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PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA - CIÊNCIA DO SOLO

TESE

Indicadores de Qualidade do Solo em Sistemas Agroflorestais

Priscila Silva Matos

2021

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INSTITUTO DE AGRONOMIA
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INDICADORES DE QUALIDADE DO SOLO EM SISTEMAS AGROFLORESTAIS

PRISCILA SILVA MATOS

Sob a Orientação do Professor
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e Coorrientação do Professor
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Tese submetida como requisito parcial para obtenção do grau de **Doutora**, no Programa de Pós-Graduação em Agronomia - Ciência do Solo, Área de Concentração em Manejo do Solo e Qualidade Ambiental.

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TESE APROVADA EM: 31/08/2021.

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Felipe da Costa Brasil. Dr. UFRRJ

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RESUMO GERAL

MATOS, Priscila Silva. **Indicadores de qualidade do solo em Sistemas Agroflorestais**. 2021. 109f. Tese (Doutorado em Agronomia - Ciencia do Solo). Instituto de Agronomia, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, 2021.

Os sistemas agroflorestais, devido à sua multifuncionalidade, têm sido considerados uma excelente estratégia para aumentar a produção de alimentos, ao mesmo tempo, em que cumprem com os objetivos sociais e ambientais, atendendo aos objetivos do desenvolvimento sustentável (ODS), principalmente os ODS 13 e 15. Estes sistemas podem melhorar a qualidade do solo, aumentando a matéria orgânica do solo (MOS), alterando a estrutura do solo, a fertilidade e as propriedades biológicas. Os objetivos desta tese são: i) avaliar as propriedades químicas, físicas e biológicas do solo em uma pastagem não manejada, diferentes sistemas agroflorestais e floresta secundária; ii) compreender as relações entre a qualidade da serapilheira, matéria orgânica do solo (MOS) e principais parâmetros da qualidade do solo; e iii) Avaliar a sensibilidade de índices de qualidade do solo para detectar as diferenças causadas pela conversão do uso do solo. O solo, os macroinvertebrados e a serapilheira foram coletados em abril e setembro de 2018 sob cinco usos do solo, incluindo três sistemas agroflorestais, uma pastagem não manejada e uma floresta secundária em Sapucaia-RJ, Brasil. De acordo com os resultados, as correlações entre a qualidade da serapilheira, MOS e parâmetros do solo sugerem que as entradas de serapilheira de alta qualidade (isto é, baixa relação C: N) juntamente com a MOS são essenciais para estimular à atividade biológica. No segundo capítulo, se observa que as práticas de manejo influenciaram à esporulação de fungos micorrízicos arbusculares (FMA) e o número total de espécies em sistemas agroflorestais e que a comunidade de FMA está correlacionada com outros importantes parâmetros de solo. Além disso, a glomalina contribui para o aumento do conteúdo do carbono organico do solo (COS), principalmente em sistemas agroflorestais e em áreas de pastagem. No terceiro capítulo, foi constatado que os efeitos da adoção de sistemas agroflorestais nas frações de carbono do solo foram percebidos nas camadas mais superficiais (0-5, 5-10 cm), principalmente na fração de particulada. A sazonalidade influencia a dinâmica do COS e suas frações. O índice de manejo de carbono (IMC) foi sensível para detectar mudanças por mudança no uso do solo e mostrou que a pastagem acumula carbono no solo mesmo com sinais de degradação. No capítulo quatro, o indice Soil Management Assessment Framework (SMAF) foi sensível para detectar mudanças na qualidade do solo causadas pela conversao de usos do solo.

Palavras-chave: Sustentabilidade. Sistemas agrícolas. Saúde do solo.

GENERAL ABSTRACT

MATOS, Priscila Silva. **Soil quality indicators in Agroforestry Systems**. 2021. 109p. Thesis (Doctor in Agronomy - Soil Science). Instituto de Agronomia, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, 2021.

Agroforestry systems, due to their multifunctionality, have been considered an excellent strategy to increase food production while at the same time complying with social and environmental objectives, meeting the objectives of sustainable development (SDGs), especially the SDGs 13 and 15. These systems can improve soil quality by increasing soil organic matter (MOS), altering soil structure, fertility, and biological properties. The objectives of this thesis are: i) to evaluate the chemical, physical and biological properties of the soil in an unmanaged pasture, different agroforestry systems, and secondary forest; ii) understand the relationships between litter quality, SOM, and main soil quality parameters; and iii) Assess the sensitivity of soil quality indices to detect differences caused by land use conversion. Soil, macroinvertebrates, and litter were collected in April and September 2018 under five land use, including three agroforestry systems, an unmanaged pasture, and a secondary forest in Sapucaia-RJ, Brazil. The correlations between litter quality, SOM, and soil parameters suggest that high-quality litter inputs (i.e., low C: N ratio) along with SOM are essential to stimulate biological activity. In the second chapter, it is shown that management practices influenced arbuscular mycorrhizal fungi (AMF) sporulation and the total number of species in agroforestry systems, and that the AMF community is correlated with other essential soil parameters. In addition, glomalin contributes to increasing soil organic carbon (SOC) content, especially in agroforestry systems and pasture areas. In the third chapter, it is shown that the effects of adopting agroforestry systems on soil carbon fractions were perceived in the more superficial layers (0-5, 5-10 cm), mainly in the particulate fraction. Seasonality influences the dynamics of the SOC and its fractions. The carbon management index (CMI) was sensitive to detect changes due to changes in land use and showed that pasture accumulates carbon in the soil even with signs of degradation. In chapter four, the Soil Management Assessment Framework (SMAF) index was sensitive to detect changes in soil quality caused by land use conversion.

Key words: Sustainability. Agricultural systems. Soil health.

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SUMMARY

1 GENERAL INTRODUCTION

Agroforestry systems have been a central theme in political forums and agendas. They have received international scientific recognition for their contribution to sustainable development, including the Convention to Combat Desertification (UNCCD), Convention on Biological Diversity (CBD), United Nations Framework Convention on Climate Change (UNFCCC) and, the UN Decade (2021-2030) on Ecosystem Restoration (UN-DER). In 2015, Brazil signed the international climate change mitigation commitments (Paris agreement), known as Nationally Determined Contributions (NDCs). It declares the goals to reduce CO2 emissions by 43% by 2030, giving special attention to the recovery of degraded areas. Agroforestry systems have been classified as one of the low-carbon agriculture plan (Plano ABC+) strategies by the Brazilian government as an eligible form of land use to achieve these targets. In addition, several studies suggest agroforestry interventions for sustainable agriculture, the restoration and maintenance of soil health, and soil fertility (CHERUBIN et al., 2018; MATOS et al., 2020; AWAZI AND AVANA, 2020; TSUFAC et al., 2021).

The state of Rio de Janeiro has an excellent vocation for the development of sustainable agroforestry practices. The State of the Southeast Region with the highest percentage of Atlantic Forest still preserved and has been highlighted by the reduction in deforestation rates verified in recent years and having highly favorable geography for the development of agroforestry systems providing a diversified agriculture base and sustainable. The most significant remnants are found in conservation units, and the remainder is sprayed on rural properties (INEA, 2021). In this sense, INEA Resolution 134/2016 defines criteria and procedures for implementing, managing, and exploiting agroforestry systems and the practice of fallow in the State of Rio de Janeiro. Furthermore, Resolution 143/2017 that institutes the State System for Monitoring and Evaluation of Forest Restoration (SEMAR) and establishes guidelines, guidelines, and criteria on the preparation, execution, and monitoring of forest restoration projects in the state of Rio de Janeiro, for the which also applies to agroforestry systems which are considered a forest restoration technique.

Soil quality addresses the issues of productivity and sustainability simultaneously that’s why it has become so indispensable for developing countries. Land use type and agricultural management can be considered the significant factors that affect soil quality due to the change it brings on the soil’s physical, chemical, and biological properties (CARAVACA et al., 2002). Tese changed properties, in turn, affect land productivity. That is why Hartemink (2003) stated that soil degradation is the principal component of land degradation, and almost all land degradation is caused by soil degradation. Currently, Brazil is the second-largest global supplier of food and agricultural products, and the country is poised to take leadership when responding to additional global demand (FAO, 2015). However, the agricultural expansion to produce commodities leads to severe erosion of arable land, nutrient loss, some overgrazing, environmental problems, and loss of biodiversity. According to predictions from 2015 to 2070, Brazil will be substantially affected by soil erosion processes (BORRELI et al., 2020). Moreover, the manifold risks created by pollution, landslides, drought, and pandemics (e.g., COVID-19 in which recovery rates hypothetically correlate with a healthy diet and thus to soil quality, because soils with optimal nutrients, water, and air produce healthy crops) are aggravated by the skyrocketing human population, lifestyle changes, and inapt technology use (LANDRIGAN et al., 2018).

The increase in soil quality under agroforestry systems is mainly due to the litter deposition, responsible for significant inputs to the soil's contents of organic matter (OM)

(HERGOUALC'H et al., 2012; TUMWEBAZE et al., 2012). The quality of litter and the organic material deposited in the soil through pruning (common management practice in these systems) determines the carbon dynamics in these systems, which is an essential strategy for increasing soil carbon sequestration (MÜLLER & GAMA-RODRIGUES, 2012; CHAZDON, 2014). The assessment of chemical, physical and biological properties is crucial to assess the progression of soil quality as a function of adopting these systems.

In this context, the dissertation was built with the aim in the first chapter to address the correlations between litter quality and the biological, chemical, and physical properties of the soil and the organic matter of the soil and other key parameters. In the second chapter, we showed the results of how different land uses have influenced the community of arbuscular mycorrhizal fungi (AMF), glomalin production, and their relationship with other soil parameters. In the third chapter, we studied the responses of SOC content in different soil fractions to the short-term implementation of agroforestry systems and how seasonality can influence the dynamics of SOC and its fractions. Also, we tested if the carbon management index (CMI) is sensitive to detecting management practices quality across the unmanaged pasture, different agroforestry systems, and a reference area (forest). Finally, in chapter four, we proposed using a soil quality index, Soil Management Assessment Framework (SMAF), one of the most advanced analytical schemes to assess soil health for soil quality assessment in the evaluated systems.

2 CHAPTER I

LINKAGES AMONG SOIL PROPERTIES AND LITTER QUALITY IN AGROFORESTRY SYSTEMS OF SOUTHEASTERN BRAZIL

Article published in “MDPI - Sustainability Journal”: Matos, P. S.; Fonte, S. J.; Lima, S. S.; Pereira, M. G.; Kelly, C.; Damian, J. M.; Fontes, M.A.; Chaer, G.M.; Brasil, F.C.; Zonta, E. Linkages among Soil Properties and Litter Quality in Agroforestry Systems of Southeastern Brazil. Sustainability 2020, 12, 9752.

2.1 RESUMO

Os sistemas agroflorestais têm sido propostos como uma solução para lidar com o conflito entre os esforços para manter a conservação ambiental e a necessidade de aumento da produtividade agrícola nas pequenas propriedades no Brasil. No entanto, o impacto da mudança do uso do solo de pastagens degradadas para sistemas agroflorestais nas propriedades do solo permanece obscuro. Os objetivos desta pesquisa foram: 1) avaliar as propriedades químicas, físicas e biológicas do solo em diferentes usos da terra (pastagens degradadas, sistemas agroflorestais e floresta secundária); e 2) compreender as relações entre a qualidade da serapilheira, a matéria orgânica do solo (MOS) e os principais parâmetros de qualidade do solo na Mata Atlântica brasileira. O solo, os macroinvertebrados e a serapilheira foram coletados em abril e setembro de 2018 sob cinco usos do solo, incluindo: três tipos de sistemas agroflorestais, uma pastagem degradada e uma floresta secundária em Sapucaia-RJ, Brasil. Os resultados mostraram que as propriedades do solo separaram claramente os três sistemas agroflorestais (AS1, AS2, AS3) da floresta e pastagem. Além disso, a qualidade da serapilheira e a MOS provavelmente influenciam as propriedades biológicas e físico-químicas múltiplas do solo sob sistemas agroflorestais e floresta secundária. Os resultados sugerem que os sistemas agroflorestais podem melhorar as propriedades biológicas, químicas e físicas do solo e que a qualidade da serapilheira pode ser um importante impulsionador de seus efeitos e contribuições potenciais para a restauração do solo na região.

Palavras-chave: Relação C:N. Enzimas. Macroinvertebrados. Pasto. Restauração. Floresta secundária. Degradação do solo. Matéria orgânica do solo. Qualidade do solo.

2.2 ABSTRACT

Agroforestry systems have been promoted as a solution to address trade-offs between environmental conservation efforts and the need for increased agricultural productivity on smallholder farms in Brazil. However, the impact of land use change from degraded pasture to agroforestry on soil properties remains unclear. The objectives of this research were to: 1) assess soil chemical, physical and biological properties across distinct land uses (degraded pasture, agroforestry, and secondary forest); and 2) understand relationships between litter quality, soil organic matter (SOM), and key soil quality parameters in the Brazilian Atlantic Rainforest. Soils, macroinvertebrates and litter were collected in April and September of 2018 under five land uses, including: three types of agroforestry systems, a degraded pasture and a secondary forest in Sapucaia-RJ, Brazil. The results showed that soil properties clearly separated the three agroforestry systems plots (AS1, AS2, AS3) from the forest and pasture plots. Moreover, litter quality and SOM likely influence multiple biological and physiochemical soil properties under agroforestry systems and secondary forest. Thefindings suggest that agroforestry systems can help support soil biological, chemical and physical properties, and that the litter quality may be an important driver of their effects and potential contributions to soil restoration in the region.

Key words: C:N ratio. Enzymes. Macroinvertebrates. Pasture. Restoration. Secondary forest. Soil degradation. Soil organic matter. Soil quality.

2.3 INTRODUCTION

Degradation of agricultural lands around the globe threatens food security and the resilience of agricultural systems in the face of climate change (BRANCALION et al., 2019) In Brazil, data from the Ministry of the Environment indicate that there are about 52.3 million ha of degraded pasture (FGV, 2015), representing over half of the total pasture area in Brazil (DIAS FILHO, 2014; RIBEIRO-JUNIOR et al., 2017). The main driver of soil degradation in Brazilian agricultural lands is water erosion, followed by acidification, compaction, salinization, pollution, and desertification in semiarid areas (FAO, 2015). Agroforestry systems have been recommended as a means to restore degraded lands (SCHULZ, 2014; DAGAR, 2016; LEWIS et al., 2020), with some studies suggesting that agroforestry systems containing native tree species can help facilitate secondary succession, similar to what happens in secondary forests (SCHULZ, 2014; MORESSI et al., 2014; LENZ et al., 2019).

In 2015, Brazil signed the Paris Agreement and committed to a 47% reduction in greenhouse gas emissions by 2030. A substantial portion of these climate change mitigation commitments relies on highly ambitious targets - restoring 15 million ha of degraded forests and 12 million ha of degraded pastures. Among the technologies suggested in this plan are agroforestry systems for recovery of degraded pasture (UN, 2015). In addition to GHG reduction targets, recent environmental legislation (law number 12.651 / 12) requires rural landowners to maintain a portion of their lands (20% cover for areas within the Atlantic Forest Biome) with perennial vegetation cover (legal reservation). In this new legislation, agroforestry systems are recognized as a means to help farmers meet this requirement, while providing multiple socio-economic benefits.

Different types of agroforestry systems and practices lead to varying impacts on ecosystem services and soil quality. Simple agroforestry systems (with few tree species) may not meet restoration criteria as established by Brazilian law due to low levels of biodiversity and structural complexity that may not adequately provide the desired level of ecosystem services. Other more complex systems can be quite effective in supporting a range of ecological and economic functions (MMA, 2005). In this regard, agroforestry systems with high biodiversity or ‘successional’ agroforests are often preferred over simpler farming systems. Some studies indicate positive effects of high species diversity and functional heterogeneity in agroforestry systems on soil chemical, physical and biological properties (BINI et al., 2013; WARTENBERG et al., 2017; CHEN et al., 2019); however, evaluation of different agroforestry systems remains scarce and merits further research.

More complex agroforestry systems exhibit notable similarities to natural forests due to their extensive tree cover and presence of a more developed litter layer (OLIVEIRA et al., 2018). Litter deposition in agroforestry systems is critical for maintenance soil organic matter (SOM) (HERGOUALC’H et al., 2012; TUMWEBAZE et al., 2012). Leguminous species that are generally used in agroforestry systems contribute to natural regeneration because of their association with nitrogen (N) fixing bacteria. Nitrogen fixing plants increase the performance and fertility of agroforest soils by producing high quality leaf litter (i.e., low C:N ratio), which favors the release of N to the soil (DUARTE et al., 2013). Practices associated with mixed agroforestry systems, such as the inclusion of vegetation that is structurally and taxonomically diverse, as well as continuous soil cover, are often associated with soil biological activity, including enhanced abundance and diversity of soil macrofauna (ROUSSEAU et al., 2013; KAMAU et al., 2017). Additionally, agroforestry systems can contribute to C sequestration in agricultural lands via storage of C in tree biomass and SOM (SHI et al., 2018; ZARO et al., 2019; KEARNEY et al., 2019).

Given the current widespread conversion of degraded pastures to agroforestry systems across Brazil, it is imperative to more fully understand how agroforestry systems may influence overall soil health and fertility (JIAN et al., 2020). This shift to agroforestry systems is likely associated with improved nutrient cycling and greater soil biological activity, with implications for multiple soil functions, but more research is needed. To address this knowledge gap, this study considered an experimental farm in southern Brazil, where diverse management practices had been implemented on plots with similar soil properties and management history prior to the establishment of three distinct agroforestry systems. In this context, we aimed to: 1) assess soil chemical, physical and biological properties across degraded pasture, different agroforestry systems and secondary forest, and 2) understand relationships between litter quality, SOM, and key soil quality parameters.

2.4 MATERIAL AND METHODS

2.4.1 Site description and land uses

This study was conducted at the Arca de Noé Farm, an agroecological research station located near the city of Sapucaia, Rio de Janeiro, Brazil (21º 59' 42" S, 42º 54' 52" W; Figure 1). At roughly 800 m elevation, the site is characterized by dry winters and temperate summers (Cwb in the Köppen Climate Classification system), with mean monthly temperatures that vary between 17°C and 32°C (June and January; respectively) and a mean annual rainfall of 1,451 mm. Soils at this site are predominantly Ultisols (USDA, 2014) with a clay-loam texture. The region is largely comprised of massifs of highland hills and cliffs, with a natural vegetation generally dominated by the Atlantic Forest, which is characterized as Dense Ombrophylous Forest (IBGE, 2012).

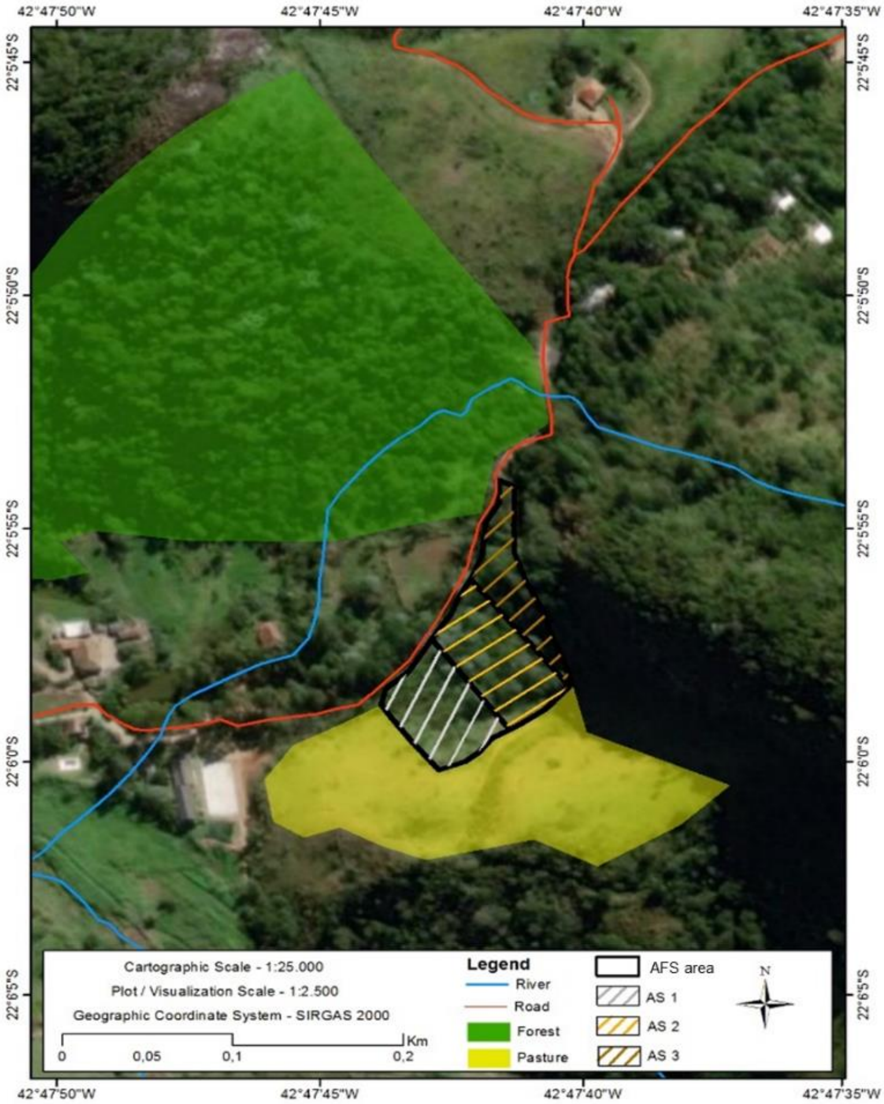

Figure 1. Arial view of the agroecological experimental station Arca de Noe Farm with the five studied land uses overlaid on top of the image. The farm is in the county of Sapucaia – RJ, Brazil.

In this study, we considered five existing land uses on the farm (Figure 1): 1) secondary forest (FOREST) dominated by semi-deciduous tree species (*Tibouchina granulosa*, *Lecythis pisonis* Cambess., *Centrolobium tomentosum* Guillem. ex Benth., *Inga* spp., *Schizolobium parahyba* (Vell.) Blake, *Peltophorum dubium*, *Hymenaea courbaril*, *Aspidosperma olivaceum* Müll. Arg., *Dalbergia nigra* (Vell.) Allemão ex Benth.); 2) pasture replanted with the grass *Urochloa decumbens*, under extensive grazing (PASTURE); 3) an agroforestry system characterized by the integration of banana and coffee with a mix of other fruit and timber species and other species to provide shade, biomass production, and pollination services (AS1); 4) an agroforestry system focused on bananas and energy production (which also includes fruit trees and a mix of other trees and plants (AS2); and 5) a third agroforestry system focused on bananas and other fruits (AS3; see Table 1 for detailed species lists). Pasture was established by removal of native vegetation in 1995. In 2010, the agroforestry systems were planted on a portion of this existing pasture. These systems received a single application of rock phosphate (fertilizer permitted in organic production) and cattle manure to the banana tree roots at the time of establishment. The secondary forest was included here as a reference and had an age of about 30 years since previous deforestation. All plots considered in this study were located on the same soil type and textural class and had similar slopes of roughly 30º.

Table 1. Plant species present in each of the three agroforestry systems established in 2010 at the Arca de Noé farm, Sapucaia, Rio de Janeiro state, Brazil.

System	Scientific name	Family	Commom name	Function	Introduction
AS1	*Musa paradisiaca* L.	Musaceae	banana	fruit production	planted
	Coffea arabica	Rubiaceae	coffee	grain production	planted
	Carica papaya L.	Caricaceae	papaya	fruit production	planted
	Morus nigra L.	Moraceae	black mulberry	fruit production	planted
	Eugenia uniflora L.	Myrtaceae	Brazilian cherry	fruit production	planted
	Inga edulis Mart.	Fabaceae	ice-cream bean	fruit production	planted
	Myrciaria glazioviana (Kiaersk.) G. M. Barroso ex Sobral	Myrtaceae	“cabeludinha”*	fruit production	planted
	Malpighia glabra L.	Malpighiaceae	“acerola”*	fruit production	planted
	Mangifera indica L.	Anacardiaceae	mango	fruit production	planted
	Psidium guajava L.	Myrtaceae	guava	fruit production	natural/ regenerated
	Hymenaea courbaril L.	Fabaceae	“jatobá”*	timber production	planted
	Tithonia diversifolia (Hemsl.) A. Gray	Arecaceae	mexican-sunflower	biomass production	planted

Be continued…

Table 1 - Continuation.

System	Scientific name	Family	Commom name	Function	Introduction
AS1	*Solanum mauritianum* Scop	Solanaceae	“fumo-bravo”*	shade and biomass production	natural/ regenerated
	Trema micrantha (L.) Blume	Cannabaceae	“trema”*	shade and biomass production	natural/ regenerated
	Sapium glandulatum (Vell.) Pax	Euphorbiaceae	“burra-leiteira”*	shade and biomass production	natural/ regenerated
	Vernonia polycephala Less.	Asteraceae	“assa-peixe”*	pollination services	natural/ regenerated
AS2	*Musa acuminata*	Musaceae	banana	fruit production	planted
	*Musa paradisiac*a L.	Musaceae	banana	fruit production	planted
	Jatropha curcas L.	Euphorbiaceae	physic nut	energy production	planted
	Persea Americana Mill.	Lauraceae	avocado	fruit production	planted
	Morus nigra L.	Moraceae	black mulberry	fruit and biomass production	planted
	Inga edulis	Fabaceae	ice-cream bean	fruit and biomass production	planted
	Eriobotrya japonica (Thunb.) Lindl.	Rosaceae	“nêspera”*	fruit production	planted
	*Carica papay*a L.	Caricaceae	papaya	fruit production	planted
	Mangifera indica L.	Anacardiaceae	mango	fruit production	planted
	Eugenia uniflora	Myrtaceae	cherry	fruit production	planted
	Tithonia diversifolia (Hemsl.) A. Gray	Arecaceae	Mexican-sunflower	biomass production	planted
	Solanum mauritianum Scop	Solanaceae	“fumo-bravo”*	shade and biomass production	natural/ regenerated*
	Piper aduncum L.	Piperaceae	“aperta-ruão”*	biomass production	natural/regen erated
	Vernonia polycephala Less.	Asteraceae	“assa-peixe”*	pollination services	natural/regen erated
AS3	*Musa acuminata*	Musaceae	banana	fruit production	planted
	Musa paradisiaca L.	Musaceae	banana	fruit production	planted
	Mangifera indica L.	Anacardiaceae	mango	fruit production	planted

Be continued…

Table 1 - Continuation.

