Disentangling elevational and vegetational effects on ant diversity patterns


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ABSTRACT

When aiming to assess the effects of elevation on animal diversity, many studies have been carried out in different vegetation types occurring across elevational gradients. Thus, it remains unclear if any changes observed in species richness are caused by factors directly associated with elevation or are caused by vegetation change across the gradient. Here, we disentangled the effects of elevation from changes in vegetation by assessing ant diversity patterns along an elevational gradient. We analyzed patterns of ant diversity utilizing two different sampling approaches across the elevational gradient: (1) a standardized sampling including only forest formations and (2) a non-standardized sampling including forest (low elevational bands) and grasslands (high elevational bands). We sampled ants at eight elevational bands of Atlantic Forest in Brazil, and the highest three bands were sampled at both forest and grassland habitat. We found that the two approaches produce contrasting patterns of alpha and beta diversity, but the same pattern of gamma diversity. However, in the non-standardized sampling approach, the regression analysis produced a reduced explanation of the species richness gradient and a decrease in the elevational effect size. Different patterns found in the two approaches could be due to distinct environmental conditions in these habitats. In conclusion, our results highlight the potential bias of non-standardizing vegetation type across elevational gradients when assessing elevational patterns of species diversity.

1. Introduction

Two centuries have passed since von Humboldt’s seminal study on diversity patterns across elevational gradients (von Humboldt, 1849); yet the subject is still being explored by ecologists and biogeographers around the world. Aiming to improve ecological theories, which underpin biodiversity conservation strategy, elevational gradients have been used to assess species richness patterns (Peters et al., 2016; Rahbek, 2005), species distributions ranges (Stevens, 1992; Wen et al., 2018) interspecific interactions (Roslin et al., 2017), community phylogenetic structure (Smith et al., 2014) and climate change impacts on biodiversity (Colwell et al., 2008). Elevational gradients are interesting because they present a high variability of environmental factors on a relatively small spatial scale (Körner, 2007; Sundqvist et al., 2013). They also present well established ecological patterns based on both historical and current factors, that shape species distribution (Colwell and Rangel, 2010; Rahbek, 2005) and mirror ecological patterns of latitudinal gradients (Rahbek, 2005).

There are many factors intrinsically linked to elevational gradients such as temperature, area availability, atmospheric pressure, and UV-B radiation (Körner, 2007). The change in these factors across environmental gradients can affect the physiology of plants, metabolic processes, body size, and distribution, due to different selection pressures (Körner, 1998, 2007). Such adaptations to these pressures can be notably dramatic, especially when we consider the drastic change in vegetation type at the treeline of mountains. The treeline was...
characterized by Harsch and Bader (2011) as “an ecotone delimited at the upper end by the tree species limit, the uppermost elevation or latitude at which tree species occur as trees, or krummholz, regardless of height, and at the lower end by continuous forest > 3 m tall”; for example, the shift from forest habitat to grassland habitats formations in tropical mountains. High levels of turnover of plant species occur throughout the elevation gradient (Sundqvist et al., 2013; Kraft et al., 2011) that could also form zonations of multilayered forests in tropical mountains (Hemp, 2006). However, such changes in vegetation type, in terms of forests and grassland or bushland formations, are not necessarily directly linked to the elevation gradient, and therefore should not be treated as an intrinsic elevational variable (unlike temperature, atmospheric pressure, etc.). Indeed, studies have shown that the occurrence of the treeline, and the vegetation types found above it, are not driven by climatic factors that are usually linked to elevation (Pompeu et al., 2018; Safford, 2001).

Even though vegetation type has been demonstrated to affect diversity on elevational gradients (e.g. Axmancher and Fielder, 2008; Axmancher et al., 2009; Carneiro et al., 2014), many studies designed to assess elevation effects have still been carried out in different vegetation types located at distinct altitudes (e.g. Classen et al., 2015; Nakamura et al., 2015; Peters, 2016; Tello et al., 2014; Wen et al., 2018). Körner (2007) highlighted the importance of separating factors that are directly linked to elevational gradients from those that vary regionally but are not specific to elevation (i.e. precipitation, seasonality and wind velocity), in order to reduce confounding bias and obtain results that reflect only the elevation gradient. In this sense, it is possible to discern the effects of elevational gradients conducted on different vegetation types by repeating the same survey and thus standardizing a survey to the same vegetation type, shifting sampling points among different vegetation types along an elevational gradient may influence the resulting patterns found in elevational gradient studies.

