Soil acidification controls invasive plant species in the restoration of degraded Cerrado grasslands

Running Head: Soil Acidification Controls Invasive in Cerrado

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Abstract

The Cerrado, South America's largest savanna, features acidic, nutrient-poor soils. Its native plant species are well adapted to these conditions. However, abandoned pastures historically undergo changes in chemical properties due to interventions like liming and fertilizer use. This often favours the growth of invasive African grasses, hindering native plant species' growth and impacting restoration efforts. In a Cerrado grassland undergoing restoration study, we used 56 plots across 14 blocks to test whether soil acidification could reestablish soil chemical conditions closer to the native state, controlling invasive species growth in these nutrient-poor soils. Our experiment aimed to assess the impact of reduced soil nutrients by soil acidification on invasive species biomass. We hypothesized that decreased soil pH and nutrient availability would reduce invasive species biomass compared to natives, enhancing diversity. We show that acidification decreased the total aboveground biomass of invasive species by 71%, significantly lowering their dominance over natives. The acid-amendment negatively affected invasive species while sparing natives. Maintaining nutrient-poor soil conditions can help to control invasive grasses in restoration projects, as fertilizer application can favour invasive species establishment. The effectiveness of soil acidification depends on local soil nutrient levels. In areas with high soil cation content, larger quantities of amendment may be required. Restoration strategies in nutrient-poor ecosystems should aim recovering historical soil nutrient levels particularly if invasive grasses are an obstacle, to control invasive growth and support slow-growing natives, aiding ecological restoration of these ecosystems.

Keywords: Invasive species control, Cerrado restoration, soil acidification, nutrient availability, species diversity, fertilizer impacts

Implication for Practice

• Fertilizer application risks Cerrado restoration by favouring invasive plant species over natives. Maintaining low-nutrient soils is crucial for Cerrado restoration and native vegetation persistence.

• Soil acidification shows promise for controlling invasive grasses and facilitating native species restoration in the Cerrado by altering environmental filters that favour natives over invasives.

• The effectiveness of soil acidification depends on site-specific conditions like baseline nutrient levels. Higher treatment concentrations may be needed in some areas based on soil cation levels.

• Ongoing monitoring of community dynamics is important following interventions to track longer-term outcomes over multiple seasons and guide adaptive management.

Introduction

Ecological restoration is crucial for biodiversity conservation and provisioning of ecosystem services. It involves identifying degradation causes, controlling legacy effects, and selecting suitable species for the restoration site (Gann et al. 2019). Restoration success involves recoveringmultiple ecosystem properties such as community structure, diversity, and composition (Gann et al. 2019; Ruiz-Jaen & Mitchell Aide 2005). Nonetheless, restoration effectiveness may be impeded by both biotic and abiotic factors that represent ecological filters (Buisson et al. 2018; Funk 2021; Szitár et al. 2016). While abiotic factors such as nutrient availability can play a crucial role in shaping ecosystem processes and community assembly by filtering species establishment (Baer et al. 2019; Bustamante et al. 2012; Lira-Martins et al. 2022), invasive exotic species (hereafter named invasive species) can represent a strong biotic filter that impedes the establishment of native target species (Norton 2009; Weidlich et al. 2020).

Nutrient availability greatly influences community structure and ecosystem processes, and that is highly relevant to restoration success where soils are naturally nutrient-poor, such as in the Cerrado – the Brazilian Savanna (Furley & Ratter 1988; Lira-Martins et al. 2022; Oliveira et al. 2021). These conditions favour slow-growing plant species adapted to low soil nutrients(Haridasan 2008).

Moreover, Cerrado soils have high concentrations of Al, which further limits nutrient availability, particularly phosphorus (Brenner et al. 2018; Lopes & Guilherme 2016; Singh et al. 2017). However, native species may possess adaptations that enable them to cope with high soil Al. These adaptations include site-specific Al accumulation within certain organs to detoxify Al (Bressan et al. 2016; Malta et al. 2016). In addition, whilst some species are capable of accumulating Al in their leaves (e.g. several species of Vochysiaceae) (Haridasan 1982), others can actively exclude Al through the release of carboxylates into the soil (de Castro et al. 2022). Despite these favourable properties for slow-growing species, an imbalance in nutrients or increased availability can negatively impact community assembly and diversity, favouring faster-growing, resource-acquisitive, or invasive exotic species (Lannes et al. 2016; Muehleisen et al. 2023).

Changes in nutrient availability often occur when native vegetation is replaced by crops or pastures with exotic grasses. In the Cerrado, native grasslands thrive in acidic and nutrient-poor soils (Lira-Martins et al. 2022; Silveira et al. 2020). However, converting this vegetation to exotic pasture or crops requires intensive liming and fertilizer addition to enhance productivity (Yamada 2005). Soil legacy, influenced by historical land use, plays a pivotal role in shaping the ecological balance of ecosystems. When lime is applied to soil, it elevates the levels of Ca and Mg and releases inorganic phosphate, which is typically bound to Al and Fe in acidic soils (Haynes 1982). Such alterations in soil chemistry, forming part of the soil's legacy, can inadvertently favour the success of invasive species in regions such as the Cerrado. Studies have already showed that human induced increased nutrient availability can favour plant invasion (Liu et al. 2017; Liu & van Kleunen 2017; Parepa et al. 2013), thus highlighting the influence of historical soil chemical alteration on the proliferation of non-native species. These invasive species benefit from higher soil resources, leading to increased dominance (Ren et al. 2019; Vitti et al. 2020).

