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The role of native species as biome resistance factors on molasses grass (*Melinis minutiflora* Beauv.) invasion in rupestrian fields in Minas Gerais State, Brazil

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Dedicatória

À minha tia Valdeniz (*in memoriam*)

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Introdução Geral

Espécies exóticas podem se disseminar pelo meio ambiente, atuar como uma espécie invasora e ocupar nichos que antes eram ocupados por nativas, substituindo o seu papel no ecossistema (Beck et al. 2008) e não apenas competindo com elas, mas sim causando mudanças em todos os processos relacionados a sua sobrevivência (Vitousek et al. 1997). A União Internacional para Conservação da Natureza – UICN (1999) define espécie invasora como uma exótica que se estabeleceu em habitats ou ecossistemas naturais causando mudanças e ameaçando a biodiversidade nativa. A invasão biológica consiste na obtenção de vantagens por uma espécie após o desaparecimento dos obstáculos naturais a sua proliferação, o que permite sua rápida disseminação para novas áreas dentro dos ecossistemas invadidos, nos quais se torna dominante (Valery et al. 2008). A transformação de uma espécie exótica em invasora é um caso especial de mudança da composição da comunidade vegetal onde uma espécie substitui uma ou mais nativas, causando sérias consequências à conservação e utilização de serviços ecossistêmicos (Ehrenfeld 2003).

O processo de invasão é amplamente conhecido como uma das maiores ameaças à biodiversidade (Wilcove et al 1998, Mack et al. 2000) . Segundo Pysek et al. (2011) as espécies exóticas podem alterar os habitats invadidos através de uma variedade de mecanismos que tem o potencial de repercutir sobre a hierarquia ecológica e afetar os processos ecológicos. Os impactos causados pela invasão são muitos: redução da riqueza, abundância e diversidade das espécies nativas locais (Pysek et al. 2011), alteração dos processos ecossistêmicos como produtividade primária (Vitousek et al. 1997, Pysek et al. 2010), decomposição, hidrologia, geomorfologia, ciclagem de nutrientes, regimes de perturbação (Vitousek et al. 1997, Richardson et al. 2000), competição por recursos (Lavergne et al. 1999, Martin 1999), alterações na população microbiana do solo (Grayston & Campbell 1998, Grierson & Adams 2000, Sanon et al. 2009) e principalmente favorece alterações no regime do fogo, especialmente na propagação do mesmo (Asner & Beatty 1996). Não existe uma resposta ambiental padrão a invasão porque as alterações dependem das interações de cada espécie invasora com o ecossistema invadido. Em alguns casos, a mesma espécie pode ter diferentes efeitos quando áreas com diferentes características são comparadas (Ehrenfeld 2003).

No estado de Minas Gerais, Brasil, existe um tipo particular de vegetação denominada campo rupestre que ocorre sobre afloramento rochoso de canga hematítica, que são solos ricos em minério de ferro onde há predominância de campo graminoso na região conhecida como Quadrilátero Ferrífero (Rizzini 1979), que por causa da mineração é um dos ecossistemas mais ameaçados do Estado (Matias et al. 2009). Essa vegetação de Campos Rupestres Ferruginosos ocorre em altitudes superiores a 1000m (Giulietti & Pirani 1997) e pertence ao bioma Cerrado, um dos mais ameaçados do mundo (Myers et al. 2000). Essa vegetação ocorre entre frestas dos afloramentos constituindo ilhas de vegetação de diferentes tamanhos onde funcionalmente se estabelecem os nichos ecológicos. O intemperismo das rochas propicia a formação de ilhas, sendo que nas maiores há maior pulverização das rochas e formação de solo, contrastando com as de menor tamanho. Áreas com incidência de solo ocorrem sobre a canga nodular e apresentam uma vegetação mais densa, especialmente o campo graminoso (Conceição et al. 2007). O solo encontrado em cangas pode ser caracterizado como raso, deficiente em estrutura e nutricionalmente pobre (Matias et al. 2009). Apesar do ambiente hostil, a flora encontrada sobre estas formações é diversa, incluindo espécies ameaçadas e endêmicas. Devido a grande biodiversidade encontrada no Quadrilátero Ferrífero esta região é classificada como sendo de extrema importância biológica (Viana & Lombardi 2007).

O Parque Estadual da Serra do Rola Moça (PESRM) é um dos mais importantes redutos de preservação de campos rupestres no estado de Minas Gerais (Plano de Manejo do Parque Estadual da Serra do Rola Moça 2007) cuja área tem sofrido com a invasão da gramínea africana *Melinis minutiflora* Beauv., conhecida como capim-gordura ou capim-meloso (Hoffmann et al. 2004). Esta gramínea exótica é conhecida pelo seu cheiro adocicado e exudados oleosos presentes nos tricomas foliares (Parsons 1972). A introdução de *M. minutiflora* no Brasil ocorreu para o seu uso na criação de gado, onde era utilizado para a nutrição dos animais (Morosini & Klink 1997) devido as suas características nutricionais (Parsons 1972). Atualmente esta espécie tem sido usada para revegetação de cavas de minas o que favoreceu a disseminação de suas sementes pela água e vento, permitindo assim que a invasão atinja em torno de 60% da área do parque (Scotti et al. 2012).

A instalação de *M. minutiflora* favorece a incidência de fogo devido às resinas e óleos foliares, fazendo com que seja altamente inflamável até mesmo em condições

ambientais em que a umidade relativa do ar está alta (D'Antonio & Vitousek 1992). Com maior incidência e intensidade do fogo um feedback positivo é criado onde a espécie beneficiada é a invasora devido ao seu rápido reestabelecimento e dispersão após o incêndio (Hughes et al. 1991). Essa situação leva a uma tendência de dominância de capim meloso sobre as espécies nativas, causando a perda de espécies vegetais (D'Antonio & Vitousek 1992).

A resistência do bioma é um fenômeno que se refere a fatores ambientais que servem para limitar a disseminação e crescimento de espécies invasoras (D'Antonio & Thomsen 2004), sendo particularmente importante no início do processo de invasão quando o invasor ainda está se instalando (Vermeij 1996).

Este estudo teve como objetivo analisar os diferentes mecanismos utilizados pelas espécies nativas do campo rupestre presente no PESRM para alterar e tentar conter o processo de invasão por *M. minutiflora.*

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Limiting effects of plant community on molasses grass (*Melinis minutiflora* Beauv.) invasion in rupestrian fields

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Abstract

Exotic species can spread in the environment, act as an invasive, occupy a niche that was of native species before and replace their role. The impacts caused by invasive species on the native ones show in many ways: decreasing species richness and abundance of native biota, and reducing their local species diversity. In Minas Gerais State, Brazil, a particular kind of vegetation occurs associated with rich iron ore, composed mostly by grass species with representatives of Fabaceae, Asteraceae, Loganiaceae and Malpiguiaceae plants, among others. The native legume *Periandra mediterranea* (Vell.) Taub. is found spread at the area coexisting with native and alien species. The Parque Estadual Serra do Rola Moça (PESRM) is one of the main strongholds for rupestrian fields' preservation and has been suffering gradual invasive process by the African grass *Melinis minutiflora* Beauv. what has favored fire incidence. Plants can reveal resistance to invading species population growth and works as barrier. In our work we studied the effects of the invasive grass *M. minutiflora* on plant community and their resistance to invasion in order to subsidize the management of the invasive species *M. minutiflora*. To evaluate these effects we analyzed: relative frequency of species and families, community richness and composition. We could see a clear resistance to invasion by vegetal community at the lowest levels of occupancy by *M. minutiflora* as shown by the species: *Sporobolus aeneus* (Poaceae), *Chamaecrista secunda* (Fabaceae), *Paspalum polyphyllum* (Poaceae), *Peixotoa tomentosa* (Malpiguiaceae), *Achyrocline satureioides* (Asteraceae) and *Periandra mediterraneae* (Fabaceae). The latter was a key element for this resistance; having its presence inhibited only when the alien grass became dominant. The data of this study is very important to understand the impacts of invasion and the mechanisms of resistance presented by rupestrian fields at PERSM, showing the negative effects of *M. minutiflora* on native plant species diversity and also suggesting the resistance and tolerance of *P. mediterranea* at initial invasion phase.

Keywords

Biome resistance; species richness; *Periandra mediterranea*; *Melinis minutiflora*.

Introduction

An exotic species can spread in the environment, begin to act as an invasive and occupy a niche that was of native species before, replacing their role in the ecosystem (Beck et al. 2008) and not only competing with them but leading to changes in all processes related for its survival (Vitousek et al. 1997). The International Union for Conservation of Nature – IUCN (1999) defines alien species as exotic species which are established in natural habits or ecosystems causing changes and threatening native biodiversity. The transformation of an exotic species into an invasive one is a special case of plant community composition change where a species substitute one or more natives causing serious consequences to conservation and ecosystems services utilization (Ehrenfeld 2003).

Invasion by exotic species is broadly known as one of the biggest threats to biodiversity (Wilcove et al 1998, Mack et al. 2000). They can alter ecosystem processes such as primary productivity, biodiversity and regional biota (Pysek et al. 2010). Exotic plant invasion is a serious ecological and conservation threat to native plant communities (Vitousek et al. 1996). The impacts caused by invasive species on the native ones are shown in many ways by decreasing species richness and abundance of native biota and reducing their local species diversity (Pysek et al. 2011).

In Minas Gerais State, Brazil, there is a particular kind of vegetation that occur associated with rich iron ore at a region known as Quadrilátero Ferrífero (Rizzini 1979) and because of mining it is one of the most endangered ecosystems of the state (Matias et al. 2009). This vegetation is called Rupestrian Fields and grows in altitudes higher than 1000m on the substrate mentioned above and is characterized as a complex of slow-growing herbaceous shrubs and tortuous and sclerophyllous plant communities, with a high diversity of plant species and endemism (Giulietti & Pirani 1997). This vegetation occurs in ecological niches surrounded by rocky surfaces often aggregated onto soil, called "soil islands" (Conceição & Pirani 2007). The soil from "canga" (rocky outcrops) can be categorized as shallow, deficient in structure and nutrient-poor, but the samples from "soil islands", where vegetation is found, have better fertility (Matias et al. 2009).

