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Assessing the restoration of a seasonally flooded riparian forest through soil carbon and nitrogen cycling indicators and soil microbial communities

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The restoration of a six-year-old, seasonally flooded riparian forest planted with woody species native to the Brazilian Cerrado biome within a buffer zone system was evaluated based on soil carbon and nitrogen cycling parameters and soil microbial communities. Soil organic carbon, humic and fulvic acids, phospholipid fatty acid profiles of total bacteria and fungi, and selected functional microbial groups, such as aerobic/anaerobic and denitrifying communities, were used as indicators of soil quality and fertility. Restoration was progressing towards the soil conditions of a preserved riparian forest (PS) compared to a disturbed site (DS). However, a higher soil ammonium concentration was observed at the experimental site (ES) and DS than at PS, which was attributed to impaired nitrification due to periodic flooding. Principal component analysis confirmed that soil organic matter, cation exchange capacity, NO_3^-, and the denitrifying, anaerobic/aerobic, actinomycetes, and Gram-negative groups are suitable indicators for riparian site restoration. In contrast, soil NH_4^+ accumulation emerged as the main environmental impact hindering riparian forest recovery.

Keywords: Ammonium, Flooding, Humic Acids, Organic Carbon, Phospholipid Fatty Acid (PLFA)

Introduction

The Brazilian Cerrado biome (tropical savannah) has undergone intense deforestation over the last few decades, with a gradual replacement of its native vegetation by cropland and grazing areas, particularly in riparian zones (Caballero et al. 2023). The riparian forests of the Velhas River, located in the São Francisco River basin within the Cerrado biome, have been degraded by anthropogenic, hydrologic, and geomorphological processes (Kimura et al. 2017). The Sabará stream, a tributary of the Velhas River, is considered one of the most polluted rivers in this basin (Junqueira & Campos 1998), and its riparian forest is subject to periodic flooding (Kimura et al. 2017). Deforestation of the riparian forests along the Sabará stream floodplain, associated with periodic flooding, has led to erosion and destabilization of the stream margins, resulting in landscape degradation and stream siltation.

Riparian forests provide several ecosystem services related to erosion control, soil stabilization, and drainage, which are mediated by soil organic matter (SOM – Six & Paustian 2014), including soil humic substances (HSs – *e.g.*, humic and fulvic acids). These are the final decay products of lignin-rich plants. HSs, plant root systems, soil microbial communities, clays, ionic bridging and polyvalent cations promote soil aggregation (Tisdall & Oades 1982), which is primarily responsible for soil stabilization (Gupta & Germida 2015), improving soil fertility and reducing the risk of erosion (Piccolo & Drosos 2025). Land management and environmental impacts (*e.g.*, deforestation, flooding, and fire) may disrupt soil aggregate stability by altering the soil microbial community, decomposition processes, HS formation, and soil carbon (C) accumulation (Olk et al. 2019). Therefore, some soil physicochemical properties, such as soil C, fulvic and humic acids, and soil aggregation, are considered soil health indicators (Six & Paustian 2014, Piccolo & Drosos 2025) and are reliable for riparian forest restoration (Kimura & Scotti 2016, Kimura et al. 2017, Santos & Scotti 2018, Gomes et al. 2024).

HSs may also play an important role in nitrogen (N) cycling, as they can retain and fix soil ammonium, the first product of organic N mineralization, due to its negative charge (Piccolo & Drosos 2025). However, flooding may cause alterations in N cycling, changing the soil oxygen availability (Reddy & DeLaune 2008) and the soil microbial community (Unger et al. 2009), especially those participating in the N cycle (Saint-Laurent et al. 2014, Ribbons et al. 2016), and consequently influencing key processes, such as nitrification and denitrification (Reddy & DeLaune 2008). Such biogeochemical processes are mediated by soil microorganisms whose activity depends on microbial community size (microbial biomass) and community composition, which may be modified by land use. Profiling of phospholipid fatty acids (PLFAs) in the microbial community structure has been widely used to assess changes in microbial communities in the soil, particularly those related to C and N availability in the soil (Gomes et al. 2024, Grayston & Prescott 2005), and environmental stressors, such as flooding (Bossio & Scow 1998, Unger et al. 2009). This method also enables the assessment of changes in some functional microbial groups, such as arbuscular mycorrhizal fungi and aerobic/anaerobic (Bossio & Scow 1998) and denitrifying (Fu et al. 2015) microbial communities.

