 100 cm depth is considered during sampling, denoting the importance of deep soil to the overall C storage.

The decrease in SOC along the soil profile is not always accompanied by a decrease in roots. All land uses presented an increase in root density in some layer below topsoil. For example, natural regeneration presented an increase in root density from 0.08±0.03 in the 150-200 cm layer to 0.13±0.10 g cm^{-3} in the 200-300 cm layer (Table 8-4, Appendix B).

3.3.3. *Carbon stock changes after land restoration*

Comparing the carbon stocks to agricultural land, the restoration efforts show an increase of total ecosystem C, i.e., carbon sequestration took place. As most of the C was stored in soil, also the C sequestration mainly occurred as SOC, except in the reforestation sites. In the intermediate and advanced agroforestry system, SOC sequestration (0-300 cm) represented roughly three fourths and in natural regeneration approximately two thirds of total carbon sequestration. In contrast, when reforestation was implemented, only 5% of the total carbon sequestration occurred in the soil, due to the high C accumulation in the aboveground biomass coupled with a loss of 27 Mg ha^{-1} of deep SOC (40-300 cm) (Figure 3-3).

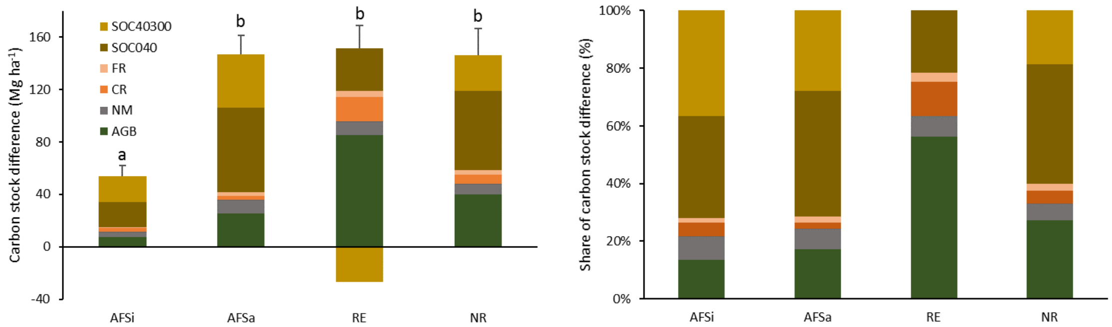

Figure 3-3. Left: Carbon sequestration (carbon stock difference compared to agriculture, in Mg ha^{-1}) after land restoration with different approaches. Error bars represent standard error of the sum of all pools (AGB + NM + CR + FR + SOC040 + SOC40300). Different letters indicate statistical difference in the carbon stock difference among the land uses. Right: Share (%) of carbon sequestration in the different pools (only considering positive differences). AFSi: agroforestry system in intermediate stage; AFSa: agroforestry system in advanced stage; RE: reforestation; NR: natural regeneration. AGB = aboveground living biomass; NM = aboveground necromass; CR = coarse roots (diameter > 2.0 mm); FR = fine roots (diameter ≤ 2.0 mm) (sum of the whole soil profile = 0-300 cm); SOC040 = soil organic carbon in the 0 to 40 cm layer; SOC40300 = soil organic carbon in the 40 to 300 cm layer.

Aboveground necromass was responsible for a significant share of carbon accrual, ranging from 6% of total C accumulation in natural regeneration sites to 8% in the reforestation ones. On the other hand, fine roots represented only a small share of the amount of C that accumulated, contributing only 2 to 4% of total carbon sequestration.

3.3.4. Soil organic carbon stock changes after land restoration

Even though the overall clay content was not different between the land restoration approaches and the negative reference (agriculture) (Figure 8-1, Appendix B), slight variations in clay content may still affect the reported overall differences in SOC (Figure 8-2, Appendix B). Thus, for the changes of SOC stock (difference from agriculture), we report the measured as well the corrected results, i.e., without and with adjustment by the soil clay content, respectively.

Soil organic carbon accumulation mainly occurred in the topsoil (0-40 cm), regardless correction by clay content or not. In natural regeneration, it ranged from 60 to 50 Mg ha^{-1} whereas in the reforestation approach it ranged from 33 to 50 Mg ha^{-1}, depending on whether SOC stock changes were presented as measured (first value of the range) or adjusted (detrended) to similar clay contents (second value of the range). In all land covers there was a change in the magnitude of topsoil carbon accrual when clay was included as a covariate in the model. However, in deep soil (40-300 cm), there was even a shift in the direction of change, i.e., accumulation when not adjusted and loss when detrended. In the natural regeneration sites, there was a carbon sequestration of 27 Mg ha^{-1} when clay was not included in the model, but a loss of 4 Mg ha^{-1} when values were mathematically adjusted to clay content (not significant, $p<0.05$). This pattern of presenting an accumulation of SOC in the 40-300 cm layer when not adjusted by clay, and a loss when the results are adjusted, was observed for the other restoration approaches, except for the reforestation site. In this site, there was a loss of 27 or 26 Mg ha^{-1} (without and with detrending for variations in clay content, respectively), depicting that the loss of subsoil carbon with reforestation was consistent regardless of any changes in soil clay content (Figure 3-4). This indicates that reforestation includes the risk of deep soil C losses.

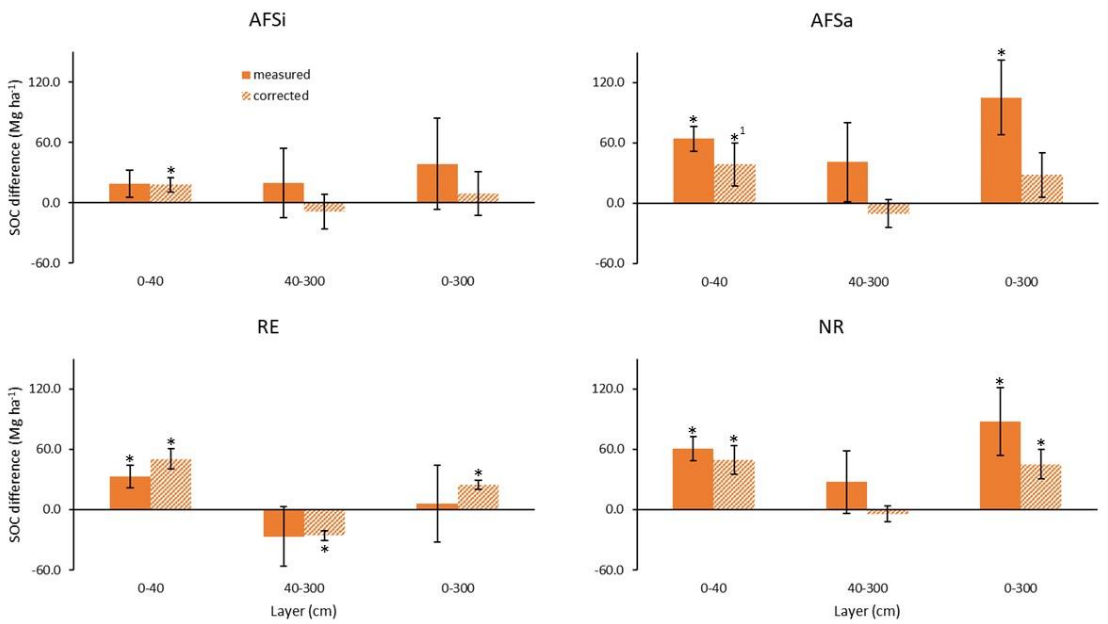

Figure 3-4. Soil organic carbon difference after land use change (agriculture as reference) in the topsoil (0-40 cm), deep soil (40-300 cm) and whole soil profile (0-300 cm). Solid colour bars are the measured values (not adjusted by clay content) and hatched colour bars are the corrected values (adjusted by clay content). Asterisks indicate that the SOC difference is statistically different to agriculture (LSD test, $p<0.05$). In the case of *[1], $p=0.0519$. AFSi: agroforestry system in intermediate stage; AFSa: agroforestry system in advanced stage; RE: reforestation; NR: natural regeneration.

3.4. Discussion

3.4.1. Carbon stocks and pools relationships

We showed that reforestation presented the highest aboveground carbon, but due to a loss in deep soil carbon, all the restoration approaches resulted in similar ecosystem carbon sequestration. The aboveground carbon stocks in our study presented similar values as in the Amazon (Asner et al., 2010; Longo et al., 2016). Also, our results are in similar magnitude as carbon stocks earlier found in the Atlantic Forest, though in the lower range (Alves et al., 2010; Brancalion et al., 2021). Variations are likely due to the high degree of forest fragmentation found in the Atlantic Forest and particularly in the study area (Lima et al., 2020; Rosa et al., 2021), which leads to a reduction in the carbon stocks due to an edge effect (Chaplin-Kramer et al., 2015; Magnago et al., 2017; Pyles et al., 2022).

Soil organic carbon has the highest shares in the total carbon stock of the Atlantic Forest. In a preserved Forest Reserve, Vieira et al. (2011) found that SOC in 0-100 cm soil depth was twice that of the aboveground living biomass. In a forest fragment, Zanini et al. (2021) found that the SOC stock in the 0-20 cm layer only was even 3 times larger than that of aboveground biomass. Hence, our study supports the important contribution of SOC to the overall carbon stock. Deep soil C is usually not reported, which might lead to an underestimation of ecosystem carbon stock by 32 to 62%, as indicated from the data of the reforestation and agroforestry system intermediate, respectively.

3.4.2. Soil organic carbon stocks and depth distribution

The inclusion of soil carbon stocks below topsoil is essential for the estimate of ecosystem carbon stocks (Duarte-Guardia et al., 2020; Jobbagy & Jackson, 2000; Kögel-Knabner & Amelung, 2021). In a forest reserve in the Amazon region, Strey et al. (2017) found that 78% of the SOC was found below 30 cm in a 1,000 cm deep pit. In different locations in the Amazon region, Telles et al. (2004) found that more than 50% of the SOC stock was found below 40 cm in 210 cm soil profiles. In the Atlantic Forest, assessments of SOC stocks reach up to 100 cm, but already depict the importance of including layers below topsoil (Santos et al., 2019; Vicente et al., 2016; Vieira et al., 2011). In our study, the sole assessment of soil up to 40 cm only as suggested by the "4 per 1000" Initiative would have led to an underestimation of 50 to 70% of SOC depending on the land cover, similar to a 60% underestimation reported by Wade et al. (2019).

3.4.3. Carbon stock changes after land restoration

Restoration changed substantially the carbon stock in the different pools assessed. Changes in the aboveground necromass seem to follow the aboveground living biomass. However, the changes in the SOC pool does not follow the aboveground living biomass pattern, especially for deep soil (40-300 cm), where there was a loss of stock in areas with the highest aboveground living biomass. Therefore, our data does not support our hypothesis.

Acknowledging carbon increments or losses below topsoil after land use change might shift the overall results interpretation (Mobley et al., 2015). When restricting our assessment to the top 40 cm of soil only as recommended by the "4 per 1000" Initiative (Minasny et al., 2017) or even solely to 30 cm as suggested by IPCC (2019), we could have concluded that reforestation is the restoration approach with highest carbon sequestration potential. However, due to the loss of deep soil (40-300 cm) carbon in this approach, we did not find difference in the ecosystem carbon sequestration among the different restoration approaches. Therefore, reforestation, natural regeneration and agroforestry system can be used interchangeably when the goal is to promote carbon sequestration.

Previous studies found a loss of SOC below topsoil under agriculture production (Steinmann et al., 2016), after paddy rice establishment on marsh soils (Kalbitz et al., 2013), after conversion from natural forest to forest plantation and agriculture (Sheng et al., 2015) and even after conversion from agriculture to forest plantation (Mobley et al., 2015). Differently, our study found a decrease of SOC below topsoil after land restoration with mixed native tree species. Moreover, in all the studies, the maximum soil depth evaluated was 100 cm, whereas we found a loss of SOC in the 40-300 cm layer. To the best of our knowledge, this is the first study to find deep SOC loss after land restoration in the Atlantic Forest Biome.

Noteworthy, the loss of subsoil carbon took place in reforestation areas, where higher aboveground carbon growth was found. In a way this is the reverse results of Sandhage-Hofmann et al. (2021), who reported reduced aboveground biomass as induced by elephants, leading to elevated SOC stock. Apparently, SOC storage below topsoil cannot be easily inferred from aboveground biomass data, which has consequences when trying to upscale SOC stocks from biome satellite monitoring (Dube et al., 2018; Gomes et al., 2019).

The loss of carbon from deep soil in reforestation areas can in part be attributed to a priming effect due to fresh C inputs from root exudates and turn over (Fontaine et al., 2007). Indeed, Gaudel et al. (2022) found in a global meta-analysis that deep soil C is more susceptible to a priming effect than topsoil C. In order to overcome limited nutrient availability for the rapid aboveground biomass growth in the reforestation areas, plants may have invested in root growth, which caused a decomposition of organic matter and consequently nutrient release that, in turn, supported aboveground growth (Hartley et al., 2010; Terrer et al., 2021). Besides, microbial activity could be enhanced when subsoil C and microorganisms were exposed to increased aeration and decreased wetness following the introduction of new roots by the planted trees (Cheng & Kuzyakov, 2005). In principle it is also possible that site selection for reforestation by chance favoured sites with less subsoil C. However, according to interviews with local farmers and authorities this is unlikely, as there are no local preferences for any of the restoration efforts. Moreover, lower subsoil C is possibly also still a temporal observation, as on the longer-term (several decades and beyond) increased C inputs from root debris should overcompensate these effects.

The priming effect caused by an external source of C can be reduced with nitrogen (N) addition (Hartley et al., 2010; Wang et al., 2014). The introduction of leguminous trees such as *Mimosa tenuiflora*, *Mimosa caesalpiniifolia* and *Gliricidia sepium* have the potential to increase N content in the soil in restoration projects with mixed species (Chaer et al., 2011; Macedo et al., 2008), and *Acacia mangium* in eucalyptus plantation (Voigtlaender et al., 2012). In our study area, leguminous trees such as *Anadenanthera sp*, *Piptadenia gonoacantha* and *Enterolobium contortisiliquum* were planted on the reforestation areas in small numbers. Therefore, the implementation of reforestation projects with higher share of leguminous trees can contribute to a reduction in the priming effect of the deep SOC.

Gross et al. (2022) pointed to the importance of necromass to the carbon sequestration when trees are inserted in croplands. In our study, even though necromass represents a small portion of ecosystem carbon, it is worth noting its relative contribution to the overall carbon sequestration, reaching up to 10% of carbon accrual after land use change. In addition, carbon from litter and deadwood will be incorporated mainly in the topsoil, contributing to an increase in the SOC stock in the long run (Fang et al., 2021; Gross & Harrison, 2019).

In addition to C sequestration, future work should also account for C emissions or at best for a full life-cycle assessment of each measure, in order to have a more precise mitigation potential of different restoration approaches (Amelung et al., 2020). In the reforestation approach, there are C emissions associated with the planting activities that do not occur during a natural regeneration process, like seedling production and transport to the area, and emissions with mechanized activities such as soil preparation and weeding (Lefebvre et al., 2021). Such emissions are not present in the natural regeneration approach, which might favor its adoption over the reforestation.

3.4.4. *Implications for land restoration in the Atlantic Forest*

Reforestation is by far the most used restoration approach in the Atlantic Forest, followed by natural regeneration (Brancalion et al., 2016; Guerra et al., 2020). The preference for this restoration approach might be due to the higher growth performance when aboveground biomass is used as an indicator (Brancalion et al., 2021; Ferez et al., 2015). However, as first demonstrated by our study in the Atlantic Forest, there was a loss of deep soil carbon that partially offsets the higher aboveground biomass accumulation achieved by this approach. This implies that passive restoration with natural regeneration of the Atlantic Forest yields similar amounts of total ecosystem carbon, even with smaller aboveground growth. Therefore, land restoration via natural regeneration can be scaled up in the Atlantic Forest without compromising ecosystem carbon sequestration.

The choice of a particular restoration approach can also have other positive or negative trade-offs, e.g., for biodiversity. Crouzeilles et al, (2017), for instance, showed that restoration success for biodiversity was better with passive restoration than with active one, likely due to lower land disturbance. Such considerations are clearly in favour of natural regeneration compared with agroforestry systems or reforestation in the long run. Yet, agroforestry systems might have a greater appeal for small and family farmers, since they can provide financial benefits (Lamb et al., 2005). In the agroforestry systems commonly established in the study region, farmers can harvest commercial crops between the planting lines in initial stages, and fruits in intermediate and more advanced stages of the system's development. In its final stages, the area will resemble the structure of a secondary forest, which can then be accounted as preservation areas according to the Brazilian legislation (Miccolis et al., 2019; Tubenchlak et al., 2021; Vieira et al., 2009). Hence, successional agroforestry systems can be a viable option of land restoration for small farmers, since the income generated during the restoration process can compensate the restoration costs after a short period after implementation (Martinelli et al., 2019; Padovan et al., 2022).

3.5. Conclusion

We included the deep soil carbon pool into the assessment of different land restoration approaches on total ecosystem carbon stocks. We found that land restoration with reforestation using mixed native tree species promoted higher aboveground biomass growth, which, however, was not accompanied by a gain in soil organic carbon stock in deep soil layers. Hence, losses of deep subsoil C may offset C gains in aboveground biomass. However, natural regeneration and agroforestry systems promoted a carbon accrual in deeper soil horizons, yet with lower aboveground carbon stock. In the end, all restoration approaches contributed to C sequestration and related climate change mitigation by promoting total ecosystem carbon stocks in similar manner. Not considering deep subsoil C combined with aboveground C, however, would have led to a false ranking of the success of the land restoration practices.

4. CHAPTER 4: IMPACT OF LAND RESTORATION ON THE TEMPERATURE SENSITIVITY OF DEEP SOIL RESPIRATION

4.1. Introduction

Land restoration holds promise for enhancing SOC stocks in deeper soil layers (Kaonga & Bayliss-Smith, 2009; Lan et al., 2021; Tian et al., 2023), offering a potential avenue for climate change mitigation (Bossio et al., 2020; S. L. Lewis et al., 2019). However, the long-term efficacy of soil carbon sequestration relies on the stability of organic matter under a future warmer climate (Pries et al., 2023; Schmidt et al., 2011). At present, conclusions regarding deep SOC temperature sensitivity, i.e., its decomposability under increasing temperature, remained controversial (Fierer et al., 2003; Pries et al., 2017; Li et al., 2020; McGrath et al., 2022). In particular for restored lands, the temperature sensitivity of subsoil and deep SOC respiration remains largely unexplored, thus complicating assessments of its potential for climate change mitigation. Investigating the stability of deep soil carbon under warming conditions in restored lands may thus provide better insights into its capacity for climate change mitigation and contributions to the global carbon cycle.

It is generally assumed that complex molecular structures require higher activation energies to be decomposed, which leads to a higher temperature sensitivity of soil respiration than for rather simple organic molecules (Davidson & Janssens, 2006). Since subsoil and deep soil organic matter is usually more altered than topsoil C, (Han et al., 2016; Marin-Spiotta et al., 2009; Zhang et al., 2015), they should present higher temperature sensitivity to decomposition, as has been observed in some studies (Fierer et al., 2003; Li et al., 2020; Mu et al., 2016; Soong et al., 2021). However, other studies also reported a declining (Gillabel et al., 2010; McGrath et al., 2022; Wordell-Dietrich et al., 2017) or constant temperature sensitivity along the soil profile (Pries et al., 2017), indicating that there are additional factors influencing the response of soil organic matter to warming. To account for such confounding variables, the term apparent temperature sensitivity has been introduced (Davidson & Janssens, 2006; Pries et al., 2023).

Soil profiles exhibit considerable variations in horizon and thus organic matter properties with depth (Jobbágy & Jackson, 2001; Kögel-Knabner & Amelung, 2021), suggesting that variations in temperature sensitivity will be site-specific. Beside recalcitrant organic moieties affecting SOM turnover (Xu et al., 2012), organic matter stability is enhanced by mineral protection (Gillabel et al., 2010; Ladd et al., 1993; Li et al., 2020) or the lacking supply of other available C sources (Fontaine et al., 2007), of nutrients such as nitrogen (Bernal et al., 2016; Z. Liang et al., 2019), or phosphorus (Cleveland et al., 2002; Feng & Zhu, 2019; Wang et al., 2022) or both (Meyer et al., 2018; Peixoto et al., 2021). Hence, a combined analysis of organic matter quality and soil properties is needed to understand the factors that influence the temperature sensitivity of soil organic matter degradation.

