

# Distribution of non-native invasive species and soil properties in proximity to paved roads and unpaved roads in a quartzitic mountainous grassland of southeastern Brazil (rupestrian fields)

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**Abstract** One of the most important disturbances of roads is the facilitation of the increase of non-native invasive species into adjacent plant communities. The rupestrian fields of *Serra do Cipó*, a montane grassland ecosystem in southeastern Brazil, are recognized for their enormous richness of species and endemism rates. The presence of non-native invasive species in this ecosystem could threaten the existence of the native flora and its associated organisms. The aim of this study is to understand how non-native invasive species and native species are distributed along paved and unpaved roads, in a montane grassland ecosystem such as the Brazilian rupestrian fields. The two road surfaces provide differing gradients from their edges with respect to nutrients, soil chemical aspects and plant species diversity. High content of calcium at the roadside in the paved road resulted from the paving process, in which limestone gravel is used in one of the several paving phases. In these newly created habitats the

toxicity of aluminum is drastically reduced and nutrient enriched, hence representing favorable sites from where non-native invasive species are capable to colonize and grow for undetermined period waiting the chance to invade the adjacent pristine habitats. Disturbances provoked by any natural or human-caused event can provide the opportunity for the non-native invasive species to colonize new plant communities.

**Keywords** Biological invasion · Road ecology · Rupestrian fields · *Serra do Cipó* · Montane grasslands

## Introduction

The construction of roads created a new kind of freedom to humankind and encouraged economic prosperity to contemporary society (Lay 1992). However, despite all the benefits, these constructions can cause several ecological impacts on the biodiversity and ecosystem function of native habitats (e.g., Sarbello and Jackson 1985; Thurber et al. 1994; Cole et al. 1997; Forman and Alexander 1998; Bengston and Fan 1999; Forman 2000; Christen and Matlack 2009). One of the most important impacts is the facilitation of the invasion by non-native invasive species in adjacent plant communities (e.g., Parendes and Jones 2000; Trombulak and Frissell 2000; Pauchard and Alaback 2004; Christen and Matlack

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2006, 2009). Biological invasions by non-native invasive species are a primary cause of changes to biodiversity, working synergistically with other components of global change, such as climate change and habitat destruction (Dukes and Mooney 1999; Ludsins and Wolfe 2001).

Roads differ from other kinds of disturbances by being a linear structure, acting as corridors (Christen and Matlack 2006, 2009) or facilitating the access of non-native species, many of which establish and become invasive. The construction and maintenance of roads create safe areas for non-native invasive species to germinate and establish, by removing native species (and its seed bank) and adding new layers of soil, creating areas of soft and deeper soil (Frenkel 1970; Schmidt 1989; Lonsdale and Lane 1994; Greenberg et al. 1997; Trombulak and Frissell 2000). Along the roadsides, non-native invasive species of plants find lower competition relative to the dense native vegetation, and usually receive higher amounts of light and water (see Wester and Jurvik 1983; Parendes and Jones 2000). Moreover, the roadside trimming may favor non-native invasive species of plants since they are less sensitive to pruning than native species (Forman and Alexander 1998; Benefield et al. 1999). These corridors act by dispersing plant propagules (intensified by increased traffic of human and animal vectors) or opening up new habitats for native species that become abundant. Also, many native plants are capable of behaving as invasive species, such as *Baccharis dracunculifolia* (Asteraceae) and *Solanum lycocarpum* (Solanaceae; Rodarte et al. 1998, Gomes and Fernandes 2002), a native species of South America with strong invasive potential.

The different types of roads differ in their level of disturbance to the environment. Paved roads are those that impose more impacts to the adjacent ecosystems, followed by graded roads, graded unpaved roads, and four-wheel-drive tracks (off-road) (Tyser and Worley 1992; Parendes and Jones 2000; Gelbard and Belnap 2003). The edges of the paved roads are under frequent disturbance caused by vehicles and track maintenance. Gelbard and Belnap (2003) postulated a higher level of biological invasions by non-native invasive plants on paved roads (those with a higher degree of improvement), mainly due to the creation of the soil texture and to different depth and chemical characteristics of its edges, besides the invasion

promoted by the construction and maintenance. In addition, paved roads are designed to drain water from the rain from their edges, which can increase the vulnerability to these invasions, due to increased moisture and nutrient availability (Holzapfel and Schmidt 1990). According to Davis et al. (2000) the disturbances associated with an increased nutrient inflow are the main factor potentiating invasions by non-native invasive plants.