Despite the known role of C and N bio-

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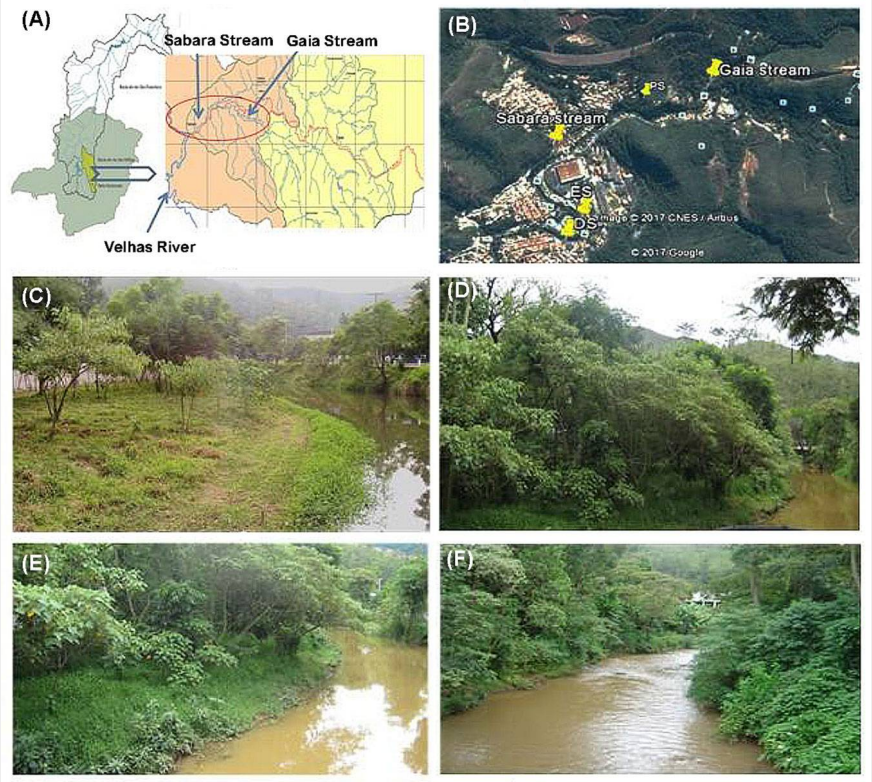

Fig. 1 - (A): Location of the Velhas River basin, highlighting Sabará and Gaia streams; (B): Location of experimental (ES), disturbed (DS) and preserved (PS) sites; (C): ES before revegetation; (D): ES, 4 years after restoration; (E): ES before flooding; (F): ES during a flooding event.

geochemical cycles in the functioning of riparian zones, few studies have attempted to use these soil biogeochemical elements as indicators of riparian forest restoration (Card & Quideau 2010, Kimura et al. 2017, Santos & Scotti 2018, Gomes et al. 2024). In this study, we assessed the restoration progression of a six-year-old riparian forest planted with native species in the Velhas River basin, subject to periodic flooding, compared with a degraded site and a preserved riparian forest.

Material and methods

Study sites

The study sites (Fig. 1A, Fig. 1B) consisted of urban and peri-urban riparian sites, located on the right bank of the Sabará stream, a tributary of the Velhas River, in the São Francisco basin (Fig. 1A), in Sabará, Minas Gerais State, Brazil (19° 52′ 53.33″ S; 43° 47′ 38.76″ W). The Sabará stream receives domestic waste from Sabará and from its tributary, Gaia stream, which is polluted by domestic and industrial waste from different cities (Fig. 1A, Fig. 1B). The Experimental Site (ES) is an urban riparian site that was previously used for grazing activity (Fig. 1C) and later subjected to restoration procedures (Fig. 1D). The predominant native vegetation in the region is tropical savannah (Brazilian Cerrado). The mean annual temperature is 22-23 °C, with dry winters and summer rainfall. The total annual rainfall ranged from 1200 to 1600 mm, and the margins of the Sabará stream are affected by annual floods (Pinheiro 1997, Godim Filho et al. 2004), increasing the flow rate from approximately 2810 (Fig. 1E) to 8000 $m^3 s^{-1}$ (Fig. 1F).

The studied sites were as follows: (i) experimental site (ES, 120 × 15 m), where the restoration procedures were adopted using native species (Fig. 1D, Fig. 1E) from the Cerrado biome; (ii) preserved site (PS), a fragment of a riparian forest (120 × 15 m) that was chosen as a positive reference for biological, physical and chemical integrity, located at a preserved park (19° 52′ 47″-19° 52′ 34″ S; 44° 07′ 44″-43° 47′ 30″ W – Fig. 1B), situated nearly 1000 m away from ES; (iii) disturbed site (DS, 120 m × 15 m), adjacent to ES with similar conditions to the ES prior to being converted to plantation (Fig. 1B), also formerly used for grazing. The dominant vegetation in DS (negative control) consisted of pioneer and invasive species (*e.g.*, *Brachiaria decumbens* and *Ricinus communis*), with no woody vegetation.

Experimental design

The experimental site (120 × 15 m) was revegetated with species from the Brazilian Cerrado biome using the buffer zone system described by Kimura et al. (2017) to restore the lost ecosystem functions of drainage, soil stability and soil fertility (Fig. 1D). In Zone I (5 m from the river), phreatophyte and flood-tolerant species (*e.g.*, *Morus nigra, Rapanea guianensis, Miconia* sp., *Eugenia uniflora, Psidium rufus, Inga edulis, Croton urucurana, Eritrina speciosa* and *Nectandra lanceolata* [cf]) were planted. In Zone II (10 m from the margin), woody biomass-producing species (*e.g.*, *Croton floribundus, Anadenanthera peregrina, Centrolobium tomentosum, Machaerium villosum, Piptadenia gonoacantha, Luehea divaricate,* and *Samanea tubulosa*) were planted to improve litter and organic matter formation. In Zone III (15 m from the margin), herbaceous, shrub, and woody species (*e.g.*, *Hymenea courbaril, Mimosa bimucronata, Tradescantia* sp., and *Piper umbellatum*) were planted to ensure drainage and the control of surface and subsurface erosion (Fig. 1D).