In the Cerrado, invasive African grasses often limit restoration effectiveness by inhibiting the establishment of native species, thus impeding Cerrado regeneration (Coutinho et al. 2019; Schmidt et al. 2019). An example is the invasion of Cerrado areas by the African grass *Melinis minutiflora* P. Beauv, which significantly increases biomass and negatively affects native seedling survival, particularly in nutrient-richer soils (Hoffmann & Haridasan 2008). Many invasive African grasses are widespread due to their extensive use in pastures and croplands, and they often outcompete native Cerrado species (Eller & Oliveira 2017; Pivello et al. 1999). This competitive advantage is particularly prominent when soil fertilization reduces native plant community diversity (Bustamante et al. 2012). Increased nutrient input promotes rapid growth and biomass accumulation, exacerbating the impact of invasive African grasses (Williams & Baruch 2000). Moreover, invasive grass species can alter belowground ecosystem processes and soil nutrient stocks to their advantage (Garcia et al. 2022; Rossiter-Rachor et al. 2009). For example, *Urochloa decumbens* (Stapf) R.D.Webster was found to increase soil carbon and nitrogen content, potentially creating a positive feedback that further facilitates invasion (Garcia et al. 2022). Given the pervasive presence of invasive African grasses in the Cerrado, effective management should not solely rely on species removal but also involve modifying environmental conditions to reduce their establishment and spread.

To mitigate the adverse impact of altered soil chemical conditions on ecological restoration, one potential approach is to manipulate soil chemical properties (Owen & Marrs 2000; Tibbett et al. 2019; van der Bij et al. 2018). Using an acidifier, like iron sulphate, for soil acidification is a widely adopted strategy to reduce soil pH effectively(Chhabra 2021). This depletes cations from the soil exchangeable pool and further immobilizes inorganic phosphate (Duddigan et al. 2021; Tibbett & Diaz 2005). This serves to restore soil chemical properties to their previous state of higher acidity and lower nutritional status thus inhibiting the establishment of invasive species and facilitating the development of native species adapted to nutrient-poor and low-pH soil conditions (Owen et al. 1999; Owen & Marrs 2000). Studies manipulating soil pH report impacts on ecological factors like the abundance and diversity of soil biota, mediated by changes in nutrient availability and inter-species interactions (Zhalnina et al. 2015), thus resulting in competitive exclusion of some microbial (Rousk et al. 2010) and plant species

(Zhalnina et al. 2015). Nevertheless, impacts of soil pH manipulation on ecological parameters of tropical plant communities are yet to be understood.

We conducted a soil manipulation experiment to control the invasion of exotic grasses in Cerrado grassland restoration. While previous studies have focused primarily on the use of acid amendments in calcareous soils to lower pH levels by 1 or 2 units to improve plant nutrient availability (Heydarnezhad et al. 2012; Lee et al. 2021), our study takes a different approach. Our objective was to assess whether reducing soil pH and nutrient status through soil manipulation can control the biomass of invasive grasses. We hypothesized that a decrease in soil pH and nutrient availability would reduce the biomass of invasive grasses, thereby promoting the establishment of native species and increasing species diversity, thus enhancing restoration effectiveness. In this study we conducted a soil acidification experiment specifically focused on restoring tropical savanna grasslands restoration by actively managing soil pH.

Methods

Study site

The study occurred in Chapada dos Veadeiros National Park, central Brazil (14º 05’ S, 47º 38’ W), with grassland areas of different stages undergoing restoration. Rainfall is concentrated between October and April, averaging 1,324 mm annually, with a mean temperature of 24 °C. The area mainly comprises Ferralsol and Cambisol soils (Correia et al. 2001). The experiment was conducted in grasslands undergoing restoration within the park, which were previously abandoned pastures occupied by African grasses. A 50-hectare underwent a restoration intervention in 2016, involving native herb and shrub seeding (Sampaio et al. 2019). The area was originally an open Cerrado characterized by a continuous grassy layer, dominated by herbaceous species with woody layer being minimal (Sampaio et al. 2019), and with this vegetation being dominant at the specific site. Initially, invasive African grasses, such as *Urochloa decumbens*, *Urochloa humidicola*, *Andropogon gayanus*, *Hyparrhenia rufa, Urochloa brizantha, and Melinis minutiflora*, dominated the ground cover. It is unclear when the native grassland vegetation was first converted into pasture, but according to the local community, this likely occurred over 30 years ago. Before restoration efforts began, the area consisted of abandoned pasture with no active management. Most of the exotic grasses likely colonized the area passively, due to the widespread use of these species in the surrounding region. The restoration intervention involved burning, three rounds of soil tilling, and direct seeding of native species. However, four years after the intervention, the area was once again dominated by invasive species..

In February 2021, we established 14 experimental blocks, each covering 100 m². The blocks were systematically distributed to capture the full range of environmental variation in the study area. Within each block, we set up four 1 m² plots, where a seed mix of native grasses and shrubs—similar to the 2016 initial seeding—was sown on bare soil 15 days after soil acidification

Experimental design

The 14 experimental blocks were distributed across the study area, with each block placed at least 10 metres apart to ensure independence. Environmental variation within the area was accounted for in the experimental design and modelled using mixed-effects models, as detailed in the statistical analysis section. Within each block, four 1 m² plots were established. Two plots served as controls, with no acid applied, while the other two received acidification treatments. To maintain independence, the plots within each block were spaced at least 7 metres apart. This design resulted in each treatment being replicated 28 times across the study area.

In February and September 2021, we applied 1 kilogram of iron sulphate ($FeSO_4 \cdot H_2O$) per plot, equivalent to 10 tonnes per hectare. This standard soil amendment, used for alkali and saline soils (Chhabra 2021), was applied following preliminary field trials to ensure effective acidification. The aim was to impact exotic plants while monitoring potential adverse effects on native species.

Iron sulphate was applied with care to avoid contact with aboveground vegetation and minimise leaching risks. Plant leaves were lifted to prevent direct contact, and the iron sulphate was applied uniformly to the soil. The application was spread evenly, and the soil was ensured to absorb the solution fully to avoid superficial runoff.

After setting up the experiment, we sowed seeds with the same species composition used in the initial restoration effort. Following the completion of the second phase, our experiment comprised 56 distinct 1 m^2 plots, each treated as an individual sampling unit in our analyses. This design allowed for replication and provided a detailed understanding of the effects of soil acidification within the experimental framework.

