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Research paper

Recovery of below-ground associations in restored Brazilian Atlantic Forest

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ABSTRACT

Soil consists of abiotic and biotic components that sustain biodiversity and forest ecosystem functioning. However, soil restoration has been understudied, and restoration efforts often overlook it. Here, we apply an ecological coupling approach using correlation-based network analysis to assess the joint recovery of key abiotic, biotic, and functional soil components during forest restoration in the Brazilian Atlantic Forest. We analyzed soil microbial composition as the biotic component, soil physico-chemical characteristics as the abiotic component, and microbial biodiversity, carbon stocks, and greenhouse gases as functional components. Two restoration methods were studied: (i) active restoration on former sugarcane fields and (ii) assisted restoration on former pasturelands. We examined chronosequences of early-, intermediate-, and late-stage restored forests, comparing them to three reference forests. We show that active forest restoration on former sugarcane fields initially disrupted soil associations, but this disruption was overcome as the forest further developed. Active restoration increased the coupling between soil components and created a co-occurrence network with strong linkages between abiotic and functional soil components. However, the late-stage restored forest remained different from all three reference forests. Assisted forest restoration on pastures already resulted into coupling during the transition from pasture to forest, while coupling and network structure of late-stage restored forest was not different from the native conserved reference forest. The observed coupling was the result of strong links between biotic and functional soil components. Both methods facilitated soil recovery, but coupling in assisted restoration was not different from the native conserved reference forest, while actively restored soils remained distinct from both native conserved and degraded reference forests. We propose that actively restored forests may need more time to converge with reference forests or that active restoration fosters a novel forest soil state.

1. Introduction

Ecological restoration of global biodiversity hotspots is critical to halt and reverse loss of biodiversity and climate change by land degradation (Shin et al., 2022). The Brazilian Atlantic Forest is such a global biodiversity hotspot that has been reduced to 12–16 % of its original area due to urbanization, agriculture, and logging (De Lima et al., 2015; Rodrigues et al., 2009). In the past 3–4 decades, legislation has led to protect remaining forest fragments and promote restoration of degraded lands (Ribeiro et al., 2011). In the Brazilian Atlantic Forest, most restoration projects have focused on restoring iconic species or aboveground species richness (Londe et al., 2020; Rodrigues et al., 2009; Werden et al., 2022). However, belowground microbes may also require restoration, as they are essential for nutrient cycling in ecosystems, and influence aboveground plant growth and biodiversity (Kardol and Wardle, 2010; Ramirez et al., 2018). In addition, belowground microbes

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are crucial for ecosystem stability in combination with soil chemistry (Jansson and Hofmockel, 2020). Therefore, we tested how forest restoration influences the associations among soil microbes, soil chemistry, soil C stocks, and soil-borne greenhouse gas fluxes.

Soil plays a fundamental role in supporting root development, organic matter decomposition, and providing nutrients for plant growth (Baldrian et al., 2023; Gongalsky et al., 2021; Liu et al., 2023; Morriën, 2016). Soil microbes are key players that drive nutrient cycling, with prokaryotes and fungi breaking down recalcitrant materials, thereby releasing nutrients for plants and other microbes. The feeding of protists and other predators on microbes releases nutrients that are trapped in the microbial biomass (Kardol and Wardle, 2010; Morriën, 2016). Many of these soil food web relationships depend on abiotic soil properties, which in turn are influenced by soil microbes and other soil biota. For example, pH affects the availability of soil nutrients and, consequently, modifies microbial growth and composition, while soil aggregation influences how pore spaces may be filled by air and water, which enables microbial life to grow and survive (Indoria et al., 2020; Lammel et al., 2018a, 2018b). Therefore, understanding the outcomes of feedback interactions between physical, chemical, and biological soil properties requires an analysis of how to restore the complex associations between soil organisms and their abiotic environment (Geisen et al., 2019; Mendes et al., 2019).

Restoration depends on site-specific characteristics, such as land-use legacies, proximity to conserved forest fragments, and the human and material resources available for restoration (Rodrigues et al., 2009; Safar et al., 2020). Previous land use, including agriculture, logging, and urban development, leaves legacies that affect both above- and belowground components, with severely degraded areas often requiring more intensive interventions and longer recovery periods than low-degraded areas (Holl and Aide, 2011; Meli et al., 2017). In such degraded areas with limited natural regeneration potential, active forest restoration is typically applied, requiring the removal of disturbance factors such as perennial crops and cattle. It also involves soil preparation activities, including tilling, liming, and fertilization, to support the planting of native species (Crouzeilles et al., 2017; Holl and Aide, 2011). Conversely, when a site shows potential for self-regeneration, forests may recover by more passive methodologies that are based on the capacity of natural forest recovery. Assisted restoration, for instance, involves removing disturbance factors and planting native species in gaps within existing forest fragments to accelerate natural forest recovery of extensively used grasslands or derelict forest fragments (Meli et al., 2017; Shono et al., 2007; Werden et al., 2022).

Tropical forests may take over 100 years to fully mature (Poorter et al., 2021a, 2021b), and co-occurrence-based ecological networks enable to assess how soil components are associated in the restored forests. Co-occurrence-based network modeling involves correlating soil components and representing them as nodes, with their associations depicted as edges (Baguette et al., 2013; Matchado et al., 2021; Peterson et al., 2013). Co-occurrence networks are applied as a multivariate tool for modeling and identifying patterns in complex datasets, while also providing insightful graphical representations. By analyzing these networks, patterns among soil components can be recognized by analyzing co-occurrence of critical components within communities (e.g., species, genes, nutrients, functions). From these patterns, possible implications may be derived about resilience to disturbances and other ecosystem properties (Baguette et al., 2013; Matchado et al., 2021; Peterson et al., 2013). Additionally, co-occurrence-based network analysis may be applied to represent soil food webs, highlighting the complex associations between soil organisms and the environments they inhabit (Guseva et al., 2022; Ramirez et al., 2018). However, co-occurrence based networks indicate not more than that two components are present together, which may mean that they interact, but they may also respond similarly to the same environmental changes (Goberna and Verdú, 2022).

In co-occurrence networks, the distance refers to the average number of steps or connections required to traverse between two nodes, which may be bacterial species, or nutrients, for example. From an ecological perspective, a shorter distance between nodes can facilitate rapid information exchange, allowing for quick responses to environmental changes (Jordán and Scheuring, 2004). Modularity indicates how well a network is divided into distinct clusters, where nodes within the same cluster are more strongly connected than those in other clusters. Modularity is often associated with niche partitioning, where different species or functional groups occupy specific roles or niches (Deng et al., 2012). Network density refers to actual connections as a proportion of the total possible connections. A denser network suggests a more robust exchange of resources, energy, or information, which can help maintain ecosystem functions and improve resilience to disturbances (Wu et al., 2024). Also, key nodes identified by their higher number of strong links, are often used to identify components, for example, species, genes, soil characteristics, or functions, which are critical for community stability (Faust, 2021).

The proportion of significant correlations among soil components that are not occurring by chance, informs about the order, or disorder, within the system; this concept has been referred to as ‘coupling’ (Ochoa-Hueso et al., 2021), which is based on natural species co-occurrences. As a result of species extinctions, species cease to co-occur, which leads to reduced associations among soil components, resulting in decreased coupling values and loss of soil functioning (Ochoa-Hueso et al., 2021). Greater coupling indicates greater internal organization, and increased efficiency in the transfer of energy and matter within ecosystems (Ochoa-Hueso et al., 2021). By applying the coupling framework, it has been possible to assess the levels of system organization and elucidate the consequences of defaunation on soil communities and functions (Eisenhauer et al., 2023), as well as the effects of altered rainfall regimes on soil (Yang et al., 2023). In both cases, decreasing coupling values were associated with losses in soil biodiversity, leading to deficits in soil decomposition, diminished control of soil-borne diseases, and lowered plant biomass production. Conversely, when the coupling framework was used to evaluate soil system organization in restored grasslands, restoration was shown to enhance the internal organization of soil systems, bringing them to levels comparable to reference sites (Resch et al., 2022).

In the present study, we evaluated the recovery of associations among soil organisms, physicochemical properties, and functions in the Brazilian Atlantic Forest in response to two restoration methods: (i) active forest restoration on sugarcane fields, and (ii) assisted forest restoration on former pastures. The effects of both restoration methods were analyzed using a chronosequence of restored forests. Then, late-stage restored forests were compared to reference forests with varying degradation levels (secondary degraded, native degraded, and native conserved). We tested the hypothesis that (1) during assisted restoration, soil associations will become more similar to old-growth forests, and (2) active restoration will result in different network associations and coupling than the references.

2. Methodology

2.1. Site selection

We selected representative semi-deciduous Atlantic Forests on clay soils in São Paulo state, Brazil. The sites were selected based on their land-use history, the method of forest restoration, and the time since the start of restoration. We acquired this information through interviews with restoration practitioners and landowners, complemented by satellite data (LandSat/Copenicus/Google Earth software v. 7.3.4.8248). Details about the location and climate characteristics of the sampled sites are described in Supplementary Fig. S1 and Supplementary Table S1, respectively.

The selected sites were forest fragments restored by active and assisted methodologies over the past 30 years, complying with the Brazilian environmental legislation (Rodrigues et al., 2009). The restoration methodology applied to the selected sites was contingent on previous land use: (i) sugarcane fields showed no potential for self-regeneration into forest, so they underwent active restoration, involving sugarcane removal, soil preparation with liming and N-P-K fertilizer, followed by extensive planting and seeding of native species. In contrast, (ii) pastures with natural regeneration potential were subjected to assisted restoration, involving fewer interventions like fencing, removing exotic grasses, and introducing fast-growing species to occupy spaces without plants.

We categorized the selected restored sites into three temporal developmental stages based on the time since the start of restoration: Sites restored less than 8 years are named 'early'; between 8 and 16 years are named 'intermediate'; and sites that started to be restored more than 16 years ago were named 'late'. Sugarcane fields were selected as starting points for active restoration time series, and pastures as a start of time series of assisted restoration. Three forests were selected as references: a secondary degraded forest, a degraded native forest, and a conserved native forest. Supplementary Table S2 provides additional details on land use, restoration methodologies, and the initial year of restoration.

2.2. Plot delimitation and soil sampling

For each forest type (e.g., early-stage actively restored forest), we selected three forest fragments ranging from 20 to 30 ha. In each fragment, we delimited one 30 × 30 m plot located within a representative section of the forest. Additionally, plots were established in sugarcane and pasture fields (Supplementary Fig. S2). The selection criteria for plot placement included forest characteristics that matched the dominant conditions of the fragment, excluding areas with clearings, grass dominance, or lianas, and ensuring that plots were at least 30 m away from the edges.