System	Scientific name	Family	Commom name	Function	Introduction
AS3	*Artocarpus heterophyllus*	Moraceae	jack fruit	fruit production	planted
	Citrus sp.	Rutaceae	cravo lemon	fruit production	planted
	Plinia trunciflora (O. Berg) Kausel	Myrtaceae	jabuticaba	fruit production	planted
	Campomanesia phaea (O. Berg.) Landrum	Myrtaceae	cambuci	fruit production	planted
	Solanum mauritianum Scop	Solanaceae	“fumo-bravo”*	shade and biomass production	natural/ regenerated
	Piper aduncum L.	Piperaceae	“aperta-ruão”*	biomass production	natural/ regenerated
	Tithonia diversifolia (Hemsl.) A. Gray	Arecaceae	mexican-sunflower	biomass production	planted

*natural / regenerated: species that were already in the area when the system was implemented. Species identified according with Flora do Brasil (2018).

2.4.2 Soil, litter and arthropod sampling

Sampling was conducted in 2018 at two separate time points, rainy season (April) and dry season (September), to assess a suite of soil biological, chemical, and physical properties within each land use (e.g., forest, pasture and agroforestry systems). One transect was laid out in each of the land use types, and four sampling plots (6 × 8 m) were established approximately 15 m apart along the transect. The four plots within each land use were considered replications. While we recognize that the lack of true replication limits interpretation of our findings, we note that similar sampling designs have been used in previous studies evaluating the effect of land use on soil properties (ASSUNÇÃO et al., 2019; FERREIRA et al., 2020; FRANCAVIGLIA et al., 2017; LAGOMARSINO et al., 2011) and that this approach is important for studying unique management systems where replicated, randomized field trials are not feasible. Within each sampling plot, four sub-samples of soil (0-10 cm depth) were collected using a shovel (~5 m spacing between sub-samples) and combined to generate one composite sample per sampling plot per season. A portion of each composite sample was kept cool for transport to the laboratory at the Federal Rural University of Rio de Janeiro (Seropédica, Brazil), where it was stored at 4°C (for <2 weeks) until analysis of microbiological parameters. The rest of each composite sample was air-dried, sieved to 2 mm, and analyzed for chemical properties.

Physical parameters were evaluated only in the rainy season (April). Bulk density (BD) was measured at four sub-samples per sampling point by inserting a metal cylinder ring (5 cm diameter) vertically into the soil to a 10 cm depth. Soil from within each ring was returned to the lab for separation into soil, rocks and large roots, and then dried at 105°C, and weighed (TEIXEIRA et al., 2017). For evaluation of water-stable aggregation, four soil cores (10 cm diameter) were collected to a depth of 10 cm in each sampling plot and combined into one composite sample. Field moist soil was passed through an 8 mm sieve by gently breaking soil clods along natural planes of fracture, and then air-dried for subsequent analyses.

The litter and soil dwelling arthropods were evaluated at both sampling times using pitfall traps adapted from Moldenke (1994). The traps consisted of plastic containers (10 cm diameter) that were inserted 10 cm deep into the soil such that they were level with the surface level. Two replicate traps were installed in each plot, for a total of eight traps per land use system per sampling time. For both sampling times, traps remained in the field during a 9-day period and specimens collected in each trap were returned to the lab, stored in 70% ethanol and identified to the level of order, class or family, based on Gallo (1988) and Dindal (1990). Abundance for each taxa was averaged across two traps in each sampling plot and reported as individuals $trap^{-1}$ day^{-1}. Diversity was evaluated using both species richness (S, number of taxonomic groups) and the Shannon Index (H) (SHANNON, 1948) for each plot considering the number of unique taxonomic groups encountered by the two traps per plot, that is average number of species per sampling plot in each land use system. A total of 40 traps were used to assess litter and soil dwelling arthropods in all land uses at each sampling time. The litter biomass was quantified by collecting the organic material at the soil surface that had not decomposed following the procedures proposed by Sanqueta (2002). A wooden square with internal area of 0.25 m^2 was placed ~1 m away from the pitfall traps within each sampling plot, for a total of eight samples per land use system at each sampling time. This material was dried at 65°C to constant weight, weighed and crushed in a mill for further chemical analysis.

2.4.3 Soil microbial measurements

For analysis of microbial biomass, Microbial Biomass Carbon (MBC) and Microbial Biomass Nitrogen (MBN), refrigerated soil (stored at 4°C) was passed through a 2 mm sieve and two sub-samples (20g each) were weighed for each sampling point. One of these sub-samples was fumigated with chloroform by 24 h and then shaken for 30 min with K_2SO_4 (0.5 mol L^{-1}), while the other was not fumigated and submitted to the same extraction procedure (VANCE, 1987; TATE et al., 1988). The estimation of C in microbial biomass was done with colorimetric determination (BARTLETT; ROSS, 1988). Quantification of N in microbial biomass was performed according to the methods of Brookes et al. (BROOKES et al., 1985) by steam distillation (Kjeldahl), followed by acid-base volumetry with sulfuric acid as a titrator.

Microbial activity, Soil basal respiration (Sbresp), for each sampling plot was assessed using soil respiration on duplicate 50 g sub-samples of refrigerated soil (JENKINSON; POWLSON, 1976). Samples were stored in 100 ml flasks and incubated in glass jars with volume of 3 L together with 10 ml of 1 mol L^{-1} NaOH solution for 143 h (April) and 162 h (September). After the incubation period, the CO_2 trapped by the NaOH solution was precipitated with 2 ml of barium chloride (10%) in water and titrated with HCL (0.5 mol L^{-1}), using phenolphthalein (1%) as an indicator in alcoholic medium. The values of accumulated CO_2 were expressed in µg of C per g of dry soil.

Enzyme activity was assessed via quantification of β-glucosidase (C-cycle) and acid phosphatase (P-cycle). In addition, the total enzyme activity was evaluated by analyzing the hydrolysis of fluorescein diacetate (FDA). β-glucosidase activity was analyzed according to (TABATABAI, 1994), using 1.0 g of fresh soil and the substrate p-nitrophenyl-β-D-glucoside (0.05 mol L^{-1}). Analysis of acid phosphatase activity was conducted with 1.0 g fresh soil, using p-nitrophenyl-sulfate as a substrate (0.05 moI L^{-1}) (TABATABAI, 1994). Colorimetric determination of was conducted in a spectrophotometer at 410 nm. Results were expressed in µmol·g-1 h-1 p-nitrophenyl. Analysis of fluorescein diacetate (FDA) hydrolysis was conducted according to Schnürer and Rosswall (SCHNURER, 1982) and modified by Dick et al. (1996), using 1.0 g soil fresh and FDA stock solution. Samples were read in a spectrophotometer at 490 nm to determine the amount of hydrolyzed fluorescein and results expressed in µg fluorescein g^{-1} soil h^{-1}.

2.4.4 Soil physicochemical analyses

Soil organic carbon (SOC) was quantified by the oxidation of organic matter using a solution of potassium dichromate in acid medium, with an external source of heat (YEOMANS; BREMNER, 1988). Available P and K were evaluated using a Mehlich-1 extractant (H_2SO_4 0.0125 mol L^{-1} + HCl 0.05 mol L^{-1}), while exchangeable Ca^{2+}, Mg^{2+} and Al^{3+} were extracted with KCl (1 mol L^{-1}). The concentrations of these elements in the soil samples were determined by titration (P, Al^{3+}, K, Ca^{2+} and Mg^{2+}). Cation exchange capacity (CEC; cmolc kg^{-1}) and pH was analyzed in a 1:5 suspension of soil and deionized water (TEIXEIRA et al., 2017) Analysis of permanganate oxidizable carbon (POXC) was conducted on 2.5 g air-dried soil based on the method of (YEOMANS; BREMNER, 1988).

Water-stable aggregation (WSA) was determined using a Yoder wet-sieving apparatus (Yoder, 1936). For the evaluation of the aggregate distribution, 25 g of the air-dried, 8-mm sieved soil was transferred to the top of a set of sieves with 2.00, 1.00, 0.50, 0.25 and 0.105 mm mesh sizes, moistened with spray and subjected to vertical oscillation in the Yoder apparatus, for 15 min. The material retained on each sieve was then rinsed into separate Petri dishes and dried in an oven at 65°C. Mean weight diameter (MWD) of the aggregates was calculated according to van Bavel (1950) by summing the proportions of soil in each size class multiplied by the corresponding average size of aggregates in each class.

Soil texture was determined by the pipette method (TEIXEIRA et al., 2017). In the first step, chemical dispersion was performed using NaOH 0.1 mol L^{-1} as a dispersing agent, following the methodology used by Ruiz (2005). The second step consisted of mechanical dispersion by shaking at 60 rpm for a period of 16 hours. Total clay (diameter <0.002 mm) and sand (diameter 2 to 0.05 mm) contents were obtained, respectively, by pipetting and sieving, while the silt content (diameter between 0.05 to 0.002 mm) was calculated by the difference.

2.4.5 Litter nutrient analysis

Litter samples were ground and evaluated for total C and N by dry combustion of 5.0 ± 0.1 mg samples using an elemental analyzer Perkin Elmer 2400 CHNS. Litter macro- and micronutrient concentrations were evaluated via the digestion method USEPA 3051A, which was conducted in a closed system using microwave radiation in a MARS Xpress® device. All analyses were performed in triplicate and used high purity acids (P.A.) and Milli-Q water for dilution. The concentrations of P, K, Mg and Ca in the extracts resulting from digestion were determined P by colorimetry, K by flame photometry, Ca and Mg by atomic absorption spectrometry using a Varian SpectrAA 55B device.

2.4.6 Statistical analyses

One-way ANOVA with Tukey tests were used to compare soil properties and macrofauna communities among the five land uses separately for the rainy season (April) and dry season (September) sampling times. Data was ln transformed as needed to meet the assumptions of homoscedasticity and normality. All univariate tests were carried out using R statistical software (R Development Core Team, 2019). Given that the five land uses were not replicated across multiple treatment plots, we considered the four sampling plots within each management type as replicates to explore differences between land use plots. While we understand that that experimental design limits broad causal inferences about management, valuable insight can be gained by exploring differences between the land uses and relationships between the variables measured.

For each data set (soil chemical, physical properties, and microbiological properties), principle component analysis (PCA) together with between-class PCA were used to explore relationships between variables within a data set and multivariate differences between land uses at each time point. Highly collinear variables were omitted from the PCA and associated land use comparisons. Ordination and visualization of soil fauna communities was conducted using nonmetric multidimensional scaling (NMDS). Bray-Curtis distances were calculated between samples using the dominant soil taxa. Nonmetric multidimensional scaling (NMDS) ordinations were plotted for the distance matrices, and correlations between environmental variables and NMDS axes were calculated and included as arrows in plots if significant ($P < 0.05$). Treatment effects were tested using the ADONIS method of permutational multivariate analysis with 999 permutations. All multivariate analyses were completed in R using the vegan package (OKSANEN et al., 2018) and ade4 library within the R environment (DRAY et al. 2007).

In order to understand the role of SOM and litter quality in driving multiple soil quality parameters, we used multiple linear regression with each soil variable as the response, and the following model terms: SOM, sampling time, and the sampling time by SOM interaction. Analysis was repeated with litter C:N ratio replacing SOM. With these models we were able to understand the relationships between SOM, litter C:N, and multiple soil biological, chemical and physical parameters, while to accounting for differences between the April and September evaluations. Data were ln transformed as need to meet model assumptions and these analyses were conducted in JMP 14.0 statistical software (SAS, 2018).

2.5 RESULTS

2.5.1 Soil chemical and physical properties

The land use plots differed significantly in soil physical and chemical properties for both seasons evaluated. In the rainy season, the agroforestry systems generally contained higher levels of SOM, available P, Ca, Mg, K, CEC, pH than soils under forest or pasture management. In the dry season the same tendencies were apparent, but differences were only significant for available P, Ca and Mg (Table 2). Additionally, pH tended to be lower and Al^{3+} levels higher in pasture and forest. The pasture system also presented higher bulk density than the agroforestry systems and secondary forest. Aggregate stability (MWD) in the rainy season was generally higher in the forest compared to the other land uses, but only significantly higher than AS3 (Table 2).

Table 2. Mean values for soil chemical and physical properties sampled on an experimental farm in the county of Sapucaia – RJ, Brazil at two time points, in the rainy season (April) and dry season (September) of 2018.

	Rainy Season (April)						Dry Season (September)					
Soil variable	**Forest**	**Pasture**	**AS1**	**AS2**	**AS3**	***p-value***	**Forest**	**Pasture**	**AS1**	**AS2**	**AS3**	***p-value***
pH	4.5^{c}	4.6^{c}	5.7^{a}	5.3^{ab}	5.2^{b}	< 0.001	4.5^{a}	4.8^{a}	5.1^{a}	5.2^{a}	4.8^{a}	
	0.1	*0.1*	*0.3*	*0.2*	*0.1*		*0.4*	*0.1*	*0.2*	*0.4*	*0.4*	
SOC (g kg^{-1})	23.5^{ab}	23.1^{b}	28.0^{a}	25.3^{ab}	26.4^{ab}	0.039	21.6^{a}	21.4^{a}	24.2^{a}	22.6^{a}	24.5^{a}	
	0.44	*1.9*	*2.26*	*2.12*	*3.4*		*0.858*	*1.14*	*4.46*	*4.45*	*1.13*	
POXC (mg kg^{-1})	653^{a}	653^{a}	788^{a}	1092^{a}	826^{a}		786^{ab}	576^{b}	1121^{a}	747^{ab}	660^{b}	0.023
	333	*371*	*126*	*112*	*197*		*234*	*101*	*38.7*	*225*	*323*	
Avail.P (mg kg^{-1})	26.5^{ab}	22.5^{b}	29.5^{a}	31.5^{a}	28.5^{a}	0.002	20.4^{bc}	18.8^{c}	25.6^{a}	27.7^{a}	24.2^{ab}	< 0.001
	0.6	*0.6*	*4.7*	*3.1*	*1*		*2.4*	*1.03*	*2.3*	*1.6*	*1.1*	
Ca^{2+}(meq/100mg)	0.8^{b}	0.6^{b}	3^{a}	2.9^{a}	3^{a}	< 0.001	0.8^{c}	0.7^{c}	2.4^{b}	4.4^{a}	3.4^{ab}	< 0.001
	0.2	*0.1*	*0.4*	*0.7*	*0.4*		*0.3*	*0.2*	*0.3*	*0.9*	*0.6*	
Mg^{2+}(meq/100mg)	0.7^{b}	0.5^{b}	2.0^{a}	2.0^{a}	2.1^{a}	< 0.001	0.7^{b}	0.4^{b}	2.2^{a}	1.7^{a}	1.4^{a}	< 0.001
	0.2	*0.1*	*0.2*	*0.2*	*0.3*		*0.3*	*0.1*	*0.1*	*0.5*	*0.1*	
K^{+}(meq/100mg)	0.2^{d}	0.1^{d}	0.6^{b}	0.5^{c}	0.7^{a}	< 0.001	0.2^{c}	0.1^{c}	0.6^{a}	0.5^{a}	0.4^{b}	< 0.001
	0.08	*0.01*	*0.08*	*0.03*	*0.04*		*0.1*	*0.02*	*0.1*	*0.1*	*0.04*	
Na^{+}(meq/100mg)	0.1^{a}	0.1^{a}	0.1^{a}	0.1^{a}	0.1^{a}		0.03^{b}	0.04^{a}	0.04^{a}	0.04^{a}	0.03^{ab}	< 0.001
	0	*0.01*	*0.01*	*0*	*0.01*		*0.01*	*0.04*	*0.01*	*0*	*0.01*	
Al^{3+}(meq/100mg)	1.5^{a}	1.6^{a}	0.3^{b}	0.3^{b}	0.2b	< 0.001	1.7^{a}	1.6^{a}	0.3^{c}	0.4^{b}	0.4^{bc}	< 0.001
	0.2	*0.06*	*0.2*	*0.2*	*0.2*		*0.4*	*0.1*	*0.1*	*0.1*	*0.1*	
CEC (cmol/Kg)	16.9^{ab}	15.8^{b}	16.7^{ab}	18.2^{ab}	19.0^{a}	0.019	16.5^{a}	14.2^{a}	13.8^{a}	16.1^{a}	16.1^{a}	
	1	*0.9*	*2.1*	*1.2*	*0.8*		*1.5*	*0.5*	*1.4*	*1.4*	*1.4*	
BD (g m^{-3})	1.5^{b}	1.8^{a}	1.6^{b}	1.5^{b}	1.5^{b}	< 0.001						
	0.04	*0.04*	*0.1*	*0.1*	*0.04*							
Clay (%)	28.1^{a}	30.3^{a}	30.5^{a}	33.8^{a}	31.4^{a}							
	3.3	*3.3*	*1.1*	*5.1*	*4.7*							
Sand (%)	55.8^{a}	55.7^{a}	40.01^{a}	46.9^{a}	49.6^{a}							
	14.1	*5.9*	*3.8*	*5*	*8.2*							
MWD (mm)	4.7^{a}	4.5^{ab}	4.5^{ab}	4.5^{ab}	4.3^{b}	0.025						
	0.04	*0.1*	*0.3*	*0.02*	*0.1*							

Values in italics below each mean represent the standard error from four measurements in each plot. Means with different letters have significantly different values according to Tukey tests. Abbreviations: Avail., available; SOC, soil organic carbon; POXC, permanganate oxidizable carbon, CEC, cation exchange capacity; BD, bulk density; MWD, mean weight diameter; AS1: agroforestry system 1; AS2:agroforestry system 2; AS3: agroforestry system 3.

Similar to the findings from ANOVA, multivariate differences, using between-class PCA, of soil chemical and physical properties clearly separated soils in the three agroforestry systems plots (AS1, AS2, AS3) from the forest and pasture plots in rainy season (P = 0.001 by Monte Carlo Permutation test; 56.32% of randtest observation; Figure 2A). In the rainy season, the first and second principal component (PC) axes explained 39.72% and 20.87% of the variability, respectively. The agroforestry systems plots were separated from the other land uses mainly along PC1 and were associated with higher values of pH, P, and SOC, while forest was associated with higher MWD, and pasture was associated with higher values of BD (Figure 2A). In Dry season PC 1 was associated with available P, pH and soil texture and explained 43.67% of the variability, while PC 2 was associated with SOC and CEC explained 20.66% of the variability. The agroforestry systems were associated higher values of pH, P and clay and mainly separated from the forest and pasture plots along PC 1 (P = 0.002 by Monte Carlo Permutation test; 43.47% of randtest observation; Figure 2B).

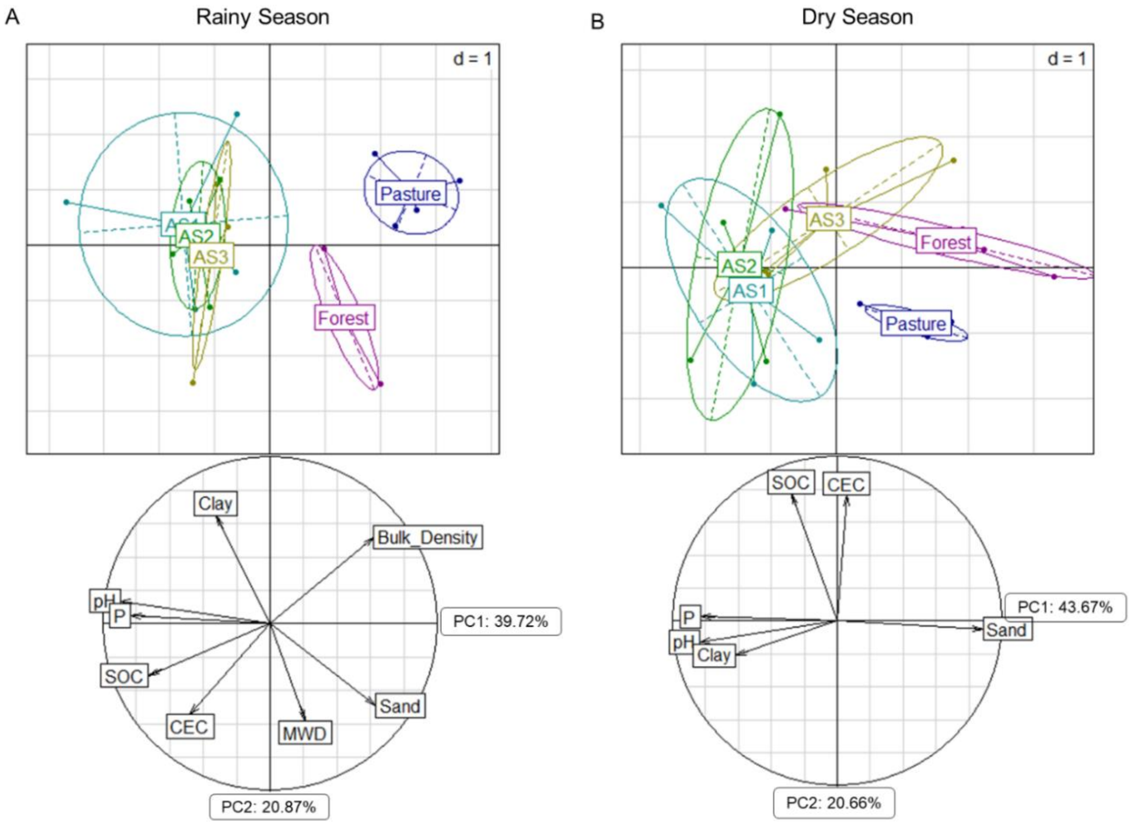

Figure 2. Between-class analysis of the 5 different land use using soil chemical and physical properties in (a) rainy season and (b) dry season (P = 0.001 and P = 0.002, for group separation in each time period, respectively, by Monte Carlo permutation test). Variable-correlation circle of soil chemical and physical properties in (a) rainy season and (b) dry season. See Table 2 for additional explanation of abbreviations.

2.5.2 Microbiological properties

The land uses differed significantly in soil microbiological properties in the two periods evaluated (Table 3). In the rainy season, agroforestry systems and pasture showed higher levels of MBC than the forest. The highest values of MBN were observed for AS3, pasture, forest and lowest values in AS1 and AS2. Regarding the activity of enzymes, β-glucosidase had higher values in agroforestry and forest plots than in the pasture plot and acid phosphatase was higher in the forest than in other land uses. Total enzymatic activity (FDA) was higher in pasture and forest than in agroforestry systems in the rainy season. In the dry season, agroforestry systems indicated an increase in microbial biomass, greater microbial activity (Sbresp) and FDA than in forest and pasture. Additionally, phosphatase tended to be higher under forest than agroforestry systems and pasture. The other variables evaluated showed no difference between treatments in the dry season (Table 3).

Table 3. Mean values for soil microbiological properties sampled on an experimental farm in the county of Sapucaia – RJ, Brazil at two time points, in the rainy season (April) and in the dry season (September) of 2018.

Rainy Season (April)							**Dry Season (September)**					
Microbiological variables	**Forest**	**Pasture**	**AS1**	**AS2**	**AS3**	***p-value***	**Forest**	**Pasture**	**AS1**	**AS2**	AS3	*p-value*
MBC (mg microbial C kg $^{-1}$ soil)	339^{d}	507^{b}	530^{ab}	429^{c}	571^{a}	< 0.001	667^{b}	580^{c}	708^{a}	716^{a}	727^{a}	< 0.001
	22.5	*20.6*	*26.6*	*27.4*	*22.1*		*20.2*	*13.5*	*14*	*27.9*	*6.9*	
MBN (mg microbial N kg^{-1} soil)	42.9^{b}	51.7^{ab}	29.6^{c}	28.9^{c}	63.2^{a}	< 0.001	70.6^{a}	66.6^{a}	72^{a}	87.3^{a}	75.1^{a}	
	4.1	*5.9*	*4.3*	*6.1*	*6.9*		*15.6*	*7.8*	*5.2*	*6.3*	*15.2*	
Sbresp (µg C-CO_2 g^{-1} h^{-1})	1.9^{a}	1.7^{a}	2.3^{a}	2.1^{a}	1.9^{a}		2.2^{bc}	1.9^{c}	3.3^{a}	2.8^{ab}	2.7^{abc}	< 0.001
	0.2	*0.5*	*0.4*	*0.2*	*0.4*		*0.2*	*0.5*	*0.3*	*0.1*	*0.4*	
FDA (µg fluorescein g^{-1} soil h^{-1})	114^{b}	128^{a}	96.2^{c}	102.4^{bc}	113^{b}	< 0.001	101.2^{b}	88.2^{b}	130^{a}	126^{a}	126^{a}	< 0.001
	5.2	*3.22*	*2.7*	*5.4*	*9.8*		*9.1*	*8.2*	*11.9*	*11.6*	*7.7*	
β-glucosidase (µmol g^{-1} h^{-1} p-nitrophenyl)	6.5^{ab}	5.7^{b}	8.1^{a}	7.1^{ab}	7.1^{ab}	0.072	9.9^{a}	8.5^{a}	10.5^{a}	9.9^{a}	10.2^{a}	
	1.02	*0.8*	*1.3*	*1.1*	*1.02*		*1.02*	*0.8*	*1.3*	*1.1*	*1.02*	
Acidic Phosphatase (µmol g^{-1} h^{-1} p-nitrophenyl)	8.6^{a}	5.2^{b}	5.6^{b}	4.9^{b}	5^{b}	< 0.001	13.9^{a}	5.7^{b}	6.1^{b}	6.3^{b}	6.7^{b}	0.008
	0.3	*0.3*	*0.7*	*0.5*	*0.4*		*4.5*	*2.4*	*2.9*	*2.4*	*2.8*	

Values in italics below each mean represent the standard error from four measurements in each plot. Means with different letters have significantly different values according to Tukey tests. Abbreviations: MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; Sbresp, Soil Basal respiration; FDA, fluorescein diacetate hydrolysis.

