Applied Soil Ecology 196 (2024) 105316

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Functional genes related to N and P cycling in degraded and restored areas from Brazilian drylands

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ARTICLE INFO

Keywords:
Soil functions
Microbial ecology
N and P cycles
Drylands

ABSTRACT

There has been widespread application of grazing exclusion in soil conservation, particularly in semiarid regions. However, it is unclear how grazing exclusion reduces the negative effects of overgrazing on functional genes related to essential nutrient cycles, such as N and P, in the Caatinga biome. This study evaluated the impact of long-term overgrazing and grazing exclusion on the soil microbial functional pools related to N and P cycling compared to native Caatinga soil. There were significant variations in the abundance of functional genes across the three areas ($p < 0.05$). In the dry season, both native and restored areas showed notably higher copy numbers per gram of dry soil of *nif*H and *nir*K genes (ranging from 4.90E+05 to 6.86E+06) compared to the degraded area (ranging from 2.88E+05 to 1.97E+06). Copy numbers of archaeal *amo*A, *nir*S, and *nos*Z genes exhibited no significant variation among areas, except for bacterial *amo*A, which was higher in the native area (~ 5.43E+05). Similar results were observed in the rainy season; however, *nir*S gene abundance was higher in the degraded area (~1.38E+08), and *nos*Z showed higher abundance in the native area (~3.37E+05). The high abundance of *nos*Z and *nir*S genes, coupled with the concurrent lower levels of *amo*A and *nif*H genes associated with nitrification and biological nitrogen fixation in areas under desertification, indicates an augmented denitrification activity. This suggests an escalated degradation of organic nitrogen, potentially leading to reduced availability of N inputs for soil organisms. Consequently, It may exacerbate N losses within the soil. Overall, these findings underscore a potential imbalance in the N cycle, particularly during the dry season. The results also substantiate that restoration improved the soil properties highlighting the potential of grazing exclusion as a restoration strategy for degraded soils in the Caatinga biome.

1. Introduction

As the largest seasonally dry tropical forest found in South America, the Caatinga biome takes up 70 % (0.85/1.2 million km^2) of the Brazilian semiarid (Souza et al., 2021). The Caatinga is considered an important hotspot of biodiversity owing to the presence of endemic plants and animal species (Leal et al., 2005; Pereira et al., 2021; Silva et al., 2022). This biome presents soils with high vulnerability to desertification due to their environmental conditions, such as geology and climate, limiting the suitability for different land uses. According to CGEE (2016), approximately 70,000 km^2 of the Brazilian semiarid areas are undergoing a process of desertification, leading to the loss of soil biodiversity and unbalanced ecosystem functions.

Overgrazing is an important and unsuitable practice that has accelerated the desertification in the Caatinga biome, and it has decreased the diversity and changed the structure of soil microbial communities

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https://doi.org/10.1016/j.apsoil.2024.105316
Received 22 June 2023; Received in revised form 26 January 2024; Accepted 29 January 2024
Available online 1 February 2024
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(Pereira et al., 2021). Grazing exclusion has been shown to be effective in restoring soil properties, mainly microbial status and diversity, in the long term (Oliveira Filho et al., 2019; Pereira et al., 2021). Worldwide, grazing exclusion has been reported to be an effective practice method in restoring soil properties (Xun et al., 2018; Liu et al., 2020; Byrnes et al., 2018).

Therefore, studies have shown that overgrazing has negatively affected the soil microbial communities while grazing exclusion could reduce these negative effects and restore microbial properties (Pereira et al., 2021). This restoration in soil microbial communities is essential, given that microorganisms perform several ecosystem functions related to the cycling of nutrients, such as N and P, essential to plant productivity (Naylor et al., 2022). For example, N is a structural component of amino acids, proteins, and enzymes (Trovato et al., 2021), essential for plant growth. Regarding P in soils, this element is critical in major biological systems associated with energy storage, cell replication, and protein synthesis (Wu et al., 2019). Although important to soil and plants, both nutrients' dynamics and availability depend on soil organic matter (SOM) and microbial communities. Thus, these features are concerning since the process of desertification has depleted the soil organic matter content (Oliveira Filho et al., 2019; Silva et al., 2022).

The transformation of organic nutrients from SOM into bioavailable forms to plants involves several microbial genes and their expressed enzymes (Meng et al., 2022). For example, the *pho*D gene is related to the mineralization of organic P, being a sensitive biomarker to investigate the biological function of the alkaline phosphatase (Zheng et al., 2021; Lang et al., 2021). Regarding N cycling, different processes are carried out by the microbial community and modulated by several functional genes that can be used as markers for the presence of certain steps of the process, such as biological nitrogen fixation (*nif*H), ammonium oxidation (*amo*A) for archaea (AOA) and bacteria (AOB), nitrate reduction (*nir*S and *nir*K), and nitrous oxide reduction (*nos*Z) (Wallenstein and Vilgalys, 2005). Recent studies conducted in other parts of the world demonstrated the impact of overgrazing on soil microbial communities and functional gene abundance. For instance, a significant decrease in the abundance of key functional genes involved in nitrogen cycling in response to overgrazing was detected in Mongolia soils (Wang et al., 2021a). Similarly, studies in Guyuan City, China, and Mongolia, reported an increase in nitrogen functional genes under long-term grazing exclusion (Song et al., 2019; Wang et al., 2021b).

Although the above microbial functional genes have been well-studied in several soils and environmental conditions (Philippot et al., 2013; Ding et al., 2023; Chen et al., 2021; Li et al., 2022), studies assessing the effect of overgrazing and grazing-exclusion on the microbial functional genes in soils from Caatinga the tropical dry forest have yet to be reported. Here, we assessed the dynamics of *amo*A, *nif*H, *nir*K, *nir*S, *nos*Z, and *pho*D genes in soils under desertification (overgrazing) and restoration (grazing-exclusion) in the Brazilian semiarid region. We hypothesized that the desertification process reduces the abundance of genes related to N and P cycles while grazing exclusion could recover them. To address this hypothesis, we analyzed functional genes using quantitative PCR with DNA extracted from soils collected in areas under desertification (overgrazing), restoration (twenty years of grazing exclusion), and native forests in the Brazilian drylands region.

2. Material and methods

2.1. Study site

The study was conducted in Irauçuba, Ceará, Brazil (3°44′ 46"S e 39°47'00"W) (Fig. 1). The yearly precipitation is approximately 454 mm (Funceme, 2022), mainly concentrated between January and May (Fig. 2). The climate in this region is hot semiarid (BSh) according to Köppen's classification system, with the mean temperature ranging from 26 °C to 28 °C (Alvares et al., 2013). The soils were classified as Planosols, according to WRB/FAO (Oliveira Filho et al., 2019).