Four-month-old seedlings grown under nursery conditions were planted in the field with a 3 × 3 m spacing. Fertilization was performed according to Somasegaran & Hoben (1985).

Soil samples were collected at a depth 0-20 cm along 3 transects (100 m each) crossing the area at 6 years after transplantation at each study site (ES, PS, and DS). A total of 27 mixed soil samples (3 soil samples/transect × 3 transects/site × 3 sites), which consisted of a composite sample formed by 5 subsamples collected within a 10-m radius (see Fig. S1 in Supplementary material), were used for all chemical and biological analyses. The study site (Fig. 1E) experienced annual floods over the six years following transplantation (Fig. 1F).

Soil chemical analysis

Soil samples were sieved with a 2-mm mesh and analyzed to determine their chemical properties (EMBRAPA 1997). The experimental site was compared with the control sites for cation content, cation exchange capacity (CEC), base saturation, organic matter content, and soil pH, among other parameters. The soil samples were kept cool (4 °C) during sampling and transport and were maintained at 2 °C until processing. The total N level was determined in field samples using the Kjeldahl digestion technique, while ammonium and nitrate contents were determined using the steam distillation method (Bremner & Keeney 1965), with 10 g of soil extracted with 2 mol L^{-1} KCl.

Fractionation of soil organic matter

The fractionation of sequestered C from SOM was performed as described by Dabin (1971). Briefly, 15 g of sieved soil was processed to obtain different C fractions, representing humic and fulvic acids. This method is based on acid-base reactions that extract HSs, yielding an insoluble fraction (humic acid, HA) and a soluble fraction (fulvic acid, FA). The determination of organic C in the HA and FA fractions was performed as described by Dabin (1971).

Microbial biomass and carbon determination (C_{mic})

Soil samples were sieved through a 2-mm mesh screen and dried overnight at 105 °C to determine the moisture content. They were then used to evaluate the biomass (C_{mic}) following the fumigation method described by Vance et al. (1987).

Phospholipid fatty acid (PLFA) profiles

PLFAs were extracted from lyophilized soil following the method described by Bligh & Dwyer (1959). The total lipids were then fractionated into glyco-, neutral, and polar lipids, as outlined by Gehron & White (1983). The fatty acid methyl esters were obtained from the elution of the phospholipid fraction using methanol, followed by mild alkaline methanolysis (White et al. 1979). The extracts were analyzed using gas chromatography with flame ionization detection (Hewlett-Packard model 5890 series 2 chromatograph). Fatty acid chemical structures were verified by chromatography/mass spectrometry (Hewlett-Packard model 5890 series 2 gas chromatograph and Hewlett-Packard model 5971 mass selective detector), and PLFAs were expressed as equivalent peak responses to the internal standard. Peak areas were converted to PLFA μg^{-1} dry soil (absolute abundance). Fatty acid nomenclature is in the form of A:B ωC, in which A indicates the total number of C atoms, B indicates the number of double bonds, and C indicates the position of the first double bond from the aliphatic (ω) end of the molecule. The suffixes "c" and "t" indicate *cis* and *trans* geometry. The prefixes "i", "a" and "me" refer to iso, anteiso and mid-chain methyl branching, and "cy" refers to cyclopropyl rings (Frostegard et al. 1993, Frostegard & Baath 1996). Approximately 40 individual PLFAs were identified as the sum of signatures as described in Tab. S1 (Supplementary material).

Statistical analysis

The variables studied were compared among sites (PS, DS, and ES) using analysis of variance (ANOVA) for normally distributed variables and the Kruskal-Wallis test for non-normally distributed variables. Multiple comparisons of normal and non-normal variables were performed using Tukey and Nemenyi tests, respectively. Pearson correlations were computed for soil chemical and microbial data, and principal component analysis (PCA) was used to identify the most significant variables based on the variance explained. The analysis presented the variables in terms of the first two components, and the influence of each variable on the restoration process at each site was demonstrated using a scatter plot in MINITAB v. 1.5. The significance level was set at 5% ($P \leq 0.05$) for all analyses.

Tab. 1 - Soil chemical properties in different sites. Means followed by the same letter in a row are not significantly different after the Tukey test ($p < 0.05$). (SB): sum of bases; (CEC): cation exchange capacity; (BS): base saturation.