Restoring degraded soil may be key in sequestering C and thus contributing to climate change mitigation (Amelung et al., 2020). Land restoration can affect the properties along the profile including deep soil (Kong et al., 2022; Lan et al., 2021; Tuo et al., 2018); hence, it is likely that also the temperature sensitivity of deep soil respiration is affected by land restoration practices. To address these knowledge gaps, we evaluated soil respiration and its temperature sensitivity along the soil profile of different land restoration approaches and related it to soil properties. We selected the Atlantic Rainforest (Brazil) for sampling of topsoil (0-20 cm), subsoil (40-100 cm) and deep soil (150-200 cm), as here we could find a gradient of soil restoration from arable soil to patches of secondary forest on same soil, via different restoration approaches (Quartucci et al., 2023). Samples were then incubated in the lab at increasing temperatures. We hypothesized that i) the respiration responses to warming decreases with soil depth, but that ii) the degree of this effect declines with restoration progress. To account for confounding variables, we additionally determined soil texture, pH and the contents of total C and N and available P.

4.2. Material and methods

4.2.1. Study area

The study was performed on samples from a rural area in Iperó, state of São Paulo, Brazil. The climate is humid subtropical with a total annual rainfall of 1,219 mm. The area belongs to the Atlantic Forest Biome (Alvares et al., 2013; ICMBio, 2017). Mean annual temperature is 19.6 °C, being February the hottest month with 22.7 °C, and July the coldest month with 15.8 °C (Alvares et al., 2013). During the last ten years the instant temperatures ranged from a minimum of 0.6°C to a maximum of 39.1°C (INMET, n.d.). The region is dominated by highly weathered soils, mainly Ferralsols, followed by Acrisols (ICMBio, 2017). The land history of the area goes back to the 19th century when large scale deforestation took place for coffee cultivation, and then coffee was gradually substituted by sugarcane and pasture in the 20th century (Carlucci et al., 2021; ICMBio, 2017; Oliveira & Carvalho, 2017). Currently, the area is characterized by small farms (8-20 ha) that have been established since 1996 when a rural settlement was established (ICMBio, 2017; INCRA, n.d.). In addition to cropland, farmers maintain small patches (0.5-10 ha) of secondary forest that were present when they started managing the land (ICMBio, 2017; Oliveira & Carvalho, 2017). Moreover, small portions (0.5 to 5 ha) of cropland area have been converted to native vegetation using different restoration approaches in order to comply with the legislation (Rother et al., 2018; Tubenchlak et al., 2021). This resulted in a mosaic of cropland, converted areas at different levels of succession and old secondary forest.

4.2.2. Study design

The study consisted of six paired-site land uses, each sampled in triplicate, totalling 18 areas: 1) agriculture (AG) - areas established since 1996 that undergo regular soil preparation in the topsoil layer (0–20 cm) using plows and harrows. They receive intermittent liming and mineral fertilization at low rates. Fallow periods of 1-3 years follow 3-5 years of cropping, with common crops including maize, common bean, cassava, okra, eggplant, and pepper; 2) agroforestry system in intermediate stage (AFSi, 5.7 ± 0.6 years old) - established on former agricultural land, involves planting a mix of native and exotic trees. Soil preparation includes plowing, harrowing, and manually preparing pits for tree seedlings. Initially, crop production between tree lines is prioritized, transitioning to fruit production after canopy closure (0-4 years). Common fruit-bearing species include banana, mango, guava, avocado, citrus, lychee, and black mulberry; 3) agroforestry system in advanced stage (AFSa, 19.0 ± 1.7 years old) - in advanced succession, the agroforestry system follows earlier management practices, shifting from crops to fruits. Management now includes pruning larger trees until they reach about 5 m in height. Although over 15 years old, some fruit trees continue to produce under the canopy, albeit fewer in number, including guava, mango, and avocado; 4) reforestation with mixed native species (RE, 16.0 ± 2.0 years old) - active restoration that involves planting mixed native tree species on former arable land. Soil preparation includes plowing, harrowing, and manual pit preparation. Planting occurs with spacing of 3.0 m between rows and 2.0 m between seedlings, with 20-50 species randomly distributed. Initial weeding encourages canopy growth, followed by fencing to promote natural regeneration; 5) natural regeneration (NR, 17.0 ± 2.6 years old) - passive restoration that involves vegetation recovery without human intervention after fencing off arable land. Initially, grasses and shrubs invade the area, followed by small trees shading the grasses within 0-2 years. Over time, larger trees establish, leading to the formation of a multi-strata secondary forest, and; 6) old secondary forest (SF) - forested areas since settlement establishment (1996) have undergone no specific management, such as timber or non-timber forest product harvesting. These preservation areas are set aside by farmers to meet legal requirements. For more details on site properties see Quartucci et al. (2023).

4.2.3. Sampling and respiration measurements

In each area, we collected three soil samples from three different depths using a mechanized hand soil corer: 0-20, 40-100 and 150-200 cm (hereafter topsoil, subsoil, and deep soil, respectively), totalling 162 samples. The samples were first air dried and then 2.0 mm sieved and homogenized. After homogenization, we took a sub-sample of 20 g of soil (dry weight equivalent) of each field replicate, placed it in a vessel and added deionized water to 40% of their maximum water holding capacity, mixing with a sterilized spatula to allow a homogenous moisture content. Rewetting and sieving can result in an elevated CO_2 release from the soil during the first days (Fierer & Schimel, 2003). Thus, the samples were pre-incubated at 22 °C for 120 hours to stabilize the respiration rate, allowing to minimize possible effects of soil mixing and water addition (Blagodatsky et al., 2000). Moreover, we expect that subsoil and deep soil exposure to ambient O_2 concentrations had negligible impact on our results, since O_2 concentration over 5% has little influence on soil respiration (Salomé et al., 2010), and O_2 concentration rarely drops below 5% even in deeper layers (Teh et al., 2005).

After pre-incubation, the vessels were placed in an automated respirometer (Respicond VIII, Nordgren Innovations AB, Sweden), which measured continuously CO_2 released by the soil by trapping in a KOH solution (Nordgren, 1988). The CO_2 entrapment causes a decrease in the KOH conductivity, which was measured every half hour, and were converted to CO_2 based on equation 1:

$$Ct = A\ x\ \frac{R0 - Rt}{R0}$$

Where, *Ct* is the absorbed CO_2 at time t, *A* is a proportionality constant relating the decrease in conductivity to absorbed CO_2, *R0* is the conductance of KOH measured at *t=0*, and *Rt* is the conductance at time *t*.

Soil samples were incubated in a range of 10 to 30 °C, in 5 °C intervals, for 24 hours each temperature. The first 12 hours of each interval was considered as equilibrium time for microorganisms to adapt to the new temperature ranges and discarded from the analysis (Koch et al., 2007; Meyer, et al., 2018). We included three vessels without soil as control, and subtracted the values of soil respiration of each vessel by the average of the three controls. The sequential temperature incubation approach may lead to preferential depletion of labile carbon (Schädel et al., 2020), potentially influencing Q10 estimates. However, pre-incubation period for 5 days prior to the main incubation experiment promotes initial mineralization of the most labile carbon pool (Blagodatsky et al., 2000; Fierer et al., 2003), minimizing this effect and ensuring a more accurate Q10 estimate.

We then calculated the average respiration (CO_2 production) of the 24 Respicond measurements (two measurements per hour over 12 hours) for each sample, totalling 972 respiration data points (162 samples x 5 temperatures + 162 with glucose). Out of this total, 6% (57 data points) presented high variation during measurements and were removed from the dataset. However, the exclusion did not prevent us from running statistical analysis, since we further calculated the average respiration in each depth, and each temperature, based on respiration of the three samples (or two samples when one was excluded). Since soil respiration has a high linear correlation with SOC concentration (Figure 9-1 - Appendix C; see also McGrath et al., 2022), we opted to report soil respiration rates as µg CO_2 g^{-1} SOC h^{-1} to mitigate potential confounding effects caused by variations in SOC content among depths (Wordell-Dietrich et al., 2017). This approach enables a more direct comparison of the respiratory activity relative to the organic carbon content in each depth, thereby enhancing the interpretability of our results (Fierer et al., 2003; Wordell-Dietrich et al., 2017). To do so, we divided the respiration rates (in µg CO_2 g^{-1} soil h^{-1}) by the SOC concentration (g kg^{-1}), resulting in µg CO_2 g^{-1} SOC h^{-1}.

At the end of the fifth day (30 °C), we added 6 mg of glucose per g of dry soil in each vessel, mixed with a vortex shaker to guarantee a homogeneous distribution, and incubated at 30 °C for 24 hours. We used the instantaneous respiration after glucose addition (lag phase) as a measure of the soil microbial biomass (substrate induced respiration SIR) (Blagodatskaya & Kuzyakov, 2013; Reischke et al., 2014). Contrary to specific respiration rate, we reported substrate induced respiration results in µg CO_2 g^{-1} soil h^{-1} to capture the entirety of microbial activity, since carbon is no longer a limiting factor. We also calculated the basal-to-substrate induced respiration ratio (Qr = Rs / SIR) as a measure of the physiological state of soil microorganisms (Blagodatskaya & Kuzyakov, 2013). We used the soil respiration at 30 °C as the basal respiration since glucose was added at this temperature.

4.2.4. Soil temperature sensitivity (Q10) determination

We then calculated Q10, which represents the factor by which the rate of a biological process, such as soil respiration, changes with a 10°C increase in temperature. For this, we used the average specific respiration rate, and calculated following equation 2:

$$Q10 = \exp^{(10*b)}$$

Parameter b in the equation above was found by fitting an exponential equation with our respiration data in the temperature range, following equation 3:

$$Rs_t = a*\exp^{(b*T)}$$

Where Rs_t is soil respiration at a given temperature, *a* and *b* are the fitted parameters, and *t* is temperature (°C).

4.2.5. Soil chemical and physical analysis

We also determined chemical and physical properties in the 162 soil samples following Raij et al. (2001) methodology: pH ($CaCl_2$), Phosphorus (P-resin), and cation exchange capacity (CEC – result of sum of bases extracted with resin plus potential acidity extracted with SMP buffer) in all samples, and texture (sand, silt and clay – hydrometer method) in one composite sample per area. For C and N contents and stocks, we retrieved the data from Quartucci et al. (2023). Likewise, carbon stocks in the aboveground biomass, litter and roots were also retrieved from Quartucci et al. (2023). The results of soil properties are presented in Table 4-1.

Table 4-1. Average soil properties (standard error in parenthesis) in the topsoil (0-20 cm), subsoil (40-100 cm) and deep soil (150-200 cm). C: organic carbon content (g kg^{-1}); N: organic nitrogen content (g kg^{-1}); P: phosphorus content (mg dm^{-3}); pH: pH ($CaCl_2$); Clay: clay content (g kg^{-1}). AG: agriculture; AFSi: agroforestry system in intermediate stage; AFSa: agroforestry system in advanced stage; RE: reforestation; NR: natural regeneration; SF: secondary forest.

Depth (cm)				C (g kg^{-1})			
	AG	AFSi	AFSa	RE	NR	SF	Mean
0-20	10.4 (1.8)	12.1 (1.8)	25.7 (2.3)	17.3 (2)	18.5 (1.2)	30.7 (11.8)	19.1 (2.5)
40-100	5.3 (1.2)	5.5 (0.8)	8.5 (1.3)	4.2 (1.2)	7.3 (1.4)	7.6 (3.9)	6.4 (0.8)
150-200	1.8 (0.8)	2.6 (0.9)	3.3 (1.3)	1.3 (0.5)	2.5 (0.7)	4.9 (3.6)	2.7 (0.6)
				N (g kg^{-1})			
	AG	AFSi	AFSa	RE	NR	SF	Mean
0-20	0.92 (0.19)	1.07 (0.13)	2.17 (0.28)	0.14 (0.19)	1.52 (0.16)	2.66 (0.74)	1.63 (0.19)
40-100	0.47 (0.1)	0.48 (0.07)	0.75 (0.05)	0.37 (0.1)	0.66 (0.07)	0.71 (0.12)	0.57 (0.05)
150-200	0.22 (0.06)	0.30 (0.08)	0.41 (0.06)	0.18 (0.04)	0.36 (0.03)	0.51 (0.11)	0.33 (0.04)
				P (mg dm^{-3})			
	AG	AFSi	AFSa	RE	NR	SF	Mean
0-20	11.3 (5.7)	6.1 (1.1)	8.7 (2.3)	8.5 (2.4)	6.8 (1.2)	9.0 (1.9)	8.4 (1.1)
40-100	2.0 (0.4)	2.1 (0.3)	2.0 (0.3)	2.2 (0.3)	3.5 (0.9)	3.0 (0.5)	2.5 (0.2)
150-200	2.6 (0.5)	2.1 (0.4)	2.1 (0.4)	2.7 (0.8)	5.5 (2.5)	3.3 (0.3)	3.0 (0.5)
				pH ($CaCl_2$)			
	AG	AFSi	AFSa	RE	NR	SF	Mean
0-20	5.1 (0.2)	5.0 (0.3)	5.2 (0.1)	4.7 (0.3)	4.7 (0.2)	5.4 (0.4)	5.0 (0.1)
40-100	4.4 (0.2)	4.7 (0.2)	4.6 (0.2)	4.3 (0.2)	4.4 (0.2)	4.1 (0.1)	4.4 (0.1)
150-200	4.3 (0.2)	4.3 (0.1)	4.2 (0.2)	4.1 (0.1)	4.5 (0.2)	4.1 (0.1)	4.2 (0.1)
				Clay (g kg^{-1})			
	AG	AFSi	AFSa	RE	NR	SF	Mean
0-20	254 (78)	254 (65)	333 (82)	230 (66)	318 (32)	359 (89)	291 (27)
40-100	349 (88)	397 (86)	504 (73)	323 (76)	446 (46)	426 (103)	407 (31)
150-200	271 (107)	410 (79)	419 (98)	297 (70)	409 (49)	365 (138)	362 (35)

4.2.6. Statistical analysis

We first performed a multiple linear regression, with soil respiration as the dependent variable, and depth, temperature and land use as independent variables. We analysed the main effects of each dependent variable and the interaction among them. Since the data presented heteroscedasticity after performing the Breusch & Pagan (1979) test, we used robust estimates of standard errors (Willians, 2000). We then performed LSD test ($p<0.05$) to check statistical differences in the factors and interactions. We repeated the same procedure a second time with Q10 as the dependent variable, and depth and land use as independent variables. Then, we performed a simple linear regression with the respiration and Q10 as the dependent variable and different independent variables that could explain the restoration progress: living aboveground carbon stock, SOC stock, litter organic carbon stock and age. For the age, we regarded cropland as zero and old secondary forest as thirty, since it was not possible to determine the last disturbance of the forest cover (see section 2.2 for the ages of the restored lands). To identify the factors that additionally influenced respiration rates and Q10, we performed stepwise multiple linear regression analyses with Q10 as dependent variable, and soil CN ratio as a proxy for carbon quality (Saviozzi et al., 2001; St. Luce et al., 2014); soil nitrogen content (N, in %) and soil phosphorus content (P, in mg dm^{-3}) as proxies for soil fertility (Dodd & Sharpley, 2015; Kopittke et al., 2017; Vitousek et al., 2010); soil pH (pH $CaCl_2$), soil cation exchange capacity (CEC, in mmolc dm^{-3}), soil silt content (g kg^{-1}) and soil clay content (g kg^{-1}) as proxies for the amount and strength of organo-mineral associations (Jones & Singh, 2014; Lavallee et al., 2020); fine roots carbon stock (FR, in Mg ha^{-1}), fine roots CN ratio (FR_{CN}) and soil sand content (g kg^{-1}) as proxies for the amount and quality of particulate organic carbon (Lehmann & Kleber, 2015; Rumpel & Kögel-knabner, 2011; Witzgall et al., 2021). Variance inflation factor (VIF) was used to detect collinearity among variables. We found that sand and silt presented strong collinearity ($VIF>10$), and excluded these variables in further analysis. Finally, the effect size (eta-squared estimates) of each selected variable was calculated for each depth (Smithson, 2001). All analyses were performed using Stata 16.1 (StataCorp LLC, College Station, USA).

4.3. Results

4.3.1. Soil respiration at different soil depth, land use and temperature

There was a significant influence of depth on the soil specific respiration rate ($p<0.0001$). Deep soil presented the fastest respiration rate, with 7.2 (± 0.5) µg CO_2 g^{-1} SOC h^{-1}, and that of topsoil (4.1 ± 0.1 µg CO_2 g^{-1} SOC h^{-1}) exceeded that of subsoil (3.2 ± 0.2 µg CO_2 g^{-1} SOC h^{-1}), respectively (Table 4-2). Each depth was influenced differently by the temperature ($p<0.0001$). The topsoil respiration was the most influenced by a temperature increase, while this effect was lacking in subsoil and deep soil, the latter being more variable (Table 4-2).

Table 4-2. Specific respiration rate (µg CO_2 g^{-1} SOC h^{-1}) with increasing temperatures of the topsoil (0-20 cm), subsoil (40-100 cm) and deep soil (150-200 cm). Numbers in parentheses are standard error. Different lower-case letters indicate statistical difference among the temperatures within the same depth, and different upper-case letters indicate statistical difference in the mean respiration rate among the depths by LSD post hoc test ($p<0.05$).

	Temperature °C					
Depth (cm)	10	15	20	25	30	Mean
0-20	1.9 (0.1) a	2.8 (0.2) b	3.6 (0.2) c	5.3 (0.2) d	6.8 (0.3) e	4.1 (0.1) B
40-100	2.7 (0.4) a	3.7 (0.4) a	3.2 (0.5) a	3.2 (0.4) a	3.2 (0.4) a	3.2 (0.2) A
150-200	7.4 (1.3) ab	9.0 (1.2) b	6.6 (0.8) ab	6.9 (1.3) ab	6.0 (0.7) a	7.2 (0.5) C

There was a statistically significant interaction between land use and depth on mean soil respiration rate ($p=0.0003$): it was slowest in arable topsoil and fastest in the secondary forests under natural regeneration, the other sites being intermediate (Figure 4-1). This pattern was not sustained in the subsoil, and finally more or less reverted in the deep soil: now, respiration was fastest for the arable cropped sites, and slowest in the agroforestry, natural regeneration and old secondary forest sites. Hence, land restoration enhanced deep soil C stability (Figure 4-1).