Soil analysis

We began by collecting soil samples from the top 0-10 cm layer using an auger in each 25 m^2 plot within the designated blocks before initiating the soil acidification process. In each plot, we took two separate soil samples and combined them to create a single composite sample for laboratory analysis. After phase 2, we obtained one soil sample (0-10 cm depth) from each 1 m^2 plot for additional analysis. Analyses of chemical properties followed (Raij et al. 2001). Soil pH was determined in a $CaCl_2$ solution. Labile P and cations K, Ca, and Mg were determined by an extraction method involving a mixture of cationic and anionic exchange resins, saturated with sodium bicarbonate (Raij et al. 2001). P was determined using a photocolorimeter, Ca and Mg were determined by atomic absorption spectrophotometry and K determination used atomic emission photometry. The sum of bases (SB) is calculated as SB = [Ca] + [Mg] + [K]. Al saturation is determined as Al saturation = [Al]/(SB+[Al]) *100.

Plant species diversity

In February 2022, four months after the last application, we surveyed the 56 plots to identify and classify all plant species found as either invasive or native. February is the peak of the rainy season when the plant community is at the peak of the flowering period, thus facilitating identification.

Furthermore, this four-month interval aligns with the time constrain of restoration practices, where practitioners face tight schedules, especially for soil preparation and invasive species management. We aimed to assess the immediate effects of soil acidification on invasive species, and benefits to the quick growth of herbaceous species after the beginning of the wet season. This period is suitable to observe initial responses of plant herbaceous communities to altered soil conditions. We visually estimated the cover of each species in each plot. Based on these data, we used the vegan package (Oksanen et al. 2022) to calculate Shannon's index (*H'*) of diversity. The Shannon index is calculated taking into account both the abundance of each species and the number of species, providing a more comprehensive measure of diversity that reflects not just how many species are present, but also how evenly their individuals are spread across those species.

Biomass sampling

In April 2022, at the end of the rainy season, the plant communities were well-developed. We used a 0.25 m^2 quadrat to sample the aboveground biomass of native (AGB_n) and invasive (AGB_i) species within each 1 m^2 plot. All biomass was clipped and stored in paper bags, then oven-dried at 50 °C to constant weight, and weighed on a precision balance.

Statistical analyses

We used linear mixed-effect models to assess the impact of acid addition on soil properties, AGB_i, and AGB_n. 1m^2 plots were sampling units. Stepwise backwards selection helped identify the best model for evaluating soil variables' influence on AGB_i, AGB_n, and AGB_i:AGB_t (proportion of AGB_i to plot total AGB). A full model with non-correlated variables was constructed, progressively eliminating variables with P >0.05) (Zuur et al. 2009). Treatment in phase 1 (25m^2 control and acid plots) nested in blocks was a random intercept. Soil chemical parameters and AGB variables were response variables for testing soil acidification effects. Principal component analysis (PCA) assessed associations between soil parameters. For analysing AGB_i:AGB_t in the 1m_2 plots, mixed-effects models with a binomial family distribution were employed. Mixed-effect models with the same random structure but different independent variables (soil chemical features) were tested against AGB_i, AGB_n and AGB_i:AGB_t. A square root transformation was applied to AGB_i and AGB_n to meet residual distribution assumptions. Models were implemented using the lme4 package (Bates et al. 2015), with *P* values obtained via Satterthwaite's degrees of freedom method from the lmerTest package (Kuznetsova et al. 2017) in R version 4.2.1 (R Core Team 2023).

We investigated the direct and indirect effects of soil properties on species biomass and diversity. Decreasing soil nutrient levels were expected to directly impact AGBi reduction. Considering invasive species' rapid growth and dominance, AGB_i was expected to have a direct negative effect on AGB_n and a positive direct effect on species diversity (*H'*). Hence, soil nutrient status indirectly influences species diversity. Structural Equation Modelling (SEM), incorporating the most significant soil variable from the model testing soil chemical properties and AGB_i, AGB_n, and *H'*, was used to test this hypothesis. The piecewiseSEM package (Lefcheck 2016) and mixed-effect models were employed for this analysis.

Results

Soil properties

The treatment with acid addition (+A) effectively induced a systematic effect on soil chemical properties. The PCA indicated a clear distinction in terms of soil nutrient status among acidified and non-acidified plots, where the first axis indicates a positive association with nutrients and a negative with Al saturation and the second axis is associated with the variation in soil texture (**Fig. 1**). There was a consistent decrease in soil pH from 4.13 to 3.91, representing a 70% increase in H+ concentration. This acidification also led to a decrease in nutrient availability, such as a 66% reduction in the sum of bases and a 26% increase in Al saturation (Table 1). Potassium was the only element that did not show a significant decrease. Notably, the acidification treatment resulted in soil values that were similar to those of reference native grassland areas located near the experimental site (Table 1).

Aboveground biomass of invasives and natives

Invasive species were represented in the plots by the African grasses *Andropogon gayanus, Hyparrhenia rufa, Melinis minutiflora*, *Urochloa brizantha, Urochloa decumbens and Urochloa humidicola*. *Urochloa brizantha, Melinis minutiflora*, and *Urochloa decumbens* were the most frequent invaders across the experiment, with *U. brizantha* and *U. decumbens* showing the highest mean coverage (**Table S1**). Some native abundant species were *Andropogon leucostachyus, Aristida setifolia, Axonopus aureus, Echinolaena inflexa, Lepidaploa aurea* and *Loudetiopsis chrysothrix.* Soil acidification had a systematic negative effect on AGB_i in the experimental plots (P < 0.001) and represented a 70.7% (CI=59.0-86.7) reduction in AGB_i across plots (**Fig. 2a**). There was a wide variation in AGB_i explained by differences between blocks as captured by the random term of the model and this was associated with soil [Ca] and [Mg] before acidification (**Fig. S1**). The mixed model framework not only captures the fixed effects of acidification on AGB_i but also allows for an exploration of how pre-acidification soil characteristics (e.g. [Ca] and [Mg] levels) interact to influence the vegetation's response to acidification.