Variables	Disturbed site (DS)	Experimental site (ES)	Preserved site (PS)
pH	7.20 ± 0.06^a	7.33 ± 0.22^a	6.23 ± 0.15^b
P (mg dm^{-3})	51.33 ± 1.86^a	33.67 ± 2.03^b	44.67 ± 1.45^a
K^+ ($mmol_c\ dm^{-3}$)	3.77 ± 0.09^a	2.20 ± 0.23^b	2.52 ± 0.06^b
Ca^{2+} ($mmol_c\ dm^{-3}$)	56.67 ± 0.33^c	154.67 ± 0.88^a	146.67 ± 1.2^b
Mg^{2+} ($mmol_c\ dm^{-3}$)	5.33 ± 0.33^a	8.33 ± 1.45^a	7.67 ± 0.67^a
SB ($mmol_c\ dm^{-3}$)	66.00 ± 0.00^c	165.33 ± 0.33^a	157.00 ± 1.0^b
CEC ($mmol_c\ dm^{-3}$)	75.67 ± 0.33^c	173.67 ± 0.88^a	166.00 ± 0.58^b
BS (%)	87.67 ± 0.33^b	95.33 ± 0.88^a	94.67 ± 0.33^a

Results and discussion

Soil carbon metabolism

The planting of native species in ES improved soil fertility after six years (Tab. 1). Soil fertility indicators, including cation exchange capacity (CEC) and sum of bases (SB), were comparable between ES and PS (Tab. 1). In contrast, soil pH and phosphorus (P) and potassium (K) concentrations were relatively higher in DS than in ES and PS. These differences may be attributed to the historical use of fertilization associated with grazing management. Additionally, flooding may have influenced soil pH and the availability of P and K, suggesting that the restored sites (ES and PS) exhibited greater resilience to these environmental conditions.

The contribution of woody species to soil fertility can be estimated by the improvement in SOM and SOC (Tab. 2). The soil C metabolism variables, as expressed by SOC, humic acid, and fulvic acid, showed the following trend: PS > ES > DS (Tab. 2). ES showed an increase in soil C and HSs (Tab. 2) at 6 years after planting, as also observed by Podwika et al. (2018) following afforestation. Given that SOM and HS are widely considered indicators of soil health and fertility (Piccolo & Drosos 2025), the quantitative increase in these parameters in ES at 6 years after planting highlights improved soil quality at this site. Although there was a significant increase in both humic and fulvic acids, fulvic acid levels were higher than those of humic acid (Tab. 2). HSs are the final product of the decomposition process of plant residues rich in lignin (Stevenson 1994). Humic acids and humin have higher molecular weights and constitute a more condensed fraction of HSs, whereas fulvic acids have more oxidized substances of lower molecular weights (Hertkorn et al. 2002). Therefore, the planting of native woody species in ES contributed to litter inputs, resulting in high-quality SOM in this still young, 6-year-old forest (Card & Quideau 2010).

One of the primary ecosystem services

Tab. 2 - Comparison among the three sites (PS: Preserved site; ES: Experimental site; DS: Disturbed site) for nitrogen and carbon parameters. Pairwise comparisons between sites were based on one-way ANOVA (a) or Kruskal-Wallis (b) tests, followed by Tukey or Nemenyi *post hoc* adjustments, respectively (significance at $p \leq 0.05$).

Parameters	DS (mean ± SD)	ES (mean ± SD)	PS (mean ± SD)	DS × PS (p-value)	DS × ES (p-value)	PS x ES (p-value)
C - Organic (g kg^{-1})(a)	13.70 ± 0.9	18.92 ± 1.07	23.47 ± 2.1	<0.0001	<0.0001	0.0002
C - Humic acid (g/kg^{-1})(a)	0.98 ± 0.2	1.70 ± 0.2	2.37 ± 0.18	<0.0001	0.0002	0.0003
C - Fulvic acid (g/kg^{-1})(a)	2.21 ± 0.3	3.22 ± 0.3	4.08 ± 0.23	<0.0001	<0.0001	0.0006
C Mic. biomass (mg kg^{-1})(a)	264.83 ± 17.0	544.67 ± 43.9	870.98 ± 37.3	<0.0001	<0.0001	<0.0001
Total N (g kg^{-1})(b)	0.98 ± 0.07	1.23 ± 0.09	2.03 ± 0.1	<0.0001	0.0003	<0.0001
$N\text{-}NH_4^+$ (g kg^{-1})(a)	54.85 ± 2.0	55.55 ± 21.8	33.60 ± 7.5	0.0069	0.99	0.0053
$N\text{-}NO_3^-$ (g kg^{-1})(b)	96.09 ± 6.2	129.89 ± 38.4	209.58 ± 32.9	<0.0001	0.057	<0.0001

