



Invasion of the Brazilian campo rupestre by the exotic grass *Melinis minutiflora* is driven by the high soil N availability and changes in the N cycle

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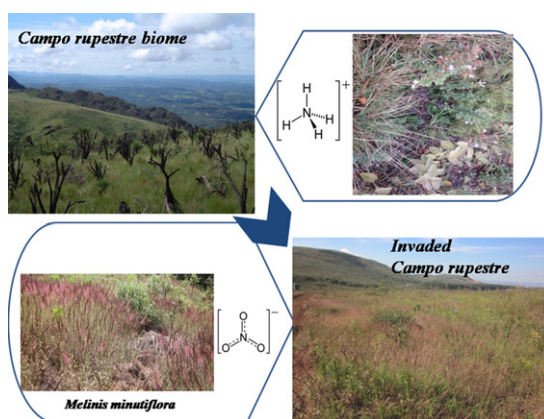
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HIGHLIGHTS

- *Melinis minutiflora* invasion threatens the biodiversity of a campo rupestre biome.
- The site was assessed using leaf C, N, and $\delta^{15}\text{N}$, and soil C, N, NO_3^- and NH_4^+ .
- Soil had a high level of NH_4^+ and a high N-based fertility from N_2 fixation.
- NO_3^- increased in invaded soils through the stimulation of nitrifying bacteria.
- N fertilisation in restoration procedures may increase the invasiveness degree.

GRAPHICAL ABSTRACT



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ABSTRACT

The Serra do Rola Moça State Park (PESRM) in Minas Gerais State, Brazil is a preserved site representative of the campo rupestre biome over an ironstone outcrop that has a high level of plant diversity. Almost 60% of this grassy field has been invaded by the exotic molasses grass (*Melinis minutiflora*), which constitutes a severe threat to the biodiversity and survival of this biome, particularly due to the impacts of annual fires and inappropriate restoration interventions. Many invasive species exhibit a high demand for nitrogen (N). Hence, this work aimed to study the N cycle alterations promoted by *M. minutiflora* in a site of the campo rupestre, where the leguminous species *Mimosa pogocephala* was prevalent. The biome's soils exhibited a high natural N fertility and low C:N ratio. The main N source in this biome resulted from the biological N fixation performed by *M. pogocephala* associated with *Burkholderia nodosa*, as evidenced by the total leaf N content, leaf $\delta^{15}\text{N}$ signature, nodule occupation and bacterial molecular identification analyses. The displacement of native species by molasses grass was associated with changes in the soil N forms, namely the nitrate increased as the ammonium decreased. The latter was the dominant N form in the native species plots, as observed in the soil analysis of total N, ammonium and nitrate contents. The dominant ammonium form was changed to the nitric form by the stimulation of ammonia-

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oxidising bacteria populations due to the invasive species. Therefore, the key mechanism behind the invasiveness of the exotic grass and the concomitant displacement of the native species may be associated with changes in the soil N chemical species. Based on this finding and on the high N-based soil fertility found in the campo rupestre N fertilisation procedures for restoration of invaded areas should be strictly avoided in this biome.

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1. Introduction

Biological invasions threaten worldwide biodiversity (Davis, 2003) by decreasing species richness, diversity and evenness (Hejda et al., 2009). In general, the effects on biodiversity occur due to strong modifications in community composition and community dynamics through alterations in key ecosystem processes (Ehrenfeld, 2003) such as biogeochemical cycles and soil chemistry (Ehrenfeld and Scott, 2001). Several invasive species are able to modify soil organic matter and nutrient stocks, especially nitrogen cycling pools (Ehrenfeld, 2003; Hawkes et al., 2005; Stark and Norton, 2015). Such changes may be promoted by augmenting ecosystem N through fixation of atmospheric N, increasing N loss rates or altering the N-chemical form (Corbin and D'Antonio, 2004; Evans et al., 2001). Furthermore, invaders have been reported to alter soil moisture and water loss (Zavaleta, 2000) and change fire regimes (D'Antonio, 2000; D'Antonio et al., 2011).

However, the impacts on the species diversity and composition of invaded communities differ among invaders (Hejda et al., 2009). The invasive mechanism of the species *Melinis minutiflora* has been related to N soil alterations that result from complex positive feedbacks among vegetation, soil microbes, and nutrient cycling (Corbin and D'Antonio, 2004; Hoffmann and Haridasan, 2008; Hawkes et al., 2005; Lazzaro et al., 2014; Stark and Norton, 2015; Suding, 2013), which ultimately benefit the invasive over competitors (Suding, 2013). Furthermore, *Melinis minutiflora* invasion has been documented to be associated with fires promotion (Suding, 2013; Bustamante et al., 2012; Yelenik and D'Antonio, 2013), resulting in the depletion of native plants and the acceleration of nitrogen-cycling rates as more soil nitrogen is released to the soil. This additional N soil input increases the growth of exotic grasses and inhibits the recovery of native species (Bustamante et al., 2012).

The Serra do Rola Moça State Park (PESRM), located in Quadrilátero Ferrífero in Minas Gerais State, was created to protect a preserved site representative of the Brazilian campo rupestre biome over an ironstone outcrop that is surrounded by six iron ore mines. The soils under campo rupestre are ironstone outcrops (canga) that are formed by alterations in primary minerals through the actions of weathering and/or lichenic acid production (Resende et al., 1996), and they have been considered by several authors as nutrient-impoverished (Carvalho-Filho et al., 2010; Messias et al., 2013).

The vegetation of the campo rupestre biome is dominated by grass, herbaceous and shrub species of Poaceae, Asteraceae, Malpighiaceae and Fabaceae (*Periandra*, *Chamaecrista*, *Mimosa*) as described by Viana and Lombardi (2007). A single leguminous species generally dominates each area of the campo rupestre depending on factors such as substrate type and altitude. In our study area, the prevalent leguminous species was *Mimosa pogocephala* Benth., which is considered endemic in this region. However, the notable and diverse vegetation in the campo rupestre biome (Giulietti and Pirani, 1988; Viana and Lombardi, 2007) appears to be incongruent with the assumed nutrient-impoverished soils of this area.

The campo rupestre biome has been threatened by intense iron ore mining activities over centuries. Mine companies located around the PESRM have extensively used exotic species such as the African pasture grass *Melinis minutiflora* (P. Beauv) in rehabilitation procedures. As a result, nearly 60% of the campo rupestre in the park has been invaded by

M. minutiflora, which constitutes a severe threat to the biodiversity and survival of the native vegetation that is periodically reached by fires (Scotti et al., 2012).

Besides, some restoration efforts in some invaded areas of the PESRM have been empirically conducted by environmental associations and non-governmental organisations by using native species with conventional NPK fertilisation techniques. Unfortunately, such interventions have increased the degree and extent of *M. minutiflora* invasion in these managed areas (Scotti et al., 2012). This finding is in agreement with Bustamante et al. (2012), who showed that fertilisation with N and P has increased *M. minutiflora* invasion.

Molasses grass requires high levels of nutrients, particularly nitrogen (N), for growth (Asner and Beatty, 1996; Barger et al., 2003). As the campo rupestre soils are considered to show low fertility levels, it is unclear how such a highly nutrient-demanding plant such as molasses grass has invaded the PESRM, particularly to such a great extent.

One of the main invasive strategies documented for exotic species is the alteration in soil N availability for their own benefit by modifying N fixation and nitrification/denitrification processes involving N cycle-related soil microbial communities (Corbin and D'Antonio, 2004; Evans et al., 2001; Hawkes et al., 2005; Lazzaro et al., 2014; Stark and Norton, 2015). Considering that invasion success depends on soil nutrients, particularly N availability, we studied the soil fertility of the campo rupestre, the alterations in the N cycle promoted by the invasive species *M. minutiflora* on the campo rupestre substrate and the impact of *M. minutiflora* on *M. pogocephala*. Our findings will assist to formulate and support future management plans.

2. Material and methods

2.1. Study area

The study was carried out at the Parque Estadual da Serra do Rola Moça (PESRM), located in the central-south region of Minas Gerais State, Brazil (20°3'20"S, 44°1'11"W) and covering the municipalities of Belo Horizonte, Brumadinho, Ibirité and Nova Lima. The regional weather is type "Cwa" (Köppen classification), presenting a well-defined rain regime characterised by dry winters and rainy summers. The PESRM is located at the "Quadrilátero Ferrífero" in the southern region of the Espinhaço mountain range, with an altitude ranges from 650 to 1500 m above sea level. This site is economically important because it hosts two world-class iron-ore mines of the Proterozoic Minas Supergroup. This supergroup consists of two types of banded iron formations; the dolomitic and quartz itabirites (Spier et al., 2007), with a canga surface covering friable and compact haematite; and a phyllite base (Spier et al., 2007). The dominant clay type rock consists mainly of iron and aluminium oxides and is classified as perferic soil based on the Brazilian Soil Classification (Spier et al., 2007). This rock is rich in Fe₂O₃ (95.8–97.8%) and other elements, such as K₂O (0.06%), MgO (18.5%), CaO (26.19%) and P₂O₅ (0.12%), as described by Spier et al. (2007).