Despite this hostile environment, the flora found above these formations is diverse, including endangered and endemic species. Because of the high biodiversity

found at Quadrilátero Ferrífero this region is classified with extreme biological importance as showed by Plano de Manejo do Parque Estadual da Serra do Rola Moça (2007) and Viana & Lombardi (2007). The vegetation is composed mostly by grass species such as *Axonopus pressus*, *Echinolaena inflexa, Paspalum spp.* with frequent subshrubs from Fabaceae (*Chamaecrista spp.*, *Periandra mediterranea*), Asteraeceae (*Aspilia foliaceae*, *Baccharis spp.*) and Malpiguiaceae (*Syrsonima spp.*, *Peixotoa* tomentosa) families (Viana & Lombardi 2007). The native legume species studied in this work was *Periandra mediterranea* (Vell.) Taub. which is distributed in neotropical regions occupying fields, cerrado biome and especially rupestrian fields of Serra do Espinhaço, Serra Dourada and Chapada dos Veadeiros, in altitudes that range from 400 to 1800m. In general this species grows in sandy soils, oxisol and rocky outcrops. The flowering and fructification periods vary with area, which occur from January to May in the studied site (Funch & Barroso 1999).

The PESRM, or Parque Estadual da Serra do Rola Moça, covers the municipalities of Belo Horizonte, Ibirité, Nova Lima and Brumadinho, totaling 4544,04 ha at the Quadrilátero Ferrífero, in the southern portion of the Espinhaço Chain (Plano de Manejo do Parque Estadual da Serra do Rola Moça). The PESRM acquires great importance in global biodiversity conservation because protects two hotspots: Atlantic Forest and Cerrado – in addition to being inserted into the Biosphere Reserve of the Espinhaço (MMA 2006). Besides is considered of special biological importance due to the high species richness (Drummond et al. 2005), PESRM is one of the main strongholds for rupestrian fields' preservation at Minas Gerais State. The park area has been suffering gradual invasive process by the African grass *Melinis minutiflora* Beauv. known as molasses grass (Hoffmann et al. 2004, Scotti et al. 2012). This alien grass is known for its sweet scent and oil exudates present in the trichomes on its leaves (Parsons 1972). The introduction of molasses grass in Brazil was for forage use in the dairy cattle raising (Morosini & Klink 1997) for its great nutritional characteristics for cattle diet (Parsons 1972). This species has been used in revegetation of mine piles what favored seed dissemination by water and wind what resulted in PESRM invasion. Currently the invasion by *M. minutiflora* reached 60% of the park area (Scotti et al. 2012). Molasses grass favors fire incidence because of the resin and oils that carries in its leaves, making them highly inflammable even in environmental conditions with 95% of relative humidity (D'Antonio & Vitousek 1992). With more fire incidence and

intensity a positive feedback is created where the benefited species is *M. minutiflora* since it rapidly reestablishes and disperses after fire (Hughes et al. 1991). This situation leads to a predominance tendency of molasses grass over the native species, causing the loss of plant species (D'Antonio & Vitousek 1992). One of the factors that favor the invasion by this species in conservation areas is the spaces open by fire (D'Antonio et al. 2011).

However plants reveal tolerance to many different biotic and abiotic environmental factors what is known as ecosystem resistance that works limiting the growth of an invading species population (D'Antonio & Thomsen 2004). Invasibility, defined as the intrinsic susceptibility of an area to invasion (Richardson 2001), has been shown to differ between biomes (Londsdale 1999), what can be also attributed to the properties of native species and community structure (von Holle & Simberloff 2005).

The present study intended to analyze and comprehend the effects of the invasive grass *M. minutiflora* on plant community of rupestrian fields and their resistance to invasion in order to subsidize the management of the invasive species *M. minutiflora*.

Material and methods

Study area

The study was developed in areas with rupestrian ferruginous fields located inside the Parque Estadual da Serra do Rola Moça (PESRM) (Plano de Manejo do Parque Estadual da Serra do Rola Moça 2007) located in Minas Gerais State, in the cities of Belo Horizonte, Brumadinho, Ibirité and of Nova Lima (Meyer et al. 2004).

The regional weather is the Cwa type by Köppen classification which presents well-defined rain regime characterized by dry winters and rainy summers (Brandão et al. 1997) and the vegetation is characterized as rupestrian fields (Plano de Manejo do Parque Estadual da Serra do Rola Moça 2007).

Experimental design

The experimental design was composed by 2 blocks with 4 treatments with 3 replicates (2 x 4 x 3 = 24), resulting in a total of 24 plots of 4m² each. The treatments were described as follows: T1 – no invasion; T2 – no invasion with the presence of *Periandra mediterranea* (Vell.) Taub.; T3 – ≤50% of invasion plus *P. mediterranea*; T4 – >50% of invasion plus *P. mediterranea*, as exemplified in Figure 1. Samples were collected between October 2013 and July 2014.

Figure 1 – **Examples of plots per treatment**. Legend: **T1** – no invasion; **T2** – no invasion with the presence of *P. mediterranea*; **T3** – ≤50% of invasion plus *P. mediterranea*; **T4** – >50% of invasion plus *P. mediterranea*.

Estimative of native species distribution

After the demarcation of plots in field, species occurrence was measured using the method proposed for Toledo & Shultze-Kraft (1982). This method consists of using

a 1m² quadrant composed of 100 squares 0,01m² each. The quadrant was placed inside de plot and then the vegetation of each square was identified.

Data analysis

A table with all species collected in the studied area was elaborated in alphabetical order of family aiming to elucidate all species found. The relative frequency of species and families was plotted in bar graphs for each treatment by using the formula:

$$Species\ A\ relative\ frequency = \frac{Species\ A\ frequency\ \times\ 100}{Sum\ of\ all\ species\ frequency}$$

Only the 15 more abundant species and families for each treatments where plotted in graphs.

The community richness was analyzed by Analysis of Variance (ANOVA) and community composition was analyzed using first a Nonmetric Multidimensional Scaling (NMDS) with Bray-Curtis Indices, which uses abundance as a comparison factor, to build a graphic representation of the dissimilarity between treatments. After we used an Analysis of Similarities (ANOSIM) in order to test statistically if there were significant differences between the treatments. And finally we used Similarity Percentage (SIMPER) to identify the taxa primarily responsible for the dissimilarity between the groups. These analyses were performed using the program Past 2.01 (Hammer et al. 2001).

Results and discussion

Families distribution according with relative frequency (Figure 2) shows that in all treatments Poaceae family presented absolute dominance, characterizing grassy field upon rocky outcrops as was also shown by Viana & Lombardi (2007) when characterized rupestrian fields, evidencing the extreme importance of this family for floristic composition. In non invaded sites after the plants of Poaceae family, the ones of Fabaceae, Loganiaceae, Malpiguiaceae and Lamiaceae families were more representative in T1 plots and the ones of Fabaceae, Malpiguiaceae, Asteraceae and

Lamiaceae families in T2 plots. The presence of the native legume *P. mediterranea* without the presence of the invasive grass (T2 plots) favored Malpiguiaceae family. In plots with invasion the abundance of all families was reduced (Figure 1), Malpiguiaceae family was reduced but occurrence of Asteraceae remained similar in all levels of invasion. The distribution of Fabaceae, Asteraceae, Malpiguiaceae and Loganiaceae families showed relative resistance to invasion independently of its level (T3 and T4). On the contrary, the other families were inhibited. On the other hand, Figure 2 shows an increase of biodiversity on T3 plots in relation to other treatments contrasting with the representativeness of all families, including Fabaceae that was reduced in the presence of the *M. minutiflora*, in plots with higher levels of invasion (T4).
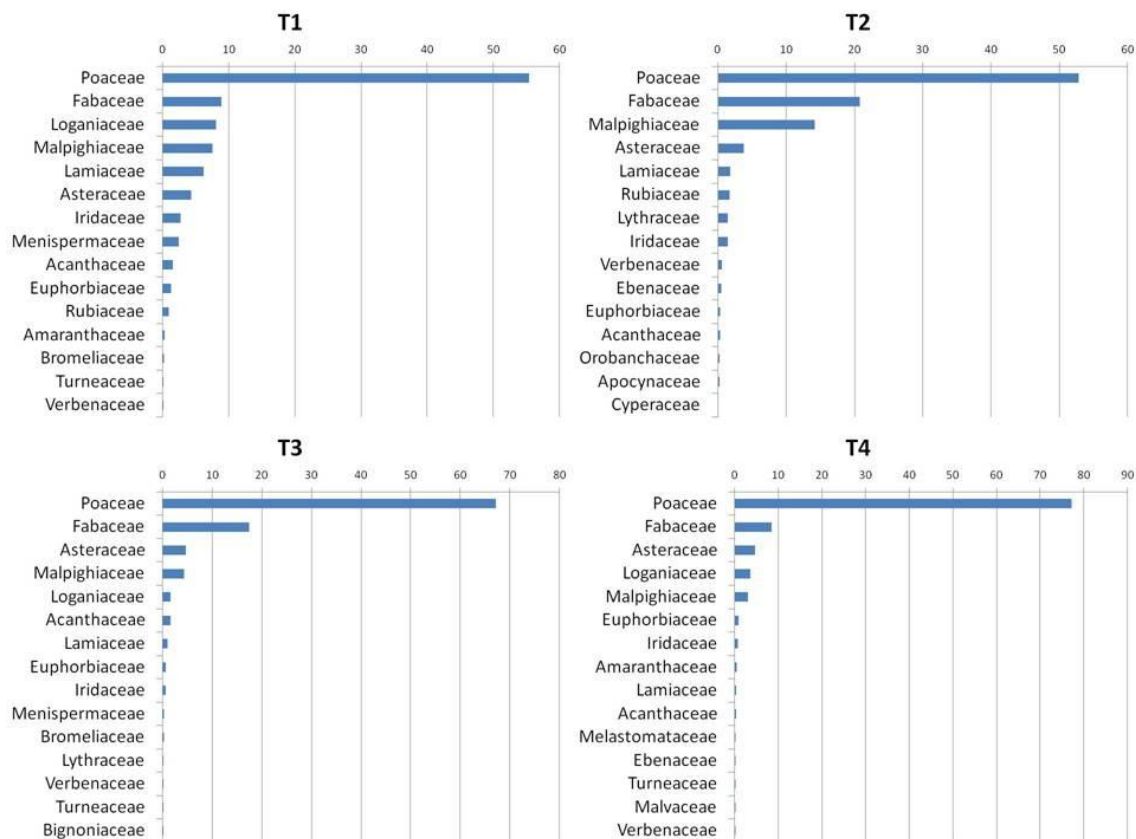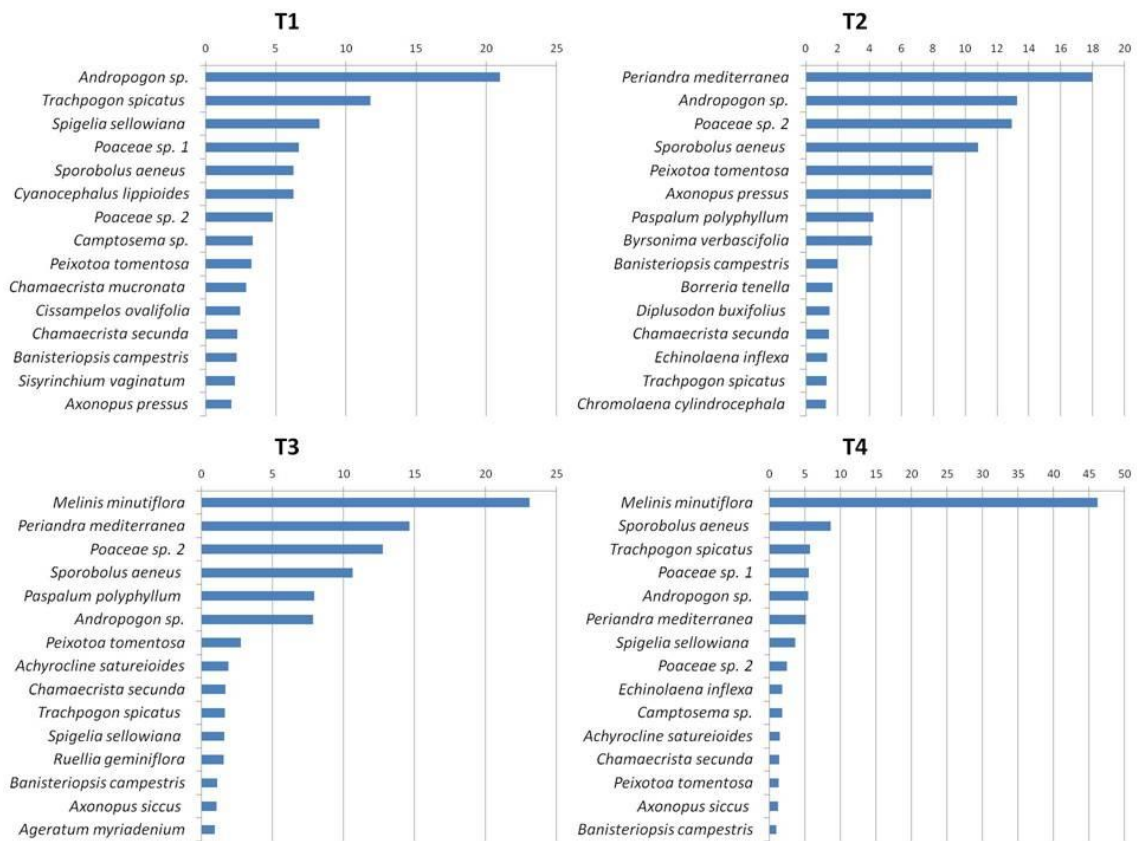