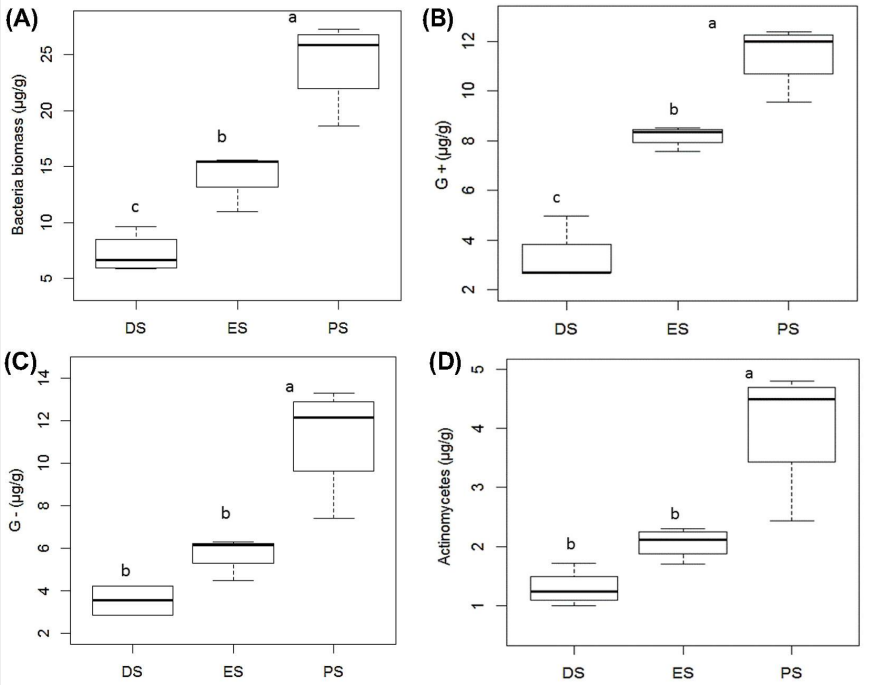

Fig. 2 - Comparisons of soil phospholipid fatty acid (PLFA) bacterial biomarkers (PLFA Bacteria biomass[1], PLFA G+[1], PLFA G-[2], PLFA Actynomycetes[2]) among sites. Analysis of variance (ANOVA[1] or Kruskal-Wallis[2]) was applied to compare mean differences among sites (significance at $p \leq 5\%$). (1) Tukey test; (2) Nemenyi test.

provided by HSs is their promotion of soil aggregation, consequently affecting soil stabilization and permeability (Bronick & Lal 2005, Piccolo & Drosos 2025), particularly in riparian sites (Kimura et al. 2017, Santos & Scotti 2018, Gomes et al. 2024, Barros et al. 2024). Thus, the increase in soil HSs in restored sites enhances the recovery of flood-regulating ecosystem services, such as soil stabilization and permeability (Kimura et al. 2017, Santos & Scotti 2018, Barros et al. 2024), contributing to the soil's physical structure (Piccolo & Drosos 2025) and a higher tolerance to the flooding impact.

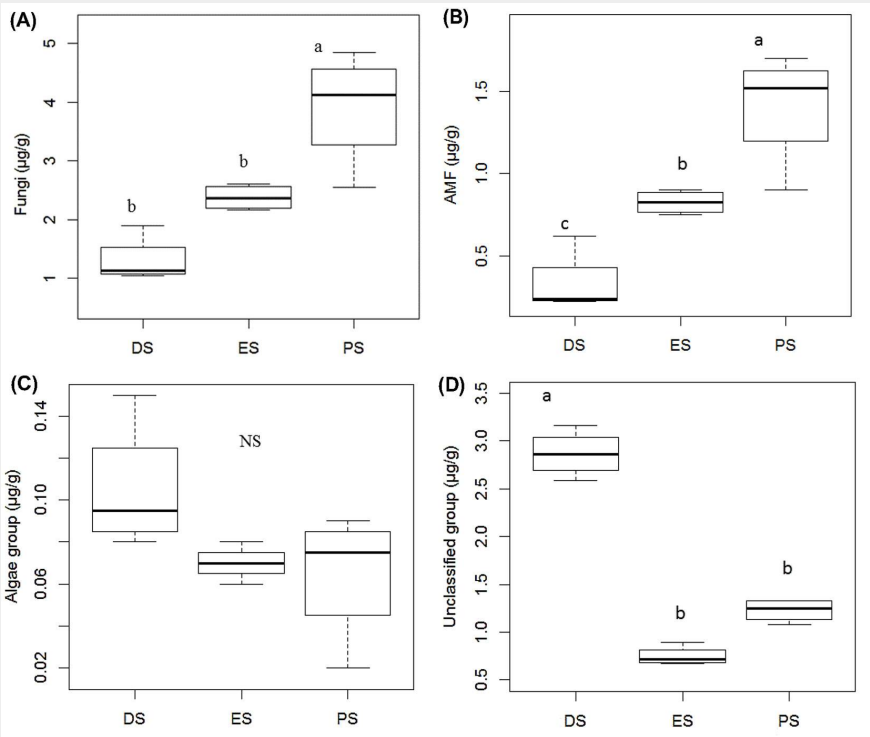

Fig. 3 - Comparisons of soil phospholipid fatty acid (PLFA) microbial biomarkers (PLFA Fungi[1], PLFA AMF[1], PLFA Algae[1], PLFA unclassified[2]) among sites. Analysis of variance (ANOVA[1] or Kruskal-Wallis[2]) applied to compare mean differences among sites (significance at $p \leq 5\%$). (1) Tukey test; (2) Nemenyi test.

Soil nitrogen metabolism

Flooding cycles typically affect N metabolism in the soil, causing accumulation of soil NH_4^+, stimulating NH_3 volatilization, and increasing denitrification (White & Reddy 2009). The distribution of total soil N at the study sites followed the same pattern as SOC (PS > ES > DS – Tab. 2), as confirmed by Podwika et al. (2018) following afforestation. However, the N ammonium content was higher at DS and ES compared to PS (PS < DS = ES – Tab. 2), and the nitrate content was higher at PS than at the other sites (PS > ES = DS).

