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

Flores, O.[1,2]; Seoane, J.[2]; Hevia, V. [3]; Azcárate, F.M. [2]

[1] Corresponding author:

Department of Biogeography and Global Change, National Museum of Natural Sciences, Spanish National Research Council (MNCN-CSIC), Spain

omarf@mncn.csic.es

Phone: +34 917452500 ext 980805

[2] Terrestrial Ecology Group, Department of Ecology, Universidad Autónoma de Madrid, Spain

[3] Social-ecological Systems Laboratory, Department of Ecology, Universidad Autónoma de Madrid, Spain

Abstract

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. Here we analyzed the response of ant species richness and assemblage composition to 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.

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 significantly 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 were observed over time but not between the two study regions.

We concluded 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 (spatio-temporal factors) 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 temperature and water availability gradients in space and time, which determines 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 probable 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 basin 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

67 herbivore abundance, drive relevant top-down effects, and are often used as biological
68 control agents of insect pests and fungal pathogens [16]. Furthermore, ant distribution
69 and abundance patterns provide information to address specific environmental
70 management issues [17, 18]; consequently, ants are particularly useful bioindicators.

71 Previous research on ant diversity documented variable responses to altitudinal
72 gradients, which showed a certain dependency on macroclimate. For instance, studies
73 reporting monotonic decreases in species richness, were frequent in temperate
74 mountains [19-21], while monotonic increases were observed in arid environments [22,
75 23]. Longino and Colwell [24] and Nowrouzi *et al*. [25] indicated more complex
76 patterns in tropical climates, which often combined a monotonic decrease at high
77 elevations with a plateau at low elevations. Mid-elevation peaks were reported in
78 tropical areas [26, 27], although also frequent at other latitudes [28, 29]. These patterns
79 were partially attributed to geometric constraints, such as the mid-domain effect and
80 area availability [30, 31] or the Rapoport rescue effect [32]. However the role of
81 climatic factors, including temperature [19-21] and water availability [22, 29, 33, 34] as
82 direct drivers of ant diversity is also unequivocal. Moreover, several studies
83 demonstrated these climatic variables also indirectly modulated ant diversity, for
84 example, by affecting primary productivity [33, 35-37].

85 Understanding how ant diversity responds to altitudinal gradients is not sufficient,
86 however, to predict how environmental changes (e.g. rising temperatures) will affect
87 species geographic ranges, because knowledge on species composition patterns is also
88 required. Species assemblages and their distributions along ecological and geographical
89 gradients result from species tolerances to climatic (abiotic) factors and biotic
90 interactions, which interplay with niche evolution to determine community composition

91 [38-40]. Kodric-Brown and Brown [41] defined a structured community as one where
92 the organization is not due to randomness [41], and all situations in which communities
93 are not random can be explained by spatial turnover, nestedness, or combinations of
94 both [42]. Nestedness of species assemblages occurs when site biotas with smaller
95 species numbers are subsets of the biotas at richer sites [43, 44]. If environmental and
96 habitat filtering and not interspecific competition is responsible for nestedness, most
97 species can coexist. Therefore, under increasing temperatures, species could migrate or
98 expand their distribution elsewhere from the species former range, without necessarily
99 implying other resident species will be displaced by the newcomers. On the contrary,
100 spatial turnover implies the replacement of some species by others, exhibiting gains and
101 losses of species from location to location globally [45]. This is a sign that some species
102 cannot coexist, because of different climatic requirements, competitive exclusion, or
103 both. In general, global warming will extend insect species ranges to higher altitudes
104 [46], therefore with spatial turnover patterns, species will migrate up an altitudinal
105 gradient and those at higher altitudes might suffer a subsequent reduction in geographic
106 distribution range, and even disappear from the area. However, Maguire *et al*. [47]
107 acknowledged both processes and community patterns might vary with scale, and
108 exhibit spatio-temporal variation, which raises concerns on our capacity to generalize
109 the results based on local studies. Indeed, transferability of results is increasingly
110 considered in analyzing the effects of global change on species distributions, although
111 such efforts are rarer in studies of community patterns [48-50].