The vegetation (Fig. 1a), is characterised as a grassy field over an ironstone outcrop (Rizzini, 1979), which is composed of herbaceous and shrubby species and occurs at altitudes above 1000 m. This

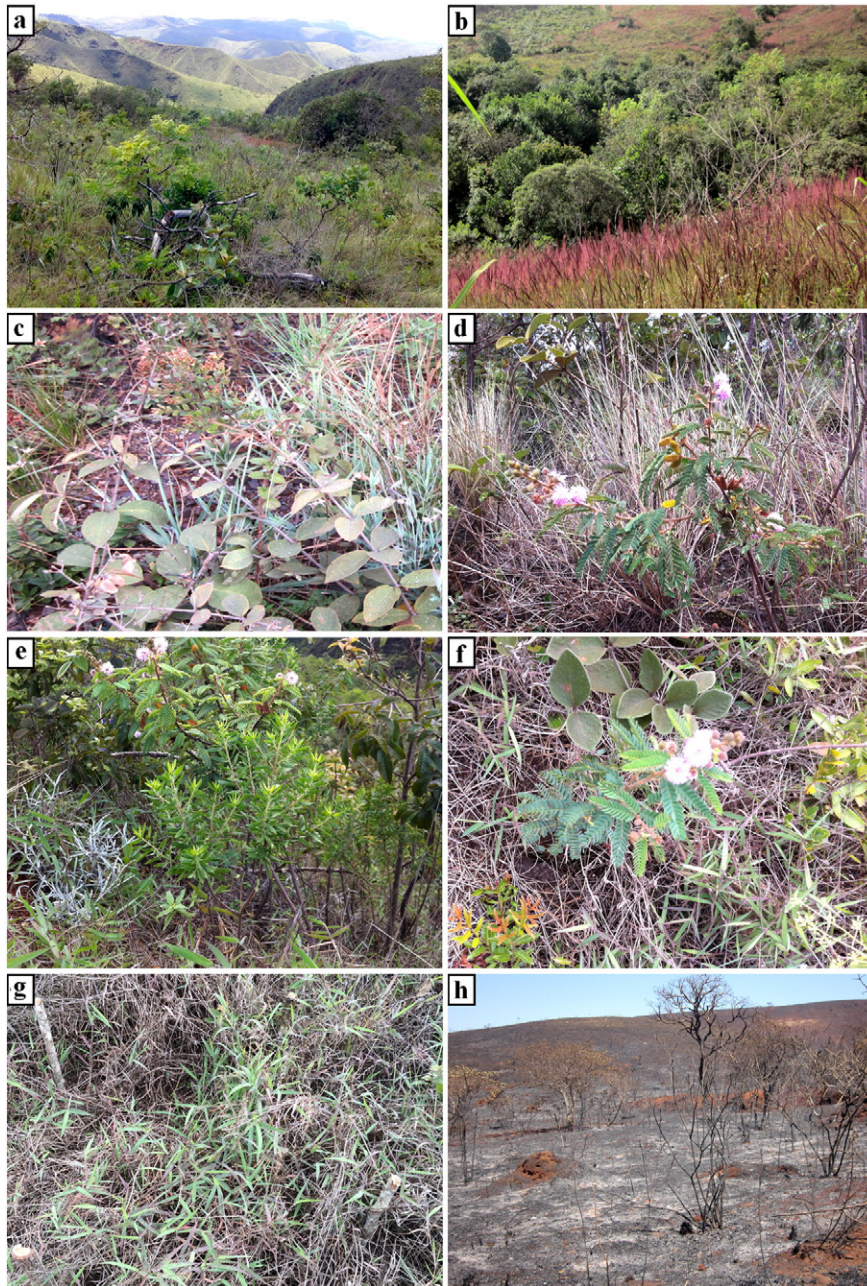


Fig. 1. Parque Estadual da Serra do Rola Moça (PESRM), Minas Gerais state, Brazil. Study site: (a) campo rupestre vegetation; (b) *Melinis minutiflora* invasion in PESRM; (c) treatment 1 (T1): native species without *Mimosa pogocephala* and no invasion; (d) treatment 2 (T2): native species plus *M. pogocephala* and no invasion; (e) treatment 3 (T3): native species plus *M. pogocephala* and $\leq 50\%$ invasion; (f) Treatment 4 (T4): native species plus *M. pogocephala* and $> 50\%$ invasion; (g) Treatment 5 (T5): full invasion of *M. minutiflora* without *M. pogocephala*; and (h) campo rupestre reached by fire (2011).

vegetation has been invaded by *M. minutiflora* (Fig. 1b), which is the main cause of fire propagation in the PESRM (Fig. 1h).

2.2. Experimental design

The experimental site was established as 600 m², or 0.06 ha, of a grassy field of campo rupestre at 1440 m above sea level (20°03'22" S, 44°01'09" W). The experimental design consisted of five treatments or plots (4 m² per plot) and three replicates (blocks), forming three plots/treatment/block and a total of nine plots/treatment. The treatments were randomly distributed in each block (200 m²). The five treatments (Fig. 1) were as follows: (T1) plots

with native vegetation, without the studied legume species (*M. pogocephala*) and no invasion (Fig. 1c); (T2) plots with native vegetation plus *M. pogocephala* and no invasion (Fig. 1d); (T3) plots with native vegetation plus *M. pogocephala* and $\leq 50\%$ invasion by *M. minutiflora* (Fig. 1e); (T4) plots with native vegetation plus *M. pogocephala* and $> 50\%$ invasion by *M. minutiflora* (Fig. 1f); and (T5) full invasion by *M. minutiflora* without *M. pogocephala* (Fig. 1g).

For the leaf analyses, samples were collected from plants belonging to the Malpighiaceae, Asteraceae and Poaceae families, and *M. pogocephala* and *M. minutiflora* species. The leaf samples were collected from three individuals/family or species/treatment/block. The

plots sampled were only from T1–T4 because T5 did not contain any native species.

2.3. Species distribution

After the demarcation of plots in the field, species occupation and occurrence were measured using the method proposed by Toledo and Schultze-Kraft (1982). This method uses a 1 m² quadrant composed of 100 squares of 0.01 m² each. The quadrant was placed inside the plots and then the vegetation in each square was identified.

2.4. Soil analysis

Each soil sample consisted of two mixed samples/plot (5 cm depth or 200–300 g). The chemical analysis was performed according to Embrapa (1997). The soil samples were kept cool (4 °C) during sampling and transport and were maintained at 2 °C until processed. Field soil samples had their total N level determined by semimicro-Kjeldahl digestion (Bremner, 1960), and N ammonia and nitrate contents were determined using the steam distillation method of Bremner and Keeney (1965) after extracting 10 g of soil with 2 mol L⁻¹KCl.

2.5. Leaf analysis

Leaf samples were taken in the field with three replicates from three individuals of each family or species (Malpighiaceae, Asteraceae and Poaceae families; *M. pogocephala* and *M. minutiflora* species)/treatment/block. The samples were dried at 70 °C for at least 48 h and, after reaching a constant weight, were ground to a fine powder in a ball mill (Glen Creston Ltd.). They were then analysed for their total carbon (C), N and $\delta^{15}\text{N}$ contents using an isotope ratio mass spectrometer (Finnigan MAT Delta E, Thermo Electron, Bremen, Germany) coupled to an EA 1110 elemental analyser (Thermo Electron, Milan, Italy), where.

$$\delta^{15}\text{N}(\text{‰}) = \left(\frac{\text{sample atom}\%^{15}\text{N} - 0.3663}{0.3663} \right) \times 1000$$

i.e., the abundance of ^{15}N is expressed in “delta” notation (δ), which is the deviation per thousand (‰) of ^{15}N abundance of the sample in relation to the international standard (i.e. atmospheric N_2), which has an atom% of $^{15}\text{N} = 0.3663$ (Unkovich et al., 2008).

2.6. Identification of *M. pogocephala* nodule bacteria

M. pogocephala effective nodules (internal pink colour) were grown on yeast mannitol agar according to Vincent (1970). The 16S rRNA genes were amplified as described by Rivas et al. (2007) and nearly complete sequences (>1400 bp) were obtained by Macrogen Corporation (Amsterdam, The Netherlands). The sequences obtained were compared with those on the EzTaxone-server. The sequences were aligned using ClustalW software. Phylogenetic trees were constructed with the neighbour-joining method (Saitou and Nei, 1987) using Kimura (1980) two-parameter model in Mega5 software. Confidence in the nodes of the trees was determined by bootstrap analysis using 1000 repetitions.

