

RESEARCH ARTICLE

# Spatial patterns of species richness and nestedness in ant assemblages along an elevational gradient in a Mediterranean mountain range

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## Abstract

### Background

The study of biodiversity spatial patterns along ecological gradients can serve to elucidate factors shaping biological community structure and predict ecosystem responses to global change. Ant assemblages are particularly interesting as study cases, because ant species play a key role in many ecosystem processes and have frequently been identified as useful bioindicators.

### Methods

Here we analyzed the response of ant species richness and assemblage composition across elevational gradients in Mediterranean grasslands and subsequently tested whether these responses were stable spatially and temporally. We sampled ant assemblages in two years (2014, 2015) in two mountain ranges (Guadarrama, Serrota) in Central Spain, along an elevational gradient ranging from 685 to 2390 m a.s.l.

### Results

Jackknife estimates of ant species richness ranged from three to 18.5 species and exhibited a hump-shaped relationship with elevation that peaked at mid-range values (1100–1400 m). This pattern was transferable temporally and spatially. Elevation was related to ant assemblage composition and facilitated separation of higher elevation assemblages (> 1700 m) from the remaining lower elevation species groups. Ant assemblages were nested; therefore species assemblages with a decreased number of species were a subset of the richer assemblages, although species turnover was more important than pure nestedness in all surveys. The degree of nestedness changed non-linearly as a cubic polynomial with elevation. These assembly patterns coincided more clearly over time than between the two study regions.

**Competing interests:** The authors have declared that no competing interests exist.

## Discussion

We suggest double environmental stressors typical of Mediterranean mountains explained species richness patterns: drought at low elevations and cold temperatures at high elevations likely constrained richness at both extremes of elevational gradients. The fact that species turnover showed a dominant role over pure nestedness suggested current ant assemblages were context-dependent and highly vulnerable to global change, which threatens the conservation of present day native ant communities, particularly at high elevations.

## Introduction

Predicting the response of biodiversity to the main drivers of global change has become a primary goal of modern ecology [1–3]. Consequently, the analysis of species richness and assembly patterns along latitudinal and altitudinal gradients provide clues to project possible effects of climate change on communities [4, 5]. The Mediterranean mountains are particularly suitable for this purpose, given a characteristic combination of high temperature and low water availability in summer (one to several months with drought limiting primary productivity), and irregular rainfall concentrating on autumn and spring. Overall, Mediterranean mountains present a progressively cooler and wetter environment at higher elevations while valleys suffer more severe summer drought conditions [6, 7]. In addition, the Mediterranean Basin will probably face particularly marked increases in aridity, temperature and frequency of extreme climatic events [8–10]; therefore it is critical to advance our knowledge of how communities respond to more severe abiotic factors.

Ants are diverse and ubiquitous, exhibit strong interspecific interactions, and perform many functional processes in ecosystems [11]; therefore, ants are considered a viable indicator group of ecosystem change by many community ecologists. Ants are also dominant terrestrial community members [12] and play an important role in plant community dynamics, acting as seed harvesters [13], dispersal agents [14], and influencing soil nutrient status and plant growth [15]. As predators, ants control herbivore abundance, drive relevant top-down effects, and are often used as biological control agents of insect pests and fungal pathogens [16]. Furthermore, ant distribution and abundance patterns provide information to address specific environmental management issues [17, 18]; consequently, ants are particularly useful bioindicators.

Previous research on ant diversity documented variable responses to altitudinal gradients, which showed a certain dependency on macroclimate. For instance, studies reporting monotonic decreases in species richness with elevation were frequent in temperate mountains [19–21], while monotonic increases were observed in arid environments [22, 23]. Longino and Colwell [24] and Nowrouzi *et al.* [25] indicated more complex patterns in tropical climates, which often combined a monotonic decrease at high elevations with a plateau at low elevations. Mid-elevation peaks were reported in tropical areas [26, 27], although also frequent at other latitudes [28, 29]. These patterns were partially attributed to geometric constraints, such as the mid-domain effect and area availability [30, 31] or the Rapoport rescue effect [32]. However, the role of climatic factors, including temperature [19–21] and water availability [22, 29, 33, 34] as direct drivers of ant diversity is also unequivocal. Moreover, several studies demonstrated these climatic variables also indirectly modulated ant diversity, for example, by affecting primary productivity [33, 35–37].

Understanding how ant richness responds to altitudinal gradients is not sufficient to predict how environmental changes (e.g. rising temperatures) will affect species geographic ranges,

because knowledge on species composition patterns is also required. Species assemblages and their distributions along ecological and geographical gradients result from species tolerances to climatic (abiotic) factors and biotic interactions, which interplay with niche evolution to determine community composition [38–40]. Kodric-Brown and Brown [41] defined a structured community as one where the organization is not due to randomness [41], and all situations in which communities are not random can be explained by spatial turnover, nestedness, or combinations of both [42]. Nestedness of species assemblages occurs when site biotas with smaller species numbers are subsets of the biotas at richer sites [43, 44]. If environmental and habitat filtering (i.e., the sorting of species by abiotic factors and habitat types) and not inter-specific competition is responsible for nestedness, most species can coexist. Therefore, under increasing temperatures, species could migrate or expand their distribution elsewhere from the species' former range, without necessarily implying other resident species will be displaced by the newcomers. On the contrary, spatial turnover implies the replacement of some species by others, exhibiting gains and losses of species among locations [45]. This is a sign that some species cannot coexist, because of different climatic requirements, competitive exclusion, or both [42]. It is expected that global warming will shift ectotherm species ranges to higher altitudes where they could find temperatures within their fundamental niches, as have often been advanced for arthropods [4, 46]. Therefore, with spatial turnover patterns, species will migrate up an altitudinal gradient and those at higher altitudes might suffer a subsequent reduction in geographic distribution range, and even disappear from the area. However, Maguire *et al.* [47] acknowledged both processes and community patterns might vary with scale, and exhibit spatio-temporal variation, which raises concerns on our capacity to generalize the results based on local studies. Indeed, transferability of results is increasingly considered in analyzing the effects of global change on species distributions, although such efforts are rarer in studies of community patterns [48–50].