112 In the present study, we explored patterns of ant species diversity and community
113 composition in Mediterranean mountain grasslands, integrating spatio-temporal
114 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) and Serrota (maximum 2294 m a.s.l.). The two mountains are ~ 100 km apart with similar climatic, geologic, and biotic characteristics. Mean annual temperatures range from ~ 14 ºC at 600 m to ~ 4 ºC at the summits (2300 – 2428 m); and mean annual rainfall ranges from 550 mm to 1500 mm, with severe summer drought [51]. 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, facing southeast to southwest with gentle slopes (< 5%), and not affected by anthropogenic disturbance (i.e., not close to buildings or main paths). We sampled 18 grasslands in 2014 (in Guadarrama range) and 12 in 2015 (six in Guadarrama range and six in Serrota range). 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 in July, during stable anticyclonic meteorological periods (Guadarrama: 17 - 23 July 2014 and 9 - 16 July

138 2015; Serrota: 1 - 8 July 2015), taking advantage of the seasonal ant activity peak
139 during summer in Central Spain [52]. Traps were collected after one week and ant
140 specimens were sorted to species, excluding winged ones.

141 Pitfall trapping is considered more objective and unbiased than other methods for
142 sampling ground ants [53, 11]. We chose pitfall traps previously tested in other studies
143 in Central Spain, which demonstrated success at capturing the complete species pool
144 and the entire ant size range variability present in the sampled area [52, 54, 55].

145

146 Statistical analyses

147 We examined the relationship between species richness, taxonomic composition, and
148 nestedness of grassland ant communities with elevation. Our analytical strategy was to
149 build descriptive models for the data gathered during the most intensive sampling in
150 Guadarrama 2014, and to validate the patterns detected with data gathered in a subset of
151 the Guadarrama grasslands during 2015 (temporal validation) and with data gathered in
152 the Serrota range during 2015 (spatial validation).

153 *Species richness*

154 First, we assessed the relationship between species richness and elevation. We estimated
155 the species pool size at each locality with the incidence-based first order jackknife
156 estimator. The relationship shape between estimates of richness and elevation was
157 explored applying a generalized additive model (GAM) with Gaussian errors and a thin
158 plate regression spline for elevation. Models were built with data from Guadarrama
159 2014 sampling and validated with data from the Guadarrama 2015 and Serrota 2015
160 samplings. Squared Pearson correlation between jackknife estimates of richness and

161 jackknife estimates predicted by the model were used to assess species richness pattern
162 constancy between years and sites.

163 *Taxonomic composition of assemblages*

164 We explored how species assemblages differed based on taxonomic composition by
165 applying non-metric multidimensional scaling (NMDS) to the hemi-matrices of binary
166 Bray-Curtis distances among grasslands derived from species occurrences in pitfall
167 traps. In addition, a model-based analysis was conducted by building separate negative
168 binomial regressions for each species abundance data (as the multivariate response
169 variable) by elevation, year, and interaction (as the explanatory variables) [56].
170 Significance of the interaction (assessed by resampling) would suggest that patterns of
171 species composition along an elevational gradient changed between years. Likewise, for
172 Guadarrama 2014 and Serrota 2015, a multivariate model was built by fitting negative
173 binomial regressions for individual species abundance, elevation, location, and
174 interaction [56]. Significance of the interaction would suggest how assemblages
175 changed with elevation were context-dependent.

176 *Nestedness patterns*

177 To assess assemblage nestedness among grasslands surveyed on each mountain and
178 year we calculated the matrix temperature (T) [57] and the NODF (Nestedness metric
179 based on Overlap and Decreasing Fill) [58]. While T has traditionally been the most
180 commonly used metric for assessing overall nestedness [59], the more recent NODF
181 index is claimed to exhibit more robust statistical properties, and separate contributions
182 to nestedness of columns (due to species incidence) and rows (due to site composition)
183 can be quantified [58, 60]. T decreases and NODF increases with nestedness.