Figure 2 – **Families abundance per treatment**. Legend: **T1** – no invasion; **T2** – no invasion with the presence of *P. mediterranea*; **T3** – ≤50% of invasion plus *P. mediterranea*; **T4** – >50% of invasion plus *P. mediterranea*.

Table 1 – **Inventoried species at PESRM.** Legend: **T1** – no invasion; **T2** – no invasion with the presence of *P. mediterranea*; **T3** – ≤50% of invasion plus *P. mediterranea*; **T4** – >50% of invasion plus *P. mediterranea*.

Family	Species	T1	T2	T3	T4
Acanthaceae	*Ruellia geminiflora* Kunth	1,56%	0,27%	1,56%	0,28%
Amaranthaceae	*Gomphrena agrestis* Mart.	0,25%	-	-	0,16%
	Pfaffia gnaphaloides (L.f.) Mart.	-	-	-	0,26%
Apocynaceae	*Mandevilla pohliana* (Stadelm.) A.H.Gentry	-	0,19%	-	-
Asteraceae	*Achyrocline satureioides* (Lam.) DC.	1,07%	0,30%	1,90%	1,47%
	Ageratum myriadenium (Sch.Bip. ex Baker) R.M.King & H.Rob.	-	-	0,93%	0,75%
	Aldama tenuifolia (Gardner) E.E.Schill. & Panero	-	0,27%	0,09%	0,30%
	Aspilia foliacea (Spreng.) Baker	0,53%	-	-	0,05%
	Asteraceae sp.	-	-	0,07%	0,05%
	Baccharis erioclada DC.	-	-	0,07%	-
	Baccharis reticularia DC.	0,23%	-	0,05%	-
	Calea clausseniana Baker	0,12%	0,08%	0,18%	0,14%
	Chaptalia integerrima (Vell.) Burkart	-	-	-	0,05%
	Chromolaena cylindrocephala (Sch.Bip. ex Baker) R.M.King & H.Rob.	0,38%	1,25%	0,22%	-
	Chromolaena multiflosculosa (DC.) R.M.King & H.Rob.	0,58%	-	0,30%	0,87%
	Chrysolaena obovata (Less.) Dematt.	-	-	0,53%	0,33%
	Conyza bonariensis (L.) Cronquist	-	0,56%	-	-
	Lucilia lycopodioides (Less.) S.E. Freire	-	-	0,10%	-
	Mikania purpurascens (Baker) R.M.King & H.Rob.	-	-	0,12%	-
	Senecio pohlii Sch.Bip. ex Baker	-	-	0,05%	-
	Symphyopappus angustifolius Cabrera	-	0,33%	0,04%	-
	Symphyopappus brasiliensis (Gardner) R.M.King & H.Rob.	-	0,86%	0,07%	0,55%
	Trichogonia salviifolia Gardner	1,39%	0,08%	-	0,06%
Bignoniaceae	*Jacaranda caroba* (Vell.) DC.	-	-	0,07%	-
Bromeliaceae	*Dyckia consimilis* Mez.	0,16%	-	0,22%	-
Cyperaceae	*Bulbostylis consanguinea* (Kunth) C.B.Clarke	-	0,08%	-	-
Ebenaceae	*Diospyros hispida* A.DC.	-	0,5%	-	0,21%
Euphorbiaceae	*Croton antisyphiliticus* Mart.	0,14%	0,32%	0,39%	0,63%
	Croton campestris A.St.-Hil.	0,07%	-	0,22%	0,06%
	Croton serratoideus Radcl.-Sm. & Govaerts	0,74%	-	-	-
	Euphorbia potentilloides Boiss.	0,29%	-	0,05%	-
	Sapium haematospermum Müll.Arg.	-	-	-	0,16%
Fabaceae	*Camptosema* sp.	3,34%	1,04%	0,90%	1,76%
	Chamaecrista cathartica (Mart.) H.S.Irwin & Barneby	0,21%	0,25%	0,06%	-
	Chamaecrista desvauxii (Collad.) Killip	0,2%	-	0,14%	0,25%
	Chamaecrista mucronata (Spreng.) H.S.Irwin & Barneby	2,89%	-	-	-
	Chamaecrista secunda (Benth.) H.S.Irwin & Barneby	2,24%	1,44%	1,68%	1,32%
	Periandra mediterranea (Vell.) Taub.	-	18,02%	14,66%	5,12%
Iridaceae	*Sisyrinchium vaginatum* Spreng.	2,08%	0,59%	0,47%	0,66%
	Trimezia juncifolia Benth. & Hook.f.	0,64%	0,87%	0,18%	-

Lamiaceae	Cyanocephalus lippioides (Pohl ex Benth.) Harley & J.F.B. Pastore	6,23%	0,88%	0,89%	0,30%
	Eriope crassipes Benth.	-	0,92%	0,03%	-
Loganiaceae	Spigelia sellowiana Cham. & Schltdl.	8,11%	-	1,58%	3,58%
Lythraceae	Diplusodon buxifolius (Cham. & Schlechtd.) DC.	-	1,47%	0,19%	-
Malpighiaceae	Banisteriopsis campestris (A. Juss.) Litle	2,2%	2,02%	1,10%	0,96%
	Byrsonima verbascifolia (L.) DC.	1,1%	4,17%	0,40%	0,76%
	Peixotoa tomentosa A.Juss.	3,27%	7,96%	2,76%	1,24%
	Tetrapterys microphylla (A.Juss.) Nied.	0,96%	-	-	-
Malvaceae	Sida glaziovii K.Schum.	-	-	-	0,05%
Melastomataceae	Cambessedesia hilariana (Kunth) DC.	-	-	0,03%	0,23%
Menispermaceae	Cissampelos ovalifolia DC.	2,46%	-	0,22%	-
Orobanchaceae	Buchnera lavandulacea Cham. & Schltdl.	-	0,25%	-	-
Poaceae	Andropogon sp.	20,96%	13,26%	7,86%	5,44%
	Axonopus pellitus (Nees ex Trin.) Hitchc. & Chase	0,12%	-	-	-
	Axonopus pressus (Nees ex Steud.) Parodi	1,84%	7,86%	0,42%	0,09%
	Axonopus siccus (Nees) Kuhlm	-	-	1,04%	1,14%
	Echinolaena inflexa (Poir.) Chase	1,24%	1,34%	0,72%	1,80%
	Eragrostris articulata (Schrank) Nees	-	0,95%	0,9%	-
	Melinis minutiflora Beauv.	-	-	23,1%	46,2%
	Paspalum polyphyllum Nees	0,19%	4,22%	7,95%	0,26%
	Poaceae sp. 1	6,65%	-	-	5,52%
	Poaceae sp. 2	4,78%	12,92%	12,76%	2,43%
	Poaceae sp. 3	-	0,18%	0,12%	-
	Sporobolus aeneus (Trin.) Kunth	6,27%	10,82%	10,63%	8,62%
	Trachpogon spicatus Kuntze	11,74%	1,28%	1,65%	5,65%
	Tristachya leiostachya Nees	1,63%	-	0,07%	-
Rubiaceae	Borreria tenella (Kunth) Cham. & Schltdl.	0,82%	1,66%	-	-
	Declieuxia oenanthoides Mart. & Zucc. ex Schult. & Schult.f.	0,12%	-	0,03%	-
Turneaceae	Turnera oblongifolia Cambess.	0,12%	-	0,07%	0,16%
Verbenaceae	Lippia seriacea Cham.	0,07%	0,54%	0,10%	0,04%

Figure 3 shows that *P. mediterranea* modifies species distribution and so does *M. minutiflora* but in two different ways. In plots without invasion (T1 and T2) different species of Poaceae occurred (Figure 3) with dominance of the genera *Andropogon.* The presence of the alien grass, even in lower densities (T3) reduced the distribution of all species indiscriminately but some species remained their representativeness such as *P. mediterranea* (Fabaceae), *Sporobolus aeneus* (Poaceae), *Paspalum polyphyllum* (Poaceae), *Peixotoa tomentosa* (Malpiguiaceae), *Achyrocline satureioides* (Asteraceae), *Chamaecrista secunda* (Fabaceae).

