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Passive restoration of subtropical grasslands leads to incomplete recovery of ant communities in early successional stages

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ABSTRACT

Understanding how land use and management practices affect biodiversity is essential for developing effective restoration and conservation strategies. Here, we used ant communities to evaluate the effects of historical land uses (former agriculture or tree plantation) and current management (grazing, burning, mowing, and fertilizing) in subtropical grasslands. We also examined how ant species richness and composition are affected through changes in resource diversity and habitat structure. We classified 80 study sites in southern Brazil into two groups: permanent and secondary grasslands. Permanent grasslands presented high, medium or low management intensities that had never been under other land use. Secondary grasslands are former grasslands that were used for agriculture or pine plantations in the past, followed by passive restoration. We sampled ant communities with D-Vac suction in three multiplots per site in 2013–2015. We found that secondary grasslands had lower ant species diversity and different community composition than permanent grasslands. Ant diversity did not differ among permanent grasslands, but composition was markedly different, especially when comparing medium and high management intensities with low. Additionally, ant richness in secondary grasslands was mainly mediated by a decreased resource diversity (i.e., plant species richness) and grass cover, while burning and mowing management increased ant richness by increasing resource diversity. Our results indicate that passive recovery of grasslands in early successional stages after other land uses leads to incomplete recovery of ant communities, as important resource and structure-related drivers of ant species remain altered. In contrast, traditional management practices promote ant diversity.

1. Introduction

Natural and semi-natural grasslands are biodiversity hotspots and support essential ecosystem services but are severely threatened by land-use changes (Hoekstra et al., 2005; Overbeck et al., 2015). Conversion of complex natural grasslands to simplified land-uses, such as agriculture and tree plantations, leads to biodiversity losses (Marriott et al., 2004), and grasslands that were cleared, cropped and then abandoned do not easily recover their original biodiversity (Buisson et al., 2019). In consequence, biological communities becoming established on such abandoned sites tend to differ significantly from reference ecosystems (e.g., Koch et al., 2016), and it may require decades or even centuries for restoration (i.e., full recovery of native biota and ecosystem functions; Gann et al., 2019). The success of passive restoration, as a process of natural or spontaneous regeneration, might therefore depend on the level of soil degradation and the distribution of natural habitat remnants (Fensham et al., 2016), even when under the adequate regime of grassland management (Buisson et al., 2019).

Another common threat to grassland biodiversity is the alteration of traditional management (Uchida and Ushimaru, 2014). Grassland ecosystems are evolutionarily shaped by disturbances, such as grazing and fire (Milchunas et al., 1988; Bond and Keeley, 2005), and thus

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appropriate management is essential for grassland conservation (Overbeck et al., 2007; Veldman et al., 2015). Intermediate levels of disturbances (i.e., through medium or low impact management approaches) usually maximize species diversity and productivity (Overbeck et al., 2005; Nabinger and de Faccio Carvalho, 2009; Uchida and Ushimaru, 2014). Both management extensification (i.e., cessation or reduction of cattle grazing, mowing, or fire) and intensification (increase of grazing stocking rates, overseeding with exotics, and fertilization) change abiotic properties and favour the dominance of more competitive or disturbance-tolerant species, thus, decreasing diversity and causing biotic homogenization at the landscape scale (Gossner et al., 2016). Understanding how land-use change and management intensity affect biodiversity is essential given the urgent need to develop effective restoration and conservation strategies.

In south Brazil, grasslands managed through medium-intensity grazing are considered reference systems for conservation because they maintain high plant diversity (Koch et al., 2016; Ferreira et al., 2020). Degradation of grasslands and the associated losses of biodiversity and ecosystem functions are prevalent in South America (Leidinger et al., 2017; Staude et al., 2018; Tiscornia et al., 2019), in particular after land-use change. South Brazilian grasslands have suffered massive changes in recent decades, mainly due to the expansion of soybean cultivation and exotic tree plantations (Oliveira et al., 2017; Torchelsen et al., 2019). Even when intensive land use is abandoned, the secondary grasslands are depauperated and differ from permanent grasslands (Koch et al., 2016). In addition, the type and intensity of management in permanent grasslands have been shown to alter the grassland communities and their ecological processes (Koch et al., 2016; Leidinger et al., 2017; Ferreira et al., 2020). While biodiversity reduction in degraded grasslands has been described for plant communities, knowledge about effects on other trophic levels is insufficient.

Terrestrial invertebrates have been widely used for monitoring land-use impacts because they are abundant, diverse, easily sampled, and fulfil important functional roles in the ecosystems (e.g., Andersen and Majer, 2004; Gerlach et al., 2013). Among invertebrates, ants are a globally dominant faunal group recognized as a robust bioindicator due to their sensitivity to environmental change (Andersen, 2019; Philpott et al., 2010). Moreover, ants are social insects with sessile colonies nesting in the soil or vegetation (Hölldobler and Wilson, 1990), which can be an advantage as opposed to organisms that are sampled in a site but actually are just passing through. In a recent meta-analysis, Casimiro et al. (2019) have reinforced the performance of ants in the monitoring of ecological restoration, demonstrating that natural regeneration of degraded lands is indeed able not only to restore ant diversity but also to enhance it above reference levels. Therefore, ant communities should be suitable indicators to analyze the effectiveness of grassland recovery and the impacts of different management intensities. Earlier studies on south Brazilian grasslands showed that these ecosystems support a highly diverse ant fauna (Dröse et al., 2017), and that environmental filtering is an important process structuring ant communities at local scales (Dröse et al., 2019). However, studies on the effects of land-use change and grassland management on ants in these ecosystems are still missing (but see Rosado et al., 2013).