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 [61]. 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 [62]. 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…, 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). Detection of a high correlation between observed (in 2015) and 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). Detection of a high correlation between observed and 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 (β_{SOR}), Simpson-based multiple-site dissimilarity (β_{SIM}), and nestedness-resultant multiple-site dissimilarity (β_{NES}). β_{SIM} accounts for spatial turnover and β_{NES} integrates dissimilarity due to nestedness, while β_{SOR} expresses the total dissimilarity between communities and equals the sum of β_{SIM} and β_{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 3.3.2; R Core Team 2016) and specialized packages vegan (v. 2.4-5) [63], mvabund (v. 3.12-3) [56], and betapart (1.5.0) [64].

Results

We detected 37 species, all recorded in Guadarrama (35 in 2014 and 26 in 2015, 24 were shared between years) and 20 species in Serrota (all species shared with Guadarrama). 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 1**). 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 = 3.96, F = 12.94, $P < 0.0001$, $D^2 = 83.8\%$, **Fig 1**). This pattern of species richness was similar between years and between mountain ranges (correlations for estimates of richness and richness predicted by the model were r = 0.92, t = 4.68, df = 4, $P = 0.0095$, $R^2 = 85\%$ for Guadarrama 2015 data; and r = 0.86, t = 3.42, df = 4, $P = 0.0267$, $R^2 = 75\%$ for Serrota 2015 data).

Table 1. **Observed (S) and first-order jackknife estimator (J ± SE) of ant species richness in dry grasslands in central Spain**.

ID	Sampling	S	J±SE	N	X	Y	Elevation (m)
G1	G2014	7	7±0	13	437876	4494480	685
G1	G2015	7	7.9±0.9	10	437876	4494480	685

G2	G2014	8	8±0	12	434821	4496549	768
S1	S2015	14	18.5±2.4	10	351711	4474792	808
G3	G2014	14	16.7±1.6	11	440394	4503515	865
G4	G2014	14	16.8±1.6	12	435541	4506094	1012
G5	G2014	14	15.8±1.3	12	431692	4513777	1044
G5	G2015	11	11.9±0.9	12	431692	4513777	1044
S2	S2015	11	14.7±2.3	12	357653	4481333	1086
G6	G2014	13	14.8±1.3	9	414145	4507982	1171
G7	G2014	13	18.5±2.6	12	438808	4523233	1285
G8	G2014	15	15.9±0.9	11	416330	4511347	1330
G8	G2015	14	17.5±2.2	8	416330	4511347	1330
S3	S2015	13	15.8±1.6	12	340207	4476196	1374
G9	G2014	9	9±0	12	436806	4524537	1487
G10	G2014	10	12.8±1.6	12	400821	4495703	1625
S4	S2015	4	4±0	11	326465	4482790	1646
G11	G2014	3	3±0	19	400543	4496999	1675
G12	G2014	5	5.9±0.9	12	419833	4519838	1786
G12	G2015	5	5.9±0.9	12	419833	4519838	1786

G13	G2014	5	5±0	12	419076	4519012	1830
G14	G2014	4	4.9±0.9	12	418737	4519177	1920
S5	S2015	2	2±0	12	323719	4482788	2000
G15	G2014	3	3±0	12	419392	4520829	2026
G15	G2015	3	3±0	8	419392	4520829	2026
G16	G2014	3	3.9±0.9	12	417883	4521249	2139
G17	G2014	4	5.8±1.9	12	418676	4521128	2266
S6	S2015	3	3.9±0.9	12	323944	4485467	2286
G18	G2014	3	3±0	12	419420	4522544	2390
G18	G2015	3	3.9±0.9	12	419420	4522544	2390

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). Geographic coordinates (X and Y) were provided as UTM European Datum 1950.

Fig 1. **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.

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 2**). Multivariate GLMs for species abundances revealed significant elevation effects (P = 0.001) and a significant region effect (P = 0.047), but significant effects were not detected for year or any elevation interactions (**Table 2**). These results suggested taxonomic composition varied mainly with elevation, but differences between regions cannot be excluded.

Fig 2. 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.