Experimental studies in grassland ecosystems have shown a negative correlation between diversity and soil nutrient status, due to the increased dominance of a few highly competitive species, whether native or non-native (Huenneke et al. 1990; Tilman 1993, 1997). Therefore, ecosystems with poor soils, such as the rupestrian fields (montaneous grasslands) in Brazil and serpentine grasslands in the United States, allow the coexistence of many plant species, perhaps due to low productivity which, in turn, leads to reduced competitive dominance. Armstrong and Huenneke (1992) reported on the increased abundance of non-native grasses in places where the  $\text{Ca}^{2+}/\text{Mg}^{2+}$  ratio was increased. In addition, Harrison (1999) found higher proportions of non-native species into higher levels of  $\text{Ca}^{2+}$ , while the number of native species showed an opposite response. Contrasting with the traditional approach to understanding invasions, an alternative approach is to focus on the physical environmental conditions that affect invasive species fitness, which are the same for native species (e.g., Huston 1979, 2004). Low resource availability and abiotic stresses caused by extreme climatic conditions are one of these environmental conditions that can be found in regions on quartzitic mountain tops in *Serra do Cipó*, Brazil. The rupestrian fields of *Serra do Cipó*, in southwestern Brazil, are recognized for their enormous species richness and endemism that reaches more than 30% of total plants species (Giulietti et al. 1987, 1997; Rodarte et al. 1998; Pirani et al. 2003). Just in the only completely surveyed area, a corridor of  $5\text{ m} \times 30\text{ km}$  ( $150\text{ km}^2$ ), Giulietti et al. (1987) reported more than 1,600 species of plants.

The presence of non-native invasive species in this ecosystem could modify the processes that lead to biodiversity and ecosystem services. In a recent publication, González et al. (2010) supports the idea that non-native invasive terrestrial plants are able to outperform natives in low-resource environments like the rupestrian fields. The aim of this study is to

understand how non-native invasive species and native species are distributed along paved and unpaved roads, in a montaneous grassland ecosystem such as the Brazilian rupestrian fields. Patterns of change in soil chemical factors in the vicinity of the roads will be observed in an attempt to determine the chemical factors that may be facilitating the establishment of non-native species (e.g., Wester and Jurvik 1983; Gelbard and Belnap 2003).

## Methods

*Serra do Cipó* is located in the southern portion of *Espinhaço* range, a group of predominantly quartzitic mountains, about 1,100 km long, in southeastern Brazil. The altitude in the study area varies from 1,000 to 1,250 m a.s.l. This area is predominantly covered by low stature woody vegetation with shrubs and small trees and abundant grasses and sedges, excepting the neighboring watercourses, where gallery evergreen forests occur. These highlands are known as rupestrian fields (*campos rupestres*), a montaneous grassland ecosystem, and is surrounded by the *cerrado* (savanna) ecosystem. The area has a Köppen climate type Cwb (mesothermic climate with mild summers and rainy season in summer), with average temperatures ranging from 17.4 to 19.8°C, being lower than 22°C in the warmer month (Galvão and Nimer 1965). The region's annual rainfall is ca. 500 mm, with a 3–4 months dry winter, and a wet period of 7–8 months (Madeira and Fernandes 1999). The soil is sandy, shallow, with low water holding capacity and nutrient poor, with high aluminum concentration (Ribeiro and Fernandes 2000; Benites et al. 2007; Negreiros et al. 2008). The stretch of paved road where the work was done crosses the protected area called *Morro da Pedreira* and the northwestern portion of the national park of *Serra do Cipó*. This road was constructed in the early 1940's as an unpaved road and the pavement of this stretch was finished in 2004.

The study was conducted between October 2007 and January 2008. In attempt to answer the questions of this study, nine 100 meter long and 1 meter wide transects (100 × 1 m), were traced perpendicularly to the paved road (from km 103, W43°35'55"/S19°17'35.4" to km 107, W43°34'00"/S19°17'25"), 500 meters distant from each other; while nine transects

of same size were traced perpendicularly to an unpaved road in the vicinity (4 km from the coordinates W43°36'02.6" e S19°17'07.5"). Each transect was subdivided into 20 subplots of 5 × 1 m. The richness and abundance of plant species were recorded at every interval of 5 m. All species were collected and identified by specialists. Species with invasive potential were determined from literature (Kissmann and Groth 1995a; Groth and Kissmann 1995b; Mitchell et al. 1999; Staples et al. 2000; Wu et al. 2003; Bakar 2004; Martins et al. 2004; Womack and Burge 2006; Munhoz and Felfili 2007; Henry et al. 2007) and from field observation of their frequency in the area.