Habitat structure and resource diversity are key factors driving indirect ant responses to land use. Disturbances that cause a direct impact on soils (e.g., clearing, tillage, cropping) have a stronger impact on ant communities than those primarily removing plant biomass (e.g., grazing, burning, mowing; Hoffmann and Andersen, 2003). Land-use intensification affecting soil compaction and moisture can also influence ant nest architecture, thus affecting population persistence for different ant species (Schmidt et al., 2017; Heuss et al., 2019). Furthermore, variations in vegetation structure can result in ant species turnover due to preferences for either shady or open habitats, potentially affecting ant population and community dynamics (Hoffmann, 2010; Andersen, 2019). Bare soil (Silva et al., 2020), grass and herbaceous vegetation cover (Queiroz et al., 2017), vegetation height (Dröse et al., 2019), and shrub encroachment (Wiezik et al., 2013) have already been described as ecological drivers of ant communities in grasslands. Plant species richness is often used as a proxy for food resource diversity for ants, and a positive relationship between plant richness and ant diversity has been found (Ribas et al., 2003; Vasconcelos et al., 2019).

Here, we specifically asked (1) whether ant species diversity and community composition differ between secondary (i.e., early stages of spontaneous grassland recovery previously converted to cropland or tree plantations) and permanent grasslands in south Brazil. We expected ant communities in secondary grasslands to be impoverished and altered. We further ask (2) whether ant species diversity and community composition differ among permanent grasslands with different management intensities. We expected that both extensification and intensification of grassland management lead to ant diversity losses and community change, while grasslands managed at intermediate levels with traditional disturbance, such as grazing and fire, should sustain higher ant diversity. To elucidate how ant diversity responds to land-use change and management intensity, we used an integrative approach considering the potential indirect pathways of altered habitat structure and resource diversity. Additionally, we further unmasked ant interspecific association to grassland types, as 'habitat condition indicators' (Bakker, 2008), shedding light on relationships between species life history and habitat structure to complement broad community composition patterns.

2. Material and methods

2.1. Study area

The study was carried out in the highland region in southern Brazil, Rio Grande do Sul state (Fig. 1A). Study sites were located in the municipalities of Cambará do Sul (29°02′ S, 50°34′ W), Jaquirana (28°53′ S, 50°21′ W), and São Francisco de Paula (29°26′ S, 50°34′ W), within a region of ca. 4800 km^2. Altitude ranges from 900 to 1200 m a.s.l. Climate is humid subtropical, i.e., Cfb type according to the Köppen climate classification (Alvares et al., 2013), with temperate summers, frosts during winter, and no dry season. Mean annual temperature is 14–16 °C, and mean annual precipitation 1500–1700 (2500) mm (Almeida, 2009).

The highlands are originally characterized by natural grasslands in mosaic with *Araucaria* forests (Andrade et al., 2019). While the currently humid and warm climate is suitable for forest expansion (Behling, 2002), fire and grazing have contributed to the maintenance of natural grasslands and their biodiversity (Overbeck et al., 2007). However, accelerated transformation of natural areas by agriculture and silviculture, and different grassland management intensities, have resulted in a mosaic of different land uses in the region.

2.2. Sampling design

We studied 80 grassland sites with a minimum size of 1 ha each and most of them with more than 10 ha, distributed throughout the region (Fig. 1A). At all sites, at least one natural grassland site under medium- or low-intensity management (see below for definitions) was situated within a radius of 2 km, so that source sites for ant populations were available. All appropriate ethic and environmental approvals were supplied by the Environmental Secretariat of Rio Grande do Sul state (SEMA, Brazil) for sites within conservation units and by landowners in sites within private lands.

With the help of conservation authorities and landowners, as well as satellite images (Google Earth), we gathered information on land-use history for each site (i.e., whether a grassland was historically converted to another land use, or not) and on recent grassland management (i.e., grazing intensity, burning frequency, mowing, fertilizing, overseeding). We then classified the grasslands into two types: (1) permanent, sites with an uninterrupted grassland history (n = 46), or (2)