The use of natural pastures is a frequent practice by farmers in this region. In 2000, nine grazing-exclusion systems were implemented in

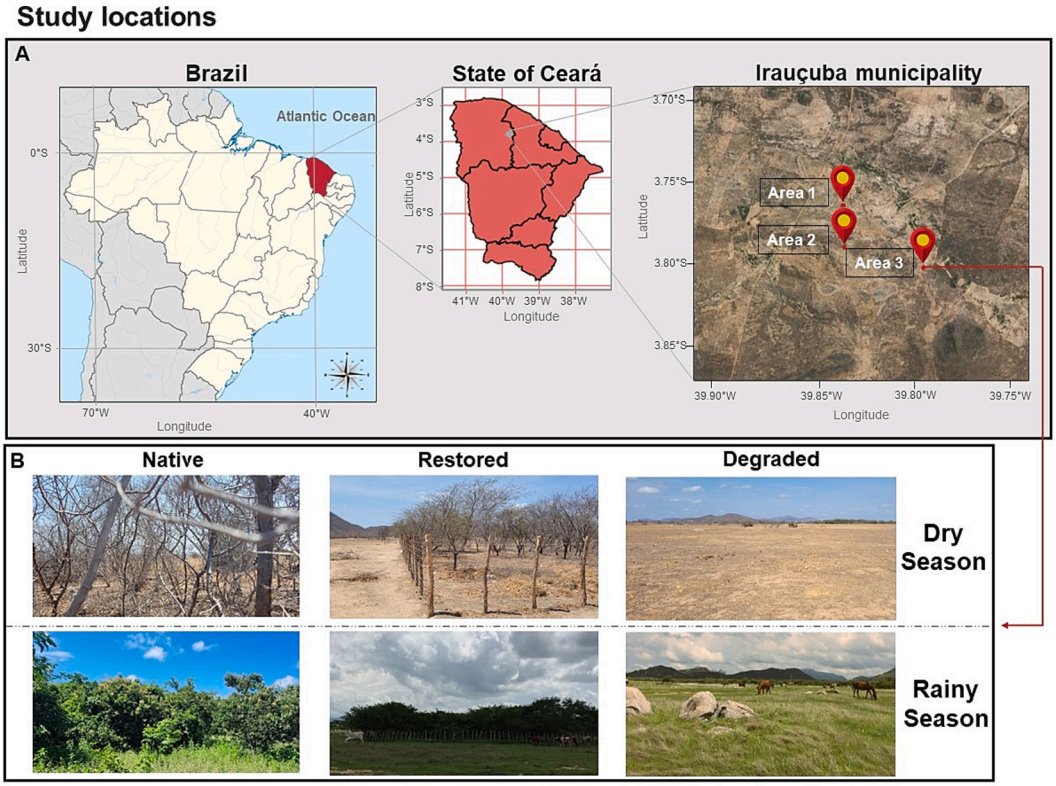

Fig. 1. A - Areas 1, 2, and 3 of soil sampling in Irauçuba Municipality, Ceará State, Brazil. B - Caatinga native vegetation, degraded areas due to overgrazing and restored areas due to grazing exclusion during the dry and rainy seasons of Area 3.

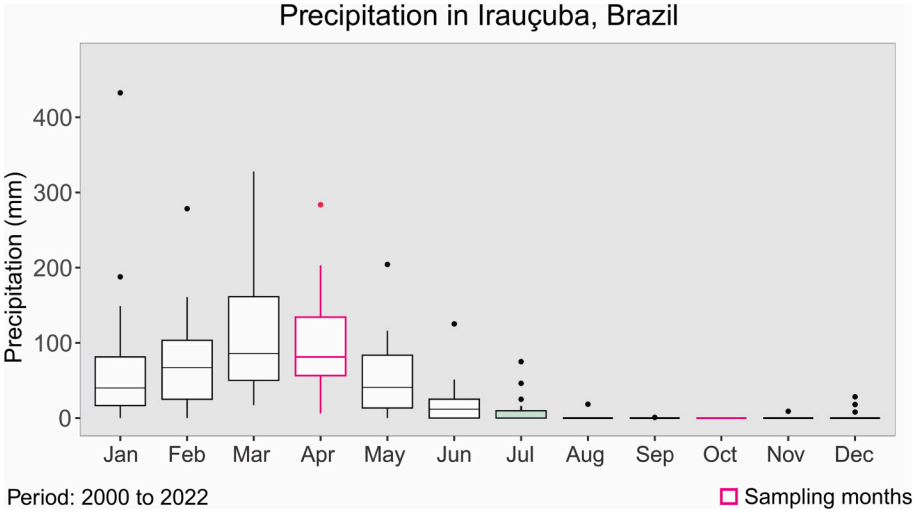

Fig. 2. Monthly averages of precipitation for the last 22 years in Irauçuba, Brazil. Sampling periods are highlighted in pink (i.e., April and October). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

50 m × 50 m (2,500 m^2), separated by fences to avoid animal grazing (Fig. 3). To avoid edge effects, we sampled in an area of 40 m × 40 m.

We collected 27 soil samples in the dry season (Oct/2021) and 27 in the rainy season (Apr/2022. Soil samples were collected at a depth of 0–10 cm depth in three different scenarios, as follows: native Caatinga vegetation (Native), 20 years of grazing exclusion by fencing (Restored) and areas of advanced desertification by overgrazing (Degraded). These scenarios were repeated in three large areas, separated by about 2 km. At each sampling point, we collected nine sub-samples, which were homogenized to create a composite sample. Thus, we analyzed 54 samples (3 areas×, 3 scenarios×, 3 composite replicates×, and 2 seasons). The experimental design was detailed extensively in Pereira et al. (2021) and Silva et al. (2022).