To assess chemical properties and nutrient availability, in each subplot sampled for plant species, a soil composite sample was collected (approximately 500 g) at a depth of 20 cm, representing a valid estimate of average nutrient levels of five 100 g single samples (modified from Binkley and Vitousek 1989), totaling 90 soil composite samples for each road (paved and unpaved;  $n = 180$  samples). The sampling was done according to procedures described by Dick et al. (1996). Soils were analyzed by the Soil Department of the Universidade Federal de Viçosa. The water pH was measured in water, KCl and  $\text{CaCl}_2$  using the proportion 1:2.5 (v/v) of soil: solution. The exchangeable  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Al}^{3+}$  were extracted by KCl solution of 1 mol/L determined in extracts and the levels of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  by titration with EDTA 0.01 mol/L and the concentration of  $\text{Al}^{3+}$  by titration with NaOH 0.025 mol/L as in Silva et al. (1999). The elements P and K were extracted by Mehlich 1 solution. The potential acidity (Hydrogen + Aluminum) was extracted with solution of calcium acetate, 0.5 mol/L at pH 7.0 and determined by alcalimetric titration of extract (Silva et al. 1999). The sum of bases (SB), effective cation exchange capacity ( $t$ ), the aluminum saturation ( $m$ ) and saturation of bases ( $V$ ) were calculated, respectively, according to the expressions:  $\text{SB} = (\text{K} + \text{Ca}^{2+} + \text{Mg}^{2+})$ ;  $t = \text{SB} + \text{Al}^{3+}$ ;  $100.\text{Al} = m/t$ ,  $V = 100.\text{SB}/\text{SB} + (\text{H} + \text{Al})$  (Alvarez et al. 1999).

To test whether the nutrient availability was higher at the edges of both roads and whether the soil chemical variables were different along the transects and between roads, we used multivariate analysis of variance (MANOVA), where the independent variables were the distance and type of road (paved or

unpaved) and the dependent variables were the nutrients (K, P, N, Ca, Al, Mg) and chemical parameters of soil. In case of significant effects in the multivariate test, univariate  $F$ -tests (ANOVA) were used to identify the specific dependent variables that were significant in the general model. To determine possible differences in richness and abundance of native and non-native invasive species along the gradient away from the road, Spearman correlation tests were carried out, as the residuals did not fit the assumptions of homoscedasticity after attempts to change (Montgomery et al. 2006). All statistical analyses were performed using the software R (R Development Core Team 2007).

## Results

### Soil gradient status

Soil nutrient status varied significantly with the distance from the edge in both paved and unpaved roads (MANOVA, Wilk's lambda = 0.55,  $F = 1,826$ ,  $df = 9.00$ ,  $P < 0.001$ ). The same pattern was found for the soil chemical properties analyzed (MANOVA, Wilk's lambda = 0.474,  $F = 1.7037$ ,  $df = 9.00$ ,  $P < 0.001$ ). Significant differences between the two road types (paved or unpaved) regarding overall soil nutrients (MANOVA, Wilk's lambda = 0.744,  $F = 8.88$ ,  $df = 1.00$ ,  $P < 0.001$ ) and soil chemical aspects (MANOVA, Wilk's lambda = 0.866,  $F = 2.96$ ,  $df = 1.00$ ,  $P < 0.001$ ) were found, indicating that the type of the road (paved or unpaved) and the distance from the road side had a significant effect on nutrients availability and chemical aspects of the soil. Below we discuss the variables which influenced the overall effects (all data presented in Appendix A).