We found no effect of acidification on AGB_n (P = 0.885, **Fig. 2a**). We further evaluated the effect of the acidification on AGB_i:AGB_t. According to model estimates, acidification reduced the proportion of AGB_i to 30% (CI=6.5-73.2) which is a 53% reduction compared to the 64% (CI =23.1-91.0) in control plots (P = 0.006, **Fig. 2b**). It should be noted that, plots exhibiting a reduced effect of acidification on AGB_i have intrinsically higher soil nutrient status (**Fig. S1**). This suggests that between plot differences in soil properties resulted in the variability of the measured effects.

No significant differences were observed in the cover of invasive species (**Fig. S2**). However, we found a positive correlation between the proportion of invasive species biomass relative to total biomass (AGB_i:AGB_t) and *Urochloa decumbens* (Fig. S3). This suggests that *U. decumbens* made a significant contribution to the relative biomass of exotic species in the study

Relationships between soil properties, biomass and diversity

We detected important effects of soil chemical properties associated with the variation in AGB_i. The best model exhibited Al saturation (P < 0.001) as having a negative relationship with $AGB_{i.}$ (**Fig. 3a**). Specifically, we observed that plots with higher aluminium saturation consistently exhibited lower AGB_i values, while those with lower aluminium saturation were associated with higher AGB_i values. This negative relationship persisted across all experimental plots, independent of the applied treatments, as treatment effects were not incorporated into this analysis. It is noteworthy that the observed gradient in aluminium saturation across plots is a direct consequence of the varying acidification management strategies implemented in our study design.

The best model testing the effect of soil variables on the proportion of AGB_i indicated Al saturation as exhibiting a negative relationship with the proportion of AGB_i (**Fig. 3b).** Species diversity, as estimated by the Shannon index (*H'*), showed a quadratic relationship with AGB_n (**Fig. 3c**). A quadratic relationship implies that the species diversity initially increases with AGB_n but begins to decline as AGB_n continues to increase, suggesting that intermediate values AGB_n corresponded to the higher values of diversity. We found no significant effect of AGB_i on species diversity. Furthermore, we detected that this pattern is associated with a decrease in species diversity (*H'*) (**Fig. S4**).. It should be noted that we did not identify a significant difference in species richness per plot between treatments, with control plot showing an average of 7.9 species while acidified plots exhibiting 8.2 species (P=0.709).

Direct and indirect effects of soil nutrient status on plant biomass and species diversity

The structural equation model showed a systematic effect of soil chemical-induced variation in the aboveground structure and diversity (**Fig. 4**). The model indicated a strong direct negative effect of increasing Al saturation on AGB_i. As we predicted, an increase in AGB_i resulted in a negative effect on AGB_n. In addition, an increase in AGB_n had a positive effect on species diversity (*H'*), and this was associated with the rising number of native species (**Fig. S4**). Based on the test of directed separation in the piecewiseSEM package, we included an error correlation between Al saturation and *H'* that indicated a negative correlation between the error of these variables.

Discussion

In the restoration of Cerrado ecosystems, the dominance of invasive grass species presents a significant barrier, requiring early detection and effective control to decrease their spread and ensure restoration efficacy. Our research examined the impact of induced soil acidification on reducing soil nutrient availability and decreasing the biomass of invasive plant species. The data revealed that this intervention markedly controlled the biomass of invasive species, consequently increasing the native: invasives biomass ratio increasing community equability and, thus, raising the diversity index. These findings emphasize the strategic advantage of manipulating soil chemistry to limit nutrient availability, thereby inhibiting the growth of invasive grasses and facilitating the recovery of native Cerrado vegetation.

Effects of soil manipulation on invasive biomass

The acid-induced nutritional reduction in soil is reflected in the measured decrease in invasive species biomass. Nevertheless, plots exhibiting a reduced effect of acidification on AGB_i have intrinsically higher soil nutrient status. This observation suggests that a more concentrated acidification approach at these specific locations might have yielded a more effective control over invasive species. This pattern of variability across blocks strengthens the general conclusion regarding the importance of soil nutrients influencing the establishment and spread of invasive grasses. We designed the study and modelled the data to account for variability both within and between blocks. Thus, the observed variability was indeed intended to refer to differences across blocks. Many of these invasive grasses exhibit a high nutritional demand (Oliveira et al. 2022) (e.g. *Urochloa brizantha*), and significant reductions in these mineral resources can exert a detrimental impact on their proliferation. Furthermore, a decrease in soil nutrient availability can represent a significant step towards reducing invasion, especially in the context of native species restoration, as elevated nutrient availability often facilitates invasion (Cleland et al. 2013). Thus, this study reinforces that soil acidification has a strong potential to effectively decrease soil cation availability (Chhabra 2021), positioning it as a promising and strategic approach to address the challenge of invasive species management following the implementation of restoration measures in neotropical grasslands and savannas. Its potential to alter the competitive advantage of invaders by limiting soil nutrient availability highlights its importance among restoration strategies (Duddigan et al. 2021; Dunsford et al. 1998; Owen & Marrs 2000; Tibbett et al. 2019; Tibbett & Diaz 2005; van der Bij et al. 2018). However, it is important to consider that the lasting impact of iron sulphate on soil is influenced by various factors, including the dosage applied and the soils texture (Chhabra 2021). These elements are important to determining the duration and effectiveness of soil acidification as a method for controlling invasive species.

Experiments often indicate that the performance of invasive exotic species is favoured over native species at high nutrient availability (Daehler 2003; Ren et al. 2019). The response of invasive grasses to nutrient availability has been experimentally reported for the Cerrado. Bustamante et al. (2012) detected a substantial increase in the biomass of the African grass *M. minutiflora* following savanna fertilization , especially with the addition of phosphorus. Similarly, Lannes et al. (2016) reported that nutrient addition in another experiment in Cerrado, increased the biomass of the African grasses *U. decumbens* and *M. minutiflora*, highlighting the significant effect of phosphorus on invasive species' response. Furthermore, other nutrients, such as boron, may also stimulate the biomass accumulation of certain African grasses in Cerrado (Lannes et al. 2020). Altogether, these results reinforce the importance of nutrient imbalance in affecting the invasion of nutrient-poor ecosystems by exotic species (Sardans et al. 2017).