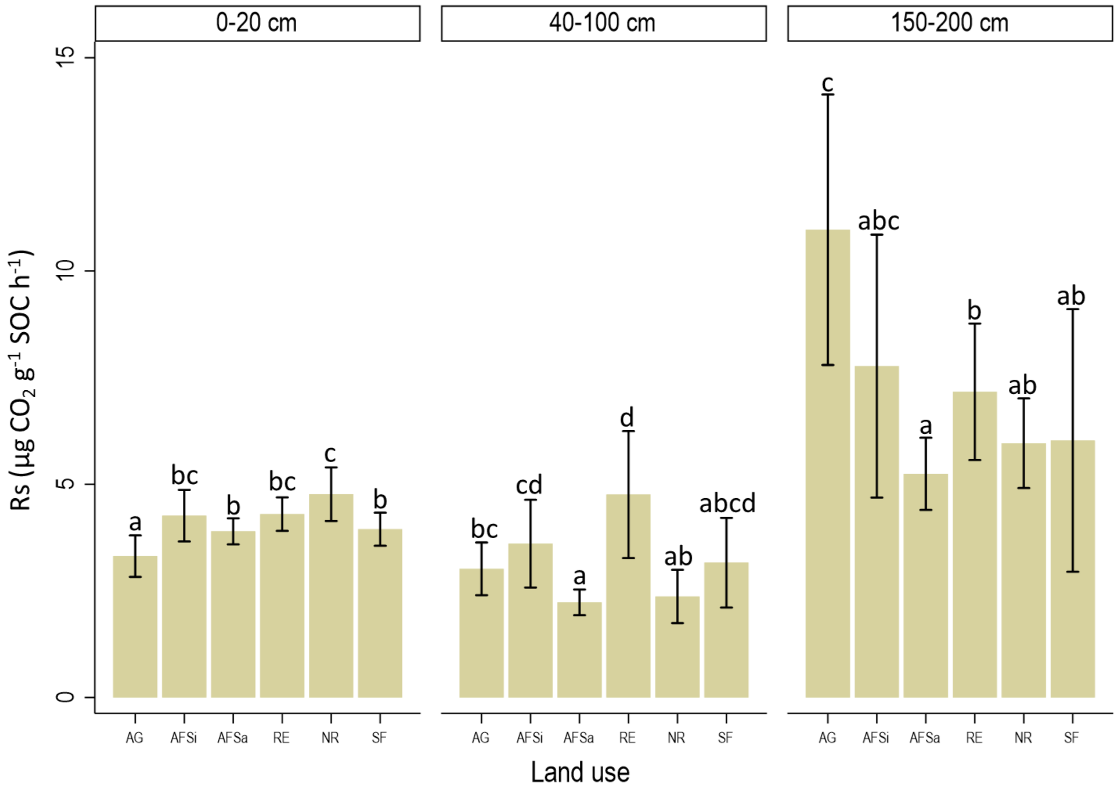

Figure 4-1. Average specific respiration rate (Rs, in µg CO_2 g^{-1} SOC h^{-1}) of the five temperatures in each land use, in the topsoil (0-20 cm), subsoil (40-100 cm) and deep soil (150-200 cm). Error bars are 95% confidence interval. Different letters indicate statistical difference among the land uses within the same depth by LSD post hoc test ($p<0.05$). AG: agriculture; AFSi: agroforestry system in intermediate stage; AFSa: agroforestry system in advanced stage; RE: reforestation; NR: natural regeneration; SF: secondary forest.

Respiration rate in the subsoil and deep soil decreased with increasing SOC stock, indicating that as the regeneration process advanced, carbon stability increased. This trend was not observed in the topsoil (Figure 4-2). The combined analysis of all layers also showed a decreasing trend of respiration rate with increasing soil carbon stocks (Figure 9-2 – Appendix C). Aboveground litter carbon stock was also negatively correlated with soil respiration rate in subsoil and deep soil, although with lower Pearson correlation coefficient than SOC stock. Yet, the aboveground carbon stock and restoration age had low correlation with the respiration rate for the topsoil, but both were statistically significantly correlated in the deep soil (Table 9-1 – Appendix C). Accordingly, all the studied parameters were statistically significant in the deep soil layer, two in subsoil, whereas none were significant in the topsoil.

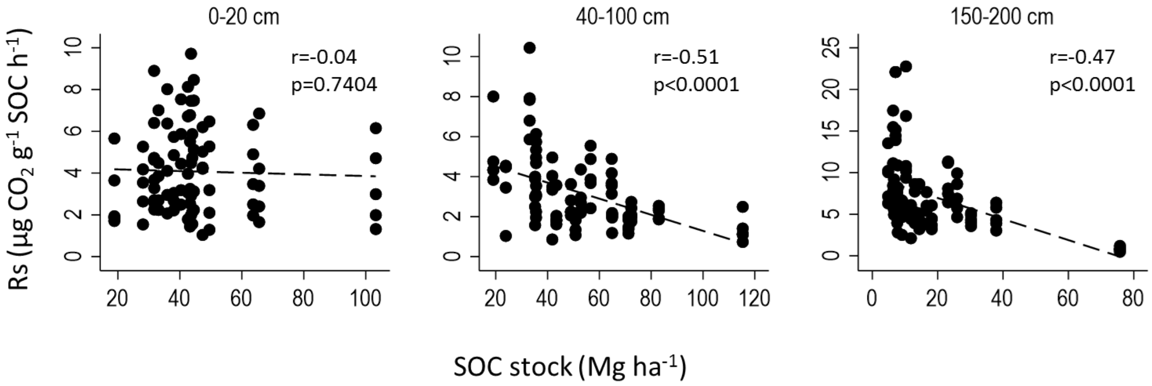

Figure 4-2. Relationship between soil specific respiration rate (Rs, µg CO_2 g^{-1} SOC h^{-1}) and soil organic carbon stock (SOC, Mg ha^{-1}) in the topsoil (0-20 cm), subsoil (40-100 cm) and deep soil (150-200 cm). r and p are the Pearson correlation coefficient and p value, respectively.

4.3.2. Substrate induced respiration and ratio basal-to-substrate induced respiration

Substrate induced respiration (SIR) was also influenced by depth ($p<0.0001$). Topsoil had a four-fold higher respiration rate than subsoil and 12-fold higher than deep soil after glucose addition. Subsoil and deep soil also presented a statistically significant difference between them, with 6.6 (± 0.9) and 2.1 (± 0.3) µg CO_2 g^{-1} soil h^{-1}, respectively (Figure 4-3).

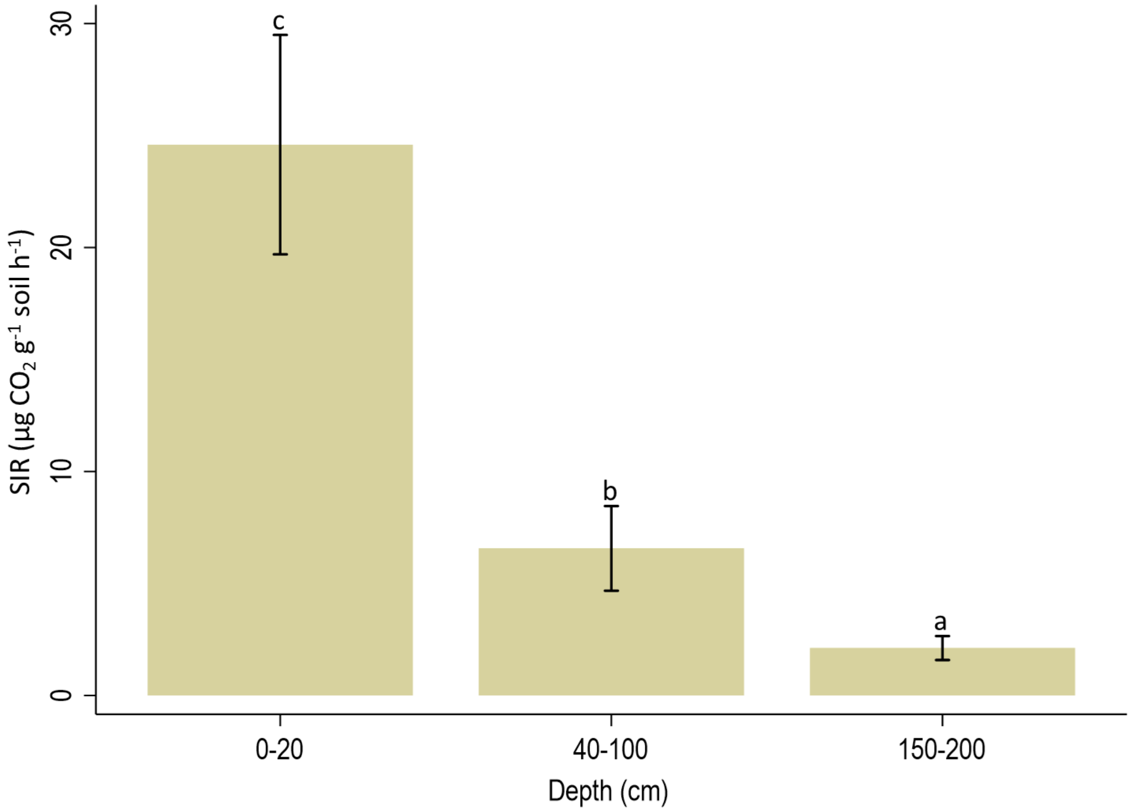

Figure 4-3. Soil respiration rate (µg CO_2 g^{-1} soil h^{-1}) after glucose addition (substrate induced respiration, SIR) in the topsoil (0-20 cm), subsoil (40-100 cm) and deep soil (150-200 cm). Error bars are 95% confidence interval. Different letters indicate a statistically significant difference among the depths by LSD post hoc test ($p<0.05$).

The ratio basal-to-substrate induced respiration (Qr) was influenced by depth ($p<0.0001$) and there was a significant interaction between depth and land use ($p=0.0001$). The ratio in the subsoil was statistically smaller than in the topsoil and deep soil (Figure 9-3 – Appendix C). In the topsoil, agriculture presented statistically significant lower ratio than all land uses, except for the agroforestry system in intermediate stage. In the subsoil, reforestation and secondary forest differed statistically. In the deep soil, reforestation and secondary forest presented the lowest values (Figure 4-4). Hence, consideration of depth again almost reverted the impact of land-use on Qr, as it did for respiration (Figure 4-1).

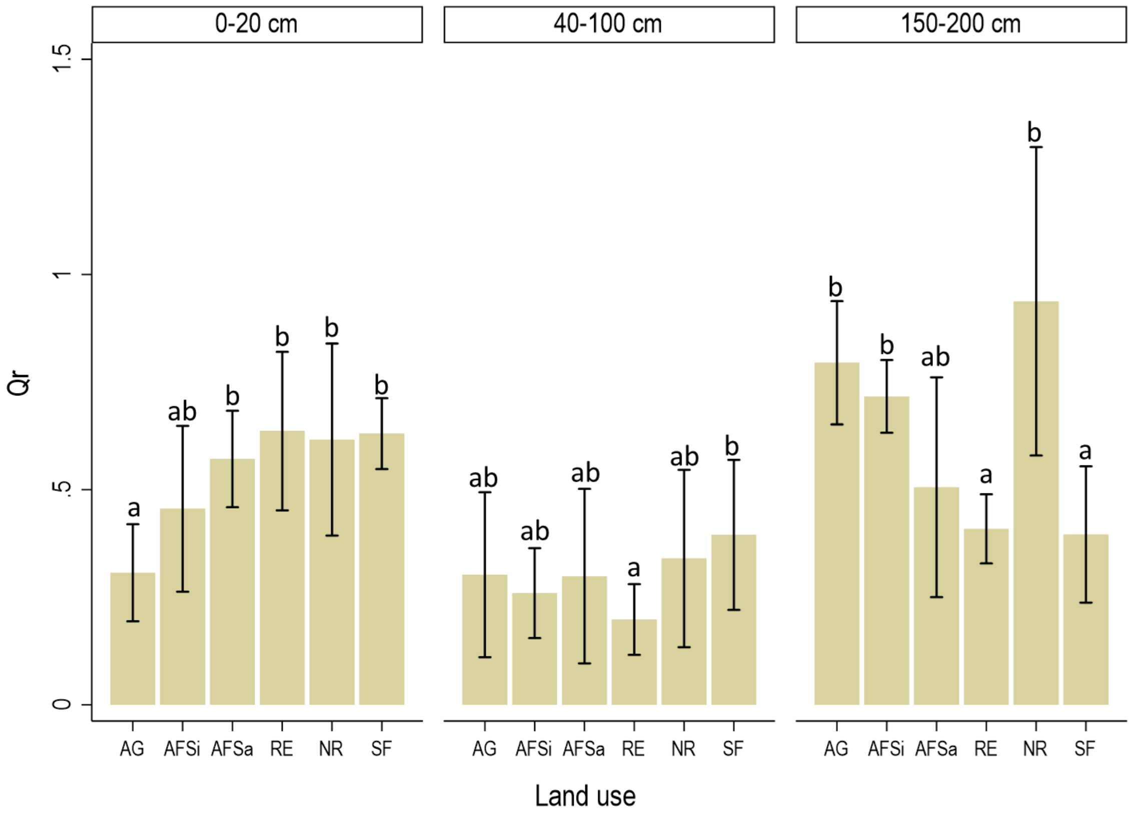

Figure 4-4. Basal-to-substrate induced respiration ratio (Qr) in each land use, in the topsoil (0-20 cm), subsoil (40-100 cm) and deep soil (150-200 cm). Error bars are 95% confidence interval. Different letters indicate statistical difference among the land uses within the same depth by LSD post hoc test (p<0.05). AG: agriculture; AFSi: agroforestry system in intermediate stage; AFSa: agroforestry system in advanced stage; RE: reforestation; NR: natural regeneration; SF: secondary forest.

4.3.3. Temperature sensitivity Q10

There was an influence of depth on the temperature sensitivity of soil respiration (Q10) ($p<0.0001$). Opposite to the specific respiration rate (Table 4-2), the temperature sensitivity of deep soil was the lowest, although without difference to the subsoil, whereas topsoil presented the highest value (Figure 4-5).

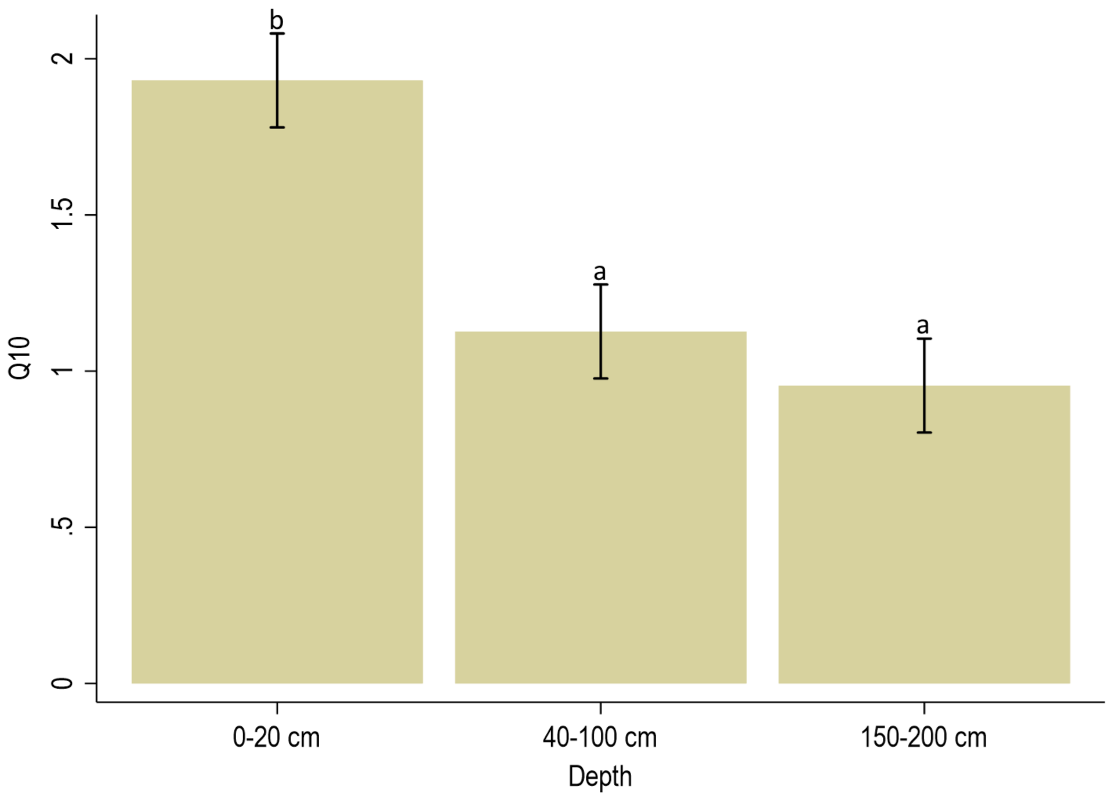

Figure 4-5. Temperature sensitivity (Q10) of the topsoil (0-20 cm), subsoil (40-100 cm) and deep soil (150-200 cm). Error bars are 95% confidence intervals. Different letters indicate a statistically significant difference by LSD post hoc test ($p<0.05$).

The method of restoration did not influence Q10 ($p=0.2118$). Nevertheless, there was a positive and statistically significant correlation between Q10 and restoration age in the topsoil and deep soil (Figure 4-6). Soil organic carbon stock, aboveground carbon stock and litter carbon stock did not present statistically significant correlation with Q10 in any depth (Table 9-2 – Appendix C).

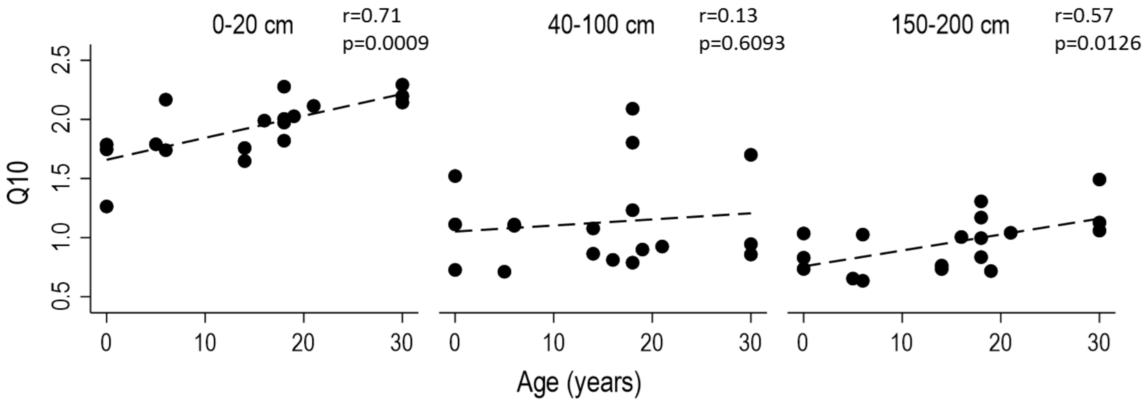

Figure 4-6. Relationship between temperature sensitivity (Q10) and restoration progress (age, in years) in the topsoil (0-20 cm), subsoil (40-100 cm) and deep soil (150-200 cm). Age zero are areas under agriculture and age 30 are under secondary forests. r and p are the Pearson correlation coefficient and p value, respectively.

The results of the stepwise regression showed that different factors influence Q10 in each depth. In the topsoil, fine roots carbon stock and soil pH had a positive influence on the temperature sensitivity, with fine roots being the parameter with higher influence. In the subsoil, phosphorus content presented the highest influence, followed by the CN ratio. In deep soil, nitrogen content was the most influencing factor, followed by soil clay content and cation exchange capacity, that were negatively correlated with Q10 (Table 4-3).

Table 4-3. Stepwise multiple linear regression for the prediction of Q10 values in topsoil (0-20 cm), subsoil (40-100 cm) and deep soil (150-200 cm). Variables: Fr = fine roots carbon stock; pH = pH $CaCl_2$; P = soil phosphorus content; CN = soil CN ratio; N = soil nitrogen content; Clay = soil clay content; CEC = soil cation exchange capacity. Numbers in parenthesis in the coefficient column are standard errors.

Depth (cm)	Variables	Coefficient	t	P>t	Eta-squared
0-20	Fr	0.189 (0.05)	3.59	0.003	0.46
	pH	0.318 (0.12)	2.74	0.015	0.33
40-100	P	0.290 (0.08)	3.83	0.002	0.49
	CN	0.455 (0.02)	2.00	0.064	0.21
150-200	N	18.1 (7.1)	2.53	0.024	0.31
	Clay	-0.00173 (0.0007)	-2.39	0.031	0.29
	CEC	-0.0025 (0.001)	-1.91	0.076	0.21

4.4. Discussion

4.4.1. Organic carbon stability in the profile

Soil respiration measured through incubation reflects both the resistance and accessibility of organic carbon to microbial decomposition (Schädel et al., 2020). Thus, the higher the respiration, the lower the stability. Specific respiration rate presented statistically different averages among the three studied layers, indicating different carbon stability at present temperatures along the soil profile. Deep soil (150-200 cm) showed the highest values, although there was not a continuous increasing trend with soil depth, because specific respiration rate in the topsoil (0-40 cm) was higher than in the subsoil (40-100 cm) (Table 4-2). Wordell-Dietrich et al. (2017) also found that carbon was more stable in the topsoil that at 130 cm depth in a beech forest in Germany due to larger proportions of labile carbon coupled with lower carbon use efficiency in deep soil. In my study, although deep soil contained lower microbial biomass, as indicated by lower substrate-induced respiration (SIR) (Figure 4-3), it presented higher respiration, also pointing to a lower carbon use efficiency (CUE) of microorganisms in these depths (Agnelli et al., 2004; Spohn et al., 2016; Wordell-Dietrich et al., 2017). As the degree of soil organic matter composition also increases with depth, i.e., labile C sources are increasingly missing, microorganisms cannot easily incorporate the carbon into their biomass (Marín-Spiotta & Hobley, 2022; Rumpel & Kögel-Knabner, 2011). Consequently, a higher proportion of carbon is respired rather than being used for growth (Soares & Rousk, 2019).