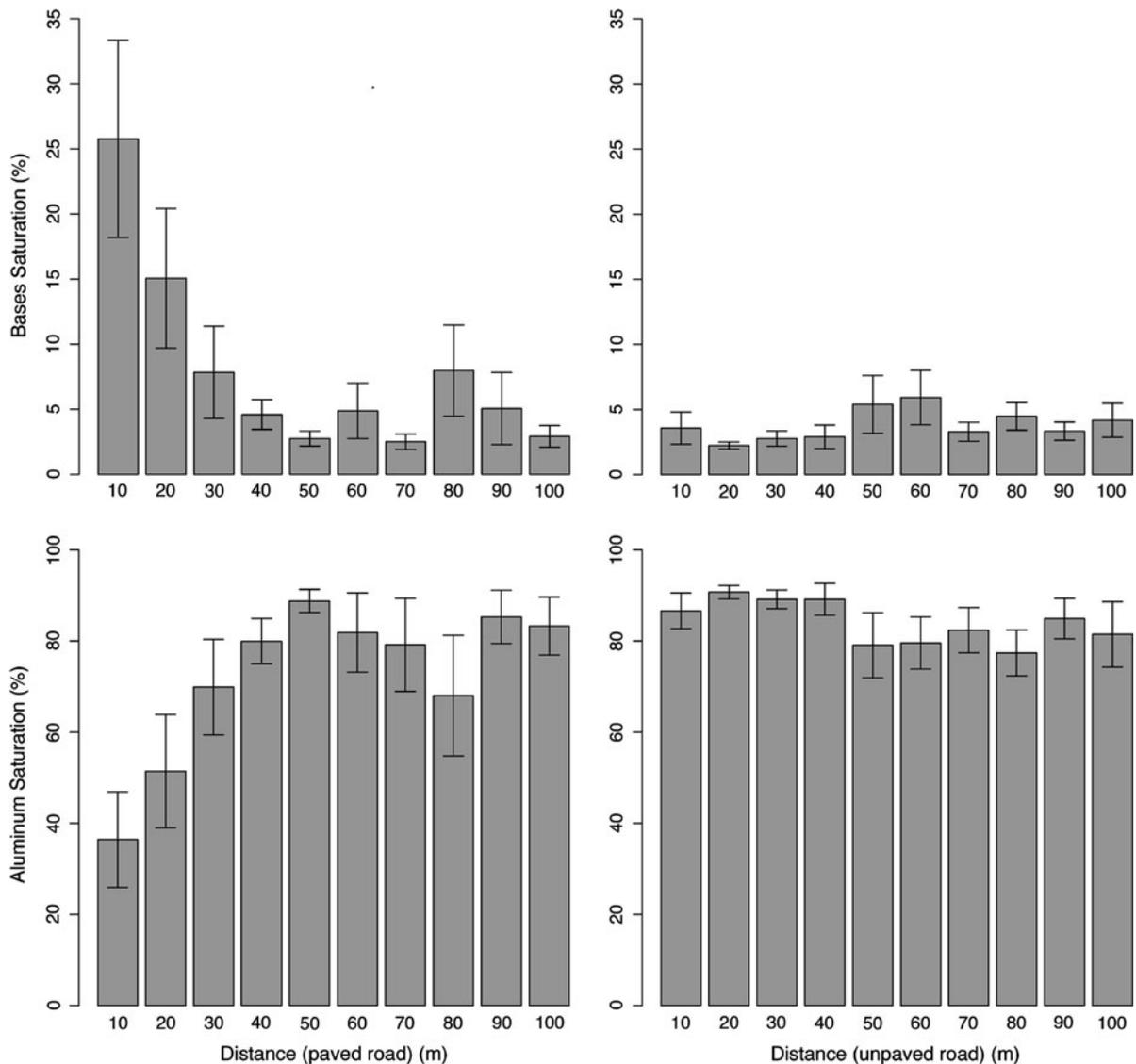
Among the two soil chemical variables that contributed to the effects of distance from the roadside and type of road, the indices of base saturation in the paved road ( $25.77 \pm 7.6\%$ ) were almost tenfold higher than the roadside of the unpaved road ( $3.57 \pm 1.24\%$ , Fig. 1). Moreover, the aluminum saturation index was lower at the roadside of the paved road ( $36.4 \pm 10.49\%$  for paved and  $86.61 \pm 3.92\%$  for unpaved road, Fig. 1). These data can be explained by the high levels of  $\text{Ca}^{2+}$  found at the roadside of the paved road, which in combination with  $\text{Al}^{3+}$ , K and N are the nutrient variables

contributing to the effects of distance and type of road (Fig. 2). High levels of  $\text{Ca}^{2+}$  on the roadside of the paved road contributed to the change in the composition of exchangeable bases, withdrawing  $\text{Al}^{3+}$  cationic sites. This is visible with the decline of aluminum saturation with the increase in  $\text{Ca}^{2+}$  levels. The levels of  $\text{Al}^{3+}$  at the roadside declined in both roads; however, presenting lower values in the paved road. The level of  $\text{Al}^{3+}$  averaged  $0.31 \pm 0.09$   $\text{cmol}_e/\text{dm}^3$  in the border of the paved road; less than half the average value found at the side of the unpaved road ( $0.75 \pm 0.11$   $\text{cmol}_e/\text{dm}^3$ ). The content of K and N were low in the side of both road types, but always at higher degree in the paved one. The content of Mg and P did not vary statistically with the distance from the roadside in both road types (Appendix A). Although the pH did not contribute to the global effect in the analysis, it showed a little increase in the first 15 meters of paved road, only surpassing the threshold of 5.5 in these areas (Appendix A).