In each plot, soil samples were collected from five fixed positions: one at the center and four at the corners (Supplementary Fig. S2). Therefore, we had 33 plots (11 typologies × 3 replicates of each forest typology) and 5 samples per plot, yielding 165 soil samples. The samples were taken from the top 10 cm of soil, where microbial abundance, activity, and diversity are highest (Bieluczyk et al., 2023; Fierer et al., 2003). All tools used for soil sampling were thoroughly cleaned between sampled points to prevent cross-contamination. For chemical and texture analyses, 1200 g of soil per sample was collected in plastic bags and stored in a cold chamber for later analysis. Samples for microbiological analyses were stored in 50 mL sterile falcon tubes, transported in coolers with dry ice, and preserved at −80 °C until further use. Undisturbed soil samples for density and porosity analysis were extracted using a 30 mm diameter auger and metal rings, wrapped in cling film, placed in cardboard boxes, and sent for external laboratory analysis.

2.3. Soil physical-chemical analysis and soil C stocks

Soil nutrients were measured based on the standard methodology for Brazilian tropical soils (Cantarella et al., 1998). Soil samples were homogenized using a 2 mm sieve, air-dried, and then weighed for each specific analysis using the gravimetric method. Soil pH was measured in a 0.01 M $CaCl_2$ solution. Soil organic carbon was measured using the Walkley-Black method and multiplying the results by 0.58 to estimate the soil organic matter content. P-phosphate (P) was extracted using ion exchange resins and quantified through a colorimetric method (Van Raij et al., 2001). Carbon (C) and nitrogen (N) were determined using an automatic C and N analyzer, connected to a continuous-flow isotope ratio mass spectrometer (Thermo Scientific, model Delta V Advantage; Milan, Italy). Soil porosity analysis involved saturating samples with water, followed by stabilization in Richard's pressure chamber, drying in forced air, and weighing (Klute and Dirksen, 1986; Smith and Mullins, 1991). Soil bulk density was measured by drying the undisturbed sample in forced air and weighing it (Teixeira et al., 2017).

Soil C stocks (Megagram ha^{-1}) were calculated by multiplying the contents of soil C (%), bulk density (mg m^{-3}), and the thickness of the soil layer (cm). Since the soil bulk density was higher in sugarcane, pasture, and restored sites compared to the references (the secondary and the two native forests), non-corrected C stocks would be systematically overestimated in the selected sites. Then, the stocks were corrected by an equivalent soil mass method and using an average of the soil densities of the three mature forests as a reference.

2.4. Greenhouse gas assessments

Methane (CH_4) and carbon dioxide (CO_2) fluxes were measured as evolving from the soil. One gas measurement was performed next to every collected soil sample. The base dimensions of the static chambers were 19.5 cm (diameter) × 20 cm (height). This base was inserted 2–3 cm in the soil and connected to a lid with a volume of 2 L, totalizing approximately 7 L of air when the chamber was closed. The chamber lid contained two inlets for flexible tubes (6 mm diameter), which were connected, for 10 min incubation time, to an ultraportable CH_4-CO_2 gas analyzer (model U-GGA-915, LGR-ICOS instruments, United States). During the incubation, the vacuum of the gas analyzer moved the air from the chamber in a closed circulation continuous flow. CH_4 and CO_2 concentrations were measured every 10 s, in situ and in real time. The output of the portable gas analyzer provided the gas parameters needed for calculating the total C-CH_4 (μg) and C-CO_2 (mg) inside the chamber. The calculation was done using the Clapeyron ideal gas law: $pV = nRT$, which relates absolute pressure p to absolute temperature T, with volume V of the container holding the gas and the amount n (in moles) of gas contained in there, and R is the molar gas constant. Then, the C-CH_4 and C-CO_2 fluxes were quantified by the first derivative relating concentrations and time, excluding the measurements of the first 60 s of chamber incubation to avoid any stabilization biases. Using the extrapolation from second to hour and from the chamber area (0.0266 m^2) to a plot meter, we obtained the fluxes in μg C-CH_4 m^{-2} h^{-1} and milligram C-CO_2 m^{-2} h^{-1}.

2.5. Soil DNA extraction, amplicon sequencing

Total genomic DNA was extracted from 0.25 g of soil per sample using the DNeasy PowerSoil DNA Isolation Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The quality and quantity of the extracted DNA were evaluated using three complementary approaches: (i) a Qubit fluorometer (Invitrogen, Carlsbad, USA) for DNA concentration, (ii) 1 % sodium boric acid agarose gel electrophoresis to assess DNA integrity (Brody and Kern, 2004), and (iii) a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, USA) to evaluate purity based on A260/A280 ratios, with values between 1.8 and 2.0 considered acceptable.

Amplicon sequencing was conducted to characterize the soil prokaryotic, fungal, and protist communities. All sequencing procedures were performed at the Genome Québec Innovation Centre (Montreal, Canada). For prokaryotic communities, the V4 region of the *16S rRNA* gene was amplified using the primer pair 515F (5′-GTGYCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACNVGGGTWTCTAAT-3′) (Caporaso et al., 2011), generating a fragment of 300 bp. For protist communities, the V4–V5 region of the *18S rRNA* gene was amplified using the primer pair 616*f (5′-TTAAARVGYTCGTAGTYG-3′) and 1132r (5′-CCGTCAATTHCTTYAART-3′) (Hugerth et al., 2014), generating a 504 bp fragment. For fungal communities, the internal transcribed spacer region (ITS1) was amplified using the primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) (Gardes and Bruns, 1993; Smith and Peay, 2014). The amplicon length varied depending on the fungal taxonomic group. Detailed PCR amplification temperatures and cycles for each of the amplicon barcodes can be found in Supplementary Table S3. After amplification, all PCR products were purified using the Quantabio PCR

Purification Kit (Quantabio, Beverly, MA, USA). Library preparation was carried out using the Illumina SP Reagent Kit v1.5, following the manufacturer's protocol, and paired-end sequencing (2 × 250 bp) was performed on an Illumina NovaSeq 6000 platform.

2.6. Data analysis

The obtained amplicon sequencing data were processed using bash and the Qiime2 pipeline (Bolyen et al., 2019). For *16S rRNA* and ITS data, sequences were merged, primers removed, low-quality sequences were filtered and trimmed. The Deblur plugin clustered *16S rRNA* sequences into ASVs (Amir et al., 2017), while the v-search plugin clustered ITS sequences into OTUs (Rognes et al., 2016). Taxonomic inference used the SILVA database for prokaryotic communities using the *16S rRNA* (Quast et al., 2012) and UNITE databases for soil fungi communities using ITS (Kõljalg et al., 2020). Abundance matrices were generated, rarefied, and filtered. The *18S rRNA* data followed the same processing steps as ITS data, but only forward sequences were used. The PR^2 database was employed for taxonomy assignment specific to protist communities (Guillou et al., 2012), followed by manual filtration of the compositional matrix to retain protist groups and subsequent rarefaction. All statistical analyses and graphical outputs were performed using R version 2024.04.2 (Team, 2021). The community structures of prokaryotes, fungi, and protists were assessed based on the obtained compositional matrices using Principal Coordinate Analysis (PCoA). Bray-Curtis distances were calculated from the microbial community data to generate the PCoA multivariate visualization. Sample coordinates from axis 1 and axis 2 of the PCoA were then extracted. Additionally, the Shannon index was calculated from the compositional matrices to represent the soil microbial diversity for prokaryotes, fungi, and protists.

We accessed the establishment of soil associations along two restoration methods: (i) forests restored by the active method, categorized as early (<8 years), intermediate (8–16 years), and late (>16 years) stages, with sugarcane fields as the start point, and (ii) forests restored by the assisted method, similarly categorized and compared to pastures as the start point. Then, both late-stage restored forests were compared to reference forests with varying degradation levels (secondary degraded, native degraded, and native conserved). For each category of restored forests, starting points, and reference forests, we selected abiotic physicochemical soil characteristics (soil pH, organic matter, nitrogen, phosphorus, bulk-density, and total-porosity), biotic communities (PCoA axes 1 and 2 from prokaryotic, fungal, and protist communities), and functional soil characteristics (diversity indices from prokaryotic, fungal, and protist communities, soil carbon stocks, along with CH_4 and CO_2 greenhouse gas fluxes).

In our study, coupling represented the proportion of significant correlations between soil variables. The analysis was based on correlating the selected soil variables using nonparametric Spearman rank correlations, pooling the 15 samples from the three sampled squares for each category of restored forests, starting points, and reference forests. We also assessed the proportion of significant correlations characterized as positive and negative. In addition, coupling was also analyzed for abiotic-biotic, abiotic-functional, and biotic-functional soil categories. To ensure the robustness of observed coupling values, a null model was generated by randomly subsampling 15 samples per category of restored forests, starting points, and reference forests, calculating Spearman correlations, and counting significant ones ($p < 0.05$) over 1000 iterations. The p-value was determined based on the proportion of simulated correlations higher than the observed value. The 0.25 and 0.75 quantiles of the random correlations were also calculated to assess the distribution's spread.

Additionally, for each category of restored forests, starting points, and reference forests, undirected network graphs were created to represent soil associations. The nodes represented selected soil characteristics, while the edges represented significant correlations between these characteristics. In each network, blue edges indicated positive correlations, red edges indicated negative correlations, and the thickness of edges represented the strength of associations between soil components. Node size indicates the degree, revealing the number of connections per node. We also calculated several network topologies with ecological significance: distance, which measures the path length between nodes to assess how quickly or easily associations occur between soil properties; modularity, which shows how the network is organized into modules or clusters, indicating how closely soil processes or components interact; density, reflecting the proportion of potential connections between nodes, providing insight into how interconnected the soil system is; and key nodes, represented by the PageRank index, which highlights the most important nodes based on the number and strength of correlations. Finally, to assess the structure of associations within each network and to compare networks across categories of restored forests, starting points, and reference forests, the PageRank results from each network were correlated with one another and presented as a correlogram.

3. Results

3.1. Assisted forest restoration on pasture fields

We evaluated soil coupling by measuring the proportion of significant correlations among all soil components, along with how many of these correlations were positive and negative. The soil's total coupling in pasture and early-stage restored forests was 16 % for both, followed by an increase to 29 % in the intermediate stage, and then a drop to 14 % in the late stage. The percentage of positive coupling followed the same trend, with pasture and early-stage restored forests showing 10 % and 12 %, followed by an increase to 27 % in the intermediate stage, and a drop to 10 % in the late stage (Fig. 1A). The percentages of total coupling and positive coupling were significantly different from the null model (Supplementary Figs. S3 and S4), while the proportion of negative coupling was not, suggesting that negative coupling may have occurred by chance (Supplementary Fig. S5).