Microorganisms CUE is also related to their physiological state (Blagodatskaya & Kuzyakov, 2013). Our results showed that the basal-to-substrate induced respiration ratio (Qr) did not differ between topsoil and deep soil (Figure 9-3 – Appendix C), although the results might represent different physiological states. On the one hand, topsoils exhibit larger proportions of microbial biomass in an active state, but due to high carbon availability, there is limited additional activity with the addition of C (glucose), leading to a high basal-to-substrate induced respiration ratio. On the other hand, the high Qr in deep soil might be the result of stress conditions caused by long-term starvation due to low availability of carbon, nitrogen and phosphorus (Blagodatskaya & Kuzyakov, 2013; Meyer et al., 2018), and, in our soils, also more acid conditions (Table 4-1). This environment induces a physiological state where most of the microbial biomass is dormant and unresponsive to additional carbon inputs (Blagodatskaya & Kuzyakov, 2013; Reischke et al., 2014).

Land restoration influenced carbon stability differently below topsoil layers (Figure 4-1). Carbon was more stable under agroforestry systems, followed by natural regeneration, whereas reforestation promoted the most vulnerable carbon. A previous study in the study area has shown that the reforestation sites had lower deep soil (40-300 cm) organic carbon stocks than paired agroforestry systems in advanced stages and natural regeneration and also lower than agricultural sites (Quartucci et al., 2023). Thus, natural regeneration and especially agroforestry systems do not only promote deep soil carbon sequestration but also lead to higher stability of the deep soil C in the Atlantic Forest, offering a higher climate change mitigation potential than reforestation practices.

The low respiration rate in deep soil (Figure 4-1) coupled with high Qr (Figure 4-4) values suggests that the microorganisms in natural regeneration areas are utilizing available substrates more efficiently, indicating a more mature and stable microbial community that is well-adapted to the prevailing environmental conditions (Hu et al., 2020). Tree species composition in naturally regenerated areas closely resembles the original forest composition, because regeneration occurs from seeds in the seed bank and nearby forests (Bechara et al., 2016; Crouzeilles et al., 2017; Miao et al., 2016). In contrast, reforestation involves planting species that may not have historically occurred in the region (Rodrigues et al., 2009). As a result, soil microorganisms in naturally regenerated areas are likely more adapted to the current conditions compared to those in reforested areas (Hu et al., 2020; Kong et al., 2022; Liu et al., 2019; Singh Rawat et al., 2023).

4.4.2. Relationship between soil carbon stability and restoration progress

We found an inverse relation between soil respiration and SOC stock in subsoil and deep soil (Figure 4-2), denoting a higher C stability as land restoration and C sequestration progresses in these layers. At first glance, this finding is surprising, since frequently C accrual in soil goes along with higher accumulation of less stable C such as particulate organic matter (Amelung et al., 1998; Cotrufo et al., 2019). Indeed, higher CN ratio as typical for POM was observed with increasing SOC stock in subsoil and deep soil (Figure 9-4 – Appendix C). Only if the carbon has been used for anabolism and incorporated into microbial necromass and other complex structures, which may be later on also stabilized by minerals (Liang et al., 2017), an increased resistance of SOM against decomposition can be expected (George et al., 2010; Lange et al., 2023). Yet, microbial residues have low CN ratios, thus contrasting the rise in CN with progressing restoration in our sites. Unless no specific stable constituents of root debris such as lignins and suberins hinder decomposition, other factors likely contributed to the lower biodegradability of deep soil C than simply a different biodegradability of the substrate, such as the low pH value and the above-mentioned limitations in C and P (Bernal et al., 2016; Blagodatskaya & Kuzyakov, 2013; Meyer et al., 2018).

4.4.3. Soil temperature sensitivity along the profile

Our hypothesis that the temperature sensitivity (Q10) would decrease along the soil profile was only partly confirmed as we did not find a continuous gradient from topsoil to deep soil; rather, we found a decrease from the topsoil to the subsoil, but not from the subsoil to deep soil (Figure 4-3). Our results contradict several studies that pointed to an increase in the temperature sensitivity with depth (Fierer et al., 2003; Li et al., 2020; Mu et al., 2016; Soong et al., 2021), although there are also other studies finding a decrease (Gillabel et al., 2010; McGrath et al., 2022; Pang et al., 2015; Wordell-Dietrich et al., 2017). In a global estimation of the effects of warming on soil carbon losses, Wang et al. (2022) also found that the temperature sensitivity of deep soil was smaller than topsoil. Here we show that the absence of temperature sensitivity can reach twice the depth reported for tropical environments (100 cm; McGrath et al., 2022) and still deeper than the deepest depth reported in the literature to our knowledge for mineral soils (130-160 cm; Wordell-Dietrich et al., 2017). Overall, it seems that temperature sensitivity varies throughout the soil profile. Consequently, climate models that assume constant temperature sensitivity at different depths will fail to estimate future soil carbon emissions (García-Palacios et al., 2021), at least for the longer runs, when also subsoil C is taking part of soil C turnover with land-use change (Lan et al., 2021; Makumba et al., 2007; Quartucci et al., 2023).

4.4.4. Relationship between soil temperature sensitivity and restoration progress

We hypothesized that the temperature sensitivity would decrease with progressing restoration, being smaller in the areas with longer-lasting restoration due to higher OM protection. However, the opposite trend was observed, with significant increase in the topsoil and deep soil (Figure 4-6). If more complex molecular structures accumulated in soil with increased input of root debris (Han et al., 2016; K. Zhang et al., 2015), the higher temperature sensitivity would be reasonable (Davidson & Janssens, 2006); however, as outlined in section 4.2., the wider CN ratio suggests an accumulation of POM and root debris (see also Quartucci et al., 2023) rather than the formation of larger portions of stable, mineral associated SOM. We thus attribute our findings to two main processes. On the one hand, the soils sequester mainly C but less N and P, i.e., there are increasing nutrient limitations with restoration success (Table 4-1), which may hinder organic matter degradation and, possibly similar to effects from increasing SOM complexity, thus increase Q10 (Cleveland et al., 2002; Liang et al., 2019; Peixoto et al., 2021). Bai et al. (2023) also found in their meta-analyses that reduced N supply and larger CN ratio due to increased root biomass resulted in larger sensitivity of SOC loss to warming, corresponding to larger Q10 ratios. On the other hand, we have to reconcile that our incubations only lasted for a few days and not years, while the mean residence time of particulate organic matter is in the range of months to years but not in the range of days (Balesdent et al., 1988; Derrien & Amelung, 2011). Hence, assessing the respiratory response to warming occurs at a time scale too short to significantly affect the turnover of POM, i.e., the latter might not have been captured within the timeframe of our study and the relationship between Q10 and POM might be less straightforward than assumed (Fierer et al., 2003).

4.4.5. Factors influencing subsoil and deep soil temperature sensitivity

Soil texture generally influences temperature sensitivity in subsoil and deep soil, given the effect of physical protection of organic matter in the clay fraction (Kögel-Knabner & Amelung, 2021; McGrath et al., 2022). Although clay content influenced deep soil temperature sensitivity, soil N content had a slightly higher influence. Contrary to Bai et al. (2023), who found that N limitations promoted soil carbon transformations with warming in the uppermost soil layer, our study showed that temperature sensitivity increased with increasing N content in deep soil. Since topsoil and deep soil usually present contrasting N content (Batjes, 2014; Krull & Skjemstad, 2003; Zhao et al., 2014), the influence of soil N on the temperature sensitivity might depend on the N concentration. Hence, under very N-limited conditions such as deep soil, and with another microbial community present there than in the topsoil, an increase in N availability might also have a positive effect on respiration due to the alleviation of nutrient limitation (Cleveland & Townsend, 2006; Liang et al., 2019; Meyer et al., 2018), differing from the results of Bai et al. (2023) which covered a broader N range.

In the subsoil, i.e., in-between topsoil and deep soil studied here, the P content was the main factor influencing soil temperature sensitivity. Cleveland et al. (2002) found that topsoil respiration in a tropical forest with highly weathered soil was strongly constrained by P. In a meta-analysis of the effect of phosphorus addition on soil respiration, Feng and Zhu (2019) found that respiration increased with rising temperatures, indicating that alleviating phosphorus limitation promotes carbon losses at current temperatures, with even greater losses anticipated in a warmer climate. In addition to the limitation of nitrogen and phosphorus individually, Meyer et al. (2018) identified a co-limitation of these nutrients in layers below the topsoil, and Peixoto et al. (2021) found similar limitations at depths of 500 cm. When P limitations are overcome, also soil respiration increases, and so did here Q10.

In general, subsoil and deep soil layers in tropical and subtropical climates are marked by low nutrient contents (Jobbágy & Jackson, 2001). Soil nutrients are vital for microbial activity because they support essential physiological processes like enzyme production and metabolic functions (Knelman et al., 2014); deficiencies in nutrients like nitrogen and phosphorus limit these processes, thereby reducing microbial growth and carbon decomposition rates, particularly below the topsoil (Liang et al., 2019). Thus, the low soil fertility in tropical and subtropical climates can exert a positive effect of sub- and deep soil carbon stability under a warmer climate. Conversely, the stability of carbon in subsoil and deep soil may be compromised in a future scenario with higher nitrogen and phosphorus depositions and elevated temperatures, so that higher carbon accumulation on the aboveground biomass might be offset by deep soil carbon loss (Kwon et al., 2021; Menge et al., 2023; Terrer et al., 2021).

4.4.6. Implications for land restoration

The reasons to implement land restoration are manifold: soil fertility improvement, food production, legislation compliance, carbon sequestration and climate change mitigation (Hagger et al., 2017; Rother et al., 2018). However, where, and how land restoration is implemented has to be considered in order to guarantee maximum benefits (Brancalion et al., 2019; Strassburg et al., 2020; Zanini et al., 2021).

We found that temperature sensitivity increases with higher P (subsoil) and N (deep soil) content. Since soils in tropical environments usually have lower nutrient contents than in temperate climates (Batjes, 2014; Yang et al., 2013), soil temperature sensitivity of tropical climate tends to be lower. Indeed, Li et al. (2020) found that tropical forests had the lowest temperature sensitivity in the 0-100 cm soil layer across a wide geographic distribution spanning different forest ecosystems in China. Globally, Koven et al. (2017) concluded that warm climates have lower temperature sensitivity than cold climates in the 0-100 cm layer. Thus, land restoration in tropical and subtropical climates might provide higher climate change mitigation benefits than colder climates due to higher deep soil carbon stability in a future warmer scenario.

In the Atlantic Forest in Brazil, there is a goal to restore 15 Mha by 2050 without competing with profitable agriculture or cattle raising (Brancalion et al., 2013; Melo et al., 2013; Pinto et al., 2014). Of this total, 1.4 Mha has already been successfully restored (Crouzeilles et al., 2019). Given that the land restoration approach can significantly influence both the magnitude of deep soil carbon sequestration (Kaonga & Bayliss-Smith, 2009; Lan et al., 2021; Quartucci et al., 2023) and its stability, as demonstrated in this study (Figure 4-1), selecting the most suitable approach is crucial for maximizing climate change mitigation benefits. Currently, reforestation has been the predominant method employed in the Atlantic Forest (Brancalion et al., 2016; Guerra et al., 2020). In this study we showed higher carbon stability in subsoil and deep soil under the agroforestry systems approach. Additionally, our findings suggested that microbial community in the natural regeneration approach exhibited greater adaptability to the restored environment and more efficient carbon utilization. Consequently, there is a room for higher climate change benefits with land restoration in the Atlantic Forest if other approaches are used other than reforestation. There is not a universal formula for choosing the best restoration approach and local conditions have to be taken into account (Rodrigues et al., 2011), but natural regeneration could be implemented in high resilient areas (Gillson et al., 2021; Stefanes et al., 2016) and agroforestry systems could be a solution for small holder farmers, reconciling carbon sequestration and stability benefits with food production during the restoration process (Martinelli et al., 2019; Padovan et al., 2022).

4.5. Conclusion

We showed that deep soil (150-200 cm) presented lower carbon stability than topsoil (0-20 cm) at current temperatures, but higher stability with increasing temperatures, in the Atlantic Forest in Brazil. Subsoil (40-100 cm) carbon stability was more similar to topsoil at current temperatures, but will respond similarly to deep soil with rising temperatures. As land restoration progresses, carbon stability increased in both subsoil and deep soil. Hence, current measures are successful in mitigating climate change.

The temperature sensitivity is influenced by different factors along the soil profile: P content was the most influencing factor in subsoil, and N in deep soil. Hence, stability of soil carbon and its temperature sensitivity are depth and nutrient dependent. Adequate management of nutrient supply is thus also critical for optimizing soil C accrual for climate change mitigation.

Among the different restoration approaches, agroforestry systems presented the highest carbon stability throughout the soil profile, whereas reforestation, the most used approach in the region, presented the lowest C stability in the subsoil and deep soil. Thus, land restoration in the tropics may include sustainable land management when considering subsoil and deep soil, with best climate mitigation benefits achieved with the implementation of natural regeneration and prolonged agroforestry management.

5. CHAPTER 5: FINAL DISCUSSION

5.1. Summary of the research objectives and results

Deep soil layers store most of the organic carbon within the soil profile (Borchard et al., 2019; Harper & Tibbett, 2013; Strey et al., 2017). Although deep soil carbon has a lower mean residence time than that near the soil surface, the deep soil carbon pool is susceptible to changes and thus plays an active role in the global carbon cycle (Shi et al., 2020). Thus, deep soil can thus act as carbon sinks or sources (Chen et al., 2018; Fontaine et al., 2007; Jackson et al., 2002). These functions change after the introduction of trees into the environment, when roots reach deep soil layers (Adriano et al., 2017; Canadell et al., 1996; Li et al., 2019; Yunusa et al., 2012) and alter carbon dynamics through rhizodeposition and fostered turnover processes (Bernal et al., 2017; Billings et al., 2018; Lan et al., 2021). Thus, studying deep soil layers is crucial for determining the potential climate change mitigation of land management alternatives. However, in the case of land restoration in the Atlantic Forest areas in Brazil, it is still unclear to which extent deep soil layers can influence the overall ecosystem carbon dynamics.

This study sought to elucidate the carbon sequestration potential and stabilization of soil carbon compounds through land restoration efforts by integrating deep soil layers into the assessment framework. Initially, a global analysis was conducted to investigate the influence of land use, with and without trees, across various climatic conditions on deep soil carbon stocks. Subsequently, employing a paired-site case study approach within the Atlantic Forest region of Brazil, deep soil layers were integrated into the analysis of land restoration interventions to evaluate both carbon sequestration potential and carbon stability in a future warmer environment. Regarding my research questions outlined in chapter 1, section 3, I summarize the following results:

1. How do climate, land use, and soil type influence deep soil carbon stocks?

The majority of carbon stored globally is stored within the soil pool (Canadell et al., 2022), with deep soil layers containing the largest proportion (Borchard et al., 2019; Strey et al., 2017). Understanding the distribution of deep soil carbon is crucial for developing management strategies aimed at minimizing losses and enhancing carbon stocks. I hypothesized that 1) updated soil carbon information would affect our understanding of overall ecosystem C storage, particularly for tropical forests and cropland, where new data have been generated in the last two decades; 2) deep soils in colder climates contribute less to the overall C pool due to larger topsoil SOC stocks, while the opposite is true for soils in arid climates, and; 3) forest ecosystems comprise larger SOC stocks in the whole profile than croplands and grasslands, although with a smaller share of deep layers due to larger contributions from topsoil. I created a database of more than 12,000 soil profiles reaching 200 to 500 cm deep and assessed carbon stocks according to land use, soil type, and climate. I found that carbon stocks in the 100-300 cm layer amounted to 83 and 96% of the 0-100 cm layer stock for forestlands and croplands, respectively, contrary to 56 and 57% reported by Jobbagy and Jackson (2000). The differences between my findings and those of the former two authors are most likely due to the fact that 35% of the profiles of my database were investigated after 2000. Thus, these updated deep soil carbon data changed our understanding of forest and cropland C stocks, corroborating the first hypothesis. In relation to climate, the 100-300 cm layer represented 52% of the 0-100 cm stock in cold climates, whereas it reached in dry climates, thus corroborating the second hypothesis. The results of the updated database also showed that total SOC stock (0-300 cm) in forestlands was 264 Mg ha^{-1}, larger than in croplands (242 Mg ha^{-1}) and grasslands (228 Mg ha^{-1}). However, in the 200-300 cm layer, the SOC stock in croplands was 5% larger than that in forestlands. Thus, the third hypothesis was only partly corroborated. The total SOC stock (0-300 cm) was largest in forestlands, although in the 200-300 cm layer it was smaller than that in croplands. Overall, these findings showed that the contribution of deep soil carbon stocks to total soil carbon stock is larger than previously estimated, underscoring their importance to the global carbon cycle.

2. What is the carbon sequestration potential of land restoration in the Atlantic Forest?

The magnitude of soil carbon sequestration depends on the restoration approach (Brancalion et al., 2021), and deep soil layers are responsive to land restoration (Lan et al., 2021). I compared the carbon sequestration potential of common approaches of land restoration in the Atlantic Forest (Brancalion et al., 2016; Guerra et al., 2020; Padovan et al., 2022) by sampling aboveground (standing living, standing dead and litter) and belowground (roots and soil) carbon pools. Contrary to most restoration studies in the Atlantic Forest that include only topsoil layers (Ferez et al., 2015; Mendes et al., 2019; Zanini et al., 2021), I sampled soil up to 300 cm depth. I hypothesized that soil carbon stock changes would follow the aboveground carbon stock pattern. I found that the living aboveground carbon sequestration of reforestation areas with mixed native species were 85 ± 15 Mg ha^{-1}, statistically larger than natural regeneration sites (40 ± 13 Mg ha^{-1}) and agroforestry systems (25 ± 5 Mg ha^{-1}). Conversely, reforestation resulted in a loss of 27 Mg ha^{-1} in the 40-300 cm soil layer when compared to the negative reference (cropland), whereas natural regeneration and agroforestry resulted in the sequestration of 27 and 41 Mg ha^{-1}, respectively. Accordingly, the carbon sequestration of deep soil (40-300 cm) was inversely correlated with the aboveground living biomass, thus refuting the hypothesis. In the end, the three approaches used for land restoration resulted in the same level of carbon sequestration and can be used interchangeably when the restoration goal is to promote carbon sequestration. Yet, using only aboveground carbon would have led to a false ranking of the approach with the highest carbon sequestration potential. Thus, including deep soil is crucial for accurate assessment of restoration practices in the Atlantic Forest.

3. What is the effect of land restoration on the stability of soil carbon along the soil profile under a future warmer climate?

The stability of soil carbon can be assessed using soil respiration measurements obtained through incubation experiments (Schädel et al., 2020). Soil temperature sensitivity, quantified by the Q10 factor, denotes the extent to which soil respiration increases in response to a 10°C temperature rise (Kirschbaum, 1995; Meyer et al., 2018). To understand how the SOC of restored lands will respond to climate change, I incubated topsoil (0-20 cm), subsoil (40-100 cm), and deep soil (150-200 cm) at increasing temperatures and measured heterotrophic respiration. I hypothesized that 1) the temperature sensitivity Q10 decreases along the soil profile and, 2) the degree of this effect declines with restoration progress, meaning that the older the restored area, the lower the temperature sensitivity. I found that Q10 in the topsoil layer was 1.9 (± 0.06), which was significantly higher than subsoil (1.1 ± 0.09) and deep soil (1.0 ± 0.06), thus partially confirming the first hypothesis, since no difference was found between subsoil and deep soil. Yet, I also found that Q10 was not influenced by the restoration approach, thus confirming the second hypothesis. Nevertheless, and unlike hypothesized, Q10 was positively correlated with restoration age in both topsoil and deep soil layers. Finally, I revealed a nuanced interplay of factors affecting Q10 values. Specifically, in the subsoil layers, phosphorus content emerged as the primary determinant of Q10, whereas in the deep soil layer, nitrogen levels and clay content exhibited similar influence. Overall, these results show that subsoil and deep soil temperature sensitivity do not follow that of topsoil, and highlight the significant impact of soil fertility alongside soil texture on temperature sensitivity below the surface soil layers.