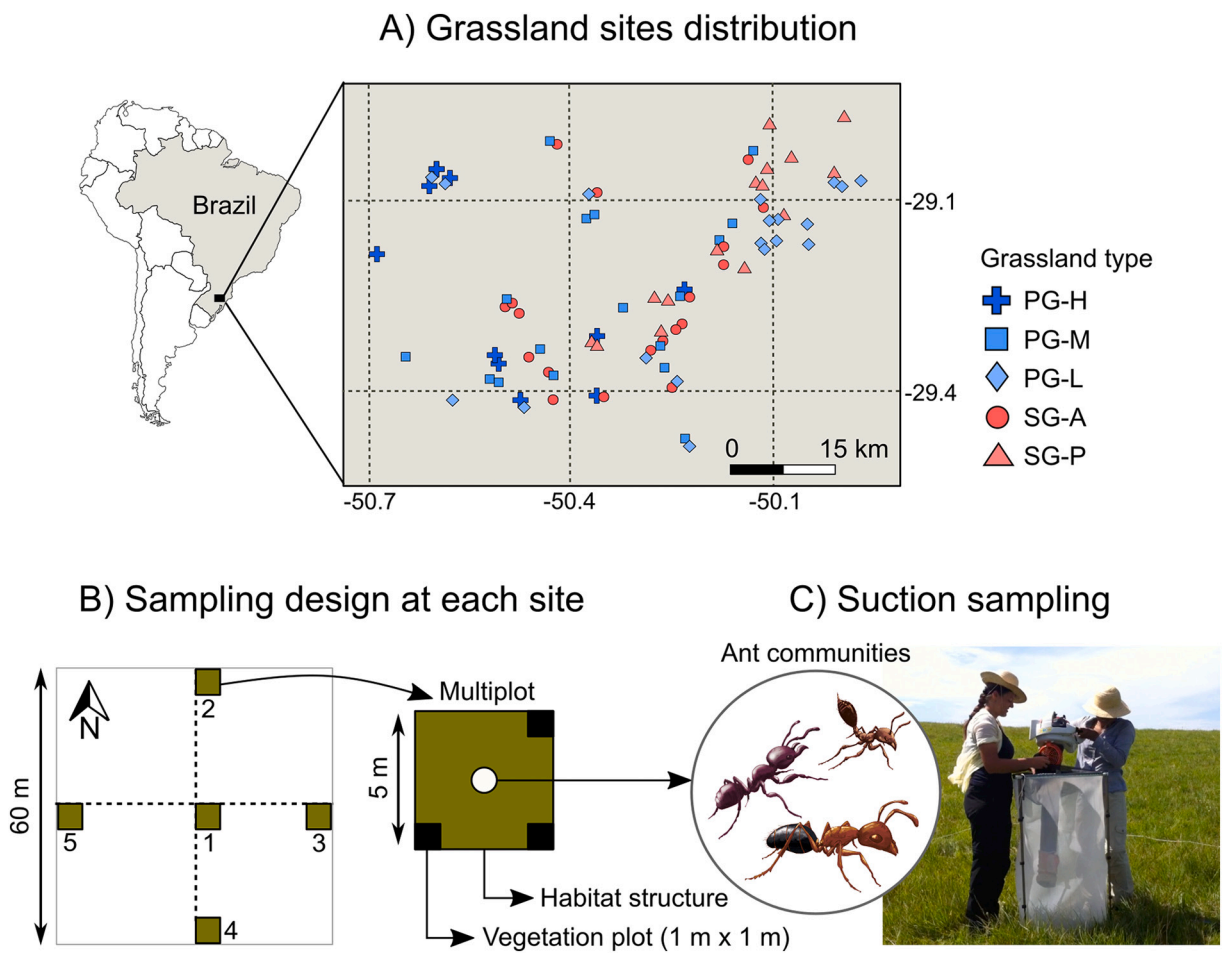

Fig. 1. (A) Study site distribution in the highland region in southern Brazil. Grassland types: permanent grasslands with high (PG-H), medium (PG-M), and low (PG-L) intensity management; secondary grasslands after conversion to agriculture (SG-A) or pine plantation (SG-P). (B) Sampling design at each study site with five 5 m × 5 m multiplots and fifteen 1 m × 1 m vegetation plots. Habitat structure variables were sampled in all five multiplots. (C) D-Vac device (Stihl SH 86) used to sample ground- and vegetation-dwelling ant communities using a fine-meshed gauze cage (0.25 m^2) at multiplots 1, 2, and 4.

secondary, sites previously converted to arable fields or tree plantations, then abandoned and undergoing natural recovery ($n = 34$).

Permanent grasslands were further classified according to the intensity of recent management practices: (i) high (PG-H; $n = 10$), (ii) medium (PG-M; $n = 17$), and (iii) low-intensity (PG-L; $n = 19$). PG-M grasslands are traditionally managed under intermediate cattle stocking rates (average of 6.0 heads of cattle month, per ha and year, ± 0.4 SE), and burned every 1–2 years commonly at the end of winter by the landowners as a management tool to remove dry biomass and favour high-quality vegetation resprouting; they are considered our reference models. PG-H grasslands present high cattle stocking rates (15.6 ± 3.9), and are frequently fertilized and over-seeded with exotic forage. PG-L grasslands present low cattle stocking rates (12.3 ± 1.5) and only occasional burnings. Both PG-H and PG-L have suffered from management intensification and extensification (respectively) for more than 10 years (Appendix A: Table A1; Koch et al., 2016; Leidinger et al., 2017).

Secondary grasslands were divided into two categories: (iv) former agriculture (SG-A; $n = 19$), and (v) former pine plantations (SG-P; $n = 15$). SG-A grasslands presented in average 6 years (±1.1) of arable use (time as non-grassland) with tillage use and intensive fertilization. Abandonment of agriculture occurred about 3 years (±0.5) before our sampling, and the early-successional secondary grasslands then sustained relatively high cattle stocking rates (13.8 ± 3.0). SG-P grasslands had on average 23.8 years (±2.8) of silvicultural use, and about 4.3 years (±0.9) of abandonment and passive restoration with currently low cattle stocking rates (2.4 ± 0.3). Fire was not recorded in secondary grasslands (Appendix A: Table A1; Koch et al., 2016; Leidinger et al., 2017). We further refer to all these five categories as grassland types.

Sampling was conducted in two seasons: November 2013 to February 2014 (sites 1–40), and November 2014 to January 2015 (sites 41–80). We selected sites randomly in each season to avoid sampling bias regarding grassland types. We systematically established a 60 m × 60 m plot at each site, with four 5 m × 5 m multiplots oriented on a cross along a north-south axis, and one multiplot at the plot centre (Fig. 1B).

2.3. Ant communities

At each site, we systematically sampled grassland ant communities by suction sampling (Brook et al., 2008) in three multiplots (multiplots 1, 2, and 4; Fig. 1B). At each multiplot, we randomly threw a cage of 0.25 m^2 over the vegetation, fixed it to the ground, and sampled all arthropods from within the cage (i.e., from the ground and vegetation) with a D-Vac device (Stihl SH 86) twice for 2.5 min each, with a small break in between (Fig. 1C). The cage was closed by gauze material at the top to minimize individuals escaping during sampling. Sampled arthropods were stored in plastic bags with ethyl acetate and then preserved in 80% ethanol before sorting to taxonomic groups at the Laboratório de Ecologia de Interações (LEIN) of the Universidade Federal do Rio Grande do Sul (UFRGS).

We identified ants to genus level based on Baccaro et al. (2015), and determined species with specific literature (e.g., Gonçalves, 1961; Wilson, 2003; Longino and Fernández, 2007) and in comparison with

specimens deposited in scientific collections at LEIN and Entomological Collection Padre Jesus Santiago Moure at the Universidade Federal do Paraná (DZUP). Vouchers were deposited both at LEIN and DZUP.