In the present study, we explored patterns of ant species diversity and community composition in Mediterranean mountain grasslands, integrating spatio-temporal transferability. Specifically, we addressed the following questions: (1) what is the relationship between ant species richness and elevation; (2) how does elevation affect ant species composition; (3) what are the relative contributions of spatial turnover and nestedness in ant community composition; and (4) are our findings transferable spatially and temporally?

## Methods

### Study areas and sampling design

We studied grassland ant communities in two mountains in the larger Sistema Central range, Sierra de Guadarrama (reaching 2428 m a.s.l. at its highest peak, Peñalara: 40°51'01"N 3°57'17"O) and Sierra de la Serrota (maximum 2294 m a.s.l., Serrota: 40°29'57"N 5°04'43"O). The two mountains are ~ 100 km apart with similar climatic, geologic, and biotic characteristics. Mean annual temperatures range from ~ 14 °C at 600 m to ~ 4 °C at the summits, and mean annual rainfall ranges from 550 mm to 1500 mm, with severe summer drought [51, S1 Table]. Soil temperature decreases with elevation ( $R^2 = 0.80$ ,  $F_{1,16} = 62.23$ ,  $P < 0.0001$  for maximum temperatures and  $R^2 = 0.96$ ,  $F_{1,16} = 371.2$ ,  $P < 0.0001$  for minimum temperatures; own data based on temperature loggers buried at ground level during the sampling period in 2014, S3 Fig). Substrata are primarily composed of granites, and pasturelands are distributed along the complete elevational gradient, largely as the result of traditional livestock grazing.

Sample sites were interspersed along the elevational gradient trying to keep a difference in elevation of 100 m between consecutive sites. The closer sampling sites were at least 1 km apart. Sites faced southeast to southwest, had gentle slopes (< 5%), and were not heavily grazed

nor affected by anthropogenic disturbance (i.e., they were not close to buildings or main paths). With this sampling restrictions, we strived to control the heterogeneity of sampling units by considering similar annual-dominated dry grasslands. We sampled 18 grasslands in 2014 (in Guadarrama range) and 12 in 2015 (a subset of six sites from the original sample in Guadarrama range and another six in Serrota range; [S1 Table](#)). On each grassland, we randomly placed a 4 x 3 grid of pitfall traps, 2.5 cm diameter x 5 cm deep, with 5 m spacing between traps, which were filled with a 3:1 ethanol/ monoethylene glycol mixture. Ant sampling was conducted once in each sampling period under stable anticyclonic conditions, in July, to take advantage of the seasonal ant activity peak during summer in Central Spain (Guadarrama: 17–23 July 2014 and 9–16 July 2015; Serrota: 1–8 July 2015) [[52](#)]. Traps were collected after one week and ant specimens were kept in ethanol, sorted in the laboratory and identified to species level with a binocular microscope, excluding the occasional winged ones, which could have dispersed from elsewhere.

Pitfall trapping is considered more objective and unbiased than other methods for sampling ground ants [[53](#), [11](#)]. Pitfall traps have been used successfully in other studies in central Spain to capture the complete local species pool and ant size ranges [[52](#), [54](#), [55](#)]. The Consejería de Medio Ambiente, Administración Local y Ordenación del Territorio of the Autonomous Community of Madrid granted official permits to accessing sites and trapping.