Ammonium availability in the soil is favored in acidic soils and under anaerobic or low-redox conditions (Ismunadji & Dijkshoorn 1971). Thus, due to the elevated redox potential found under aerobiosis, NH_4^+-N is oxidized to NO_3-N. Flooding commonly induces a rapid decline in redox potential and subsequent accumulation of NH_4^+, impairing nitrification (Pinay et al. 1995). Such a phenomenon appears to occur at ES and DS but not PS, suggesting better resilience in the latter (Ou et al. 2019). In addition, ammonium accumulation at DS and ES, located downstream of a pollution point source (Fig. 2A), may also be attributed to water pollution in the Sabará stream (Junqueira & Campos 1998), suggesting land degradation.

Soil microbial communities

Periodic flooding may also change aerobic-anaerobic conditions, thereby altering biochemical reactions (Unger et al. 2009, Rousk et al. 2010, Ou et al. 2019). Such fluctuations in the water table in riparian zones, which drive cycles of anaerobic and aerobic conditions, exert selective pressure on soil microbial communities (Card & Quideau 2010), thereby altering C availability (Reddy & DeLaune 2008, Ou et al. 2019). The total soil microbial biomass followed the same pattern as SOC (PS > ES > DS – Tab. 2). Such an increase in total microbial biomass at the site under restoration may be attributed to the increase in SOM and SOC, which are known as reliable soil quality indicators at restored sites (Correa et al. 2015, Medeiros et al. 2023), particularly in riparian zones (Card & Quideau 2010, Kimura et al. 2017, Santos & Scotti 2018, Gomes et al. 2024). However, the differences in total microbial biomass between ES and PS can be attributed to the early stage of the forest restoration process (Card & Quideau 2010) and the effects of periodic flooding (Unger et al. 2009, Randle-Boggis et al. 2018).

Changes in soil microbial communities have been assessed by analyzing the ester-linked PLFA composition, which enables the detection of shifts in specific microbial

communities under environmental stress (Frostegard et al. 1993, Frostegard & Baath 1996). The analysis of the PLFA profile of total bacterial biomass showed a pattern similar to that of total microbial biomass and total C, with PS > ES > DS (Fig. 2A), as predicted by Frostegard & Baath (1996). Within the bacterial population, the Gram-positive bacteria (Fig. 2B) showed the same distribution pattern (PS > ES > DS), but for the Gram-negative bacteria (Fig. 2C), a larger population was observed at PS than at ES and DS (PS > ES = DS). The greater abundance of Gram-negative bacteria in PS was associated with soil C accumulation at older sites (Card & Quideau 2010). The inhibition of this bacterial category in ES and DS may suggest selective pressure caused by flooding in this group, which is known to be more associated with aeration (Ponder Jr & Tadros 2002). However, Gram-positive bacteria can be favored under anaerobic conditions, as putative indicators of the impact of flooding (Bossio & Scow 1998), and the balanced Gram-negative and Gram-positive populations at PS may suggest better resilience of this site to flooding.

Actinomycetes (Fig. 2D) and the total fungi (Fig. 3A) followed the same pattern as Gram-negative bacteria (PS > ES = DS), which may reflect the greater sensitivity of these microbial communities to anaerobic flooding conditions (Bossio & Scow 1998, Unger et al. 2009). Therefore, PLFAs of actinomycetal and fungal origins may be considered indicators of drier sites (Card & Quideau 2010). However, the arbuscular mycorrhizal fungi (AMF – Fig. 3B) showed a pattern similar to that of the soil C profile (PS > ES > DS), likely because AMF play a functional role in soil aggregation and stabilization at restored riparian sites (Rillig et al. 2010, Kimura & Scotti 2016, Gomes et al. 2024). In contrast to the PLFA algal group, which did not differ among sites (Fig. 3C), a large population of unclassified microbial community (Fig. 3D) was observed at DS compared with PS and ES (DS > PS > ES), possibly serving as a potential microbial indicator of land degradation in riparian areas.

Functional microbial groups

The abundance of the aerobic PLFA functional group (Fig. 4A) followed the pattern observed for SOM (PS > ES > DS), and the anaerobic microbial population (Fig. 4B) was larger at PS than at ES and DS (PS > ES = DS). This suggests that the anaerobic group was not fully recovered in ES. The dominant anaerobic PLFA signature across all study sites was C16:0, which is characteristic of anaerobic bacteria (Verbaendert et al. 2011). Fu et al. (2015) considered PLFA C16:0 and C:150i signatures as biomarkers of anaerobic denitrifying microbial populations. Similarly, Hoefman et al. (2014) found an abundant composition of both biomarkers in *Methylomonas lenta* isolated from a denitrification tank. Our results (Fig. 4C) showed that these microbial PLFA biomarkers (C:150i and C16:0) increased at PS and ES, but not at DS (PS > ES > DS). Although the entire anaerobic group had not recovered at ES compared to DS, the specific anaerobic denitrifying populations were enhanced in ES, suggesting that this physiological activity might be improved at the site under restoration, as a putative indicator of flooding.