2.4. Habitat variables

We assessed grassland habitat structure variables and plant species richness in all five multiplots at each site (Fig. 1B); these data are fully described in Koch et al. (2016). As variables to describe grassland structure and habitat openness (Podgaiski et al., 2014; Andersen, 2019; Dalle Laste et al., 2019), we estimated bare soil, shrub and graminoid cover at the multiplot scale. These variables were responsive to the different grassland types (Koch et al., 2016). In three 1 m × 1 m vegetation plots per multiplot (Fig. 1B), we recorded the identity and visually estimated the cover for each plant species with the Londo scale. Plant species richness (average of the 15 vegetation plots per site) was used as a proxy for ant resource diversity (Ribas et al., 2003; Vasconcelos et al., 2019).

2.5. Data analysis

We performed all statistical analyses using R (R Version 3.6.3, R Development Core Team, 2020). Ant catches from the three multiplots were pooled to provide site-level ant data.

2.5.1. Ant species diversity

To compare ant diversity among grassland types at the regional scale, we calculated rarefaction and extrapolation curves with Hill numbers $q = 0$ (species richness), $q = 1$ (the exponential of Shannon's entropy index), and $q = 2$ (the inverse of Simpson's concentration index) using the framework suggested by Chao et al. (2014). The higher the q value, the more weight is given to frequencies of abundant species (Chao et al., 2014). In our study, grassland types varied in the number of sampling sites. Thus, we constructed incidence-based inter/extrapolation curves with 95% confidence intervals based on 50 bootstraps using the package *INEXT* (Hsieh et al., 2016). Extrapolation was set up to 19 sites, which is the largest sampling size of a grassland type (i.e., PG-L and SG-A), to allow comparisons.

At the local scale, we tested for differences in average ant species richness among grassland types using generalized linear mixed models (GLMMs). Sampling season was used as a random factor. Species richness best fitted a negative binomial distribution, based on maximum likelihood estimation with the *fitdist* function in the *fitdistrplus* package (Delignette-Muller and Dutang, 2015). We then performed GLMM with the *glmer.nb* function of the *lme4* package (Bates et al., 2015). The models were submitted to analysis of residuals to check model performance. In case of significant grassland type effects on ant richness, we performed post-hoc Tukey tests to compare categories with the *multcomp* package (Hothorn et al., 2008).

We further tested for direct and indirect effects of conversion (i.e., former conversion to agriculture or pine plantation) and current management intensity (i.e., grazing, burning, mowing, and fertilizing) that may lead to different effects of grassland vegetation (Koch et al., 2016) on local ant species richness, considering all the 80 sites. Burning, mowing, and fertilizing were used as binary variables (presence/absence), while grazing intensity was represented by heads of cattle months per ha per year. We used Structural Equation Modeling (SEM) and included direct and indirect effects of former conversion to agriculture or pine plantation, and only indirect effects of grazing intensity, burning, mowing and fertilizing on ant species richness (Appendix A: Fig. A1). Several studies have already reported that the major effects of disturbances on ants are indirect through the altered habitat structure, microclimate, resource availability, or competitive interactions – see a review in Andersen (2019) – and that direct effects, if existent, are of minor importance. Here we tested these indirect effects of conversion or current management practices on ant richness through changes in resource diversity and habitat structure variables (Appendix A: Fig. A1). Plant species richness was used as a proxy for resource diversity, while bare soil, shrub, and grass cover were chosen as habitat structure variables, due to their variation across grassland types (Koch et al., 2016).

We checked multicollinearity among the proposed indirect drivers, plant species richness, bare soil, shrub and grass cover, using the Variance Inflation Factor (VIF) (Akinwande et al., 2015) with the *fmsb* package. All variables presented VIF ≤ 1.20, which indicates insignificant multicollinearity and were maintained in the models. SEM were performed with the *piecewiseSEM* package (Lefcheck, 2016), which allows including a random effect (i.e., sampling season). The goodness-of-fit tests were conducted using Fisher's C and p-value. Contrary to the most tests, a non-significant p-value indicates a good probability that a model fits the data, indicating consistency between observed data and the hypothesized model.

2.5.2. Ant species composition

To investigate if ant species composition varies among grassland types we first performed a permutational multivariate analysis of variance on Bray-Curtis dissimilarity of ant species data. As a measure of each species abundance per site, we considered the number of samples per plot in which each species occurred (i.e., 0 to 3). As ants are social organisms, these data approximate the number of ant colonies per species at each site (Gotelli et al., 2011). We used the *adonis* function, with 999 permutations. To check pairwise differences between grassland types we used the function *pairwise.perm.manova* from the package *RVAideMemoire* (Hervé and Hervé, 2020), with 999 permutations. To visualize patterns in ant community composition among grassland types we performed non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities.

To investigate potential effects of resource diversity and habitat structure driven by conversion history and current management practices on ant species composition, we used partial distance-based redundancy analysis (dbRDA). We considered plant species richness, and bare soil, shrub, and graminoid cover, as predictors in the model. Environmental variables were standardized to 0 average and 1 standard deviation using the *decostand* function in the *vegan* package previous to the analysis. The sampling season was added as a conditional argument in the model.

To test whether particular ant species are indicators for particular grassland types, we conducted an indicator species analysis (ISA – Dufrêne and Legendre, 1997). This method considers the indicator value (*IndVal*) of each species, combining values of habitat specificity (ecological preferences) and fidelity within a specific habitat (species-site association strength). Species with *IndVal* of 100 are perfect indicators. We also used ant incidence data per site as an abundance measure, and performed the analyses with the *multipatt* function of the *indicspecies* package using 999 permutations.