The soil components were grouped into abiotic, biotic, and functional categories, and the percentage coupling among these categories was analyzed. We observed an increase from pasture sites to intermediate-stage restored forests. Specifically, abiotic-biotic coupling ranged from 17 % to 33 %, abiotic-functional coupling from 15 % to 29 %, and biotic-functional coupling from 15 % to 26 %. However, all these coupling percentages had decreased in late-stage forests, with biotic-functional coupling at 21 %, abiotic-biotic at 17 %, and abiotic-functional at 8 % (Fig. 2A). All coupling percentages among soil categories were significantly different from the null model (Supplementary Figs. S6, S7, and S8).

Regarding network topologies, the network distance decreased from pasture sites (1.93) to intermediate-stage restored forests (0.90), followed by an increase in late-stage forests (1.65). Modularity of networks followed a similar trend, decreasing from pasture (0.43) to intermediate-stage forests (0.10), and then increasing in late-stage restored forests (0.32). The density of the networks showed the opposite pattern, decreasing from pasture sites (0.16) to intermediate-stage forests (0.29), and further decreasing in late-stage forests (0.14; Fig. 3A).

The nodes with the most connections, also referred to as key nodes, also changed over time since the start of assisted forest restoration. The key nodes in the pasture ecological network were soil organic matter, carbon stocks, protist, and fungal diversities, and the prokaryotic PCoA 1 axis. In the network from the early-stage restored forest, soil total-porosity, bulk-density, and both prokaryotic PCoA axes were the key nodes. In the intermediate-stage restored forest network, the key nodes were protist PCoA 2 and prokaryotic diversity. In the network from the late-stage restored forest, the key nodes were the prokaryotic and fungal PCoA axes (Fig. 4A).

We compared the different soil networks by correlating their key

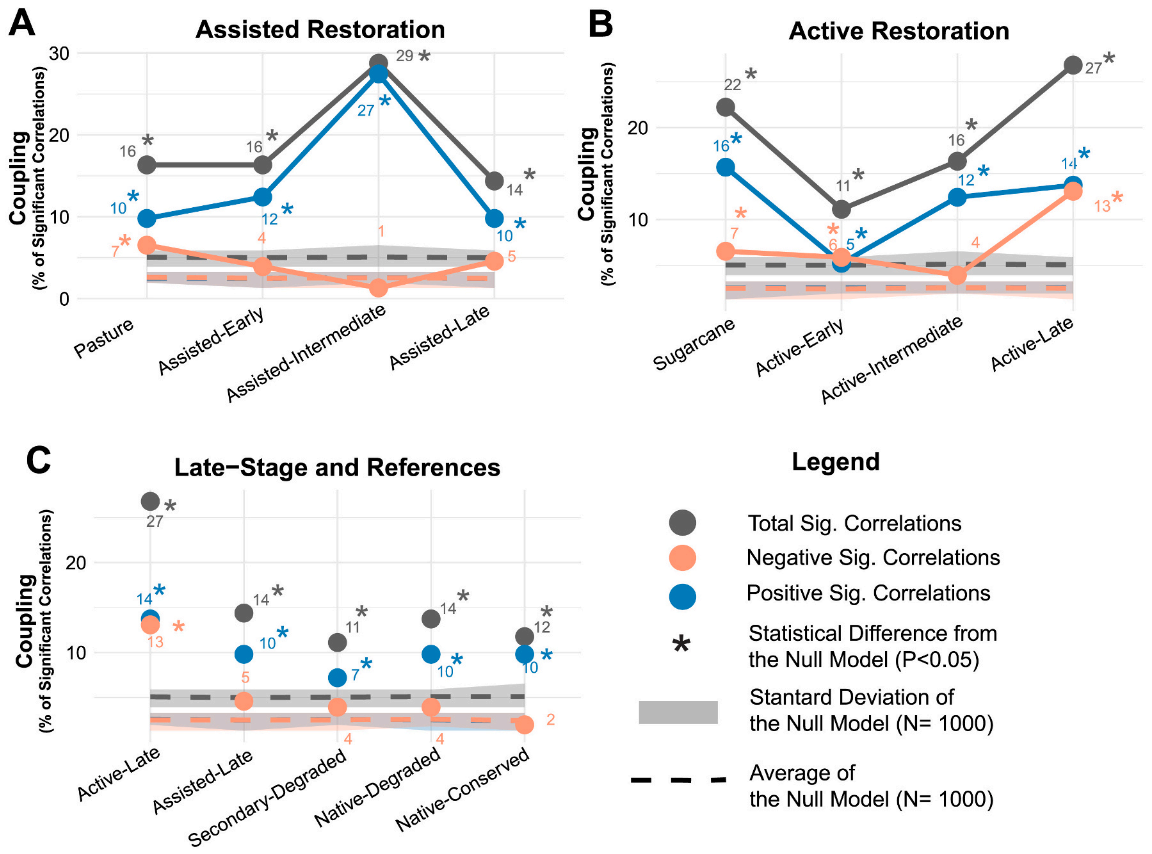

Fig. 1. Coupling development expressed as the percentage of significant total, positive, and negative correlations among soil components. (A) shows the development of coupling in chronosequences of assisted restored forests on former pasture lands, (B) active restored forests on former sugarcane fields, and (C) late-stage restored forests compared to reference forests. The analysis is based on significant Spearman rank correlations ($P < 0.05$) out of 153 correlations. A null model, generated using bootstrapping ($n = 1000$), was used to determine if the observed correlations could occur by chance. The model's average is represented by dashed lines, with the shaded area showing the 0.25 to 0.75 variance intervals.

nodes and we noticed that pasture sites and assisted restored forests were positively correlated (Fig. 5A).

3.2. *Active forest restoration on sugarcane fields*

We found that the coupling of soils decreased from 22 % to 11 % during the conversion of sugarcane fields into early-stage restored forest. This initial disruption was mostly due to a decline in the percentage of positive coupling from 16 % to 5 %. As the active restored forest aged, the total coupling steadily increased in the late-succession stage, reaching 27 %, with 14 % positive coupling and 13 % negative coupling (Fig. 1B). Most of the coupling percentages differed significantly from the null model (Supplementary Figs. S3, S4, and S5).

A decrease in coupling among soil categories was observed following the conversion of sugarcane fields to early-stage restored forests. Specifically, abiotic-biotic coupling dropped from 29 % to 12 %, abiotic-functional coupling from 21 % to 12 %, and biotic-functional coupling from 20 % to 9 %. As the forest aged to the late-succession stage, coupling among those soil categories steadily increased to 30 % for abiotic-functional, 27 % for abiotic-biotic, and 21 % for biotic-functional (Fig. 2B). All these coupling percentages were significantly different from the null model (Supplementary Figs. S6, S7, and S8).

Network topological properties showed distinct trends during the conversion of sugarcane fields into restored forests. The network distance between nodes, which represents how quickly information can travel within the network, decreased from 1.29 in sugarcane fields to 1.18 in early-stage restored forests, followed by increases at the intermediate and late stages (1.98 and 1.27, respectively). Network density, representing the strength of interconnectedness of the network, decreased from 0.22 in sugarcane fields to 0.11 in early-succession stage forests but increased at the intermediate and late stages (0.16 and 0.27, respectively). Modularity, which measures the extent to which the network is divided into distinct clusters or modules, increased from 0.34 in sugarcane fields to 0.54 in early-stage restored forests before declining at the intermediate and late stages to 0.41 and 0.35, respectively (Fig. 3B).

The nodes with the most connections, also referred to as key nodes, also changed during the development of actively restored forest. In the sugarcane ecological network, the key-nodes were soil P concentration and the PCoA 1 axis from protist and fungal communities. In the network of early-stage actively restored forest, key-nodes included P and N concentrations, soil organic matter, soil bulk-density, the PCoA 1 axis from prokaryotic communities, soil carbon stocks, and CH_4 fluxes. For the ecological network from intermediate-stage forest, the key nodes were soil pH, the PCoA 1 axis from prokaryotic communities, and N concentration. In the network from late-stage forests, soil bulk-density, and the PCoA 1 axis from prokaryotic and protist communities were the key nodes (Fig. 4B).

We compared the different soil networks by correlating their key nodes and noticed that sugarcane fields were negatively correlated to the active restored forests (Fig. 5B).

3.3. *Late-stage restored forests development to reference forests*

The total soil coupling in the late stage of assisted restored forest was 14 % and approached that of the native degraded forest (14 %), while the total soil coupling of the late stage of actively restored forest surpassed all forests (27 %; Fig. 1C). The percentage positive coupling for