Between-class PCA analysis of soil microbiological properties reinforced univariate ANOVA findings, clearly separating the agroforestry systems plots from the forest and pasture in rainy season (P = 0.001 by Monte Carlo Permutation test; 72.43% of randtest observation; Figure 3A) and in Dry Season (P = 0.001 by Monte Carlo Permutation test; 59.35% of randtest observation; Figure 3B). In the rainy season there was a clear separation of all management plots (randtest simulated p-value = 0.001). PC1 explained 41.49% of the variability, was associated with microbial biomass N (MBN), β-glucosidase activity, microbial activity (Sbresp) and total enzymatic activity (FDA) and separated the agroforestry system plots from the pasture plots. PC2 (explaining 30.24% of the variability) was strongly associated with phosphatase activity and microbial biomass C (MBC) and separated all the management plots from the forest (Figure 3A). In the dry season, PC1 and PC2 explained 48.66% and 18.24% of the variability, respectively (Figure 3B). Again, PC1 was associated with β-glucosidase activity, Sbresp, and FDA, with the addition of MBC for this time point, and separated the agroforestry systems plots from the forest and pasture (Figure 3B).

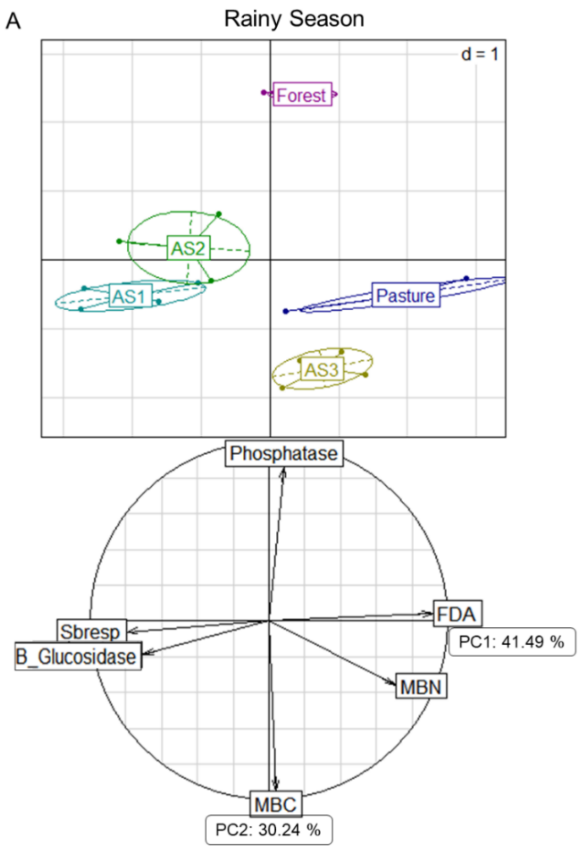

Figure 3. Between-class analysis of the 5 different land use using soil microbiological properties in (a) rainy season and (b) dry season (P = 0.001 by Monte Carlo permutation test). Variable-correlation circle of soil chemical and physical properties in (a) rainy season and (b) dry season. See Table 3 for additional description of abbreviations.

2.5.3 Litter and soil dwelling arthropods and diversity indices

A total of 7,306 individuals were collected in the pitfall traps in the rainy season and another 6,901 individuals in the dry season. These were separated into 7 taxonomic groups, mainly at order and class level. In the rainy season, the most abundant organisms were Formicidae (ants, 39.9%), Collembola (19.8%), and Diptera (true flies, 14.7%). Ground dwelling arthropods varied considerably across land use plots (Table 4). The highest total abundance was found under the pasture (42.1 ind. trap-1 day-1) and the lowest in AS2 (11.8 ind. trap-1 day-1). Taxonomic richness ranged from 6.25 to 6.88 on average per sampling point basis in each land use type; although there was no statistical difference, the richness tended to be lower in pasture and higher in agroforestry systems (AS1, AS3, AS2) and forest.

Species diversity based on the Shannon Index (H) ranged from 1.20 to 1.99 and was highest in the agroforestry systems and forest and lowest in pasture. In the dry season, the most abundant organisms were Collembola (29.6%), Formicidae (ants, 23.3%), Diptera (true flies, 13.5%), Acari (8.2%), Araneae (spiders, 7.4%), Coleoptera (5.9%) across all land uses. The highest total activity was again found under pasture (31.6 ind. trap-1 day-1) and the lowest in the forest (15.3 ind. trap-1 day-1). Taxonomic richness ranged from 6.43 to 7 on average per sampling point basis in each land use type. Although there was no statistical difference, richness tended to be lower in pasture compared to forest and agroforestry systems. Species diversity based on the Shannon Index (H) ranged from 1.40 to 2.04 and was highest in the agroforestry systems and forest and lowest in pasture (Table 4). The groups that accounted for less than <5% of total abundance were included in Others at both times.

Table 4. Number of individuals per trap per day of the epigeal fauna communities, abundance and diversity on average per sampling point basis in each land use type on an experimental farm in the county of Sapucaia – RJ, Brazil at two time points, in the rainy season (April) and in the dry season (September) of 2018.

	Rainy Season (April)						**Dry Season (September)**					
Epigeal fauna (Ind. trap^{-1} day^{-1})	**Forest**	**Pasture**	**AS1**	**AS2**	**AS3**	***p-value***	**Forest**	**Pasture**	**AS1**	**AS2**	**AS3**	***p-value***
Acari	0.27^{b}	0.53^{b}	0.76^{b}	0.94^{b}	1.96^{a}	< 0.001	0.65^{c}	0.71^{bc}	2.38^{ab}	1.89^{abc}	2.68^{a}	< 0.01
	0.2	*0.6*	*0.6*	*0.7*	*0.8*		*0.3*	*0.6*	*1.9*	*0.7*	*1.4*	
Araneae	1.11^{a}	1.68^{a}	1.09^{a}	0.44^{a}	0.63^{a}		1.07^{a}	2.22^{a}	1.75^{a}	0.68^{a}	1.86^{a}	
	0.7	*2.2*	*0.5*	*0.4*	*0.6*		*0.5*	*3.5*	*1.4*	*0.5*	*0.8*	
Coleoptera	1.22^{a}	0.39^{b}	1.06^{ab}	0.65^{ab}	0.57^{ab}	0.013	2.37^{a}	0.33^{c}	1.36^{b}	0.81^{bc}	0.98^{bc}	< 0.001
	1.2	*0.4*	*0.6*	*0.4*	*0.5*		*0.2*	*0.3*	*0.7*	*0.6*	*0.5*	
Collembola	4.02^{ab}	6.46^{a}	3.36^{ab}	4.46^{ab}	2.25^{b}	0.051	4.03^{a}	7.6^{a}	5.57^{a}	7.19^{a}	5.65^{a}	
	1.2	*3.6*	*2.8*	*3.5*	*1.3*		*1.5*	*3.9*	*3.6*	*1.9*	*2.2*	
Diptera	2.83^{ab}	1.17^{b}	4.14^{ab}	1.42^{b}	5.76^{a}	0.016	4.15^{a}	1.6^{b}	2.42^{ab}	2.81^{ab}	2.51^{ab}	0.073
	1.9	*0.9*	*2.4*	*1.3*	*5.3*		*1.7*	*1.1*	*2.2*	*1.9*	*0.7*	
Formicidae	1.1^{b}	30.3^{a}	2.40^{b}	1.89^{b}	4.9^{b}	0.002	1.21^{b}	17.8^{a}	2.39^{b}	1.92^{b}	1.44^{b}	< 0.001
	0.4	*32*	*1.8*	*1.5*	*7.9*		*1*	*17.2*	*1.2*	*0.8*	*0.9*	
Others	1.98^{b}	1.56^{b}	2.82^{ab}	2.01^{b}	4.92^{a}	0.006	1.82^{a}	1.38^{a}	3.74^{a}	2.64^{a}	2.44^{a}	
	0.42	*1.01*	*2.20*	*1.04*	*2.95*		*1.19*	*0.68*	*2.67*	*1.22*	*1.32*	
Total Abundance	12.5^{b}	42.1^{a}	15.6^{b}	11.8^{b}	21^{ab}	0.005	15.3^{b}	31.6^{a}	19.6^{ab}	17.9^{ab}	17.6^{ab}	0.048
	4.1	*32.3*	*6.8*	*6.2*	*15.2*		*3.9*	*21.4*	*9.01*	*4.8*	*3.7*	
Richness (S)	6.86^{a}	6.25^{a}	6.88^{a}	6.62^{a}	6.88^{a}		6.88^{a}	6.43^{a}	6.62^{a}	6.75^{a}	7.00^{a}	
	0.38	*1.16*	*0.35*	*0.52*	*0.35*		*0.35*	*0.79*	*1.06*	*0.46*	0.00	
Shannon (H)	1.99^{a}	1.2^{b}	1.93^{a}	1.83^{a}	1.96^{a}	< 0.001	1.9^{a}	1.4^{b}	1.99^{a}	1.9^{a}	2.04^{a}	< 0.001
	0.1	*0.5*	*0.2*	*0.2*	*0.3*		*0.2*	*0.3*	*0.4*	*0.2*	*0.1*	

Values in italics below each mean represent the standard error from four measurements in each plot. Means with different letters have significantly different values according to Tukey tests.

The results of the NMDS and PERMANOVA analyses showed that agroforestry plots and forest were separated from pasture. In April, the separation was largely associated with differences in Formicidae, Collembola, Araneae (Figure 4A). In September, the analysis again showed a clear separation between the land uses, with pasture clearly separated from forest and agroforestry systems. This separation was also related to Formicidae, Araneae as well as that of Coleoptera (Figure 4B).

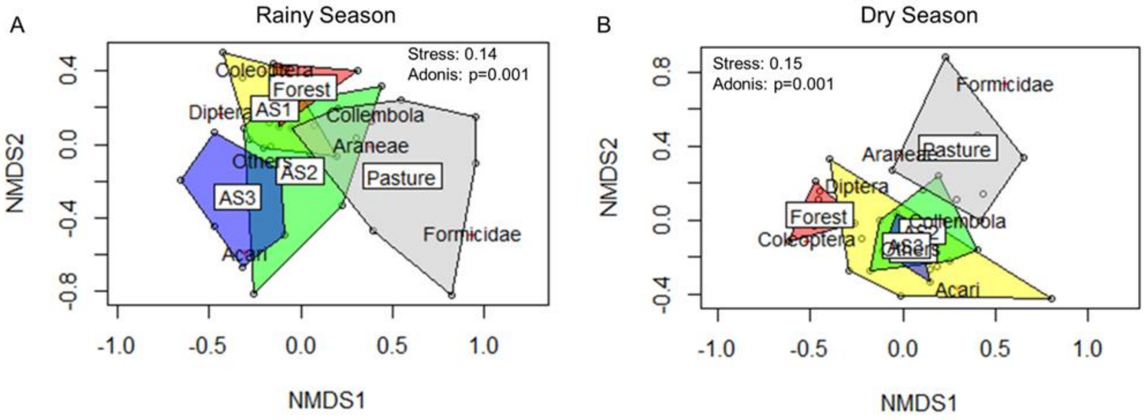

Figure 4. Nonmetric multidimensional scaling (NMDS) relating the soil fauna groups that representing more than 5% of total abundance, from plots sampled in rainy season (a) and dry season (b), respectively. Forest (red), Pasture (grey), AS1 (yellow), AS2 (green), AS3 (blue).

2.5.4 Litter chemical properties

Litter biomass and chemistry from forest and agroforestry systems differed significantly in April, such that litter nutrient content (P, Ca^{2+}, Mg^{2+} and K^{+}) of agroforestry systems was higher in relation to forest, while the forest presented the highest C:N ratio. In September there was a significant difference between treatment litter only for N content and C:N ratio. Litter biomass was highest in AS3 and forest in April, while no treatment differences where apparent in September (Table 5).

Table 5. Mean values for litter chemical properties sampled on an experimental farm in the county of Sapucaia – RJ, Brazil at two time points, in the rainy season (April) and in the dry seasons (September) of 2018. Samples were collected from five land uses: secondary forest, degraded pasture, and three agroforestry systems (AS1, AS2, and AS3).

	Rainy season (April)					**Dry season (September)**				
Litter variables	**Forest**	**AS1**	**AS2**	**AS3**	***p-value***	**Forest**	**AS1**	**AS2**	**AS3**	***p-value***
C:N	28.4^{a}	25.4^{ab}	21.5^{b}	22.9^{b}	0.026	23.4^{a}	18.6^{b}	18.3^{b}	19.1^{b}	0.028
	1.6	3.9	4.7	5.9		*1.8*	*1.4*	*0.7*	*1.2*	
P (mg kg^{-1})	0.05^{b}	0.11^{a}	0.11^{a}	0.09^{a}	< 0.001	0.03^{a}	0.06^{a}	0.06^{a}	0.06^{a}	
	0.02	*0.02*	*0.02*	*0.02*		*0.01*	*0.02*	*0.01*	*0.01*	
Ca^{2+}(mg kg^{-1})	94.65^{b}	156.7^{a}	148.7^{ab}	136.9^{a}	0.009	99.3^{a}	129^{a}	101^{a}	101^{a}	
	9.7	*5.2*	*15.4*	*15.6*		*29.1*	*9.7*	*12.7*	*12.4*	
Mg^{2+} (mg kg^{-1})	18.2^{b}	26.2^{ab}	23.2^{ab}	41.3^{a}	0.017	23.2^{a}	28.1^{a}	20.0^{a}	25.5^{a}	
	3.3	*8.9*	*2.5*	*7.5*		*7.8*	*2.9*	*7.3*	*7.2*	
K^{+} (mg kg^{-1})	9.99^{b}	16.4^{a}	10.4^{b}	15.3^{ab}	0.008	17.2^{a}	29.9^{a}	20.3^{a}	39.3^{a}	
	0.7	*3.1*	*1.9*	*4.5*		*7.7*	*3.6*	*4.2*	*27.6*	
Biomass (kg ha^{-1})	1578^{a}	1366^{b}	1112^{c}	1499^{ab}	< 0.001	1410^{a}	1086^{a}	1261^{a}	1193^{a}	
	64.8	*60.6*	*56.3*	*79.1*		*64*	*149*	*208*	*264*	

Values in italics below each mean represent the standard error from four measurements in each plot. P-values for one-way ANOVA are presented to the right of means. Means with different letters have significantly different values according to Tukey tests.

2.5.5 Relationships between litter quality, SOM and key soil quality parameters

When accounting for the different sampling times, litter quality and SOM were significantly related to a number of soil biological, chemical and physical parameters (Table 6). For example, litter C:N ratio was negatively correlated with invertebrate abundance, MBC, available K and P, pH, and BD, and was positively correlated with phosphatase activity and aggregate stability (MWD). The effect of litter C:N ratio on the soil microbial parameters FDA and Sbresp appeared to depend on sampling time (interaction $p \leq 0.010$), such that FDA increased (and Sbresp did not change) with increasing C:N ratio for samples collected in the rainy season (April) and both parameters decreased with C:N in the dry season (September). SOM was positively related to MBC, Sbresp, β-glucosidase activity, available K, pH and CEC, and negatively correlated with BD. There were also marginally significant ($p < 0.07$) positive relationships between SOM and macrofauna richness, Shannon diversity, and available P. Significant interactions between sampling time and SOM content ($p < 0.05$), suggest that the relationship with FDA and pH depend on the sampling time in question (Table 6). Sampling time was an important predictor of many of the response variables considered, both when looking at relationships with litter quality and SOM content (Table 6).

Table 6. Model results depicting the relationships between litter quality (C:N ratio) and SOM with soil quality variables from soils collected from five land uses and two sampling times (Rainy or dry season) on an experimental farm in the county of Sapucaia - RJ, Brazil.

Soil Response Variable	C:N ratio	C:N effect direction*	Sampling Time	C:N x Time	SOM	SOM effect direction*	Sampling Time	SOM x Time
Abundance	0.029	-	ns	ns	ns		ns	ns
Richness	ns		ns	ns	0.061	+	ns	ns
Shannon	ns		ns	ns	0.058	+	ns	ns
MBC	0.025	-	<0.001	ns	0.009	+	<0.001	ns
MBN	ns		<0.001	ns	ns		<0.001	ns
FDA	0.030	Apr +, Sep -	ns	<0.001	ns	Apr -, Sep +	ns	0.002
Sbresp	0.003	Apr 0, Sep -	ns	0.010	0.034	+	<0.001	ns
β-glucosidase	ns		<0.001	ns	<0.001	+	<0.001	ns
Phosphatase	0.003	+	<0.001	ns	0.093	-	ns	ns
K	0.002	-	0.001	ns	<0.001	+	ns	ns
CEC	ns		0.017	0.010	0.035	+	0.009	ns
pH	0.045	-	0.009	ns	0.002	Apr +, Sep 0	ns	0.021
P	0.021	-	<0.001	ns	0.057	+	0.023	ns
BD	0.030	-	ns	ns	0.012	-	ns	ns
MWD	0.022	+	0.088	ns	ns		ns	ns
POXC	ns		ns	ns	ns		ns	ns
SOM	ns		0.081	ns	NA		NA	NA

* refers to the direction of the correlation overall or for the different sampling times (in case of significant interaction); Positive relationship (+) or negative relationship (-).

2.6 DISCUSSION

2.6.1 Soil chemical and physical properties across different land uses

Clear differences in soil chemical properties were evident between the forest, pasture and agroforestry systems plots. Soils under forest and pasture generally had lower fertility (in terms of pH, SOC, POXC, available K) concentrations than the agroforestry system plots (Table 2). While not always significant for the univariate tests, multivariate comparisons showed a high degree of separation between agroforestry systems and the pasture and forestry plots (Figure 2A-B). The agroforestry systems also tended to have higher levels of available P and CEC than pasture, thus contributing the overall higher soil fertility in the agroforestry plots. We note that the agroforestry systems received an initial addition of rock phosphate to the banana planting pits during the first year of establishment. This may have contributed to the higher P availability and pH observed in those systems (UTOMO, 1995), but these initial P inputs may have also contributed to SOM by increasing overall system productivity and the return of organic residue to the soil, thus further contributing to soil fertility impacts. Another factor contributing to the higher levels of soil fertility under agroforestry system is the high diversity of tree species and improved litter quality derived from these trees in the agroforestry system plots. For example, Tithonia diversifolia, which was included in all of the treatments, is known to accumulate high concentrations of N, P and K in its leaves (JAMA et al., 2005) Residue from such plants can contribute to soil fertility status through nutrient mobilization and return as well as by contributing to SOM, which can increase the availability of P in acid soils by blocking the P adsorption sites on mineral surfaces (AYAGA et al., 2005) Additionally, the greater presence of legumes under the agroforestry systems likely contributed to litter quality, as is evidenced by C:N ratios of forest vs. agroforestry systems litter. Residues that are rich in N are thought to enhance the C use efficiency of decomposer organisms (COTRUFO et al., 2013; MANZONI et al., 2017), resulting in a higher microbial biomass (COTRUFO et al., 2013) and eventual stabilization of SOC. Our findings thus lend support to the potential role of agroforestry systems for sequestering C and supporting overall soil fertility in agricultural lands (ZARO et al. 2019; KEARNEY et al., 2019).

When considering soil physical properties, the forest had the highest aggregate stability and among the lowest bulk density values, suggesting that these soils may support higher water infiltration rates and improved erosion control (NCIIZAH et al., 2015; NUNES et al. 2015). Meanwhile, the pasture had the highest bulk density, indicating potential compaction issues (Table 2). This is likely associated with poor grazing and soil fertility management, as it had received no fertilization and had been under continuous grazing for many years. The lack of nutrient inputs and other management interventions to maintain both above- and belowground productivity has been shown to negatively affect soil structure and overall fertility in other tropical pasture systems (FONTE et al., 2013). This degraded condition is likely representative of many Brazilian pastures, since 80% have been considered to be in some state of degradation (ZARO et al., 2019). At the same time, we note that the agroforestry systems plots all had bulk densities that were more similar to the forest soil, suggesting potential amelioration or avoidance of compaction issues since establishment of the agroforestry systems on degraded pastures eight years prior. However, as stated above, due to the lack of true replication in this study, we cannot draw firm conclusions about management impacts soil properties. Nevertheless, we note that prior to the establishment of the agroforestry systems, these plots were under a nearly identical management regime as the adjacent pasture plot.

Also, given that there are no significant differences in soil texture between plots, it is likely that the soils began in a similar state prior to agroforestry systems implementation and thus likely that many of the differences observed between plots are due at least in part to management. While important insights can be gained from this study, our findings and any causal inferences suggested here should be interpreted with caution.

2.6.2. Soil biological properties

Beyond differences in soil physiochemical properties, the management plots evaluated here indicated clear differences in soil biological properties. For example, the agroforestry systems supported generally higher levels of microbial biomass C and soil respiration (Table 3), especially in the dry season (September), several weeks after pruning. A similar trend was also apparent for FDA and β-glucosidase activity, suggesting that the high inputs of relatively high-quality residues in agroforestry systems encouraged microbial growth and activity. Soils under secondary forest generally had intermediate values between agroforestry systems treatments and pasture for many microbial parameters. While the forest, comprised largely of semi-deciduous species, also deposited high amounts of residues as senescent litter in the dry season (as indicated by high standing litter biomass), this material was of much lower quality than the agroforestry systems residues from pruning (Table 5). This senesced litter is not likely to decompose as rapidly and stimulate microbial activity to the same extent as green leaves (FONTE et al., 2004). At the same time, the forest soil showed the highest values of acid phosphatase at both sampling times, and this was a key factor differentiating the forest from the other management plots (Figure 3A-B). We suspect that this is associated with the generally lower levels of available P in the forest system and the high inputs of low-quality litter, since both plants and microbes under such conditions are likely to respond by producing phosphatase to stimulate P mineralization (OLANDER; VITOUSEK et al., 2000). Among the agroforestry systems, AS3 displayed the highest microbial biomass (Table 3). This may be related to the higher density of the biomass species *T. diversifolia* in this system. Jama et al. (2000) working in tropical Africa, found this species to support increased microbial biomass and soil biological activity. Overall, we note that the three agroforestry systems supported relatively similar microbial properties relative to the forest or pasture plots considered here.

The assessment of ground dwelling arthropods demonstrated clear differences between land use systems, especially between the forest and pasture plots (Figure 4A-B). In general, there was higher diversity in plots with trees than was observed in the pasture, while the pasture had the highest abundance of arthropods, comprised mostly of ants (Table 4). We suggest that this may related with diversity of food resources. When there is a reduction in the diversity of food resources, some ground-dwelling arthropod groups can establish themselves quickly and dominate the community, as is often observed with social insects, such as ants (SANABRIA et al., 2016). The complexity of the litter structure is often a good predictor of the abundance and diversity of soil litter fauna. In a survey of soil quality parameters, including ground-dwelling arthropods in Nicaragua, Rousseau et al. (2013) found forests to have higher diversity than nearby pasture systems, while a maize-bean agroforestry system displayed intermediate levels of diversity. This same study found that another social insect, termites, were significantly higher in the pasture than in secondary forest. They related these findings to increased habitat complexity and organic matter inputs in the tree-based systems (ROUSSEAU et al., 2013). Soils with high litter biomass and diversity (from multiple tree species) are expected to have a higher diversity and abundance of soil fauna groups since they allow for higher numbers of microhabitats and therefore increase niche differentiation between groups (SANTONJA et al., 2017). We suspect that the high diversity of ground dwelling invertebrates observed in the agroforestry systems is related to the similarity of these systems, in terms of vegetative structure and the presence of a developed litter layer, to secondary forest. Beyond effects on overall diversity and the dominant groups, we noted clear differences in key functional groups of macrofauna. For example, Coleoptera (beetles) tended to be more abundant in the forest than in the other systems, and it may be that this group is more sensitive to disturbance and thus a good indicator of forest restoration (WORK et al., 2008).

2.6.3. Linkages between litter quality, SOM and soil quality parameters

We found evidence for linkages between litter quality, SOM and several important soil quality metrics, highlighting the importance of litter input type and processing to ecological functioning. Specifically, we found the C:N ratio was negatively correlated with microbial biomass C and the abundance of ground dwelling arthropods (Table 6), both important indicators of soil biological activity. These negative correlations suggest that high quality litter inputs (i.e., low C:N ratio) are important for stimulating decomposition (PARTON et al., 2007) and mineralization processes (COTRUFO et al., 2013) and the activity of a range of soil organisms (CARRILO et al., 2011; FUJII et al. 2018) that depend on the resulting increases in nutrient availability. Changes to nutrient availability, and implications for overall system productivity, are further evidenced by increased availability of P and K with decreasing C:N. Surprisingly, we found that aggregate stability (MWD) tended to increase with C:N ratio. This was somewhat surprising since aggregation is often associated with increased soil biological activity (SIX et al., 2004) It may be that higher C: N litter stimulated increased growth of fungal hyphae in soils, which can be important for the formation of larger macroaggregates (HAYS; WATSON, 2019; LEHMANN et al. 2017).

Similar to the effects of litter quality, SOM was related to soil biological activity and multiple soil properties. SOM was positively related to microbial biomass C, respiration, and β-glucosidase activity and also showed marginally significant relationships with the diversity of ground dwelling arthropods. This finding supports the general notion that, together with litter inputs, SOM is an essential resource base for not only decomposer organisms, but entire soil food webs (LAVELLE et al. 2001; MOORE et al. 2004) and is thus a key driver of multiple soil biological processes. We also note that the observed positive correlations between SOM and available K and P, as well as CEC, point to the importance of SOM in both water and nutrient cycling that has been reported elsewhere (CRASWELL et al., 2001; KING et al., 2020).

Given the marked impacts of SOM and litter quality on soil functioning suggested by our findings, both represent key management objectives in the development of agroforestry systems to restore degraded lands. Litter quality, in particular, can be readily managed via tree species selection and might play an important role in facilitating soil restoration through effects on biological communities and the functions they regulate. The use of leguminous tree species for biomass production in these systems is particularly relevant since many of them are associated with N_2-fixing bacteria, suppling N to the system and enhancing nutrient cycling and soil fertility through high-quality litter that supports soil biological communities. Additionally, pruning management is another practice that regulates the quality and amount of litter inputs entering the system and strategic timing is needed in order to balance litter inputs and nutrient cycling with productivity.

2.6 CONCLUSIONS

The physicochemical and biological assessment of these land uses reveal that soil under agroforestry systems generally had higher soil fertility status, as well as SOM and biological activity than pasture or forest. The linkages between litter quality, SOM and soil parameters suggest that high quality litter inputs (i.e. low C:N ratio) together with SOM are important for stimulating biological activity and multiple soil properties with future implications for soil restoration.