Figure 3 – **Abundance of species per treatment.** Legend: **T1** – no invasion; **T2** – no invasion with the presence of *P. mediterranea*; **T3** – ≤50% of invasion; **T4** – >50% of invasion.

A rupestrian field characteristic is the presence of a legume in the composition of niche or "vegetation islands" (Matias et al. 2009). When we analyze Table 1 and Figure 3, we can see that in plots without *P. mediterranea* (T1) species of the genera *Chamaecrista* occur distributed in all 4 species found in the studied area: *C. cathartica*, *C. desvauxii*, *C. mucronata,* and *C. secunda.* When *P. mediterranea* is the dominant legume (T2) only *C. cathartica* and *C. secunda* was registered. At initial invasion phase of *M. minutiflora* (T3) the distribution of *P. mediterranea*, *C. cathartica* and *C. secunda* were not seriously affected but the presence of another species of this genera (*Chamaecrista desvauxii*) was favored. Only in higher levels of invasion (T4) we could see the inhibition of *P. mediterranea.* These data suggest that this legume shows ability to limit *M. minutiflora* invasion which when is overcome results in the dominance by this grass (T4). This resistance was strengthened by the presence of the species *P. tomentosa*, demonstrating the known Biome Resistance.
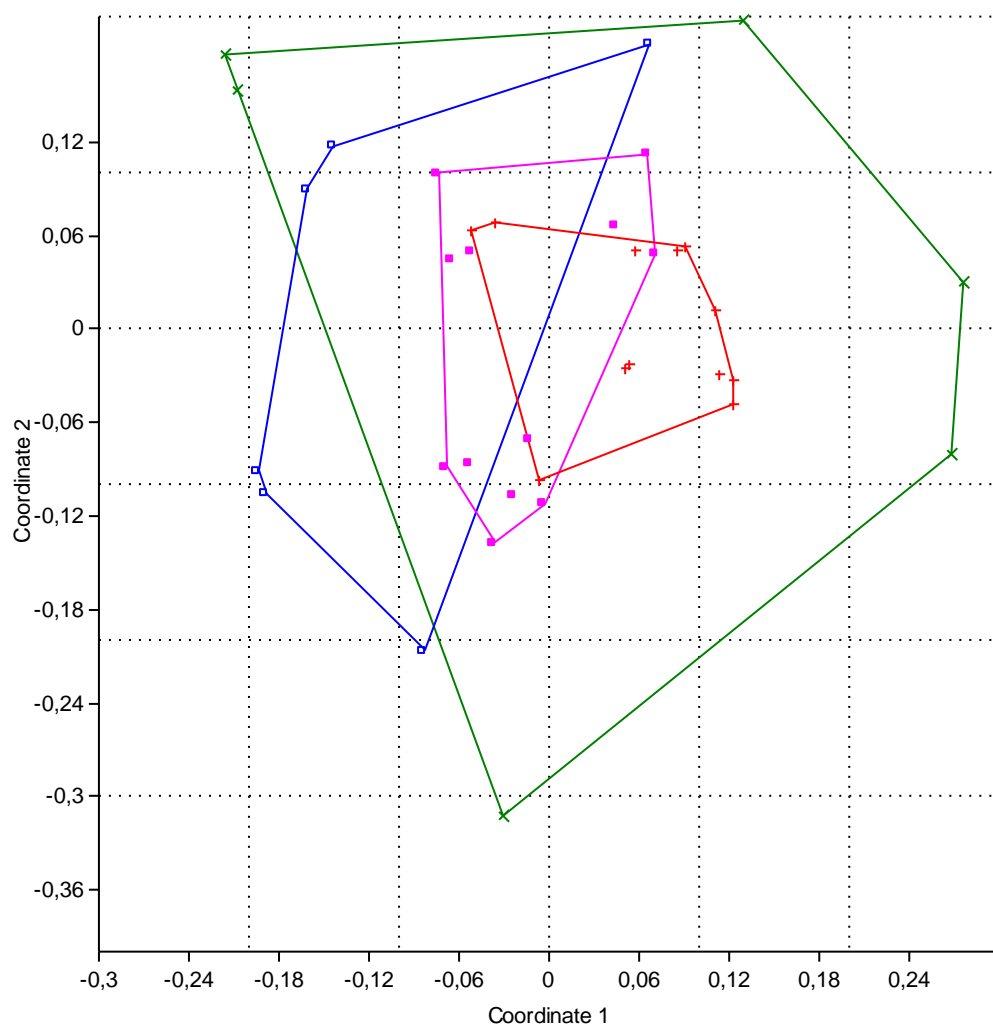
Figure 4 – NMDS Analysis. Green area = T1 (no invasion); Blue area = T2 (no invasion with the presence of *P. mediterranea*); Pink area = T3 (≤50% of invasion); Red area = T4 (>50% of invasion). P_{anosim} <0,001.

Differently than other studies that showed that invasion in most cases caused significant reduction in native species richness (Richardson et al. 1989, Pysek et l. 2011), we didn't find this effect on *M. minutiflora* invasion in the studied site ($F_{3,32}$ = 0,42035; p = 0,73964) over this parameter. But when considering species diversity we found negative effects of invasion on plant community.

According to the graphic results of the NMDS analysis (Figure 4) the distribution of native species, as demonstrated by T1, comprehended bigger species diversity, as expected. The template species distribution in T2 plots with *P.*

mediterranea plus native species was partially modified until 50% of invasion and strong alteration and detachment of species in relation to template T3 was verified when *M. minutiflora* reached higher levels of dominance. This difference in species diversity between treatments was statistically proved by ANOSIM ($p = <,0001$) and the SIMPER analysis showed that the invasive species *M. minutiflora* is the major responsible for the observed dissimilarity, contributing for this difference in 22,38%.

Negative interactions between native plant community and alien species are the most common consequences of invasion, and that's the reason why these species can be considered ecosystem engineers, causing modifications in habitats (Crooks 2002). Even if the mechanisms used in the process of invasion are poorly understood, it's well agreed that exotic plant species will eventually cause a decline in native diversity (D'Antonio & Vitousek 1992).

Several studies noticed the loss of native plant diversity as a consequence of exotic invasion at the same time of the invader establishment and the increase its abundance (Crooks 2002, MacDougall & Turkington 2005, Vilà et al. 2011). Hejda et al. (2009) studied the effects of 13 invasive species on native community and found negative effects of invasion on species richness and diversity in most cases.

Both success and impacts of an invader depends on its biological attributes, environmental characteristics of the invaded site and interactions with the natural community (Vilà & Weiner 2004). Once an invader achieves an appreciable density it will have effects on the invaded locality (Shea & Chesson 2002) and if it can outcompete native species it can become a community dominant (Houlahan & Findlay 2004). This dominance can be considered the largest effect of alien species leading to homogenization of the invaded habitat (McKinney & Lockwood 1999, Houlahan & Findlay 2004, Shwartz et al. 2006). The homogenization threaten is alarming when we follow the evolution of *M. minutiflora* invasion at PESRM.

Biome resistance is the sum of factors that act as a barrier protecting the habit from the growth of alien species (D'Antonio & Thomsen 2004). Environmental resistance is very important to control invasion process, especially when the invader is still being installed at the biome (Vermeij 1996). We could see a clear resistance to invasion by vegetal community at the lowest levels of occupancy by *M. minutiflora* (T3 plots) as shown by the species: *Sporobolus aeneus* (Poaceae), *Chamaecrista secunda*

(Fabaceae), *Paspalum polyphyllum* (Poaceae), *Peixotoa tomentosa* (Malpiguiaceae), *Achyrocline satureioides* (Asteraceae) and *Periandra mediterraneae* (Fabaceae). The latter was a key element for this resistance; having its presence inhibited only when the alien grass became dominant (T4 plots). Biotic resistance is strongly important for repealing invaders and maintains ecosystem processes (Levine & D'Antonio 1999, Vinebrooke et al. 2004). The data of this study allow us to understand the impacts of invasion and allow to describe the mechanisms of resistance presented by rupestrian fields species at PERSM, showing the negative effects of *M. minutiflora* on native plant species diversity and also suggesting the resistance and tolerance of *P. mediterranea* at initial invasion phase (such hypothesis is strengthened by metabolic indicators presented in Chapter 2).