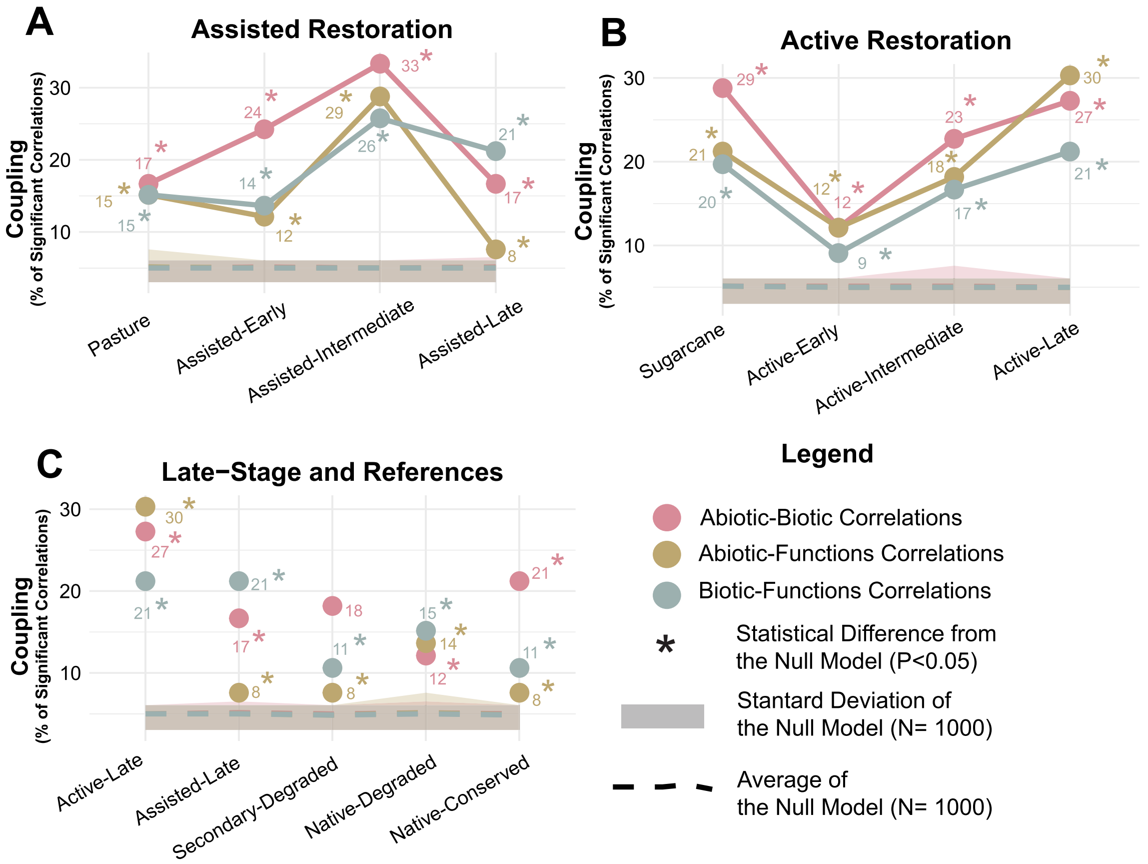

Fig. 2. Coupling development expressed as the percentage of significant total correlations among abiotic, biotic, and functional soil component categories. (A) shows the development of coupling in chronosequences of assisted restored forests on former pasture lands, (B) active restored forests on former sugarcane fields, and (C) late-stage restored forests compared to degraded and reference forests. The analysis is based on significant Spearman rank correlations ($P < 0.05$) out of 66 correlations. A null model, generated using bootstrapping ($n = 1000$), was used to determine if the observed correlations could occur by chance. The model's average is represented by dashed lines, with the shaded area showing the 0.25 to 0.75 variance intervals.

the assisted late-stage was 10 % and matched those of the native degraded and conserved references, which were both 10 %, whereas the late stage of the actively restored forest was 14 %, thereby surpassing all other forests (Fig. 1C). The percentages of total and positive coupling were significantly different from the null model (Supplementary Figs. S3 and S4), except for the proportion of negative coupling, which could have occurred by chance (Supplementary Fig. S5).

In the late-stage actively restored forest, most couplings were organized around abiotic-function variables, representing 30 % of the total couplings, followed by 27 % abiotic-biotic and 21 % biotic-function couplings. Interestingly, these percentages surpassed those of all reference forests. In the late-stage assisted restored forest, biotic-function couplings were the most prominent, making up 21 % of significant couplings, followed by 17 % abiotic-biotic and 8 % abiotic-function couplings. The percentage of biotic-function couplings also surpassed all values of reference forests, while the abiotic-biotic couplings were not different from the secondary degraded forest (18 %). The abiotic-function couplings matched those of the secondary degraded and native conserved forests, which were both at 8 % (Fig. 2C). All these couplings percentages were significantly different from the null model (Supplementary Figs. S6, S7, and S8).

The distance as topology of ecological network at the active restored forest at late-stage (1.23) was close to the native conserved (1.14), while the assisted forest (1.65) was similar to the native degraded reference (1.61; Fig. 3C). The network modularity in the active (0.35) and assisted late-stage forests (0.32) were close to the native degraded (0.34; Fig. 3C). Network density was highest in the active forest (0.27), while the assisted late-stage (0.14) was similar to the native degraded (0.14; Fig. 3C).

Within the network of late-stage actively restored forests, soil bulk density, and the PCoA 1 axis from prokaryotic and protist communities were the key nodes. In the network of late-stage assisted restored forests, the key nodes were the prokaryotic and fungal PCoA axes. In the network of the secondary degraded reference forest, the key nodes were the fungal and protist PCoA axes. Within the native degraded reference network, the key nodes were soil organic matter, soil carbon stocks, pH, nitrogen concentration, the prokarya PCoA 1 axis, and fungal diversity. In the native conserved reference, the key nodes were soil pH, total porosity, and the prokarya and fungal PCoA axes (Fig. 4C).

When the soil networks of late-stage forests were compared to the references, we observed that active restored forests were positively correlated to a secondary-degraded reference, while assisted restored forests were positively correlated to a native-conserved reference forest (Fig. 5C).

4. Discussion

We examined the effects of the temporal development of two forest restoration methodologies in the Brazilian Atlantic Forest on soil component associations. Specifically, we assessed assisted restoration, applied to abandoned pastures with natural regeneration potential, and active restoration, applied to more degraded sugarcane fields requiring full planting. The aging of assisted restoration in former pasture sites resulted in a soil network with a similar number of associations compared to old-growth reference forests, but with stronger links between biotic components (such as microbial community composition)

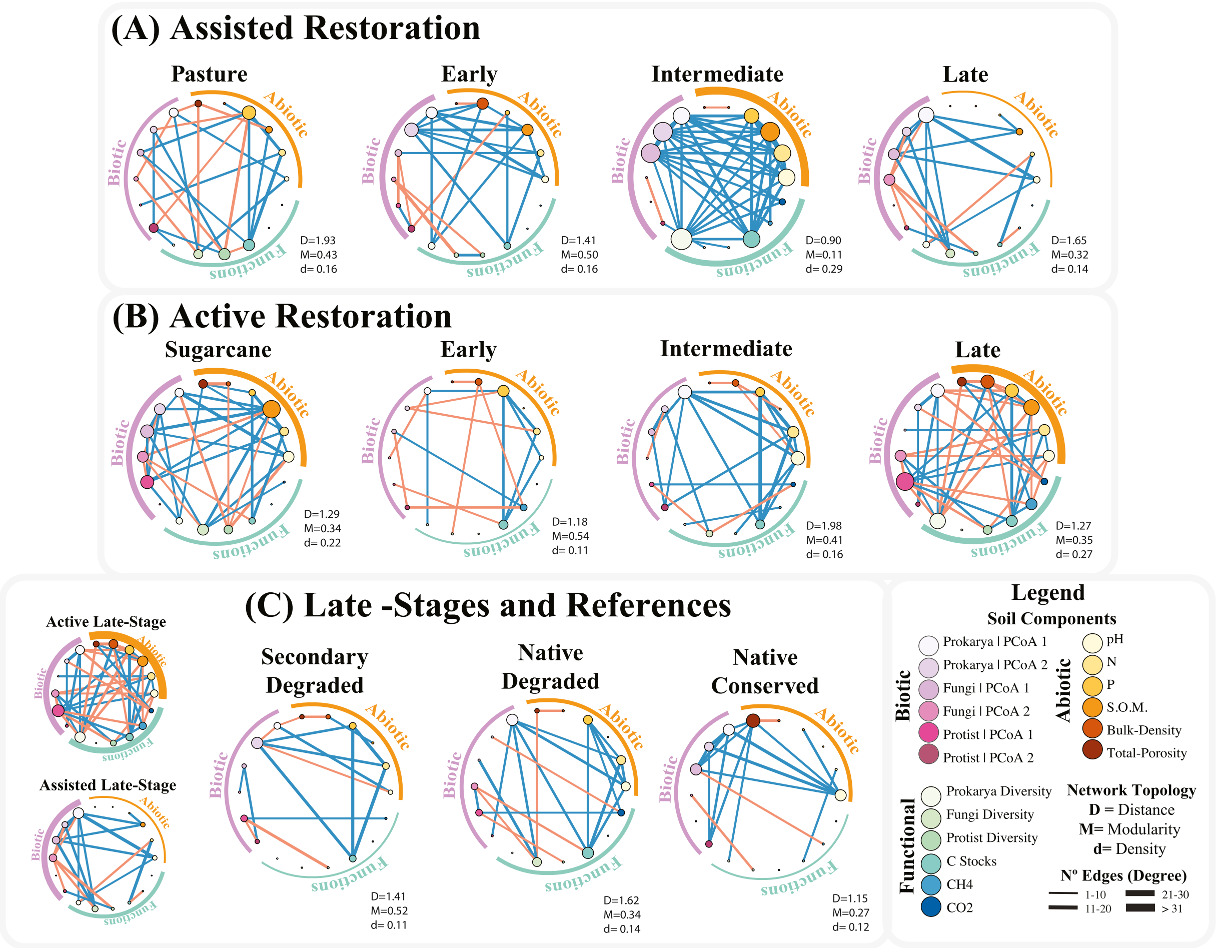

Fig. 3. Ecological network analysis based on soil components. (A) shows the network from chronosequences of assisted restored forests on former pasture lands, (B) active restored forests on former sugarcane, and (C) late-stage restored forests compared to degraded and reference forests. In each network, the nodes represent the soil components, while blue edges indicate positive correlations and red edges indicate negative correlations.

and functional components (such as greenhouse gas emissions and carbon stocks). Our results are in agreement with other studies showing that soil microbial communities are key components in maintaining terrestrial ecosystem functioning (Delgado-Baquerizo et al., 2016; Wagg et al., 2014). In active restoration of former sugarcane fields, soil microbial coupling was initially disrupted. However, as restoration progressed toward a more mature forest stage, soil microbes gradually became more connected, even showing more associations among soil components than reference forests. Additionally, late-stage actively restored forests exhibited a network where abiotic soil characteristics were strongly linked to soil functional components, likely because residual abiotic heterogeneity such as soil compaction and nutrient content continued to shape microbial associations. These findings align with other studies suggesting that human-modified soils may foster novel environments where past land-use legacies co-exist with new ecological changes (Hobbs, 2016; Perring et al., 2013). Our results suggest that assisted forest restoration on less degraded soils leads to a more complete recovery of soil microbial associations toward reference forest conditions than active restoration on more degraded soils.

4.1. *The soil associations in the reference forests*

The soil networks of the reference forests revealed few correlations of soil microbes with abiotic and functional soil components, and none of these components dominated the co-occurrence networks. Our results suggest that within the native forests, dominant correlations were minimized, resulting in a well-distributed network. One possible explanation is that old-growth forests promote more homogeneous abiotic soil conditions. In contrast, heterogeneous soil characteristics in agricultural systems, such as variations in pH and nutrient availability, often drive shifts in microbial communities and foster strong correlations (Fraterrigo et al., 2005; Lammel et al., 2018a, 2018b). Competition for specific limiting resources may lead communities to adopt similar strategies to acquire them, thereby fostering stronger correlations (Goberna and Verdú, 2022). This was not the case in our reference forests, suggesting that soil functions there do not depend on numerous or strongly correlated associations. We interpret this as an indication of a more stable soil system under these mature forests. Such stability in mature terrestrial ecosystems has been observed before and is often linked to the slowing turnover of plant communities as forest succession advances (Jangid et al., 2013).