2.7. Quantitative PCR (qPCR)

The soil abundance of ammonia-oxidising bacteria (AOB) was determined using qPCR to estimate the number of soil bacteria expressing the ammonia monooxygenase genes (*amoA*). The MoBio (Carlsbad, CA, USA) Power Soil DNA extraction kit was used on each soil sample (0.5 g), following the recommended protocol. DNA concentration and purity were assessed using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and the PCR reactions were carried

out in an Applied Biosystems Quant Studio Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) with an iQ SYBR Green Supermix from Bio-Rad (Hercules, CA, USA). Bacterial *amoA* gene quantification was performed using the *amoA*-1F (5'-GGGGTTTCTACTGGTG GT-3') and *amoA*-2R (5'-CCCTCGGGAAGCCTTCTTC-3') primers (Rotthauwe et al., 1997) with the following thermocycling conditions: 30 s at 95 °C, followed by 40 cycles of 30 s at 95 °C, 34 s at 60 °C, and 60 s at 72 °C. A calibration curve was obtained using a 10-fold serial dilution of a known concentration of positive control DNA (ranging from 10² to 10⁸). The C_T values obtained from each sample were then compared with the standard curve to determine the DNA concentration of the original sample.

2.8. Data analysis

All data were appraised by analysis of variance (ANOVA). Means were compared using either Tukey's test at 5% probability level or the Kruskal–Wallis non-parametric test (at 5% probability level) when the assumptions of ANOVA were not met. All analyses were performed with R statistical software (version 3.2.2).

3. Results and discussion

3.1. Site occupation and plant invasion

Fig. 2 shows the species occupation indices of plots under different treatments. The occupation index of the native species in the study site was ~80% and the remaining 20% consisted of rocky outcrop (T1). In the plots where *M. pogocephala* was included (T2, T3 and T4) the occupation index was ~5% and was independent of the treatment. The *M. minutiflora* invasion process occurred by initially displacing the native species (T3), then occupying all of the open spaces (T4 and T5), reaching almost 100% occupation. Thus, the invasive plant produced more biomass than the native species, as previously described for other invasive plants (Hoffmann and Haridasan, 2008). The invasive behaviour shown by *M. minutiflora* might be associated with a nutritional demand (Barger et al., 2003; Hoffmann and Haridasan, 2008), particularly for N, which is the limiting nutrient in the majority of biomes (Laungani and Knops, 2009) and is considered the main nutritional demand for *M. minutiflora* plants (Barger et al., 2003).

3.2. Biological nitrogen fixation (BNF) as the biome N source.

M. pogocephala formed effective root nodules in the campo rupestre, and the nodules from the experimental site were predominantly occupied by *Burkholderia* strains closely related to *Burkholderia nodosa* (Fig. 3), a nodulating *Mimosa* species only found, to date, in Brazil (Moulin et al., 2015). Indeed, dos Reis et al. (2010) found that several *Mimosa* species from the Cerrado biome are nodulated by *Burkholderia* species, but this is the first report of nodulation in the endemic species, *M. pogocephala*.

The high biomass produced by the invasive grass depends largely on soil N availability (Laungani and Knops, 2009) because species of Poaceae show an elevated leaf C:N ratio compared to Fabaceae (James, 2008). The invasive species showed the lowest leaf N (T3: $p < 0.001$ and T4: $p = 0.001$) and C (T3: $p = 0.0195$) contents among the studied species (Figs. 4a and b). In contrast, *M. pogocephala* presented a higher C content (T2: $p = 0.020$; T3 = 0.008) than the Poaceae plants, as well as the highest (1.5–2.0%) N content (T2: $p < 0.001$; T3: $p < 0.001$). The leaf C:N ratio (Fig. 4c) was significantly lower in *Mimosa* leaves for T2, T3 and T4. However, the C:N ratio found in *M. minutiflora* leaves was high and comparable to the previous results of Asner and Beatty (1996) in Hawaii. As expected, the presence of *Burkholderia* in nodules resulted in the highest N leaf content and the lowest C:N ratio of the Fabaceae, highlighting the role of this species in maintaining the fertility of the campo rupestre biome. Thus, this species is likely to be a pivotal source

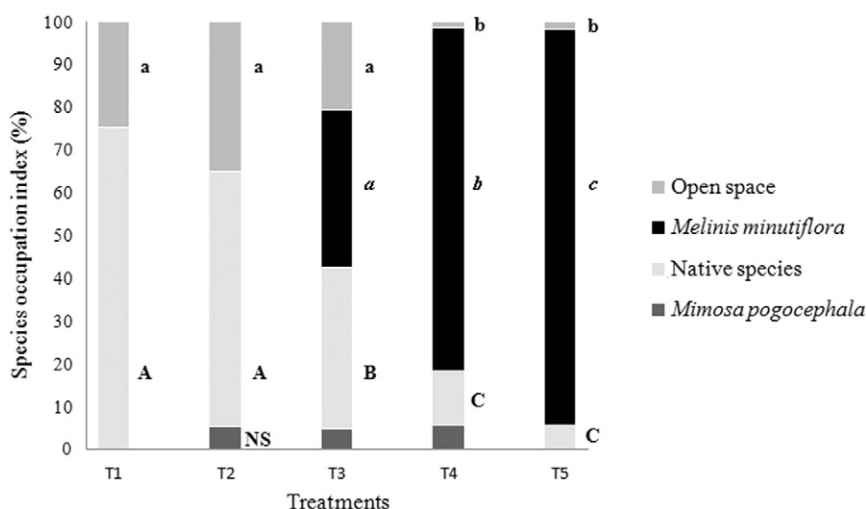


Fig. 2. Vegetation occupation index of open spaces in the plots treated as follows: (T1) native species without *Mimosa pogocephala* and no invasion; (T2) native species plus *M. pogocephala* and no invasion; (T3) native species plus *M. pogocephala* and $\leq 50\%$ invasion; (T4) native species plus *M. pogocephala* and $> 50\%$ invasion; and (T5) full invasion of *Melinis minutiflora* without *M. pogocephala*. Means with different letters are significantly different, according to the Mann-Whitney test at 5% confidence level ($p \leq 0.05$).

of N-organic matter for all the species in the study site, possibly constituting a positive feedback mechanism for *M. minutiflora* invasion (Laungani and Knops, 2009; Mangla et al., 2011).

The $\delta^{15}\text{N}$ differences recorded between the leguminous (*M. pogocephala*) and reference (non-leguminous) species suggested that the N content in *M. pogocephala* leaves (Fig. 5) probably originated from BNF. The $\delta^{15}\text{N}$ values of N-fixing species are relatively more

negative than reference species due to the isotopic atmospheric discrimination promoted by N-fixing bacteria (dos Reis et al., 2010). Therefore, measuring the leaf $\delta^{15}\text{N}$ abundance under natural conditions can assist distinguishing the potential N_2 -fixing plants from non-fixing plants (dos Reis et al., 2010). This way, the field results of intense depleted $\delta^{15}\text{N}$ signals in *M. pogocephala* leaves, the presence of *Burkholderia* strains in its root nodules, as well as the higher leaf N