### Plant community

We found 377 plant species belonging to 65 families in the 1,800  $\text{m}^2$  studied ( $n = 18$  transects). The most representative families were Asteraceae (62 spp.), Poaceae (40 spp.), Cyperaceae (32 spp.), Fabaceae (31 spp.), and Melastomataceae (23 spp.). Almost fifty percent (48.3%) of all species were represented by less five individuals. Nine species were recognized in the literature as potentially invasive: *Melinis minutiflora* (Martins et al. 2004), *Mimosa pigra* (Kissmann and Groth 1995a), *Cajanus cajan* (Staples et al. 2000; Wu et al. 2003), *Mimosa pudica* (Kissmann and Groth 1995a), *Sida glaziovii* (Kissmann and Groth 1995b), *Pteridium aquilinum* (Mitchell et al. 1999; Womack and Burge 2006), and *Melochia* sp. (Bakar 2004). In addition, *Paspalum notatum* (Henry et al. 2007), and *Andropogon bicornis* (Munhoz and Felfili 2007) were considered invaders to rupestrian fields due to their natural absence in these areas. All of these species were non-native introductions to the rupestrian fields of Serra do Cipó, although they are common to the cerrado (savanna) of Brazil.

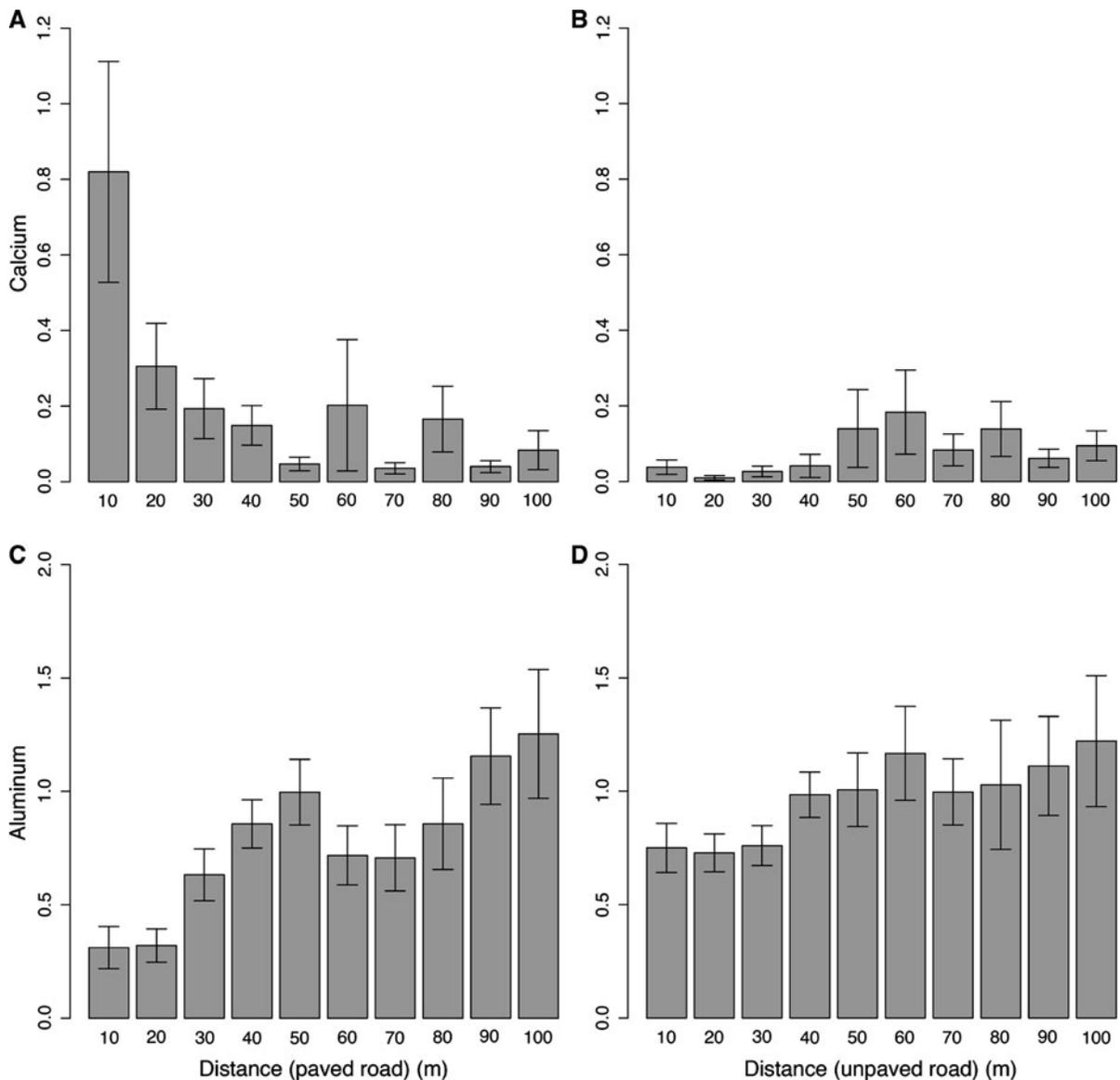
The richness of non-native invasive species correlated negatively with the distance of the roadside from the paved road ( $r = -0.31$ ,  $P < 0.001$ , Fig. 3a). Moreover, no variation in species richness of non-native invasive species was observed in the unpaved