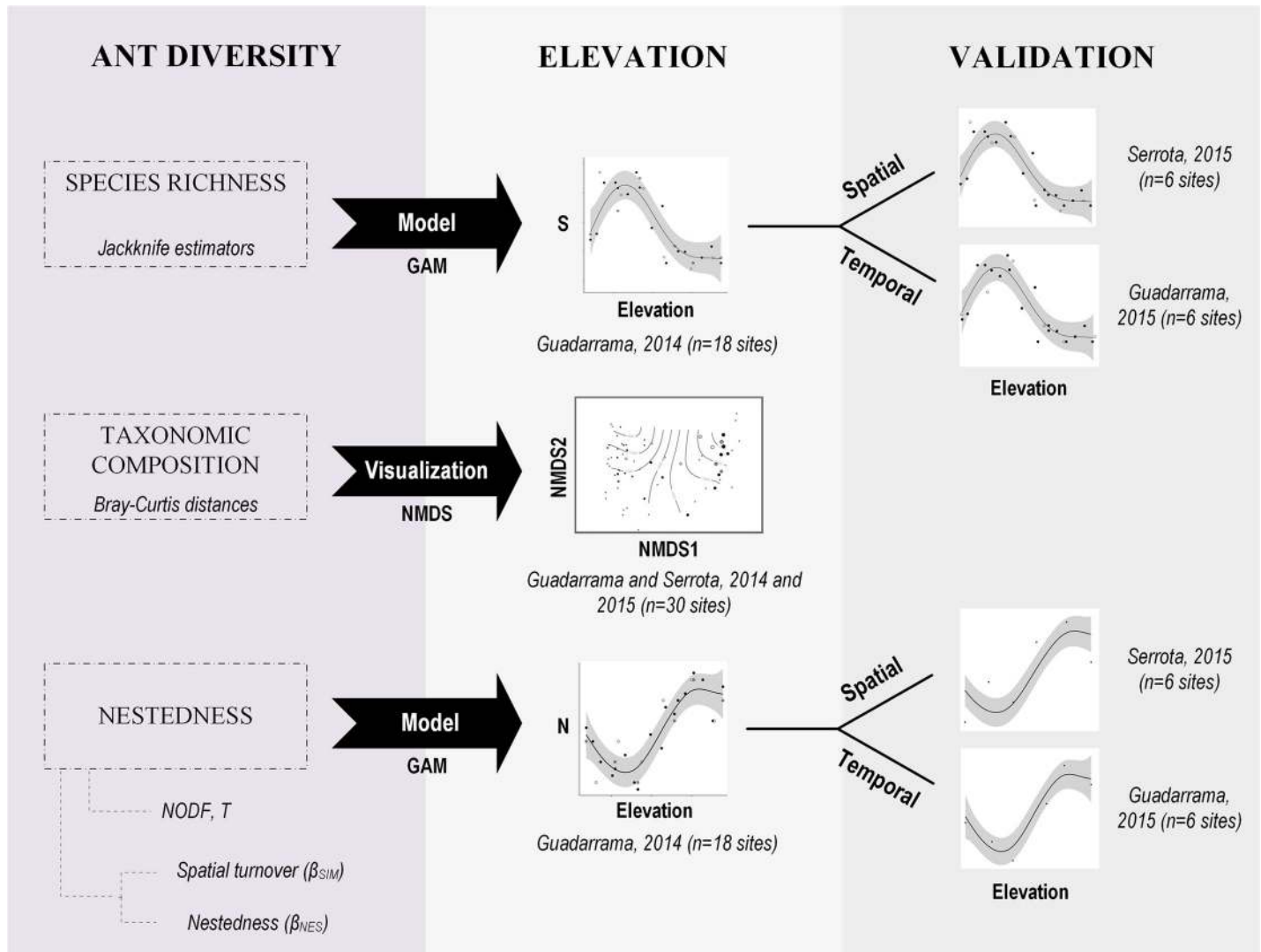
## Statistical analyses

We examined the relationship between species richness, taxonomic composition, and nestedness of grassland ant communities with elevation ([Fig 1](#)). Our analytical strategy was to build descriptive models for the data gathered during the most intensive sampling in Guadarrama 2014 ( $n = 18$  sites), and to validate the patterns detected with data gathered in a subset of the Guadarrama grasslands during 2015 ( $n = 6$  sites, temporal validation) and with data gathered in the Serrota range during 2015 ( $n = 6$  sites, spatial validation).

**Species richness.** First, we assessed the relationship between species richness and elevation. We estimated the species pool size at each locality with the incidence-based first order jackknife estimator, which is traditionally considered a robust method that render conservative estimates [[56](#)]. The relationship shape between estimates of richness and elevation was explored applying a generalized additive model (GAM) with Gaussian errors and a thin plate regression spline for elevation. GAMs are often used to explore the shape of the relationship between response (richness) and explanatory (elevation) variables, avoiding to impose a polynomial relationship beforehand [[57](#), [58](#)]. Models were built with data from Guadarrama 2014 sampling and validated with data from the Guadarrama 2015 and Serrota 2015 samplings. The mean absolute error of jackknife estimates predicted by the models was calculated to assess the constancy of species richness pattern between years and sites.

**Taxonomic composition of assemblages.** We explored how species assemblages differed based on taxonomic composition by applying non-metric multidimensional scaling (NMDS) to the hemi-matrices of binary Bray-Curtis distances among grasslands derived from species occurrences in pitfall traps.

**Nestedness patterns.** To assess assemblage nestedness among grasslands surveyed on each mountain and year we calculated the matrix temperature (T) [[59](#)] and the NODF (Nestedness metric based on Overlap and Decreasing Fill) [[60](#)]. While T has traditionally been the most commonly used metric for assessing overall nestedness [[61](#)], the more recent NODF index is claimed to exhibit more robust statistical properties, and separate contributions to nestedness of columns (due to species incidence) and rows (due to site composition) can be quantified [[60](#), [62](#)]. T decreases and NODF increases with nestedness.



**Fig 1. Analytical pathway followed in this study.** Three elements of ant diversity were assessed along an elevational gradient in Guadarrama and Serrota mountain ranges in Central Spain: species richness (estimated with jackknife), taxonomic composition (described with Bray-Curtis distances) and nestedness (described by indexes of nestedness). The relationships of these elements to elevation was inspected with ordination (non-metric multidimensional scaling) and models (generalized additive). Models were built with the primary dataset obtained in Guadarrama in 2014 and their predictions evaluated with smaller validation datasets.