Acknowledgements

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The role of *Periandra mediterranea* (Vell.) Taub. over invasion of molasses Grass (*Melinis minutiflora* Beauv.) on rupestrian fields: nitrogen biogeochemical cycling implications

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Abstract

Rupestrian fields are found at Minas Gerais State over iron rich soils. This vegetation comprehends several endemic species that are endangered by intense mining activity. Parque Estadual Serra do Rola Moça (PESRM) is an important protected area for rupestrian ferruginous fields' conservation which nowadays is threatened by the presence of the invasive African grass *Melinis minutiflora* Beauv. Alien species can cause alterations in invaded habitats through a variety of mechanisms affecting from community composition to nutrient cycles. Nitrogen is often the most limiting resource for plant species and because of this it is usually affected by invasion. In our work we studied the effects of *M. minutiflora* on nitrogen biogeochemical cycle at PESRM. Considering that *Periandra mediterranea* (Vell.) Taub. is a dominant legume, the experimental design was based on the presence of it or not with invasive species. To evaluate these effects we analyzed: mineral soil nitrogen, enzymatic activity, ammonifiers and nitrifiers populations, %C, %N and C:N ratio in soil and in plant material. The dominant N form in soil of native species was ammonium, what suggests the preference for this N-form in their nutrition. *M. minutiflora* appears to be highly N-dependent for its growth using both studied inorganic form but specially nitrate. Since this grass showed low N foliar content, we could expect the observed field pattern to follow the native legume which is the main source of N in this ecosystem. Proteolytic activity was found in native plots as well as in the invaded ones, what suggests the uptake of organic forms of N by all species. However, the studied N-forms were reduced at initial phase of invasion, indicating competition. In spite of this, where native species were dominant the C:N ratio in legume species was very low, contrasting with the very high ratio found in *M. minutiflora* leaves. Rupestrian fields showed resistance to invasion through N competition. The invasive grass broke biome resistance by modifying the dominant N-form in soil from ammonium to nitrate, which is not required by native species.

Keywords

Ammonium; nitrate; biome resistance; proteolytic activity.

Introduction

Minas Gerais State has the largest iron ore deposit in Brazil, the Quadrilátero Ferrífero, in which grows a type of vegetation called Rupestrian Fields, which belongs to the Brazilian Cerrado biome, one of the most endangered biomes in the world (Myers et al. 2000) due mining activity (Matias et al. 2009). Parque Estadual Serra do Rola Moça (PESRM) is an important protection area that is being threatened by the presence of the African grass *Melinis minutiflora* (Beauv.). In addition to causing the reduction of biodiversity it also produces a large quantity of biomass that works as fuel leading to annual fire incidences with considerable dimensions. Actually about 60% of the Park area is invaded by molasses grass (Bomediano et al. 2013). Ecologists have long recognized that vegetation can exert a strong influence over soil properties, and that individual plant species play an important role in determining soil fertility in natural ecosystems (Zinke 1962). Plant species distribution is at the same time a cause and an effect of patterns of nutrient cycling in natural ecosystems (Hobbie 1992). They have the ability to alter the substrate they live in changing crucial components of biogeochemical cycling, driving mineral cycles and altering the hydrology of the soil (Kelly et al. 1998). The effects that plants can exert on nutrient cycling is influenced by litter quality (as predicted by C:N and lignin:N ratios): species from low-nutrient environments produce poor-quality litter that decomposes slowly, further reducing nutrient availability, compared with species from high-nutrient environments (Flanagan & Van Cleve 1983).

Alien species can alter invaded habitats through a variety of mechanisms that have the potential to cascade through the ecological hierarchy and affect ecosystem level processes. At the community level, invasive species often exhibit an ability to outcompete their native counterparts for resources (Lavergne et al. 1999, Martin 1999). Their introduction is a modification whose nutritional change potential is wide because it can affect components such as carbon, nitrogen, water and ecosystem cycles. There is not a standard environmental response to invasion because the changes depend on the interaction of each species with the invaded area. In some cases the same species can have different effects when areas with different characteristics are compared (Ehrenfeld 2003).

Even though Dassonvile et al. (2008) noticed that invasive species usually alter the nature of the soil in a way to show opposite characteristics after invasion: more nutrients in usually poor sites and less in the rich ones, Liao et al. (2008) showed that invasive plant dominance is often correlated with changes in ecosystem processes in soil which frequently are measured as increases in available nutrients and nutrient pools. The frequent increases in soil nutrient concentrations that are observed over time with many invaders has been proposed as a sort of feedback process in which elevated concentrations of nutrients, especially nitrogen, promote the greater success of invaders relative to natives (Weldenhamer & Callaway 2010).

Nitrogen is often the most limiting resource after water for most of soils, especially in semi-arid and arid grasslands (Vasquez et al. 2008). Mangla et al. (2011) found that annual grasses become more competitive when compared to native species in increasing nitrogen situations, supporting their hypothesis that increasing nitrogen would increase the competitive effects of invasive over natives.

An increase in soil nutrients availability not necessarily means an improvement for the affected ecosystem. In the case of oligotrophic soils or early successional ecosystems increased nutrients may lead to further invasion (Vitousek et al. 1987, Vitousek & Walker 1989). Plants can affect nutrient cycling direct through uptake, use and loss of nutrients, and indirectly by influencing microbial activity (Hobbie 1992).

Nutrient cycles can be altered by exotic invasive species because they have traits with potential to increase rates of decomposition and nutrient cycling, such as high specific leaf areas, growth rates and leaf nutrient concentrations (Allison & Vitousek 2004). Invaders were correlated with much higher litter decomposition rates and increases in soil nitrogen mineralization and nitrification (Ashton et al. 2005) what is explained by low lignin:N ratio, since Poaceae plants showed high cellulose and low lignin contents (Berg 2000). The changes in the resources cycle processes can be a reflex of the changes in soil microbial community influenced by the differences in the inputs amount and quality received by the soil by different plant species (Grayston & Campbell 1998, Grierson & Adams 2000).

Sanon et al. (2009) found that microbial communities may have a role in the ecosystem-scale changes in soil biochemistry caused by invasive plants. They found increased concentration of nitrogen, carbon and phosphorus correlated with increases in bacterial abundance and soil microbial activity. However, arbuscular mycorrhizae fungi

and rhizobial development were severely reduced. Assuming that litter quality determines litter decay rate and thus nitrogen mineralization rate (Hobbie 1992) and that most of the studies show that the presence of alien species contributes to a nutrient increase (Liao et al. 2008) we can presume that Alisson & Vitousek (2004) conclusions were correct when assumed that invaders normally present higher littler decay rates. These differences between decomposition rates must be caused by differences in leaf tissue of invaders compared to native species (Weldenhamer & Callaway 2010).

There is no pattern when it comes to soil nitrogen changes caused by invasive species being observed increase, decrease and no effect at all (Ehrenfeld 2003) even though most of studies appoint to an increase in nutrient cycling.

The invasive species studied in this work was *Melinis minutiflora* Beauv. which is an aggressive invasive African grass in many regions of the American continent (Parsons 1972, Blydenstein 1976, Smith & Tunison, 1992). This Poaceae was primarily introduced for cattle grazing (Morosini & Klink 1997) but it rapidly overtook these barriers and became an invasive species increasing fire frequency (D'Antonio & Vitousek 1992). Studies in Hawaii shrubland showed that one of the mechanisms used by this species for invasion is related to changes on nitrogen biogeochemical cycle increasing inorganic N pools and N mineralization rates to improve the process of establishment (Asner & Beatty 1996).

The studied site shows severe environmental conditions, such as soils scarcity and nutrient deficiency, high daily temperature oscillations and intense irradiation, restricting the occupation by plants (Shure & Ragsdale, 1977). The dominant vegetation belongs to the biome Brazilian Cerrado, called "campo rupestre" (rupestrian field), one of the most endangered biomes in the world (Myers et al., 2000). This vegetation grows in altitudes higher than 1000m on the substrate mentioned above and is characterized as a complex of slow-growing herbaceous shrubs and tortuous and sclerophyllous plant communities, with high plant diversity and endemism (Giulietti & Pirani 1997). This vegetation occurs in ecological niches surrounded by rock surfaces often aggregated onto soil, called "soil islands" (Conceição et al. 2007). Soil from "canga" (rocky outcrops) can be categorized as shallow, deficient in structure and nutrient-poor, but the samples from "soil islands", where vegetation is found, have more humic substances and better fertility (Matias et al. 2009).

M. minutiflora is widely spread at the Parque Estadual da Serra do Rola Moça becoming a serious threat to biodiversity (Plano de Manejo do Parque Estadual da Serra do Rola Moça 2007). Scotti et al. (2012) showed that this species presented high inhibitory allelophatic effect over native species.

However, some native species showed a degree of tolerance to *M. minutiflora* extract. One of these species was the native legume *Periandra mediterranea* (Vell.) Taub., which is distributed in neotropical regions occupying fields, cerrado biome and especially rupestrian fields in altitudes that range from 400 to 1800m. This species grows in sandy soils, oxisol and rocky outcrops with its flowering and fructification periods varying with area, ranging from January to May in the studied site (Funch & Barroso 1999). *P. mediterranea* tolerance to *M. minutiflora* extract was related to seed germination as that reached about 60%. Plants can present tolerance to many different biotic and abiotic environmental factors such as: drought (Yordanov et al. 2000), aluminum (Ma et al. 2001), contaminant (Minkina et al. 2012), among others. Biome resistance is a known phenomena that refers to environmental factors that limit population growth of invading species (D'Antonio & Thomsen 2004). Invasiveness, defined as the intrinsic susceptibility of an area to invasion (Richardson 2001), has been shown to differ between biomes (Lonsdale 1999) what can also be attributed to the properties of native species and community structure (von Holle & Simberloff 2005). The objective of this study was to evaluate the role of *P. mediterranea* above *M. minutiflora* degree of invasiveness and on nitrogen biogeochemical cycling.

Material and methods

Study area

The study was developed at the Parque Estadual da Serra do Rola Moça (PESRM), located in the central-south region of Minas Gerais state, Brazil, in the cities of Belo Horizonte, Brumadinho, Ibirité and Nova Lima (Meyer et al. 2004),occupying an area of 3.941,09 ha (Fernandes 2003).

The regional weather is the Cwa type by Köppen classification which presents well-defined rain regime characterized by dry winters and rainy summers (Brandão et

al. 1997) and the vegetation is characterized as rupestrian ferruginous fields (Plano de Manejo do Parque Estadual da Serra do Rola Moça 2007).

This vegetation occurs associated with rich iron substrate at a region known as Quadrilátero Ferrífero (Rizzini 1979) and grows in altitudes superior to 1000m above rocky outcrops or primary soils with predominant vegetation composed of herbaceous species with sparse taller plants (Plano de Manejo do Parque Estadual da Serra do Rola Moça 2007). Despite this hostile environment, the flora found above these formations is diverse, including endangered and endemic species (Viana & Lombardi 2007).

Experimental design

The experimental design was composed by 3 blocks with 4 treatments or plots with 3 replicates/block (3 x 4 x 3 = 36), resulting in a total of 36 plots of 4m² each. The treatments were designed in a way to represent different levels of invasion of *M. minutiflora*: T1 − no invasion without the presence of the native legume *Periandra mediterranea* (Vell.) Taub.; T2 − no invasion with the presence of *P. mediterranea*; T3 − ≤50% of invasion plus *P. mediterranea*; T4 − >50% of invasion plus *P. mediterranea*, as exemplified in Figure 1.