**Fig. 1** Index of bases saturation and aluminum saturation along a gradient of 100 m from the paved road and unpaved road. These soil chemical variables contributed to the overall effects of distance from the roadside and type of road

road ( $r = -0.14$ ,  $P = 0.18$ , Fig. 3b). Clearly, the higher difference in species richness of non-native invasive species with distance from the roadside is observed in the first 5 m, indicating a strong effect of the road paving. The same trend was observed for the abundance of non-native invasive species. A negative correlation of non-native invasive species abundance and distance was found from the roadside in the paved road ( $r = -0.31$ ,  $P < 0.001$ , Fig. 3c) while no statistical correlation was detected in the unpaved road ( $r = -0.17$ ,  $P = 0.10$ , Fig. 3d).

The richness of native species did not vary significantly with the distance from the roadside in both paved ( $r = -0.16$ ,  $P = 0.13$ , Fig. 3e) and unpaved roads ( $r = -0.19$ ,  $P = 0.07$ , Fig. 3f), perhaps indicating the short time the impact was installed. On the other hand, while the abundance of native species did not correlate with the distance from the roadside in the paved road ( $r = 0.05$ ,  $P = 0.61$ , Fig. 3g), the abundance of native species correlated negatively with the distance from the roadside in the unpaved road ( $r = -0.27$ ,  $P < 0.01$ , Fig. 3h).



**Fig. 2** Calcium rates (cmol<sub>e</sub>/dm<sup>3</sup>) and Aluminum rates (cmol<sub>e</sub>/dm<sup>3</sup>) along a gradient of 100 m from the paved road and unpaved road. High content of calcium at the roadside in

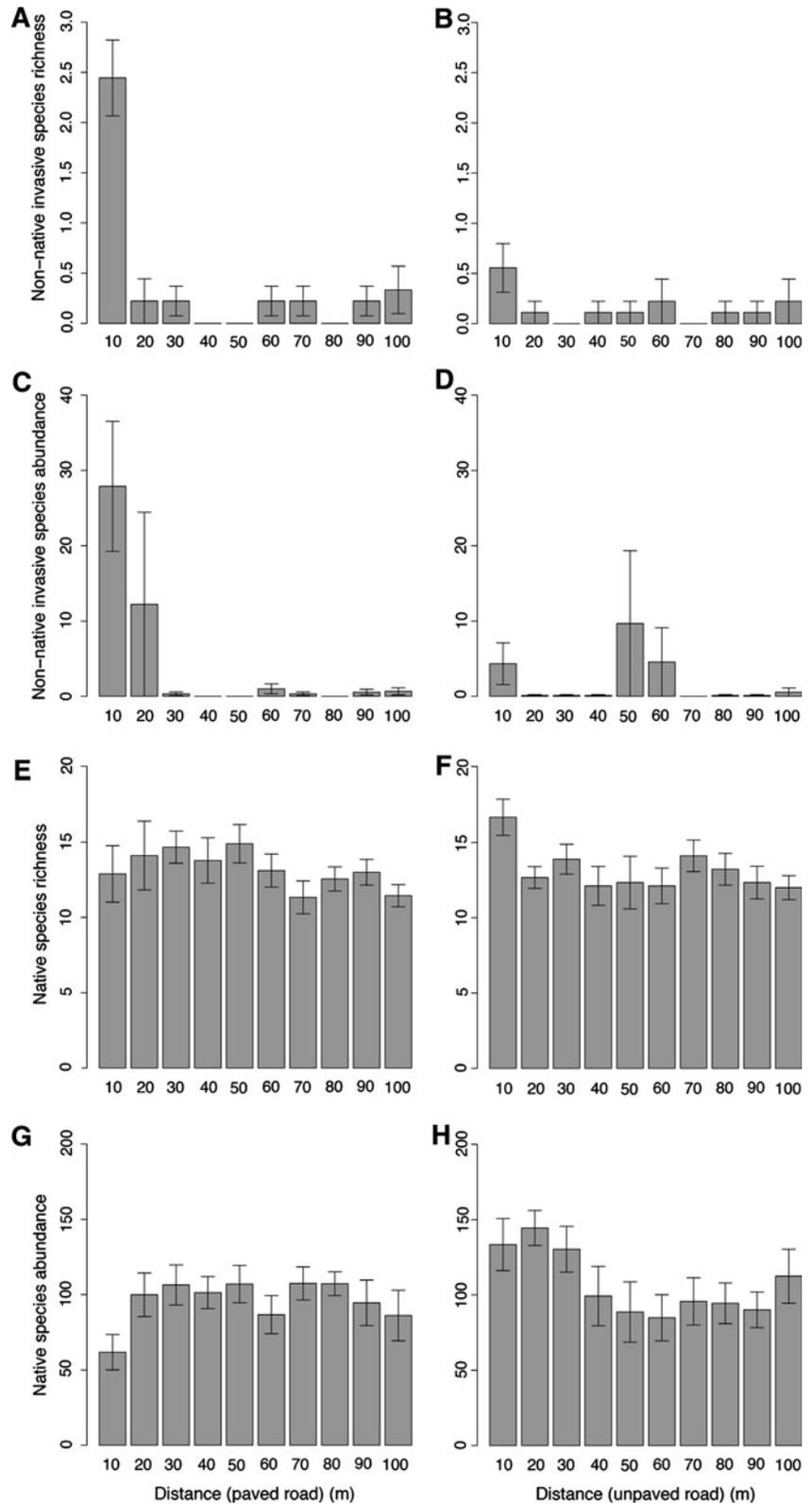
the paved road resulted from the paving process, in which limestone gravel is used in one of the several phases of the paving