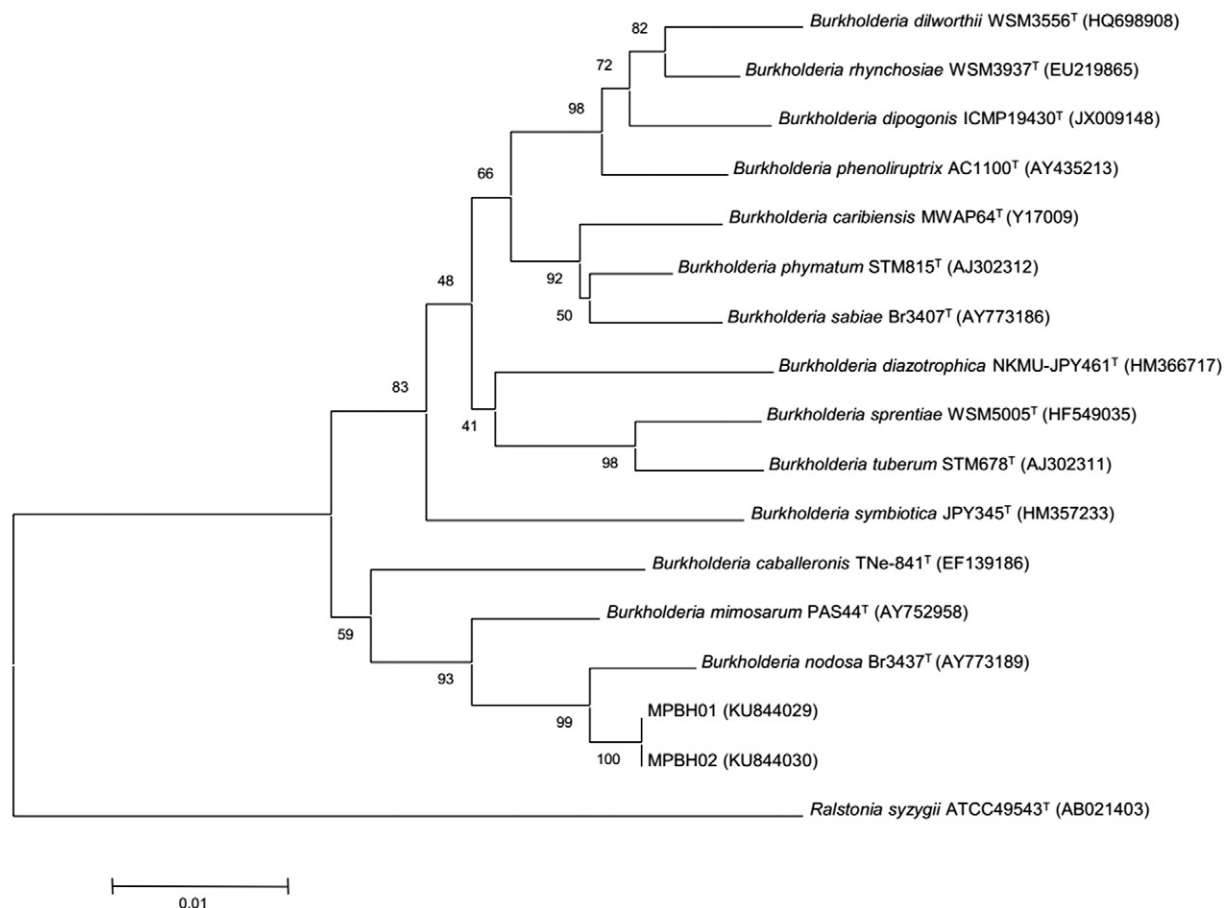


Fig. 3. Maximum likelihood phylogenetic tree based on 16S–23S rDNA intergenic spacer (ITS) sequences (604 nt) showing the position of strains isolated from nodules within the genus *Burkholderia*. Bootstrap values were calculated for 1000 replications. Bar. 1 nt substitution per 100 nt.

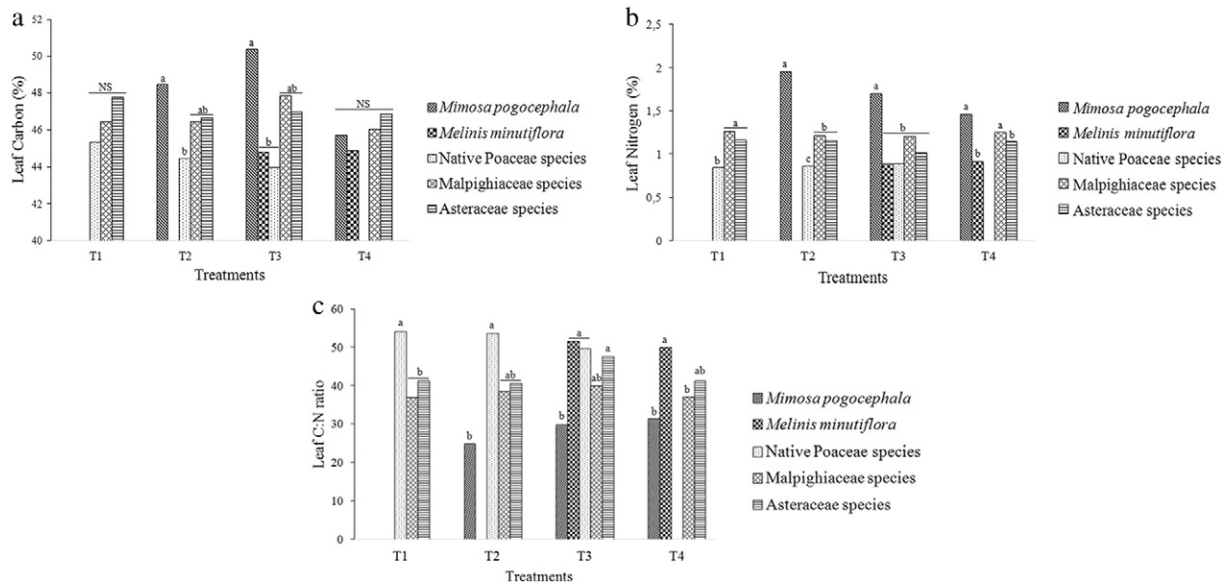


Fig. 4. Contents of nitrogen (a), and carbon (b), and the carbon-to-nitrogen ratio (c) in leaves of *Mimosa pogocephala*, *Melinis minutiflora*, Poaceae native species, Malpighiaceae and Asteraceae species from treatments T1-T4. The bars represent the means $\pm$ SEM (standard error of the mean). Treatments: (T1) native species without *M. pogocephala* and no invasion; (T2) native species plus *M. pogocephala* and no invasion; (T3) native species plus *M. pogocephala* and $\leq 50\%$ invasion; and (T4) native species plus *M. pogocephala* and $>50\%$ invasion. Means with different letters are significantly different according to Tukey's multiple range test at 5% confidence level ($p \leq 0.05$).

content and lower C:N ratio indicate the contribution of BNF to the growth of *M. pogocephala* and its role in maintaining the N-related fertility of campo rupestre soils.

3.3. Soil fertility and plant invasion

The low soil fertility of campo rupestre (Carvalho-Filho et al., 2010; Messias et al., 2013) is incongruent with the high plant biodiversity and endemism (Viana and Lombardi, 2007), as well as the rampant presence of an invasive exotic plant. Such species generally exhibit a high nutrient demand and reach dominance in more productive sites (Barger et al., 2003). Indeed, our results (Table 1) show that the soil from the study site cannot be considered as infertile because its nutrient content is similar to soils from most of the preserved Brazilian biomes. The low soil fertility of campo rupestre (Carvalho-Filho et al., 2010; Messias et al., 2013) is incongruent with the high plant biodiversity and endemism (Viana and Lombardi, 2007), as well as the rampant presence of an invasive exotic plant. Such species generally exhibit a high nutrient demand and reach dominance in more productive sites (Barger et al., 2003). Indeed, our results (Table 1) show that the soil

from the study site cannot be considered as infertile because its nutrient content is similar to soils from most of the preserved Brazilian biomes (Table 2). Whereas the P content in campo rupestre soil was similar to that in Caatinga and Cerrado biome soils, the K and Ca contents were comparable to those of Cerradão forest. Only Mg was found low in the study site but comparable to Cerradão forest. The soil pH at the study site was similar to that of Atlantic forest, which is attributed to the high organic matter content. The organic matter (OM) and Cation exchange capacity (CEC) were unexpectedly high in the campo rupestre biome, which suggests that OM is the main source of nutrients in this biome. A high soil OM level was similarly found by Carvalho-Filho et al. (2015) and our team (Baião, 2012), who discovered elevated amounts of humic acid in the vegetation islands of the campo rupestre.

The similarity of soil nutrient profiles among campo rupestre soils and various natural and undisturbed ecosystems in Brazil suggests that this biome is unlikely to be considered infertile.

Therefore, the inorganic nutrient sources in these soils are mostly associated with organic matter decomposition as evidenced by the high soil organic matter content (Table 1), allied with the rock weathering effect. In fact, banded iron formations from campo rupestre contain mineral elements, such as K_2O , MgO , CaO and P_2O_5 (Spier et al., 2007), and are highly colonised by lichens (Baião, 2012). These lichens can produce lichenic acid that dissolves rock (Resende et al., 1996), slowly resulting in continuously available nutrients. The nutrients arising from litter decomposition and rocks may be sufficient to sustain the native vegetation. Indeed, at the start of the invasion process, the nutrient availability (particularly for the scarce calcium and magnesium) of T3 increased (Table 1), suggesting the contribution of organic matter decomposition that might have been stimulated by the invasive species (Schaeffer et al., 2012). This increase was followed by an intense depletion of soil nutrients (T4, Table 1), suggesting a nutrient immobilisation effect by the invasive plant. These immobilised nutrients returned to the soil with the leaf fall in the dry season (T5, Table 1), again becoming available to the plants.