Effects of soil manipulation on native biomass

Although acid addition had an impact on the biomass of invasive species, we did not detect a significant direct effect on the biomass of native species. This is most likely a reflection of these species' adaptation to low nutrient content. Native species adapted to low soil nutrient status seem to show limited response to reduced resource availability (Davidson et al. 2011).In addition, we acknowledge that the short assessment period following application may have limited our ability to evaluate the long-term effects on native species biomass. However, the importance of this treatment lies on its indirect effect through the management of AGB_i control, where low soil nutrient availability decreases the biomass of invasives.

Such indirect edaphic control over AGB_n has an important effect on species diversity. While native species are adapted to acidic soils with low nutrient availability, they are inferior competitors when it comes to using higher amounts of available nutrients to grow, compared to invasive African grasses (Eller & Oliveira 2017). Thus, the reduction in the dominance of invasive grasses through acid addition increases community equability and may potentially enhance the diversity of herbaceous communities.

These findings suggest that acid addition controlling invasive plant species biomass, rather than directly influencing native biomass, could be a viable strategy in the ecological restoration of Cerrado grasslands. This approach may effectively restore native chemical soil conditions and enhance the control of invasive grass species control, thereby supporting broader restoration objectives.

Underlying causes of soil properties effect on exotic species and plant community diversity

Higher soil nutrient availability fosters the establishment of invasive species, as evidenced in the results showing an increase in Al saturation leading to a decrease in nutrient availability and thus reducing invasive biomass. An increase in aboveground biomass of invasives (AGB_i) under higher nutrient availability can limit the biomass of native species within the ecosystem. Consequently, this phenomenon leads to a reduction in species diversity in the community (*H'*), and soil acidification appears to alleviate this pressure on community diversity by limiting the dominance of invasives, even if the number of native species did not change. However, it's important to note that the relationship between AGB_n and species diversity in the community is highest at intermediate AGB_n levels, and extreme AGB_n values may also reduce species diversity in the community. This suggests that the dynamics of native species and their contribution to diversity can vary depending on the extent of their aboveground biomass. Despite the soil nutrient depletion's role in reducing invasives, some plots still exhibited high AGB_n levels. In these plots, the abundant cations were not sufficiently counterbalanced by the experiment's acid concentration. The positive associations of [Ca] and [Mg] with invasive species further underscore the significance of soil nutrient status as a critical environmental factor influencing Cerrado grasslands restoration.

Under conditions of high nutrient availability, invasive species can further restrict the below-ground resources available to species with lower competitiveness for soil resources (Harris & Facelli 2003), potentially leading to a decrease in overall diversity. Consequently, a more limited group of highly competitive, resource-acquisitive species, such as the African grasses in the study area (Williams & Baruch 2000), may become dominant. At higher dominance levels, these invasive species can employ strategies that contribute to a positive feedback loop in their favour. For example, species like *Melinis minutiflora* can replenish the soil seed bank by producing many seeds that remain viable in the soil for extended periods (Carmona & Martins 2010; Xavier et al. 2021). Additionally, some of these invasive grasses tend to respond positively to fire, as seen with *Urochloa sp.* (Damasceno & Fidelis 2020), *A. gayanus* (Rossiter et al. 2003), potentially promoting a grass-fire cycle (D'Antonio & Vitousek 1992) where they continually increase in abundance (Silvério et al. 2013), outcompete native species, and reduce overall diversity in the area.

We do acknowledge that the four-month assessment period following acidification may seem relatively short for predicting a fully established community composition over longer timeframes. Nevertheless, this period provides valuable insights into the effects of soil acidification on controlling invasive species and the potential impact of this method on native species, particularly in the context of addressing the primary challenge in Cerrado restoration.

It should be noted that, while certain soil amendments serve to induce acidification, their efficacy and duration of impact can vary significantly. Persistent acidification is important for ensuring the extended efficacy of restoration strategies in these naturally acidic soils, as it supports the maintenance of low nutrient availability and low acidity levels. For instance, a study comparing the long term effects of different soil amendments found that elemental sulphur exhibited a long-lasting effect on soil pH, which was still discernible after 14 years (Tibbett et al. 2019). Iron sulphate also was effective in acidifying the soil, although it had a less sustained acidifying effect for the same duration.

This difference in the longevity of the acidifying effects has important practical implications for restoration activities and more research should be developed on testing these effects with different soil amendments. When planning soil amendment strategies, it is important to consider the longevity of the acidifying impact, in addition to other factors, as the selection of an amendment with a prolonged acidifying effect is decisive for maintaining low nutrient levels and high acidity in the soil, as highlighted in the present study.

The use of acid amendments

Here, the proposed goal is to evaluate the effect of soil acidification in suppressing invasive species or decreasing their proportion in the Cerrado grassland. Iron sulphate II is a commonly used acid agent. It will oxidize in the soil to iron III, precipitating as iron hydroxide, which is common in Cerrado soils (Lopes & Guilherme 2016). Iron oxidation also decreases P availability (Lasisi et al. 2023) and cations are leached away with sulphate in soil (Marchi et al. 2020). Other possible acidifying agents can be used such as elemental sulphur, pyrite (Iron di-sulphide, FeS_2) and aluminium sulphate ($Al_2(SO_4)_3.18H_2O$) (Brownrigg et al. 2022; Chhabra 2021). Furthermore, low molecular weight organic acids such as citric and oxalic acids can be applied to alter soil pH and nutrient availability. These are produced at an industrial scale by fermentation processes of fungi employing renewable carbohydrates such as sugar cane (Mottakin et al. 2022) and even coconut (Costa et al. 2020).