2.2. Soil chemical and physical characterization

The extraction methods for the nitrogen forms (total N, NH_4^+, NO_3^-) were extracted by the steam distillation method proposed by Kjeldahl (Nelson and Sommers, 1982; Vezzani et al., 2001; Freitas et al., 2013). Available phosphorus (P) was extracted through the ion exchange resin following van Raij et al. (2001). The pH was measured in a $CaCl_2$ solution (0.01 $molL^{-1}$). Total organic carbon (TOC) was extracted by organic carbon oxidation in organic form with potassium dichromate ($K_2Cr_2O_7$) and determination by colorimetry. The NH_4^+ and NO_3^- fractions were determined following the aerobic incubation method proposed by Hart et al. (1994). Soil (Aluminum [Al^{3+}], calcium [Ca^{2+}], and magnesium [Mg^{2+}]) were extracted using KCl solution (1 mol L^{-1}), while sodium (Na^+) was extracted by $Mehlich^{-1}$ solution (EMBRAPA, 2009). Electrical conductivity (EC) was measured using an electrical conductivity-meter. Cation exchange capacity (CEC) was determined

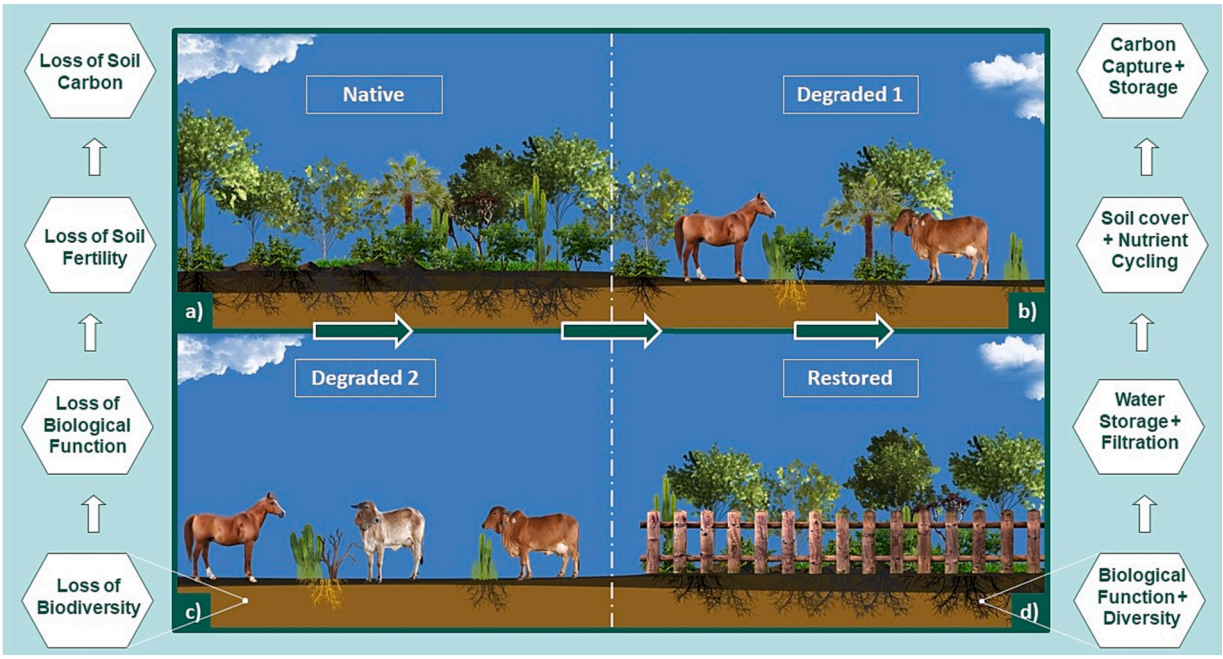

Fig. 3. Representation of native Caatinga vegetation (A - Native), soil degradation through overgrazing (B and C - Degraded) and 20 years of grazing exclusion by fencing (D – Restored).

following the method of van Raij et al. (2001), described for tropical soils. A pipette method was used to measure the clay fraction (Gee and Bauder, 1986), while sieving was used to measure the sand fraction, and the silt fraction was determined by subtracting the sand and clay fractions from the total mass. A gravimetric method with oven drying was used to determine soil moisture, in which wet soil samples were weighed and then dried in an oven at 105 °C, until they reached a constant mass. The samples were weighed again in the following step, and the mass difference represented that of the water. The results of soil physical and chemical characteristics can be found in Table 1.

2.3. Alkaline phosphatase activity and microbial biomass carbon

The alkaline phosphatase (Alk, EC 3.1.3.1) activity was determined following the procedures described in Weaver et al. (1994). Assays were conducted in triplicate by incubating 1 g of fresh soil with 1 mL of the ρ-nitrophenol phosphate (0.05 M). Samples were incubated at 37 °C for 1 h and the reaction was stopped by addition of $CaCl_2$ (0.5 M) and NaOH (0.5 M). The extracts were filtered in Whatman's paper and the resulting colour intensities were measured at 410 nm in a spectrophotometer (EZ Read 400, Biochrom). Values are expressed as µmol of PNP produced per g of soil (dry weight equivalent) during 1 h. Enzyme activities were calculated based on a standard curve developed with a ρ-nitrophenol solution.

Microbial biomass carbon (MBC) was determined by the chloroform fumigation-extraction method using a correction factor of 0.54. A total of 10 g of soil from each sample was fumigated with chloroform (99.8 %) and digested with potassium dichromate for 30 min at 100 °C. Extracts were determined by titration using iron sulfate and ammoniacal ferrous sulfate (Brookes et al., 1985; Vance et al., 1987).

2.4. Quantitative PCR

Genomic soil DNA was extracted from 0.25 g of soil (fresh weight) using the DNeasy PowerSoil Pro Kit (QIAGEN Inc., USA), following the manufacturer's instructions. Quantitative PCR (qPCR) was performed to enumerate copies of functional genes involved in soil phosphorus and nitrogen cycling (*pho*D, *amo*A (AOA), *amo*A (AOB), *nif*H, *nir*K, *nir*S and *nos*Z (Table S1). All reactions were run in duplicate, 20 µL, each reaction comprising 10 µL of 2× Sso advanced Universal SYBER Green Supermix (Bio-Rad, Inc., USA), 1 µL of a forward/reverse primer mixture (10 pmol μL^{-1} each) of each primer (Table S1), 2 µL of genomic soil DNA (5 ng μL^{-1}), and 6 µL of nuclease-free sterile water. The protocol for phoD was as follows: 3 min at 95 °C, followed by 45 cycles of 10 s melting at 95 °C and 30 s anneal and elongation at 58 °C. The protocol for all the nitrogen genes was as follows: 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 30 s with an additional 15 s at 80 °C. PCR-grade water (no template) was used as a negative control. To verify the specificity of the qPCR, a melting curve procedure followed by a gel-based analysis of amplification products were performed for each functional gene. Standard curves (10^1 to 10^9 copies) were generated with plasmid DNA containing partial fragments of each gene; *nif*H, *amo*A (AOA), *amo*A (AOB), *nir*K, *nir*S, and *nos*Z. Reactions were performed on a 96-well PCR plate using the CFX ConnectTM Real-Time System (BIO-RAD).