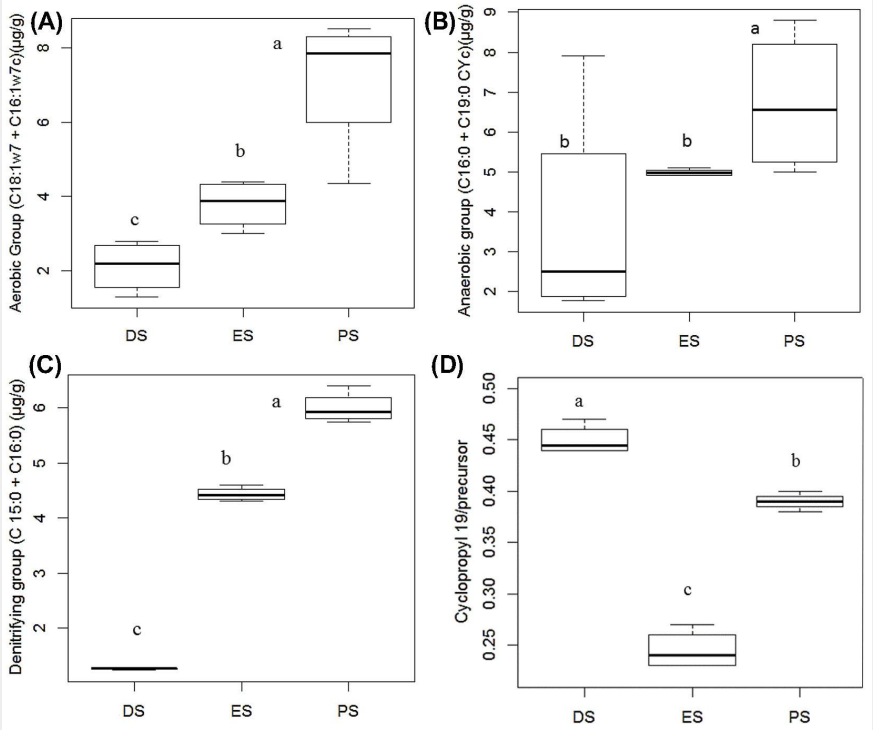

Fig. 4 - Comparisons of soil phospholipid fatty acid (PLFA) microbial functional groups (PLFA aerobic[1], PLFA anaerobic[1], PLFA denitrifying[1], PLFA stress ratio[2]) among sites. Analysis of variance (ANOVA[1] or Kruskal-Wallis[2]) was applied to compare mean differences among sites (significance at $p \leq 5\%$). (1) Tukey test; (2) Nemenyi test.

In addition, the Cyclopropyl 19/precursor stress ratio (Fig. 4D), as expressed by the PLFA cy19:00/ C18:1w7c signatures (Bossio & Scow 1998), differed among sites. The former PLFA marker corresponds to the anaerobic microbial population and the latter to the aerobic population (Unger et al. 2013). We observed the following pattern for this stress ratio (Cyclopropyl 19/precursor): DS > PS > ES. This may indicate strong

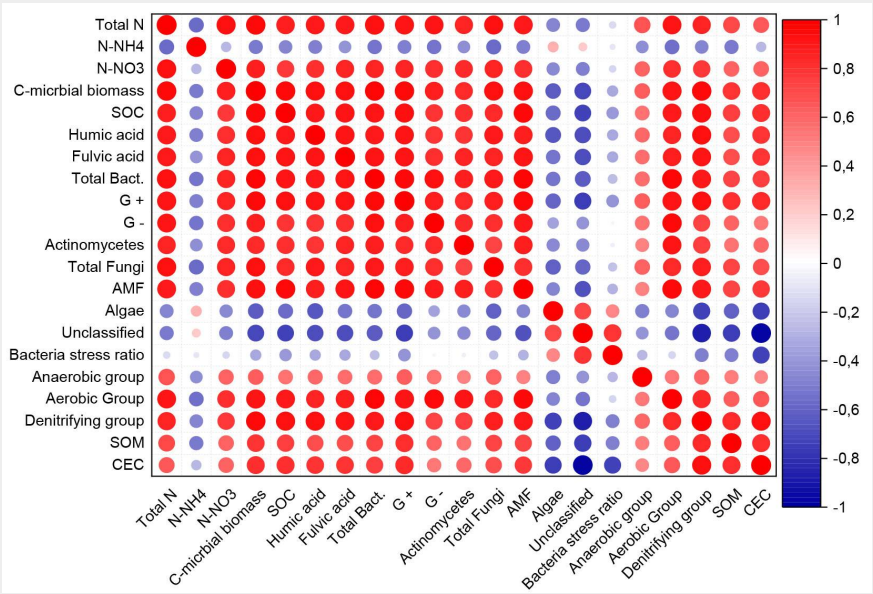

Fig. 5 - Pearson's correlation analysis of soil chemical and microbial parameters from the studied sites. (G+): Gram-positive bacteria; (G-): Gram-negative bacteria.