5.2. Synthesis and outlook

Our results indicate that deep soil layers play an important role in assessing carbon accrual and potential carbon losses after land restoration in the Atlantic Forest. In the following, I further complement this analysis by deriving it into six main sections. First, I extend the analysis of the importance of deep soil layers to the total SOC stock worldwide. Second, I show that deep soil layers not only passively store a large amount of carbon but also actively participate in the global carbon cycle. In the third section, I explore the relationships of the deep SOC pool and the aboveground and topsoil carbon pools. In the fourth part, I re-asses my soil data in a sequential manner, i.e., including deeper layers in a progressive way and running statistical analysis of each step. Here, I show how the inclusion of deep soil layers can change the study conclusion. In the fifth section, I revisit some studies that estimated the potential carbon sequestration of land restoration but did not account for deep soil carbon sequestration. In the last part, I combine all the results and recommend a strategy that can enhance the benefits of climate change mitigation in the Atlantic Forest.

5.2.1. Extended analysis of the relevance of deep soil layers to the ecosystem C stocks

The total depth of a soil refers to the vertical distance from the surface to the bedrock and can vary widely, ranging from a shallow layer of a few centimeters to several meters deep. Deeper soils are associated with intense sedimentation or high degrees of weathering, a process influenced by a variety of factors, including climate, parent material composition, age, and microbial activity (Huggett, 2023; Modenesi-Gauttieri et al., 2011; Sangmanee et al., 2022). In regions subject to intense weathering over extended periods, soils can reach 500 cm deep, occasionally surpassing depths of 1,000 cm (Harper & Tibbett, 2013; Strey et al., 2017; Yang et al., 2022). Nevertheless, an examination of more than 1,000 studies in the soil science literature reveals an average depth of 23 cm on soil carbon studies (Yost & Hartemink, 2020), highlighting a notable disparity from the potential maximum soil depth.

To give a broader perspective on deep soil C worldwide, I extended the analysis by selecting the profiles that reached 300 cm from the database produced in Chapter 2, and assessed topsoil (0-40 cm) and deep soil (40-300 cm) carbon stocks along a latitudinal gradient. Although there is no universally accepted definition of deep soil and studies in the literature offer a wide range of depths to characterize it (Li et al., 2019; Richter et al., 1995; Taylor et al., 2002; Wang et al., 2015), the delineation adopted here aligns with the parameters outlined in chapter 3. Moreover, this analysis excluded organic soils and higher latitudes (<-40° and >50°) with few profiles.

An examination of 1,720 soil profiles revealed a trend of increasing total SOC from both low and high latitudes towards the equator, with a notable exception observed within the 0-5° band, which exhibited markedly lower values (Figure 5-1). Furthermore, my analysis revealed that the deep SOC exhibits greater variability along the latitudinal gradient than to top SOC. While the variation around the average stock across latitudes was 31% in topsoil, it notably increased to 59% in deep soil, indicating a heightened sensitivity of deep soil to latitudinal changes in environmental factors. Importantly, my findings also underscored a spatial disparity in the deep SOC distribution, with the southern latitudes exhibiting higher deep SOC stocks than their northern counterparts: the average deep SOC content ranged from 237 Mg ha^{-1} in latitudes from -40 to 0°, whereas it decreased to 151 Mg ha^{-1} in latitudes from 0 to 50°. This difference highlights the need to implement management strategies to mitigate potential deep SOC losses in the global south.

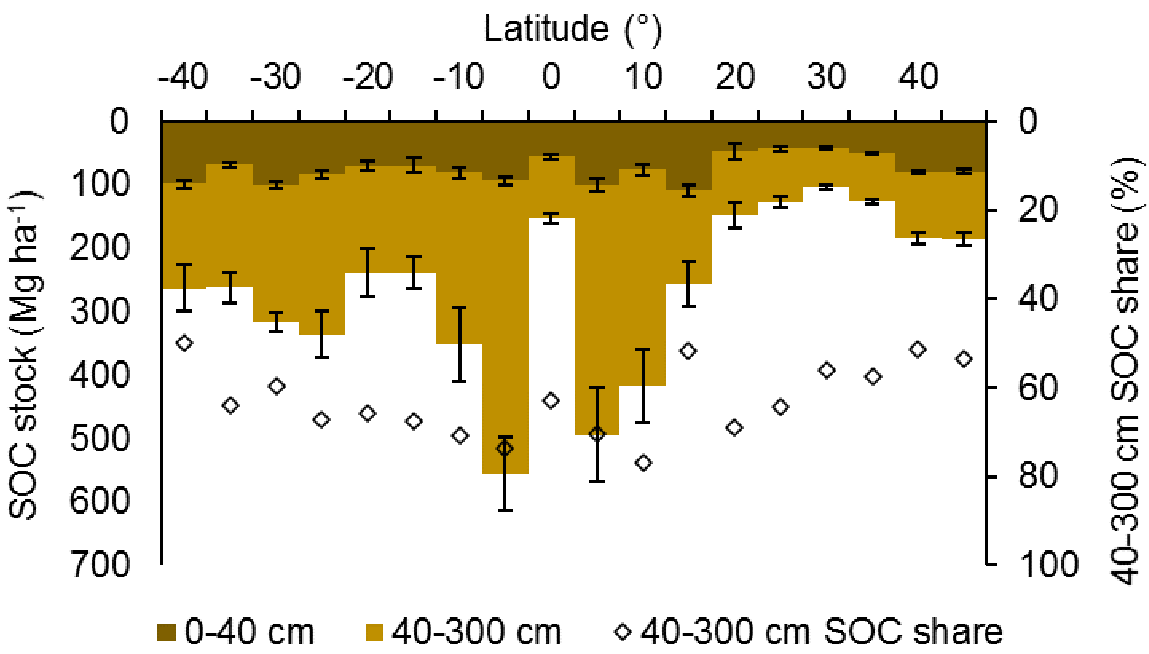

Figure 5-1. Soil organic carbon stock (SOC, Mg ha^{-1}) in the topsoil (0-40 cm) and deep soil (40-300 cm) along a latitudinal gradient. The error bars indicate the standard error. ◊ indicate the share of deep (40-300 cm) SOC stock (additional y axis, in %). The latitudes in the x axis are grouped into classes of 5 degrees, e.g., latitude -40 represents a range from -40 to -35.

Although carbon concentration notably decreases below the topsoil, it is present in substantial quantities within deep soil layers, resulting in the total soil carbon stock below the topsoil exceeding that of the topsoil itself (Harper & Tibbett, 2013; Krull & Skjemstad, 2003; Veldkamp et al., 2003). The larger deep SOC stock in the southern hemisphere can be attributed to the influence of deep roots (Pierret et al., 2016). Vegetation types associated with the southern hemisphere usually present deeper rooting depth than northern hemispheres, as for example tropical grasslands with roots possibly reaching more than 2,000 cm, whereas in boreal forests roots are usually present in the first 200 cm of the soil profile (Canadell et al., 1996). In addition to maximum rooting depth, the share of roots below topsoil layers is also higher in the southern hemisphere (Jackson et al., 1996), which contributes to larger C inputs from root exudates and turn over, which in turn forms SOM more efficiently than leaf litter inputs (Hu et al., 2016; Sokol et al., 2019). Moreover, intrinsic OM protection mechanisms of soils in the southern hemisphere, such as binding at the surface of the mineral phase in Ferralsols or aggregate protection in Nitisols, may favor the stabilization of organic carbon, and decreasing potential losses from respiration (Kögel-Knabner & Amelung, 2021; Mathieu et al., 2015). Consequently, deep SOC stock can be modified by the introduction of deep rooted plants such as trees and shrubs (Button et al., 2022; Lorenz & Lal, 2005; Peixoto et al., 2020).

The deep soil organic carbon share (40-300 cm) presented the same increasing trend towards the equator as total SOC (Figure 5-1). On average, 63% of the total SOC content (0-300 cm) was found in the deep soil layers, varying from 61% in the northern hemisphere to 65% in the southern hemisphere. Particularly, the profiles in the Atlantic Forest (chapter 3) presented a slightly lower share of deep SOC, with an average of 59% among all land uses. Despite intrinsic variations worldwide, the significant contribution of deep SOC therefore emerges as a consistent ecosystem characteristic across all latitudes. Hence, studies that assess SOC stocks by only examining topsoil layers are missing more than half of the relevant data or scope of their investigation.

5.2.2. Deep soil layers actively participate in the ecosystem carbon dynamics

Deep soil layers not only store large amounts of carbon globally, as demonstrated so far, but also participate actively in the carbon cycle via sequestration and emission, as will be demonstrated in this section. Until a couple of decades ago, deep soil carbon had been regarded as being stable and slow-cycling, thus contributing little to the global carbon cycle (Button et al., 2022; Pries et al., 2023; Krull & Skjemstad, 2003). The predominance of shallow sampling depths in soil science studies suggests a limited recognition of the importance of deep soil layers among researchers (Yost & Hartemink, 2020). Yet, some studies have already shown that deep soil carbon stock can undergo substantial alterations, especially after land-use change. Jackson et al. (2002) conducted a study on native grasslands and paired grasslands invaded by woody plants across a precipitation gradient in the USA. Their findings revealed that deep soil experienced an increase in carbon stock in arid regions but a decrease in more humid areas. Later, Fontaine et al. (2007) experimentally showed that deep soil carbon was unstable after the introduction of fresh labile carbon (cellulose), and that the increase in respiration triggered by the newly added carbon promoted priming of SOC, leading to a decrease in SOC stock. More recently, Meyer et al. (2018) found that the stability of deep soil carbon can be disrupted by the addition of nitrogen and phosphorus, raising concerns about the long-term storage efficacy of forest environments under increased nutrient deposition (Kwon et al., 2021; Menge et al., 2023). Different field investigations revealed that land restoration can promote changes in deep soil carbon. Deep SOC was found to increase after the introduction of *Gliricidia* spp. (Fabaceae) into maize crops in Malawi (Makumba et al., 2007) and after the implementation of irrigated cropland in desert areas in China (Li et al., 2010). Long-term land restoration through the introduction of trees was found to significantly change soil carbon concentration down to 2,000 cm depth, with either higher or lower C concentrations than agricultural areas depending on the tree species introduced and the depth evaluated (Lan et al., 2021).The findings presented in Chapter 3 further corroborate these observations, showing that deep SOC stock can either increase or decrease depending on the restoration approach used. This demonstrates that deep soil carbon stock can fluctuate after land use change and that is essential to incorporate deep soil layers into the assessment of carbon sequestration potential of different land management approaches.

As we recognize the dynamic nature of deep soil carbon stocks, it becomes equally important to explore how these stocks respond to changes in temperature. Given the dynamic nature of the soil organic matter along the soil profile and its interactions with the soil matrix (Han et al., 2016; McGrath et al., 2022), we might expect variations of Q10 with depth. Some studies have investigated soil Q10 values in subsoil and deeper layers. However, the findings from these studies have shown some degree of divergence, highlighting the complexity and variability of soil respiration dynamics at greater depths. Li et al. (2020) found higher Q10 in the 70-100 cm layer compared to the 0-10 cm in a forest ecosystem in China. Similarly, Fierer et al. (2003) found higher Q10 in deep soil (50-200 cm) than topsoil (0-50 cm) in a grassland in USA. Lower Q10 in deep soil compared to topsoil was also found in a cropland in Belgium (Gillabel et al., 2010) and in a forest in Hawaii (McGrath et al., 2022). Yet, Mu et al. (2016) found high variability in Q10 along a 400 cm soil profile in a permafrost region in China. While a higher temperature sensitivity is expected in deep soil due to the presence of more complex organic matter structures (Davidson & Janssens, 2006; Han et al., 2016), it appears that the Q10 values of deep soil are influenced by factors beyond organic matter structure alone, rendering them more context-dependent than those of topsoils (Pries et al., 2023). Although these studies suggest divergent trends in the temperature sensitivity of deep soil compared to topsoil (i.e., either increasing or decreasing), it is important to note that the Q10 value of deep soil differs from that of the topsoil. Since the amount of soil organic carbon below topsoil layers is significant (Strey et al., 2017), the emissions associated to deep soil will have a significant contribution to total emissions (Soong et al., 2021; Veldkamp et al., 2003). Hence, global estimations that do not include deep soil might underestimate the soil contribution to the global carbon cycle (Bond-Lamberty et al., 2018; Lei et al., 2021; Nissan et al., 2023). Moreover, models that include deep soil, but rely on a constant Q10 value across the soil profile may inaccurately estimate soil emissions, thus potentially leading to erroneous conclusions (Lynch et al., 2017; Todd-Brown et al., 2013).

5.2.3. Relationship between deep soil carbon and other pools

The soil profile is far from homogeneous, exhibiting substantial changes with depth. The quantity, characteristics, and age of organic matter vary significantly (Jobbagy & Jackson, 2000; Rumpel & Kögel-knabner, 2011; Shi et al., 2020), nutrient content tends to decrease with increasing depth (Jobbágy & Jackson, 2001), and clay content and type can shift, leading to different stabilization mechanisms (Heckman et al., 2022; Schweizer et al., 2021). Moreover, the carbon sources differ: topsoil carbon primarily originates from aboveground litter, whereas deep soil carbon is predominantly derived from root litter and exudates (Hu et al., 2016; Rasse et al., 2005). Consequently, topsoil and deep soil will most likely respond differently to land use changes such as land restoration. Therefore, the response of deep soil cannot be directly inferred from our knowledge of topsoil.

To the best of my knowledge, this study represents the first effort in land restoration research within the Atlantic Forest of Brazil to integrate deep soil assessments. Among the three restoration approaches examined, reforestation resulted in the highest aboveground carbon accumulation but a loss of deep SOC (see Table 3-2 and Figure 3-4, Chapter 3). This suggests that deep soil layers do not consistently mirror the aboveground sequestration trends, which contradicts original hypothesis outlined in chapter 3. To gain a deeper understanding of how deep soil responds after land restoration, I conducted an analysis to explore its relationship with aboveground and topsoil pools. This involved a graphical examination using scatterplots and quantifying the strength of the correlation using Pearson's correlation coefficient. I conducted this analysis using the carbon sequestration per year, i.e., I divided the carbon sequestration results calculated in chapter 3 by the age of the restoration intervention.

Soil organic carbon sequestration (Mg ha^{-1} $year^{-1}$) in the topsoil (0-40 cm) had a weak and not significant correlation with living aboveground biomass carbon sequestration (AGC, Mg ha^{-1} $year^{-1}$). In contrast, deep soil (40-300 cm) exhibited a strong, negative, and significant correlation, indicating that increased aboveground biomass carbon sequestration is associated with decreased deep soil carbon sequestration (Figure 5-2). Furthermore, in areas of rapid aboveground biomass increment, not only was deep soil carbon sequestration reduced, but it can even be negative, i.e., there was a loss of carbon (as already shown in chapter 3). Using the linear equation that describes this relationhsip, aboveground carbon sequestration rates above a threshold (in my analysis >4.3 Mg ha^{-1} $year^{-1}$) leads to a loss of deep SOC. Lastly, the relationship between SOC sequestration in the topsoil and deep soil did not show any trend in restored areas of the Atlantic Forest. These findings suggest that deep soil layers, instead of mirroring the aboveground sequestration trends, exhibit the opposite trend. Moreover, deep soil and topsoil carbon dynamics seem to be decoupled after land restoration.

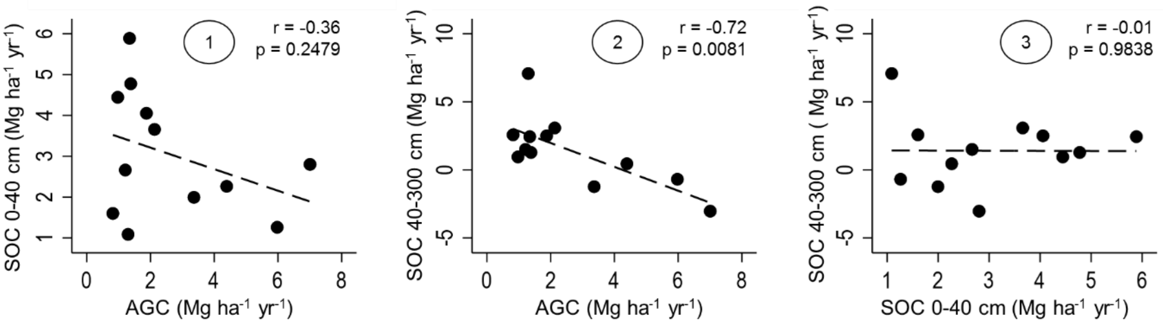

Figure 5-2. Relation between: 1) topsoil (0-40 cm) organic carbon sequestration (SOC 0-40 cm, Mg ha^{-1} $year^{-1}$) and living aboveground carbon sequestration (AGC, Mg ha^{-1} $year^{-1}$) (top-SOCseq = 3.739 -0.264*AGCseq); 2) deep soil (40-300 cm) organic carbon sequestration (SOC 40-300 cm, Mg ha^{-1} $year^{-1}$) and living aboveground carbon sequestration (AGC, Mg ha^{-1} $year^{-1}$) (deep-SOCseq = 3.731 – 0.877*AGCseq), and; 3) deep soil (40-300 cm) organic carbon sequestration (SOC 40-300 cm, Mg ha^{-1} $year^{-1}$) and topsoil (0-40 cm) organic carbon sequestration (SOC 0-40 cm, Mg ha^{-1} $year^{-1}$) (deep-SOCseq = 1.445 – 0.011*top-SOCseq). r and p in the figure are Pearson correlation coefficient and p value, respectively.

In an analysis of 108 experiments assessing changes in SOC and aboveground C stocks, Terrer et al. (2021) also found an inverse relationship between these two pools, contrary to their hypothesis that an increase in aboveground C stock would be accompanied by an increase in SOC stock. I also hypothesized that SOC stocks would follow aboveground patterns, but as detailed in chapter 3, areas exhibiting rapid aboveground carbon accumulation presented a decline in deep soil carbon. In addition, a general strong negative correlation between deep SOC and aboveground carbon sequestration. These differences may be attributed to the nutrient acquisition strategy of the trees, which is shaped by their symbiotic associations with soil fungi. Ectomycorrhizae (ECM) associated plants demonstrate enhanced N acquisition efficiency compared to arbuscular mycorrhizae (AM) associated plants on soils with low N levels (Lin et al., 2018; Yang et al., 2022). ECM-associated plants increase their aboveground biomass by mining soil nutrients present in soil organic matter (Lin et al., 2018;

Terrer et al., 2021). This leads to the priming of SOC, i.e., the decomposition of existing SOC and consequently a reduction in the SOC stocks (Sulman et al., 2017; Zak et al., 2019). On the other hand, AM-associated plants have reduced aboveground growth in soil with limited nutrients, but belowground carbon inputs from roots turn over and rhizodeposition lead to an increase in SOC stock (Wang et al., 2016; Wilson et al., 2009). Moreover, AM-associated plants enhance mineral-associated organic matter formation (Agnihotri et al., 2022), which limits SOC decomposition (McGrath et al., 2022). My results show that reforestation resulted in higher aboveground growth and deep soil C losses (chapter 3) and also caused higher soil respiration in the 40-100 cm and 150-200 layers than agroforestry systems and natural regeneration approaches (Figure 4-1, chapter 4). Conversely, agroforestry systems presented low rates of aboveground biomass increment but high rates of SOC sequestration (chapter 3), and lower soil respiration (chapter 4). Additionally, the physiological state of microorganisms in deep soil was also altered following land restoration (Figure 4-4, chapter 4), consistent with previous findings in deep soil layers after restoration (Kong et al., 2022). This indicates that land restoration may have altered microbial communities through the introduction of different tree species in the reforestation and agroforestry system approaches. Thus, in restoration approaches which involve deliberate species selection like reforestation and agroforest systems, the choice of species should seek to strike a balance between AM- and ECM-associated plants to guarantee optimal rates of both aboveground and SOC sequestration.