2.5. Statistical analyses

Data were tested for residuals normality and homoscedasticity, using the Shapiro–Wilk's and the Breusch–Pagan tests, respectively. Once data were observed to meet the Nested ANOVA criteria, post-hoc tests were utilized as Tukey's test (5 %) to compare the mean groups. A redundancy analysis, as (RDA) triplots were used to compare soil physico-chemical and biological parameters to functional genes (Yamaura et al., 2019). The data analyses were performed in the RStudio (version 3.6.3) and Canoco® software (v. 4.5) for Windows.

3. Results

3.1. Degradation and restoration on soil properties

Soil pH ($CaCl_2$) was generally acid (ranging 4 to 5), with degraded areas more acidic ($p < 0.05$) than restored and native areas in dry and rainy seasons. Soil organic carbon (SOC), Ca^{2+}, and EC were higher in both native and restored areas, independently of seasons (dry and rainy). During the dry season, the restored area showed higher available P contents, when compared with native and degraded. However, the native showed the highest P content than all other treatments during the rainy season. The highest total N content was found in native and restored, while degraded areas showed the lowest. There was a seasonal difference in restored and degraded treatments, with higher N content in the dry season. There was no difference in NO_3^- contents between areas during the rainy season, and there was no seasonal difference for any of the areas, except to degrade during dry season, that showed the lowest values. Ammonium (NH_4^+) had similar levels in all areas, except for degraded area, with higher contents in the dry season.

The Na^+ content was lower in the native (dry) area, and showed no significant difference between the other areas or seasons. In all areas, moisture was higher during the rainy season, but the degraded area presented the lowest values. Exchangeable sodium percentage (ESP)

Table 1
Soil characterization in a gradient of desertification in the Caatinga biome in dry and rainy seasons.

	Native		Restored		Degraded	
	Dry	Rainy	Dry	Rainy	Dry	Rainy
pH	5.64 ^{Aa}*	5.48 ^{Aa}	4.84 ^{Aa}	4.67 ^{Ba}	4.62 ^{Ba}	4.75 ^{Ba}
P (mg kg^{-1})	7.52 ^{Bb}	17.81 ^{Aa}	16.63 ^{Aa}	7.16 ^{Bb}	6.44 ^{Ba}	6.60 ^{Ba}
Ca^{2+} (mmol$_c$ dm^{-3})	74.67 ^{Aa}	63.81 ^{Aa}	59.22 ^{Aa}	41.68 ^{Aa}	22.16 ^{Ba}	21.41 ^{Ba}
SOC (g kg^{-1})	23.65 ^{Aa}	23.99 ^{Aa}	29.02 ^{Aa}	18.16 ^{Bb}	9.5 ^{Ba}	7.75 ^{Ca}
Total N (mg kg^{-1})	1258.3 ^{Aa}	1093 ^{Aa}	1130.8 ^{Aa}	937.8 ^{Ab}	684.2 ^{Ba}	533.9 ^{Bb}
NH_4^+ (mg kg^{-1})	38.08 ^{Aa}	28 ^{Aa}	34.34 ^{Aa}	35.9 ^{Aa}	34.08 ^{Aa}	20.3 ^{Bb}
NO_3^- (mg kg^{-1})	38.88 ^{Aa}	31.2 ^{Aa}	33.83 ^{Aa}	30.7 ^{Aa}	19.20 ^{Ba}	21.21 ^{Aa}
Na^+ (mmol$_c$ dm^{-3})	1.02 ^{Ba}	2.49 ^{Aa}	2.28 ^{Aa}	1.57 ^{Aa}	2.92 ^{Aa}	3.38 ^{Aa}
Al^{3+} (mmol$_c$ dm^{-3})	0.61 ^{Aa}	2.94 ^{Aa}	0.76 ^{Aa}	0.99 ^{Aa}	2.26 ^{Aa}	2.30 ^{Aa}
EC (mmol$_c$ dm^{-3})	106.99 ^{Aa}	127.04 ^{Aa}	90.53 ^{Aa}	116.43 ^{Aa}	47.08 ^{Bb}	73.14 ^{Bb}
Moisture (%)	1.55 ^{Ab}	7.28 ^{Aa}	1.16 ^{Ab}	6.14 ^{Aa}	0.75 ^{Ab}	4.28 ^{Ba}
ESP (%)	2.11 ^{Ba}	11.78 ^{Ba}	1.44 ^{Ba}	1.44 ^{Ba}	3.89 ^{Ab}	20.78 ^{Aa}
MBC (mg C g^{-1})	6.79 ^{Aa}	7.55 ^{Aa}	7.35 ^{Aa}	8.23 ^{Aa}	4.47 ^{Aa}	5.06 ^{Ba}

*Means followed by the same letter do not differ by Tukey's test (5 %). The capital letters in the line indicate how soil management differs within each season; the lowercase letters indicate how soil management differs within seasons. pH: potential of hydrogen ($CaCl_2$), P: available phosphorus, Ca^{2+}: calcium, SOC: soil organic carbon, N: total nitrogen, NO_3^-: nitrate ion, NH_4^+: ammonium ion, Na^+: sodium, ESP: exchangeable sodium percentage, Al^{3+}: aluminum, EC: electrical conductivity and MBC: microbial biomass carbon.

increased more in degraded areas, independent of seasons. The Al^{3+} contents showed no effect of sol management treatments or time (seasons). Moisture was higher during the rainy season in all treatments, but the degraded areas presented the lowest values (Table 1).

Microbial biomass of C (MBC) was higher in native and restored areas, mainly in the rainy season. Degraded sites presented the lowest MBC content, mainly during rainy season (Table 1). Native and restored (grazing exclusion) presented higher levels of alkaline phosphatase activity, whereas desertified soils (overgrazed) showed lower values of phosphatase activity in both dry and rainy seasons.