The low fertility attributed to soils from natural ecosystems may be based on the fertility criteria used for agronomic purposes. Indeed, if we use the current agronomic classification of the soil fertility reference in Brazil (Embrapa, 1997), most or even all natural ecosystem soils would be considered infertile. Assuming that agriculturally managed sites cannot be compared to a balanced and nutrient-ratified soil

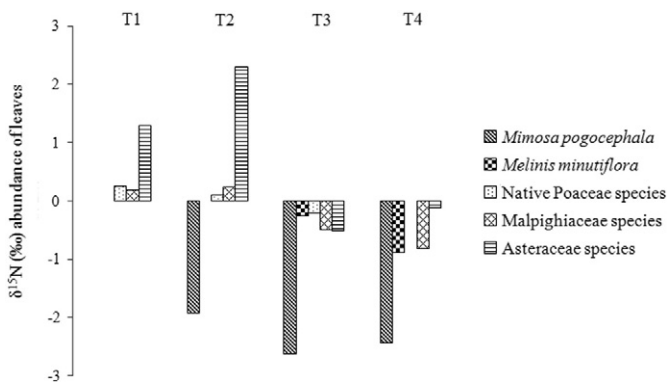


Fig. 5. Natural abundance of $\delta^{15}N$ in leaves of *Mimosa pogocephala*, *Melinis minutiflora*, native Poaceae, Malpighiaceae and Asteraceae species in the treatments as follows: (T1) native species without *M. pogocephala* and no invasion; (T2) native species plus *M. pogocephala* and no invasion; (T3) native species plus *M. pogocephala* and $\leq 50\%$ invasion; and (T4) native species plus *M. pogocephala* and $>50\%$ invasion.

Table 1

Physical and chemical analysis of soil samples from following treatments: T1 – Native species without *Mimosa pogocephala* and no invasion; T2 – Native species plus *M. pogocephala* and no invasion; T3 – Native species plus *M. pogocephala* and invasion $\leq 50\%$; T4 – Native species plus *M. pogocephala* and invasion $> 50\%$; T5 – full invasion without the presence of *M. pogocephala*. Means with different letters are significantly different as determined by Tuckey multiple-range test at the 5% confidence level ($p \leq 0.05$). NS: Do not differ at $p < 0.005$ by same test.

Treatments	pH	OM (%)	P (ppm)	K (cmol/dm ³)	Ca	Mg	CEC (meq/100 g)	C (%)	N	C:N
T1	4.2 ^b	8.5 ^{NS}	3.5 ^{NS}	0.11 ^a	1.567 ^b	0.176 ^b	8.5 ^{NS}	4.96 ^{NS}	0.30 ^{ab}	15.5 ^{NS}
T2	4.3 ^b	8.2	3.5	0.09 ^b	1.384 ^c	0.18 ^b	7.8	4.7	0.33 ^a	14.5
T3	4.5 ^b	8.6	3.3	0.11 ^a	1.920 ^a	0.36 ^a	8.8	4.9	0.31 ^{ab}	18.3
T4	4.6 ^b	5.1	3.0	0.09 ^b	1.167 ^d	0.22 ^b	6.2	2.99	0.24 ^b	12.4
T5	5.3 ^a	5.8	3.3	0.07 ^b	2.047 ^a	0.4 ^a	5.6	3.3	0.26 ^{ab}	14.0

Soil organic matter content (OM). Phosphorus (P). Potassium (K). Magnesium (Mg). Calcium (Ca). Cation exchange capacity (CEC). Total carbon. (C). Total nitrogen (N).

from natural vegetation under structural nor functional aspects, another measure of soil fertility must be considered. As defined by Marrs (1993), fertility is a function of the combined effects of all ecosystem processes that produce the supply of essential nutrients for plant uptake.

N is considered to be the most limiting soil nutrient (Laungani and Knops, 2009). Thus, the C:N ratio has long been considered an important tool to assess the soil fertility in both agricultural or natural ecosystems (Lange et al., 2014). Based on this parameter, although most natural ecosystems in Brazil are N-limited, they show a low C:N ratio (Table 3). Accordingly, we also recorded a low C:N ratio at the study site (Table 1), which is compatible with other undisturbed soils (Table 3), particularly tropical forest soils. The lowest values were found in plots where the native plants were associated with *M. pogocephala* (T2) with concomitant high soil N (± 3.0 g/kg) and C (8%) contents, which were much higher than those recorded in other Brazilian biomes (Table 3). These high soil N and C (organic matter) contents could be attributed to the decomposition of vegetation litter, particularly *M. pogocephala* litter. Therefore, the greater soil fertility promoted by the presence of this leguminous species may explain the aggressive invasion of the agropastoral *M. minutiflora* species that has a high nutritional demand, particularly for N (Barger et al., 2003). The N-associated fertility of campo rupestre was able to support a high degree of invasion.

3.4. Changes in the N cycle promoted by the invasive plant

The total soil N (Fig. 6a) was only depleted in T4 when molasses grass occupied $> 50\%$ of the site. However, while the soil ammonium (Fig. 6b) was significantly higher in the plots with native species (T1 and T2), particularly in the presence of *M. pogocephala* (T2), its content was depleted in T3 when the *M. minutiflora* invasion occurred. These results indicate that the slow-growing native species may parsimoniously use the ammonium, contrasting with the fast-growing invasive species that showed a more aggressive ammonium uptake (T3, T4 and T5). Similar results were recorded by Evans et al. (2001), who found that a decrease in inorganic N availability was caused by a *Bromus tectorum* invasion. Indeed, the vegetation type can influence soil N dynamics by various mechanisms (Ye et al., 2015).

The root uptake systems of nitrate and ammonium in plants show different affinities (Xu et al., 2012) due to the expression of different

protein transporters, which depends on the form and concentration of soil N (Nacry et al., 2013). Soil microorganisms show a substantially faster initial uptake of all N forms, hence, these communities may compete with plants for this nutrient (Kuzaykov and Xu, 2013). In undisturbed sites, the first chemical species produced is ammonium as a result of the decomposition of organic matter; therefore, this compound is likely to be the target of competition among plants and microorganisms. Indeed, the assimilation of ammonium is more energetically efficient when compared with that of nitrate because ammonium can be directly incorporated into glutamate through an ammonium assimilation pathway. Nitrate, however, has higher mobility (Brady and Weil, 1999). Thus, the environmental conditions in undisturbed sites may favour the uptake of ammonium by plants and microorganisms, particularly under grassy vegetation. Accordingly, our results confirmed that *M. pogocephala* litter is the major N source in this biome and ammonium is the prevalent N form in the soil of plots covered by native plants.

However, the plots invaded by *M. minutiflora* showed a very low ammonium content (T3, T4 and T5), independent of the degree of invasiveness, suggesting an aggressive and rapid ammonium uptake by this species since the beginning of the invasion process. Conversely, a high nitrate concentration was found in the invaded plot (T3, Fig. 6c) followed by a nitrate depletion (T4, Fig. 6c) indicating that the invasive plant may use both the N forms. The ability to use nitrate appears to be a competitive advantage of the invasive species because this N-based form seems to be intrinsically associated with the invasion process. A strong correlation exists between plant invasiveness and changes in the N cycle, generally including an increase in ammonium and/or nitrate availability in the soil after the invasion (Corbin and D'Antonio, 2004; Lazzaro et al., 2014; Sperry et al., 2006). However, the direction and extent of such effects vary extensively depending on species-specific interactions among invasive species, invaded communities and environmental factors, such as the soil type (Ehrenfeld, 2003).

While ammonium was reported as a positive feedback signal in invasive plants (Mangla et al., 2011), we found that nitrate was the N form increased by *M. minutiflora* at the beginning of the invasive process (T3). When the invasive grass reached its highest degree of invasiveness (T4 and T5), both soil nitrate and ammonium contents decreased what explain the decline in soil fertility (high C:N), promoted by the rampant invasion and plant uptake, as suggested by D'Antonio et al. (2011).

Table 2

Soil nutrient content under different Brazilian undisturbed vegetation types.

References	Moreno and Schiavini, 2001			Moraes et al., 1999	Correa et al., 2015	Results from present study
Variables/vegetation type	Gallery forest Cerrado	Tropical forest Cerrado	Cerradão	Atlantic Forest	Caatinga	Campo rupestre
pH	5.20	5.80	5.08	3.2	5.80	4.30
OM (dag/kg ou %)	4.00	3.20	2.40	5.90	1.20	8.00
P (mg/dm ³ ou ppm)	5.34	2.25	1.65	5.29	3.66	3.50
K (cmol/dm ³)	0.20	0.28	0.17	0.19	0.22	0.10
Mg (cmolc/dm ³)	1.18	1.44	0.49	0.78	0.35	0.18
Ca (cmol/dm ³)	3.92	3.86	1.06	5.10	2.03	1.48
CEC (cmolc/dm ³)	5.48	5.60	2.10	4.71	3.70	8.00

Soil organic matter content (OM). Phosphorus (P). Potassium (K). Magnesium (Mg). Calcium (Ca). Cation exchange capacity (CEC).