5.2.4. Integrating deep soil layers into the decision-making process

Decision-making theory emphasizes the importance of incorporating comprehensive information to reach informed and accurate conclusions. Yet, in many cases decisions are based on incomplete data, which can lead to suboptimal outcomes (Citroen, 2011). This principle is highly relevant in the context of evaluating different management alternatives, such as soil carbon sequestration potential across different land uses (Kumar et al., 2017). In most cases, assessments have focused primarily on topsoil layers (Yost & Hartemink, 2020), leading to conclusions that may not fully reflect the full carbon sequestration potential of different land use practices. By integrating deeper soil layers into the decision-making process, we can improve land use decisions and enhance climate change mitigation strategies.

An analysis of 152 land restoration studies in the Atlantic Forest found that less than half reported any soil data (Mendes et al., 2019), emphasizing the importance of including soil pools in restoration assessments for a comprehensive analysis of the entire system. Yet, including only topsoil layers will not be enough for a thorough assessment of carbon sequestration potential, and neglecting deep soil layers could potentially lead to false conclusions about the best restoration approaches. Thus, I re-assessed the SOC sequestration data used in chapter 3, including each sampled layer in a sequential manner, and then I presented the conclusion of the best restoration approach after each step of the analysis. For this analysis, I included only restoration approaches with similar average age (agroforestry system in advanced stage, reforestation with mixed native species and natural regeneration). Further, I provide examples from the literature on how including deep soil layers may change the overall conclusions of such studies.

The analysis of SOC sequestration showed that the decision on the restoration approach with the highest sequestration potential changed threefold as deeper layers were included in the decision process (Figure 5-3). When only the first layer (0-20 cm) or a combination of the first and second layers (0-40 cm) were analyzed, all restoration approaches showed the same sequestration potential. However, when I included the 40-100 cm layer (thus comparing the cumulative sequestration in a 0-100 cm profile) I found that agroforestry systems sequestered more carbon than reforestation approaches. Further including the 100-150 cm layer (thus comparing the cumulative sequestration in a 0-150 profile), I found that both the agroforestry system and natural regeneration outperformed the reforestation approach. Adding more layers below 150 cm increased the magnitude of carbon sequestration but did not affect the ranking of the different approaches. Hence, had I sampled only topsoil layers (0-40 cm) or even to a depth of 100 cm, I would have come to an incorrect conclusion about the soil sequestration potential of different restoration approaches in the Atlantic Forest.

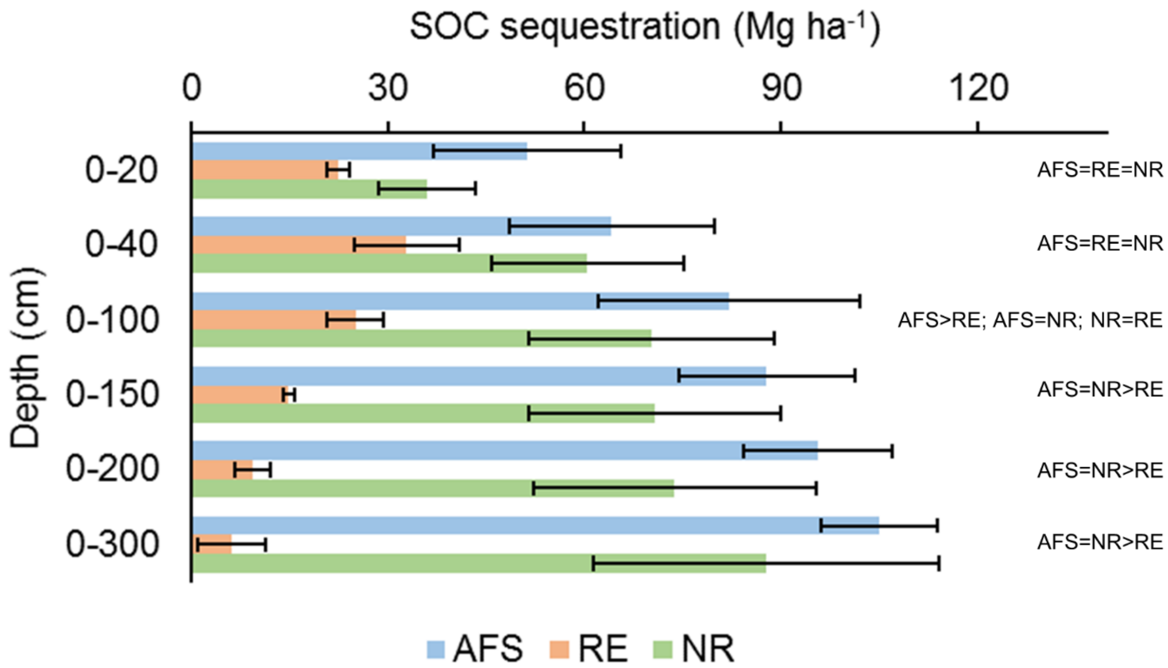

Figure 5-3. Soil organic carbon sequestration (SOC, Mg ha^{-1}) in cumulative depths (cm) after land restoration with agroforestry system (AFS), reforestation with mixed native species (RE)

and natural regeneration (NR). Error bars are standard error (n=3). The comparisons on the right side of the graph means the decision of the sequestration potential using the statistical significance of a linear model and post hoc LSD test ($p<0.05$).

Although the agroforestry system and natural regeneration approaches resulted in an increasing trend of SOC accumulation along the soil profile, a discernible inflexion point emerged below a depth of 40 cm in the reforestation approach (Figure 5-3): below this threshold, reforestation caused a loss of SOC (see Figure 3-3, chapter 3); hence the cumulative SOC started to decrease. Several studies have demonstrated that surface and deep soil layers exhibit contrasting responses to agricultural practices, highlighting the role of soil sampling depth in shaping the interpretation of results. For instance, Olson and Al-Kaisi (2015) studied the carbon dynamics of no-tillage treatment in 5-cm depth intervals and found that the SOC stock was 9% higher in the 0-5 cm soil layer, but 13% lower in the 0-75 cm soil layer after 20 years compared to the baseline scenario. Tautges et al. (2019), in a 19-year experiment of a maize-tomato rotation system, found that the addition of winter cover crops to the system resulted in an increase of 3.5% in the SOC in the 0-30 cm layer, but a decrease of 10.8% in the 30-200 cm layer, resulting in an overall loss of SOC. Yet, in western Germany, repeated monitoring of agricultural soils showed that SOC concentration increased from 9.4 to 9.8 g kg^{-1} from 2005 to 2013 in the topsoil (Ap horizon), whereas it decreased from 4.1 to 3.5 g kg^{-1} in subsoil (starting from the B horizon to 60 cm (Steinmann et al., 2016). Also in Germany, results of a long-term experiment in agricultural areas showed that 20% of the organic carbon increment caused by improved management practices took place in the 30-100 cm layer (Skadell et al., 2023). Investigations of land use change following tree introduction have similarly revealed divergent trends in the sequestration of surface and deep SOC. In a 40-year old pine forest planted on a cotton field, Mobley et al. (2015) found a net increase in the SOC stock in the 0-35 cm layer, but a decrease in the 35-60 cm layer, resulting in a net negative in the 0-60 cm layer. In an assessment of six paired sites with woody plant invasion on grasslands, Jackson et al. (2002) found that four sites had opposite SOC sequestration between top (0-100 cm) and deep soil (100-300 cm), i.e., topsoil had positive values and deep soil negative values, or topsoil had negative and topsoil positive values. Lastly, Chen et al. (2022), assessing the impact of sampling stratification on SOC stock of grazed alpine meadows, found that the SOC stock increase in a 120 cm soil profile occurred mainly below 40 cm. Overall, these studies demonstrate that shallow sampling can lead to wrong conclusions about best management practices and potential soil carbon sequestration.

If the correlations among topsoil, deep soil, and aboveground carbon pools (Figure 5-2) persist in other restored areas within the Atlantic Forest Biome, it could lead to potential new interpretations regarding total ecosystem carbon sequestration (comprising aboveground, topsoil, and deep soil carbon) of previous studies. For example, Zanini et al. (2021) found higher carbon sequestration in tree biomass after 5 years of restoration with mixed native species, although no difference in topsoil, compared with natural regeneration. Similar results were found across a 60 years chronosequence study (Brancalion et al., 2021), where reforestation presented 50% higher aboveground carbon stocks, and no statistical differences in topsoil. Yet, Ferez et al. (2015) found that a high-input silvicultural system for reforestation in the Atlantic Forest resulted in a 3-fold increase in aboveground carbon stock compared to a low-input system after 6 years, but no difference in topsoil organic carbon stock. If deep soil does not follow topsoil carbon sequestration, and deep soil presents an inverse trend of aboveground carbon sequestration (Figure 5-2), the general interpretation of these studies might be masked by a smaller growth (or even decrease) in deep soil carbon. Thus, the inclusion of deeper layers into the assessment can change the overall interpretation of the respective findings.

The IPCC Tier 1 guidelines advocate a sampling depth of 30 cm for land converted to forest use (IPCC, 2019), which closely aligns with the average depth of soil carbon studies of 23 cm (Yost & Hartemink, 2020). Although it is challenging to determine the optimum sampling depth for all scenarios, a general guideline advises sampling to at least the depth of the active rooting zone (Nepstad et al., 1994; Olson & Al-Kaisi, 2015). In land use change studies after tree introduction this depth usually exceeds 100 cm after a few years (Adriano et al., 2017; Pinheiro et al., 2016; Sommer et al., 2000). In our study, the root C stock in the 150-300 cm layer in the agroforestry system in the intermediate stage was significantly higher than that of the paired agricultural sites (Table 3-2, Chapter 3), demonstrating that roots actively penetrate deep soil layers after six years of land restoration in the Atlantic Forest. Therefore, to ensure a comprehensive coverage of significant SOC changes in studies on land restoration with trees, the IPCC should provide an explicit recommendation for extending sampling depth beyond the conventional 30 cm.

5.2.5. Revisiting potential carbon sequestration in land restoration studies

Natural climate solutions - land management practices that promote carbon sequestration and reduce emissions - have great potential for sequestering carbon from the atmosphere and mitigating global temperature rise (Griscom et al., 2017). Cost-effective approaches within this framework can offset up to 100% of a country's emissions, particularly in tropical regions (Griscom et al., 2020). Among these natural solutions, land restoration is a highly promising option due to its high sequestration potential per unit of land and potential land availability (Paustian et al., 2016). The Bonn Challenge, a global commitment to restore world forests, identified 1,200 Mha of land suitable for restoration worldwide and launched a goal of implementing restoration on 350 Mha by 2030 (Lewis et al., 2019). However, existing estimates of land restoration carbon sequestration potential often focus solely on living biomass (aboveground and coarse roots), overlooking other critical carbon pools such as necromass, fine roots, and soil (Lewis et al., 2019). Consequently, the carbon sequestration potentials provided by these studies are likely underestimated.

In this section, I revisit existing studies on carbon sequestration potential through land restoration, incorporating C sequestration values from other pools into the published estimates. To approximate C sequestration in pools not quantified in these studies, I used the share of carbon sequestered in each pool after land restoration with natural regeneration as a reference (see chapter 3), i.e., 27% in the living aboveground biomass, 5% in the coarse roots, 6% in the necromass, 2% in fine roots, 41% in the topsoil (0-40 cm), and 19% in the deep soil (40-300 cm). Notably, my results of C sequestration in the living aboveground biomass and coarse roots were similar to earlier estimates from Latin America (Bernal et al., 2018).

If all the areas pledged by the Bonn Challenge are effectively restored and maintained, the accumulated carbon in the living aboveground biomass and coarse roots will total 36 Pg by the end of the century (Lewis et al., 2019). Considering other pools not included in this analysis, there is an additional 77 Pg of C stored, of which 21 Pg are located in the deep soil. In Latin America and the Caribbean, carbon sequestration on living aboveground biomass with natural regeneration in the next 40 years is 8.5 Pg of C, equivalent to the region's fossil fuel and industrial emissions between 1993 and 2014 (Chazdon et al., 2016). Adding potential carbon sequestration to other pools would increase the sequestration to 31.1 Pg; therefore an additional 22.6 Pg of C could be stored. Out of this, 18.7 Pg would be sequestered in the soil, whereas 5.8 Pg would be sequestered in the deep soil (40-300 cm). In an optimistic scenario of natural regeneration in degraded areas in the Atlantic Forest, 0.46 Pg of C could be sequestered in the aboveground biomass by 2035, with additional 0.70 Pg and 0.32 Pg in topsoil and deep soil, respectively (Crouzeilles et al., 2020). Another study in the Atlantic Forest found that restoring moderately and severely degraded pastures could capture 0.53 Pg of C in the living aboveground and coarse roots over 100 years (Barros et al., 2023). Considering the other pools not included in this study, an additional 1.14 Pg could be sequestered, with 0.31 Pg from the deep soil. These additional amounts correspond to approximately four times the annual emissions associated with land-use change in Brazil in 2022 (Tsai et al., 2023). Lastly, the "4 per 1,000" is an international initiative aimed at implementing soil practices to promote an increase in SOC stocks by 0.4% (or 4 ‰), which would be sufficient to compensate for the annual rise in CO_2 atmospheric concentrations on a global scale (Minasny et al., 2017). However, these figures are solely based on topsoil estimates, not taking into consideration contributions from subsoil and deep soil layers.

Although the estimation concept is based on best agricultural practices that usually affect topsoil layers, practices such as adoption of agroforestry systems have the potential to increase SOC stocks below the topsoil layers, as shown in chapter 3 and elsewhere (Cardinael et al., 2015). Thus, 4 per 1,000" initiative is probably higher than its initial estimates.

The additional carbon estimates provided here are approximations derived from the share of sequestered carbon following land restoration with natural regeneration, as detailed in chapter 3. It is important to recognize that different restored areas may exhibit varying proportions of carbon pools, thus affecting these estimations. Additionally, my calculations were based on areas with an average age of 17 years, and those in the final stage of restoration may display different carbon pool distributions (Brancalion et al., 2021; Lewis et al., 2019). Furthermore, soil carbon sequestration depends on the initial carbon content, reflecting the degree of degradation at the time of restoration intervention (Tian et al., 2023). Nevertheless, existing studies fail to fully account for the comprehensive sequestration potential of restored areas by neglecting the soil pool. Incorporating soil carbon sequestration in topsoil and deep soil has the potential to at least double the sequestration estimates of published studies, highlighting the unparalleled mitigation potential of land restoration (Griscom et al., 2017).

5.2.6. Land restoration in the Atlantic Forest: what is next?

Approximately 80% of the restoration in the Atlantic Forest of Brazil is conducted via reforestation with mixed native species (Brancalion et al., 2016). This preference is likely due to higher aboveground and topsoil carbon increments as a consequence of reforestation implementation, as shown in many studies (Brancalion et al., 2021; Ferez et al., 2015; Zanini et al., 2021) and in my own results (Chapter 3). However, as discussed in section 2.4 of this chapter, the inclusion of deep soil layers into the assessment can change the results. By including deep SOC changes, I showed that the three restoration approaches sequestered similar amounts of carbon, i.e., reforestation resulted in the highest aboveground biomass increment but lost deep soil carbon, agroforestry system the lowest aboveground growth, but the highest soil carbon sequestration, and natural regeneration had intermediate results for aboveground and soil. Thus, aboveground and deep soil carbon sequestrations were negatively correlated (Figure 5-2). Moreover, I also assessed the soil carbon stability and found that overall, reforestation caused higher decomposability (lower stability) in subsoil and deep soil, whereas agroforestry systems resulted in lower vulnerability, and intermediate results for natural regeneration. In the end, if the restoration goal is to promote carbon sequestration, the three restoration approaches yield similar results. However, the vulnerability of carbon pools must be considered when considering their long-term mitigation potential (Goldstein et al., 2020). Here, agroforestry systems emerged as the approach with the highest mitigation potential, whereas reforestation has the lowest, and natural regeneration showed intermediate results. Agroforestry systems have the lowest aboveground biomass carbon stock - a pool generally more susceptible to losses than soil carbon (Goldstein et al., 2020) - and exhibit the highest soil carbon stability. In contrast, reforestation has the highest aboveground carbon and the most vulnerable soil carbon. Therefore, a combined analysis of sequestration potential, including deep soil and the vulnerability of soil carbon compounds, provides a new perspective for land restoration in the Atlantic Forest, with the potential to enhance the climate change mitigation benefits by increasing the adoption of agroforestry systems and natural regeneration.

In addition to carbon sequestration potential, factors such as costs and other environmental services should be considered in land restoration projects. When low costs are intended, natural regeneration should be favored due to the lower number of activities involved in the restoration process (Brancalion et al., 2021; Crouzeilles et al., 2020). Regarding biodiversity, both reforestation and natural regeneration seem to produce similar outcomes in the Atlantic Forest (Romanelli et al., 2022). Yet, since successional agroforestry systems can produce food while restoring the land, such an approach can be an interesting strategy to attenuate land competition, partly compensating for the decrease in food production caused by the expansion of forests on agricultural lands (Martinelli et al., 2019; Padovan et al., 2022). Thus, scaling up restoration through natural regeneration and agroforestry system in the Atlantic Forest not only increases climate change mitigation potential but also reduces costs and promotes additional ecosystem services.

The history of reforestation in the Atlantic Forest shows that there has been a focus on tree species selection to promote biodiversity and aboveground biomass growth (Rodrigues et al., 2009). Future research should emphasize species selection that optimizes nutrient acquisition from nitrogen and phosphorus-deficient deep soils (Aoyagi et al., 2022; Dierks et al., 2021). Moreover, long-term restoration experiments involving reforestation should include deep soil carbon pool assessments to gain a better understanding of the relationships between aboveground and deep soil carbon dynamics and to ascertain whether my findings hold true in other reforestation projects (Brancalion et al., 2021; Ferez et al., 2015; Zanini et al., 2021). Meanwhile, there is significant potential to increase the use of agroforestry systems and natural regeneration as substitutes for reforestation, thereby enhancing climate change mitigation benefits.

5.3. Conclusion

This study demonstrated that integrating deep soil layers into the evaluation of climate change mitigation potential in land restoration efforts within the Atlantic Forest of Brazil leads to a revised understanding of the optimal restoration approach. Reforestation with mixed native species is currently the most common restoration approach, and it promotes the highest carbon sequestration when aboveground biomass and topsoil are assessed. Yet, by including deep soil layers, I showed that the high aboveground biomass increment of reforestation interventions was offset by losses of deep soil carbon, resulting in a net ecosystem carbon sequestration comparable to other restoration approaches used in the region. Moreover, reforestation resulted in the highest deep soil respiration, indicating lower stability of soil carbon compounds. In contrast, agroforestry systems resulted in the lowest aboveground carbon sequestration and the highest deep soil carbon sequestration and stability. Natural regeneration, the second most implemented restoration approach, showed intermediate results in terms of carbon sequestration and stability. The adoption of agroforestry systems and natural regeneration has the potential to scale up land restoration in the Atlantic Forest. These approaches offer compelling advantages for landowners, including opportunities for food production and reduced implementation costs, thereby facilitating their broader adoption Thus, transitioning from reforestation to agroforestry systems or natural regeneration, where feasible, enhances the effectiveness of climate change mitigation efforts by bolstering carbon stability, in addition to reducing food production pressure and restoration costs.

To the best of my knowledge, this study is the first to incorporate deep soil into land restoration assessments of the Atlantic Forest of Brazil. Hence, the inclusion of deep soil layers into new studies or future assessments in long term experiments is suggested. Emphasis should be placed on analyzing how different restoration approaches and species selection within the same approach influence nutrient acquisition from deep soil, and how these interactions affect soil carbon sequestration and stabilization in deep soil.