3. Results

We sampled 11,384 ants from 62 species, 23 genera and seven subfamilies (Appendix A: Table A2). *Pheidole* was the richest genus (12 species), followed by *Solenopsis* (eight species), *Camponotus* and *Hypoponera* (six species each). The most common species were *Solenopsis invicta* (present in 58 out of 80 sites – 72.5%), *Solenopsis* sp. 5 (66.3%), *Linepithema micans* and *Pheidole obtusopilosa* (55.0% each), and *Pheidole pampana* (48.8%).

3.1. Ant species diversity

Sample-based rarefaction and extrapolation curves indicated that accumulated ant species richness ($q = 0$) was similar across all five grassland types (Fig. 2A). However, with increasingly weighing more abundant species (Shannon diversity, $q = 1$ and Simpson diversity, $q = 2$) the differentiation between permanent (PG-H, PG-M, PG-L) and

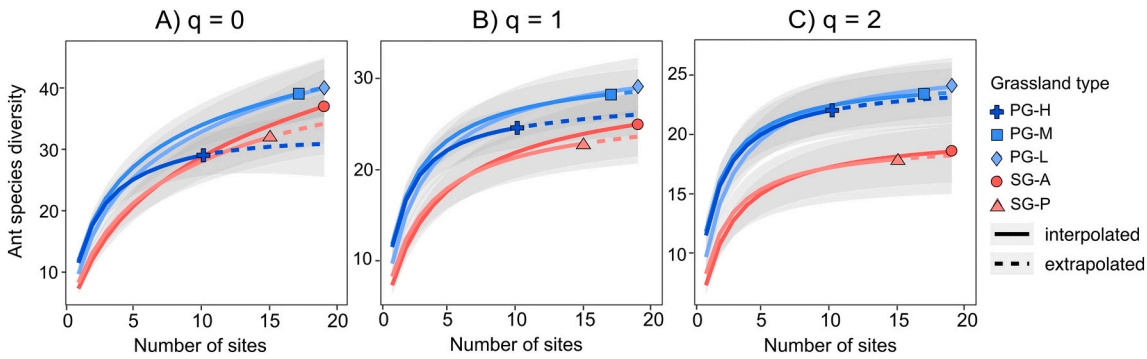

Fig. 2. Sample-based rarefaction and extrapolation curves with 95% confidence intervals (grey areas) comparing ant Hill numbers among grassland types. Hill numbers: $q = 0$ (species richness), $q = 1$ (exponential of Shannon's entropy index), and $q = 2$ (inverse of Simpson's concentration index). Grassland types: permanent grasslands with high (PG-H), medium (PG-M), and low (PG-L) intensity management; secondary grasslands after conversion to agriculture (SG-A) or pine plantation (SG-P). Solid lines are interpolation, and dashed lines are extrapolation curves. Extrapolation extends up to 19 sites for each grassland type.

secondary grasslands (SG-A, SG-P) became clearer (Fig. 2B), showing significant differences when using Simpson diversity (Fig. 2C).

Average species richness per site varied significantly among grassland types ($\chi^2 = 23.1$; $p < 0.01$; $R^2_{(c)} = 0.24$; Fig. 3). Pairwise comparisons indicated that both secondary grassland types (SG-A, SG-P) had lower richness than PG-M, and SG-A lower than PG-H. The three types of permanent grasslands did not differ among themselves, neither did the two secondary grasslands (Fig. 3).

Structural equation modelling indicated that both conversions to agriculture or pine plantation did not directly affect ant species richness in the recovering grasslands. However, the main negative effect of former grassland conversion on ant richness was mediated by a reduction in plant species richness and grass cover (Fig. 4). Conversion to pine plantation also favoured shrub cover, which positively affected ant richness. Considering current management practices, permanent grasslands with burning and mowing increased ant richness as mediated by increased plant species richness. Although conversion to agriculture, pine plantation and grazing intensity led to higher bare soil cover, the latter did not significantly affect ant species richness in the recovering grasslands. Fertilizing did not predict any relationships.

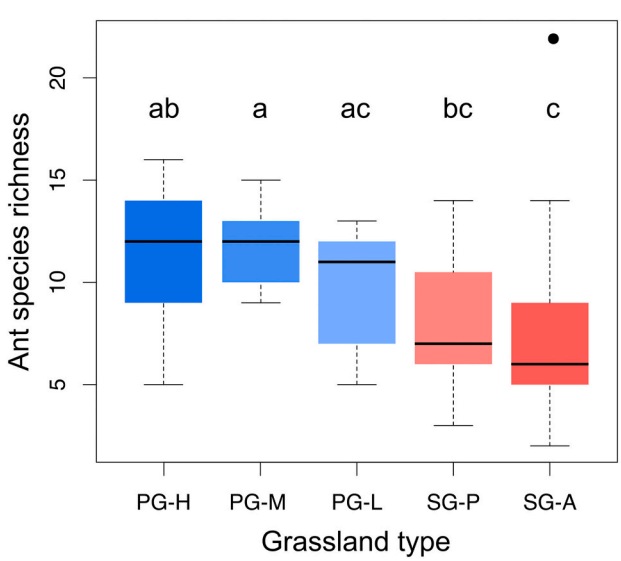

Fig. 3. Local ant species richness from different grassland types (generalized linear mixed model results). Boxplots show median, 25–75% quantiles, and minimum-maximum values of ant species richness. Lowercase letters indicate significant differences measured with post-hoc Tukey tests. Grassland types: permanent grasslands with high (PG-H), medium (PG-M), and low (PG-L) intensity management; secondary grasslands after conversion to agriculture (SG-A) or pine plantation (SG-P).

3.2. Ant species composition

Ant species composition significantly differed among grassland types (Adonis: $F = 5.22$; $p < 0.01$). PERMANOVA pairwise comparisons indicated that all pairs of grassland types differed from each other, and NMDS ordination plot showed a clear distinction especially between ant communities from permanent vs. secondary grasslands (Fig. 5A). Among permanent grasslands, PG-L sites were remarkably more distant from PG-H and PG-M sites, which were closer to each other.