In general, there is no universal formula for soil acidification that can be applied to all situations. The selection and combination of various technologies should be based on local soil conditions, technical and economic feasibility, and the level of social involvement among stakeholders. Acknowledging the environmental impacts of soil amendments is essential. Therefore, the efficacy, safety, and cost-effectiveness of these amendments should be thoroughly evaluated in field trials prior to application. Our results show significant variability in the effectiveness of controlling invasive species across different soil nutrient conditions, suggesting that acidification strategies should be customized based on site-specific soil properties. Future research should assess the effectiveness of various acidifying agents, their interactions with soil conditions, and their environmental impacts to develop more sustainable practices. .

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Impacts of soil acidification on species biodiversity

Iron sulphate soil amendments have diverse effects on biodiversity, which vary based on the specific environmental context and the mix of other materials used in the amendment process. Soil microbial communities significantly differ with changes in soil pH (Bååth & Anderson 2003). Research shows that bacterial communities are more abundant and diverse at higher pH levels, whereas fungal communities do not exhibit significant changes with pH variations (Rousk et al. 2010).

In Cerrado soils, soil micriobiota also show variation in species composition and abundance with soil pH. For instance, some groups of bacteria such as Acidobacteria and Actino bacteria are more frequently associated with higher soil pH whilst Probacteria are more common in nutrient rich and less acidic soils (Procópio & Barreto 2021). Such differences can be also found for fungi where Basidiomycota are more abundant in low pH soils, Ascomicota can be found more towards higher soil pH (Procópio & Barreto 2021). Furthermore, soil pH alteration driven by land use change can affect the soil biota in the Cerrado (de Souza & Procópio 2021; Lammel et al. 2015), and it is expected also from acidification aiming at restoration. Conventional agricultural systems richer in nutrient and pH seem to have higher number of taxonomic bacterial groups, nevertheless, lower functional groups when compared to native acidic soils in Cerrado (Souza et al. 2016; D'Angioli et al. 2022) Soil biota can also vary significantly in native areas with acid addition. In a heathland system, areas treated with iron sulphate had a decrease in bacteria relative to control soils, aligning with the natural edaphic conditions of native heathlands (Tibbett et al. 2019). It is therefore likely that there are indirect effects of soil acidification on plant success, via alterations to the soil microbial community, which were not studied here, but should be a priority for future research.

Soil fertilization in nutrient poor ecosystems

Restoring functional ecosystems requires carefully considering the distinctions of community dynamics and environmental factors that shape species assemblages (Hulvey & Aigner 2014; Palmer et al. 1997). This is especially critical in environments characterized by low nutrient status soils, such as the Cerrado. While soil fertilization is a common practice in restoration efforts under the assumption that added nutrients universally benefit plant growth, results from this study challenge that notion within the context of the Cerrado and similar nutrient poor ecosystems.

In line with previous studies of Lannes et al. (2016) and Bustamante et al. (2012) that documented how increased nutrient availability can favour fast-growing exotic species at the expense of native vegetation adapted to nutrient-poor conditions, the findings in the present study indicate that routinely applying fertilizers can shift competitive dynamics towards invasive African grasses over native vegetation adapted to low nutrient conditions. Thus, in the Cerrado, rather than enhancing restoration outcomes, fertilization can negatively impact efforts to reestablish native plant communities. The key priority should therefore focus on maintaining or restoring the characteristically acidic and low nutrient soils that enable native species to persist over exotic plants, which are not as well adapted to such conditions.

This insight highlights that the prevalent use of fertilizer amendments in Cerrado restoration projects poses a significant risk of undermining restoration efforts in the Cerrado and other low-nutrient ecosystems. . Instead, working to simulate the soil conditions to which native species are adapted should provide a more sound ecological approach for enhancing restoration and habitat recovery. The study results provide an important lesson on adopting ecological knowledge to advance restoration methodology in Cerrado.

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Author's contributions

DLM, ROX, GGM, EM, LR, RSO conceived the ideas and design methodology; DLM, ROX, GGM, MNF, LSV, TA, FVB, BS conducted experimental set-up and collected the data; DLM analysed the data and wrote the first draft, and all authors contributed critically to the drafts and revised the manuscript.

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Table 1. Results of soil chemical properties of control plots with no acid addition (-A, n=28) compared to acidified plots (+A, n=28). *P* values were obtained using the Satterthwaite's approximation for degrees of freedom. The 95% confidence intervals are in parentheses. The "Change" column represents the percentage difference in each specific soil property between the treatment and control groups. The "Reference" column shows the baseline soil values from 5 reference sites of native grassland near the experimental site within the National Park Chapada dos Veadeiros. Values between brackets in "Reference" column represent range. Al nutrients are in their available form.

Soil variable	-A	+A	*P value*	*Change (%)*	*Reference*
pH	4.1 (4.1-4.2)	3.9 (3.8-4.0)	< 0.001	5.4	3.9 (3.9-4.0)
Ca ($mmol_c$ kg^{-1})	3.4 (2.4-4.4)	1.2 (0.2-2.2)	0.002	65.3	1.4 (1.0-2.0)
K ($mmol_c$ kg^{-1})	1.4 (1.2-1.5)	1.2 (1.1-1.4)	0.147	9.2	0.6 (0.4-0.8)
Mg ($mmol_c$ kg^{-1})	1.7 (1.4-2.1)	1.0 (0.7-1.3)	0.004	42.3	1.0 (1.0-1.0)
P labile (mg kg^{-1})	4.9 (4.6-5.3)	2.5 (1.9-2.6)	< 0.001	54.3	2.4 (2.0-3.0)
Sum of bases ($mmol_c$ kg^{-1})	6.0 (4.5-7.6)	2.1 (0.5-3.6)	< 0.001	65.9	3.0 (2.6-3.5)
Al saturation (%)	72.3 (67.5-77.2)	91.4 (86.5-96.2)	< 0.001	26.4	80.0 (75-93)