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The significance of nestedness indices was estimated by comparison with suitable binary null models, which were simulated by randomizing the original matrix transformed to presence/absence binary data. Two null models were built to encompass the range from maximally liberal (equitable) to maximally conservative (constrained) null models [63]. The first was an equiprobable model built by randomizing rows and columns and therefore maintaining only the number of species presences ('r00' algorithm). The second was a proportional resampling model constructed by constraining randomization to maintain row and column totals (site richness and species incidence, respectively), while using marginal column frequency to select species ('quasiswap' algorithm). Thus, the first null model was more liberal and only accounted for matrix fill (the incidence of the total species set), while the second null model was more stringent, because it imposed additional structure on the data, accounting for among-site

differences (e.g. different carrying capacities) and among-species differences (e.g. different rarities). Subsequently, significant nestedness was attributed to variation in observed species richness or species incidence, if evaluated with the equiprobable model, or to variation beyond that observed, if evaluated with the proportional resampling model [64]. One thousand randomizations of the original matrix were applied.

The relative degree of nestedness, i.e. how much a grassland was nested within the set of sampled grasslands in the same geographic area and time period, was evaluated by a nestedness rank, according to T. Poorer grasslands, i.e. those having lower species diversity which were a subset of richer grasslands, were assigned higher ranks. These ranks were estimated as the ordinate in nestedness plots built with the T index, which were calculated as  $(k-0.5)/n$  for  $k = 1 \dots n$  rows (e.g. the bottom row in the graph, where  $k = 18$  was the more nested site, which for the 2014 sampling in Guadarrama had a rank of  $(18-0.5)/18 = 0.97$ ).

The shape of the relationship between nestedness and elevation during the 2014 sampling in Guadarrama ( $n = 18$ ) was explored using GAM with Gaussian errors and a thin plate regression spline for elevation. This model was validated using Guadarrama 2015 data (temporal validation). A low mean absolute error of predicted ranks would suggest the nestedness rank of plots did not vary between years. Similarly, the model built with Guadarrama 2014 sampling data was validated using the Serrota 2015 data (spatial validation). A low mean absolute error of predicted ranks would suggest the nestedness rank of plots did not vary between regions.

The contributions of spatial turnover and nestedness to the distribution pattern in Guadarrama 2014 data were calculated using three beta diversity indices: Sørensen-based multiple-site dissimilarity ( $\beta_{\text{SOR}}$ ), Simpson-based multiple-site dissimilarity ( $\beta_{\text{SIM}}$ ), and nestedness-resultant multiple-site dissimilarity ( $\beta_{\text{NES}}$ ).  $\beta_{\text{SIM}}$  accounts for spatial turnover and  $\beta_{\text{NES}}$  integrates dissimilarity due to nestedness, while  $\beta_{\text{SOR}}$  expresses the total dissimilarity between communities and equals the sum of  $\beta_{\text{SIM}}$  and  $\beta_{\text{NES}}$  [42]. *P*-values were estimated through the equiprobable ('r00') null model (the proportional resampling null model was not useful to assess this partitioning of beta diversity).

Statistical analyses were performed using R (v 3.5.0; R Core Team 2018) and specialized packages *vegan* (v. 2.5–1) [65], and *betapart* (1.5.0) [66].

## Results

We detected 37 species, all recorded in Guadarrama (35 in 2014 and 26 in 2015, 25 were shared between years) and 20 species in Serrota (all species shared with Guadarrama; note the different sample sizes). A total of 15 species were common among the three surveys (S1 Table).

Jackknife estimates of ant species richness in Guadarrama 2014 ranged from three (SE = 0) species in most of the highest grasslands to 18.5 (SE = 2.6) at intermediate elevations (Table 1). Estimates of richness related to elevation were positive below 1100 m a.s.l. and negative until a lower limit was reached at ca. 2000 m a.s.l. (Fig 2). Overall, richness exhibited a hump-shaped relationship with elevation, which we described using GAM with a spline for elevation, roughly equivalent to a third-order degree polynomial (equivalent  $df = 4.43$ ,  $F = 16.01$ ,  $P < 0.0001$ ,  $D^2 = 83.8\%$ , Fig 2). This pattern of species richness was more similar between years than between mountain ranges (mean absolute errors of predictions were 1.4 species for Guadarrama 2015 data; and 2.6 species for Serrota 2015 data).

A NMDS solution was built that satisfactorily summarized the Bray-Curtis distances among grasslands in both years and study regions (stress = 0.10, linear  $R^2$  between Bray-Curtis and NMDS distances = 0.95). This scaling separated grasslands roughly according to elevation, but not regions or years and suggested an elevation threshold of 1700 m a.s.l. was a suitable elevation to distinguish the two groups (Fig 3).