About 13% of the ant composition variation was explained by resource diversity and habitat structure ($F = 2.56$; $p = 0.001$) in the dbRDA model. All the environmental descriptors we used were significant in describing ant composition patterns (Fig. 5B). Axis 1 was mainly associated with grass cover ($F = 2.72$; $p < 0.01$; positive values) and bare soil ($F = 2.42$; $p < 0.01$; negative values), separating permanent from secondary grasslands, and Axis 2 with plant richness ($F = 3.49$; $p = 0.001$; negative values) and shrub cover ($F = 1.60$; $p = 0.05$; positive values), separating PG-M from PG-L and SG-A from SG-P.

Indicator species analysis detected eight ant species associated exclusively with one grassland type. PG-H had one exclusive indicator species (*Pheidole* sp. 2), and PG-M and PG-L had two indicator species each (PG-M: *Solenopsis* sp. 17 and *Camponotus* sp. 4; PG-L: *Solenopsis* sp. 3 and *Myrmelachista gallicola*). We found two species associated with SG-A (*Acromyrmex ambiguus* and *Dorymyrmex pyramicus*) and only one indicator species for SG-P (*Camponotus rufipes*); more details about indicator species are available in Appendix A: Table A3.

4. Discussion

Our study provides novel insights into how grassland degradation affects ant communities, a dominant and ecologically important faunal group, in an extremely biodiverse and threatened ecosystem in southeastern South America. Our results for ant communities add evidence that land-use changes in grasslands have legacy effects, as already indicated for plant communities (Koch et al., 2016), and ecosystem processes (Leidinger et al., 2017).

4.1. Ant communities in secondary vs. permanent grasslands

We showed that former agricultural and silvicultural sites, which are in the early stages of spontaneous grassland recovery, have locally impoverished ant communities. At the regional scale, both types of secondary grasslands yielded significantly lower Simpson diversity of ant communities than permanent grasslands. This reflects the dominance of a single species (fire ant *Solenopsis invicta*) and the high

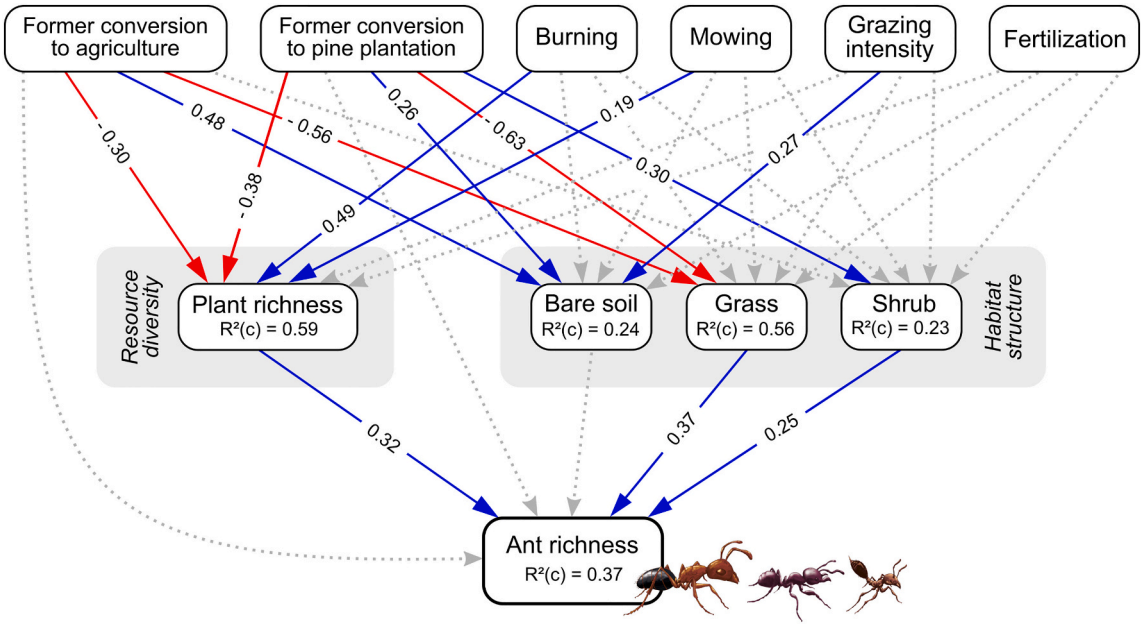

Fig. 4. Structural equation model testing for the effects of grassland conversion and current management practices on ant species richness through changes in resource diversity and habitat structure. Blue arrows represent positive significant relationships and red arrows represent negative significant relationships ($p \leq 0.05$). Grey arrows represent non-significant relationships ($p > 0.05$). $R^2_{(c)}$ indicates the total variation in a dependent variable that is explained by the combined independent variables and the random factor (sampling season); global goodness-of-fit: Fisher's $C = 24.7$; df = 20; $p = 0.21$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