3.2. Degradation and restoration on functional gene abundance

The amplification efficiency (E) was calculated according to the eq. $E = [10^{(-1/slope)} - 1] \times 100$, and for all quantified genes, the amplification efficiency of gene copies (g $soil^{-1}$) ranged from 90.1 % to 91.7 %. The coefficient of determination (R^2) for the trend lines of the linear regression models for the evaluated genes ranged from 0.9941 to 0.9990. In the dry season, *amo*A gene abundance targeting nitrifiers spanned from 2.72E+04 to 5.94E+06 copies g dry $soil^{-1}$. Bacterial *amo*A exhibited lower average abundance and experienced a more substantial decrease (by a factor of 7) compared to archaeal *amo*A. The combined abundance of *nir*K, *nir*S, and *nos*Z genes, targeting denitrifiers, ranged from 3.78E+06 to 3.91E+07 copies g dry $soil^{-1}$. The *nif*H gene, targeting biological nitrogen fixers, ranged from 2.32E+05 to 8.99E+05 copies g dry $soil^{-1}$.

During the rainy season, *amo*A gene abundance varied from 1.05E+05 to 1.62E+07 copies g dry $soil^{-1}$. On average, bacterial *amo*A was substantially less abundant (approximately 79 times) than archaeal *amo*A. The collective abundance of *nir*K, *nir*S, and *nos*Z genes ranged from 1.22E+06 to 3.90E+08 copies g dry $soil^{-1}$. The *nif*H gene abundance spanned from 2.13E+04 to 3.88E+06 copies g dry $soil^{-1}$. The *pho*D gene, targeting phosphorus mineralizers, exhibited abundance ranging from 1.44E+05 to 5.84E+06 copies g dry $soil^{-1}$ in the dry season and from 5.31E+04 to 1.24E+07 copies g dry $soil^{-1}$ in the rainy season.

In general, the abundance of functional genes varied significanly across the three areas ($p < 0.05$). During the dry season, the copy number of *pho*D was higher in the native (average 3.02E+06) as compared to both, the degraded (average 2.77E+05) and restored areas

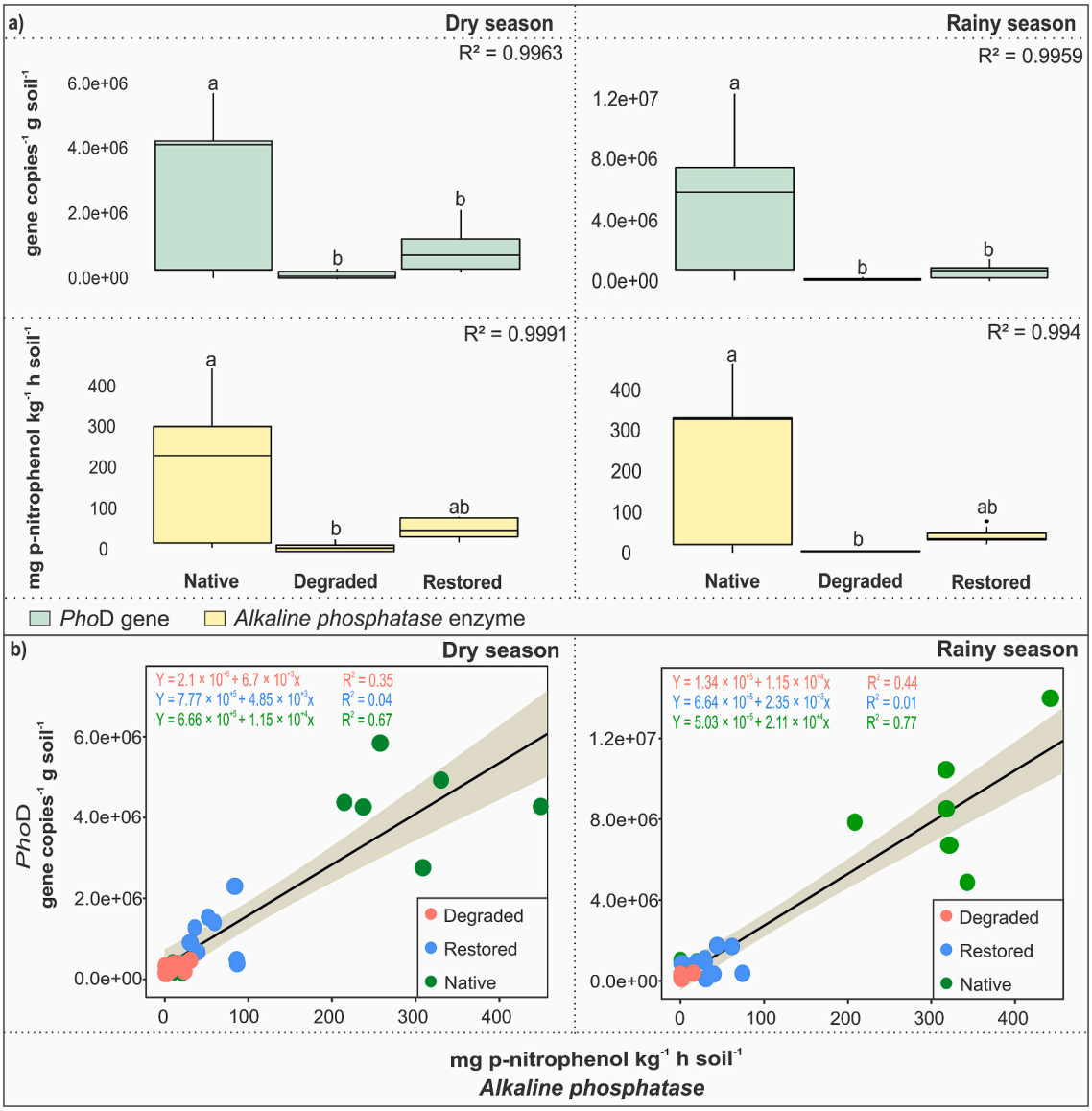

Fig. 4. Differences in *pho*D gene abundance and alkaline phosphatase activity of a desertification gradient in dry and rainy season (A). Seasonality of *pho*D gene abundance and alkaline phosphatase activity (B). Linear regression analyses between *pho*D gene and alkaline phosphatase in dry and rainy season. (C).

(average 1.04E+06) (Fig. 4A). In addition, the results showed a high correlation between the *pho*D copy number and the phosphatase activity (Fig. 4B).