4.2. *The development of soil associations of assisted restored forests toward references*

In the late stage of assisted restored forests, biotic and functional components dominated soil associations, while abiotic characteristics played a limited role in explaining microbial correlations. Apparently, in assisted restored forests, abiotic factors are relatively of minor importance as drivers of microbial networks. These associations likely underpin essential plant-microbes interactions that support forest nutrition and productivity, including processes such as arbuscular mycorrhizal

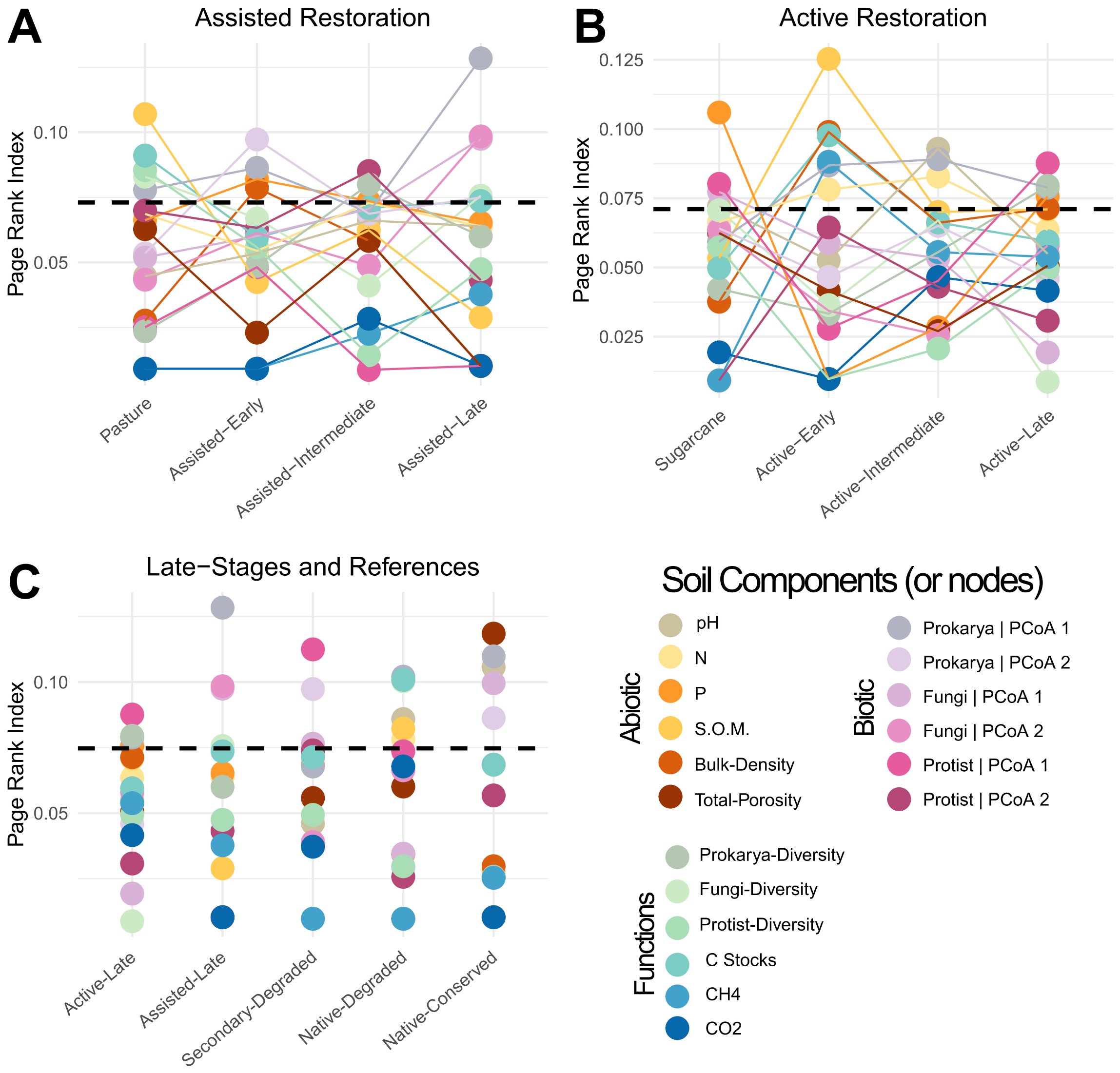

Fig. 4. Development of network key nodes. (A) shows the development of network key nodes in chronosequences of assisted restored forests on former pasture lands, (B) active restored forests on former sugarcane fields, and (C) late-stage restored forests compared to degraded and reference forests. The analysis was based on the PageRank index, which ranked the most important soil components (or nodes) within each network based on the number and strength of correlations (or edges) connected to the node. The black dashed line represents the 0.75 quantile of the PageRank values.

fungi (AMF) associations, nitrogen fixation, phosphorus mineralization and solubilization, decomposition of litter and organic matter, or even pathogenic associations (Fujii et al., 2018; Johnson and Turner, 2019).

Interestingly, in late-stage forests that result from assisted restoration, the patterns of soil microbial associations were well comparable to native conserved reference forests. All these forests were characterized by few below-ground correlations. Assisted restoration is typically applied in areas with potential for natural forest regeneration, often characterized by remnant forest species, forest patches, and connectivity to surrounding forest landscapes (Shono et al., 2007; Werden et al., 2022). Our results suggest that the soils in these areas still consider some legacy of the forest before being converted into pasture land. Additionally, pastures may also create a good starting position for forest restoration, because of extensive management based on permanent crops, with minimal soil interventions such as tilling, fertilization, or pasture renewal (Feltran-Barbieri and Féres, 2021). Consequently, under those conditions remnant soil communities and associations from former forests might have persisted, thereby likely contributing to the development of late-stage forests toward native reference conditions.

Although late-stage forests that result from assisted restoration developed soil associations resembling those in native conserved forests, the key nodes within the networks suggest that the transition from pastures to late-stage forest was not necessarily linear. Indeed, the time series of restoration sites revealed variations in time. In pastures, microbial communities were typically associated with variables of the carbon cycle, such as soil organic matter, carbon stocks, and methane fluxes. The associations with these abiotic carbon cycle-related components may have been influenced by the presence of *Brachiaria* spp., an exotic grass species widely cultivated in South American pastures (Feltran-Barbieri and Féres, 2021). *Brachiaria* spp. are characterized by its aggressive growth, investing heavily in shoot biomass and root exudation to enhance nutrient and water uptake, thereby affecting carbon storage in the soil (Carvalho et al., 2010; Merloti et al., 2023), which may explain the main key nodes in the pasture network.

In early-stage assisted forests, the variance around soil prokaryotic communities, along with changes in phosphorus levels and soil density, promoted those characteristics as key nodes in the network. These shifts likely reflect the improvement in soil aggregation due to the removal of grazing pressure and the development of a more diverse forest root system during forest development (Tulio et al., 2023). Phosphorus as a key node may reflect the nutrient variance within those soils, possibly due to its adsorption into soil particles, a characteristic typical of

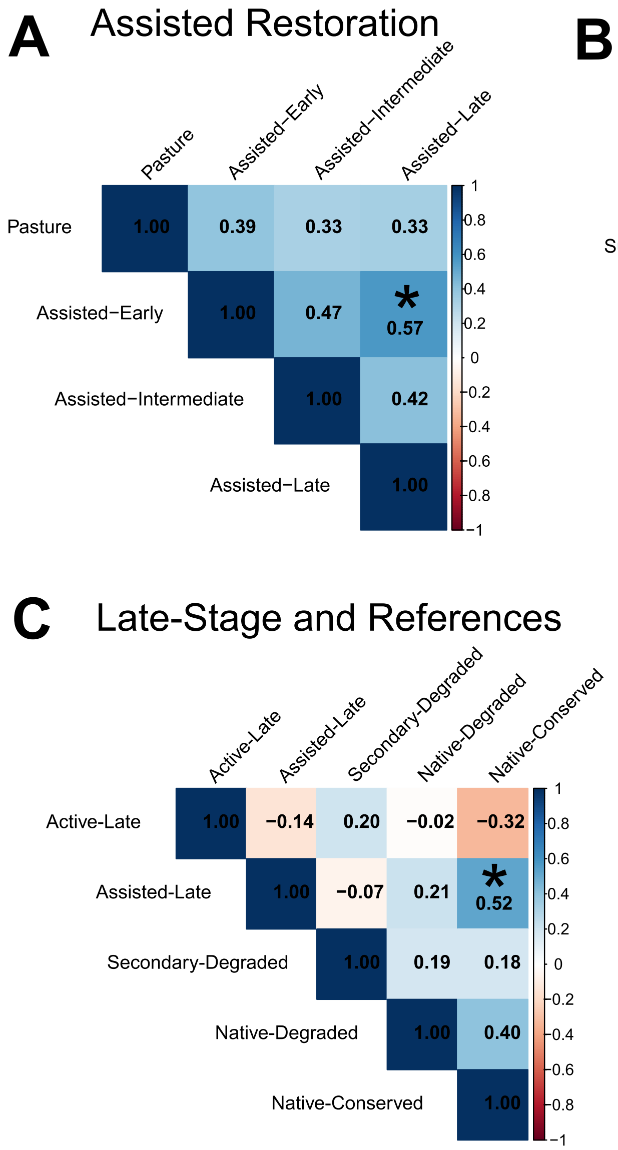

Fig. 5. Correlogram representing correlation among networks. (A) shows the correlation between networks from chronosequences of assisted restored forests on former pasture lands, (B) active restored forests on former sugarcane fields, and (C) late-stage restored forests compared to degraded and reference forests. The analysis was based on extracting the key nodes from each network and correlating them using Spearman rank to assess how equivalent the networks are to each other. Numbers within the cells indicate the strength of the correlation, with negative correlations shaded in red, positive correlations in blue, and neutral correlations in white. Asterisks (*) indicate statistically significant correlations ($P < 0.05$).

tropical soils (Baldrian et al., 2023; Pavinato et al., 2020). This variance could represent the efforts by microbial communities to release phosphorus into the soil solution by processes like P solubilization and mineralization (Baldrian et al., 2023; Pavinato et al., 2020). Interestingly, at the intermediate stage, we observed a drastic increase in associations between soil communities and primarily abiotic soil characteristics. This is likely due to the relatively high pH found in intermediate-restored forests. This may have increased nutrient availability in soil solution, which stimulates the growth of microbial communities (Lammel et al., 2018a, 2018b).