Altough causal inferences of management cannot be drawn from this study, our findings lend support to the idea that establishment of complex agroforestry systems in Brazil is likely to support soil quality and restoration goals. This further supports environmental legislation suggesting agroforestry systems as viable option for Brazil to restore degraded lands and comply with international commitments to reduce greenhouse gas emissions, move towards low-C agriculture, and consequently contribute to improving global food security.

Future studies are needed to confirm causal linkages between agroforestry management and soil quality indicators as well as to understand contributions to above and belowground C stocks, and to better quantify the potential of the agroforestry systems to restore soils across different levels of degradation.

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3 CHAPTER II

SHORT-TERM MODIFICATIONS OF MYCORRHIZAL FUNGI, GLOMALIN AND SOIL ATTRIBUTES IN A TROPICAL AGROFORESTRY

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3.1 RESUMO

A comunidade de fungos micorrízicos arbusculares (AMF) e a proteína do solo relacionada à glomalina (GRSP) que eles produzem desempenham papéis importantes na manutenção das funções do ecossistema do solo, influenciando na restauração ecológica, e são importantes para monitorar mudanças na saúde do solo devido às mudanças de uso do solo. Este estudo abordou as modificações de curto prazo na comunidade AMF e GRSP e suas associações com outras propriedades do solo em sistema agroflorestais e outros usos do solo. Os objetivos deste estudo foram responder às seguintes questões: como a comunidade de AMF e as frações de glomalina (EEG e TG) respondem a implementação de curto prazo de sistemas agroflorestais no sudeste do Brasil? 2) A mudança nas estações influencia a dinâmica das frações AMF e glomalina? 3) Os diferentes usos da terra (floresta, pastagem não manejada e diferentes sistemas agroflorestais) influenciam as relações entre a glomalina, a comunidade de FMA e os principais parâmetros de qualidade do solo? O solo foi coletado nas estações seca e chuvosa de 2018 sob cinco usos do solo, incluindo: três tipos de sistemas agroflorestais (AS1, AS2 e AS3), uma pastagem sem manejo e uma floresta secundária no estado do Rio de Janeiro, Brasil. As analises estatisticas por meios de modelos lineares de efeitos mistos e as análises multivariadas mostraram que os usos da terra influenciaram a comunidade de AMF, principalmente no nível de família. Por outro lado, a sazonalidade não se mostrou um fator essencial que modula as mudanças da comunidade de FMA e da produção de glomalina. As práticas de manejo influenciaram a esporulação de FMA e o número total de espécies em sistemas agroflorestais. A glomalina é um contribuinte potencial para SOC, principalmente em sistemas agroflorestais e áreas de pastagem. Além disso, foi encontrada correlação entre a comunidade AMF e os principais parâmetros do solo. Por exemplo, a maioria das famílias AMF e densidade de esporos foram positivamente correlacionadas com a estabilidade dos agregados do solo e SOC.Os resultados mostram que a mudança no uso da terra pode alterar a comunidade de AMF, a glomalina e sua relação com os principais parâmetros de qualidade do solo. Além disso, a adoção de sistemas agroflorestais indica a manutenção da biodiversidade e de outros parâmetros de qualidade do solo com implicações futuras para seu uso na recuperação de áreas degradadas.

Palavras-chave: Qualidade do solo. Indicadores biológicos do solo. Sazonalidade.

3.2 ABSTRACT

Arbuscular mycorrhizal fungi (AMF) community and the Glomalin-related soil protein (GRSP) they produce plays important roles in maintaining soil ecosystem functions, promoting ecological restoration, and are important for monitoring changes in soil health from land use change. This study addressed short-term changes in the AMF and GRSP community and their associations with other soil properties in agroforestry, and other land uses. The objectives of this study were to answer the following questions: how do the AMF community and glomalin fractions (EEG and TG) respond to the short-term implementation of agroforestry systems in southeastern Brazil? 2) Does the change in seasons influence the dynamics of the AMF and glomalin fractions? 3) Different land uses (forest, unmanaged pasture, and agroforestry systems) influence the relationships between glomalin, the AMF community, and the main soil quality parameters? Linear mixed-effects model and multivariate analyses showed that land uses had influenced the AMF community, meanly at the family level. On the other hand, seasonality has not proved to be an essential factor that modulates the changes of the AMF community and glomalin production. The management practices had influenced AMF sporulation and the number of total species in agroforestry systems. Glomalin is a potential contributor for SOC, mainly in agroforestry systems and pasture plots. Moreover, a correlation between the AMF community and key soil parameters was found. For example, most of the AMF families and spore density were positively correlated with the stability of soil aggregates and SOC. The findings shed light on that land-use change can shift the AMF community, glomalin and their relationship to key soil quality parameters. Moreover, the adoption of agroforestry systems indicates maintenance of biodiversity and other soil quality parameters with future implications for their use to recover degraded areas.

Key words: Soil quality. Soil biological indicators. Seasonality.

3.3 INTRODUCTION

Brazil’s environmental legislation has been extensively modified to make agriculture more sustainable and comply with international agreements to reduce CO_2 emissions in the last ten years. An example was creating the low carbon agriculture plan (plan ABC) in 2010, which goal was to promote the expansion of agroforestry systems acreage by 2.76 million ha by 2020. This plan was renewed (Plan ABC+) with new targets to be met by 2030, for example, the increase in the planted area of agroforestry systems by 100,000 hectares. In addition, Brazil's environmental legislation obliges landowners to maintain a fixed amount of native vegetation in their properties, the so-called “Legal Reserves”. It allows agroforestry systems to be used to restore the legal reserve areas in the country.

The Legal Reserve requirement in a proportion of the property varies from 80% for the Amazon biome to 35% in the transition between Amazon and Cerrado biomes and 20% in the remaining biomes (Atlantic Forest, Cerrado, Caatinga, Pantanal, and Pampa). With the introduction of this law, landowners started to be monitored by the rural environmental registry (CAR) for compliance with the legal reserve requirements (Bracalion et al., 2016), and they can restore the legal reserve with agroforestry systems. In addition, Brazil signed the Paris Agreement in 2015 to reduce CO_2 emissions by 47% by 2030, with agroforestry systems again among the technologies suggested recovering degraded pasturelands (UN, 2015). A substantial portion of these climate change mitigation commitments relies on ambitious targets – e.g., such as restoring 15 million ha of degraded forests and 12 million ha of degraded pastures. Such changes in environmental policies and legislation pushed changes in land use in all parts of Brazil, generally from degraded areas (pasture) to agroforestry systems.

Land use change can alter the abundance and diversity of arbuscular mycorrhizal fungi (AMF) species and the content of glomalin-related soil protein (GRSP) in the soil (Silva et al., 2018; Silva et al. 2016; Nogueira et al. 2016). AMF are present in the soil in the form of spores and extraradicular hyphae, as well as in plant roots with which they form a mutualistic association and are considered an essential component of various ecosystems (Hodge et al., 2001; Zhang et al., 2020). AMF delivers nutrients, mainly phosphorus (P) and nitrogen (N), to host plants in exchange for carbon (C). In addition, AMF confers many positive effects on host plants, including promoting plant growth, stabilizing soil aggregation, maintaining soil moisture, improving abiotic and biotic stress tolerance, and increasing plant biodiversity (Keymer et al., 2017; Parihar et al., 2020). Glomalin, quantified in the soil as GRSP, in turn, is an essential component present in the wall of hyphae and spores of the AMF, which is also released into the soil after the death and decomposition of these structures (Moreira and Siqueira, 2006; Gillespie et al., 2011). GRSP is considered an essential component of the soil organic carbon (SOC) pool in terrestrial ecosystems (Wright et al. 1996; Jia et al. 2016) and acts as a soil conditioner by improving fertility, aeration, water holding capacity, nutrient levels, and plant productivity (Fokom et al., 2012). The GRSP is divided into two fractions, the easily extractable soil protein related to glomalin (EE-GRSP) and total soil protein related to glomalin (T-GRSP). Given that EE-GRSP is a component of T-GRSP, they are often strongly correlated with each other. Koide and People (2013) further divided GRSP into fraction 1 and fraction 2. Hereinto, fraction 1 (EE-GRSP) was relatively labile, and fraction 2 as an older glomalin was more difficult to extract and recalcitrant in soils. Subsequently, Wu et al. (2014) proposed that the fraction 1 was called EE-GRSP, and the fraction 2 was defined as difficultly extractable glomalin-related soil protein (DE-GRSP). T-GRSP is the sum of EE-GRSP and DE-GRSP (Wu et al., 2015).

In this context, AMF and GRSP have been suggested to be used as bioindicators in the assessment of soil resources (Rillig et al., 2003; Fokom et al., 2012) since both AMF and GRSP have a positive correlation with the main edaphic attributes used for soil quality assessments (SILVA et al. 2018a); i.e., the greater the diversity of AMF species and the GRSP content, the better is the soil quality (SILVA et al. 2017; PEREIRA et al. 2018). However, the seasonality of AMF and GRSP in the soil may alter their relationships with soil parameters between dry and rainy seasons and influence their usage as an indicator of land-use impacts on soils. This aspect is still little considered in soil degradation/restoration (SILVA et al., 2018b). In this context, we aimed to 1) how AMF community and glomalin fractions (EEG and TG) respond to a short-term implementation of agroforestry systems in southeast Brazil? 2) Does change in seasons influence the dynamics of AMF and glomalin fractions? 3) The different land uses (forest, unmanaged pasture, and different agroforestry systems) influence the relationships between glomalin, AMF community, and key soil quality parameters?

3.4 MATERIAL AND METHODS

3.4.1 Site description and land uses

The study was carried out at Fazenda Arca de Noé, a farm located in the municipality of Sapucaia (21º 59' 42" S, 42º 54' 52" W), South Central Region of Rio de Janeiro state, Brazil. The vegetation is characterized by dense ombrophylous forest of the Atlantic Forest (IBGE, 2012). The predominat soil class is Ultisols (USDA, 2014) with a clay-loam texture. Argissolos according to SiBCS (SANTOS et al., 2018). The climate of the region, according to the Köppen's classification, is Subtropical highland climate (Cwb), with mean monthly temperatures that vary between 17°C and 32°C (June and January; respectively) and a mean annual rainfall of 1,451 mm.

This study was conducted in five existing land uses types:

1) Secondary forest (FOREST) that was included here as a reference and had about 30 years of regrowth following complete deforestation;

2) Pasture replanted with Urochloa decumbens under extensive grazing (PASTURE), that was established by the removal of native vegetation in 1995;

3) Agroforestry system characterized by the integration of banana and coffee with a mix of other fruit and timber species and other species to provide shade, biomass production, and pollination services (AS1).

4) Agroforestry system focused on bananas and energy production (but also includes fruit trees and a mix of other trees and plants; AS2);

5) Agroforestry system focused on bananas and other fruits (AS3; see table 1 in the first chapter for detailed species lists).

At the time of planting, all agroforestry systems received a single application of rock phosphate (fertilizer permitted in organic production) and cattle manure to the banana tree roots at the time of establishment. All plots considered in this study were located on the same soil type and textural class and had similar slopes of roughly 30º.

3.4.2 AMF and glomalin analyses

Sampling was conducted in 2018 at two separate time points, rainy season (April) and dry season (September), to assess a suite of soil biological (AMF, EEG and TG), chemical, and physical properties within each land use (e.g., forest, pasture, and agroforestry systems) at a depth of 0-10 cm. The chemical and physical properties of the soil samples were characterized (see table 2 in the first chapter).

AMF spores were extracted from 50 g of soil by means of wet sieving (GERDEMANN and NICOLSON, 1963), followed by centrifugation in a 45% sucrose solution. Spores were then quantified directly in fluted Petri dishes using a stereomicroscope to evaluate the density of spores in each sample. Subsequently, two subsamples representative of the whole variety of spores were? extracted, according to the characteristics of size, color and shape, and then mounted between blade and cover slip. Of these, a sub-sample was fixed with polyvinyl alcohol in lactoglycerol (PVLG), and the other sub-sample with the mixture of PVLG + Melzer Reagent (1:1). The spores were observed under optical microscope with light field and objective immersion light. The identification of AMF species was performed according to the morphological description available on the website of the International Culture Collection of Arbuscular Mycorrhizal Fungi (http://invam.caf.wwu. edu/) and other relevant recent literature. Based on AMF identification, we calculated species frequency (Fi) (BROWER et al., 1990) ($Fi = ji/Ke$, where ji is the number of samples containing species i, Ke the number of soil samples, Fi the species frequency i); total species richness (SR); and the Margalef index ($D\alpha = [(n-1)]/\ln$

N, where Dα is diversity, n is the number of species recorded, N is the number of individuals of any species in the sample, and ln is the natural logarithm of N).

We also evaluated glomalin-related soil protein (GRSP). Two fractions of GRSP (easily extractable glomalin - EEG; total glomalin - TG) were distinguished according to Rillig (2004). The easily extractable glomalin was obtained from the autoclave extraction using 1 g of soil sample and 8 mL of 20 mM sodium citrate solution, pH 7.4, at a temperature of 121 °C for 30 min. The total glomalin content was obtained using 1 gram of soil sample and 8 mL of 50 mM sodium citrate, pH 8.0, at 121 °C for 60 min. For extraction of the total glomalin, more than one autoclaving cycle (3 to 6 cycles depending on the sample) was required until the sample color was light yellow. In both fractions, centrifugations were performed at 5000 g for 20 min after autoclaving, and the supernatant was removed for subsequent quantification of the protein. The Bradford method quantified glomalin (WRIGHT et al., 1996) using bovine serum albumin. The GRSP concentrations for both fractions were corrected to mg g-1 of soil, considering the total volume of supernatant and the dry soil mass.

3.4.3 Statistical analyses

The differences among the studied variables were tested for their significance between the plots for each soil property in each land use (forest, pasture, AS1, AS2, and AS3) and between seasonality. The sampling pattern includes spatially multiple self-dependent plots with low variances within each. Therefore, the linear mixed-effects model (lme4 R package, v. 1.1-23 and lmerTest, v. 3.1-2) was applied to examine significant differences among land uses and seasonality variables, set as a fixed and the sampling plots as a random effect. The type II Wald X2 test and least-square mean for a pairwise t-test with false discovery rate correction for multiple comparisons (car R package v. 3.0-10) were used to explore the influence of seasonality on variables in each land use, and further, to measure the differences between land uses for each variable.

To assess the similarity between the study areas according to the frequency of occurrence of AMF species, cluster analysis was used. To perform hierarchical cluster analysis, the R software hclust function was applied (R DEVELOPMENT CORE TEAM, 2019). Ordination and visualization of AMF communities was conducted using nonmetric multidimensional scaling (NMDS). Bray-Curtis distances were calculated between samples using the dominant soil taxa. NMDS ordinations were plotted for the distance matrices, and correlations between environmental variables and NMDS axes were calculated and included as arrows in plots if significant ($P < 0.05$). Treatment effects were tested using the ADONIS method of permutational multivariate analysis with 999 permutations. All multivariate analyses were completed in R using the vegan package (OKSANEN et al., 2018) and ade4 library within the R environment (R DEVELOPMENT CORE TEAM 2019, DRAY et al., 2007). In order to understand the relationship among AMF family, spore density and key soil parameters, we used Pearson's correlation coefficient.

3.5 RESULTS

Our results showed that there was no influence of seasons on the fungal community and glomalin production. However, there was the influence of land use. For example, total glomalin (TG) and easily extractable glomalin (EEG) showed differences, in which the pasture, AS3 and AS2, respectively, presented the highest concentrations of TG; while for EEG the highest concentrations were observed in AS2, AS3 and forest, respectively in rainy season (Table 7). The contribution of EEG to TG was greater in the agroforest systems and forest compared to the pasture and the greatest contribution of TG to the SOC was verified in the pasture in AS2 and AS3, respectively (Table 7). Greater sporulation was observed in agroforestry systems and in the forest than in pasture in dry season (Table 7). The contribution of EEG to TG was greater in the pasture and in the forest compared to agroforestry systems. While the contribution of TG to the SOC was higher in Agroforestry systems and pasture than in forest (Table 7). The AMF diversity indexes were higher in the forest compared to others land use types in the rainy season. In the dry season, diversity was greater in agroforestry systems and in pasture than in forest (Table 7).

Table 7. Mean values for spore density of AMF, glomalin fractions (TG and EEG), relationship between glomalin and SOC, and total values of ecological index sampled on an experimental farm in the county of Sapucaia – RJ, Brazil at two time points, in the rainy season (April) and in the dry season (September) of 2018. Values in italics below each mean represent the standard error from four measurements in each plot. P-values for one-way ANOVA are presented to the right of means. Means with different letters have significantly different values according to Tukey tests.

	Rainy Season (April)						**Dry Season (September)**					
AMF, glomalin variables and diversity indices	**Forest**	**Pasture**	**AS1**	**AS2**	**AS3**		**Forest**	**Pasture**	**AS1**	**AS2**	**AS3**	
Number of spores/ 50 g of soil	451	689	369	498	422		408^{ab}	390^{b}	693^{a}	667^{ab}	626^{ab}	**
	290	*246*	*205*	*160*	*165*		*102*	*133*	*156*	*257*	*109*	
TG (mg g^{-1})	2.53^{c}	4.22^{a}	2.63^{bc}	3.49^{abc}	3.71^{ab}	***	2.99	3.06	3.89	3.76	3.86	
	0.382	*0.57*	*0.51*	*0.20*	*0.82*		*0.262*	*0.275*	*0.876*	*0.749*	*0.555*	
EEG (mg g^{-1})	1.28^{b}	1.50^{ab}	1.66^{ab}	1.78^{a}	1.75^{a}	**	1.68	1.52	1.36	1.54	1.54	
	0.22	*0.14*	*0.32*	*0.11*	*0.12*		*0.34*	*0.09*	*0.21*	*0.37*	*0.37*	
EEG/TG	0.507^{ab}	0.361^{b}	0.642^{a}	0.513^{ab}	0.485^{ab}	***	0.557^{a}	0.501^{ab}	0.360^{b}	0.420^{ab}	0.396^{b}	***
	0.045	*0.059*	*0.121*	*0.051*	*0.086*		*0.069*	*0.046*	*0.072*	*0.108*	*0.049*	
SOC (g kg^{-1})	23.5^{ab}	23.1^{b}	28.0^{a}	25.3^{ab}	26.4^{ab}	*	21.6^{a}	21.4^{a}	24.2^{a}	22.6^{a}	24.5^{a}	*
	0.44	*1.9*	*2.26*	*2.12*	*3.4*		*0.858*	*1.14*	*4.46*	*4.45*	*1.13*	
EEG/SOC	0.055	0.065	0.059	0.070	0.067		0.057	0.072	0.058	0.071	0.062	
	0.009	*0.011*	*0.008*	*0.005*	*0.006*		*0.013*	*0.006*	*0.013*	*0.021*	*0.012*	
TG/SOC	0.108^{b}	0.182^{a}	0.093^{b}	0.138^{ab}	0.144^{ab}	***	0.138^{b}	0.143^{ab}	0.160^{ab}	0.167^{a}	0.157^{ab}	*
	0.018	*0.021*	*0.016*	*0.011*	*0.043*		*0.007*	*0.013*	*0.012*	*0.014*	*0.017*	
Total Richness (S)	10	8	8	7	8		9	11	12	10	9	
Margalef (D)	3.45	2.70	2.73	2.22	2.67		3.01	3.52	3.87	3.19	2.86	

*** Significant at p < 0.001, ** Significant at p < 0.01* Significant at p < 0.05.

A total of 13 AMF morphotypes were collected in each season (Rainy season and Dry season) (Figure 5), belonging to five families (*Acaulosporaceae*, *Ambisporaceae*, *Diversisporaceae*, *Glomeraceae* and *Gisgasporaceae*) and eight genera (*Acaulospora*, *Ambispora*, *Dentiscutata*, *Glomus*, *Gisgaspora*, *Racocetra*, *Rhizoglomus* and *Sieverdingia*). The cluster analysis according to the frequency of occurrence of AMF species in the rainy season (Figure 5A) showed that the forest occurs in an isolated and independent way, with a greater connection distance in relation to the other treatments, where the species seem to be better distributed, and the predominant groups were *Glomus clavisporum* (generalist), *Glomus macrocarpum* (generalist) and *Racoceta persica* (exclusive). Pasture also appears isolated with a predominance of species *Glomus clavisporum*, *Glomus macrocarpum*, *Acaulospora mellea*, Ambispora leptoticha (intermediaria) and *Rhizoglomus microaggregatum*. The group formed by AS1 and AS2 has the highest frequency of *Glomus clavisporum*, *Glomus macrocarpum*, *Acaulospora mellea* and *Acaulospora scrobiculata* (generalists). In AS3 there is a predominance of species *Glomus clavisporum*, *Glomus macrocarpum*, *Glomus glomerulatum*, *Acaulospora mellea* and *Acaulospora scrobiculata* (generalist). In the dry season, treatments were distinguished in three groups according to similarity, one group was formed by forest and pasture and second group was formed by AS2 and AS3, both with greater frequency of species *Glomus clavisporum*, *Glomus macrocarpum* and *Acaulospora mellea* (Generalists). In AS1, in addition to the groups mentioned, there was a predominance of the group *Acaulospora scrobiculata* (Generalist) (Figure 5B).

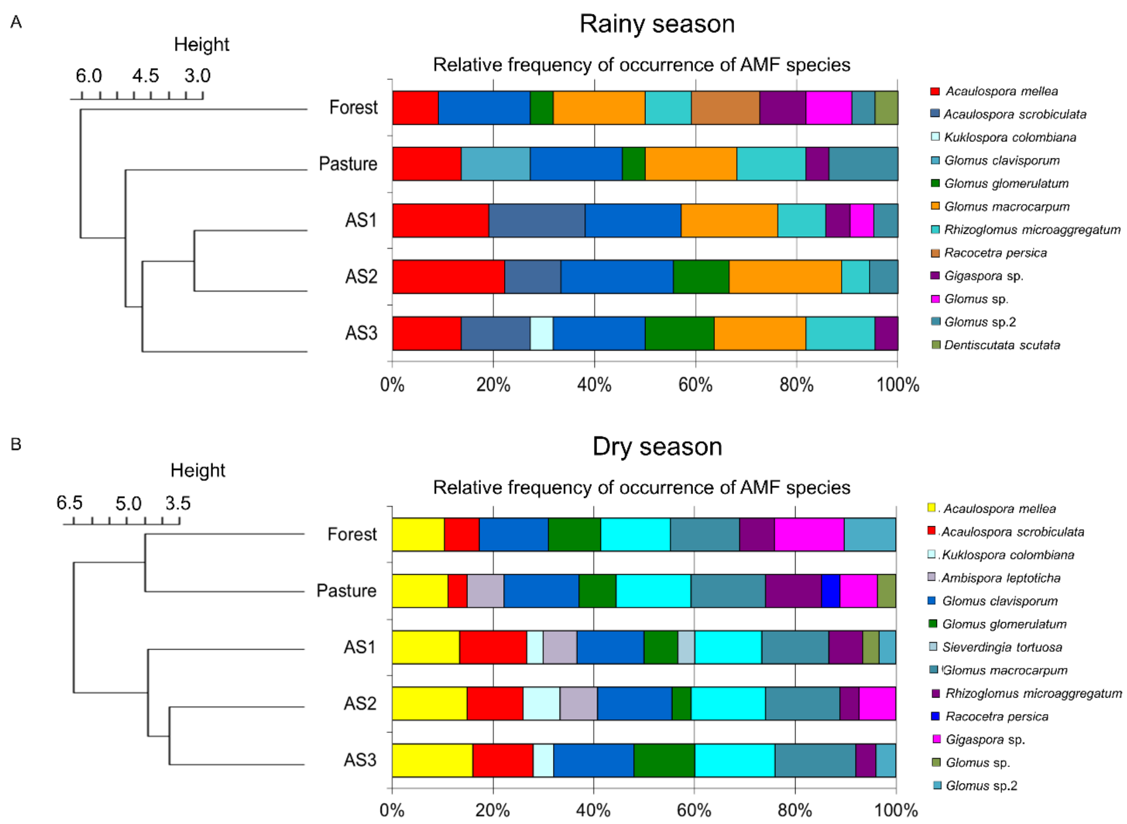

Figure 5. Cluster analysis according to the frequency of occurrence of AMF species for the rainy season (A) and dry season (B).

The results of the NMDS and PERMANOVA analyses showed that agroforestry plots were separated from forest and pasture. In rainy season, the separation was largely associated with differences in *Ambisporaceae, Gigasporaceae*, *Acaulasporaceae* (Figure 6A). In Dry season, the analysis again showed a clear separation between the land uses, with pasture clearly separated from forest and agroforestry systems. This separation was also related to *Racocetraceae*, *Ambisporaceae*, *Gigasporaceae* as well as that of *Glomearaceae* (Figure 6B).

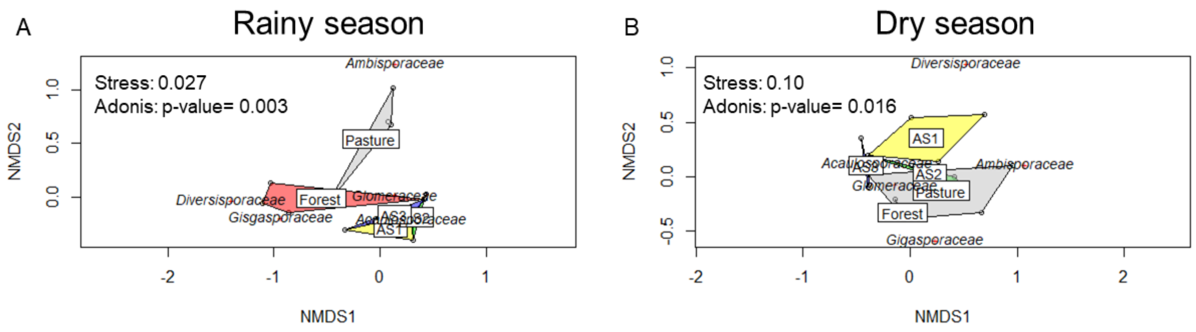

Figure 6. Nonmetric multidimensional scaling (NMDS) relating the AMF family, from plots sampled in rainy season (a) and dry season (b), respectively. Forest (red), Pasture (grey), AS1 (yellow), AS2 (green), AS3 (blue).