## Discussion

The paved and unpaved roads have different gradients from the edge with respect to nutrients, soil chemical aspects and plant species diversity. It is possible that the construction of the paved road has modified the physical and chemical conditions of the adjacent soil. Apparently, the calcium intake at the roadside of the paved road was responsible for enhanced pH at the first

5 m and for the drop in aluminum saturation. Such a trend was not found at the edges of the unpaved road. High levels of calcium were regarded as an important mechanism for the establishment of non-native invasive species (Harrison 1999). High content of calcium at the roadside in the paved road resulted from the paving process, in which limestone gravel is used in one of the several phases of the paving. We argue that use of the limestone gravel, rich in calcium, was

**Fig. 3** Richness and abundance of native and non-native invasive species along a gradient of 100 m from the paved road and unpaved road. Non-native invasive species richness for paved (a) and unpaved road (b); Non-native invasive species abundance for paved (c) and unpaved road (d); Native species richness for paved (e) and unpaved road (f); Native species abundance for paved (g) and unpaved road (h). See text for statistic values



responsible for liming the edges of the paved road. Liming resulted in increased pH up to 5.5 at the edges of the paved road. Contrarily, such phenomenon was not observed in the unpaved road. Soils presenting pH values (in H<sub>2</sub>O) lower than 5.5 can cause serious problems of aluminum toxicity to plants (e.g., Barber 1984; Machado 1997). Rupestrian fields soils are acid (Ribeiro and Fernandes 2000; Benites et al. 2007; Negreiros et al. 2008), with pH values that allow the presence of the trivalent Al<sup>3+</sup> ion. Accordingly, most of the plants not adapted to aluminum present limited growth and performance unless the pH is high, reducing the availability of Al<sup>3+</sup> in the soil (e.g., Osaki et al. 1997). Although several factors are important for the establishment of non-native invasive species, such as soil moisture and dispersion, lower levels of aluminum saturation could enable non-native invasive species to better development and deeper root growth, maximizing the use of water and scarce nutrients (e.g., Pavan et al. 1984).

Cronan and Grigal (1995) postulated that the calcium/aluminum ratio could be an indication of risk of aluminum toxicity. In our study, the average ratio of calcium/aluminum was always under 0.5 in the unpaved road, while in the paved road it was around 1.5. Using Cronan and Grigal's risk of aluminum toxicity, our data indicate that species not adapted to the toxicity of aluminum would be exposed to less than 50% of risk at the edge of the paved road, while far from road, to almost 100%. This is another indication that at the roadside in the paved road rupestrian field non-native species would be favored due to the lower levels of aluminum in the toxic soil. Many native species of *cerrado* and rupestrian fields species use strategies of Al exclusion or absorption without any detrimental effect to their growth, reproduction, or metabolic functions, while others are Al accumulators (Haridasan 1982, 1987). Another possibility is that the intake of calcium may play an important role on the imbalance of K and Mg, which may adversely affect the adjacent natural communities (Lopes 1983). *Andropogon bicornis* and *Paspalum notatum* are two species with widespread distribution within the *cerrado*, and have good ability to grow in toxic aluminum soils. Because of this, these species could better use the opened gaps at roadsides to expand into the now threatened rupestrian fields. Future studies need to address the effect

of liming in the soils of the region and its immediate effect on some native and exotic species.