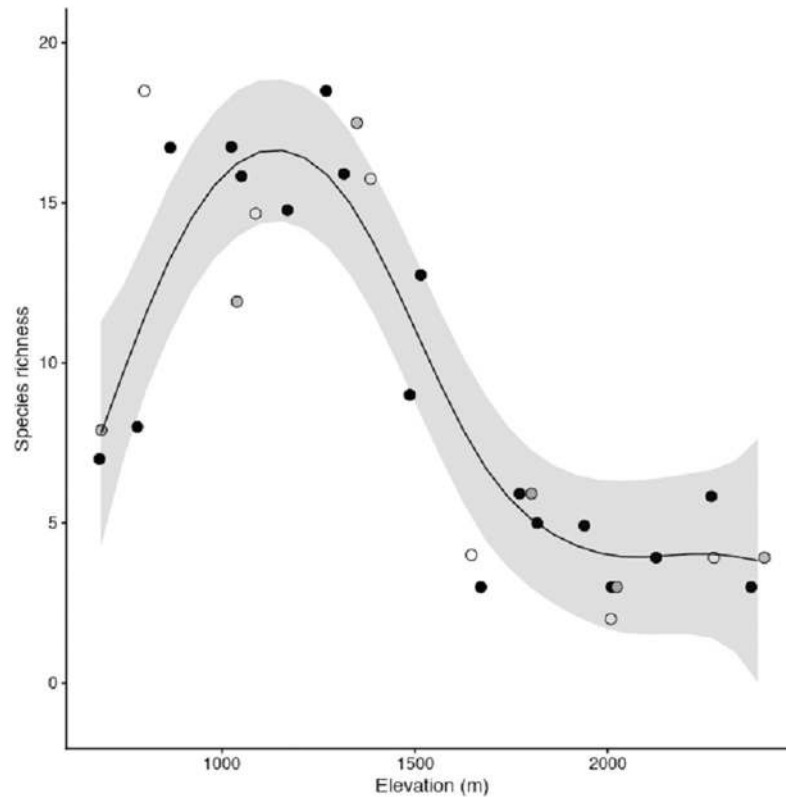
**Table 1. Observed and estimated species richness of ants in dry grasslands in central Spain.**

ID	Sampling	S	J	SE(J)	N	Elevation (m)
G1	G2014	7	7,0	0,0	13	685
G2	G2014	8	8,0	0,0	12	768
G3	G2014	14	16,7	1,6	11	865
G4	G2014	14	16,8	1,6	12	1012
G5	G2014	14	15,8	1,3	12	1044
G6	G2014	13	14,8	1,3	9	1171
G7	G2014	13	18,5	2,6	12	1285
G8	G2014	15	15,9	0,9	11	1330
G9	G2014	9	9,0	0,0	12	1487
G10	G2014	10	12,8	1,6	12	1535
G11	G2014	3	3,0	0,0	19	1675
G12	G2014	5	5,9	0,9	12	1786
G13	G2014	5	5,0	0,0	12	1830
G14	G2014	4	4,9	0,9	12	1920
G15	G2014	3	3,0	0,0	12	2026
G16	G2014	3	3,9	0,9	12	2139
G17	G2014	4	5,8	1,9	12	2266
G18	G2014	3	3,0	0,0	12	2390
G1	G2015	7	7,9	0,9	10	685
G5	G2015	11	11,9	0,9	12	1044
G8	G2015	14	17,5	2,2	8	1330
G12	G2015	5	5,9	0,9	12	1786
G15	G2015	3	3,0	0,0	8	2026
G18	G2015	3	3,9	0,9	12	2390
S1	S2015	14	18,5	2,4	10	808,4
S2	S2015	11	14,7	2,3	12	1086
S3	S2015	13	15,8	1,6	12	1374
S4	S2015	4	4,0	0,0	11	1646
S5	S2015	2	2,0	0,0	12	2000
S6	S2015	3	3,9	0,9	12	2286

Observed (S) and first-order jackknife estimator (J) with their standard errors (SE(J)) of ant species richness in dry grasslands in central Spain. Grassland plots were ordered following elevation (in m a.s.l.), identified with an ID, where G stands for Guadarrama, S stands for Serrota, and numbers represent grassland position in increasing order of elevation. Sampling: data from Guadarrama range in 2014 (G2014) and 2015 (G2015) and Serrota range in 2015 (S2015). N: samples sizes (number of pitfall traps recovered from each locality).

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Ant species assemblages were nested, showing a pattern suggesting differences in species richness among localities were more important than differences in prevalence among species (nestedness among rows was higher than nestedness among columns, [Table 2](#), [S2 Table](#)). Nestedness ranks for Guadarrama 2014 was described with a curvilinear term (spline) for elevation, roughly similar to a third-degree cubic polynomial ( $df = 3.89$ ,  $F = 15.38$ ,  $P < 0.0001$ ,  $D^2 = 85.6\%$ , [Fig 4](#)). The subset of these grasslands sampled in 2015 maintained their nestedness ranks and the model transferred to 2015 data (mean absolute error of predicted ranks was 0.080, [S1 Fig](#)). The model did not transfer between study regions (mean absolute error of predicted ranks was 0.17, [S2 Fig](#)). Largely, nestedness increased with elevation, with the exception of the lowest and highest elevation grasslands.



**Fig 2. Relationship between first-order jackknife estimates of ant species richness (see Table 1) and elevation in dry grasslands of a Mediterranean range in central Spain.** Curve and 95% IC from GAM with Gaussian errors fit to the Guadarrama 2014 survey (black circles). Richness estimates for the surveys in Guadarrama 2015 (grey circles) and Serrota 2015 (open circles) were superimposed. Elevation values were slightly jittered to avoid overlap among some points.