AMF family and total spore density were significantly related to a number of soil chemical and physical parameters (Table 8). For example, *Acaulasporaceae* was positively correlated with K, P, pH, SOC, MWD, moisture, and sand, but negatively correlated with H+Al, and clay. *Glomeraceae* was positively correlated with H+Al, and was negatively correlated with K, P and pH. *Gigasporaceae* was positively correlated with H+Al, SOC and MWD, but negatively correlated with pH. *Diversisporaceae* was positively related with H+AL, EEG, MWD and sand, but was negatively correlated with pH and moisture. *Ambisporaceae* was negatively correlated with P, K, pH and SOC, and positively correlated with clay. Finally, total spore density was positively related with SOC, EEG, MWD, CEC, sand, and negatively correlated with moisture (Table 8).

Table 8. Correlational analysis depicting the relationships among AMF family, Spore density and soil quality variables from soils collected from five land uses and two sampling times (Rainy or dry season) on an experimental farm in the county of Sapucaia - RJ, Brazil.

Soil Variables	***Acaulosporaceae***	***Glomeraceae***	***Gisgasporaceae***	***Diversisporaceae***	***Ambisporaceae***	**Spore density**
H + Al	-0.51***	0.59**	0.55**	0.57**	ns	ns
P	0.61***	-0.52***	ns	ns	-0.66***	ns
K	0.69***	-0.56***	ns	ns	-0.53***	ns
CEC	ns	ns	ns	ns	ns	0.45*
pH	0.59***	-0.54***	-0.62**	-0.51***	-0.52***	ns
SOC	0.54***	ns	0.65***	ns	-0.32***	0.49*
TG	ns	ns	ns	ns	ns	ns
EEG	ns	ns	ns	0.34**	ns	-0.55**
MWD	0.49**	ns	0.58**	0.56***	ns	0.49*
Moisture	-0.52**	ns	ns	-0.62**	ns	-0.45*
Clay	-0.32*	ns	ns	ns	0.39*	ns
Sand	0.35*	ns	ns	0.34**	ns	ns

*** Significant at $p < 0.001$, ** Significant at $p < 0.01$* Significant at $p < 0.05$.

3.6 DISCUSSION

Apparent differences in the AMF community were evident between the forest, pasture, and agroforestry systems plots. For example, the agroforestry systems supported an increase in sporulation and the total number of species, especially in the dry season (September), several weeks after pruning. It suggests that the high inputs of relatively high-quality and diverse residues in agroforestry systems encouraged microbial growth and activity (MATOS et al., 2020). In the rainy season, the forest showed high diversity compared to the others. It may be related to the fact that undisturbed systems present greater diversity in the plant community, allowing for the sporulation of different species of mycorrhizal fungi (SILVA et al., 2015). While in the pasture, the dry season appears to favor the diversity of AMF species. Possibly the lower humidity in this season may have influenced the sporulation of a more significant number of species (CAPRONI et al., 2018; COUTINHO et al., 2019).

It is important to emphasize that the technique (microscopy) used to identify species based on the spore phenotype is subjective, given that these structures undergo considerable changes with the environment, and characteristics such as color, size, and shape tend to vary with the type of soil, for example (SAGGIN-JÚNIOR et al., 2011). However, it is a widely used technique in studies to survey the diversity of arbuscular mycorrhizal fungi (OEHL et al., 2017; MAIA et al., 2020). In addition to the particularities of each land use, it may have been the other factor that influenced sporulation and AMF species groups and specific species. Favorable and even unfavorable environmental conditions may stimulate sporulation as a survival strategy of fungi (OEHL et al., 2009; VELAZQUEZ et al., 2013; SILVA et al., 2014).

Understanding the behavior of the AMF community is very important to comprehend glomalin production in each land use since the deposition of this protein in the soil occurs mainly by the decomposition of spores and hyphae (>80%) (DRIVER et al., 2005; PURIN and RILLING, 2008). The highest levels of TG were observed in pasture and agroforestry systems when compared to the forest in the rainy season; this suggests that in these land use, among other factors, there is a more significant decomposition of hyphae and spores, which consequently promoted a greater release of TG in the soil at this time. There was had a subtle alteration in the relative contribution of EEG to TG across land uses from the rainy to the dry season. In the rainy season, the contribution of EEG to TG was greater in agroforestry systems, while in the dry season, the contribution of EEG to TG was greater in the forest and pasture concerning agroforestry systems. Although statistically, the results show that there is no influence of seasonality on the production of glomalin, we suggest that these results are related, among other factors, to the more significant decomposition of spores and hyphae that may be occurring in agroforestry systems related to pasture and forest during the rainy season. While in the dry season, greater sporulation in agroforestry compared to other systems rather than decomposition may be driving these results.

Assuming the C content of glomalin to be 32% (SEGUEL et al., 2008), with respect to equivalent dry mass of soil. In our study, the greatest contribution of TG to SOC during the rainy season was about 5.69% in soils under pasture, followed by AS3 (4.5%) and AS2 (4.31%). In the dry season, the contribution of TG to SOC increased in relation to the rainy season at the agroforests and forest. This may be related to the high-quality litter input (low C: N ratio, MATOS et al., 2020) that occurred at this time through pruning in agroforestry systems and in the forest due to the fall of leaves of deciduous species, which possibly accelerated decomposition rates and increased TG concentrations (BIRD et al., 2002).

Beyond the influence of the plant species (CHAUDHARY et al., 2018), the soil characteristics (JOHNSON et al., 1992) also modulate the composition of AMF community.

We observed that AS1 and AS2 systems were very similar systems in terms of plant species composition and edaphic properties in the rainy season, which may explain the close AMF species composition in these systems. In the dry season, agroforestry systems, especially AS2 and AS3, presented similar AMF species composition and edaphic conditions. The species belonging to the genres *Glomus* e *Acaulospora* (*Glomus clavisporum*, *Glomus macrocarpum* e *Acaulospora mellea*) have high plasticity and therefore occur in all land uses. The specie Dentiscutata scutata occurred only in the forest and in the rainy season, while *Sieverdingia tortuosa* occurred only in AS1 and in the dry season. Such seasonal changes in the frequency of occurrence of AMF species do not necessarily reflect their elimination from the environment (PURIN et al., 2006).

Species considered occurring in low frequency in a season can be present in the environment in other forms, such as hyphae, colonized roots and auxiliary cells (SANTOS and CARRENHO, 2011), and so the absence of spores does not necessarily indicate the absence of root colonization and symbiotic association. Most of the species identified in our study (*Glomus clavisporum*, *Glomus macrocarpum*, *Acaulospora mellea*, *Acaulospora scrobiculata*, *Kuklospora Colombiana*, *Ambispora leptoticha*, *Glomus glomerulatum*) are characteristics of the Atlantic Forest biome (ZANGARO et al., 2010), and belong to the genres *Glomus* e *Acaulospora*, predominant Atlantic Forest (SILVA et al., 2015; SOUZA et al., 2006). According to Zangaro and Moreira (2010), some genres like *Acaulospora* have been detected as dominant in several areas of the Atlantic Forest (higher spore density and number of species in relation to other genera).

Our results showed that land uses significantly influenced the soil AMF community at the family level. For instance, the forest that is more conservative land use compared to the others favored *Gigasporaceae* which is sensitive to soil disturbance (CUENCA et al., 1998; ALLEN et al., 2003; DENISON and KIERS, 2011). On the other hand, *Acaulasporaceae* and *Glomeraceae* were associated with agroforestry systems and are considered dominant because they adapt to different conditions. The high supply of N from the litter (low C.N ratio, data not shown) in soils under agroforestry systems can change the structure of mycorrhizal communities, leading to the predominance of species with small spores, such as *Glomeraceae* (BERBARA et al., 2006). While *Ambisporaceae* (associated with pasture) possibly responded to high disturbance or low C input conditions by producing spores, making them able to persist and dominate in disturbed soils.

Significant correlations between AMF families and key soil parameters indicate a high degree of connectivity between physico-chemical and biological components of soil health. More importantly, these connections suggest that management strategies in each land use aimed at modifying one of these components is likely to have effects throughout the soil system. For example, the addition of litter inputs with high quality (low C.N ratio) through the pruning and green fertilizers increases soil fertility status, pH, and SOC in agroforestry systems (MATOS et al., 2020), consequently changing the composition of AMF communities. This change in soil quality in these land uses, probably favored families such as *Acaulasporaceae*, which was positively related with soil fertility attributes. While the *Ambisporaceae* was negatively correlated with most of the soil fertility properties including SOC, confirming the ability of individuals in this family to survive in disturbed soils. Soil pH can also affect mycorrhizal diversity directly or indirectly by compromising the availability of nutrients for the fungus or plant (MELO et al. 2017). In our research most AMF families were positively correlated with H + Al and negatively correlated with pH.

Most of the families identified in this study and total spore density are positively correlated with the stability of soil aggregates, revealing the importance that AMF have on soil structure (LEHMANN et al., 2017). Some authors claim that the action of fungi on the soil structure is related to glomalin, which is the main cementing agent of soil aggregates (Wright and Upadhyaya, 1998; Wright et al., 2007). The EEG was negatively correlated with spore density (Table 3), these results can be related with the fact that during the degradation of the AMF spores, there is an increase in the concentration of glomalin in the soil (STEINBERG and RILLIG, 2003; DRIVER et al., 2005). Moreover, we verified a positive correlation between spore density and SOC. The spore cell wall is rich in C, the spore cytoplasm has storage structures containing lipids, protein bodies and glycogen, molecules also rich in C (BONFANTE et al., 1994). Considering the structural and storage components of the spores, we can see how they contribute to C in the soil. Previous studies have demonstrated a correlation between the content of organic C in the soil and the number of spores of AMFs (YANG et al., 2011). Moisture also appears to modulate the structure of the AMF families and total sporulation; this is justified by the fact that spores are resistant structures and their existence in the system is usually reduced with high humidity when other structures such as hyphae are more abundant.

We suggest that changes in the AMF community and consequent changes in glomalin production and other soil attributes are still subtle due to the short implementation time of agroforestry systems (8 years). However, adopting land use systems with significant and continuous plant residue deposition (litter and roots) such as agroforestry systems provides an ecosystem service of considerable relevance for improving AMF activity and keeping the diversity of AMF species, as well for glomalin production and protecting C in the soil. It then makes it a viable alternative for Brazil to effectively engage in global action to mitigate the emission of greenhouse gases, reduce global warming and develop production systems for developing low carbon agriculture, in line with the United Nations (UN) 2030 Agenda of Sustainable Development Goals (SDGs) of smart agriculture for the climate (climate-smart agriculture).

3.7 CONCLUSIONS

Our research provides essential contributions for understanding short-term modifications on the AMF community and glomalin-related soil protein and linkages between soil biodiversity and key soil parameters in tropical agroforestry systems.

This work demonstrates that litter quality, plant diversity, and soil characteristics could be important factor that modulates AMF spore production and diversity. Nevertheless, these effects were detected shortly after management (only eight years after adoption of agroforestry systems); it is necessary to continue monitoring the experimental areas of this study since it will allow us to know the long-term effects and fully understand the causal links between the different land uses.

Future studies using these indicators to monitor these soils' quality and infer the sustainability of each land use are encouraged. Taken together, this study further supports environmental legislation using agroforestry system as a viable option to restore degraded lands and to comply with Brazil’s international commitments to reduce greenhouse gas emissions, move towards low-C agriculture, and consequently contribute to improving global food security.

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4 CHAPTER III

SOIL ORGANIC CARBON FRACTIONS IN AGROFORESTRY SYSTEM IN BRAZIL: SEASONALITY AND SHORT-TERM DYNAMIC ASSESSMENT

4.1 RESUMO

A adoção de usos do solo que contribuam com a entrada constante de serrapilheira no sistema, pode influenciar no armazenamento de carbono orgânico do solo (COS) em suas diferentes frações com taxas de renovação variáveis. Além disso, a sazonalidade pode influenciar a dinâmica da matéria orgânica do solo. No entanto, os mecanismos desses efeitos, particularmente em sistemas agroflorestais, ainda não estão claros. Neste estudo avaliou-se as respostas do teor de COS nas diferentes frações do solo à implementação de curto prazo de sistemas agroflorestais no sudeste do Brasil e como a sazonalidade pode influenciar a dinâmica do COS e de suas frações. Além disso, testou-se se o índice de manejo de carbono (IMC) é sensível para detectar a qualidade das práticas de manejo em pastagens (não manejadas), diferentes sistemas agroflorestais e uma área de referência (floresta). Foram avaliadas as propriedades físicas do solo, o conteúdo de SOC, as fracoes de carbono orgânico particulado (COP) e carbono orgânico associado a minerais (COAM) em três profundidades diferentes (0-5, 5-10 e 10-20 cm) em resposta à adoção de sistemas agroflorestais. Os resultados mostraram que efeitos de curto prazo da adoção de sistemas agroflorestais nas frações de carbono do solo foram observados nas camadas mais superficiais (0-5, 5-10 cm), principalmente na fração POC. A sazonalidade influenciou a dinâmica do SOC e de suas frações. O IMC foi sensível para detectar mudanças causadas pela conversão do uso do solo e mostrou que a pastagem acumula carbono no solo mesmo com sinais de degradação. Portanto, este estudo fornece informações relevantes sobre o manejo do carbono do solo em sistemas agroflorestais.

Palavras-chave: Sequestro de carbono do solo. Agricultura regenerativa. Sustentabilidade.

4.2 ABSTRACT

Adopting land uses that contribute a considerable litter input can affect soil organic carbon (SOC) pool within different soil fractions with varying turnover rates. Moreover, changes in seasons can influence the dynamic of soil organic matter. However, the mechanisms of these effects, particularly in agroforestry systems, are not clear. We studied the responses of SOC content in different soil fractions to the short-term implementation of agroforestry systems in southeast Brazil and how seasonality can influence the dynamics of SOC and its fractions. Also, we tested if the carbon management index (CMI) is sensitive to detecting management practices quality across the unmanaged pasture, different agroforestry systems, and a reference area (forest). We measured soil physical properties, TOC content in bulk soil, particular organic carbon (POC), and mineral-associated organic carbon (MAOC) fractions at three different depths (0-5, 5-10, and 10-20 cm) in response to the adoption of agroforestry systems. Our results showed that short-term effects of the adoption of agroforestry systems in soil carbon fractions were noticed in the most superficial layers (0-5, 5-10 cm), mainly in the POC fraction. Seasonality influenced the dynamics of the SOC and its fractions. The CMI was sensitive to detect changes caused by land-use change and showed that pasture accumulates carbon in the soil even with signs of degradation. Therefore, this study provides relevant information regarding the management of soil carbon in agroforestry systems.

Keywords: Soil carbon sequestration**.** Regenerative agriculture. Sustainability.

4.3 INTRODUCTION

Globally, combating climate change is one of the main challenges of the 21st century and, terrestrial soils are recognized as substantial potential in stabilizing the climate (FAO, 2019). They store twice as much carbon as the atmosphere (LAL, 2004; DAVIDSON AND JANSSENS, 2006; PAUSTIAN et al., 2016), with an estimated soil carbon storage of 1462 to 1584 Pg in the upper 100 cm of the soil profile (SINGH et al., 2018). Because of this, soils have recently become part of the global carbon agenda for climate-change mitigation and adaptation through the launch of three high-level initiatives. These include the "4p1000 initiative" launched at COP21 by UNFCC under the Lima-Paris Action Plan (LPAP) framework in Paris on December 1, 2015. The initiative's name reflects that a comparatively small proportional increase (4 ‰) of the global SOC stocks in the top 0.3–0.4 m of all non-permafrost soils would be similar in magnitude to the annual global net atmospheric CO_2 growth (RUMPEL et al., 2018). The second initiatives were the Koronivia workshops on agriculture, which included soils and SOC for climate-change mitigation and were initiated at COP23 in 2018. Finally, the FAO launched RECSOIL, a program for the recarbonization of soils (FAO, 2019). The message of all three initiatives is complementary and simple: increasing SOC can partly mitigate carbon emissions and is, at the same time, indispensable for the adaptation of agricultural systems to climate change due to the numerous co-benefits it offers.

Soil organic matter (SOM) is composed of different fractions, with different degrees of lability and recalcitrance. From a physical granulometric point of view (size), SOM comprises two types of fractions: particulate organic matter (POM) and organic matter associated with minerals (MAOM) (CAMBARDELLA AND ELLIOTT, 1992; COTRUFO et al., 2019; HADDIX et al., 2020; LAVALLEE et al., 2020). POM is predominantly made up of plant-based particulates of 0.053 mm to 2 mm in size, is mainly composed of fragmented, relatively undecomposed plant litter, is sensitive to and respond rapidly to soil quality under different condition and has a more rapid turnover time than MAOM (POEPLAU et al., 2018; LAVALLEE et al., 2020; YUAN et al. 2021). In comparison, MAOM consists of single molecules or tiny pieces of organic material leached directly from plants or been chemically transformed by the soil biota (COTRUFO et al., 2019). Their highly contrasting physical and chemical properties and mean residence times in soil determine their different responses to global pressures, such as land-use change, warming, and atmospheric CO_2 enrichment (XIAO et al., 2015).

Given this scenario, it is essential to implement sustainable and adaptive forest management practices to manage better forests to cope with future climate change challenges. Agroforestry is recognized as an afforestation activity for GHGs mitigation under the Kyoto Protocol (NAIR et al., 2009). Several studies showed Agroforestry as a potential strategy to improve soil carbon by providing organic C to the soil via branch pruning, root turnover, and exudation, and leaf litter (MONTAGNINI AND NAIR, 2004; OELBERMANN et al., 2006; KHALID et al., 2007; CHEN et al., 2017; GUO et al., 2018). A tool that has been successfully used to assess soil quality in management systems in subtropical and tropical regions is the carbon management index (CMI), proposed by Blair et al. (1995), which combines C quantity and lability in an integrated approach (ZANATTA et al., 2019). The CMI expresses the soil quality in increments in the SOC and the proportion of labile C (LC) compared to a reference soil, generally under native vegetation, which arbitrarily has a CMI of 100.

In addition, LC pools are strongly time-sensitive, and seasonal changes may play a vital role in nutrient availability and microbial activity. However, there is limited information on soil C pools' vertical and seasonal variations in agroforestry systems (GUO et al., 2018). We hypothesize that agroforestry systems, even with short implementation times, contribute to the accumulation of carbon in the soil, mainly through the POC fraction. Also, that season's changes influence the dynamics of organic matter in the soil. Furthermore, we believe that the CMI is sensitive to detecting management practices' effect on each land use. To test these hypotheses, we examine: (1) How do TOC stocks and their fractions respond to the short-term implementation of agroforestry systems in southeast Brazil? (2) Does change in seasons influence the dynamics of TOC and its fractions? (3) Is CMI sensitive to quality detection of management practices across the reference area (forest), unmanaged pasture, and different agroforestry systems?

4.4 MATERIAL AND METHODS

4.4.1 Site description and land uses

This study was conducted at the Arca de Noé Farm, an agroecological research station located near the city of Sapucaia, Rio de Janeiro, Southeastern Brazil (21º 59' 42" S, 42º 54' 52" W; 800 m.a.s.l) (Figure 7). The region is characterized by dry winters and temperate summers (Cwb in the Köppen Climate Classification system), with mean monthly temperatures that vary between 17°C and 32°C (June and January; respectively) and a mean annual rainfall of 1,451 mm. Soils at this site are predominantly Ultisols (USDA, 2014) with a clay-loam texture. Argissolos according to SiBCS (SANTOS et al., 2018). The region is primarily comprised of massifs of highland hills and cliffs, with natural vegetation generally dominated by the Atlantic Forest. In this study, we considered five existing land uses at the farm. More details related to management and plant species present in the areas can be found at Matos et al. (2020).

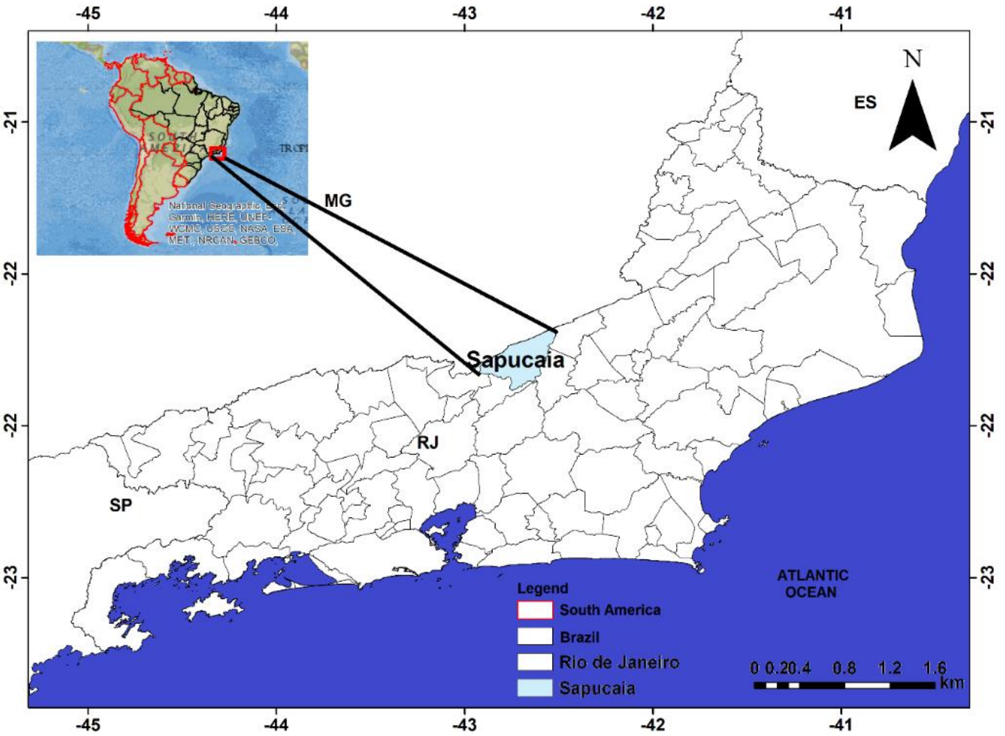

Figure 7. Location of the study area in Sapucaia, Rio de Janeiro, Brazil.

In this study, we considered five existing land uses on the farm: (1) secondary forest (FOREST) dominated by semideciduous tree species (*Tibouchina granulosa*, *Lecythis Pisonis* Cambess., *Centrolobium tomentosum* Guillem. ex Benth., *Inga* spp., *Schizolobium parahyba* (Vell.) Blake, *Peltophorum dubium*, *Hymenaea courbaril*, *Aspidosperma olivaceum* Müll. Arg., *Dalbergia nigra* (Vell.) Allemão ex Benth.); (2) pasture replanted with the grass *Urochloa decumbens*, unmanaged, under extensive grazing (PASTURE); (3) an agroforestry system characterized by the integration of banana and coffee with a mix of other fruit and timber species and other species to provide shade, biomass production and pollination services (AS1); (4) an agroforestry system focused on bananas and energy production (which also includes fruit trees and a mix of other trees and plants (AS2); and (5) a third agroforestry system focused on bananas and other fruits (AS3; see Table 1 for detailed species lists). The Pasture was established by removal of native vegetation in 1995. In 2010, the agroforestry systems were planted on a portion of this existing pasture. These systems received a single application of rock phosphate (fertilizer permitted in organic production) and cattle manure to the banana tree roots at the time of establishment. The secondary forest was included here as a reference and had an age of about 30 years since the previous deforestation. All plots considered in this study were located on the same soil type and textural class and had similar slopes of roughly 30◦.

4.4.2 Sample treatment and analyses

Sampling was conducted in 2018 at two separate time season, rainy (April) and dry (September), searching to assess a suite of soil biological, chemical, and physical properties within each land use (e.g., forest, pasture and agroforestry systems). One transects was laid out in each land use type, and four sampling plots (6 × 8 m) were established approximately 15 m apart along the transect. The four plots within each land use were considered pseudoreplicates. Physical parameters were evaluated only in the rainy season (April), since there is no significant difference in their evaluation in the two seasons (TAVARES et al., 2018; CASTIGLIONI et al. 2018; AGBESHIE et al., 2020). Soil samples from each depth (0-5, 5-10, and 10-20 cm) were air-dried and passed through a 2-mm sieve. Soil particle size was determined using the pipette method (TEIXEIRA et al., 2017) (Table 9). The volumetric ring method determined soil bulk density (TEIXEIRA et al.,2017) (Table 9).

Table 9. Particle size fractions and bulk density, up to 20 cm, across Forest, Pasture, AS1, AS2 and AS3 in Sapucaia-RJ, Brazil.

Variables	Forest	Pasture	AS1	AS2	AS3
Depth 0-5 cm					
Sand (%)	56.5	48.8	42	50	46.2
Silty (%)	10.5	13.2	27.2	19.5	19.2
Clay (%)	33	38.2	30.8	30.2	30.2
BD (Mg m-3)	1.39	1.82	1.52	1.46	1.44
Depth 5-10 cm					
Sand (%)	59.5	47.2	49.5	50.2	48.2
Silty (%)	10.5	18.8	22	18.2	17
Clay (%)	30	34	28.2	31.2	34.5
BD (Mg m-3)	1.56	1.83	1.61	1.57	1.56
Depth 10-20 cm					
Sand (%)	59	55	43	49	47.2
Silty (%)	9.75	10.2	24.8	17.8	18.5
Clay (%)	31.5	31.8	32.2	33	34.2
BD (Mg m-3)	1.72	1.72	1.83	1.75	1.82

The total organic carbon (TOC) was determined by oxidation using potassium dichromate with external heat and titration with ammonium iron sulfate, according to the modified method of Yeomans and Bremner (1988). The TOC stocks in each layer were determined by multiplying the total organic carbon in the sample by the soil density (volumetric ring method) and the layer thickness by the equivalent layer method (ELLERT AND BETTANY, 1995; CARVALHO et al., 2009; FERNANDES AND FERNANDES, 2013). The calculation of the SOC Stock of each layer sampled was calculated from equation 1:

$$SOCStock = \frac{SOC \times Ds \times \left(\frac{Dref}{Ds} \times e\right)}{10} \quad (1)$$

Where: *SOCStock*: Total organic carbon stock at a certain depth (Mg ha^{-1}); *TOC*: Total organic carbon content at the sampled depth (g kg^{-1}); *Ds*: Soil density in the depth sampled (Mg m^{-3}); *Dref*: Soil density for sampled depth in the reference area (Mg m^{-3}); e *e*: Thickness of the considered layer (cm).