Many factors lead to an accumulation of non-native species at the edge of the paved road, including greater dispersion due to traffic, higher amount of water and light, lower competition with the dense native vegetation and physical and chemical changes in soil (Wester and Jurvik 1983; Holzaphel and Schmidt 1990; Parendes and Jones 2000). These stocks of non-native invasive species may benefit from fire that is frequent in the region or even better soil conditions found in termite mounds. Rupestrian fields are mature communities which are not highly sensitive to invasion, unless disturbances occur. While some areas of these pristine fields are protected (national park), areas outside legal protected are subjected to grazing and tourism and both types of areas are likely to be burnt by frequent fires. For instance, immediate effects of fire lead to a temporary increase in exposed soil. In these gaps the concentrations of calcium, magnesium, phosphorus and potassium are increased due to ash accumulation. Furthermore, aluminum could decrease to zero for more than 40 days (Coutinho 1990; but see Nardoto et al. 2006). So, these islands of safe sites could easily be invaded by non-native invasive species which now reside along the more favorable sites created in the margins of paved roads. Disturbances provoked by any event, be it natural or human-caused, could provide the opportunity to spread into these natural habitats. Some non-native invasive species keep producing propagules at much faster rates than native species and are most equipped with evolved strategies and adaptations to colonize the new open habitats, like *Melinis minutiflora*, *Cajanus cajan* and *Mimosa pigra*, for instance. They do not only just produce more seeds but also disperse them continuously over the natural vegetation and the seeds become part of the seed bank (see Medina and Fernandes 2007). The continuous colonization of newly created disturbed sites potentially changes the landscape and ecosystem function and processes. These species behave as opportunists, which ultimately leads to large impacts that may evolve from a local to regional scale. Not surprisingly, some species can wait more than 50 years after established to become invasive (Daehler 2009). However, it should be noted that local disturbance often do not necessarily provide an

opportunity for invasive species spread in natural habitats. Future and long-term studies will address the dynamics of non-native invasive species in this mountain and test this argument.

The lack of calcium intake in the unpaved road helps to avoid a drastic decrease in the saturation of aluminum in its edges. Moreover, these areas do not have the active or incidental introduction of non-native invasive species or in which the seed bank was preserved (for details on the seed bank of the studied area see Medina and Fernandes 2007). Furthermore, the traffic is less pronounced, contributing less to the dispersion of propagules caused by human vectors than in the paved road. Hence, native species were more abundant at its borders compared to the paved road due to the union of these factors and others yet unknown.

The paving of this section of the road may have had an auxiliary role on the process of introduction and establishment of non-native invasive species in *Serra do Cipó*, acting synergistically with other factors such as increased availability of water and light and dispersion by traffic. These mountains are strategic to water conservation of southeastern Brazil, where most of the Brazilian population lives. Efforts by the Brazilian government agencies and policy makers should be aware of and monitor this invasion, given the threats posed by the phenomenon itself and increasing global climate warming. Intensive roadside monitoring and control should be implemented in order to prevent further establishment of weeds and prevent the already established ones to colonize beyond the roadsides, spreading through the native vegetations and modifying the landscape and ultimately cause serious damages to ecological services, and even extinctions of the so many narrowly distributed endemic species (see Moreira et al. 2009). Proposals for constructing paved roads in quartzitic montaneous grasslands, which are generally inhabited by a unique and highly adapted flora to live under nutritional stresses should ban the use of any products derived from lime rocks to minimize the bias of non-native species in these regions together with other actions such as environmental education and management of non-native species already introduced.

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## Appendix A

See Table 1.

**Table 1** MANOVA results for chemical variables and nutrient availability

Dependent variables	Independent variables		Contributed to overall effect?
	Distance from road (p)	Type of road (p)	
Chemical variables <sup>a</sup>			
pH	0.283	0.121	No
Al + H	0.002*	0.082	No
Sum of exchangeable bases	0.121	0.112	No
Effective cation-exchange	0.030*	0.201	No
Cation-exchange pH 7	0.008*	0.154	No
Base saturation	0.001*	0.001*	Yes
Aluminum saturation	0.031*	0.001*	Yes
Remanescent phosphorus	0.341	0.605	No
Nutrient availability <sup>b</sup>			
P	0.060	0.014*	No
K	0.000*	8.53E-07*	Yes
N	1.69E-07*	0.022*	Yes
Ca	0.005*	0.006*	Yes
Mg	0.094	0.373	No
Al	3.58E-07*	0.012*	Yes

<sup>a</sup> Wilk's lambda = 0.474,  $F = 1.7037$ ,  $df = 9.00$ ,  $P < 0.001$

<sup>b</sup> Wilk's lambda = 0.55,  $F = 1.826$ ,  $df = 9.00$ ,  $P < 0.001$

\* Significant

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