We do not attribute these results to the restoration methodology itself but rather to local soil variations that also may have played a role in driving soil microbial associations. Variations along gradients of restored forests under assisted methodologies have also been observed in plant communities (Werden et al., 2022). Local forest characteristics, such as remnant patches, often cause high variability within these forests during the restoration process, which may indicate that forest development does not necessarily follow linear trajectories to recovery (Werden et al., 2022). Based on our results, we also pointed out that microbial soil associations do not follow an exponential recovery trajectory toward reference forests, with variations observed along the forest development process due to local soil characteristics. Nonetheless, this approach still successfully achieved outcomes comparable to the reference forests.

At the intermediate-stage, we observed a drastic increase in associations between soil communities and abiotic soil characteristics. This is likely due to the relatively high pH levels found in intermediate restored forests and, consequently, the higher availability of soil nutrients in solution, which stimulates the growth of microbial communities (Lammel et al., 2018a, 2018b). We do not attribute these results to the restoration methodology itself but rather to local soil variations that also played a role in driving soil microbial associations. Variations along gradients of restored forests under assisted methodologies have also been observed in plant communities (Werden et al., 2022). Authors have noted that local forest characteristics, such as remnant patches, often cause high variability within these forests during the restoration process.

They state that while assisted restoration can achieve restoration goals, these variations mean that forest development does not necessarily follow linear trajectories of recovery (Werden et al., 2022). Based on our results, we also pointed out that microbial soil associations do not follow an exponential recovery trajectory toward reference forests, with variations observed along the forest development process due to local soil characteristics. Nonetheless, the assisted methodology still successfully promoted outcomes comparable to the native conserved reference forest.

4.3. Actively restored forest face challenges of recovery toward the references

In the late stage of actively restored forest, microbial associations with abiotic soil components accounted for most of the observed correlations. Additionally, the late-stage soil network of actively restored forests showed higher numbers of correlations than the reference forests. Factors such as phosphorus concentration and soil bulk density were associated with the majority of identified correlations. These results may reflect soil legacies from previous sugarcane cultivation, which were not entirely mitigated by active restoration. This may reflect a long-lasting legacy of previous agricultural activities, as bulk-density is significantly disrupted by sugarcane cultivation due to heavy soil tilling and harvesting machinery (Cherubin et al., 2017). The concentration of P is characterized as a limiting factor in restored forests (Van Der Sande et al., 2023), and the high associations around the element may also represent a scarcity of P in tropical soils (Pavinato et al., 2020). This is further supported by plant communities and soil microbiomes actively releasing P from mineral and organic particles (Baldrian et al., 2023).

Our results suggest that even after 30 years, active restoration has not led to fully restored soil associations to the level of reference forests, with residual soil legacies from agricultural management complicating the restoration of soil microbial associations in Atlantic Forests. Together, the soil associations in late-stage actively restored forests likely reflect a scenario where the new forest ecosystem coexists with these land-use legacies. This duality has been reported in other studies, suggesting that restoration on heavily degraded sites results in ecosystems retaining features of both the restored environment and the historical land-use practices (Hobbs, 2016; Perring et al., 2013).

The transition of sugarcane fields to actively restored forest demonstrated to follow a linear development in terms of soil associations. First, sugarcane fields had more soil coupling and more connected networks than early-stage active restored forests. Sugarcane fields undergo frequent tilling, fertilization, liming, and harvesting using heavy machinery, all within a monoculture dominated by sugarcane (*Saccharum officinarum* spp.) (Bordonal et al., 2018). These intensive agricultural practices reduce system variability, creating a more uniform environment in terms of microbial diversity and soil physical-chemical characteristics compared to natural forests (Cavalcanti et al., 2020; da Silva et al., 2012; Ferreira et al., 2015). This uniformity, in turn, could lead to more frequent and stronger co-occurrence patterns, as soil organisms respond similarly to these homogeneous conditions. The substantial local variation in P concentrations and microbial components making them as key-node within the sugarcane network provided more evidence on how the soil system components are associated. In tropical agricultural soils, P is often bound to soil particles and not readily available for uptake by microorganisms or plants (Pavinato et al., 2020). The numerous associations between soil P concentrations and soil microbial communities in sugarcane fields could represent cooperation between *Saccharum officinarum* spp. plants and the soil microbiome to mobilize and take up this limiting nutrient (Atekan et al., 2014; Awais et al., 2019).

We observed that transitioning sugarcane fields to early-stage actively restored forests strongly reduced soil coupling and led to poorly connected networks. This disruption is likely due to the intensive management practices involved in active restoration, which included removing sugarcane plants, tilling the soil, and liming to introduce native forest species (Crouzeilles et al., 2017; Morrison and Lindell, 2011). These practices likely produced a highly disturbed system where associations between soil microbiota and their components were disrupted. However, as forest restoration progressed to intermediate and late stages, the number of associations within these soil networks significantly increased, particularly among soil microbial communities, bulk density, and phosphorus. This pattern was comparable to those found in sugarcane fields and points out that intensive agricultural practices left soil legacies that could not be fully mitigated by forest progression. Interestingly, these results suggest that while active restoration is an effective way to convert degraded sites into forests, former land-use legacies impose substantial challenges to fully restoring the ecosystem to its original state.

Given that overcoming persistent soil legacy effects is a common challenge in ecosystem restoration, valuable insights can be drawn from practices elsewhere. For instance, soil removal or inversion to reduce excess nutrients has been employed in heathland restoration in the Netherlands and the UK (Benetková et al., 2022). Similarly, applying carbon sources like sawdust or biochar to stimulate soil microbiota and immobilize nitrogen has been effective in restoring tallgrass prairies in the US (Averett et al., 2004). The use of cover crops prior to forest restoration (Shono et al., 2007) to enrich nutrient-poor soils, catch crops to remove excess nutrients from the soil (In 'T Zandt et al., 2018), or even soil inoculation from conserved environments (Freitas et al., 2023; Wubs et al., 2016) are also promising management practices to restore soil system along with plant communities of highly degraded lands.

5. Conclusion

Our study demonstrates that assisted restoration of forests on abandoned pasturelands leads to effective recovery of soil microbial associations that closely resemble those in native conserved forests. These findings underscore the efficacy of restoration methods that promote natural forest recovery under conditions comparable to undisturbed ecosystems. In contrast, in actively restored forests, soil associations were predominantly shaped by abiotic factors, such as soil bulk density and phosphorus concentration. This highlights the persistence of soil legacies from previous sugarcane cultivation, which were not alleviated by forest progression. Further research is needed to elucidate the drivers and mechanisms behind these soil legacies and to develop management strategies that facilitate the recovery of not only vegetation but also soil microbial communities on degraded lands targeted for reforestation.

CRediT authorship contribution statement

Luis Fernando Merloti: Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Raúl Ochoa-Hueso:** Writing – review & editing, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Dina in 't Zandt:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **G.F. (Ciska) Veen:** Writing – review & editing. **Wanderlei Bieluczyk:** Methodology, Investigation, Data curation. **Ricardo Ribeiro Rodrigues:** Project administration, Investigation, Conceptualization. **Lucas William Mendes:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Siu Mui Tsai:** Supervision, Funding acquisition, Conceptualization. **Wim H. van der Putten:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) utilized ChatGPT-3.5 to enhance the readability of the manuscript. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2025.106408.

Data availability

Data and figures in this study are included in the main text or in the Supplementary material.

References

Amir, A., McDonald, D., Navas-Molina, J.A., Kopylova, E., Morton, J.T., Zech Xu, Z., Kightley, E.P., Thompson, L.R., Hyde, E.R., Gonzalez, A., 2017. Deblur rapidly resolves single-nucleotide community sequence patterns. MSystems 2, 10–1128.

Atekan, A., Nuraini, Y., Handayanto, E., Syekhfani, S., 2014. The potential of phosphate solubilizing bacteria isolated from sugarcane wastes for solubilizing phosphate. J. Degrad. Min. Lands Manag. 1, 175.

Averett, J.M., Klips, R.A., Nave, L.E., Frey, S.D., Curtis, P.S., 2004. Effects of soil carbon amendment on nitrogen availability and plant growth in an experimental tallgrass prairie restoration. Restor. Ecol. 12, 568–574. https://doi.org/10.1111/j.1061-2971.2004.00284.x.

Awais, M., Tariq, M., Ali, Q., Khan, A., Ali, A., Nasir, I.A., Husnain, T., 2019. Isolation, characterization and association among phosphate solubilizing bacteria from sugarcane rhizosphere. Cytol. Genet. 53, 86–95. https://doi.org/10.3103/S0095452719010031.

Baguette, M., Blanchet, S., Legrand, D., Stevens, V.M., Turlure, C., 2013. Individual dispersal, landscape connectivity and ecological networks. Biol. Rev. 88, 310–326. https://doi.org/10.1111/brv.12000.

Baldrian, P., López-Mondéjar, R., Kohout, P., 2023. Forest microbiome and global change. Nat. Rev. Microbiol. 1.

Benetková, P., Van Diggelen, R., Háněl, L., Vicentini, F., Moradi, R., Weijters, M., Bobbink, R., Harris, J.A., Frouz, J., 2022. Soil fauna development during heathland restoration from arable land: role of soil modification and material transplant. Ecol. Eng. 176, 106531. https://doi.org/10.1016/j.ecoleng.2021.106531.

Bieluczyk, W., Merloti, L.F., Cherubin, M.R., Mendes, L.W., Bendassolli, J.A., Rodrigues, R.R., de Camargo, P.B., van der Putten, W.H., Tsai, S.M., 2023. Forest restoration rehabilitates soil multifunctionality in riparian zones of sugarcane production landscapes. Sci. Total Environ. 888, 164175.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857.

Bordonal, R. de O., Carvalho, J.L.N., Lal, R., de Figueiredo, E.B., de Oliveira, B.G., La Scala, N., 2018. Sustainability of sugarcane production in Brazil. A review. Agron. Sustain. Dev. 38, 1–23.

Brody, J.R., Kern, S.E., 2004. Sodium boric acid: a Tris-free, cooler conductive medium for DNA electrophoresis. BioTechniques 36, 214–216. https://doi.org/10.2144/04362BM02.

Cantarella, H., van Raij, B., Quaggio, J.A., 1998. Soil and plant analyses for lime and fertilizer recommendations in Brazil. Commun. Soil Sci. Plant Anal. 29, 1691–1706. https://doi.org/10.1080/00103629809370060.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. 108, 4516–4522.

Carvalho, J.L.N., Raucci, G.S., Cerri, C.E.P., Bernoux, M., Feigl, B.J., Wruck, F.J., Cerri, C. C., 2010. Impact of pasture, agriculture and crop-livestock systems on soil C stocks in Brazil. Soil Tillage Res. 110, 175–186. https://doi.org/10.1016/j.still.2010.07.011.