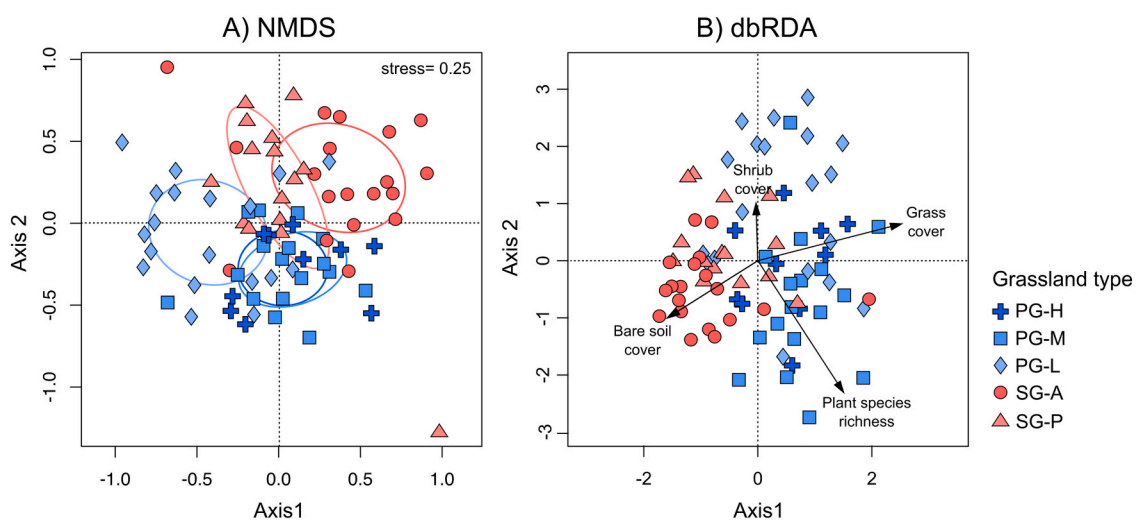

Fig. 5. Unconstrained (A) and constrained (B) ordinations of grassland sites from different grassland types based on Bray-Curtis dissimilarity of ant species incidence data. (A) Non-metric multidimensional scaling; ellipses represent one standard deviation from the centroid. (B) Distance-based redundancy analysis (dbRDA) showing effects of resource diversity and habitat structure variables. Grassland types: permanent grasslands with high (PG-H), medium (PG-M), and low (PG-L) intensity management; secondary grasslands after conversion to agriculture (SG-A) or pine plantation (SG-P).

contribution of rare species (singletons, doubletons) to ant communities in converted sites, while communities in permanent grasslands presented a higher diversity of common species (Appendix A: Table A2). Despite being one the most frequent species in South American grasslands (e.g., Calcaterra et al., 2014; Dröse et al., 2017) and present in all studied grassland types, *S. invicta* is widely known as a specialist of disturbed habitat, reaching greatest abundances in more highly disturbed ecosystems (King and Tschinkel, 2006), which corroborates our findings. At this broad scale, we did not detect conversion effects on total species richness and Shannon diversity among grassland types, possibly indicating the stronger weight rare species have on the interpolation and extrapolation of diversity curves (Chao et al., 2014).

The reduced ant diversity in secondary grasslands can be explained by historical and ecological processes. The major significant biodiversity declines occur when grasslands are converted. Grassland conversion to arable fields means complete loss of native vegetation cover, destruction of the natural litter layer, affecting the root layer, and the appearance of exposed bare soil (Andrade et al., 2015). Although studies evaluating direct effects of grassland transformation to agricultural fields on ant colony survival are scarce, we can assume that such disturbance is detrimental for ant diversity. Only a part of the ant species pool, i.e., generalist species (Pacheco et al., 2013), likely persist and inhabit simplified crop areas. Grassland conversion to tree plantations means a change from open to closed habitat, resulting in a gradual disappearance

of grassland plant species due to shading and leaf litter accumulation as trees establish (Torchelsen et al., 2019). Open-habitat adapted ant communities are negatively impacted by such transformation and usually undergo species turnover to more forest-adapted species (Queiroz et al., 2017; Andersen, 2019). When intensive land uses are later abandoned, secondary succession and reintroduction of grazing management leads to the redevelopment of native vegetation cover. However, without restoration interventions and appropriate management, this does not result in the reestablishment of typical grassland communities (Buisson et al., 2019; Thomas et al., 2019; Torchelsen et al., 2019), as shown by Koch et al. (2016) for our study sites.

We demonstrated that locally reduced ant species richness in secondary grasslands is partly mediated by the reduced diversity of resources (i.e., plant species richness) and the reduced cover of the main component of grasslands, i.e., grasses. The importance of both plant species richness and specific plant life forms for ants has been shown in previous studies. For example, patterns of ant species richness were directly associated with plant richness in neotropical savannas (Ribas et al., 2003; Vasconcelos et al., 2019), and with grass coverage in open grasslands of Brazilian Cerrado (Queiroz et al., 2017). High plant richness in grassland ecosystems may represent a wide variety of available resources to ants, for example, seeds provided by graminoid species (e.g., for harvester ants; Pol et al., 2011), fruits, seeds, floral nectar or pollen and extrafloral nectar (EFN) provided by non-graminoid plants (Costa et al., 2016), as well as leaves from graminoid or non-graminoid species serving as fungicultural substrate for leaf-cutting ants (Mueller et al., 2017). Therefore, the reduction in resource diversity through degradation (land-use history, management) can decrease niche differentiation of ant species in secondary grasslands: the diversity of ant ecological strategies will be reduced, which may then lead to increasing interspecific competition for limited resources and consequently reduced diversity (Armbrecht et al., 2004). Similarly, a reduced contribution of grasses to vegetation cover, also maintaining typical microclimatic and soil conditions in well-conserved grassland, can be a limiting factor for ant diversity recovery.

Besides lower diversity, former agricultural and silvicultural sites also presented different ant species compositions than permanent grasslands. Such differences possibly reflect intrinsic dissimilarities in ant species ecological niches, indicating their habitat requirements, ability to survive, colonize and maintain stable populations in these grasslands (Philpott et al., 2010). The higher cover of bare soil and reduced resource diversity in secondary grasslands easily explains their distinct ant species composition. These environmental characteristics favoured ant species that respond positively to disturbance, have superior competitive abilities and preference for habitat openness, as supported by the species indicator analysis. *Camponotus rufipes*, an indicator of former pine plantations, is aggressive, numerically dominant and a strong competitor for resources against other ant species. This species builds their nests mainly with dead plant material (dry straw) and can use tree stumps and deadwood as nesting sites, e.g., in former pine plantations (Ronque et al., 2016). *Dorymyrmex pyramicus* and *A. ambiguus* were indicators of former agriculture. *Dorymyrmex* is a highly thermophilic genus characteristic of open habitats reportedly associated with crop fields (Pacheco et al., 2013) and abandoned agriculture (Dalle Laste et al., 2019). *Acromyrmex*, as well as other leaf-cutting ants, has been frequently associated with disturbed habitats, especially in forest environments (Leal et al., 2014), but see Ribero et al. (2021). Overall, ant biological and ecological traits seem to explain their distinct prevalence among environments, and if local ant species were better known, a quantitative functional diversity viewpoint could be employed to demonstrate that.