6. References

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7. Appendix A – Chapter 2

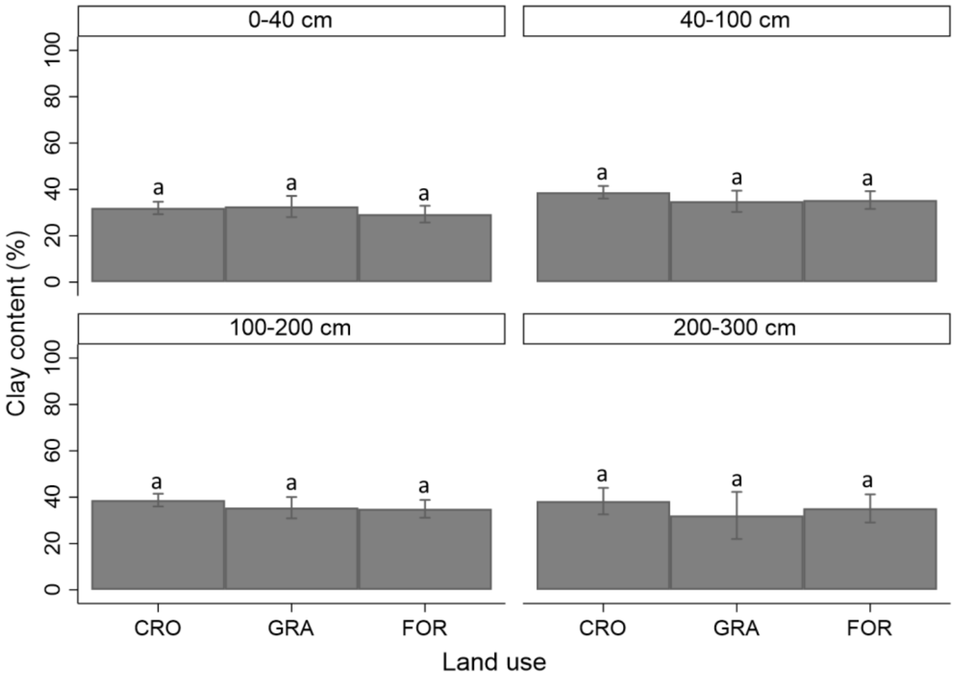

Figure 7-1. Average clay content (%) in the studied land uses at different depths. CRO=cropland; GRA=grassland; FOR=forestland. Error bars are 95% confident interval. Different letters indicate statistical difference among the land uses in the same layer (LSD test, p<0.05).

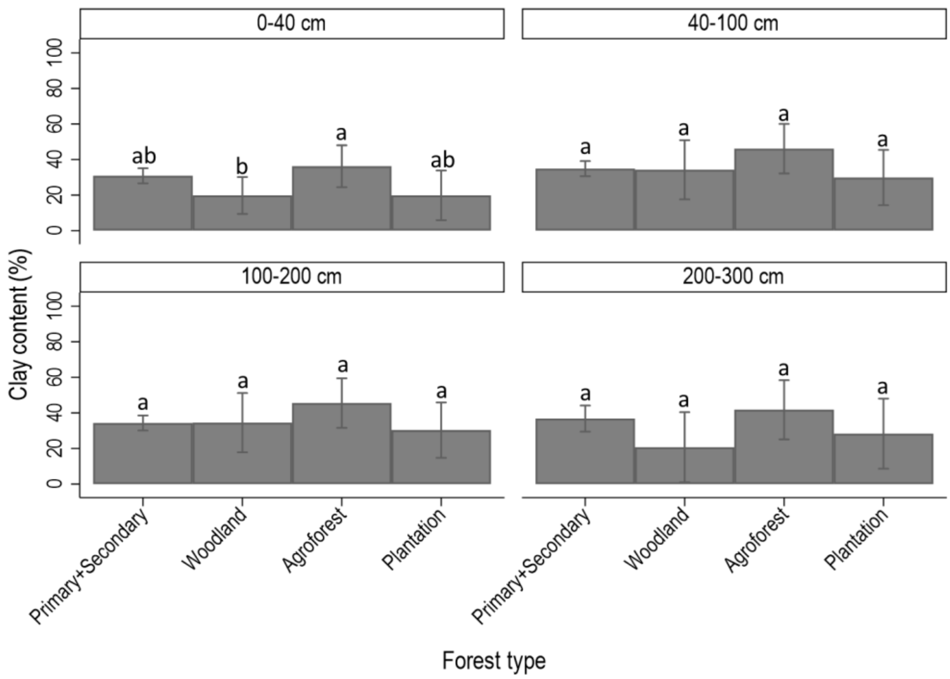

Figure 7-2. Average clay content (%) in the studied forest types at different depths. Error bars are 95% confident interval. Different letters indicate statistical difference among the forest types in the same layer (LSD test, p<0.05).

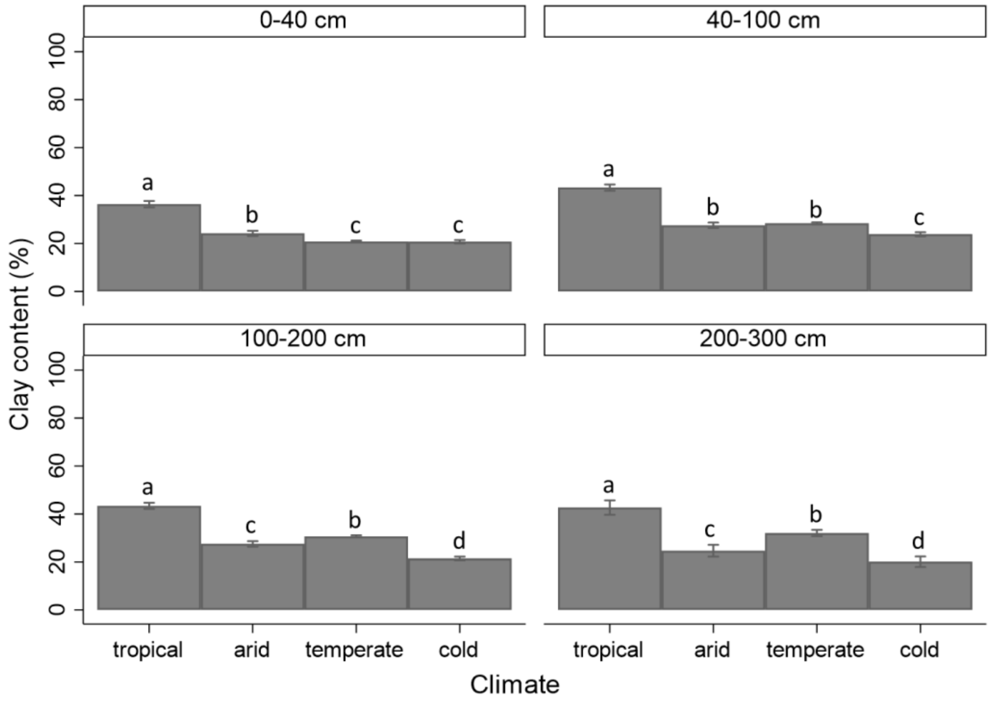

Figure 7-3. Average clay content (%) in the studied climates at different depths. Error bars are 95% confident interval. Different letters indicate statistical difference among the climate types in the same layer (LSD test, $p<0.05$). Note that from cold to tropical climates there is also a change in clay mineralogy to 1:1 minerals.

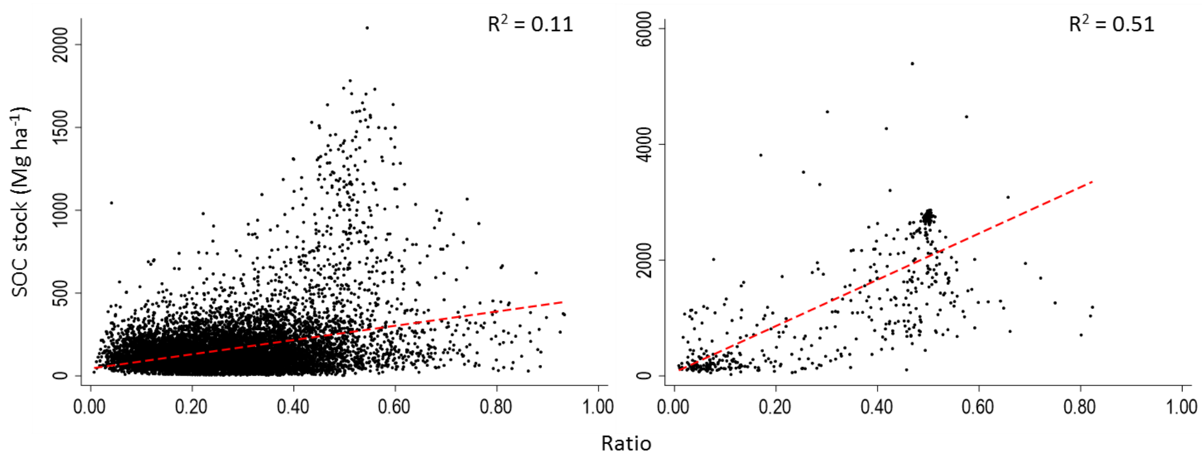

Figure 7-4. Correlation between the soil organic carbon stock in the 0-200 cm profile (Mg ha^{-1}) and the SOC stock in the 100-200 cm layer / 0-200 cm layer ratio (i.e., the share of the 100-200 cm layer in relation to the 0-200 cm layer). Left: mineral profiles (C%<15); right: organic profiles (C%>15).

Table 7-1. Number of soil profiles according to land use and climate (number in parentheses is the percentage).

Land use / Climate	Tropical	Dry	Temperate	Cold
Cropland	123 (43)	53 (19)	94 (33)	10 (4)
Grassland	45 (37)	38 (31)	27 (22)	10 (8)
Forestland	91 (38)	32 (13)	105 (44)	10 (4)
Primary+secondary	64 (46)	15 (11)	50 (36)	9 (7)
Woodland	8 (24)	7 (21)	19 (56)	0
Agroforest	16 (55)	0	13 (45)	0
Plantation	3 (8)	10 (27)	23 (62)	1 (3)

Table 7-2. Number of soil profiles according to land use and reference soil group (number in parentheses is the percentage).

Soil / Land use	Cropland	Grassland	Forestland
Acrisol	43 (15.1)	8 (6.5)	41 (17)
Albeluvisol	0	2 (1.6)	2 (0.8)
Alisol	7 (2.5)	5 (4.1)	2 (0.8)
Andosol	2 (0.7)	4 (3.3)	22 (9.1)
Anthrosol	1 (0.4)	0	0
Arenosol	4 (1.4)	8 (6.5)	4 (1.7)
Calcisol	4 (1.4)	0	0
Cambisol	35 (12.3)	19 (15.4)	26 (10.8)
Chernozem	16 (5.6)	5 (4.1)	2 (0.8)
Cryosol	0	0	0
Ferralsol	35 (12.3)	17 (13.8)	57 (23.7)
Fluvisol	28 (9.9)	3 (2.4)	3 (1.2)
Gleysol	6 (2.1)	2 (1.6)	2 (0.8)
Gypsisol	1 (0.4)	2 (1.6)	1 (0.4)
Histosol	1 (0.4)	0	4 (1.7)
Kastanozem	1 (0.4)	5 (4.1)	4 (1.7)
Leptosol	0	0	0
Lixisol	23 (8.1)	2 (1.6)	11 (4.6)
Luvisol	16 (5.6)	12 (9.8)	16 (6.6)
Nitisol	11 (3.9)	2 (1.6)	9 (3.7)
Phaeozem	6 (2.1)	9 (7.3)	0
Planosol	0	0	5 (2.1)
Plinthosol	10 (3.5)	1 (0.8)	4 (1.7)
Podzol	0	1 (0.8)	9 (3.7)
Regosol	3 (1.1)	2 (1.6)	1 (0.4)
Retisol	0	0	0
Solonchak	2 (0.7)	1 (0.8)	0
Solonetz	2 (0.7)	2 (1.6)	0
Stagnosol	0	0	0
Technosol	0	0	0
Umbrisol	4 (1.4)	0	3 (1.2)
Vertisol	17 (6)	9 (7.3)	9 (3.7)
Total	284 (100)	123 (100)	241 (100)

Table 7-3. Average share of rock fragments (%) per soil layer reported on my database.

Layer (cm)	Number of observations	Rock fragments (%)
0-40	3607	10
40-100	2185	13
100-200	3037	14
200-300	884	13
300-500	537	7

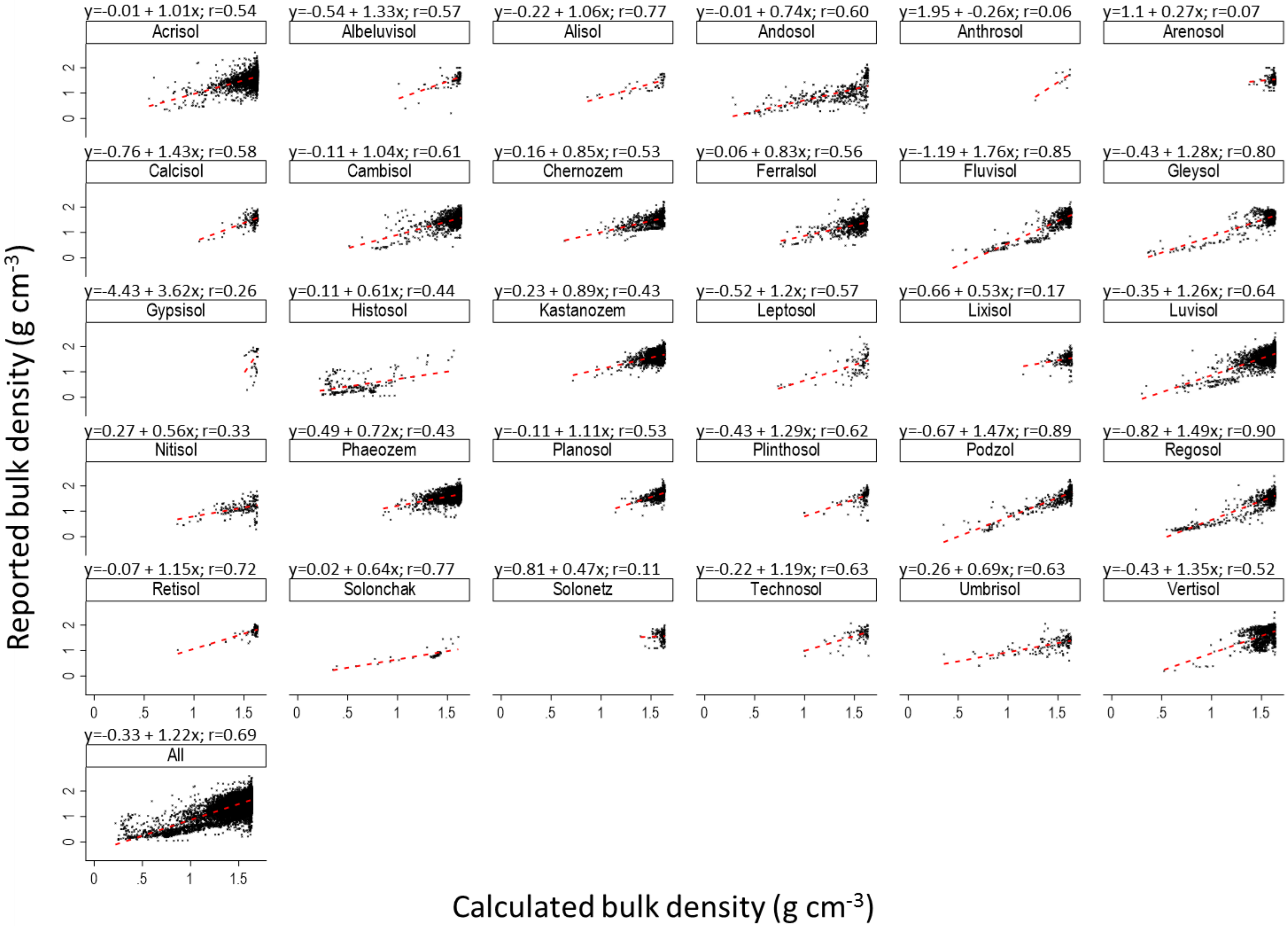

Figure 7-5. Correlation between reported and calculated soil bulk density (g cm^{-3}) in the studied reference soil groups.

List of publications included in the database (literature database – see section 2.2.1, item 4.)

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8. Appendix B – Chapter 3

Table 8-1. Mean (standard error, n=3) soil organic carbon content (g kg^{-1}) and bulk density (g cm^{-3}) in the six studied depths. AG = agriculture; AFSi = agroforestry system in intermediate stage; AFSa = agroforestry system in advanced stage; RE = reforestation; NR = natural regeneration; SF = secondary forest.

	AG	AFSi	AFSa	RE	NR	SF
Depth (cm)	Soil carbon content (g kg^{-1})					
0-20	10.3 (1.9)	12.9 (2.2)	26.4 (2.2)	17.8 (2.9)	20.3 (1.5)	28.9 (8.5)
20-40	8.2 (2.0)	8.6 (1.8)	13.3 (1.4)	10.4 (2.3)	13.3 (2.2)	13.7 (2.8)
40-100	5.3 (1.2)	5.5 (0.8)	8.5 (1.3)	4.2 (1.2)	7.3 (1.4)	7.6 (3.9)
100-150	2.8 (1.1)	3.9 (0.9)	4.7 (1.9)	2.3 (0.7)	3.9 (1.3)	6.3 (3.9)
150-200	1.8 (0.8)	2.6 (0.9)	3.3 (1.3)	1.3 (0.5)	2.5 (0.7)	4.9 (3.6)
200-300	1.0 (0.5)	1.7 (0.7)	1.8 (0.7)	0.9 (0.3)	1.7 (0.5)	3.8 (3.2)
	Soil bulk density (g cm^{-3})					
0-20	1.35 (0.04)	1.43 (0.07)	1.15 (0.03)	1.18 (0.07)	1.15 (0.02)	1.15 (0.13)
20-40	1.63 (0.07)	1.46 (0.06)	1.38 (0.04)	1.46 (0.11)	1.37 (0.08)	1.24 (0.00)
40-100	1.54 (0.07)	1.45 (0.03)	1.31 (0.02)	1.48 (0.11)	1.45 (0.07)	1.23 (0.01)
100-150	1.49 (0.14)	1.41 (0.05)	1.29 (0.05)	1.45 (0.10)	1.40 (0.05)	1.27 (0.02)
150-200	1.47 (0.13)	1.43 (0.06)	1.26 (0.03)	1.48 (0.12)	1.36 (0.06)	1.25 (0.04)
200-300	1.53 (0.11)	1.46 (0.09)	1.28 (0.01)	1.51 (0.10)	1.34 (0.07)	1.26 (0.06)

Table 8-2. Reference soil mass (Mg ha^{-1}) of each fixed depth (cm). SF = secondary forest (1, 2 and 3 are the field replicates). Since I had three field replicates, the reference soil mass of a land use was its respective reference forest. For example, the reference soil mass of agriculture (AG) in replicate 1 was SF1, and the reference soil mass of agroforestry system advanced (AFSa) in replicate 2 was SF2.

	SF1	SF2	SF3
Depth (cm)	Reference soil mass (Mg ha^{-1})		
0-20	1,924	2,793	2,188
20-40	2,468	2,495	2,478
40-100	7,535	7,227	7,395
100-150	6,409	6,509	6,118
150-200	6,233	6,550	5,895
200-300	13,061	13,165	11,466

Table 8-3. Average values (standard error, n=3) of forest structure indicators. Ind: number of alive individuals per hectare. DBH: diameter at breast height. AFSi = agroforestry system in intermediate stage; AFSa = agroforestry system in advanced stage; RE = reforestation; NR = natural regeneration; SF = secondary forest.