Here, we disentangle the effects of elevation from differences in vegetation type on ant diversity patterns along an elevational gradient. We used ants as a study model because they are well distributed in tropical areas (Moreau and Bell, 2013), sensitive to changes in the environment (Philippot et al., 2010), on vegetation types (Andersen et al., 2007) and habitat features (Meszner Pfeiffer, 2011), and have been used successfully for assessing elevational gradients patterns (e.g. Bishop et al., 2017; Szewczyk and McCain, 2016). We tested whether sampling in different vegetation types may produce different patterns on ant species richness (α and γ diversities) and beta diversity (β-diversity). We hypothesized that vegetation type can influence the assessment of elevational gradients on biodiversity by biasing some of the observed patterns, due to confounding factors not necessarily directly linked to elevation. Specifically, through this study, we show that the influence of distinct vegetation types can inhibit clear interpretations of elevational patterns on ant species diversity.

2. Material and methods

2.1. Study area

We carried out the study in Itatiaia National Park (INP), in the southeast of Brazil (22°16′– 22°28′ S and 44°34′– 44°42′ W). The oldest national park in Brazil, the INP was established in 1937 to protect important remnants of the Atlantic Forest biome. The park is located in the Itatiaia Massif, on the highest portion of the Mantiqueira mountain range. The protected area falls within 600 m.a.s.l to the peak at 2878 m.a.s.l. The treeline is situated at around 2000 m.a.s.l.

There are many vegetation types found on the Mantiqueira Mountains, ranging from lower montane forest (at 0–500 m.a.s.l.), montane forest (500–1500 m.a.s.l), and upper montane forest (1500–2000 m.a.s.l), to altitudinal grasslands (2000–2500 m.a.s.l.) (Safford, 1999). As we were interested in drastic changes in vegetation, hereafter, when referring to vegetation types in this study, we refer to the habitat formation defined simply as closed forest or open grassland. In this sense, until the treeline, there is a continuous dense Atlantic Forest formation that here we call “low elevation forests”. Above the treeline, the vegetation type changes to open grasslands, which we term “high elevation grasslands”. However, there are also some natural forest remnants that exist above the treeline that we characterize as “high elevation forests”. The low elevation forests present diverse plant families such as Areaceae, Rubiaceae, Lauraceae, Burseraceae, Rutaceae, Fabaceae, Erythroxylaceae, Myrtaceae, Salicaceae, Euphorbiaceae, Araucariaceae, Bignoniaceae, and Piperaceae. The high elevation forests are composed of plants families such as Myrtaceae, Melastomataceae, Astereaceae, Fabaceae, Proteaceae, Aquifoliaceae, and Solanaceae. The high elevation grasslands present exposed rocks and are dominated by Poaceae species (grasses), also showing some elements of herbaceous plant families as Asteraceae, Apiaceae, and Astrolemeriaceae. It also presents bogs with plant families such as Cyperaceae, Lentibulariaceae, Xyridaceae, and bryophytes.

2.2. Ant sampling

We sampled ants during the rainy season in 2015. We selected eight elevational bands that were situated at 600, 848, 1134, 1515, 1810, 2000, 2200 and 2457 m.a.s.l. As well as the elevation difference, each elevational band was located at least 1.4 km apart from each other. At each elevation, we installed a 200 m transect containing 10 sampling points spaced 20 m apart. In each sampling point, we placed four epigaeic pitfall traps in a square grid of 1.5 m × 1.5 m, resulting in a total of 40 traps at each elevation. The pitfalls were 11 cm in diameter and 11 cm in depth, filled with a 200 ml solution of water, salt (0.4%) and liquid soap (0.6%) (Bestelmeyer et al., 2000; Canedo-Júnior et al., 2016). Each trap remained operating for 48 h.