In the dry season, the copy numbers of *nif*H and *nir*K genes were significantly higher in both, the native (averaging 4.90E+05 and 6.86E+06, respectively) and restored areas (averaging 5.50E+05 and 5.52E+06, respectively), in contrast to the degraded area (averaging 2.88E+05 and 1.97E+06, respectively) (Fig. 5). The copy number of archaeal *amo*A, *nir*S, and *nos*Z genes did not vary among different areas (Fig. 5). The exception, bacterial *amo*A, that was higher ($p < 0.05$) in the native area (average 5.43E+05), as compared to the others. In the rainy season, the copy numbers of archaeal *amo*A, bacterial *amo*A, *nir*K, and *nos*Z were higher in the native (averaging 5.14E+06, 7.08E+04, 5.21E+06 and 3.37E+05, respectively), compared to both, degraded and restored areas (averaging 1.84E+05, 4.33E+03, 6.77E+05, 6.63E+04 and 1.09E+06, 5.50E+03, 2.49E+06, 7.95E+04, respectively), while *nif*H did not vary between areas. Interestingly, the gene abundance of *nir*S was higher in the degraded area (average 1.38E+08), as compared to the other areas. The native systems presented higher alkaline phosphatase activity, followed by restored areas. Degraded system presented the lowest alkaline phosphatase activity in both dry and rainy seasons (Fig. 4).

3.3. Degradation and restoration on correlations analysis

Redundancy analysis explained 54.5 and 82.0 % in the axes 1 of the dry and rainy seasons's variation, respectively (Fig. 6). The axes 2 explained 20.1 % e 4.4 % of total variations in the dry and rainy seasons's, respectively (Fig. 6). Both RDA separated the native and degraded areas and showed the restored area between them. In both seasons, the majority of the chemical and biological properties were clustered with the native area, as highlighted by total N, NH_4^+-N, NO_3^--N, phosphatase, *pho*D, *amo*A, *nif*H, and *nir*K. In contrast, *nos*Z and *nir*S clustered with the degraded areas in the dry and rainy seasons, respectively. Importantly, non-beneficial elements such as Al^{3+}, Na^+, H + Al and ESP were positively correlated with degraded areas, mainly in rainy season.

4. Discussion

Nowadays, soil degradation has been recognized as an important threat to soil biodiversity, mainly to microbial communities (Tibbett et al., 2020). Indeed, several previous studies have reported losses of important microbial groups, decreases in soil biodiversity, and changes in the composition of microbial communities (Araujo et al., 2022; Pereira et al., 2021; Silva et al., 2022; Zheng et al., 2023). Due to soil degradation of microbial communities, soil functioning can also be altered. Efforts of soil restoration would be able to ameliorate these negative effects on the microbial communities and the soil functioning. This study assessed genes related to ammonia oxidation, biological nitrogen fixation (BNF), nitrate and nitrous oxide reduction, and phosphate mineralization to address the hypothesis that soil degradation acts on reducing them. In contrast, soil restoration could recover microbial functions within the N and P cycles, partially depending on seasonality. Corroborating with this hypothesis, we observed that the abundance of some functional genes decreased with soil degradation, while presenting a recovery in their abundance, after 20 years of soil restoration, in the dry season, including *nif*H, and *nir*K, which are two important steps for biological nitrogen fixation and removal in the ecosystem.

4.1. Degradation and restoration on soil properties

Despite differences in soil moisture between the dry and rainy seasons, the highest values were recorded in the area under native forest, followed by the restored area. Moisture is a well-known factor that affects microbial activity and C dynamics (Qu et al., 2021). Thus, higher water content in native soil and grazing-exclusion creates a more conducive environment for microbial growth and activity, when compared with degraded sites. The greater proportion of soil surface covered by vegetation in these areas results in the interception of solar rays and a reduction in evaporation rate. The degraded soil presents lowest content of organic C and nutrients, such as N and P, which contributes to decrease of enzymes activity (Silva et al., 2024). Degraded soil showed a reduction of ~31 % and ~43 % in organic C, compared to the restored and native soils (Pereira et al., 2021). Therefore, the lowest activity of the alkaline phosphatase enzyme, found in degraded soil, can be correlated to this decreased availability of P as organic source (Araujo et al., 2022). Additionally, the higher apparent density in degraded soils (Oliveira Filho et al., 2019) reduces the availability of air and water in the soil, constituting yet another factor that potentially suppresses enzymatic activity.

Soil desertification, mainly caused by intensive animals grazing, severely impacts soil cover by plant removal (Oliveira Filho et al., 2019; Pereira et al., 2021). Thus, total N was lower in degraded area as compared to native and restored. Interestingly, we found a similar proportion of NO^{3-} and NH_4^+ between areas. Nitrification, i.e., the conversion of NH_4^+ into NO^{3-} has primarily been studied in near-neutral pH soils. However, recent reports demonstrated nitrification down to pH 3.0 and nitrification rates equal, or even exceed, that found in neutral soils (Li et al., 2018). More importantly, Li et al. (2018) showed that AOA generally make a greater contribution than AOB in acidic soils. Thus, it can partly explain our similar results in these fractions, since we detected acid soils in our experiments and the positive correlation of amoA (AOA) and NO_3^- during dry season. Deep molecular studies of heterotrophic nitrifier genes could contribute to expanding our understanding of the diversity of both AOB and AOA in different pH ranges of desertified ecosystems.

4.2. Degradation and restoration on functional gene abundance

The simultaneous increase in *nif*H and *nir*K can be explained by increasing microbes containing both genes, like *Bradyrhizobium japonicum* (Sánchez et al., 2010). In the rainy season, however, the soil restoration did not ameliorate the negative impact of degradation on any functional gene. The exception was the *nir*S copy number higher in the degraded area. Therefore, our results showed a seasonal effect, probably influenced by soil moisture, on the responses of functional genes to soil degradation and restoration.

Regardless of the season, our results suggest important consequences for particular soil processes, such as ammonium oxidation and BNF, being severely reduced in degraded areas. Regardless of the season and in addition to gene abundance. The levels of total N, NH_4^+-N, NO_3^--N were lower in the degraded area in both seasons. Indeed, ammonium oxidation is considered a limiting step in the nitrification process and is catalyzed by ammonia monooxygenase encoded by the *amo*A gene (Gao et al., 2016). The restoration process showed a small recovery in *nif*H, which controls the BNF process. This is important because BNF is one of the few ways nitrogen enters the soils, especially in forest environments (Sardar et al., 2023). The observed reductions in the copy numbers of the bacterial and archaeal *amo*A and *nif*H promoted by degradation may be a direct consequence of losses related to important microbial groups carrying these particular functions (Pereira et al., 2021), such as ammonia-oxidizing bacteria and diazotrophs. For instance, previous studies report that ammonia-oxidizing bacteria are highly sensitive to soil disturbance, such as high salinity and contamination (Araujo et al., 2022; Guo et al., 2020).