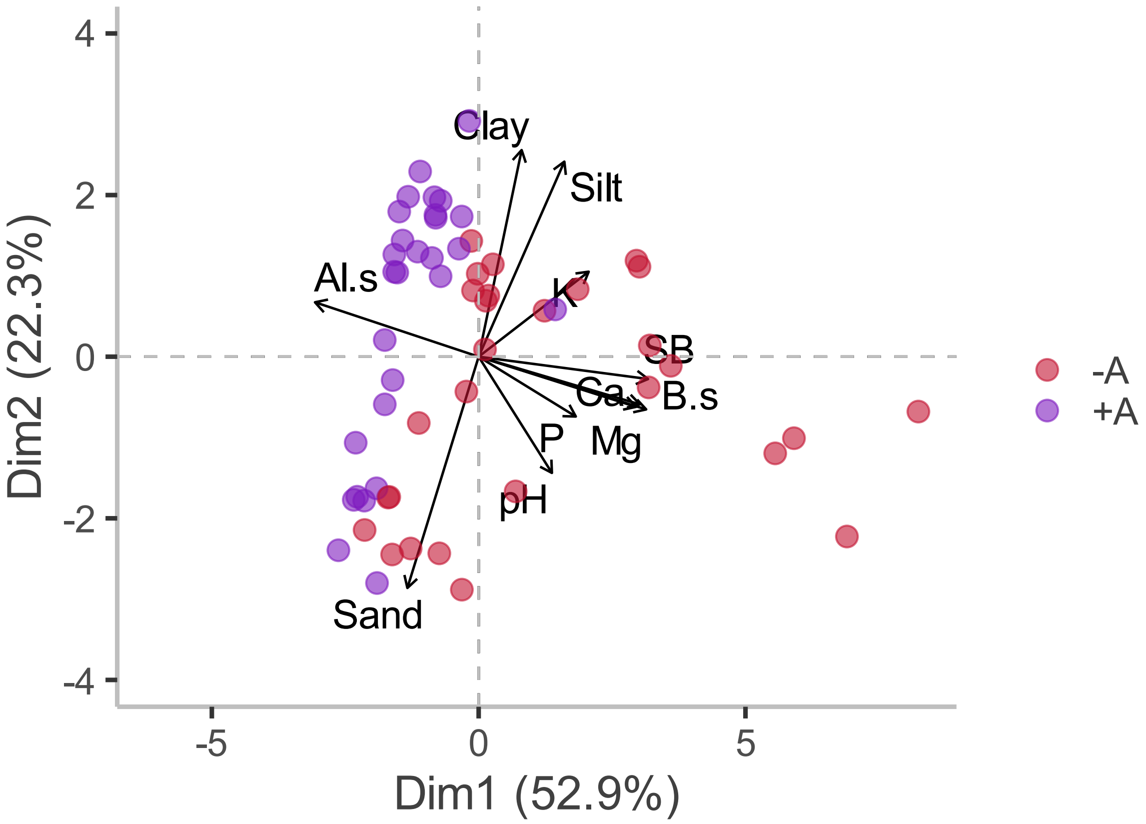

Figure 1. Principal component analysis of soil texture and chemical composition. The first dimension explained 52.9% of the variation of soil properties and is strongly associated with soil chemical conditions. The second dimension explains 22.3% of the variation and is mostly associated with soil texture. Purple points represent acidified plots (+A, n=28) and red points depict control plots(-A, n=28). Al.s = Al saturation, SB = sum of bases, B.s= Base saturation, K = potassium, Mg = magnesium, Ca = calcium.