282

283 **Table 2**. **Multivariate GLMs (negative binomial regressions) fitted to species abundances.**

	Deviance	P values
a)		
Elevation	192.91	**0.001**
Year	50.27	0.649
Elevation x Year	5.99	0.997
b)		
Elevation	178.14	**0.001**
Region	84.44	**0.047**
Elevation x Region	18.85	0.618

284 *P*-values estimated by bootstrap (n = 1000). Significant *P*-values ($P < 0.05$) are highlighted in
285 bold. a) Guadarrama 2014 vs. Guadarrama 2015. b) Guadarrama 2014 vs. Serrota 2015.

286

287 Ant species assemblages were nested, showing a pattern suggesting differences in
288 species richness among localities were more important than differences in prevalence
289 among species (nestedness among rows was higher than nestedness among columns,
290 **Table 3**, **S2 Table**). Nestedness ranks for Guadarrama 2014 was described with GAM

291 and curvilinear term (spline) for elevation, roughly similar to a third-degree cubic
292 polynomial (df = 3.89, F = 15.38, $P < 0.0001$, $D^2 = 85.6\%$, **Fig 3**). The subset of these
293 grasslands sampled in 2015 maintained their nestedness ranks and the model transferred
294 to 2015 data (Pearson's correlation between predicted and observed ranks, r = 0.96, t =
295 7.28, df = 4, $P = 0.0018$, $R^2 = 93\%$, **S3 Fig**). The model did not transfer between study
296 regions (r = 0. 74, t = 2.18, df = 4, $P = 0.10$, $R^2 = 54\%$, **S4 Fig**). Largely, nestedness
297 increased with elevation, with the exception of the lowest and highest elevation
298 grasslands.

299 **Table 3**. **Nestedness indices for dry grassland ant assemblages in Guadarrama**
300 **range 2014 surveys (18 grasslands, 35 species).**

NODF	NODFr	NODFc	T	fill
38.31: **0.001-0.015**	40.62: **0.001-0.001**	37.72: **0.001**-0.103	25.24: **0.001**-0.912	0.23

301

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

308

309 **Fig 3**. **Generalized additive model (GAM) of nestedness ranks (based on nestedness**
310 **temperature (T) index) for elevation in ant species assemblages in dry grasslands**
311 **of a Mediterranean range in central Spain.**

312 Curve and 95% IC from GAM with Gaussian errors fit to the Guadarrama 2014 survey
313 (black circles). Nestedness ranks for the Guadarrama 2015 (grey circles) and Serrota
314 2015 (open circles) surveys are superimposed. Low ranks indicate poorer species
315 community richness, where species are a subset of richer species communities.
316 Elevation values were slightly jittered to avoid overlap among some points.

317

318 Species turnover and nestedness were significant beta diversity components, although
319 species turnover among grasslands was more important than pure nestedness of
320 assemblages (β_{SOR} = 0.88, β_{SIM} = 0.79, β_{NES} = 0.09, all *P*-values = 0.001). This
321 relationship among beta diversity components did not vary among surveys (**S5 Table**).

322

323 **Discussion**

324 Ant species richness and taxonomic composition changed predictably with elevation in
325 the Mediterranean mountains we investigated, exhibiting largely consistent patterns
326 between years and among regions. These changes were more notable along mid-
327 elevations and associated more clearly with differences of species richness among sites
328 than with prevalence or abundance among species. To our knowledge, this is the first
329 study specifically designed to describe ant community species richness and taxonomic
330 composition responses to elevational changes in the Mediterranean Basin. However, we
331 restricted our study to grasslands, therefore the results might not be a suitable model

332 system for other habitat types. Notwithstanding, grasslands are among the ecosystems
333 exhibiting greater economic and ecological relevance in the Mediterranean Basin [65,
334 66].