This study is the first to report ammonia-oxidizing micro-organisms' losses in Brazilian degraded dryland soils. Previous studies have shown a decrease in *nif*H copy number and diazotrophs in soils under degradation in an ecosystem in China (Che et al., 2017; Zhang et al., 2022). Our results showed an increased abundance of the *nir*S gene in soils under degradation, and it may indicate losses of N from the soil to the

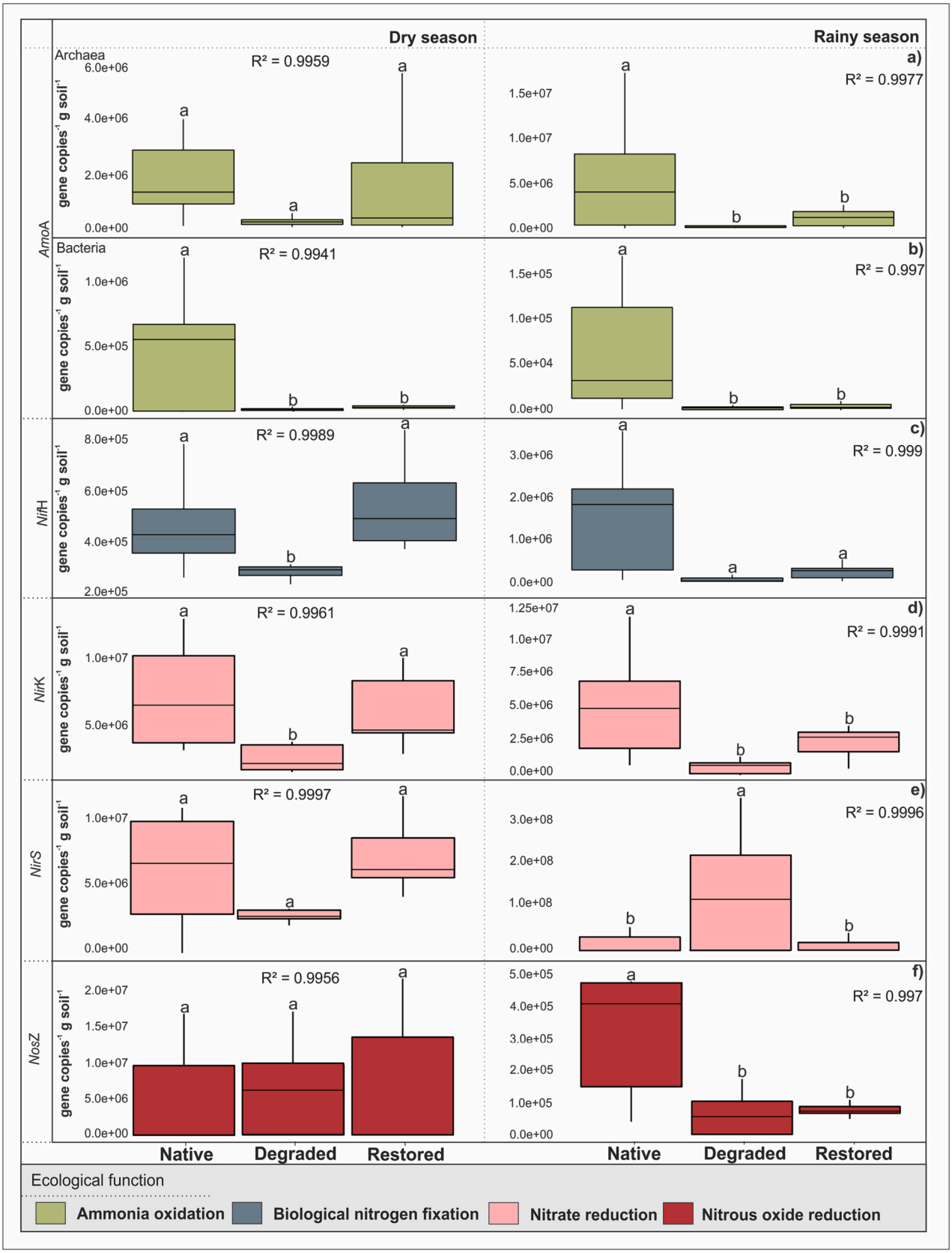

Fig. 5. Differences in nitrogen cycling genes (*amo*A [archaea, bacteria], *nif*H, *nir*K, *nir*S and *nos*Z) abundance in dry and rainy season (A), Seasonality of *amo*A, *nif*H and *nos*Z genes abundance (B).

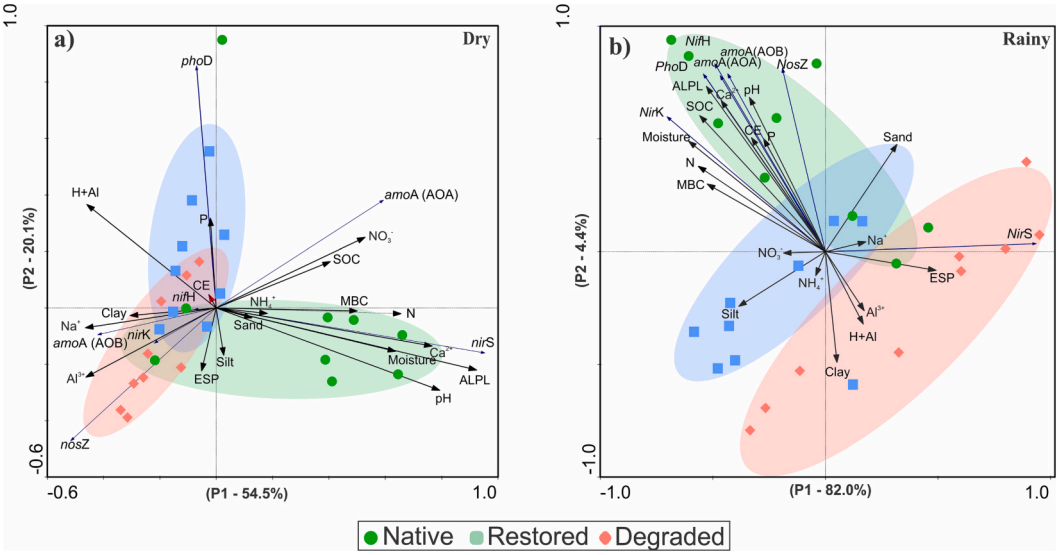

Fig. 6. Redundancy analysis (RDA), based on the correlation of soil physicochemical and biological parameters with functional genes in a gradient of desertification. a) Dry season; b) Rainy season. Data are shown for three different land use types, native, degraded and restored; red vectors for the soil chemical, physical parameters, and alkaline phosphatase activity, blue vectors for microbial genes (nitrogen and phosphorus cycling). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