4.2. Effects of grassland management regime change on ant communities

Contrary to our expectations, grassland management intensity did not affect ant diversity considerably, and this was consistent for rare and dominant species. This means that either management intensification or extensification maintains similar diversity levels to traditionally managed grasslands (medium intensity). Moreover, our path analysis revealed some interesting indirect pathways by how grassland management type affects local ant richness. For example, we detected that the occurrence of burning and mowing indirectly favours ant richness by increasing grassland plant richness. This pattern is repeatedly shown in open ecosystems worldwide where plant species richness is enhanced with habitat openness and the break of dominance (e.g., Thomas et al., 2019; López-Mársico et al., 2020), and this brings benefits that cascades to other trophic levels with more diversity of nest sites and food resources (Joern and Laws, 2013), as we showed. Fire suppression in fire-prone ecosystems leads to declines of both plant and ant species (Abreu et al., 2017; Silva et al., 2020), and in subtropical grasslands mowing have positive effects on plant diversity and grass cover (Thomas et al., 2019), highlighting the role of these managements in maintaining a biota adapted to open vegetation. However, when disturbance levels are high, mowing (Noordijk et al., 2010; Heuss et al., 2019) or fertilization (Pihlgren et al., 2010) can reduce ant diversity just as low levels of grazing (Dröse et al., 2019). A balance among disturbance modes and regimes is thus complex. In our case, a complicating issue is that all grasslands are under the same land use, i.e., grazing, albeit under contrasting intensities. Consequently, mowing, burning, and fertilization are additional management actions, and it may become harder to disentangle their individual contributions.

Although ant diversity did not markedly vary under different management regimes in permanent grasslands, species composition was different, especially under extensification. These results are in line with other studies that show that ant composition has been altered with the presence of fire (Maravalhas and Vasconcelos, 2014), grazing (Hoffmann, 2010), and mowing (Heuss et al., 2019). In our study, this change was driven by higher plant richness and grass cover under intensification and medium intensity management and by higher shrub cover under extensification. Results from indicator species analyses point out the role of shrubs in low-intensity management. For example, we found *M. gallicola* as an indicator of grasslands under extensification. *Myrmelachista* is an arboreal ant genus that builds nests in cavities and dry twigs of living trees (Nakano et al., 2013; Baccaro et al., 2015). Dröse et al. (2017) reported *M. gallicola* as an indicator species of the vegetation stratum (i.e., this species prefers dwelling in the vegetation instead of being found on the ground). Low-intensity managed grasslands in the subtropical region present taller herbaceous vegetation, higher shrub cover, and even the presence of pioneer woody species (Koch et al., 2016), and *M. gallicola* seems to respond quickly to habitat change under low-intensity use or absence of disturbance in grasslands in southern Brazil. However, due to a lack of knowledge on ant species ecological characteristics for these grasslands, additional studies on nesting sites, plant species preferences, and foraging patterns are necessary.

4.3. Conservation implications

Our results support the importance of traditional management in maintaining high biodiversity in south Brazilian grasslands. In the early stages of spontaneous recovery, ant communities in former agricultural and silvicultural sites present reduced diversity and deviating species composition compared to permanent grasslands. This change is mainly mediated by low resource richness and grass cover. Our results emphasize the consequences of natural ecosystem conversion for biodiversity, suggesting it is not sufficient to rely on passive vegetation restoration after grassland abandonment. We suggest implementing active restoration practices, either by planting seedlings or by sowing seeds to achieve vegetation establishment, which then can also help typical fauna recovery on secondary grasslands. The use of fire as a management measure for biodiversity conservation remains controversial in southern Brazil, especially in protected areas (Overbeck et al., 2016; Overbeck et al., 2018; Pivello et al., 2021). Our results also stress

that grassland management, such as burning and mowing, contributes to preserving ant diversity in natural grasslands in the studied fire-prone ecosystem. This supports recent studies that have shown that burnings in subtropical grasslands also indirectly increased the diversity of other arthropod groups such as spiders (Podgaiski et al., 2013), grasshoppers (Ferrando et al., 2016), thrips (Podgaiski et al., 2017), and insect pollinators (Goldas et al., 2021). From a conservation perspective, the presence of shrub patches in the grassland could further increase biodiversity as they provide resources for specific species absent in open grassland. These results can contribute to the discussion of restoration and conservation management strategies. They also suggest that ant communities are good indicators to evaluate land-use changes and management practices in subtropical grasslands.

CRediT authorship contribution statement

William Dröse: Conceptualization, Validation, Formal analysis, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization. **Luciana R. Podgaiski**: Conceptualization, Validation, Formal analysis, Writing - Original Draft, Writing - Review & Editing. **Martin M. Gossner**: Investigation, Data Curation, Writing - Review & Editing. **Sebastian T. Meyer**: Investigation, Data Curation, Writing - Review & Editing. **Julia-Maria Hermann**: Investigation, Data Curation, Writing - Review & Editing. **Jan Leidinger**: Investigation, Data Curation, Writing - Review & Editing. **Christiane Koch**: Investigation, Data Curation. **Johannes Kollmann**: Investigation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition. **Wolfgang W. Weisser**: Investigation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition. **Milton de S. Mendonça Jr.**: Resources, Writing - Review & Editing. **Gerhard E. Overbeck**: Conceptualization, Resources, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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