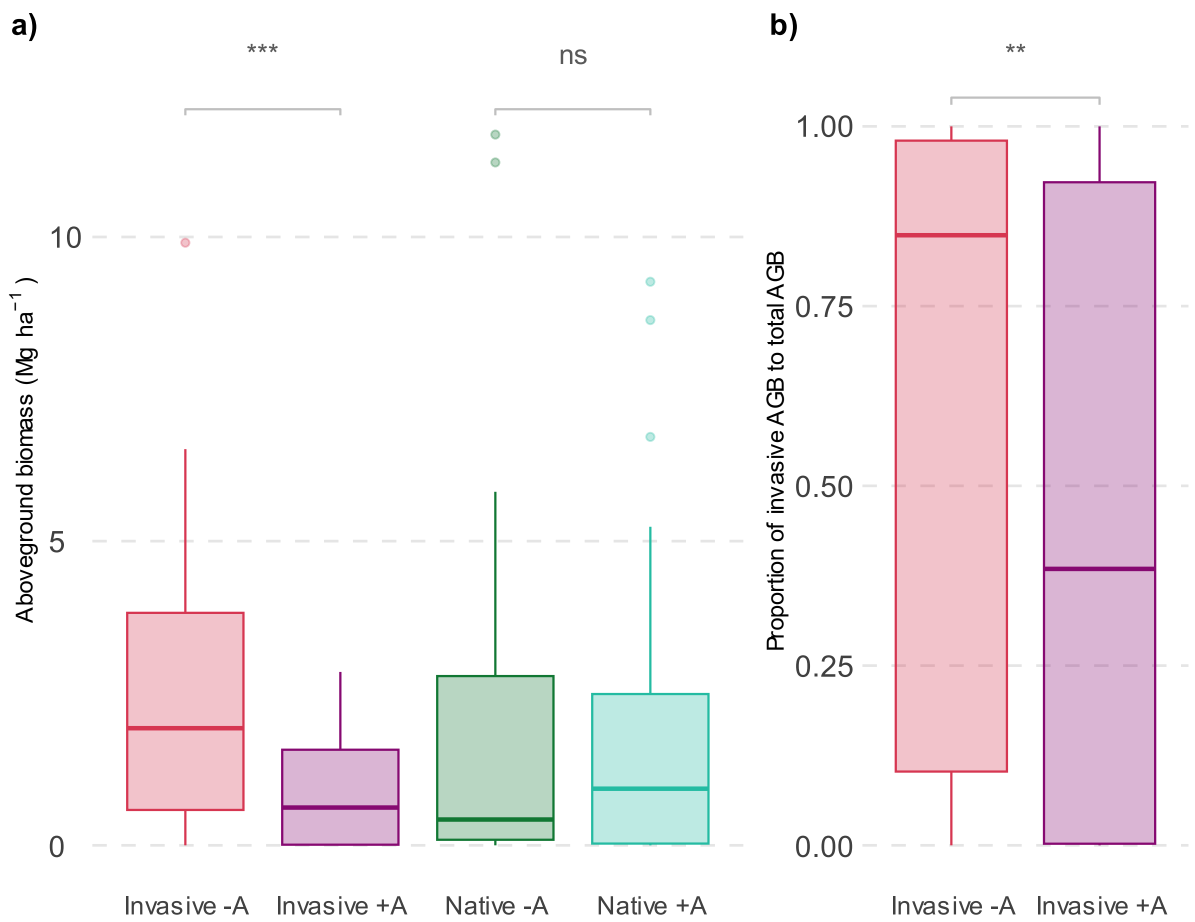

Figure 2. a) Boxplot indicating the aboveground biomass of native and invasive species within the acidified (+A, n=28) and control (-A, n=28) treatments (x axis). Models indicate no significant difference in acid treatment on native species and a significant difference in invasive aboveground biomass (ns = not significant P > 0.05, *** stands for *P* < 0.001). *P* values were obtained using the Satterthwaite's approximation for degrees of freedom. **b)** The proportion of invasive aboveground biomass (AGB_i) relative to total aboveground biomass in acidified (+A, n=28) and control (-A, n=28) plots treatments indicated in the y axis (** stands for *P* = 0.006).

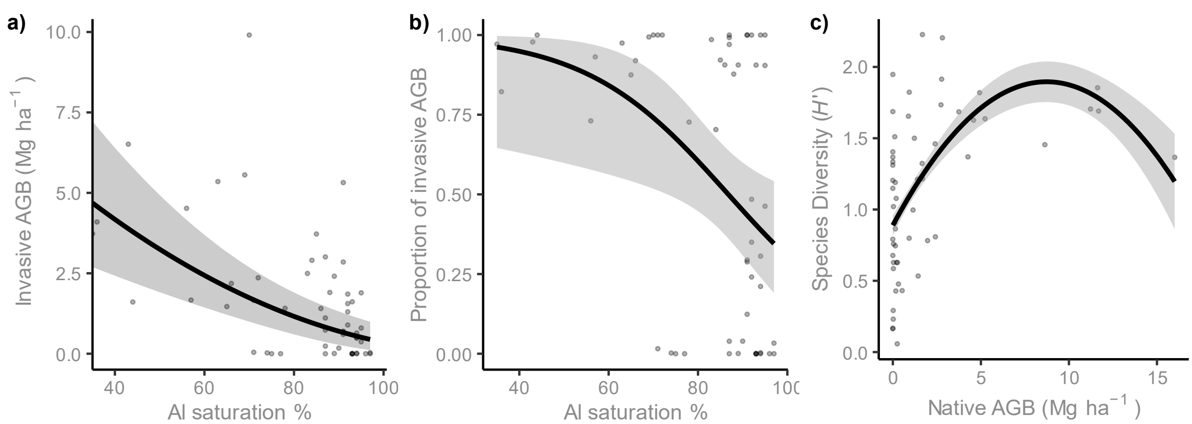

Figure 3. Mixed model predictions for **a)** Aluminium saturation effect on AGB_i ($P < 0.001$, $R^2_m = 0.23$, $R^2_c = 0.67$, where R^2_m is marginal R^2 for only fixed effects and R^2_c is conditional, which integrates fixed and random terms) and **b)** Al saturation effect on AGB_i ($P = 0.015$, $R^2_m = 0.07$, $R^2_c = 0.70$). **c)** Association between AGB_n and species diversity ($P_1 = 0.0151$, $P_2 = 0.0235$, $R^2_m = 0.17$, $R^2_c = 0.50$). *P* values for the fixed effects were obtained using the Satterthwaite's approximation for degrees of freedom. Analysis was conducted with a sample size of n=56.

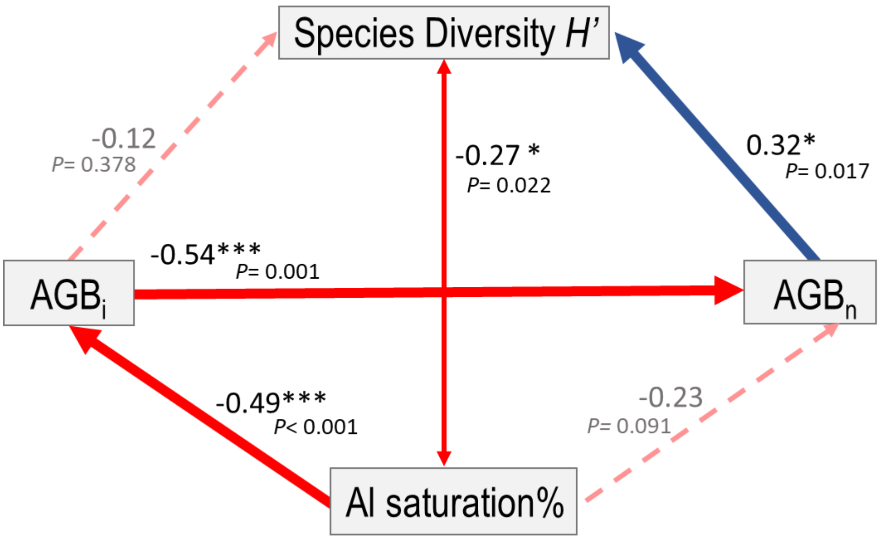

Figure 4. Mixed effect structural equation model indicating associations between soil properties (Al saturation %), invasive above ground biomass (AGB_i), native above ground biomass (AGB_n), and species diversity (Shannon's diversity index (H')). Significant path coefficients are marked with asterisks: ***, $P < 0.001$; *, $P < 0.05$. The blue arrows depict positive, and the red arrows depict negative standardized coefficients. Dashed arrows indicate nonsignificant coefficients ($P > 0.05$). *P* values for the fixed effects were obtained using the Satterthwaite's approximation for degrees of freedom. Analysis was conducted with a sample size of n=56.