Cavalcanti, R.Q., Rolim, M.M., de Lima, R.P., Tavares, U.E., Pedrosa, E.M., Cherubin, M. R., 2020. Soil physical changes induced by sugarcane cultivation in the Atlantic Forest biome, northeastern Brazil. Geoderma 370, 114353.

Cherubin, M.R., Franco, A.L.C., Guimarães, R.M.L., Tormena, C.A., Cerri, C.E.P., Karlen, D.L., Cerri, C.C., 2017. Assessing soil structural quality under Brazilian sugarcane expansion areas using Visual Evaluation of Soil Structure (VESS). Soil Tillage Res. 173, 64–74. https://doi.org/10.1016/j.still.2016.05.004.

Crouzeilles, R., Ferreira, M.S., Chazdon, R.L., Lindenmayer, D.B., Sansevero, J.B.B., Monteiro, L., Iribarrem, A., Latawiec, A.E., Strassburg, B.B.N., 2017. Ecological restoration success is higher for natural regeneration than for active restoration in tropical forests. Sci. Adv. 3, e1701345. https://doi.org/10.1126/sciadv.1701345.

da Silva, D.K.A., de Oliveira Freitas, N., de Souza, R.G., da Silva, F.S.B., de Araujo, A.S.F., Maia, L.C., 2012. Soil microbial biomass and activity under natural and regenerated forests and conventional sugarcane plantations in Brazil. Geoderma 189, 257–261.

De Lima, R.A.F., Mori, D.P., Pitta, G., Melito, M.O., Bello, C., Magnago, L.F., Zwiener, V. P., Saraiva, D.D., Marques, M.C.M., De Oliveira, A.A., Prado, P.I., 2015. How much do we know about the endangered Atlantic Forest? Reviewing nearly 70 years of information on tree community surveys. Biodivers. Conserv. 24, 2135–2148. https://doi.org/10.1007/s10531-015-0953-1.

Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Jeffries, T.C., Gaitan, J.J., Encinar, D., Berdugo, M., Campbell, C.D., Singh, B.K., 2016. Microbial diversity drives multifunctionality in terrestrial ecosystems. Nat. Commun. 7. https://doi.org/10.1038/ncomms10541.

Deng, Y., Jiang, Y.-H., Yang, Y., He, Z., Luo, F., Zhou, J., 2012. Molecular ecological network analyses. BMC Bioinformatics 13, 113. https://doi.org/10.1186/1471-2105-13-113.

Eisenhauer, N., Ochoa-Hueso, R., Huang, Y., Barry, K.E., Gebler, A., Guerra, C.A., Hines, J., Jochum, M., Andraczek, K., Bucher, S.F., Buscot, F., Ciobanu, M., Chen, H., Junker, R., Lange, M., Lehmann, A., Rillig, M., Römermann, C., Ulrich, J., Weigelt, A., Schmidt, A., Türke, M., 2023. Ecosystem consequences of invertebrate decline. Curr. Biol. 33, 4538–4547 e5. https://doi.org/10.1016/j.cub.2023.09.012.

Faust, K., 2021. Open challenges for microbial network construction and analysis. ISME J. 15, 3111–3118. https://doi.org/10.1038/s41396-021-01027-4.

Feltran-Barbieri, R., Féres, J.G., 2021. Degraded pastures in Brazil: improving livestock production and forest restoration. R. Soc. Open Sci. 8, 201854.

Ferreira, M.P., Alves, D.S., Shimabukuro, Y.E., 2015. Forest dynamics and land-use transitions in the Brazilian Atlantic Forest: the case of sugarcane expansion. Reg. Environ. Chang. 15, 365–377. https://doi.org/10.1007/s10113-014-0652-6.

Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. Soil Biol. Biochem. 35, 167–176.

Fraterrigo, J.M., Turner, M.G., Pearson, S.M., Dixon, P., 2005. Effects of past land use on spatial heterogeneity of soil nutrients in southern Appalachian forests. Ecol. Monogr. 75, 215–230. https://doi.org/10.1890/03-0475.

Freitas, A.S.D., Zagatto, L.F.G., Rocha, G.S., Muchalak, F., Silva, S.D.S., Muniz, A.W., Hanada, R.E., Tsai, S.M., 2023. Amazonian dark earths enhance the establishment of tree species in forest ecological restoration. Front. Soil Sci. 3, 1161627. https://doi.org/10.3389/fsoil.2023.1161627.

Fujii, K., Shibata, M., Kitajima, K., Ichie, T., Kitayama, K., Turner, B.L., 2018. Plant–soil interactions maintain biodiversity and functions of tropical forest ecosystems. Ecol. Res. 33, 149–160. https://doi.org/10.1007/s11284-017-1511-y.

Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol. Ecol. 2, 113–118.

Geisen, S., Wall, D.H., Van Der Putten, W.H., 2019. Challenges and opportunities for soil biodiversity in the Anthropocene. Curr. Biol. 29, R1036–R1044. https://doi.org/10.1016/j.cub.2019.08.007.

Goberna, M., Verdú, M., 2022. Cautionary notes on the use of co-occurrence networks in soil ecology. Soil Biol. Biochem. 166, 108534. https://doi.org/10.1016/j.soilbio.2021.108534.

Gongalsky, K.B., Zaitsev, A.S., Korobushkin, D.I., Saifutdinov, R.A., Butenko, K.O., De Vries, F.T., Ekschmitt, K., Degtyarev, M.I., Gorbunova, A.Yu., Kostina, N.V., Rakhleeva, A.A., Shakhab, S.V., Yazrikova, T.E., Wolters, V., Bardgett, R.D., 2021. Forest fire induces short-term shifts in soil food webs with consequences for carbon cycling. Ecol. Lett. 24, 438–450. https://doi.org/10.1111/ele.13657.

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boutte, C., Burgaud, G., de Vargas, C., Decelle, J., 2012. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. Nucleic Acids Res. 41, D597–D604.

Guseva, K., Darcy, S., Simon, E., Alteio, L.V., Montesinos-Navarro, A., Kaiser, C., 2022. From diversity to complexity: microbial networks in soils. Soil Biol. Biochem. 169, 108604. https://doi.org/10.1016/j.soilbio.2022.108604.

Hobbs, R.J., 2016. Degraded or just different? Perceptions and value judgements in restoration decisions. Restor. Ecol. 24, 153–158.

Holl, K.D., Aide, T.M., 2011. When and where to actively restore ecosystems? For. Ecol. Manag. 261, 1558–1563.

Hugerth, L.W., Muller, E.E., Hu, Y.O., Lebrun, L.A., Roume, H., Lundin, D., Wilmes, P., Andersson, A.F., 2014. Systematic design of 18S rRNA gene primers for determining eukaryotic diversity in microbial consortia. PLoS One 9, e95567.

In 'T Zandt, D., Fritz, C., Wichern, F., 2018. In the land of plenty: catch crops trigger nitrogen uptake by soil microorganisms. Plant Soil 423, 549–562. https://doi.org/10.1007/s11104-017-3540-2.

Indoria, A.K., Sharma, K.L., Reddy, K.S., 2020. Hydraulic properties of soil under warming climate. In: Climate Change and Soil Interactions. Elsevier, pp. 473–508. https://doi.org/10.1016/B978-0-12-818032-7.00018-7.

Jangid, K., Whitman, W.B., Condron, L.M., Turner, B.L., Williams, M.A., 2013. Soil bacterial community succession during long-term ecosystem development. Mol. Ecol. 22, 3415–3424. https://doi.org/10.1111/mec.12325.

Jansson, J.K., Hofmockel, K.S., 2020. Soil microbiomes and climate change. Nat. Rev. Microbiol. 18, 35–46.

Johnson, D.W., Turner, J., 2019. Tamm review: nutrient cycling in forests: a historical look and newer developments. For. Ecol. Manag. 444, 344–373. https://doi.org/10.1016/j.foreco.2019.04.052.

Jordán, F., Scheuring, I., 2004. Network ecology: topological constraints on ecosystem dynamics. Phys Life Rev 1, 139–172. https://doi.org/10.1016/j.plrev.2004.08.001.

Kardol, P., Wardle, D.A., 2010. How understanding aboveground–belowground linkages can assist restoration ecology. Trends Ecol. Evol. 25, 670–679. https://doi.org/10.1016/j.tree.2010.09.001.

Klute, A., Dirksen, C., 1986. Water retention: laboratory methods. In: Methods of Soil Analysis: Part 1—Physical and Mineralogical Methods, (methodsofsoilan1). Soil Science Society of America, American Society of Agronomy.

Kõljalg, U., Nilsson, H.R., Schigel, D., Tedersoo, L., Larsson, K.-H., May, T.W., Taylor, A. F., Jeppesen, T.S., Frøslev, T.G., Lindahl, B.D., 2020. The taxon hypothesis paradigm—on the unambiguous detection and communication of taxa. Microorganisms 8, 1910.

Lammel, Daniel R., Barth, G., Ovaskainen, O., Cruz, L.M., Zanatta, J.A., Ryo, M., de Souza, E.M., Pedrosa, F.O., 2018a. Direct and indirect effects of a pH gradient bring insights into the mechanisms driving prokaryotic community structures. Microbiome 6, 106. https://doi.org/10.1186/s40168-018-0482-8.

Lammel, Daniel R., Barth, G., Ovaskainen, O., Cruz, L.M., Zanatta, J.A., Ryo, M., de Souza, E.M., Pedrosa, F.O., 2018b. Direct and indirect effects of a pH gradient bring insights into the mechanisms driving prokaryotic community structures. Microbiome 6, 1–13.

Liu, J., Fang, K., Kou, Y., Xia, R., He, H., Zhao, W., Liu, Q., 2023. Variations in the soil micro-food web structure and its relationship with soil C and N mineralization during secondary succession of subalpine forests. Sci. Total Environ. 879, 163257. https://doi.org/10.1016/j.scitotenv.2023.163257.

Londe, V., Turini Farah, F., Ribeiro Rodrigues, R., Roberto Martins, F., 2020. Reference and comparison values for ecological indicators in assessing restoration areas in the Atlantic Forest. Ecol. Indic. 110, 105928. https://doi.org/10.1016/j.ecolind.2019.105928.

Matchado, M.S., Lauber, M., Reitmeier, S., Kacprowski, T., Baumbach, J., Haller, D., List, M., 2021. Network analysis methods for studying microbial communities: a mini review. Comput. Struct. Biotechnol. J. 19, 2687–2698. https://doi.org/10.1016/j.csbj.2021.05.001.

Meli, P., Holl, K.D., Rey Benayas, J.M., Jones, H.P., Jones, P.C., Montoya, D., Moreno Mateos, D., 2017. A global review of past land use, climate, and active vs. passive restoration effects on forest recovery. PLoS One 12, e0171368. https://doi.org/10.1371/journal.pone.0171368.