atmosphere as gases like NO, N_2O and N_2, since the *nir*S gene encodes nitrate reduction (denitrification) (Gineyts and Niboyet, 2023). Denitrification is an important process responsible for the losses of N from the soil, bringing environmental issues to the atmosphere (Ding et al., 2021). Denitrifier genes NirK and NirS are commonly used to characterize the abundance and composition of denitrifier communities in ecosystems, as a key step in denitrification for converting nitrite into nitric oxide (Kou et al., 2021). Based on the high abundance of the nirS and nirK genes and the lowest (p 0.05) NO_3^- levels found in degraded areas, it is inferred that denitrification is intensified in these areas, decreasing the pool of N in the soil and favoring nitrogen losses through gaseous forms.

The notably lower N content in degraded areas demonstrated the potential limitation on nitrogen availability, likely arising from altered soil conditions, reduced vegetation cover, and the effects of overgrazing in microbial communities (Feng et al., 2023; Hayashi, 2022; Pereira et al., 2021; Silva et al., 2022). This constraint is further evidenced by the diminished presence of specific nitrogen-related genes, including *amo*A, *nif*H, *nir*K, *nir*S and *nos*Z, within these areas. It suggests that the reduced N content in these areas may, in part, could be attributed to the diminished capacity of microbial communities to fix and convert N compounds.

Additionally, the discernible seasonal fluctuations in both N content and gene abundance offer insightful perspectives on temporal dynamics. The higher N content in native and restored areas during the dry season likely results from more favorable soil conditions and enhanced vegetation cover, which foster a diverse and active microbial community. Conversely, the degraded areas display lower N content and reduced gene abundance, indicating a potential slowdown in N cycling processes exacerbated by the persistent pressure of overgrazing.

The high abundance of *nos*Z and *nirS* genes, juxtaposed with lower levels of *amo*A and *nif*H genes associated with nitrification and biological nitrogen fixation in areas undergoing desertification in Brazil, indicates that denitrification can be an important biological process. Zhang and Zhou (2022), found a higher abundance of genes related to denitrification in an overgrazed grassland ecosystem in China and attributed it to the decrease in plant species, which consequently increases N_2O emissions. This suggests an escalated degradation of organic N, potentially resulting in diminished availability of N inputs for soil organisms, thereby potentially exacerbating nitrogen losses within the soil.

Furthermore, these results unveil a distinctive dynamic hitherto unreported in soils of the Caatinga biome experiencing desertification in Brazil. The prevalence of *nos*Z and *nir*S genes, primarily implicated in denitrification, points to a biological activity primarily focused on converting nitrate into gaseous nitrogen, potentially leading to substantial nitrogen losses from the ecosystem. Conversely, the reduced presence of *amo*A and *nif*H genes, central to nitrification and biological nitrogen fixation, implies diminished availability of N in the form of ammonia, coupled with a reduced capacity for biological nitrogen fixation within the soil.

In addressing the challenge of limited nitrogen (N) accessibility for soil organisms, which directly impacts the availability of this essential element for plant growth and metabolic processes (Gao et al., 2023), the rehabilitation of degraded areas affected by soil desertification in Brazil becomes a critical focus. Within this context, the nif*H* gene emerges as a pivotal player in the soil ecosystem. Achieving an enhancement in the function of the nif*H* gene requires strategic soil management and a thoughtful approach. This encompasses the introduction of nitrogen-fixing bacteria carrying the nif*H* gene into the soil (Dias et al., 2012). Furthermore, the careful selection of nitrogen-fixing plants, such as legumes, coupled with the incorporation of organic matter through compost, plays a crucial role in establishing a diverse and supportive microbial environment (Koudahe et al., 2022).

Regarding the P cycle, we quantified the copies of *pho*D in our three different soils, which was identified as the most frequent alkaline phosphatase gene in the microbial community (Tan et al., 2013). Our results showed a significant positive relationship between *pho*D abundance and alkaline phosphatase activity, as Fraser et al. (2015) observed in a long-term study under distinct land management techniques. As reported in previous studies, land degradation led to a significant negative effect on the soil bacterial community (Araujo et al., 2022; Pereira et al., 2021), which is important to P turnover, since bacteria are responsible for excreting phosphatases that catalyze the hydrolysis of ester-phosphate bonds (Fraser et al., 2015).

4.3. Degradation and restoration on correlation analysis

Finally, we applied the RDA to identify the correlation between chemical and biological properties to the abundance of the functional

genes (Garizi et al., 2011). This analysis showed that the native area was separated from the degraded area, showing the restored area in the transition. This indicates that the chemical and biological properties found in the restored area were closer to those of the native area, which suggests that the restoration process can improve the soil properties (Farrell et al., 2020). Interestingly, the functional variables, such as phosphatase, phoD, amoA (AOB and AOA), nifH, and nirK, were more associated with the native area and suggest maintaining soil functionality in the native area. In contrast, nosZ and nirS clustered with the degraded area, which may suggest these genes as indicators of environmental stress or degradation.

5. Conclusions

In this study, we investigated the abundance of functional genes related to the nitrogen (N) and phosphorus (P) cycles in native, degraded, and restored soils of the Brazilian Caatinga biome. Our findings suggest that degradation decreases, while restoration increases, the functional genes related to N and P processes, indicating a microbiological limitation in N and P. These results demonstrate that long-term grazing exclusion may be an effective strategy for restoring the soil functionality. However, some additional practices could be suggested, such as the use of biofertilizers, increase of soil organic matter, and replanting of native vegetation. These practices can increase the soil microbial biomass and activity, so restoring the ecological soil functions related to N and P cycling in the Caatinga biome.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2024.105316.

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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.

Data availability

No data was used for the research described in the article.

Acknowledgements

We are deeply thankful to the São Paulo Research Foundation (FAPESP) for the grants 2022/07117-0, 2021/14418-3, and 2016/18944-3 for financial support of this research. We also thank the National Council for Scientific and Technological Development (CNPq) for the grant 402646/2021–5, and their scientific support. Finally, we are grateful to all colleagues who have helped with sampling and laboratory analysis (Jaedson Mota, Eno Taniguchi, Grace Cheng, George Portela, Lucas Gouveia, Izabelly Pacífico e Yasmin Florentino).

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