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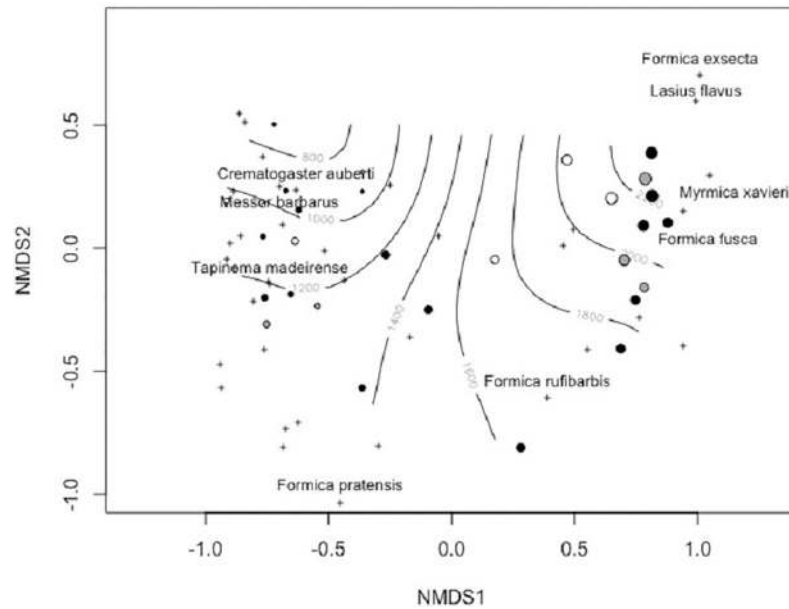
Species turnover and nestedness were significant beta diversity components, although species turnover among grasslands was more important than pure nestedness of assemblages ( $\beta_{\text{SOR}} = 0.88$ ,  $\beta_{\text{SIM}} = 0.79$ ,  $\beta_{\text{NES}} = 0.09$ , all  $P$ -values = 0.001). This relationship among beta diversity components did not vary among surveys (S3 Table).

## Discussion

Ant species richness, taxonomic composition and nestedness changed predictably with elevation in the Mediterranean mountains we investigated, exhibiting largely consistent patterns between years and among regions. These changes were more notable along mid-elevations and associated more clearly with differences of species richness among sites than with prevalence or abundance among species. To our knowledge, this is the first study specifically designed to describe ant community species richness and taxonomic composition responses to elevational changes in the Mediterranean Basin. However, we restricted our study to grasslands, therefore the results might not be a suitable model system for other habitat types. Notwithstanding, grasslands are among the ecosystems exhibiting greater economic and ecological relevance in the Mediterranean Basin [67, 68].

Species richness showed a hump-shaped relationship with elevation, peaking at ca. 1100 m a.s.l. This model depiction fit to the primary dataset (Guadarrama 2014), transferred adequately to the 2015 Guadarrama dataset, and also to the spatial validation dataset in the 2015





**Fig 3. Non-metric multidimensional scaling (NMDS) of ant assemblages based on binary Bray-Curtis distances among grasslands calculated with species occurrences from pitfall traps in a Mediterranean range of central Spain.** Circles represent data from Guadarrama (black; year 2014, grey: year 2015) and Serrota (open circles) ranges. Symbol sizes are proportional to the elevation of sampling plots. As reference, circles are superimposed to a smooth elevation surface (estimated using a GAM of elevation on a bivariate spline of NMDS scores). The positions of some illustrative species are given as reference.

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Serrota range. Despite geometric constraints as mid-domain effects were described to explain these curve types [30], more recent studies tended to downplay the importance of these mechanisms [24, 29, 67]. In fact, while mid elevation peaks in species diversity were reported as relatively common [28, 29], these observations are far from universal, suggesting regional factors play an important role in driving elevational patterns of species diversity. Interestingly, research showed monotonic increases in ant species richness were common in temperate climates [19–21], where elevation was correlated with temperature. In arid climates, monotonic decreases were observed [22, 23], where hydric stress was ameliorated with altitude. Indeed, hydric stress and temperature are the two main elevational correlates in Mediterranean climates, with water availability limiting communities at low elevations, while at high elevations cold temperature stress is more important [6, 7]. This double constraint might drive a hump-shaped spatial pattern of ant species richness in Mediterranean mountains.

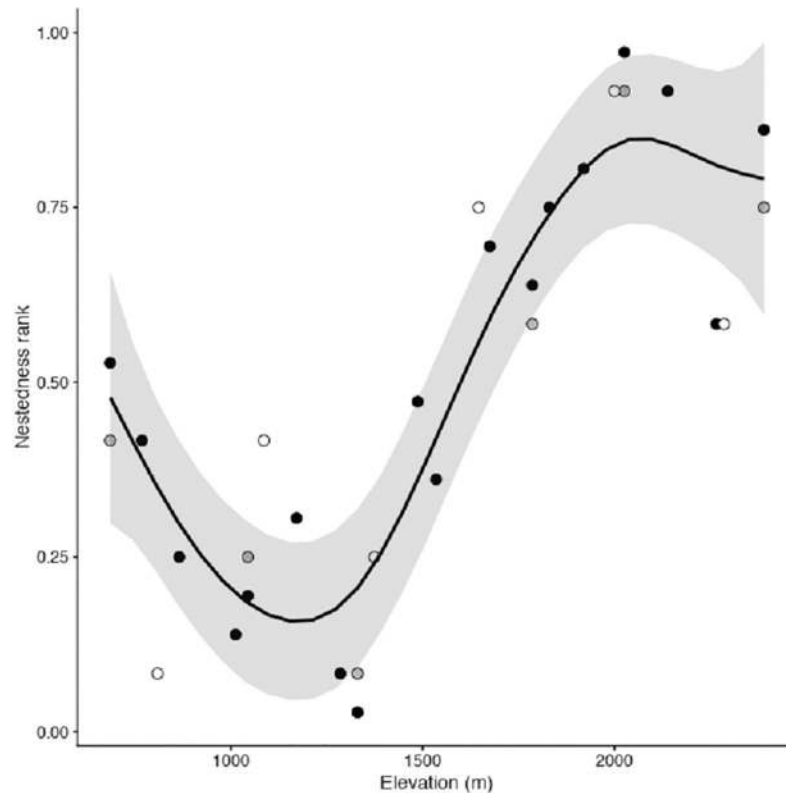
Most studies on ant communities reported species richness positively responded to temperature [19–21, 24, 26, 27, 69, 70], but a number of findings also emphasized the role of water