For the physical fractionation of the SOM, 20 g of soil samples passed in a 2.00 mm mesh were used. In each sample, 60 mL of sodium hexametaphosphate solution (5g L^{-1}) was added, then shaken for 15 hours in a horizontal shaker (CAMBARDELLA AND ELLIOT, 1992). Then, the suspension was passed through a 53 µm sieve with the aid of a water jet. The material retained in the sieve corresponds to the particulate organic carbon (POC) associated with the sand fraction. The material that passes through the 53 µm sieve corresponds to the mineral-associated organic carbon (MAOC) of the silt and clay fractions, obtained by the difference between TOC and POC. The POC was determined via wet oxidation (YEOMANS AND BREMNER, 1988).

Based on SOC changes between Forest that were considered as reference area (REF) and cropped soils, a C pool index (CPI) was calculated (CPI = SOC cropped/SOC REF) (Blair et al., 1995). Based on changes in the labile C proportion in the soil, a lability index (LI) was determined (LI = L cropped/L REF) considering the POC as the labile C, and the difference between SOC and POC estimated MAOC non-labile C. Both CPI and LI were used to calculate CMI = CPI × LI × 100 (Blair et al., 1995).

4.4.3 Statistical analyses

The differences among the studied variables were tested for their significance between the plots for each soil property in each land use (forest, pasture, AS1, AS2, and AS3) and between seasonality. The sampling pattern includes spatially multiple self-dependent plots with low variances within each. Therefore, the linear mixed-effects model (lme4 R package, v. 1.1-23 and lmerTest, v. 3.1-2) was applied to examine significant differences among land uses and seasonality variables, set as a fixed and the sampling plots as a random effect. The type II Wald X2 test and least-square mean for a pairwise t-test with false discovery rate correction for multiple comparisons (car R package v. 3.0-10) were used to explore the influence of seasonality on variables in each land use, and further, to measure the differences between land uses for each variable.

4.5 RESULTS AND DISCUSSION

4.5.1 SOC stocks and SOC fractions

Soil organic carbon stocks (SOC stocks) are determined by several site factors and also by the amount of above - and belowground litterfall, rooting depth (allocation of organic matter), and decomposition rate of organic material (chemical quality), which depends on the biological activity of the soil and also on local climatic conditions. For example, the SOC stock was higher in forest and pasture in the rainy season at a depth of 10-20 cm (Figure 8a), agreeing with the SOC content in this land uses (Table 10). It may be likely associated with high microbial activity in this land uses in this period, as shown by Matos et al. (2020). Higher microbial activity increases dissolved organic carbon release for a short period (BRYE et al., 2001; LEINWEBER et al., 2008) and induces faster turnover of C fractions. In the dry season, at a 5-10 cm depth, the forest, AS1, and AS2 had higher SOC stocks (Figure 8b). It is possibly related to the higher value of the most stable fraction (MAOC) in these land uses in this period (Table 10), which are possibly being influenced by the quality of the organic material. Our results corroborate the results found by Bieluczyk et al. (2020).

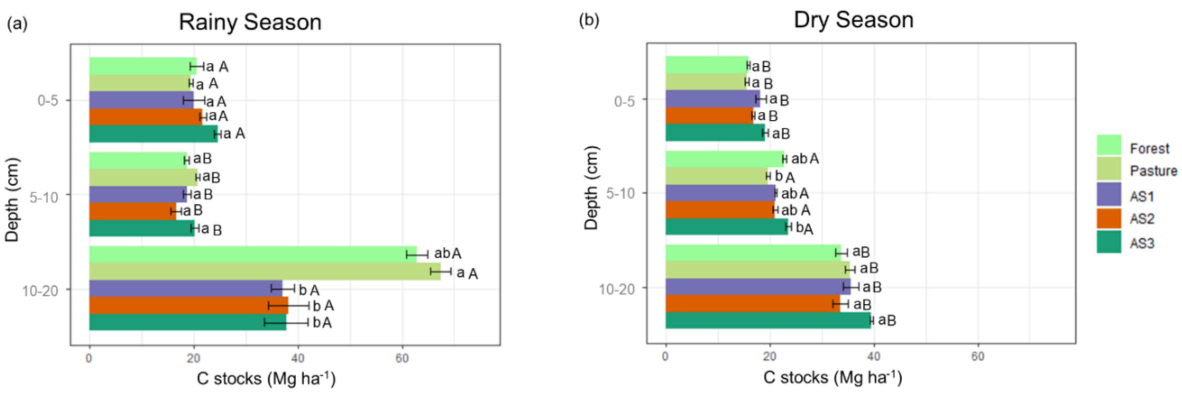

Figure 8. SOC stocks at different depths of soils across Forest, Pasture, AS1, AS2 and AS3 in Sapucaia-RJ, Brazil in rainy and dry season. Mean followed by the same letter do not differ statistically. Lowercase letters represent the variation between land uses and uppercase letters the variation between seasons.

Considering the soil under forest, pasture decreased the SOC content about 14 % in the 5-10 cm soil layer in the dry season, while agroforestry systems increased by 7.38 % (AS1), 6.69 % (AS2), and 16.33 % (AS3) related to pasture in the same layer (Table 2). It is important to emphasize that the agroforestry systems kept the soil's organic carbon content related to the forest (Table 2). These results are likely due to the more significant amounts of surface biomass in the dry season, the fall of semideciduous leaves in the forest, and the pruning that occurred in agroforestry systems, which possibly increased the inputs of organic carbon in the soil.

Table 10. Mean values of TOC, POC and MAOC sampled on an experimental farm in Sapucaia – RJ, Brazil at rainy season (April) and dry season (September) of 2018.

	Rainy season						**Dry season**					
Variables	**Forest**	**Pasture**	**AS1**	**AS2**	**AS3**	***p-value***	**Forest**	**Pasture**	**AS1**	**AS2**	**AS3**	***p-value***
Depth 0-5 cm												
SOC	29.4^{a}	28.0^{a}	29.0^{a}	31.3^{a}	33.7^{a}		22.8^{a}	22.4^{a}	26.2^{a}	24.2^{a}	27.4^{a}	
	6.89	*1.58*	*11.6*	*3.17*	*1.24*		*1.07*	*1.07*	*5.1*	*1.95*	*3.12*	
POC	7.79^{ab}	5.63^{b}	7.81^{ab}	6.71^{ab}	8.13^{a}	*	9.92^{a}	5.71^{b}	9.28^{a}	8.23^{ab}	9.18^{a}	*
	1.49	*1.33*	*0.81*	*1.13*	*0.89*		*1.92*	*2.20*	*0.72*	*0.82*	*1.27*	
MAOC	21.6^{a}	22.4^{a}	23.7^{a}	24.6^{a}	26.3^{a}		13.9^{b}	15.7^{ab}	18.1^{a}	16.0^{ab}	18.2^{a}	*
	7.28	*1.85*	*7.43*	*2.39*	*1.64*		*0.96*	*1.45*	*3.8*	*1.53*	*2.91*	
Depth 5-10 cm												
SOC	23.9^{a}	26.6^{a}	24.1^{a}	23.0^{a}	25.8^{a}		29.2^{ab}	25.1^{b}	27.1^{ab}	26.9^{ab}	30.0^{a}	*
	2.59	*2.17*	*3.53*	*4.51*	*3.35*		*2.19*	*1.22*	*1.92*	*2.91*	*1.32*	
POC	3.1^{a}	3.85^{a}	3.95^{a}	3.86^{a}	3.01^{a}		3.07^{a}	2.99^{a}	6.89^{a}	3.62^{a}	4.22^{a}	
	0.68	*0.76*	*1.10*	*0.61*	*0.55*		*0.46*	*0.29*	*5.58*	*1.02*	*0.71*	
MAOC	20.8^{a}	22.7^{a}	19.9^{a}	20.4^{a}	22.8^{a}		25.6^{a}	22.1^{a}	22.2^{a}	23.3^{a}	25.7^{a}	
	2.38	*2.62*	*2.45*	*3.59*	*2.92*		*1.66*	*1.21*	*2.25*	*2.06*	*1.88*	
Depth 10-20 cm												
SOC	36.5^{a}	39.4^{a}	21.4^{b}	22^{b}	21.9^{b}	**	19.7^{a}	20.6^{a}	20.6^{a}	19.4^{a}	23.0^{a}	
	3.08	*3.46*	*4.06*	*8.23*	*9.52*		*2.48*	*1.05*	*2.46*	*2.65*	*1.37*	
POC	1.98^{a}	1.68^{a}	1.47^{a}	1.82^{a}	1.9^{a}		2.89^{ab}	2.99^{a}	1.41^{c}	1.78^{bc}	1.76^{bc}	**
	0.99	*0.2*	*0.94*	*0.5*	*0.64*		*0.69*	*0.79*	*0.23*	*0.26*	*0.45*	
MAOC	34.5^{a}	36.9^{a}	20.0^{b}	20.2^{b}	20^{b}	**	17.0^{a}	17.6^{a}	17.5^{a}	17.9^{a}	21.3^{a}	
	3.68	*3.81*	*3.69*	*7.76*	*9.81*		*3.34*	*1.48*	*2.23*	*2.64*	*1.52*	

Values in italics below each mean represent the standard error from four measurements in each plot. Mean followed by the same letter do not differ statistically. Abbreviations: SOC: Soil organic carbon; POC: Particulate organic carbon; MAOC: Mineral-associated organic carbon. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; . $p <0.1$.

The POC is often used as an indicator of soil quality as it is a readily available source of soil nutrients, contributes to soil structure, is overly sensitive to soil management due to its relatively fast turnover rates and close link to litter input (LAVALLEE et al., 2020; POEPLAU et al., 2018). Our results showed that the variation in POC content between land uses in the two seasons at a depth of 0-5 cm, with higher values for agroforestry systems and forests (Table 1), is dependent on the addition of plant residues in quantity and quality. Thus, land uses that provide a more significant input of these residues to the soil surface, with a C/N and lignin/N ratio favorable to mineralization, influence the maintenance of POC values (COTRUFO et al., 2013). These high levels of POC in agroforestry systems and Forest, mainly on the surface, are meaningful for the natural functioning of this soil ecosystem.

The MAOC fraction in the dry season was higher in the agroforestry systems and the pasture at a depth of 0-5 cm compared to the forest (Table 10). The practices carried out in the managed areas favor the gradual increase in surface MAOC contents in the dry (statistically) and rainy (numerically) seasons. However, from a management point of view, the MAOC does not always work as a good indicator for measuring soil quality since changes in the contents of this SOM compartment take many years to be observed (CARMO et al., 2012) due to the high degree of stability of this physical fraction of SOM. At a 10-20 cm depth, the MAOC content fraction showed the same trend as SOC in the rainy season, with higher averages for Forest and pasture in depth (Table 10). As mentioned above, we suggest that this may be related to more significant microbial activity in these land uses in referred season. Moreover, the higher MAOC contents in all depths related to the POC indicated more efficient processes of stabilization and humification of the soil organic matter (SOM) in all land uses (Table 10).

High contents of MAOC are essential to ensure the supply to microorganisms, the SOM oxidation processes, and the carbon stocks, preventing soil loss and degradation processes. From a climate change mitigation perspective, increasing the soil carbon content of the MAOC fraction is more desirable than increasing the POC fraction since this represents a more stable, long-term carbon reservoir in soils (MIDWOOD et al., 2021). Nevertheless, a careful balance between the content of these two fractions is desired to ensure at the same time that some of the carbon is locking away and allowing SOC turnover to release nutrients, and plant productivity must be struck.

4.5.2 Seasonality's influence on SOC stocks and SOC fractions

Seasonality influenced SOC stocks at all depths (Figure 9). Our results corroborate Wuest (2014) and Ryan et al. (2009), who confirmed that seasonal changes influence SOC. At depths of 0-5 and 10-20 cm, the land uses had higher averages in the rainy season (Figure 8a). It may signal faster carbon mineralization, which is possibly influenced by weather conditions, along with the quality of the organic material that promotes carbon accumulation in the most stable fractions of the SOM. The increase in soil carbon contents is a slow process due to the complexity of stable organic fractions, depending on the quantity and quality of deposited organic residues and the prevailing climatic conditions that directly affect the decomposing biota community (TORRES et al., 2019). On the other hand, at 5-10 cm depth, the highest means in all treatments were found in the dry season (Figure 8b). Moreover, we founded that SOC and SOC stock had similar patterns for seasonality (Figure 8; Figure 9).

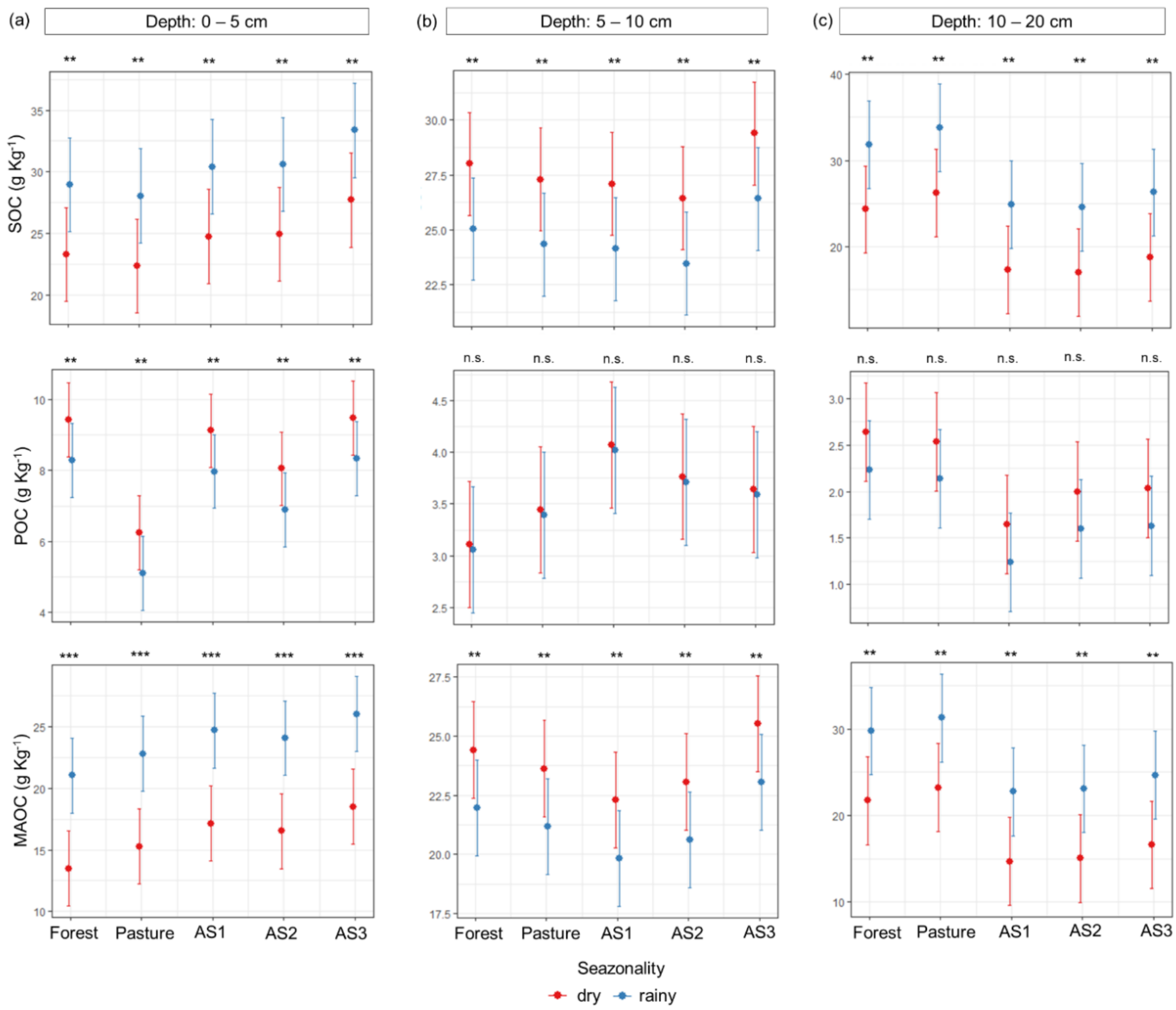

Figure 9. Effect of seasonality on SOC, TOC and MAOC in each land use in deep of (a) 0-5 cm, (b) 5-10 cm, (c) 10-20 cm. Vertical bars are confidence intervals for the means. Asterisks represent significant differences (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$) between seasonality within land uses.

The literature showed many studies about the seasonal influence on respiration and microbial factors but very few analyzing for seasonal trends in SOC (WUEST et al., 2014). However, our results showed that rapid changes in soil carbon due to seasonal inputs of plant residues, roots, and exudates or decomposition of such inputs could occur. For example, at depths 0-5 cm and 10-20 cm, the SOC was higher in the rainy season than in the dry season for all land uses (Figure 9 a, c). These results indicate that the environmental conditions verified in this period may favor the maintenance and accumulation of carbon in the more stable fractions of the SOM. Therefore, we suggest that the management of plant species pruning in the agroforestry systems studied for this time of year should prioritize plants with high phytomass production and low decomposition rate of their residues so that SOM mineralization and nutrient cycling would be slower. The MAOC fraction had the same trend as the SOC, with higher values in the rainy season at depths of 0-5 and 10-20 cm (Figure 9 a, c). At a depth of 5-10 cm more excellent values in the dry season (Figure 9 b). A different result was found by Puissant et al. (2017) in grasslands which found that the MAOC fraction did not change with the seasons. The POC fraction was significantly higher in the dry season at a depth of 0-5 cm and showed the same trend at other depths in all land uses (Figure 9 a, c). In addition to the intrinsic climatic conditions of this season that directly affect the population of decomposing microorganisms, the quantity and quality of deposited organic waste possibly influenced this result (TORRES et al., 2018). Because this time occurred pruning management in agroforestry systems and when there is a more outstanding contribution of litter in the forest due to the fall of leaves from semideciduous species, possibly soil microorganisms worked in the decomposition of litter at the level of particulate organic matter. In the pasture, this is mainly due to the death and decomposition of the raises in this season, contributing to the increase in POC.

4.5.3 Carbon indexes

The carbon pool index (CPI) to assess changes in the size of the SOC pool caused by land use is recommended (BLAIR et al., 1995). Lower CPI values indicate higher organic C loss. CPI > 1 also indicates aggradation in soil quality related to soil organic matter content and all benefits of this component for soil improvement. In our study, at depth 10-20, there was a difference between land uses for CPI, with the highest average in the pasture (1.08) compared to others in the rainy season (Figure 10a). This result reflects the highest SOC content in pasture and forest during the rainy season (Table 10). Also, there was variation for the CPI at a depth of 5-10 cm, where agroforestry systems showed the highest values in the dry season (Figure 10b). It can be possibly related to the pruning management was carried out in these land uses at this season, so a greater volume of fresh material was released onto the soil, increasing the carbon content.

There was no statistical difference between land uses in seasons for the lability index (Figure 10 c, d). In contrast, the carbon management index (CMI) was higher in pasture than others in the dry season at a depth of 10-20 cm (Figure 10f). It may be related to the robustness of the Urochloa grass root system. According to Gichangi et al. (2017), the Urochloa root system can substantially increase C storage in the soil by producing massive root biomass compared to other species. Given this, we suggest that pasture could be a component to be included in agroforestry systems to increase even more carbon in the soil.

The CMI assesses changes in SOC stocks considering aspects related to the lability of the physical carbon fractions in the soil. CMI values >100% (reference area) indicate good practices for maintaining SOM and soil quality in different management systems (ROSSI et al., 2012; GAZOLLA et al., 2015; NASCIMENTO et al., 2017). Given this, it is essential to highlight that the CMI values at depths of 0-5 and 5-10 cm in both seasons were close to or even more significant than 100 (Figure 10 e, f). We suggest that changes in soil quality, and consequently, in soil carbon accumulation, occur in the most superficial layers of the soil due to the short implementation time of agroforestry systems (8 years). Since the effects of organic material input and nutrient cycling will be seen primarily at the topsoil level, over time, these agroforestry systems will likely contribute to the accumulation of carbon in the soil in deeper layers (IWATA et al., 2012; SANG et al., 2013).

The fluctuations due to seasonal changes in the SOC, POC, and MAOC fractions in all land uses are reflected in all indices. For example, at a depth of 5-10 cm, the CPI was higher in the rainy season, while at a depth of 10 - 20 cm, it was higher in the dry season (Figure 10 a, b). The lability index was higher in the rainy season at depths of 0-5 and 5-10 cm (Figure 10 c, d). The carbon management index was higher in the rainy season at a depth of 0-5 cm (Figure 10 e, f). Using soil carbon indices can complement the understanding of organic carbon dynamics and its respective fractions under different soil-environmental conditions.

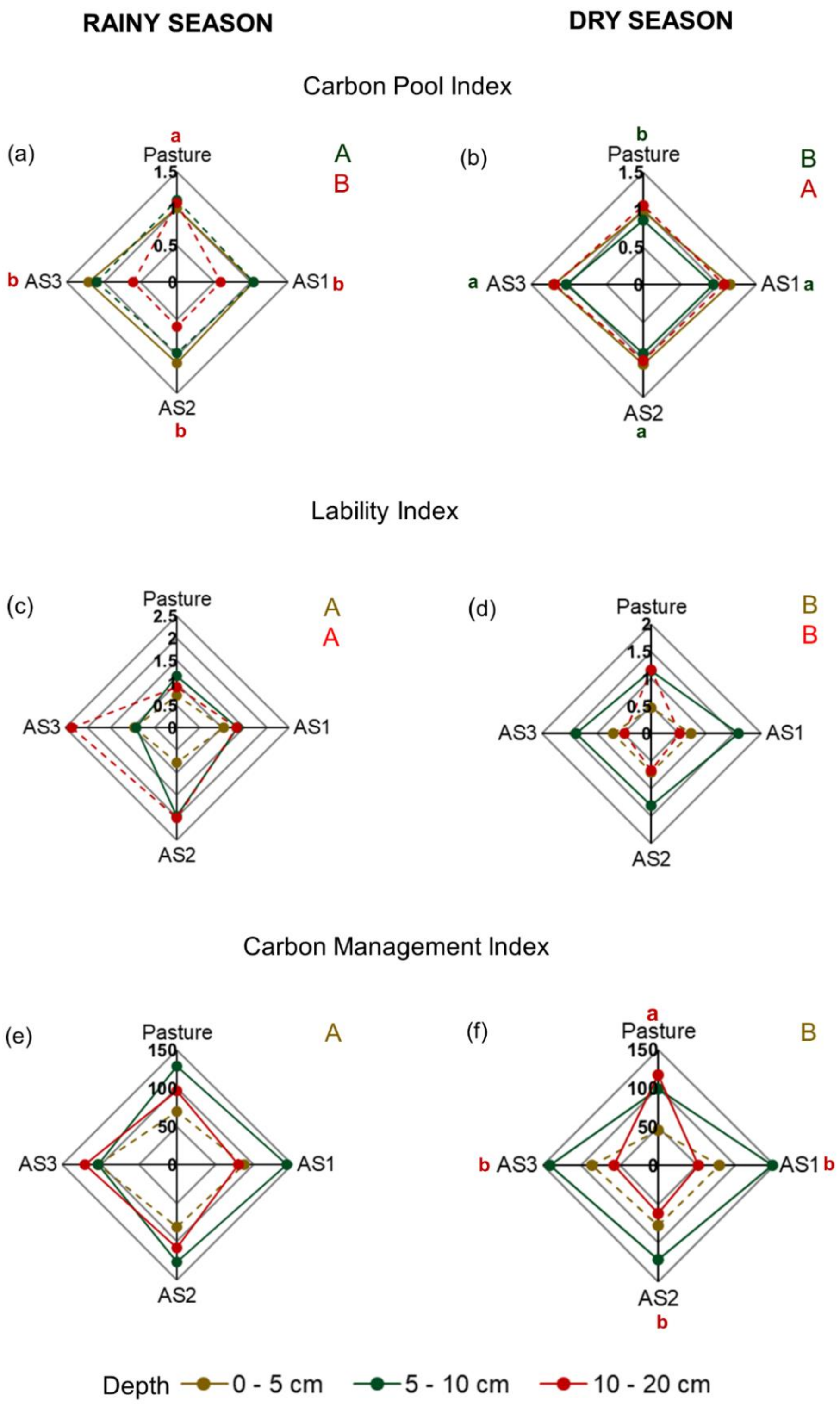

Figure 10. Carbon pool index (CPI), lability index (LI) and Carbon Management Index (CMI) at three depths (0-5, 5-10, 10-20 cm) across Forest, Pasture, AS1, AS2, AS3 in Sapucaia – RJ, Brazil in the rainy and dry seasons. Colors differentiate the depths. Dashed lines represent at what depth the land uses were influenced by seasonality. Lowercase means represent the difference between land uses. The uppercase letters represent the differences between the seasons.

4.6 CONCLUSIONS

This study showed that agroforestry systems are a viable strategy in terms of soil carbon accumulation in the most superficial layers, mainly of label fractions as POC; however, the trend is that over time there is potential for these systems to contribute more to the accumulation of soil carbon.

The farmers can monitor the quality of the litter by choosing the species that will make up the system, always combining soil quality and the system's productivity.

It was verified that seasonality influences the dynamics of SOC, POC, and MAOC in the soil.

The CMI was sensitive to detect changes caused by land-use change and showed that pasture accumulates carbon in the soil even with signs of degradation and that pasture could be a component to be included in agroforestry systems to increase even more carbon in the soil.

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5 CHAPTER IV

AGROFORESTRY SYSTEMS ENHANCE SOIL HEALTH IN SOUTHEASTERN BRAZIL

* The article is under review in Agroforestry Systems Journal.