The only different design used was for foliar analyses. The sampling method was the same but the treatments consisted in obtaining foliar material in the following treatments: A − leaves from native species collected at T1 plots (no invasion without *Periandra mediterranea*); B − leaves from *P. mediterranea* collected at T2 plots (no invasion with the presence of *P. mediterranea*); C − leaves from *P. mediterranea* collected at T3 plots (≤50% of invasion plus *P. mediterranea*); D − leaves from *M. minutiflora* collected at T3 plots (≤50% of invasion plus *P. mediterranea*); E − leaves from *P. mediterranea* collected at T4 plots (>50% of invasion plus *P. mediterranea*); F − leaves from *M. minutiflora* collected at T4 plots (>50% of invasion plus *P. mediterranea*). Samples were collected between October 2013 and July 2014.

Figure 1 – **Examples of plots per treatment**. Legend: **T1** – no invasion; **T2** – no invasion with the presence of *P. mediterranea*; **T3** – ≤50% of invasion plus P. mediterranea; **T4** – >50% of invasion plus *P. mediterranea*.

Field sampling and analysis

Soil sampling consisted of a mix of 2 samples (20 cm depth) obtained of each plot. Samples were used for further analysis such as: quantification of mineral soil nitrogen, quantification of enzymatic activity, determination of ammonifiers and nitrifiers populations, quantification of %C, %N and C:N ratio in soil and in leaves of plant material.

Quantification of mineral soil nitrogen was made using an adaptation of the methods proposed for Keeney & Nelson (1982) modified by Coelho et al. (1992). Available nitrogen pools size was determined by extracting NH_4-N and NO_3-N. The extraction was performed in 50g of soil shaken in 150 mL of 2M KCL for 1 hour at 200 rpm of speed. After decantation the supernatant was filtered and kept in - 4°C until distillation and posterior titration with 2% H_3BO_3.

The determination of microorganisms population was made using methods based on most probable number (MPN), the protocol for ammonifiers was obtained from Sarathchandra (1978) and the one used for nitrifiers from Schmidt & Belser (1994). Both of them consisted on inoculating in medium a serial dilution of the samples in 0,85% NaCl solution. The analysis of the presence or absence of these microorganisms was made after incubation for 5 days for ammonifiers and 8 weeks for nitrifiers. The population of nitrifiers was divided in two: ammonium-oxidizing and nitrite-oxidizing bacteria.

Urease activity was determined as reported by Zantua & Bremner (1975) modified according Bonmati et al. (1991). Activity was expressed as mg of $NH_4^+/g^{-1}h^{-1}$. Proteolytic activity was determined by the method described by Nannipieri et al. (1979) and modified by Bonmati et al (1991). Activity was expressed as mg of tyrosine/kg soil$^{-1}h^{-1}$. Total N was analysed using EA 1110 elemental analyser (Thermo Electron, Milan, Italy) according Schuman et al (1973).

Carbon and nitrogen leaves content were estimated through the method described by Werner & Brand (2001). Leave samples were dried at constant weight and ground to a fine powder in a ball mill (Glen Creston Ltda.). The samples were then analyzed for their total C and N content using as standard leave sample NIST peach leaves and as standard soil samples: B2150, NIIST2710, NIIST2711, B2152. Analysis was done 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).

All data were tested with Generalized Non-Linear Model to determine differences between the treatments using the program Statistica v.10. (StatSoft, Inc 2011).

Results and discussion

Although total N and C pools in soil didn't demonstrate difference between treatments (Figure 2a and b) the C:N ratio (Figure 2c) was significantly lower in the plots where *P. mediterranea* was associated with native species (T2), evidencing that *P. mediterranea* is directly related to nitrogen availability since the absence of this legume

significantly reduced soil N content. Therefore, N reduction in plots where both *P. mediterranea* and alien grass were present speaks in favor of a competition among them.

Figure 2d showed that native species (Treatment A) presented lower C and N leaves contents contrasting with *P. mediterranea* plants as shown by Figure 2d and e (Treatment B). The invasive plants presented the lowest leaves C and N contents (Figure 2d and e − Treatments D and F). N content of *M. minutiflora* leaves was not modified in plots with invasion up to 50% but it was significantly improved when invasion ratio was higher than 50%. As expected, *M. minutiflora* presented higher C:N foliar ratio (Figure 2f − Treatment D). This is justified by nodulation and N fixing capacity of *P. mediterranea* (Kirkbride-Junior 1984) which showed lower C:N ratio, favoring mineralization process. It's important to point that when invasion of *M. minutiflora* was higher than 50% (Figure 2e − Treatment C), this native legume has its %N significantly decreased with proportional increase of carbon content (Figure 2d − Treatment E).

Strengthening these results, the data of C:N ratio (Figure 2f) pointed the concomitant reduction of N content in leaves of *P. mediterranea* (Treatment E) and a significant nutrient improvement occurred in alien grass leaves (plants of treatment F - >50% of invasion). However the same effect was not registered when the invasion degree was ≤50% (Treatment D), suggesting that there was competition among them with disadvantage to the invasive plant, as *M. minutiflora* showed the lowest N concentration in leaves (Treatment D). These results suggest that *P. mediterranea* presented an ability to repel *M. minutiflora* invasion or a degree of resistance to the invasive plant which is related to nitrogen biogeochemical cycling. Invasion process was described by Lodge (1993) with three phases: arrival, establishment and spread. Vermeij (1996) emphasized the importance of environmental resistance at the earliest phase as demonstrated by *P. mediterranea* over *M. minutiflora* invasion. Levine & D'Antonio (1999) emphasized biotic resistance (ways in which resident species repeal invaders). Ecosystem resistance to a single stressor relies on tolerant species that can compensate for sensitive competitors and maintain ecosystem processes, such as primary production (Vinebrooke et al. 2004).
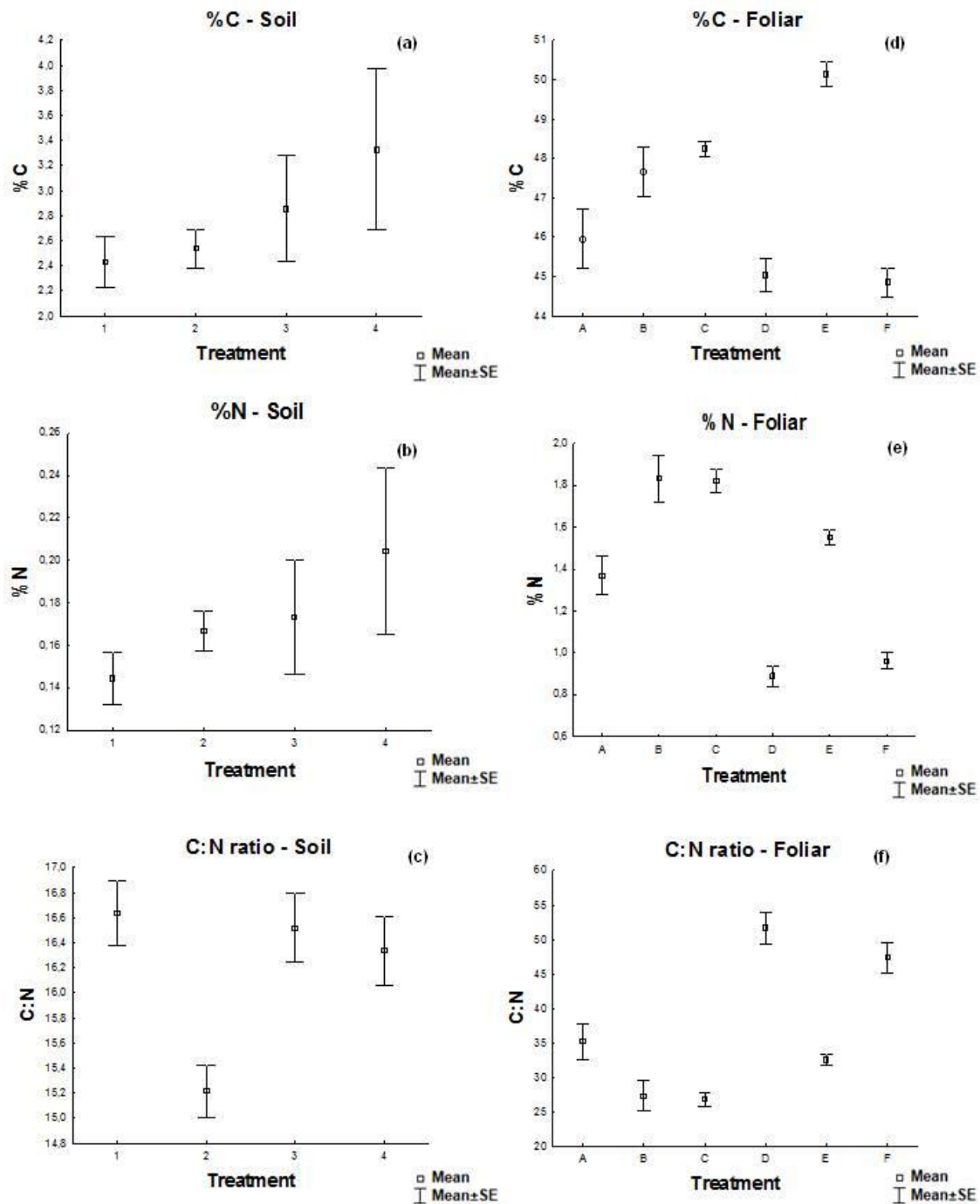
Figure 2 – Soil and foliar analysis. Quantification of %C, %N and C:N ratio in soil (a, b, c) and in foliar tissue (d, e, f). Data are means and SE. P_a =,6660; P_b =,3461; P_c =,0001; P_d =,0000; P_e =,0000; P_f =,0000.1. Legend: **T1** – no invasion; **T2** – no invasion with the presence of the native legume *Periandra mediterranea* ; **T3** – ≤50% of invasion plus *P. mediterranea*; **T4** – >50% of invasion plus *P. mediterranea*; **A** – leaves from native species collected at T1 plots; **B** – leaves from *P. mediterranea* collected at T2 plots; **C** – leaves from *P. mediterranea* collected at T3 plots; **D** – leaves from *M.*

minutiflora collected at T3 plots; **E** – leaves from *P. mediterranea* collected at T4 plots; **F** – leaves from *M. minutiflora* collected at T4 plots.