Table 3

Nitrogen soil content (mean values) in different undisturbed biomes.

Biome	Soil N content (g/kg)	C:N ratio	Reference
Campo Rupestre (undisturbed plots)	3.16	15.0	Present study
Amazon forest	2.37	12.30	Neill et al., 1997
Amazon forest	2.10	15.09	Hughes et al., 2002
Amazon basin pasture	1.60	16.07	Hughes et al., 2002
Cerrado forest	2.50	14.5	Groppa et al., 2015
Atlantic forest	2.00	19.80	Freitas et al. 2015
Cerrado pasture	1.80	15.60	Groppa et al., 2015

M. minutiflora reached its maximum invasiveness just before the dry season when the plant senesces. After, there is a return of nutrients to the soil, particularly N-chemical species (Fig. 6a) by litter decomposition (Asner and Beatty, 1996) or fire (D'Antonio, 2000). This favours the re-growth of the invasive plant and creates a positive feedback loop (Sperry et al., 2006; Stark and Norton, 2015; Suding, 2013).

The mechanism by which the molasses grass increased the nitrate availability in the soil may be associated with the increase in the nitrifying microbial community, as demonstrated by the increase in the *amoA* abundance (Fig. 7). This gene encodes ammonia monooxygenase, a key enzyme involved in the ammonia oxidation process carried out by AOB (Purkhold et al., 2000). The abundance of this microbial community increased in T3 (Fig. 7) when the invasion started and the highest soil nitrate concentration was observed. Both nitrate and AOB declined in T4 when the maximum invasion degree was reached and in T5 we could not detect AOB, possibly due to the depletion of ammonia substrate in this plot. Similar results were demonstrated by Hawkes et al. (2005),

who observed that the invasive exotic grasses doubled the gross nitrification rates by increasing the AOB soil abundance. In contrast, Ishikawa et al. (2003) observed an inhibition of the nitrification process by the invasive species *Brachiaria humidicola* due to the suppression of AOB in the soil. However, these authors failed to find that *M. minutiflora* had a similar inhibitory effect on this microbial population. The increase in the soil nitrate content promoted by *M. minutiflora* is probably due to the stimulation of the AOB through a rhizospheric effect. Thus, changes in the soil microbial community and N availability were driven by the invasive plant, favouring the nitrification process. Such alterations in soil N forms in favour of those used by *M. minutiflora* decreases the N available to native species that are displaced by the invasive species, decreasing biodiversity.

Furthermore, these results explain the increase of *M. minutiflora* invasion observed in managed areas of the park, where high N fertilisation has been applied. Therefore, based on our results, the use of chemical N fertilisation in restoration procedures in the campo rupestre, is not only

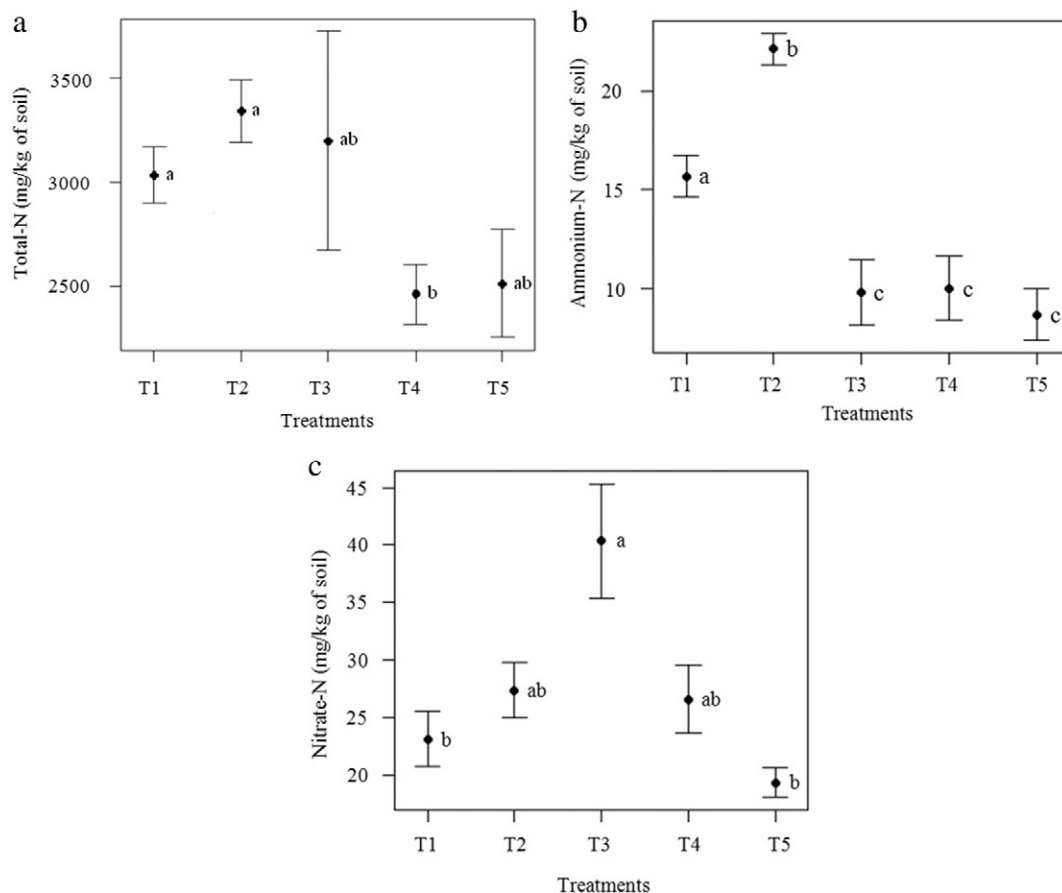


Fig. 6. Comparison between soil total N (a), ammonia-N (b) and nitrate-N (c) among treatments: (T1) native species without *Mimosa pogocephala* and no invasion; (T2) native species plus *M. pogocephala* and no invasion; (T3) native species plus *M. pogocephala* and $\leq 50\%$ invasion; (T4) native species plus *M. pogocephala* and $> 50\%$ invasion; and (T5) full invasion by *Melinis minutiflora* without *M. pogocephala*. Mean values $\pm$ SEM (standard error of the mean) according to the Mann-Whitney test at 5% confidence level ($p \leq 0.05$).

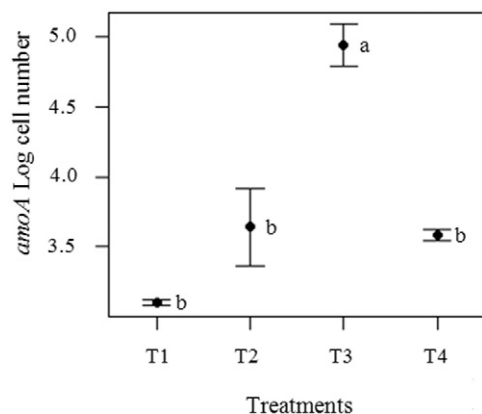


Fig. 7. Ammonia-monooxygenase bacteria abundance estimated by qPCR for the following treatments: (T1) native species without *Mimosa pogocephala* and no invasion; (T2) native species plus *M. pogocephala* and no invasion; (T3) native species plus *M. pogocephala* and $\leq 50\%$ invasion; (T4) native species plus *M. pogocephala* and $> 50\%$ invasion; and (T5) full invasion by *Melinis minutiflora* without *M. pogocephala*. Mean values $\pm$ SEM (standard error of the mean). Means with different letters are significantly different according to Tukey's multiple range test at 5% confidence level ($p \leq 0.05$).

unnecessary but should be strictly avoided because the increase in N availability would favour soil nitrification and consequently, the growth and further invasion of *M. minutiflora*.

4. Conclusions

The invasiveness of *M. minutiflora* is driven by nutritional demands, hence, the natural soil fertility of the campo rupestre can be considered sufficiently high to sustain the *M. minutiflora* invasion. The fertility levels of these soils were associated with the high N availability and low C:N ratio, which resulted from BNF promoted by *M. pogocephala* nodulated by *Burkholderia nodosa*. The ammonium content was improved in soils when the native species were dominant. The main strategy of the *M. minutiflora* invasion concerns the changes in the N chemical species in the soil through increasing nitrate and decreasing ammonium. The increase in the nitrification process by the invasive plant occurred through the stimulation of soil AOB at the beginning of the invasion. Changes in soil N forms, from ammonium to nitrate, with the depletion of the first, could explain the displacement of native species in favour of the invasive grass. Therefore, due to the high natural N-based fertility found in campo rupestre we recommend that the N fertilisation for restoration purposes is not necessary. Moreover, N fertilisation should be strictly avoided in the management of *M. minutiflora* in the PESRM because the increase in N availability would favour soil nitrification and, thus, its invasiveness.