We set one transect for each of the low elevation forest points, located from the first (660 m) to the fifth elevation band (1810 m) (i.e. below the treeline transects). At the treeline and onwards, which extended from the sixth elevation band (2000 m.a.s.l.) to the highest (2457 m.a.s.l.), we set two transects per elevation (one in each of the two habitat types), in order to sample both high elevation forests and high elevation grasslands. The transects in each vegetation type above the treeline (forest and grassland) were spatially separated by at least 160 m while maintaining both transects in the same elevation band.

Ants were identified to genera following Baccaro et al. (2015), and whenever possible, to species level, through relevant literature and by matching collected specimens with identified material in the Laboratory de Ecologia de Formigas’ ant collection at Universidade Federal de Lavras (UFLA). Voucher specimens of all collected species/morphospecies were deposited at UFLA and at the Entomological Collection Padre Jesus Santiago Moura of the Universidade Federal do Paraná (DZUP), in Curitiba, Brazil. All applicable institutional and national guidelines for the care and use of animals were followed. We had a license for all samplings, which was conceded by Sistema de Autorização e Informação em Biodiversidade (SISBIO); license number 46564–1, date 17/10/2014, authentication code: 73877888; http://www.icmbio.gov.br/sisbio/verificar_autenticidade.

2.3. Data analysis

To test whether sampling in different vegetation types in an elevational gradient may bias patterns on ant species diversity, we performed generalized linear models (GLMs) with and without mixed effects for the diversity components alpha, beta and gamma (α, β and γ, respectively) extracted from two different approaches. In the first approach, we used only closed forest transects from all elevations (low and high elevation forests) that we called single–habitat gradient. In the second approach, we used the five closed forest transects below the treeline (low elevation forests), plus the three open grassland transects above...
the treeline (high elevation grasslands). Termed mixed–habitat gradient, this approach allowed us to assess the elevational gradient across different vegetation types in terms of habitat formation (Fig. 1).

As response variables for both approaches, we considered \(\alpha\)-diversity as the number of ant species richness per pitfall traps (four pitfall traps in a 1.5 m\(^2\) grid), \(\gamma\)-diversity the overall ant species richness per elevational band (transect) and \(\beta\)-diversity as a proxy of species composition changes within each elevational band (transects). For \(\beta\)-diversity, we extracted the total beta diversity (\(\beta_{\text{total}}\)) using the 'beta.part' package, which results of turnover and nestedness mechanisms (Baselga and Orme, 2012).

For \(\alpha\)-diversity, at both approaches, we performed a generalized linear model with mixed effects (GLMM) with transect as the random and the elevation as the explanatory variable. Elevation values represent the elevation band, since there were no significant differences among sampling points in the same transect. We used the GLMM to reduce the pseudoreplication effects caused by the non–independence of pitfall traps placed in the same transects (Pinheiro and Bates, 2000). First, to investigate if \(\gamma\) diversities from two approaches are relate to elevation by a monotonic decline or hump–shaped function, we modified the Nagai (2011) function to generate linear and quadratic models for comparison from those diversity components. We verified which model would be more appropriated using the Aikake’s criteria (AICc), considering as the best model the one with lowest AIC value. After that, for \(\beta\) and \(\gamma\) diversities, we performed a generalized linear model (GLM) with the elevation also as an explanatory variable for both approaches. All GLMs and GLMM were performed using the 'lme4' package (Bates et al., 2013) and were assessed under residual analyses to obtain the adequacy of error distribution (Crawley, 2002). If both approaches (using only single or mixed habitat gradient), produced the same pattern, we performed a resampling application from 1000 bootstrap samples, replacing it in the 'boot.ci()' function from 'boot' package (Canty and Ripley, 2012). With this function, we can assess the 95% confidence intervals of a regression’s R\(^2\) and slope, and the median precision of the GLMs of the two approaches. Thus, we could determine if there was a difference in model precision and the effect size of the regressions. We performed all analysis above at R 3.01 software (R Core Team, 2013).