There was no statistical difference between treatments as regards to total N in soil (Figure 3a). Ammonium concentration was significantly high only in plots where *P. mediterranea* was associated with native species (Figure 3b – T2). Differences weren't found between native plots without *P. mediterranea* (Figure 3b – T1) and plots with invasion up to 50% (Figure 3b – T3) which showed very low ammonium content despite the presence of *P. mediterranea* (T3). These results suggested that at establishment phase *M. minutiflora* plants are competing with native species for soil stocked ammoniacal N. The invasive grass was limited by ecosystem resistance in plots where the dominant plants belong to Fabaceae, Malpiguiaceae, Loganiaceae and Asteraceae families. In T4 plots the invasive degree was increased and ammoniacal N also was increased suggesting the ability of *M. minutiflora* to take up ammonium from soil.

Nitrate ion was found significantly higher only in plots with high levels of invasion (T4). However, nitrate concentration was not modified when invasion ratio was low (T3) suggesting that *M. minutiflora* changed the strategy to N acquisition in T4 plots. On the other hand, nitrate was not available at the plots with native species. These results suggest that *M. minutiflora* can take up nitrate and this N-form didn't seem to be the preferable form of native plants. Although it is well understood that plant species differ in their ability to assimilate NO_3^- and NH_4^+ (de Graaf et al. 1998) few studies explicitly examine N preferences of species and even fewer examine nutrient uptake by exotic invaders (Hewins & Hyatt 2010). Even though *M. minutiflora* is being studied for decades in invaded habitats such as Hawaii, there is not a full pattern found when it comes to nutritional changes in ecosystem. Asner & Beatty (1996) found that *M. minutiflora* were consistently associated with elevated NH_4^+ availability in soils when occurred associated with native shrubland in Hawaii. On the other hand, Mack et al. (2001) found no effect of this invasive species on N mineralization in young woodland also in Hawaii's islands. According to Corbin & D'Antonio (2004), invasive plants can affect rates of mineralization, nitrification and N leaching losses, what depends on plant species composition.

Exotic annual grasses may benefit from the altered N cycle in their soils. Higher rates of nitrification can change plant-available N if the plant is better able to access NO_3^- than NH_4^+ or has better ability to access this ion than its competitors (Hawkes et al. 2005).
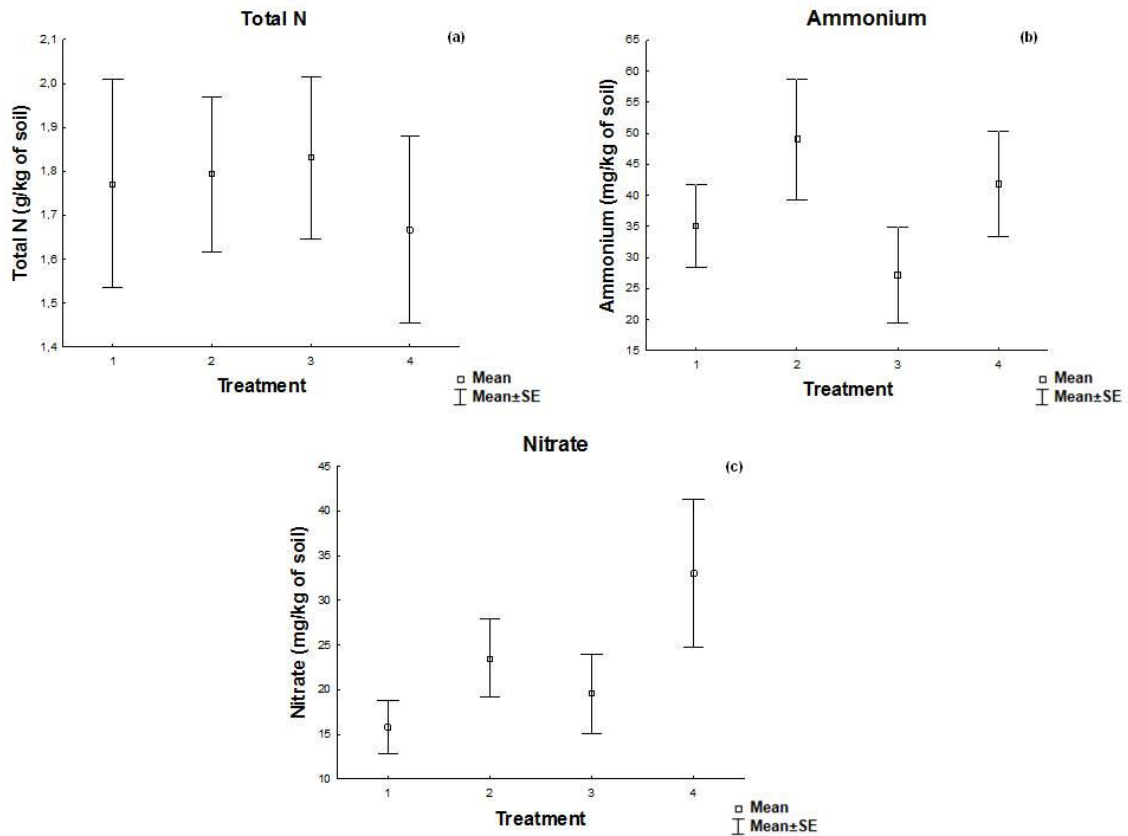
Figure 3 – Quantification of inorganic N in soil. Quantification of total N (a), ammonium (b) and nitrate (c) in soil. Data are means and SE. P_a = ,9946; P_b =,0000; P_c =,0000 . Legend: **T1** – no invasion; **T2** – no invasion with the presence of the native legume *Periandra mediterranea*; **T3** – ≤50% of invasion plus *P. mediterranea*; **T4** – >50% of invasion plus *P. mediterranea*.

While the ammonium ion was especially high in plots with native species plus *P. mediterranea*, the nitrate was produced only in T4 soils with dominance of *M. minutiflora*. On the other hand, the plots where *P. mediterranea* was associated with *M. minutiflora* ≤50% (T3) such improvement of nitrate was not registered. Besides this, ammonium production was also inhibited in T3 plots. These results suggest that in T3 plots (*M. minutiflora* ≤50%) competition to scarce resource occurred at the

establishment phase of invasion. These results speak in favor of native species resistance theory. At T4 plots the resistance was unsuccessful with alteration of dominant chemical N-form in soil. Similar results about increases in NO_3^- levels was found by Hawkes et al. (2005) studying two species of exotic grass (*Avena barbata* and *Bromus bordeaceous*) where the nitrification rates exceeded the mineralization ones in plots composed only by these species. Kourtev et al. (2003) found that all soils differed in chemical characteristics and N dynamics: NO_3^- concentrations were higher under the invasive influence, which agrees with our results.
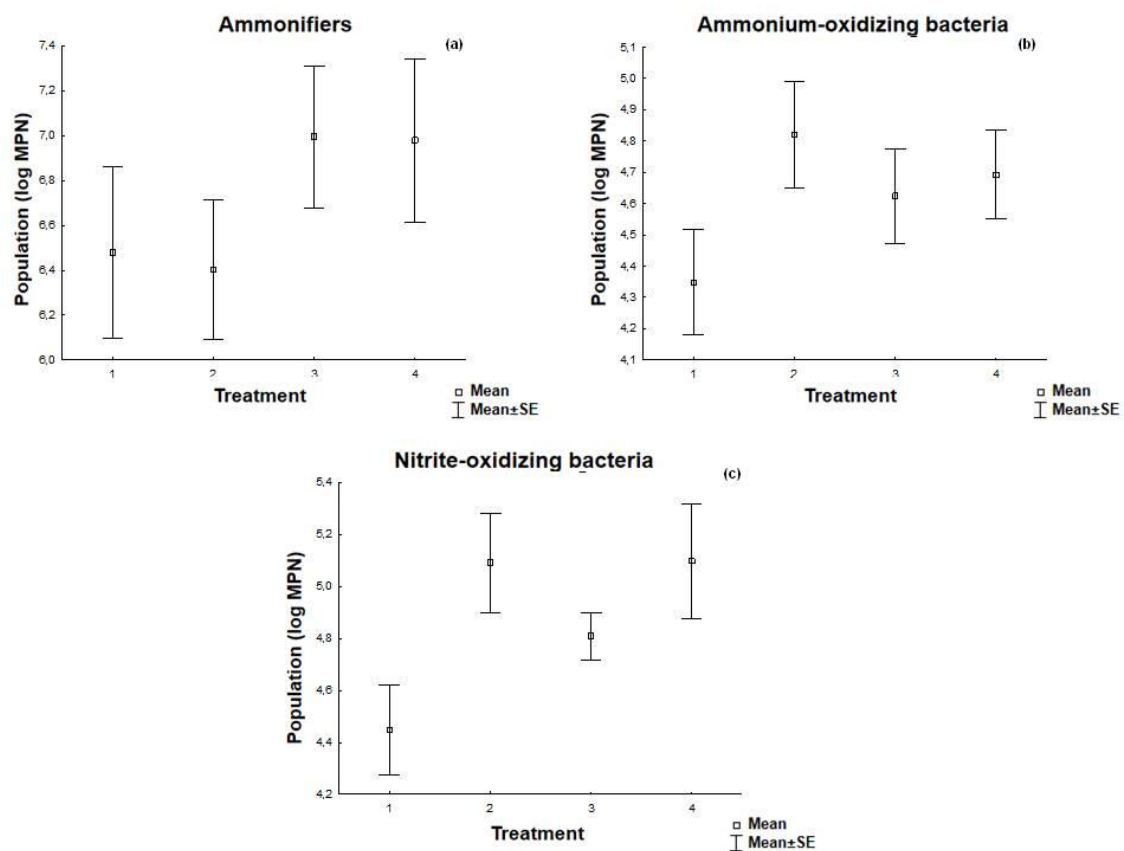

Figure 4 – **Population of microorganisms**. Quantification of ammonifiers (a), ammonium-oxidizing (b) and nitrite-oxidizing bacteria (c) population. Data are means and SE. P_a = ,9403; P_b = ,9752; P_c = ,9245. Legend: **T1** – no invasion; **T2** – no invasion with the presence of the native legume *Periandra mediterranea*; **T3** – ≤50% of invasion plus *P. mediterranea*; **T4** – >50% of invasion plus *P. mediterranea*.