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References

Asner, G.P., Beatty, S.W., 1996. Effects of an African grass invasion on Hawaiian shrubland nitrogen biogeochemistry. *Plant Soil* 186:205–211. <http://dx.doi.org/10.1007/BF02415515>.

Baião, E.B., 2012. Estudo da contribuição de *Mimosa calodendron* e da dupla simbiose de bactérias fixadoras de nitrogênio e fungos micorrízicos para fertilidade do campo

rupestre da Serra do Rola Moça (Dissertation of Universidade Federal de Minas Gerais).

Barger, N.N., D'Antonio, C.M., Ghneim, T., Cuevas, E., 2003. Constraints to colonization and growth of the African grass, *Melinis minutiflora*, in a Venezuelan savanna. *Plant Ecol.* 167:31–43. <http://dx.doi.org/10.1023/A:1023903901286>.

Brady, N.C., Weil, R.R., 1999. *The Nature and Properties of Soils*. 13th Edition. N.C. Prentice Hall, NJ 960 pp.

Bremner, J.M., 1960. Determination of nitrogen in soil by the Kjeldahl method. *Agric. Sci.* 55:11–33. <http://dx.doi.org/10.1017/S0021859600021572>.

Bremner, J.M., Keeney, D.R., 1965. Exchangeable ammonium, nitrate and nitrite by steam-distillation methods. In: Black, C.A. (Ed.), *Methods of Soil Analysis: Chemical and Microbiological Properties*. American Society of Agronomy Soil Sci Soc. Am J. Madison, pp. 595–730.

Bustamante, M.M.C., de Brito, D.Q., Kozovits, A.R., Luedemann, G., T.R.B., d.M., Pinto, A.S., Munhoz, C.B.R., Takahashi, F.S.C., 2012. Effects of nutrient additions on plant biomass and diversity of the herbaceous-subshrub layer of a Brazilian savanna (Cerrado). *Plant Ecol.* 213:795–808. <http://dx.doi.org/10.1007/s11258-012-0042-4>.

Carvalho-Filho, A., Curi, N., Shinzato, E., 2010. Relações solo-paisagem no Quadrilátero Ferrífero em Minas. Gerais. *Pesq. Agropec. Bras.* 45:903–916. <http://dx.doi.org/10.1590/S0100-204X2010000800017>.

Carvalho-Filho, A., Inda, A.V., Fink, J.R., Curi, N., 2015. Iron oxides in soils of different lithological origins in Ferriferous Quadrilateral (Minas Gerais, Brazil). *Appl. Clay Sci.* 118: 1–7. <http://dx.doi.org/10.1016/j.clay.2015.08.037>.

Corbin, J.D., D'Antonio, C.M., 2004. Effects of exotic species on soil nitrogen cycling: implications for restoration. *Weed Technol.* 18:1464–1467. [http://dx.doi.org/10.1614/0890-037X\(2004\)018\[1464:EOESOS\]2](http://dx.doi.org/10.1614/0890-037X(2004)018[1464:EOESOS]2).

Correa, E., Carvalhais, L., Utida, M., Oliveira, C.A., Scotti, M.R., 2015. Effect of plant species on P cycle-related microorganisms associated with litter decomposition and P soil availability: implications for agroforestry management. *iForest* <http://dx.doi.org/10.3832/ifor1459-0081>.

D'Antonio, C.M., 2000. Fire, plant invasions, and global changes. In: Mooney, H.A., Hobbs, R.J. (Eds.), *Invasive Species in a Changing World*. Island Press, pp. 65–93.

D'Antonio, C.M., Hughes, R.F., Tunison, J.T., 2011. Long-term impacts of invasive grasses and subsequent fire in seasonally dry Hawaiian woodlands. *Ecol. Appl.* 21: 1617–1628. <http://dx.doi.org/10.1890/1053-0703.2010.03267.x>.

Davis, M.A., 2003. Biotic globalization: Does competition from introduced species threaten biodiversity? *BioScience* 53, 481–489.

dos Reis Jr., F.B., Simon, M.F., et al., 2010. Nodulation and nitrogen fixation by *Mimosa* spp. in the Cerrado and Caatinga biomes of Brazil. *New Phytol.* 186:934–946. <http://dx.doi.org/10.1111/j.1469-8137.2010.03267.x>.

Ehrenfeld, J.G., 2003. Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems* 6:503–523. <http://dx.doi.org/10.1007/s10021-002-0151-3>.

Ehrenfeld, J.G., Scott, N., 2001. Invasive species and the soil: effects on organisms and ecosystem processes. *Ecol. Appl.* 11, 1259–1260.

EMBRAPA, 1997. *Manual de métodos de análise do solo*. EMBRAPA, Rio de Janeiro.

Evans, R.D., Rimer, R., Sperry, L., Belnap, J., 2001. Exotic plant invasion alters nitrogen dynamics in arid grassland. *Ecol. Appl.* 11:1301–1310. [http://dx.doi.org/10.1890/1051-0761\(2001\)011\[1301:EPIAND\]2.0](http://dx.doi.org/10.1890/1051-0761(2001)011[1301:EPIAND]2.0).

Freitas, A.D.S., Sampaio, E.V.S.B., Ramos, A.P.S., Barbosa, M.R.V., Lyra, R.P., Araújo, E.L., 2015. Nitrogen isotopic patterns in tropical forests along a rainfall gradient in Northeast Brazil. *Plant Soil* 391, 109–122.

Giulietti, A.M., Pirani, J.R., 1988. Patterns of geographical distribution of some plant species from Espinhaço range, Minas Gerais and Bahia, Brazil. In: Vanzolini, P.E., Heyer, W.R. (Eds.), *Proceedings of a Workshop on Neotropical Distribution Patterns*. Academia Brasileira de Ciências, Rio de Janeiro, pp. 39–69.

Groppio, J.D., Lins, S.R.M., Camargo, P.B., Assad, E.D., Pinto, H., Martins, S.C., Salgado, P.R., Evangelista, B., Vasconcellos, E., Sano, E.E., Pavão, E.R., Luna, R., Martinelli, L.A., 2015. Changes in soil carbon, nitrogen, and phosphorus due to land-use changes in Brazil. *Biogeochemistry* 12:4765–4780. <http://dx.doi.org/10.5194/bg-12-4765-2015>.

Hawkes, C.V., Wren, I.F., Herman, D.J., Firestone, M.K., 2005. Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. *Ecol. Lett.* 8:976–985. <http://dx.doi.org/10.1111/j.1461-0248.2005.00802.x>.

Hejda, M., Pšek, P., Jarošík, V., 2009. Impact of invasive plants on the species richness, diversity and composition of invaded communities. *J. Ecol.* 97:393–403. <http://dx.doi.org/10.1111/j.1365-2745.2009.01480>.

Hoffmann, W.A., Haridasan, M., 2008. The invasive grass, *Melinis minutiflora*, inhibits tree regeneration in a Neotropical savanna. *Austral Ecol.* 33:29–36. <http://dx.doi.org/10.1111/j.1442-9993.2007.01787.x>.

Hughes, R.F., Kauffman, J.B., Cummings, D.L., 2002. Dynamics of aboveground and soil carbon and nitrogen stocks and cycling of available nitrogen along a land-use gradient in Rondônia Brazil. *Ecosystems* 5:244–259. <http://dx.doi.org/10.1007/s10021-001-0069-1>.

Ishikawa, T., Subbarao, G.V., Ito, O., Okada, K., 2003. Suppression of nitrification and nitrous oxide emission by the tropical grass *Brachiaria humidicola*. *Plant Soil* 255: 413–419. http://dx.doi.org/10.1007/978-94-017-2923-9_40.

James, J.J., 2008. Leaf nitrogen productivity as a mechanism driving the success of invasive annual grasses under low and high nitrogen supply. *J. Arid Environ.* 72:1775–1784. <http://dx.doi.org/10.1016/j.jaridenv.2008.05.001>.

Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide-sequences. *J. Mol. Evol.* 16:111–120. <http://dx.doi.org/10.1007/BF01731581>.

Kuzaykov, Y., Xu, X., 2013. Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. *New Phytol.* 198:656–669. <http://dx.doi.org/10.1111/nph.12235>.