3. Results

In total, we collected 148 ant morpho–species belonging to 37 genera and nine subfamilies, with 119 species recorded in low elevation forests, 21 at high elevation forests and 46 at high elevation grasslands (see Appendix A Table A). In addition, low elevation forests shared more species with high elevation grasslands than with high elevation forests (Fig. 2). Patterns of \(\alpha\)-diversity differed between the two sampling approaches (Fig. 3a and b). Using the single–habitat gradient, elevation was found to influence \(\alpha\)-diversity, presenting an inverse relationship (\(\text{Chi} = 12.05; \ p < 0.01\)), however in a mixed–habitat gradient the effect of elevation on \(\alpha\)-diversity was not found to be significant (\(\text{Chi} = 2.23; \ p = 0.13\)). Regarding \(\beta\)-diversity, the two approaches also produced different patterns, although with contrasting results (Fig. 3c and d); in the single–habitat gradient \(\beta\)-diversity was not influenced by elevation (\(F = 1.62; \ p = 0.25\)), while in the mixed–habitat gradient elevation negatively influenced \(\beta\)-diversity (\(F = 11.32; \ p = 0.01\)). Regarding \(\gamma\)-diversity both approaches followed better a linear than a
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analyzing the mixed

4. Discussion

The standardization of vegetation type across an elevational gra-
dient has been empirically tested in this study. Our results unequiv-
 vocally show that non-standardization of vegetation type can be a
source of bias in the parameters evaluating community responses,
which is in accord with Axmancher and Fielder (2008), who verified
the vegetation type influence on geometrid moths’ diversity across an
elevational gradient. The patterns observed for \( \alpha \)-diversity and \( \beta \)-di-
versity clearly differed between the two approaches. Moreover, despite
a monotonic decline observed for \( \gamma \)-diversity in both approaches, our
analyses demonstrate that sampling in the same vegetation type leads
to stronger results in order to explain the elevational gradient effects on
species richness.

We suggest that the environmental conditions imposed by the two
vegetation types (grasslands and forests) are the main drivers of the
contrasting elevation gradient patterns observed for \( \alpha \)-diversity. Higher
elevations present lower temperatures and high levels of moisture,
which can affect ant species richness, likely producing the decrease of
alpha diversity on a single–habitat gradient. Low temperatures and high
levels of moisture affect the survival of ant colonies (for example de-
creasing foraging activity), and some species are unable to survive or
colonize areas with such conditions (Bishop et al., 2017; Bruhl et al.,
1999; Fisher, 1998; Malsch et al., 2008; Sanders et al., 2007). When
analyzing the mixed–habitat gradient, we did not detect the same de-
crease of alpha diversity, as high elevation grasslands reached almost
the same \( \alpha \)-diversity of the most speciose low elevation forests (Fig. 3a
and b). Furthermore, low elevation forests share more ant species with
high elevation grasslands than with high elevation forests (Fig. 2), even
considering that habitats from low and high elevation bands differ
considerably in species composition. Ants are sensitive to the level of
habitat openness, which could result in different species inhabiting
these areas and consequently in the different values of species richness
(Andersen et al., 2007; Botes et al., 2006; van Ingen et al., 2008). If we
compare high elevation forest and grassland sites, forests are cooler and
more humid than the low stature grassland habitat (Aparecido et al.,
2018; Körner, 2007; Safford, 1999). Consequently, the warmer condi-
tions and low levels of moisture in high elevation grasslands may per-
mit more species to occur than in high forest habitats. Indeed, in this
sense, despite inherent and notable differences, high elevation grass-
land habitat is more similar (in terms of temperature and moisture) to
forests from low elevation bands which might improve the supporting
of ant colonies in comparison to high elevational forests.

Differences in \( \beta \)-diversity patterns (Fig. 3c and d) could be caused
by different degrees of habitat heterogeneity between sites in high
elevation grasslands and forests. The decrease of \( \beta \)-diversity according
to elevation when analyzing the mixed–habitat gradient is likely due to
high elevation grasslands sites being more homogenous in comparison
to both low and high elevation forests. Such homogenization in terms
of habitat conditions (related to temperature and moisture) would po-
tentially permit ants in grass lands to colonize almost all sampling
points in the transect; while this would likely not occur in more het-
erogeneous forest sites, leading to different patterns. We hypothesize
that \( \beta \)-diversity within a single–habitat gradient may be driven by its
own habitat heterogeneity (Jankowski et al., 2009; Nunes et al., 2016),
which may not necessarily vary across the elevational gradient, at least
in the extent of elevational gradient assessed in this study.