In our study it's possible to observe that *M. minutiflora* changed its N-form preference when it occurred at different levels of density and this behavior can be a type of plasticity. Some species might be plastic in their ability to take up the two forms of N but probably within a limited range determined by phylogenetic and energetic constraints (Boudsocq et al. 2012). Ashton et al. (2010) showed that superior competitors exhibit higher resource use plasticity of chemical forms of N than the inferior competitor, which did not shift resource use. As in plots with dominance of *M. minutiflora* (>50%) NH_4^+ concentration was also increased. We can consider the hypothesis of *M. minutiflora* to use both ammonium as well nitrate in its nutrition. The NH_4^+ and NO_3^- are products of microbial action of ammonifiers in mineralization and ammonium-oxidizing and nitrate-oxidizing bacteria in nitrification process. However, the microbial population didn't differ between treatments for ammonifiers, ammonium-oxidizing and nitrate oxidizing (Figure 4). We must take into account that these results could be influenced by the technique that was used (MPN). According to Hawkes et al. (2005) microorganisms' population are better estimated by molecular methods such as qPCR but even this modern method presents discrepancy with field conditions. In spite of this, the results showed that *M. minutiflora* wins the competition when nitrification is higher than mineralization.

Even though Corbin & D'Antonio (2004) and Liao et al. (2008) observed in their studies that invasive species capacity of accelerate decomposition rates was related generally with higher plant and litter N concentrations and lower C:N and lignin:N ratios. C:N ratio in soil under the influence of *P. mediterranea* (Figure 2c) had favored organic matter decomposition and further mineralization rate. The mineralization process is conducted via microbial action and results in many different forms of nitrogen that are available to plants and microorganism. Invasive species can have profound effects upon decomposition, frequently increasing rates of this process and nutrient cycling by altering ambient decomposer communities (Ashton et al. 2005, Liao et al 2008).

The data from Figure 5 shows the enzymatic activity for protease and urease in soil. The urease activity was high in all treatments where *P. mediterranea* were present. Protease activity presented the same distribution of NH_4^+ (Figure 3) with the following tendencies: *P. mediterranea* (T2) plots = *M. minutiflora* (T4) plots (>50%) > *M. minutiflora* (T3) plots (≤ 50%) > native species (T1) plots. Therefore proteolytic activity

appears to be a significant via of mineralization of organic matter especially in the presence of *P. mediterranea*. However this activity was inhibited in initial phase of invasion process (T3) despite de presence of *P. mediterranea*. When invasion reached success (T4), protease activity was again increased. Literature shows that leguminous species can produce protease, especially under stress such as drought (Kohli et al 2012). Based on this, the protease found in T2 plots was probably produced by plants.
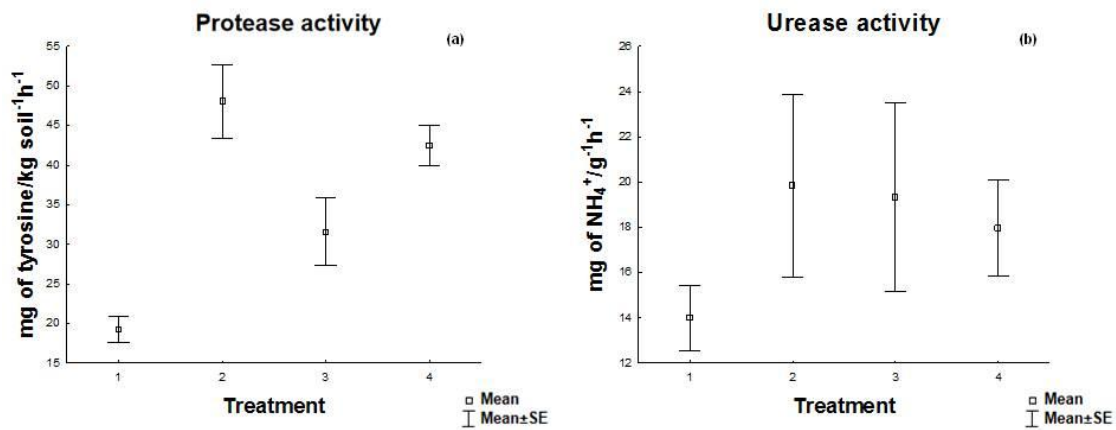
Figure 5 – **Enzymatic activity**. Quantification of protease (a) and urease (b) activity. T3 : *≤50% of invasion plus* P.mediterranea; T4 : *>50% of invasion plus* P.mediterranea;

Data are means and SE. P_a =,0000; P_b = ,0147. Legend: **T1** – no invasion; **T2** – no invasion with the presence of the native legume *Periandra mediterranea*; **T3** – ≤50% of invasion plus P. mediterranea; **T4** – >50% of invasion plus P. mediterranea.

Proteolytic activity as well as NH_4^+ production promoted by *P. mediterranea* seems to be reduced in the initial invasion process (T3) due to N competition among invasive and native species when biome resistance was expressed with native species advantage. When nitrogen became limiting to invasive plants the latter can obtain this nutrient via different mechanisms with both organic and inorganic N resulting in increased ammonium and nitrate and proteolytic activity. However, the organic matter targeted by decomposers microorganisms probably was from *P. mediterranea* litter with its low C:N ratio. The competition loss by *P. mediterranea* is signaled by increased C:N ratio in *M. minutiflora* leaves in T4 plots.

Enzymatic activity can be ruled by microorganisms and/or by plant population itself. Most N found in soil is in organic forms which is less available for plants so the first step of mineralization involves its hydrolysis by protease activity (Silva & Melo 2004). Badalucco et al. (1996) demonstrated that plant roots can stimulate bacterial growth through rhizospheric effect and consequently influence enzyme activity. The activity of enzymes involved in N acquisition appears to be more closely tied to the environmental availability of this nutrient presenting increased activity when N is a lacking resource in soil (Sinsabaugh & Moorhead 1994, Sims & Wander 2002). This affirmation could explain plant influence on enzymatic activity in rupestrian fields because of its known poor nutrient quality (Matias et al. 2009).

Kourtev et al. (2002) found higher activities of N-related enzymes in soils under invasive shrubs influence in New Jersey that also caused changes in structure and function of microbial communities. They hypothesized that these activities could be caused by soil micro biota that is competing with plant roots for N. Holly et al. (2009) had similar results for increases in enzyme activities related to litter decomposition in invaded sites by *Imperata cylindrical* when compared to the ones dominated by the native species *Andropogon glomeratus.*

However, after mineralization process it is widely assumed that microbial immobilization-mineralization ratio control N availability to plants supposing that plants use only inorganic forms of N (i.e., ammonium and nitrate) that are in excess of microbial N demand. Last decade several studies have shown that plants, especially in natural habitat, can take up amino acids from dissolved organic N (DON) directly by passing microbial mineralization step to produce simpler inorganic N forms (Harrison et al. 2007). This plant strategy has been shown to occur in many ecosystems but particularly in those that are strongly N limited as in artic and alpine tundra (Chapin et al. 1993, Henry & Jefferies 2003), boreal (Nordin et al. 2001) and low productivity grassland (Streeter et al. 2000, Bardgett et al. 2003, Weigelt et al. 2005) such as the rupestrian field where our study was done.

Coexisting plant species might be able to partition a limited N pool, thereby avoiding competition for N resources via the uptake of different chemical forms of soil N, both organic and inorganic (McKane et al. 2002, Reynolds et al. 2003). Plant species differ in their ability to take up different forms of N which create the possibility of plants to group in island (Matias et al. 2009) or niches based on N form (Miller &

Bowman 2002). Especially in N-limited environment, coexisting species show differences in the use of different N forms, allowing the dominant plant species to use the most abundant form that is present in soil, what reinforce the existence of niches of plants that were grouped based on preferential N form (McKane et al. 2002). Based on this premise and in our results it was possible to explain the dominance of some species in rupestrian field such as plants of the families Fabaceae (*Periandra mediterranea* (Vell.) Taub., *Chamaecrista cathartica* (Mart.) H.S. Irwin & Barneby, *Chamaecrista desvauxii* (Collad.) Killip.), Astearaceae (*Achyrocline satureioides* (Lam.) DC., *Baccaris reticularia* DC., *Symphyopappus angustifolius* Cabrera), Acanthaceae (*Ruellia geminiflora* Kunth), Malpiguiaceae (*Banisteriopsis campestris* (A. Juss.) Little, *Byrsonima verbascifolia* (L.) DC., *Peixotoa tomentosa* A. Juss.), Poaceae (*Axonopus pressus* (Nees ex Steud.) Parodi, *Echinolaena inflexa* (Poir.) Chase, *Trachpogon spicatus* Kuntze) which probably prefer the uptake of dominant chemical N form in soil, that in this case is ammonium. Considering protease activity in these soils, there is a possibility that these species presented the uptake of organic N forms, such as amino acids. In presence of invasive grass, all steps of N cycle (ammonium and protease activity) were reduced by competition among species. At establishment phase the ecosystem showed resistance since the native species were dominant. Besides, at this phase *P. mediterranea* showed high foliar N content and molasses grass the lowest N content in their leaves. Reinforcing the hypothesis of biome resistance is the fact that the invasive *M. minutiflora* won the competition only when was able to change the dominant N form in soil, which was not required by native plants. We consider that the alteration in dominant N form could be the cause of the rupture of resistance threshold. This alteration in N form probably is related to rhizospheric effect over microbial population involved on ammonium oxidation.

Conclusions: (I) *P. mediterranea* is responsible to N pool formation and N availability in the studied area; (II) rupestrian native species prefer to use ammoniacal N which is the dominant N form in soils; (III) rupestrian native species can use N organic forms such as amino acids due to high soil proteolytic activity; (IV) *M. minutiflora* plants can use all inorganic N forms (ammonium and nitrate) and probably organic forms too; (V) biome resistance to invasion shows by competition for ammonium and N organic forms; (VI) the invasive species can overcome biome resistance by altering the

dominant N form to nitrate; (VII) nitrate is not the N form used preferentially by native species and that is probably why biome threshold is broken.

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