**Table 2. Nestedness indices for dry grassland ant assemblages in Guadarrama range.**

NODF	NODFr	NODFc	T	fill
38.31: <b>0.001–0.021</b>	40.62: <b>0.001–0.001</b>	37.72: <b>0.001–0.143</b>	25.25: <b>0.001–0.892</b>	0.23

Nestedness indices derived from the Nestedness metric based on Overlap and Decreasing Fill (NODF), NODFr for rows (i.e. localities) and NODFc for columns (i.e. species) and matrix temperature (T). Numbers under headings for indices provide the index values followed by *P*-values estimated by equiprobable ('r00') and proportional resampling ('quasiswap') binary null models. Significant *P*-values (*P* < 0.05) are in bold face. Fill: matrix fill (sum of 1s/sum of cells). Data from 2014 in Guadarrama (18 grasslands, 35 species).

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**Fig 4. Generalized additive model (GAM) of nestedness ranks (based on nestedness temperature (T) index) for elevation in ant species assemblages in dry grasslands of a Mediterranean range in central Spain.** Curve and 95% IC from GAM with Gaussian errors fit to the Guadarrama 2014 survey (black circles). Nestedness ranks for the Guadarrama 2015 (grey circles) and Serrota 2015 (open circles) surveys are superimposed. Low ranks indicate poorer species community richness, where species are a subset of richer species communities. Elevation values were slightly jittered to avoid overlap among some points.

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availability [22, 29, 33, 34, 71]. Cold temperatures can reduce local ant species richness by direct effects that limit ant activity, such as reduced foraging time [35, 72], and, at larger biogeographical scales, cold temperatures can also limit species pools by lowering speciation rates [69]. Azcárate *et al.* [72] reported low humidity, in turn, can limit colony activation by reducing foraging possibilities, and therefore constitutes a main environmental filter. Alternatively, temperature and water availability can facilitate ant species richness indirectly, these abiotic factors affecting primary productivity and therefore the range and abundance of resources ant colonies exploit [33, 35–37]. Diversity-productivity hypothesis states that as productivity increases, so does the availability of energy and resources, so density and size of colonies also increase. These relationships should increase local species richness because the abundance of individuals lowers local extinction risks or may activate a sampling mechanism [73, 74]. In Mediterranean climates, primary productivity can be limited by cold temperatures and summer drought [75], so maximum primary productivity values likely occur at intermediate elevations, where summer drought is not too severe and temperatures are not too cold. Therefore, if species richness increases with primary productivity, a humped curve is also expected.

As anticipated, elevation also affected the taxonomic composition of ant communities. We observed a ca. 1700 m threshold separating high mountain ant species communities from those along the lower elevational gradient. This threshold seemed consistent among regions and between years. Furthermore, although nestedness was a significant component of beta

diversity, most elevational shifts among communities were attributable to species turnover. This result suggested the communities with lowest ant species diversity (high mountain communities) were also the most unique in a regional context. The singularity of high elevation ant communities was previously observed in different biomes [22, 24, 34, 35], which reinforced the conservation value of the Mediterranean mountains reported for other taxa [76–78].

The examination of community nestedness suggested current ant assemblages are context-dependent and likely highly vulnerable to global warming and other anthropogenic changes. This is because, first, species turnover exhibited a dominant role over pure nestedness, which indicated different and characteristic groups of ant species occurred along an elevational gradient, and at the very least, ant species community composition was distinct at high elevations (> 1700 m) (Fig 3). We want to emphasize that if species distributions followed gradually changing abiotic conditions (e.g. a decreasing temperature gradient with elevation), then we would expect a concomitant change in the taxonomic composition of ant assemblages, rather than the development of new, characteristic, species groups [42, 79]. It is also illuminating to consider that nestedness arises with contributions from differences among species and among sites [61]. Nestedness patterns elsewhere were explained as a simple sampling mechanism, where the smaller species group in less diverse, i.e. poorer communities were a subset of species in more diverse, i.e. richer communities, reportedly when the studied localities differed in resource availability (or productivity) and this factor determined species richness [74, 80]. However, this implied species attributes notably contributed to nestedness, because rarer species with small distributional ranges (often specialists or poor-dispersers) were not generally sampled by the least diverse, i.e. poorest communities, while the more common taxa exhibited a wide distributional range [63]. In our study, the least diverse communities were more nested than diverse ant communities, but nestedness among species was less important than among sites. We conclude the sampling mechanism did not fully explain the nested patterns in the ant communities we studied. We believe that habitat and abiotic filtering provide more plausible explanations for those patterns. However, we carefully controlled by design the habitat type (arid grasslands in gentle south facing slopes), which implied those habitat filters — if an integral component — should be fine grained. Nestedness patterns along the elevational gradient were temporally constant, but varied between mountain ranges, indicating ant community composition was spatially dependent. Finally, an historic explanation of current ant species distribution patterns in a landscape context is an important element to include. We studied a mountain range within the Mediterranean biome where major landscape changes have not occurred over the last thirty years. Extensive afforestation was conducted during 1950's and a progressive abandonment of the region took place between the 1960's and 1980's. All of our study sites were covered by grasslands since at least 1975 (a long-term time series of aerial photographs may be accessed at the Statistical Institute of the Autonomous Community of Madrid: <http://www.madrid.org/nomecalles/Inicio.icm>). Therefore, we assume all of them are equally accessible from the regional species pool. However, we cannot disregard other time-related mechanisms, which might explain community composition, such as priority effects favoring one or more species, depending on their relative order of arrival to a site [81], or metapopulation dynamics involving local extinctions and recolonizations in periods beyond this two-year study [82].