Mendes, M.S., Latawiec, A.E., Sansevero, J.B., Crouzeilles, R., Moraes, L.F., Castro, A., Alves-Pinto, H.N., Brancalion, P.H., Rodrigues, R.R., Chazdon, R.L., 2019. Look down—there is a gap—the need to include soil data in Atlantic Forest restoration. Restor. Ecol. 27, 361–370.

Merloti, L.F., Bossolani, J.W., Mendes, L.W., Rocha, G.S., Rodrigues, M., Asselta, F.O., Crusciol, C.A.C., Tsai, S.M., 2023. Investigating the effects of *Brachiaria* (Syn. *Urochloa*) varieties on soil properties and microbiome. Plant Soil 1–18.

Morriën, E., 2016. Understanding soil food web dynamics, how close do we get? Soil Biol. Biochem. 102, 10–13. https://doi.org/10.1016/j.soilbio.2016.06.022.

Morrison, E.B., Lindell, C.A., 2011. Active or passive forest restoration? Assessing restoration alternatives with avian foraging behavior. Restor. Ecol. 19, 170–177. https://doi.org/10.1111/j.1526-100X.2010.00725.x.

Ochoa-Hueso, R., Delgado-Baquerizo, M., Risch, A.C., Schrama, M., Morriën, E., Barmentlo, S.H., Geisen, S., Hannula, S.E., Resch, M.C., Snoek, B.L., Van Der Putten, W.H., 2021. Ecosystem coupling: a unifying framework to understand the functioning and recovery of ecosystems. One Earth 4, 951–966. https://doi.org/10.1016/j.oneear.2021.06.011.

Pavinato, P.S., Cherubin, M.R., Soltangheisi, A., Rocha, G.C., Chadwick, D.R., Jones, D. L., 2020. Revealing soil legacy phosphorus to promote sustainable agriculture in Brazil. Sci. Rep. 10, 15615.

Perring, M.P., Standish, R.J., Hobbs, R.J., 2013. Incorporating novelty and novel ecosystems into restoration planning and practice in the 21st century. Ecol. Process. 2, 18. https://doi.org/10.1186/2192-1709-2-18.

Peterson, E.E., Ver Hoef, J.M., Isaak, D.J., Falke, J.A., Fortin, M., Jordan, C.E., McNyset, K., Monestiez, P., Ruesch, A.S., Sengupta, A., Som, N., Steel, E.A., Theobald, D.M., Torgersen, C.E., Wenger, S.J., 2013. Modelling dendritic ecological networks in space: an integrated network perspective. Ecol. Lett. 16, 707–719. https://doi.org/10.1111/ele.12084.

Poorter, L., Craven, D., Jakovac, C.C., Van Der Sande, M.T., Amissah, L., Bongers, F., Chazdon, R.L., Farrior, C.E., Kambach, S., Meave, J.A., Muñoz, R., Norden, N., Rüger, N., Van Breugel, M., Almeyda Zambrano, A.M., Amani, B., Andrade, J.L., Brancalion, P.H.S., Broadbent, E.N., De Foresta, H., Dent, D.H., Derroire, G., DeWalt, S.J., Dupuy, J.M., Durán, S.M., Fantini, A.C., Finegan, B., Hernández-Jaramillo, A., Hernández-Stefanoni, J.L., Hietz, P., Junqueira, A.B., N'dja, J.K., Letcher, S.G., Lohbeck, M., López-Camacho, R., Martínez-Ramos, M., Melo, F.P.L., Mora, F., Müller, S.C., N'Guessan, A.E., Oberleitner, F., Ortiz-Malavassi, E., Pérez-García, E.A., Pinho, B.X., Piotto, D., Powers, J.S., Rodríguez-Buriticá, S., Rozendaal, D.M.A., Ruíz, J., Tabarelli, M., Teixeira, H.M., Valadares De Sá Barretto Sampaio, E., Van Der Wal, H., Villa, P.M., Fernandes, G.W., Santos, B.A., Aguilar-Cano, J., De Almeida-Cortez, J.S., Alvarez-Davila, E., Arreola-Villa, F., Balvanera, P., Becknell, J.M., Cabral, G.A.L., Castellanos-Castro, C., De Jong, B.H.J., Nieto, J.E., Espírito-Santo, M.M., Fandino, M.C., García, H., García-Villalobos, D., Hall, J.S., Idárraga, A., Jiménez-Montoya, J., Kennard, D., Marín-Spiotta, E., Mesquita, R., Nunes, Y.R.F., Ochoa-Gaona, S., Peña-Claros, M., Pérez-Cárdenas, N., Rodríguez-Velázquez, J., Villanueva, L.S., Schwartz, N.B., Steininger, M.K., Veloso, M.D.M., Vester, H.F.M., Vieira, I.C.G., Williamson, G.B., Zanini, K., Hérault, B., 2021a. Multidimensional tropical forest recovery. Science 374, 1370–1376. https://doi.org/10.1126/science.abh3629.

Poorter, L., Rozendaal, D.M., Bongers, F., Almeida, de J.S., Álvarez, F.S., Andrade, J.L., Arreola Villa, L.F., Becknell, J.M., Bhaskar, R., Boukili, V., 2021b. Functional recovery of secondary tropical forests. Proc. Natl. Acad. Sci. USA 118, e2003405118.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596.

Ramirez, K.S., Geisen, S., Morriën, E., Snoek, B.L., Van Der Putten, W.H., 2018. Network analyses can advance above-belowground ecology. Trends Plant Sci. 23, 759–768. https://doi.org/10.1016/j.tplants.2018.06.009.

Resch, M.C., Schütz, M., Ochoa-Hueso, R., Buchmann, N., Frey, B., Graf, U., Van Der Putten, W.H., Zimmermann, S., Risch, A.C., 2022. Long-term recovery of above- and below-ground interactions in restored grasslands after topsoil removal and seed addition. J. Appl. Ecol. 59, 2299–2308. https://doi.org/10.1111/1365-2664.14145.

Ribeiro, M.C., Martensen, A.C., Metzger, J.P., Tabarelli, M., Scarano, F., Fortin, M.-J., 2011. The Brazilian Atlantic Forest: a shrinking biodiversity hotspot. In: Zachos, F.E., Habel, J.C. (Eds.), Biodiversity Hotspots. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 405–434. https://doi.org/10.1007/978-3-642-20992-5_21.

Rodrigues, R.R., Lima, R.A., Gandolfi, S., Nave, A.G., 2009. On the restoration of high diversity forests: 30 years of experience in the Brazilian Atlantic Forest. Biol. Conserv. 142, 1242–1251.

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584.

Safar, N.V.H., Magnago, L.F.S., Schaefer, C.E.G.R., 2020. Resilience of lowland Atlantic forests in a highly fragmented landscape: insights on the temporal scale of landscape restoration. For. Ecol. Manag. 470, 118183. https://doi.org/10.1016/j.foreco.2020.118183.

Shin, Y., Midgley, G.F., Archer, E.R.M., Arneth, A., Barnes, D.K.A., Chan, L., Hashimoto, S., Hoegh-Guldberg, O., Insarov, G., Leadley, P., Levin, L.A., Ngo, H.T., Pandit, R., Pires, A.P.F., Pörtner, H., Rogers, A.D., Scholes, R.J., Settele, J., Smith, P., 2022. Actions to halt biodiversity loss generally benefit the climate. Glob. Chang. Biol. 28, 2846–2874. https://doi.org/10.1111/gcb.16109.

Shono, K., Cadaweng, E.A., Durst, P.B., 2007. Application of assisted natural regeneration to restore degraded tropical forestlands. Restor. Ecol. 15, 620–626. https://doi.org/10.1111/j.1526-100X.2007.00274.x.

Smith, K., Mullins, C., 1991. Soil Analysis. Marcel Decker.

Smith, D.P., Peay, K.G., 2014. Sequence depth, not PCR replication, improves ecological inference from next generation DNA sequencing. PLoS One 9, e90234.

Team, R.C., 2021. R: A Language and Environment for Statistical Computing. Published online 2020. Supplemental Information References S 1, pp. 371–378.

Teixeira, P.C., Donagemma, G.K., Fontana, A., Teixeira, W.G., 2017. Manual de métodos de análise de solo.

Tulio, R.H., Rachwal, M.F.G., Zanatta, J.A., Da Silva, K., Kaschuk, G., 2023. Physical, chemical and microbiological soil attributes influence soil greenhouse gases fluxes in Atlantic Forest and pine (*Pinus taeda*) plantations in Brazil. Soil Use Manag. 39, 183–197. https://doi.org/10.1111/sum.12864.

Van Der Sande, M.T., Powers, J.S., Kuyper, T.W., Norden, N., Salgado-Negret, B., Silva de Almeida, J., Bongers, F., Delgado, D., Dent, D.H., Derroire, G., 2023. Soil resistance and recovery during neotropical forest succession. Philos. Trans. R. Soc. B 378, 20210074.

Van Raij, B., de Andrade, J., Cantarella, H., Quaggio, J., 2001. Chemical Analysis for Evaluation of Fertility of Tropical Soils. Agronomic Institute, Campinas.

Wagg, C., Bender, S.F., Widmer, F., Van Der Heijden, M.G.A., 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proc. Natl. Acad. Sci. USA 111, 5266–5270. https://doi.org/10.1073/pnas.1320054111.

Werden, L.K., Zarges, S., Holl, K.D., Oliver, C.L., Oviedo-Brenes, F., Rosales, J.A., Zahawi, R.A., 2022. Assisted restoration interventions drive functional recovery of tropical wet forest tree communities. Front. For. Glob. Change 5. https://doi.org/10.3389/ffgc.2022.935011.

Wu, Z., Cheng, S., Xu, K., Qian, Y., 2024. Ecological network resilience evaluation and ecological strategic space identification based on complex network theory: a case study of Nanjing city. Ecol. Indic. 158, 111604. https://doi.org/10.1016/j.ecolind.2024.111604.

Wubs, E.R.J., Van Der Putten, W.H., Bosch, M., Bezemer, T.M., 2016. Soil inoculation steers restoration of terrestrial ecosystems. Nat. Plants 2, 16107. https://doi.org/10.1038/nplants.2016.107.

Yang, T., Zhong, X., Chen, J., Nielsen, U.N., Ochoa-Hueso, R., Qu, Y., Sui, Y., Gao, W., Sun, W., 2023. Ecosystem-level decoupling in response to reduced precipitation frequency and degradation in steppe grassland. Funct. Ecol. 37, 2910–2926. https://doi.org/10.1111/1365-2435.14425.