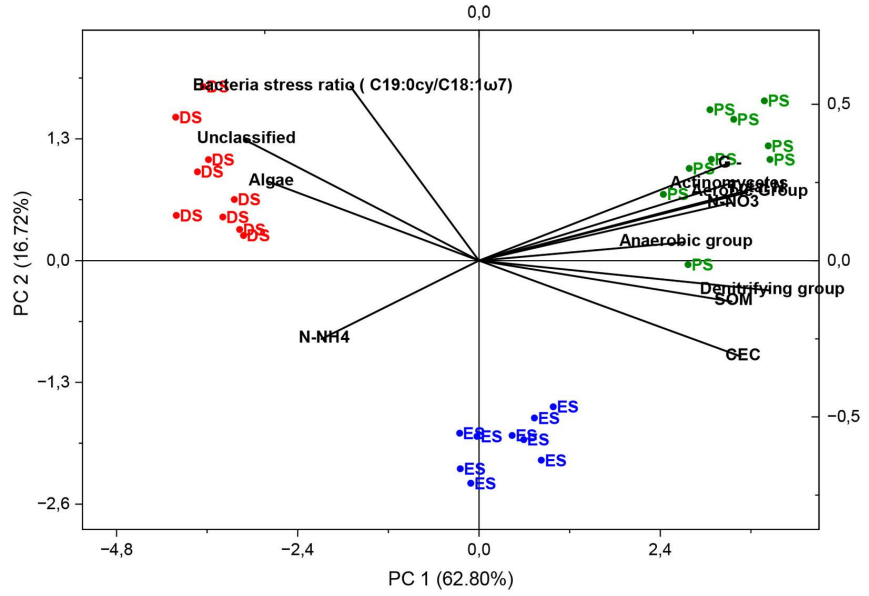

Fig. 6 - Clustering of the studied sites (PS: preserved site: ES: experimental site; DS: disturbed site) by principal component analysis (PCA) based on the most significant variables: N-NH_4^+, N-NO_3^-, cation exchange capacity (CEC), soil organic matter (SOM), Gram-negative bacteria (G-), actinomycetes, algae, unclassified PLFA group, aerobic group, anaerobic group, denitrifying group, cyclopropyl/precursor (stress ratio).

anaerobic stress at DS (Fig. 4D). In addition, the abundance of cyclopropyl fatty acids has been associated with anaerobic stress (Bossio & Scow 1998) and with different environmental stressors, such as soil pH (Moon et al. 2016). The reason for such low specificity is that cyclopropyl fatty acids (cy17:0 and cy19:0) are produced by the trans-methylation of cis-monounsaturated fatty acids when cells enter a stationary phase, thereby altering cell membrane lipid composition (Moon et al. 2016). Similarly, Bossio & Scow (1998) observed an increase in this ratio under flooding. Therefore, the increase of this bacterial stress index at DS may result from several simultaneous disturbances, such as anaerobiosis, high pH promoted by the flooding waters, and low soil fertility. In contrast, the lowest ratios at PS and ES suggest greater resilience of these sites to environmental impacts.

Correlation analysis

Pearson correlation analysis (Fig. 5) showed that all soil chemical parameters were correlated with the bacterial and fungal communities. However, the soil NH_4^+ content was not correlated with any soil parameter. Similarly, the algal and unclassified microbial groups were not correlated with the measured variables. These results support the conclusion that soil NH_4^+, algae, and the unclassified groups likely originated exogenously from polluted water.

Principal component analysis (PCA)

PCA (Fig. 6) showed that the most significant variables explained 79.52% of the total variance, with principal component (PC) 1 accounting for 62.8% and PC2 for 16.72%. In PC1, the variables most contributing were N-NO_3^-, Gram-negative bacteria, aerobic, anaerobic, actinomycetes, and denitrifying groups, CEC, and SOM. In contrast, the PLFA unclassified group, the bacterial stress ratio, N-NH_4^+, and the algae group were more strongly associated with PC2.

Site distribution in the PCA biplot (Fig. 6) showed that samples from DS (red) were separated by the PLFA unclassified group and the cyclopropyl 19/precursor ratio, highlighting the effects of anaerobic flooding at this site. PS (green) and ES (blue) were separated from DS along PC2 axis, primarily driven by N-NH_4^+. Conversely, ES (blue) and PS (green) samples were separated along PC1 axis. These results indicate that N-NH_4^+ accumulation at ES and DS is the primary environmental factor hindering soil recovery at ES.

Conclusion

Based on soil C and N cycling indicators and soil microbial communities, the 6-year-old forest planted with native species progressed toward the soil conditions of a preserved riparian site. The restoration procedure improved soil fertility, particularly with respect to SOM, SOC, CEC, humic and fulvic acids, and bacterial and fungal populations. Although SOM, CEC, NO_3^-, and certain functional microbial populations, such as denitrifying, anaerobic/aerobic, actinomycetes, and Gram-negative groups, were suitable indicators of riparian site restoration, soil NH_4^+ accumulation was the primary environmental indicator hindering riparian forest recovery. Overall, our study provides suitable indicators for assessing riparian forest restoration under flooding in tropical environments.

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Supplementary Material

Tab. S1 - PLFA profiles and Fatty acids used to determine microbial community composition according to literature.

Fig. S1 - Experimental design at site ES for mixed sample collection.

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