Lange, M., Habekost, M., Eisenhauer, N., Roscher, C., Bessler, H., Engels, C., Oelmann, Y., Scheu, S., Wilcke, W., Schulze, E.D., Gleixner, G., 2014. Biotic and abiotic properties

- mediating plant diversity effects on soil microbial communities in an experimental grassland. *PLoS ONE* 9:96182. <http://dx.doi.org/10.1371/journal.pone.0096182>.
- Laungani, R., Knops, J.M.H., 2009. Species-driven changes in nitrogen cycling can provide a mechanism for plant invasions. *PNAS* 106:12400–12405. <http://dx.doi.org/10.1073/pnas.0900921106>.
- Lazzaro, L., Giuliani, C., Fabiani, A., Agnelli, A.E., Pastorelli, R., Lagomarsino, A., Benesperi, R., Calamassi, R., Foggi, B., 2014. Soil and plant changing after invasion: the case of *Acacia dealbata* in a Mediterranean ecosystem. *Sci. Total Environ.* 497–498: 491–498. <http://dx.doi.org/10.1016/j.scitotenv.2014.08.014>.
- Mangla, S., Sheley, R.L., James, J.J., Radosevich, R.S., 2011. Intra and interspecific competition among invasive and native species, during early stages of plant growth. *Plant Ecol.* 212:531–542. <http://dx.doi.org/10.1007/s1258-011-9909-z>.
- Marrs, R.H., 1993. Soil fertility and nature conservation in Europe: theoretical considerations and practical management solutions. *Adv. Ecol. Res.* 24:241–300. [http://dx.doi.org/10.1016/S0065-2504\(08\)60044-6](http://dx.doi.org/10.1016/S0065-2504(08)60044-6).
- Messias, M.T.B., Leite, M.G.P., Neto, J.A.A., Kozovits, A.R., Tavares, R., 2013. Soil-vegetation relationship in quartzitic and ferruginous Brazilian rocky outcrops. *Folia Geobot.* 48, 509–521.
- Moraes, R.M., Delitti, W.B.C., Vuono, Y.S., 1999. Litterfall and litter nutrient content in two Brazilian tropical forests. *Rev. Bras. Bot.* 1:1–4. <http://dx.doi.org/10.1590/S0100-84041999000100002>.
- Moreno, M.I.C., Schiavini, I., 2001. Relação entre vegetação e solo em um gradiente florestal na Estação Ecológica do Panga, Uberlândia (MG). *Braz. J. Bot.* 24:537–544. <http://dx.doi.org/10.1590/S0100-84042002000300004>.
- Moulin, L., James, E.K., Klonowska, A., et al., 2015. Phylogeny, diversity, geographical distribution, and host range of legume-nodulating beta proteobacteria: what is the role of plant taxonomy? In: de Bruijn, F.J. (Ed.), *Biological Nitrogen Fixation*. John Wiley and Sons, Inc., New Jersey, pp. 177–190.
- Nacry, P., Bouguyon, E., Gojon, A., 2013. Nitrogen acquisition by roots: physiological and developmental mechanisms ensuring plant adaptation to fluctuation resource. *Plant Soil* 370:1–29. <http://dx.doi.org/10.1007/s11104-013-1645-9>.
- Neill, C., Melillo, J.M., Steudler, P.A., Cerri, C.C., Moraes, J.F.L., Piccolo, M.C., Brito, M.S., 1997. Soil carbon and nitrogen stocks following forest clearing for pasture in the south-western Brazilian Amazon. *Ecol. Appl.* 7:1216–1225. [http://dx.doi.org/10.1890/1051-0761\(1997\)007\[1216:SCANSF\]2.0.CO;2](http://dx.doi.org/10.1890/1051-0761(1997)007[1216:SCANSF]2.0.CO;2).
- Purkhold, U., Pommerening-Röser, A., Juretschko, S., Schmid, M.C., Koops, H.P., Wagner, M., 2000. Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and amoA sequence analysis: implications for molecular diversity surveys. *Appl. Environ. Microbiol.* 66:5368–5382. <http://dx.doi.org/10.1128/AEM.66.12.5368-5382.2000>.
- Resende, M.A., de Castro Rezende, G., Viana, E.M., Becker, T.W., Warscheid, T.H., 1996. Acid production of fungi isolated from stones of historical monuments of state of Minas Gerais, Brazil. Second Latin American Biodeterioration Symposium, pp. 65–67 Gramado.
- Rivas, R., García-Fraile, P., Mateos, P.F., et al., 2007. Characterization of xylanolytic bacteria present in the bract phyllosphere of the date palm *Phoenix dactylifera*. *Lett. Appl. Microbiol.* 44:181–187. <http://dx.doi.org/10.1111/j.1472-765X.2006.02050.x>.
- Rizzini, C.T., 1979. *Tratado de Fitogeografia do Brasil; aspectos sociológicos e florísticos*. Hucitec/Universidade de São Paulo, São Paulo.
- Rotthauwe, J.H., Witzel, K.P., Liesack, W.T., 1997. The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. *Appl. Environ. Microbiol.* 63, 4704–4712. doi:10.11.323.2405.
- Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4:406–425. <http://dx.doi.org/10.1093/molbev/msm092>.
- Schaeffer, S.M., Ziegler, S.E., Belnap, J., Evans, R.D., 2012. Effects of *Bromustectorum* invasion on microbial carbon and nitrogen cycling in two adjacent undisturbed arid grassland communities. *Biogeochemistry* 111:427–441. <http://dx.doi.org/10.1007/s10533-011-9668-x>.
- Scotti, M.R., Teles, I., Teixeira, A.P., Freitas, I., Ribeiro, B., Muguet, N., Uber-Buceck, E., 2012. The invasiveness of the grass *Melinis minutiflora* is favoured by fire and displaces the Endemic species in Brazil. Terrestrial invasive Plant species Conference, Sault Ste Marie Ontario, Canada. August. 2012.
- Sperry, L.J., Belnap, J., Evans, R.D., 2006. *Bromus tectorum* invasion alters nitrogen dynamics in an undisturbed arid grassland ecosystem. *Ecology* 87:603–615. <http://dx.doi.org/10.1890/05-0836>.
- Spier, C.A., Oliveira, S.M.B., Sial, A.N., Rios, F.J., 2007. Geochemistry and genesis of the banded iron formations of the Cauê Formation, Quadrilátero Ferrífero, Minas Gerais, Brazil. *Precambrian Res.* 152:170–206. <http://dx.doi.org/10.1016/j.precamres.2006.10.003>.
- Stark, J.M., Norton, J.M., 2015. The invasive annual cheatgrass increases nitrogen availability in 24-year-old replicated field plots. *Oecologia* 177:799–809. <http://dx.doi.org/10.1007/s00442-014-3093-5>.
- Suding, K.N., 2013. Ecology: a leak in the loop. *Nature* 503:472–473. <http://dx.doi.org/10.1038/nature12838>.
- Toledo, J.M., Schultze-Kraft, R., 1982. Metodologia para La evaluación agronómica de pastos tropicales. In: Toledo, J.M. (Ed.), *Manual para La evaluación agronómica. RedIntercionaldeEvaluación de pastos Tropicales (RIEPT)*. Centro Internacional de agricultura Tropical (CIAT), Cali, Colombia, pp. 91–110.
- Unkovich, M.J., Herridge, D.F., Peoples, M.B., Cadisch, G., Boddey, R.M., Giller, K.E., et al., 2008. *Measuring Plant-Associated Nitrogen Fixation in Agricultural Systems*. ACIAR, Canberra Australia.
- Viana, P.L., Lombardi, J.A., 2007. Florística e caracterização dos campos rupestres sobre canga na Serra da Calçada, Minas Gerais, Brasil. *Rodriguésia*. 58 pp. 159–177.
- Vincent, J.M., 1970. The cultivation, isolation and maintenance of rhizobia. In: Vincent, J.M. (Ed.), *A Manual for the Practical Study of Root-Nodule Bacteria*. Blackwell Scientific Publications, Oxford, pp. 1–13.
- Xu, G.H., Fan, X.R., Miller, A.J., 2012. Plant nitrogen assimilation and use efficiency. *Annu. Rev. Plant Biol.* 63:153–182. <http://dx.doi.org/10.1146/annurev-arplant-042811-105532>.
- Ye, C., Cheng, X., Liu, W., Zhang, Q., 2015. Revegetation impacts soil nitrogen dynamics in the water level fluctuation zone of the Three Gorges Reservoir, China. *Sci. Total Environ.* 517:76–85. <http://dx.doi.org/10.1016/j.scitotenv.2015.02.068>.
- Yelenik, S.G., D'Antonio, C.M., 2013. Self-reinforcing impacts of plant invasions change over time. *Nature* 503:517–520. <http://dx.doi.org/10.1038/nature12798>.
- Zavaleta, E., 2000. Valuing ecosystem services lost to *Tamarix* invasion in the United States. In: Mooney, H.A., Hobbs, R.J. (Eds.), *The Impact of Global Change on Invasive Species*. Island Press, Washington, D.C., pp. 261–300.