5.1 RESUMO

Os sistemas agroflorestais, como uma prática de manejo sustentável da terra, têm mostrado evidências sólidas de seu papel na melhoria e restauração da qualidade e saúde do solo. O Soil Management Assessment Framework (SMAF) foi testado com sucesso como uma ferramenta objetiva para quantificar o uso da terra e os efeitos do manejo sobre a saúde do solo, inclusive em algumas condições de solos brasileiros. Assim, o objetivo deste estudo foi avaliar a qualidade do solo sob cinco usos do solo, incluindo: três tipos de sistemas agroflorestais, uma pastagem degradada e uma floresta secundária no sudeste do Brasil. O estudo foi conduzido em uma fazenda experimental em Sapucaia, estado do Rio de Janeiro, onde diversas práticas de manejo foram implementadas em parcelas com propriedades de solo e histórico de manejo semelhantes antes do estabelecimento de três sistemas agroflorestais distintos. Coletamos amostras de solo nas estações seca e chuvosa e avaliamos as propriedades biológicas (carbono orgânico do solo, β-glucosidase, carbono da biomassa microbiana), químicas (pH, fósforo e potássio) e físicas (densidade aparente, macroagregação) do solo. Usando as curvas de pontuação SMAF, os valores medidos foram transformados (intervalo de 0 a 1) e um índice geral de saúde do solo (SHI) foi calculado. Encontramos valores gerais de pontuação SMAF de 0,87, 0,88 e 0,87 para AS1, AS2, AS3, respectivamente, 0,83 para pastagem e 0,82 para floresta, e os valores diferiram significativamente entre os usos da terra. O estabelecimento de sistemas agroflorestais sobre áreas anteriormente ocupadas com pastagens extensas aumenta a fertilidade do solo (12,79%) e os indicadores físicos do solo (6,5%). Nossos resultados mostram que o AS não apenas melhora o fornecimento de funções ecológicas essenciais do solo, mas também restaura a qualidade do solo de pastagens degradadas. Os sistemas agroflorestais podem, portanto, ser uma estratégia de restauração promissora para pastagens degradadas. Esses resultados são especialmente importantes no contexto de fornecer subsídios para a legislação ambiental no Brasil, no sentido de adoção de sistemas agroflorestais, que podem ser adotados para atingir as metas de desenvolvimento sustentável da agenda 2030 e também para aumentar a resiliência da propriedade agrícola indicada para o Década das Nações Unidas para a Restauração do Ecossistema (2021-2030).

Palavras-chave: SMAF. Índice de qualidade do solo. Degradação do solo. Metas de desenvolvimento sustentável.

5.2 ABSTRACT

Agroforestry systems, as a sustainable land management practice, has shown solid evidence of its role in improving and restore soil quality and health. The Soil Management Assessment Framework (SMAF) has successfully been tested as an objective tool to quantify land use and management effects on soil health, including under some Brazilian soil conditions. Thus, the objective of this study was to evaluate soil quality under five land uses, including: three types of agroforestry systems, a degraded pasture, and a secondary forest in southeastern Brazil. The study was conducted at an experimental farm in Sapucaia, state of Rio de Janeiro state, where diverse management practices had been implemented on plots with similar soil properties and management history prior to the establishment of three distinct agroforestry systems. We collected soil samples in the dry and rainy seasons, and assessed biological (soil organic carbon, β-glucosidase, microbial biomass carbon), chemical (pH, phosphorus, and potassium), and physical (bulk density, macroaggregation) soil properties. Using the SMAF scoring curves, the measured values were transformed (0 to 1 range) and an overall soil health index (SHI) was calculated. We found overall SMAF scores values of 0.87, 0.88 and 0.87 for AS1, AS2, AS3, respectively, 0.83 for pasture, and 0.82 for forest, and the values differed significantly between land uses. The establishment of agroforestry systems over areas previously occupied with extensive pasturelands enhances soil fertility (12.79%) and soil physical indicators (6.5%). Our results show that AS not only enhance the provision of key soil ecological functions, but also restore soil quality of degraded pastureland. Agroforestry systems could therefore be a promising restoration strategy for degraded pastureland. These results are especially important in the context of providing information's for environmental legislation in Brazil, towards the adoption of agroforestry systems, which can be adopted to achieve the sustainable development goals of the 2030 agenda and also to increase the farm's resilience as is nominated for the UN Decade on Ecosystem Restoration (2021-2030).

Key words: SMAF. Soil quality index. Soil degradation. Sustainable Development Goals.

5.3 INTRODUCTION

Globally, reducing soil degradation and its impacts on the environment has been one of the main challenges of the 21st century. About 25% of the world's soil is severely degraded (FAO, 2015), and the remaining 50% is moderately degraded due to various anthropogenic activities for the last few decades (SRIVASTAVA et al., 2019). The manifold risks created by pollution, landslides, drought, and pandemics are aggravated by the growing human population, lifestyle changes, and inapt technology use. Even the recovery from the COVID-19 pandemic can be accelerated by restoring soil quality and its functionality, by adopting modern innovations that strengthen the resilience of the local food production system while improving environmental quality (LAL et al., 2020). Agroforestry systems have been proven as a promising strategy for reversing land degradation through enhance soil quality and fertility because of their capacity to control soil erosion, to maintain optimum soil organic matter and different soil physicochemical properties and subsequently, to ameliorate of soil biodiversity (DOLLINGER AND JOSE, 2018; MARSDEN et al., 2019; MATOS et al., 2020).

In particular, the contribution of agroforestry to sustainable development has been recognized in international scientific and political forums and agenda, including the Convention to Combat Desertification (UNCCD), Convention on Biological Diversity (CBD), United Nations Framework Convention on Climate Change (UNFCCC) and, the UN Decade (2021–2030) on Ecosystem Restoration (UN-DER). In 2015, Brazil signed the international climate change mitigation commitments (Paris agreement), known as Nationally Determined Contributions (NDCs), where it declares the goals set to reduce CO_2 emissions by 43% by 2030 giving special attention to recovery of degraded areas. Diversified systems have been classified as one of the strategies of the low-carbon agriculture plan (Plano ABC+) by the Brazilian government as an eligible form of land use to achieve these targets. In addition, several studies suggest agroforestry interventions for sustainable agriculture, the restoration and maintenance of the soil health and soil fertility (CHERUBIN et al., 2018; MATOS et al., 2020; AWAZI AND AVANA, 2020; TSUFAC et al., 2021).

However, studies about the effectiveness of agroforestry systems in restoring soil quality is still scare under Brazilian conditions. Soil health has been defined as the continued capacity of a soil to function as a vital living ecosystem that sustains plants, animals, and humans (USDA-NRCS). It is broad concept and therefore, there is not a universal approach to evaluate soil health. In the last decades, several approaches (frameworks, models, and systems) have been developed and tested to create comprehensive soil quality indexes (BÜNEMANN et al., 2018; RINOT et al., 2019) that can be used for specific purposes and environmental conditions around the world (e.g., VELASQUEZ et al. 2007; CHERUBIN et al., 2016a; MOEBIUS-CLUNE et al., 2016; THOUMAZEAU et al., 2019; BAI et al., 2018; CHERUBIN et al., 2021). We propose in this study to use the Soil Management Assessment Framework (SMAF) (Andrews et al., 2004), one of the most advanced analytical schemes to assess soil health (KARLEN et al., 2019; LEHMANN et al., 2020). The SMAF uses non-linear scoring curves to interpret dynamic chemical, physical and biological soil indicators taking account site-specific information (soil type, climate, crop, analytical methods etc.) to assess soil health changes induced by land use and management (KARLEN et al., 2019). In Brazil, the SMAF has been tested in several conditions (e.g., CHERUBIN et al., 2016a, 2016b, 2017, 2021) but only one study used SMAF in soils under diversified systems (integrated crop-livestock system) (DA LUZ et al. 2019). Then, our study will be a pioneer on the application of SMAF for assessment of soil quality in complex agroforestry systems.

This study was designed to determine whether agroforestry systems are a viable strategy for recovering the quality of degraded soils, and if the SMAF can detect those changes in

Brazilian tropical soils. Our hypothesis was that agroforestry systems improve soil health and that these soil changes are detected by the SMAF. To test these hypotheses, this study considered an experimental farm in southern Brazil, where diverse management practices had been implemented on plots with similar inherent soil properties and management history prior to the establishment of three distinct agroforestry systems designs (AS1 - focused on banana and coffee; AS2 - focused on bananas and timber species; AS3 – focused on fruits).

5.4 MATERIAL AND METHODS

5.4.1 Site description and land uses

This study was conducted at the Arca de Noé Farm, an agroecological research station located near the city of Sapucaia, Rio de Janeiro, Southeastern Brazil (21º 59' 42" S, 42º 54' 52" W; 800 m.a.s.l). The region is characterized by dry winters and temperate summers (Cwb in the Köppen Climate Classification system), with mean monthly temperatures that vary between 17°C and 32°C (June and January; respectively) and a mean annual rainfall of 1,451 mm. Soils at this site are predominantly Ultisols (USDA, 2014) with a clay-loam texture. The region is largely comprised of massifs of highland hills and cliffs, with a natural vegetation generally dominated by the Atlantic Forest. In this study, we considered five existing land uses at the farm (Table 11). More details related to management and plant species present in the areas can be found at Matos et al. (2020).

Table 11. Description of study sites in the Arca de Noe Farm region, Sapucaia, Rio de Janeiro, Brazil.

Study site	Coordinates	Description
Forest	22°05’52,8’’S 42°47’45,3’’W	Semi-deciduous seasonal forest. The secondary forest was included here as a reference and had an age of about 30 years since previous deforestation.
Pasture	22°05’59,7’’S 42°47’43,7’’W	Pasture replanted with the grass Urochloa decumbens, under extensive grazing. Pasture was established by removal of native vegetation in 1995.
AS1	22°5'57.10"S 42°47'42.61"O	Agroforestry system characterized by the integration of banana and coffee with a mix of other fruit and timber species and other species to provide shade, biomass production, and pollination services. In 2010, the agroforestry systems were planted on a portion of this existing pasture. These systems received a single application of rock phosphate (fertilizer permitted in organic production) and cattle manure at the time of establishment.
AS2	22°5'57.93"S 42°47'41.50"O	Agroforestry system focused on bananas and energy production (which also includes fruit trees and a mix of other trees and plants. In 2010, the agroforestry systems were planted on a portion of this existing pasture. These systems received a single application of rock phosphate (fertilizer permitted in organic production) and cattle manure to the banana tree roots at the time of establishment
AS3	22°5'57.44"S 42°47'40.92"O	A third agroforestry system focused on bananas and other fruits. In 2010, the agroforestry systems were planted on a portion of this existing pasture. These systems received a single application of rock phosphate (fertilizer permitted in organic production) and cattle manure at the time of establishment.

5.4.2 Soil sampling and laboratory analyses

Sampling was conducted in 2018 at two separate time season, rainy (April) and dry (September), searching to assess a suite of soil biological, chemical, and physical properties within each land use (e.g., forest, pasture and agroforestry systems). One transects was laid out in each of the land use types, and four sampling plots (6 × 8 m) were established approximately

15 m apart along the transect. The four plots within each land use were considered replications. Within each sampling plot, four sub-samples of soil (0-10 cm depth) were collected using a shovel (~5 m spacing between sub-samples) and combined to generate one composite sample per sampling plot per season. A portion of each composite sample was kept cool for transport to the laboratory at the Federal Rural University of Rio de Janeiro (Seropédica, Brazil), where it was stored at 4°C (for <2 weeks) until analysis of microbiological parameters. The rest of each composite sample was air-dried, sieved to 2 mm, and analyzed for chemical properties. Chemical indicators included available P and K were evaluated using a Mehlich^{-1} extractant (H_2SO_4 0.0125 mol L^{-1} + HCl 0.05 mol L^{-1}), and pH was analyzed in a 1:5 suspension of soil and deionized water (TEIXEIRA et al., 2017).

Physical parameters were evaluated only in the rainy season (April), since there is no significant difference in their evaluation in the two seasons (TAVARES et al., 2018; CASTIGLIONI et al. 2018; AGBESHIE et al., 2020). Bulk density (BD) was measured at four sub-samples per sampling point by inserting a metal cylinder ring (5 cm diameter) vertically into the soil to a 10 cm depth. Soil from within each ring was returned to the lab, and then dried at 105°C, and weighed (TEIXEIRA et al., 2017). For evaluation of water-stable aggregation, four soil cores (10 cm diameter) were collected to a depth of 10 cm in each sampling plot and combined into one composite sample. Field moist soil was passed through an 8 mm sieve by gently breaking soil clods along natural planes of fracture, and then air-dried for subsequent analyses.

Wet macroaggregate stability (AGS) was determined using a Yoder wet-sieving apparatus (Yoder, 1936). For the evaluation of the aggregate distribution, 25 g of the air-dried, 8-mm sieved soil was transferred to the top of a set of sieves with 2.00, 1.00, 0.50, 0.25 and 0.105 mm, moistened with spray and subjected to vertical oscillation in the Yoder apparatus, for 15 min. The material retained on each sieve was then rinsed into separate Petri dishes and dried in an oven at 65°C. The AGS (macroaggregation percentage) was calculated by summing the aggregate mass for the >2.000 and >250 mm classes, dividing by the total soil mass, and multiplying by 100. Particle-size distribution was determined by slow dispersion for 16 hours. Soil texture was determined by the pipette method (TEIXEIRA et al., 2017). Total clay (diameter <0.002 mm) and sand (diameter 2 to 0.05 mm) contents were obtained, respectively, by pipetting and sieving, while the silt content (diameter between 0.05 to 0.002 mm) was calculated by the difference.

Biological indicators included soil organic carbon (SOC), microbial biomass carbon (MBC) and enzymatic activity. SOC was quantified by the oxidation of organic matter using a solution of potassium dichromate in acid medium, with an external source of heat (Yeomans and Bremner, 1988). For the calculations of the MBC, refrigerated soil (stored at 4°C) was passed through a 2 mm sieve and two sub-samples (20g each) were weighed for each sampling point. One of these sub-samples was fumigated with chloroform by 24 h and then shaken for 30 min with K_2SO_4 (0.5 mol L^{-1}), while the other was not fumigated and submitted to the same extraction procedure (VANCE et al., 1987; TATE et al., 1988). The estimation of C content in microbial biomass was done with colorimetric determination (BARTLETT et al., 1988). β-glucosidase (BG) activity was analyzed according to Tabatabai (1994) using 1.0 g of fresh soil and the substrate p-nitrophenyl-β-D-glucoside (0.05 mol L^{-1}). The concentration of p-nitrophenol was determined in triplicate by measuring absorbance at 410 nm in a spectrophotometer, and the results were expressed in µg g^{-1} h^{-1} p-nitrophenyl.

5.4.3 Soil management assessment framework

The SMAF was used as a tool to evaluate the land use effects on soil quality. The minimum data set included eight soil indicators (pH, P, K, BD, AGS, SOC, MBC, and BG).

The importance of each one of these indicators to soil functionality has been consistently reported in the literature (e.g., ANDREWS et al., 2004; LAL, 2016; BUNEMANN et al., 2018; VAN ES AND KARLEN, 2019; NUNES et al., 2020). The pH and available P and K contents provide information about soil acidity and nutrient availability status. Macroaggregate stability and BD indicate the soil structural and physical conditions, which affect soil aeration, water infiltration and storage, and the soil's ability to resist erosion processes. Soil organic C, MBC, and BG were chosen as biological indicators. The SOC plays a crucial role in multiple soil processes including nutrient cycling and storage, soil aggregation, and is a food source for edaphic organisms, while MBC and BG indicate the microbiological and biochemical activity of the soils. This minimum dataset has been widely used and validated for soil quality assessment through SMAF tool under different land uses and management in Brazil (e.g., CHERUBIN et al., 2016; 2017; 2021; LUZ et al., 2019; VALANI et al. 2020).

These indicators were scored by transforming the mean measured values into 0 to 1, using previously published algorithms present in the SMAF spreadsheet (ANDREWS et al., 2004; WIENHOLD et al., 2009; STOTT et al., 2010). Those algorithms account for organic matter, texture, climate, slope, region, mineralogy, weathering class, crop, sampling time, and analytical method effects on the various threshold values. For this study, the organic matter factor class (based on soil classification and used for scoring AGS, SOC, MBC, and BG) was 4 (low organic matter content) for all sites.

The texture factor class (used for scoring BD, AGS, SOC, MBC, and BG) was 4 (clay content ~35%) at all sites. The climate factor (used for scoring SOC, MBC, and BG) was 1 (≥170°C and ≥550 mm of mean annual precipitation) for all sites. The seasonal factor, impacting MBC scores, was 2 (sampling in summer - April) and 4 (sampling in summe - September) for all sites. The Fe oxide content, used for AGS scores, was 1 (Ultisols) for all sites. The mineralogy factor class, used for scoring BD, was 3 (1:1 clay and Fe and Al oxides), and the slope and weathering class factors, used for scoring P, were 5 (>15% slope) for forest and 4 (9-15%) for others, and 2 (high weathering), respectively, for all sites. The method used to measure extractable P was Mehlich^{-1} (Class 1).

Finally, all indicator scores were integrated into an overall soil quality index (SHI) using a weighted additive approach (Eq. 2).

$$SHI = \sum_{i=1}^{n} S_i W_i \tag{2}$$

Where, Si is the indicator score and Wi the weighted value of the indicators. The indicators were weighted based on chemical (pH, P, and K), physical (BD and AGS), and biological (SOC, MBC, and BG) components, so regardless of number of indicators each group had an equal weight (33.33 %) in the final index (CHERUBIN et al., 2016a).

5.4.4. Statistical analysis

Once the scores and the SHI were calculated, the differences were tested for their significance between the plots for each soil property in each land use (forest, pasture, AS1, AS2 and AS3) and between seasonality, as well as for the overall SHI and their respective physical, biological, and chemical components. The sampling pattern includes spatially multiple self-dependent plots with low variances within each. Therefore, the linear mixed-effects model (lme4 R package, v. 1.1-23 and lmerTest, v. 3.1-2) was applied to examine significant differences in soil quality components within land uses and seasonality, which were set as fixed effect, and the sampling plots as random effect. The type II Wald χ^2 test and least-square means for pairwise t test with false discovery rate correction for multiple comparisons (car R package v. 3.0-10) were used to explore the influence of seasonality on soil quality indicators in each land use, and further, to measure the differences between land uses for each group of indicators - physical, biological, and chemical - and overall SHI scores. Finally, we applied a principal component analysis (PCA; FactoMiner R package v. 2.4) integrating the complete set of variables regarding to chemical, physical and biological indicators, and through linear regression model between the extracted PC1 and SMAF scores we aimed to identify a possible gradient of soil health among land uses.

5.5 RESULTS

5.5.1 Influence of land uses in soil quality indicators

The land uses influenced the average values and the SMAF scores of the soil quality indicators (Table 12). In rainy season, the agroforestry systems showed higher means for all chemical indicators and also higher scores for pH and K compared to forest and pasture. In terms of the biological indicators, forest and AS3 soils presented the highest and lowest SOC contents, respectively, but the scores had not changed. Mean and score values of MBC were higher for both agroforestry and pasture systems and lower in the forest. The means of β-glucosidase were higher for both agroforestry systems and forest, but lower in the pasture, with no variation in the scores. For the physical indicators, the average AGS was higher in the forest and lower in AS3, with no significant variation in the scores. The highest average of soil bulk density (i.e., lowest score) was observed in the pasture.

Table 12. Mean values and SMAF scores of soil quality indicators sampled on an experimental farm in Sapucaia – RJ, Brazil at rainy season (April) and dry season (September) of 2018.

	Rainy Season (April)						**Dry Season (September)**					
Soil Indicators	**Forest**	**Pasture**	**AS1**	**AS2**	**AS3**	***p-value***	**Forest**	**Pasture**	**AS1**	**AS2**	**AS3**	***p-value***
Chemical							Mean values					
pH (H_2O)	4.5^{c}	4.6^{c}	5.7^{a}	5.3^{ab}	5.2^{b}	***	4.5	4.8	5.1	5.2	4.8	
	0.1	*0.1*	*0.3*	*0.2*	*0.1*		*0.4*	*0.1*	*0.2*	*0.4*	*0.4*	
P (mg kg^{-1})	26.5^{ab}	22.5^{b}	29.5^{a}	31.5^{a}	28.5^{a}	**	20.4^{bc}	18.8^{c}	25.6^{a}	27.7^{a}	24.2^{ab}	***
	0.6	*0.6*	*4.7*	*3.1*	*1*		*2.4*	*1.03*	*2.3*	*1.6*	*1.1*	
K (mg kg^{-1})	71^{b}	53.5^{b}	192^{a}	159^{ab}	170^{ab}	*	71.2^{bc}	48.4^{c}	185^{ab}	187^{ab}	212^{a}	**
	5.72	*4.12*	*95.4*	*77.8*	*69.3*		*12.8*	*6.55*	*59.8*	*71.3*	*92.1*	
							SMAF scores					
pH (H_2O)	0.62^{b}	0.91^{a}	0.96^{a}	0.98^{a}	0.96^{a}	***	0.59^{b}	0.94^{a}	0.92^{ab}	0.91^{ab}	0.76^{ab}	*
	0.05	*0.01*	*0.08*	*0.03*	*0.0*		*0.23*	*0.02*	*0.05*	*0.08*	*0.22*	
P (mg kg^{-1})	1	1	1	1	1		1	1	1	1	1	
	0	*0*	*0*	*0*	*0*		*0*	*0*	*0*	*0*	*0*	
K (mg kg^{-1})	0.82^{b}	0.71^{c}	0.98^{a}	0.99^{a}	0.99^{a}	***	0.82^{b}	0.67^{c}	0.99^{a}	1^{a}	0.98^{a}	***
	0.03	*0.03*	*0.03*	*0.03*	*0.00*		*0.07*	*0.05*	*0.00*	*0*	*0.05*	
Biological							Mean values					
SOC %	2.80^{a}	2.53^{ab}	2.64^{ab}	2.35^{ab}	2.31^{b}	*	2.16	2.14	2.42	2.26	2.45	
	0.22	*0.21*	*0.34*	*0.04*	*0.19*		*0.08*	*0.11*	*0.45*	*0.45*	*0.11*	
MBC (mg kg $^{-1}$)	339^{d}	507^{b}	530^{ab}	429^{c}	571^{a}	***	668^{b}	580^{c}	709^{a}	716^{a}	727^{a}	***
	22.5	*20.6*	*26.6*	*27.4*	*22.1*		*20.2*	*13.5*	*14*	*27.9*	*6.91*	
BG (µg g^{-1} h^{-1})	905^{ab}	794^{b}	1121^{a}	983^{ab}	987^{ab}	.	1379	1179	1461	1375	1426	
	142	*106*	*177*	*156*	*142*		*102*	*193*	*260*	*286*	*401*	
							SMAF scores					
SOC %	1	0.999	1	0.999	0.998		0.998	0.997	0.998	0.996	0.999	
	0	*0*	*0*	*0*			*0.001*	*0.00*	*0.001*	*0.004*	*0.000*	
MBC (mg kg $^{-1}$)	0.99b	1a	1a	1a	1a	***	1a	0.98^{b}	1^{a}	1^{a}	1^{a}	***
	0	*0*	*0*	*0*	*0*		*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	
BG (µg g^{-1} h^{-1})	1	1	1	1	1		0.998	0.997	0.998	0.996	0.999	
	0	*0*	*0*	*0*	*0*		*0.001*	*0.001*	*0.002*	*0.001*	*0.000*	

To be continued…

Table 12. - Continuation.

	Rainy Season (April)						Dry Season (September)					
Soil Indicators	**Forest**	**Pasture**	**AS1**	**AS2**	**AS3**	***p-value***	**Forest**	**Pasture**	**AS1**	**AS2**	**AS3**	***p-value***
Physical							Mean values					
BD (Mg m^{-3})	1.5^{b}	1.8^{a}	1.6^{b}	1.5^{b}	1.5^{b}	***						
	0.04	*0.04*	*0.1*	*0.1*	*0.04*							
AGS %	95.2^{a}	91.4^{ab}	91.7^{ab}	90.7^{ab}	88.7^{b}	*						
	1.97	*1.67*	*4.67*	*0.95*	*1.12*							
							SMAF scores					
BD (Mg m^{-3})	0.33^{a}	0.21^{b}	0.28^{ab}	0.31^{ab}	0.31^{ab}	*						
	0.03	*0.002*	*0.05*	*0.08*	*0.03*							
AGS %	1	1	1	1	1							
	0	*0*	*0*	*0*	*0*							

Values in italics below each mean represent the standard error from four measurements in each plot. Mean followed by the same letter do not differ statistically. Abbreviations: P: available phosphorus; K: extractable potassium; SOC: soil organic carbon; MBC: microbial biomass carbon; BG: β-glucosidase activity; AGS: macroaggregation percentage; BD: bulk density. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; $p < 0.1$.

In the dry season, greater values of P and K were observed in agroforestry systems, while the scores varied for pH with higher values in the agroforestry systems and lower in the forest. The highest K scores were also found in agroforestry systems and the lowest in pasture. Few changes were observed in the biological indicators, which only the MBC differed among land uses, with higher values in agroforestry systems and forest soils than in the pasture soil (Table 12).

The combination of all sites shows noticeable differences in the chemical, physical and biological soil indicators (Figure 11). Values observed in the forest soil differs from the other sites mainly in terms of chemical indicators (pH and K). Pasture soil had lower score for K and soil density compared to the others. Among the agroforestry systems, there is a lower score for the pH variable in AS3. The scores for the biological variables were all maximum.