Regarding \( \gamma \)-diversity we observed the same monotonic decline in
diversity in both approaches (Fig. 3e and f). Elevational gradients have
been found to produce a strong pattern on species diversity for other
insect assemblages, resulting from the influence of temperature (Mal-
sch et al., 2008; Sanders et al., 2007). Thus, even changes in vegetation
type would likely not change this pattern. According to our results,
when analyzing \( \gamma \)-diversity on a mixed–habitat gradient there was a
decrease in model robustness in terms of the proportion of explained
variance and effect size of elevation. In fact, species diversity is not
influenced by elevation per se but by factors that covary with it. Sam-
pling in different vegetation types likely means that diversity is de-
pendent on factors related to differences in the structure of habitats,
and not only limited to elevation–related factors. As Gotelli and Elliso
n (2004) have already pointed out, when many confounding factors are
included in a study’s design, it is difficult to disentangle their effects.
Thus, the higher proportion of explanation of the variation in diversity
found in a single–habitat gradient is likely due to the similarity of other
environmental factors, except those directly linked to variation in ele-
vation. Similarly, the effect size of elevation on species richness in a
mixed–habitat gradient is lower because, as well as elevation, there are
other environmental dissimilarities (as previously mentioned) that may
permit more species to survive at higher elevations in grasslands than in
the high elevation forests.

5. Conclusions

This study demonstrates that sampling in different vegetation types
across an elevational gradient produces distinct and likely biased di-
versity patterns, compared to sampling in standardized habitats when
aiming to assess exclusively elevational effects. As highlighted by
Körner (2007), there is a risk of confounding geophysical factors not
directly related to elevation, which may lead to assessments of other
environmental gradients. With this in mind, we suggest that studies
solely addressing elevational gradient effects must perform their sam-
pling in the same vegetation type, in terms of closed forest or grasslands
formation. However, when assessing only one vegetation type is not an
option, we suggest authors test overall diversity (\( \gamma \)-diversity), instead

![Fig. 2. The number of exclusive and shared ant species recorded in the three habitat types across an elevational gradient in Itatiaia National Park. The numbers within the overlapping parts of the circles represent the shared species found in the corresponding habitats, while the numbers outside of the overlap show the number of species exclusive to that habitat.](image-url)
of $\alpha$–diversity and $\beta$–diversity, as the effects of elevation may be stronger at that scale. Furthermore, it seems that $\alpha$–diversity and $\beta$–diversity responses are more sensitive to habitat changes. We also recommend that at the very least, vegetation types should be included in statistical models. The data presented here also indicate that many of published studies on elevational gradients performed on different vegetation types that aimed to assess only elevational effects, possibly confounded both elevational and vegetational effects leading to potentially biased results. In order to reinforce this argument, future studies should address the influence of other vegetation types that present intermediate differences (e.g. shrublands, open woodland) on elevational gradient diversity patterns. Finally, we conclude that removing the non–standardized sampling bias is essential to fully understand elevational gradient patterns on biodiversity.
Fig. 4. Comparison of GLM regression attributes between single and mixed-habitat elevation gradients on overall ant species richness (γ diversity). a) Proportion of variance explained (R²) and b) Effect size (slope of the regression line). Vertical dashed lines are bootstrapped confidence intervals based on 1000 bootstrap samples with replacement. Notch areas on boxplots mark the 95% confidence intervals of the median value (shown as a black horizontal line). The horizontal dotted line represent 0 value in y-axis. Black dots represent data outliers.

Author contributions
CJL, CRR, JL and ACMQ originally formulated the idea. CJL, RMF, MMGI, GPA, GBN and DQD conducted fieldwork and collected the data. CJL, CRR, JL, ACMQ and FSN performed statistical analyses. CJL wrote the article with substantial collaboration from all co-authors.

Declaration of competing interest
None.

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Appendix A. Supplementary data
Supplementary data related to this article can be found at https://doi.org/10.1016/j.actao.2019.103489.

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