Overall, species taxonomic composition varied non-linearly with elevation. The insufficiency of the sampling mechanism emphasizes that ant assemblages are highly vulnerable to global changes on climate and anthropogenic impacts, which might affect species at the habitat and/or community level. It is unlikely species' range shifts that track environmental change will maintain current ant species community composition.

## Supporting information

### **S1 Table. Ant species occurrences and environmental characteristics of sampling plots.**

IDPlot: plot identification. Survey: G2014 for Guadarrama range in 2014, G2015 for Guadarrama range in 2015 and S2015 for Serrota range in 2015. For each sampling plot we give the elevation (in m), latitude and longitude (UTM, datum European 1950), mean annual rainfall (MeanAnnualRainfall) and air temperature (MeanAnnualTemperature) according to the Atlas Climático Digital de la Península Ibérica (<http://opengis.uab.es/wms/iberia/>), and maximum and minimum soil temperatures during sampling periods measured with data loggers buried at ground level (MaximumWeekTemperatureSoil and MinimumWeekTemperatureSoil, respectively). Occurrence of the species in the plots is given in the following columns. (XLSX)

**S2 Table. Nestedness indices for dry grassland ant assemblages in surveys from all study areas in central Spain.** Guadarrama range (-G2014- year 2014: 18 grasslands, 35 species; -G2015- year 2015: 6 grasslands, 26 species) and Serrota range (-S2015- year 2015: 6 grasslands, 20 species). Indices are Nestedness metric based on Overlap and Decreasing Fill (NODF), NODFr for rows (i.e., localities) and NODFc for columns (i.e., species) and matrix temperature (T). Numbers under headings for indices provide index values followed by *P*-values estimated by equiprobable ('r00') binary null models and proportional resampling ('quasiswap'). Significant *P*-values ( $P < 0.05$ ) are in bold face. Fill: matrix fill (sum of 1s/sum of cells). (PDF)

**S3 Table. Multiple-site dissimilarities of beta diversity.** Multiple-site dissimilarities accounting for the spatial turnover ( $\beta_{SIM}$ ) and the nestedness components ( $\beta_{NES}$ ) of beta diversity, and sum of both values ( $\beta_{SOR}$ ) for dry grassland ant assemblages in all study areas in central Spain. G2014: Guadarrama range 2014; G2015: Guadarrama range 2015; S2015: Serrota range 2015. *P*-values estimated by equiprobable binary null models ('r00') are given between parentheses. Significant *P*-values ( $P < 0.05$ ) are in bold face. (PDF)

**S1 Fig. Temporal validation of the relationship between nestedness and elevation.** Generalized additive model (GAM) of nestedness ranks (based on nestedness temperature index) on elevation in ant assemblages from Guadarrama range (central Spain) fit to Guadarrama 2014 data and validated with Guadarrama 2015 data. (TIF)

**S2 Fig. Spatial validation of the relationship between nestedness and elevation.** Generalized additive model (GAM) of nestedness ranks (based on nestedness temperature index) on elevation in ant assemblages from Guadarrama range (central Spain) fit to Guadarrama 2014 data and validated with Serrota 2015 data. (TIF)

**S3 Fig. Relationships between temperature and elevation.** Linear regressions between elevation and mean annual (air) temperature (top left,  $R^2 = 94\%$ ), maximum (soil) temperature (top right,  $R^2 = 80\%$ ), minimum (soil) temperature (bottom left,  $R^2 = 96\%$ ), and mean annual rainfall (bottom right,  $R^2 = 91\%$ ). Regressions built for Guadarrama 2014 data ( $n = 18$  sites, black circles). Data for Guadarrama 2015 (dark grey circles) and Serrota 2015 (white circles) are depicted as a reference. Temperatures measured in Celsius degrees and elevation in meters. Annual climatic data from the Digital Climatic Atlas of the Iberian Peninsula (<http://opengis.uab.es/wms/iberia/>). Soil temperatures measured for the sampling period (a week) with data loggers buried at ground level. Note that 2014 was cooler than 2015, in spite of which models

for richness and nestedness built in 2014 validated well with data from 2015. (TIF)

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