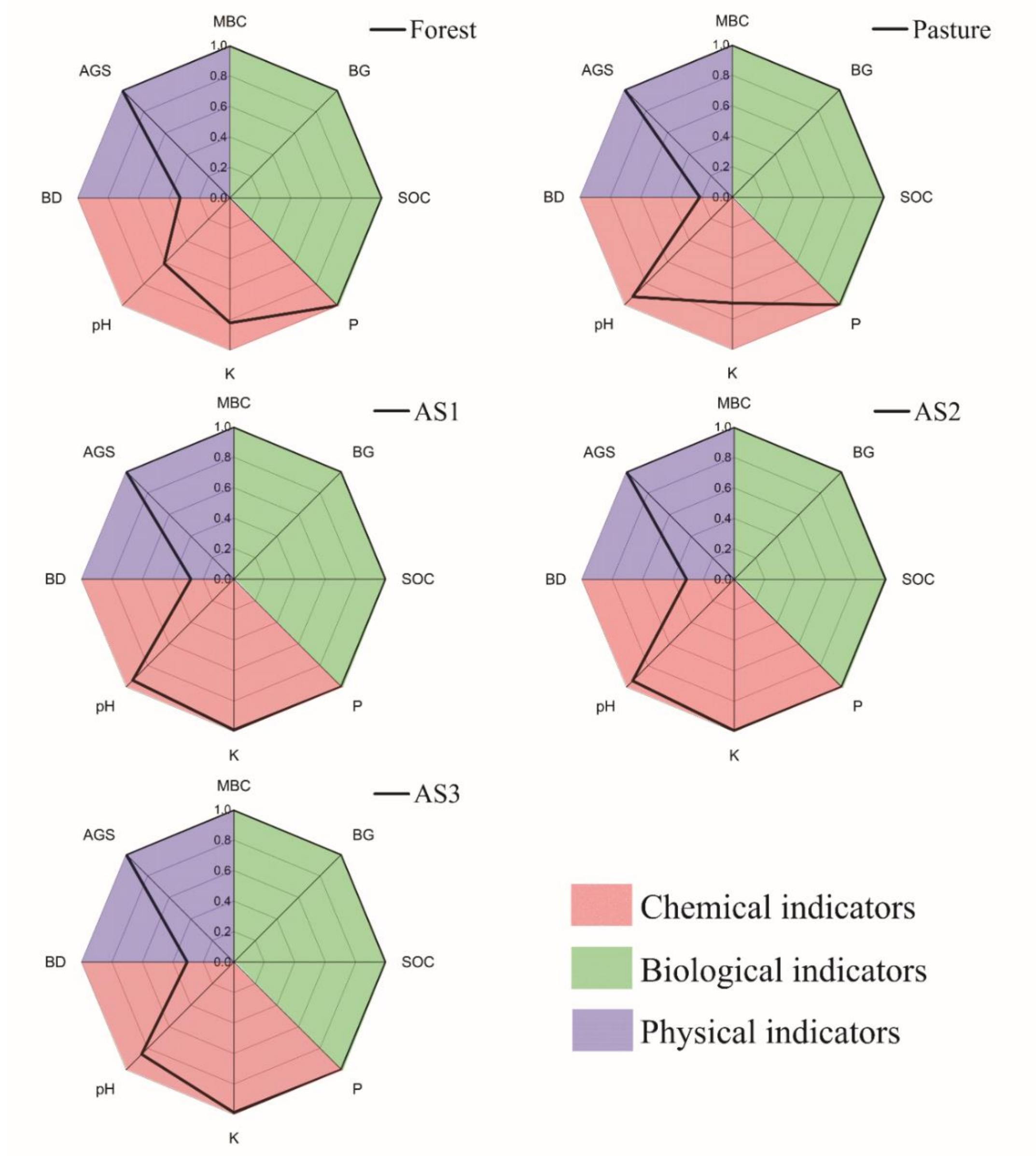

Figure 11. Individual indicator scores within each SHI component (chemical, physical, and biological) in forest, pasture, AS1, AS2, AS3 in Sapucaia – RJ, Brazil in rainy and dry season together. Values are given in unitless scores ranging from 0 to 1, based on the transformed properties' mean values. The shaded lines represent the standard deviation.

5.5.2. Seasonality effect on soil chemical and biological indicators

Our results showed seasonality affected the measured values of soil indicators (Figure 12). For example, the β-glucosidase was influenced in all the land uses, except AS1, with higher values in the dry season. Microbial biomass carbon also had higher values in the dry season, while SOC was higher in the rainy season, but those changes were observed in the forest. The chemical indicators P and pH also had higher values in the rainy season. The difference for P between the seasons was observed in all land uses, whereas pH was only different for AS1 and AS3.

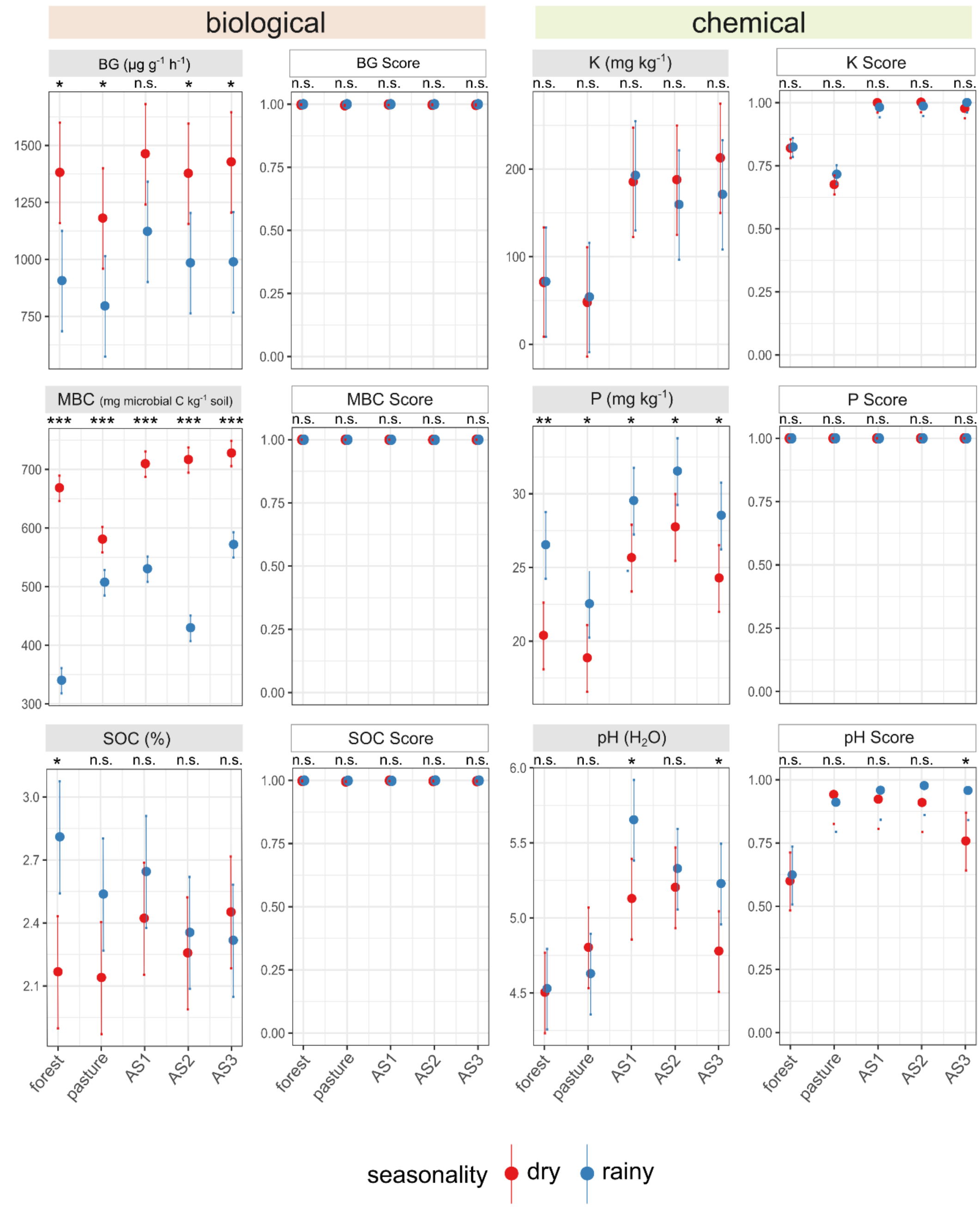

Figure 12. Overall effect of seasonality on soil quality indicators and SMAF scores in each land use. Vertical bars are confidence intervals for the means. Asterisks represent significant differences between seasonality within land uses; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; $p < 0.1$.

Nevertheless, those specific changes induced by seasonality were no detected by individual SMAF scores, except for the pH that had the highest score in the rainy season in AS3. In other words, the magnitude of the differences observed in the measured values was insufficient to print changes in the scores, which intends to represent the performance of one or more soil functions.

5.5.3 Overall soil quality index and components

Soil Health components (i.e., chemical, physical, and biological) and overall SHI for each for land use are shown in Figure 13 and Figure 14. Briefly, seasonality did not influence SMAF scores and there was no significant difference between the scores for the biological component for land uses. For the chemical component, agroforestry systems had higher scores than forest and pasture. For the physical component, the pasture had a lower score compared to other land uses. The overall SHI was higher for the agroforestry systems than others.

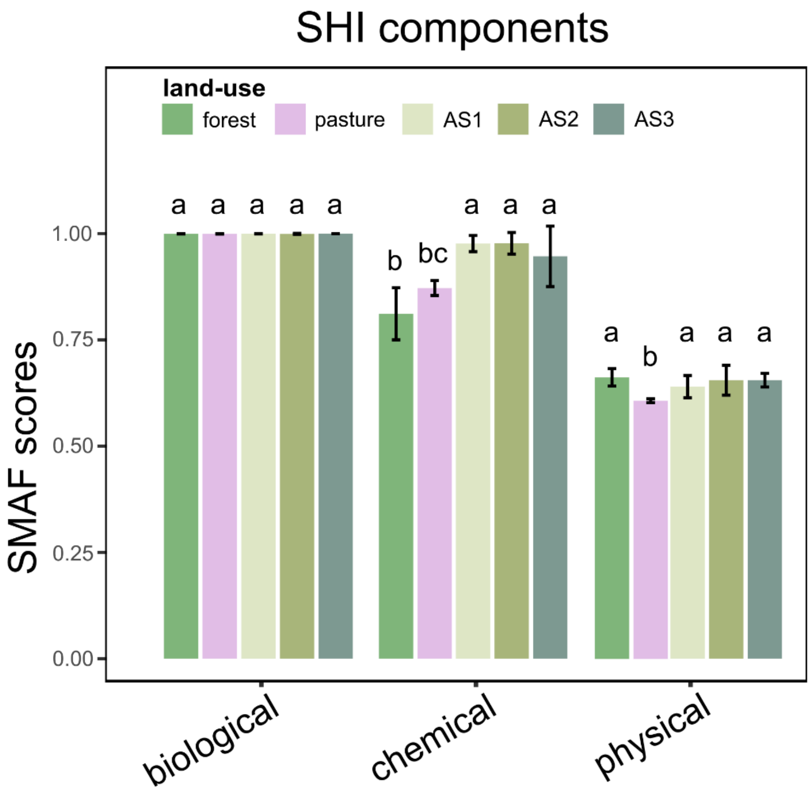

Figure 13. Soil health components (chemical, physical, and biological) scores for each land use. Error bars denote standard deviation of the mean. Mean SHI scores followed by the same letter do not differ statistically.

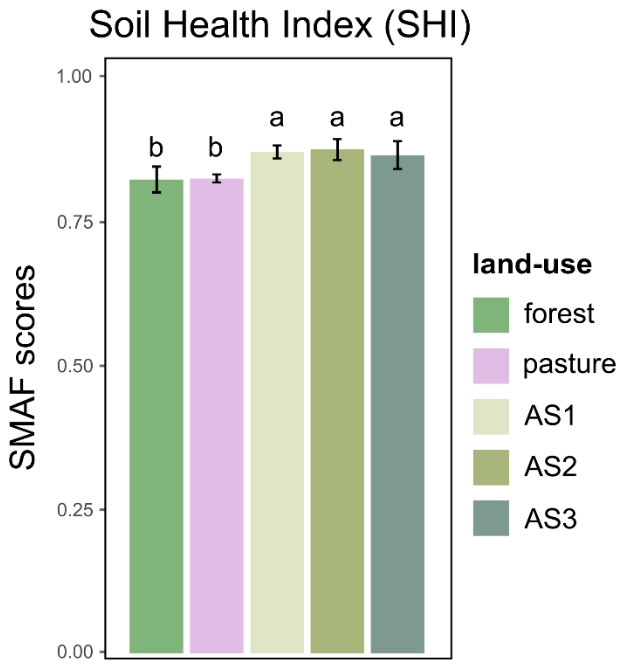

Figure 14. Overall soil heath index (SHI) scores for each land use. Error bars denote standard deviation of the mean. Mean SHI scores followed by the same letter do not differ statistically.

Correlations between the PC1 scores of the principal components analysis (PCA) and the SHI components (chemical, physical, and biological) scores, revealed that chemical component shows a positive gradient (R^2 = 0.96) in the fertility levels from forest to pasture and from pasture to AS (Figure 15). The correlation between gradient of soil health with the overall SHI followed the same pattern (R^2 = 0.78), confirming that soil chemical component is the main drivers of changes observed in the overall SHI scores.

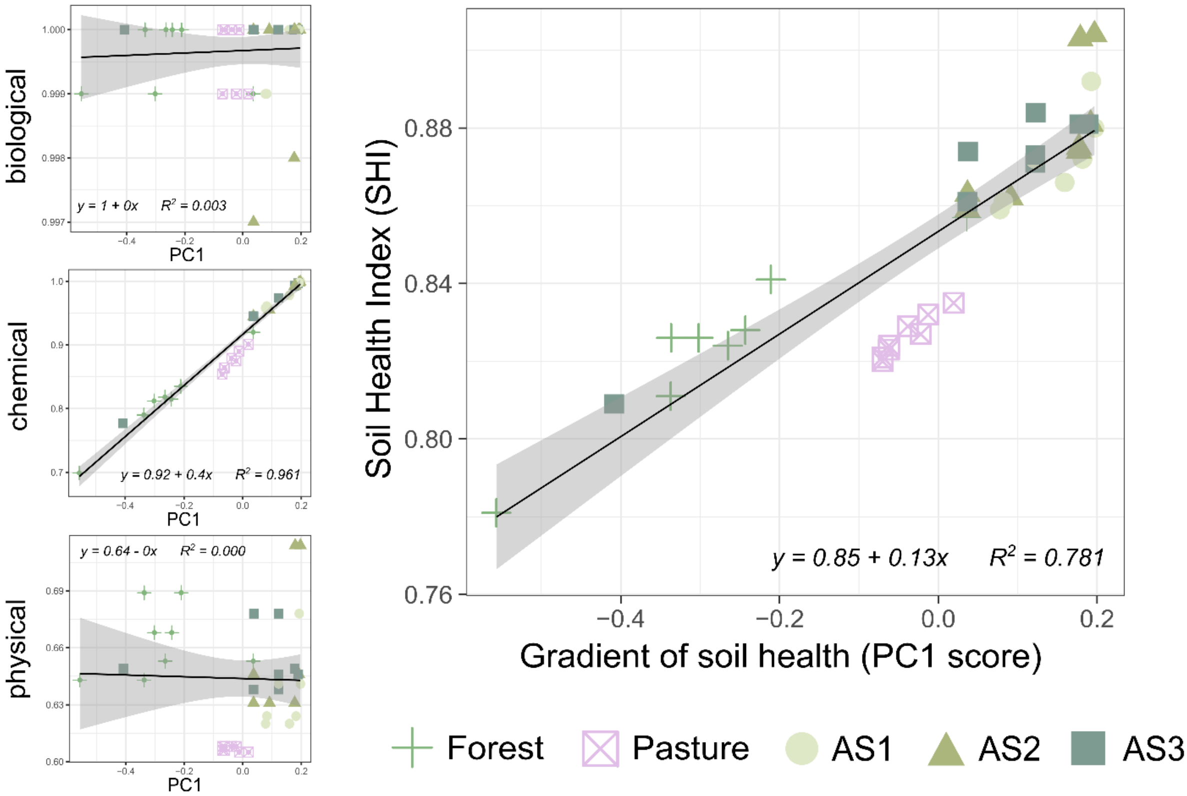

Figure 15. Variation in the importance of soil quality components across land uses. Linear regression model between PC1 scores and soil quality indices for SH components (chemical, physical, and biological) scores and overall soil health index (SHI) scores for each land use.

5.6 DISCUSSION

5.6.1. Land use effects on soil quality indicators

Long term land use changes induced substantial changes on the soil health indicators. Forest had more acidic soils (low pH), and less availability of nutrients, especially potassium, compared to agroforestry systems, highlighting the effects of land use change and inherent pedogenetic factors. The soil pH is generally acidic in forest ecosystems (GAIROLA et al., 2012). This may be due to high organic matter content and the undisturbed nature of the forest soils (ADAMS AND SIDLE, 1987). The low pH can be attributed to the accumulation and subsequent slow decomposition of organic matter, which releases organic acids (DE HANN, 1977). It has been reported that forest soils should be slightly acidic for nutrient supply to be balanced (LESKIW, 1998).

On the other hand, the continuous addition of litter to forest soils contributed to the increase in SOC, and also influencing positively the soil aggregate stability (Onweremadu et al., 2007; Durigan et al., 2017). However, using the current SMAF scoring curves, the AGS score was a non-sensitive indicator to detect land use change impacts in tropical soils, reaching a score of practically 1.0 for all land uses, as previously observed in other studies in oxidic Brazilian soils (e.g., CHERUBIN et al., 2016). The SMAF scoring curves for AGS (more-is-better sigmoidal shapes) considers differences in SOM, soil texture, and Fe oxide content (ANDREWS et al., 2004). Nevertheless, for all possible variations of these factors, the maximum score (1.0) is assigned when AGS values are >50% (the threshold value for which soil structural stability is optimum for environment protection and productivity goals). The stability of aggregates is high in tropical soils due to mineralogy's, the soils are rich in Fe and Al oxides, which in this case are the main stabilizing agents within aggregates (SIX et al., 2000; PENG et al., 2015). Therefore, additional SMAF scoring curves for AGS need to be developed for detecting smaller changes caused by recent land use and management under well-aggregated tropical soils (CHERUBIN et al., 2021).

The forest to pasture conversion induced soil compaction by increasing BD values, whith similar result founded by Cherubin et al. (2016). Studies have shown that continuous cattle trampling, absence of stocking rate control and rotational management are the major drivers for soil compaction under extensive pasture (e.g., GREENWOOD AND MCKENZIE, 2001; POLANÍA-HINCAPIÉ et al., 2021). In addition, low pasture productivity (shoots and roots) has been verified under compacted soils, reducing C inputs into the soil (MAIA et al., 2009; FRANCO et al., 2015) and contributing to an increase in soil structural degradation (POLANÍA-HINCAPIÉ et al., 2021).

Agroforestry systems have improved soil quality related to pasture and forest (Table 1, Figure 1). Some management practices adopted in agroforestry systems, such as maintenance pruning of species destined to produce green biomass (generally nitrogen-fixing species), ensure nutrient input into these soils (PENEIREIRO, 1999; SANTOS et al., 2021) and, consequently, the improvement of their chemical quality. We suggest that this practice associated with the addition of rock phosphate in the planting of the species promoted a gradual change in soil pH, as well as an increase in available nutrients. According to Omenda et al. (2021), a combination of organic input can ameliorate the P fixation of soil to enhance the chemical soil properties in acidic soils. Moreover, the species used as green manure as *Tithonia diversifolia* in AS1, AS2 and AS3, can enhance soil physical and chemical properties (Chukwuka and Omotayo, 2008); therefore, increasing nutrients in the edaphic environment. Analysis of foliar *Tithonia diversifolia* biomass shows a greater concentration of N (3.5%), P (0.37%), and K (4.10%) (JAMA et al., 2000). Recently, Matos et al. (2020) showed that the enhanced litter quality (low C: N ratio) in these agroforestry systems and continuous input of fresh material through pruning of species destined to produce green biomass increased soil biological activity, which consequently influences positively nutrient cycling. The SMAF scores for biological indicators (SOC, BG and MBC) suggested that all land uses were functioning close to their potential biological capacity.

5.6.2. Seasonality effects on soil quality indicators

Biological soil quality indicators can change quickly depending on the specific agricultural practices and land use decisions that are adopted (VAN ES AND KARLEN, 2019), also they fluctuate widely with the season (SINGH et al., 2017; SINGH et al. 2018). For example, the β-glucosidase activity (BG), and microbial biomass carbon (MBC) were higher in the dry season. These results corroborate with Silva et al. (2020) that found higher values for β-glucosidase in agroforestry systems and Atlantic Forest in the dry season in Rio de Janeiro. This result is probably associated with higher rates of leaf-litter deposition in the dry period, promoting enzyme activity and resulting in increased carbon incorporation into the soil (Matos et al., 2020). β-Glucosidase participates in the final step of cellulose decomposition, in the hydrolysis of the cellobiose residues (TABATABAI, 1994). Some studies (LOUZADA et al., 1995; PORTELA AND SANTOS, 2007; MACHADO et al., 2015) conducted in an environment of Atlantic Forest reported higher litter depositions in the dry season. High litter inputs in agroforestry systems contribute to soil microbial activity (COSTA et al., 2017).

The chemical indicators showed higher values in the rainy season. The mineralization of the organic materials to inorganic forms is associated with the release of NO_3-N, P, K, and most of the micronutrients, and is related to season and management (EPSTEIN et al., 2002). According to Cusack et al. (2019) the variation in soil P availability is related to the magnitude of soil respiration seasonality across tropical forests. In this context, it is important to highlight that greater biological activity of the soil in the dry season may be promoting the decomposition of organic matter and with it, the release of phosphorus, but only in the rainy season due to the greater water content in the soil, we have the agent of transport is the means for this phosphorus to be available and can be absorbed by plants since it has low mobility in the soil. Due to the low availability of P in tropical soils, organic P (Po) can be an important source of phosphorus (P) to plants through the decomposition and mineralization of the labile Po fraction (OLIVEIRA et al., 2014; DAMIAN et al., 2020). On the other hand, we are not clear on the reasons for the fluctuation between seasons in soil pH to AS1 and AS3 in the short term. Despite the influence of seasonality on the measured values of soil biological and chemical indicators, the SMAF scores were not sensitive to detect this effect.

5.6.3. Overall soil health index and components

Soil quality assessment for different land uses requires tools that provide easy and straightforward soil health (SH) status information. The SMAF scores detected changes in SH among land uses. The chemical component was more sensitive for capturing land uses effects on SH than the physical or biological components. The SMAF tool proved to be a good alternative to access soil quality, however some adjustments are needed such as studies to include specific crop codes for agroforestry systems and on biological indicators in tropical soils for greater accuracy of results. Our findings showed that agroforestry systems have better soil quality compared to the others. Previous studies have also shown the potential of agroforestry systems to improve soil quality (THOMAZINI et al., 2015; TAVARES et al., 2018, BATTISTI et al., 2018; DOLLINGER AND JOSE, 2018; CHERUBIN et al., 2019). We also verified a gradient in soil health, strongly derived by the chemical components, which indicates a directional transformation on soil fertility from forest to the pasture, and from pasture to AS. Soil chemical fertility improvement in agroforestry systems is likely associated with higher input of nutrients through litter and pruning (PENEIREIRO, 1999; SANTOS et al., 2021), but also to the rock phosphate used at the beginning of planting. Pruning management in these systems is an excellent tool for maintaining and/or restoring the quality of these soils. These results corroborate the findings of Celentano et al. (2020), who showed that AS are promising for nutrient cycling reestablishment in the context of small-scale farming.

Extensive and poorly managed pastureland had the lowest soil physical quality component, and overall SHI among the land uses. This is likely associated with poor grazing and soil fertility management, as it had received no fertilization and had been under continuous grazing for many years (DAMIAN et al., 2021). The lack of nutrient inputs and other management interventions to maintain both above- and belowground productivity has been shown to negatively affect soil structure and overall fertility in other tropical pasture systems (FONTE et al., 2014). This degraded condition is likely representative of many Brazilian pastures, since 80% have been in some state of degradation (RIBEIRO et al., 2017). Despite this, when we observe the gradient of soil health, which was greatly influenced by the chemical component, the pasture presents an increase in soil fertility. Probably this may be associated with the cattle manure left in the area that replaces these nutrients.

The restoration of degraded areas has been a central theme of Brazilian Environmental policy to achieve the goals stablished within international agreements (ANTONACCIO et al., 2018). Therefore, our results suggest that using SMAF to monitor SH changes within different land uses could assist farmers and their consultants make better decisions regarding sustainable management practices.

5.7 CONCLUSIONS

The SMAF was sensitive for detecting soil quality changes induced by land uses. Agroforestry systems improve soil quality, mainly driven by enhanced soil chemical component. Land-use change from forest to pasture leads to soil compaction (increased bulk density), depleting soil physical quality.

The SMAF scores for biological indicators (SOC, BG and MBC) indicated that soils are fully performing the biological functions. Despite that, future efforts are recommended to improve and adapt the SMAF scoring curves using dataset from tropical soil conditions.

Overall, agroforestry systems showed higher soil quality compared to forest and pasture. This is relevant information for the Brazilian government, which included agroforestry systems with one of the strategies of the ABC plan to recover degraded areas and to achieve the sustainable development objectives of Agenda 2030.

The tool used in this study is adequate for farmers/consultants/researchers, and decision-makers to establish priority areas for land-use change based on soil quality and, to define more sustainable management practices.

New studies including a large number of sites, soil types, and cropping systems are encouraged to validate the conclusions of this study on a regional scale, thus enabling to support decision-making towards more sustainable expansion agriculture in Brazil.

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6 GENERAL CONCLUSIONS

The soil physical, chemical and biological assessment of these land uses reveal that areas under agroforestry systems generally had higher soil fertility status and SOM and biological activity than pasture or forest. The linkages between litter quality, SOM, and soil parameters suggest that high-quality litter inputs (i.e., low C: N ratio) and SOM are essential for stimulating biological activity and multiple soil properties with future implications for soil restoration. Also, management practices like pruning in agroforestry systems are favorable for AMF spore production and diversity.

As seasonal variation appears to be a key factor for regulating the AMF community, GRSP is an important contributor to soil carbon, mainly in agroforestry systems and pasture. We founded that agroforestry systems are a viable strategy in terms of soil carbon accumulation in the most superficial layers, mainly of label fractions as POC. However, the trend is that over time there is potential for these systems to contribute more to the accumulation of soil carbon; and, that seasonality influences the dynamics of TOC, POC, and MAOC in the soil.

The CMI was sensitive to detect changes caused by land-use change and showed that pasture accumulates carbon in the soil even with signs of degradation.

The SMAF was sensitive for detecting soil quality changes induced by land uses. Agroforestry systems improve soil quality, mainly driven by enhanced soil chemical component. Land-use change from forest to pasture leads to soil compaction (increased bulk density), depleting soil physical quality. The SMAF scores for biological indicators (SOC, BG, and MBC) indicated that soils thoroughly perform biological functions. Despite that, future efforts to improve and adapt the SMAF scoring curves using a dataset from tropical soil conditions are encouraged.

While causal inferences of management cannot be drawn from this study, the findings support the idea that establishing complex agroforestry systems in Brazil is likely to support soil quality and restoration goals. It further supports environmental legislation suggesting agroforestry systems as a viable option for Brazil to restore degraded lands and comply with international commitments to reduce greenhouse gas emissions, move towards low-C agriculture, and consequently contribute to improving global food security and to achieve the sustainable development objectives of Agenda 2030.

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