	AFSi	AFSa	RE	NR	SF
Ind (nº/ha)	1,072 (59)	1,439 (204)	1,761 (318)	2,221 (871)	1,756 (297)
DBH (cm)	10.0 (0.7)	10.0 (0.2)	12.3 (0.5)	9.1 (1.3)	10.9 (0.9)
Height (m)	4.0 (0.7)	6.3 (0.8)	9.3 (0.8)	7.9 (1.1)	7.8 (0.3)

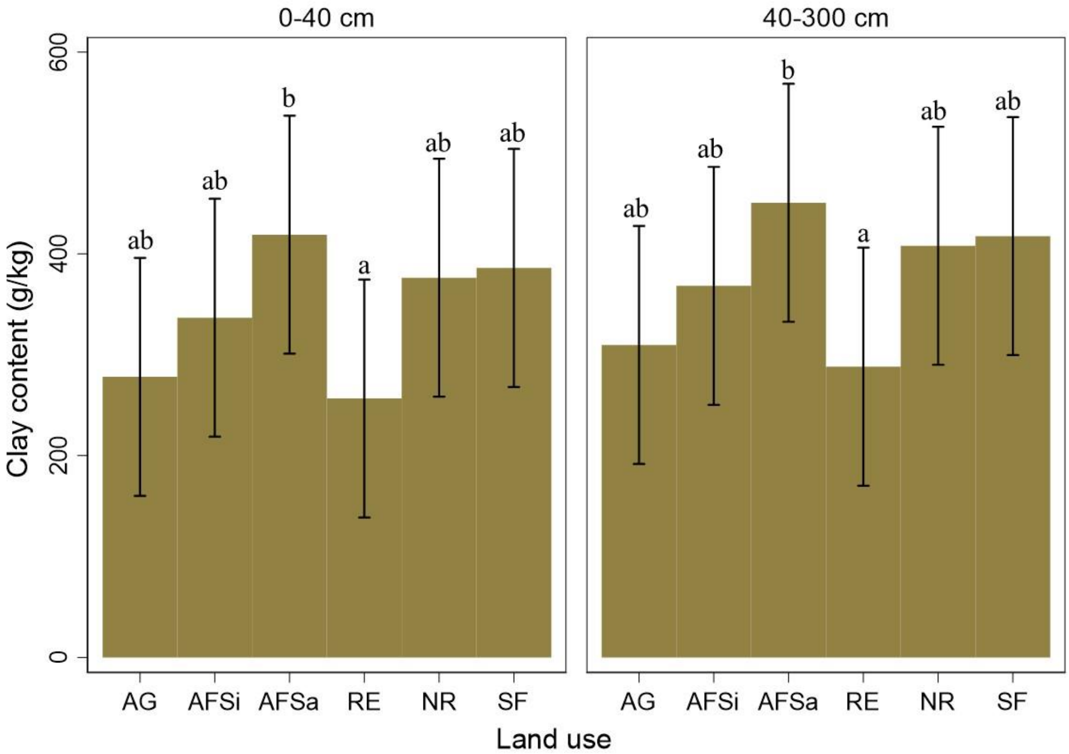

Figure 8-1. Clay content (g kg^{-1}) in all land uses assessed, in the topsoil (0-40cm) and deep soil (40-300cm). Error bars are 95% CI (n=3). Different letters indicate statistical difference within the same layer by LSD test ($p<0,05$). AG: Agriculture, AFSi: agroforestry system in intermediate stage, AFSa: agroforestry system in advanced stage, RE: reforestation, NR: natural regeneration

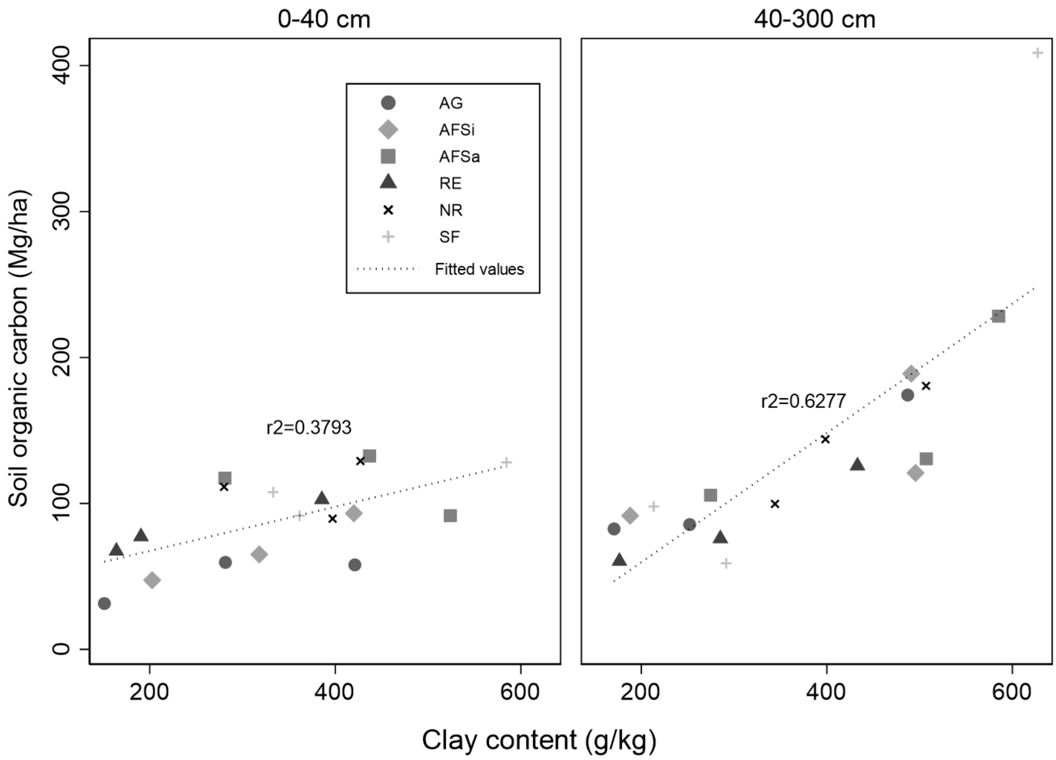

Figure 8-2. Scatterplot of SOC (Mg ha^{-1}) versus clay content (g kg^{-1}) in the topsoil (0-40cm) and deep soil (40-300cm).

Table 8-4. Average (standard error, n=3) fine root density (g cm^{-3}) in the studied land uses along the soil profile. AG: Agriculture, AFSi: agroforestry system in intermediate stage, AFSa: agroforestry system in advanced stage, RE: reforestation, NR: natural regeneration, SF: secondary forest.

Depth (cm)	AG	AFSi	AFSa	RE	NR	SF
0-20	0.421 (0.343)	0.889 (0.252)	1.920 (0.198)	2.391 (0.846)	1.716 (0.241)	2.254 (0.950)
20-40	0.056 (0.037)	0.232 (0.048)	0.511 (0.181)	1.013 (0.216)	0.452 (0.133)	0.876 (0.167)
40-100	0.009 (0.005)	0.058 (0.017)	0.223 (0.030)	0.420 (0.162)	0.220 (0.075)	0.388 (0.147)
100-150	0.006 (0.004)	0.073 (0.043)	0.096 (0.027)	0.136 (0.028)	0.354 (0.199)	0.113 (0.031)
150-200	0.004 (0.002)	0.039 (0.020)	0.108 (0.015)	0.213 (0.145)	0.078 (0.034)	0.179 (0.070)
200-300	0.007 (0.002)	0.014 (0.004)	0.049 (0.017)	0.056 (0.027)	0.127 (0.104)	0.066 (0.023)

9. Appendix C – Chapter 4

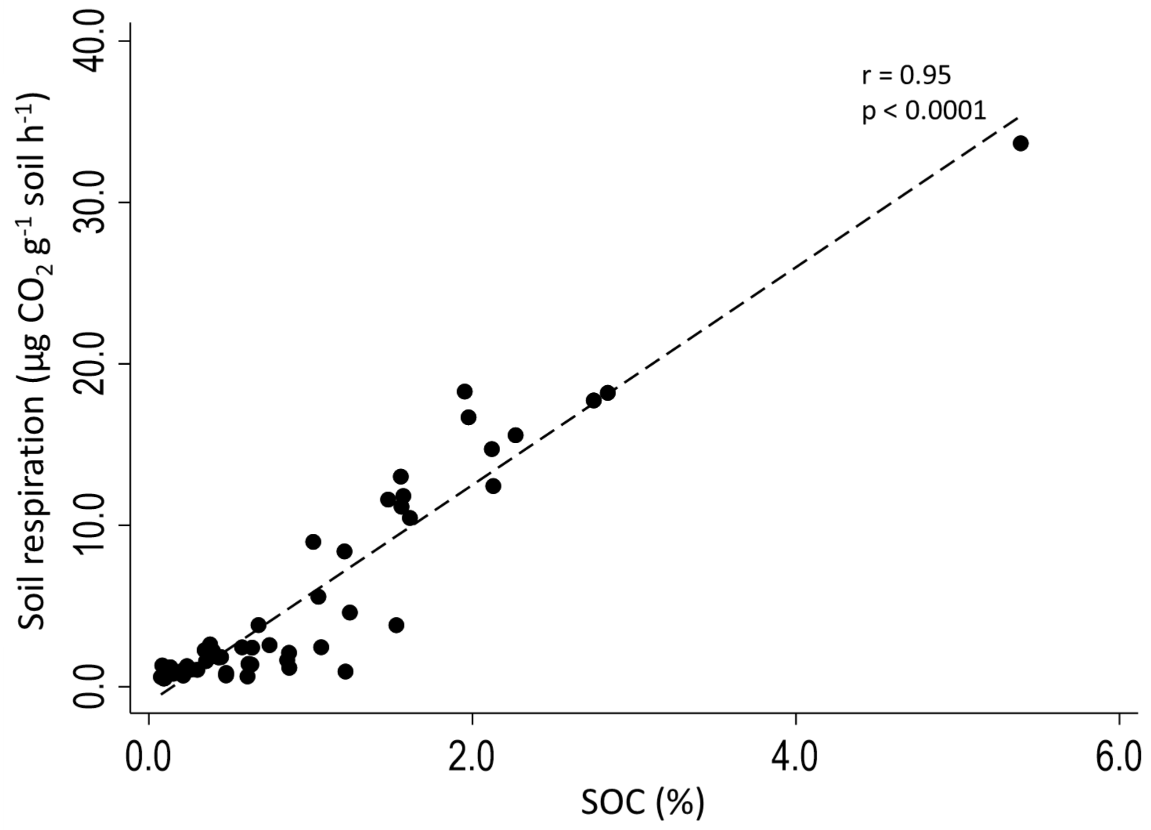

Figure 9-1. Relationship between soil respiration (µg CO_2 g^{-1} soil h^{-1}) and soil organic carbon concentration (SOC, in %) at 30 °C temperature. r and p are the Pearson correlation coefficient and p value, respectively.

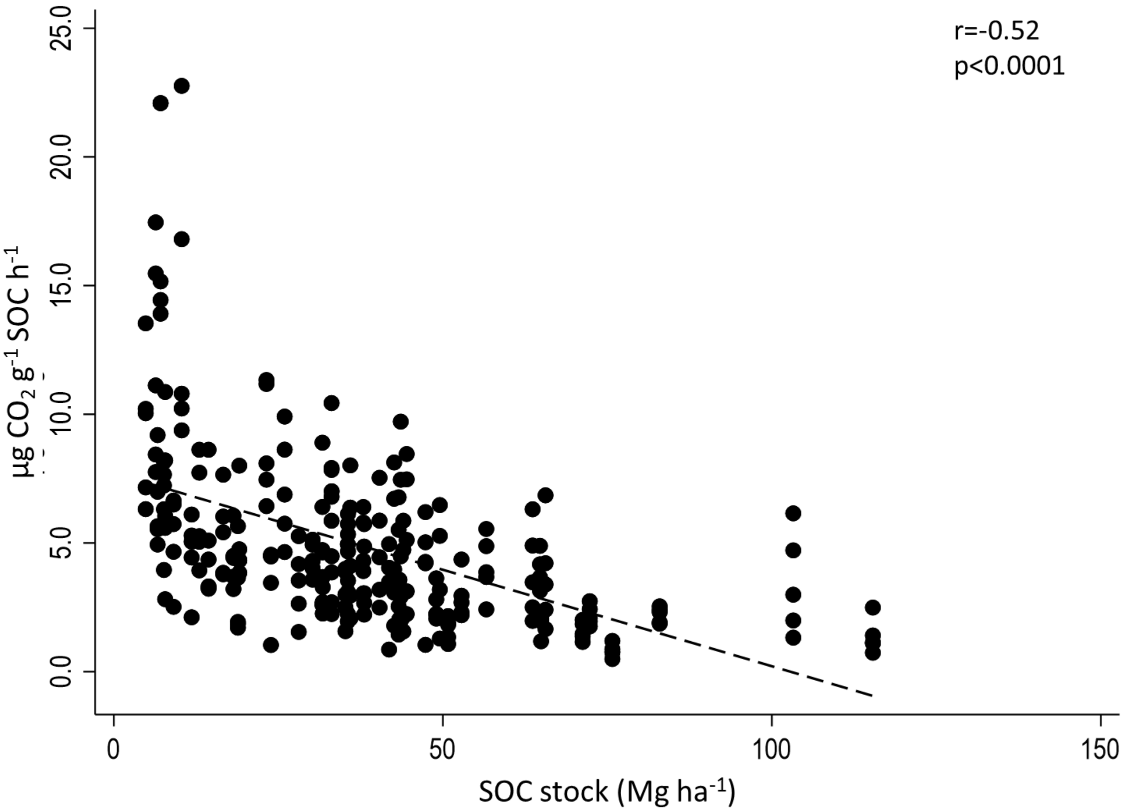

Figure 9-2. Relationship between soil specific respiration (µg CO_2 g^{-1} SOC h^{-1}) and soil organic carbon stock (Mg ha^{-1}) using combined data from topsoil (0-20 cm), subsoil (40-100 cm) and deep soil (150-200 cm) (all layers assessed in the study). r and p are the Pearson correlation coefficient and p value, respectively.

Table 9-1. Pearson correlation coefficient between soil specific respiration (µg CO_2 g^{-1} SOC h^{-1}) and different indicators depicting the restoration progress in the topsoil (0-20 cm), subsoil (40-100 cm) and deep soil (150-200 cm). SOC: soil organic carbon stock (Mg ha^{-1}); AGC: living aboveground carbon stock (Mg ha^{-1}); Litter: litter carbon stock (Mg ha^{-1}); Age: years after land use change. Correlations results highlighted in red are statistically significant.

Depth	SOC	AGC	Litter	Age
0-20 cm	-0.04 (p=0.7404)	0.01 (p=0.8928)	-0.03 (p=0.7719)	0.09 (p=0.3932)
40-100 cm	-0.51 (p<0.0001)	0.12 (p=0.2494)	-0.31 (p=0.0033)	-0.08 (p=0.4826)
150-200 cm	-0.47 (p<0.0001)	-0.25 (p=0.0190)	-0.44 (p<0.0001)	-0.35 (p=0.0006)

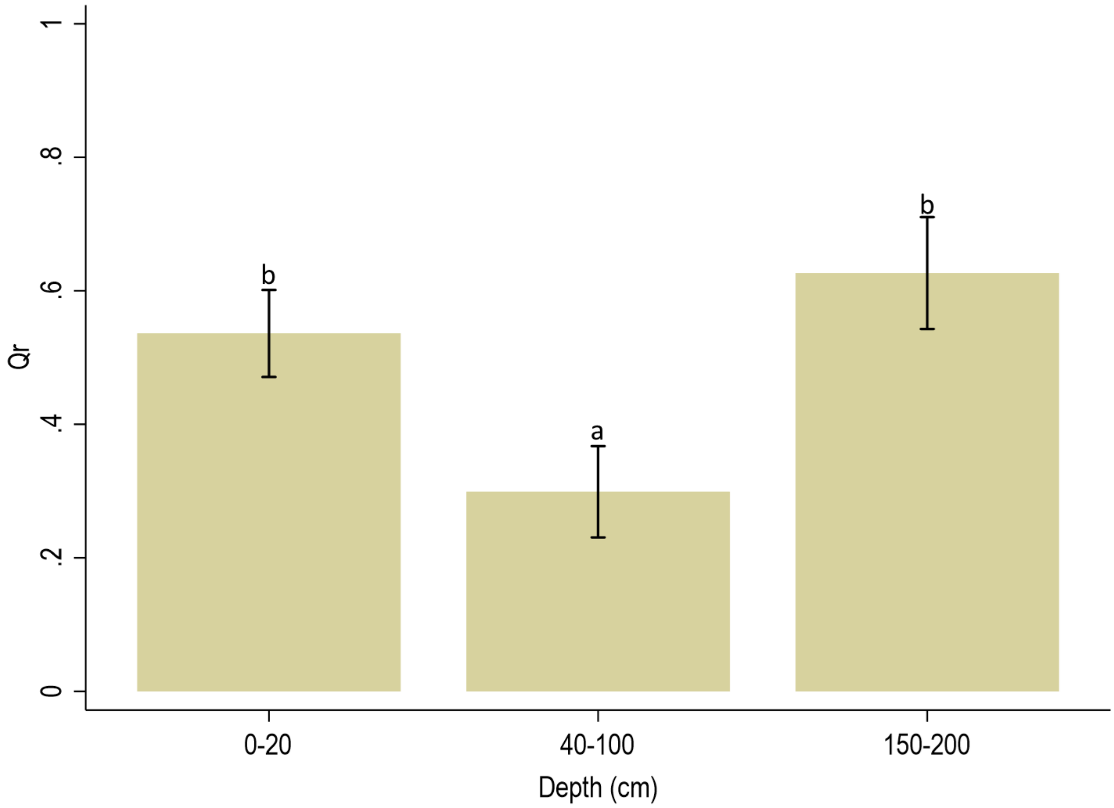

Figure 9-3. Basal-to-substrate induced respiration ratio (Qr) in the topsoil (0-20 cm), subsoil (40-100 cm) and deep soil (150-200 cm). Error bars are 95% confidence interval (n=18). Different letters indicate statistical difference among the depths by LSD post hoc test (p<0.05).

Table 9-2. Pearson correlation coefficient between soil temperature sensitivity (Q10) and different indicators depicting the restoration progress in the topsoil (0-20 cm), subsoil (40-100 cm) and deep soil (150-200 cm). SOC: soil organic carbon stock (Mg ha^{-1}); AGC: living aboveground carbon stock (Mg ha^{-1}); Litter: litter carbon stock (Mg ha^{-1}); Age: years after land use change. Correlations results highlighted in red are statistically significant.

Depth	SOC	AGC	Litter	Age
0-20 cm	0.45 (p=0.0622)	0.27. (p=0.2859)	0.39 (p=0.1140)	0.71 (p=0.0009)
40-100 cm	0.07 (p=0.7868)	-0.01 (p=0.9748)	-0.02 (p=0.9243)	0.13 (p=0.6093)
150-200 cm	0.13 (p=0.6050)	0.20 (p=0.4148)	0.21 (p=0.4043)	0.57 (p=0.0126)

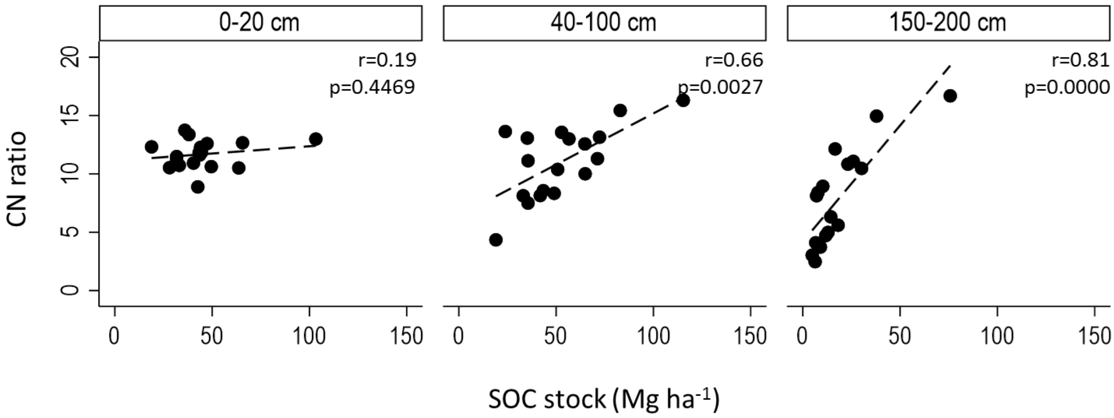

Figure 9-4. Relationship between soil CN ratio and soil organic carbon stock (SOC, Mg ha^{-1}) in the topsoil (0-20 cm), subsoil (40-100 cm) and deep soil (150-200 cm). r and p are the Pearson correlation coefficient and p value, respectively.