335 Species richness showed a hump-shaped relationship with elevation, peaking at ca. 1100
336 m a.s.l. This model depiction fit to the primary dataset (Guadarrama 2014), transferred
337 adequately to the 2015 Guadarrama dataset, and also the spatial validation dataset in the
338 2015 Serrota range. Despite geometric constraints as mid-domain effects were described
339 to explain these curve types [30], more recent studies tended to downplay the
340 importance of these mechanisms [24, 29, 67]. In fact, while mid elevation peaks in
341 species diversity were reported as relatively common [28, 29], these observations are far
342 from universal, suggesting regional factors play an important role in driving elevational
343 patterns of species diversity. Interestingly, research showed monotonic increases in ant
344 species richness were common in temperate climates [19-21], where elevation was
345 correlated with temperature. In arid climates, monotonic decreases were observed [22,
346 23], where hydric stress was ameliorated with altitude. Indeed, hydric stress and
347 temperature are the two main elevational correlates in Mediterranean climates, with
348 water availability limiting communities at low elevations, while at high elevations cold
349 temperature stress is more important [6, 7]. This double constraint might drive a hump-
350 shaped spatial pattern of ant species richness in Mediterranean mountains.

351 Most studies on ant communities reported species richness positively responded to
352 temperature [19-21, 24, 26, 27, 67, 68], but a number of findings also emphasized the
353 role of water availability [22, 29, 33, 34, 69]. Cold temperatures can reduce local ant
354 species richness by direct effects that limit ant activity, such as reduced foraging time
355 [35, 70], and, at larger biogeographical scales, cold temperatures can also limit species

356 pools by lowering speciation rates [67]. Azcárate *et al*. [70] reported low humidity, in
357 turn, can limit colony activation by reducing foraging possibilities, and therefore
358 constitutes a main environmental filter. Alternatively, temperature and water availability
359 can facilitate ant species richness indirectly, these abiotic factors affecting primary
360 productivity and therefore the range and abundance of resources ant colonies exploit
361 [33, 35-37]. Diversity-productivity hypothesis states that as productivity increases, so
362 does the availability of energy and resources, so density and size of colonies also
363 increase. These relationships should increase local species richness by abundance,
364 lowering local extinction risks or activating a sampling mechanism [71, 72]. In
365 Mediterranean climates, primary productivity can be limited by cold temperatures and
366 summer drought [73], so maximum primary productivity values likely occur at
367 intermediate elevations, where summer drought is not too severe and temperatures are
368 not too cold. Therefore, if species richness increases with primary productivity, a
369 humped curve is also expected.

370 As anticipated, elevation also affected the taxonomic composition of ant communities.
371 We observed a ca. 1700 m threshold separating high mountain ant species communities
372 from those along the lower elevational gradient. This threshold was consistent among
373 regions and between years. Furthermore, although nestedness was a significant
374 component of beta diversity, most elevational shifts among communities were
375 attributable to species turnover. This result suggested the communities with lowest ant
376 species diversity (high mountain communities) were also the most unique in a regional
377 context. The singularity of high elevation ant communities was previously observed in
378 different biomes [22, 24, 34, 35], which reinforced the conservation value of the
379 Mediterranean mountains reported for other taxa [74, 75].

380 The examination of community nestedness suggested current ant assemblages are
381 context-dependent and likely highly vulnerable to global warming and other
382 anthropogenic changes. This is because, first, species turnover exhibited a dominant
383 role over pure nestedness, which indicated different and characteristic groups of ant
384 species occurred along an elevational gradient, and at the very least, ant species
385 community composition was distinct at high elevations (> 1700 m) (**Fig 2**). We want to
386 emphasize that if species distributions followed gradually changing abiotic conditions
387 (e.g. a decreasing temperature gradient with elevation), then we would expect a
388 concomitant change in the taxonomic composition of ant assemblages, rather than the
389 development of new, characteristic, species groups [42, 76]. It is also illuminating to
390 consider that nestedness arises with contributions from differences among species and
391 among sites [59]. Nestedness patterns elsewhere were explained as a simple sampling
392 mechanism, where the smaller species group in less diverse, i.e. poorer communities
393 were a subset of species in more diverse, i.e. richer communities, reportedly when the
394 studied localities differed in resource availability (or productivity) and this factor
395 determined species richness [72, 77]. However, this implied species attributes notably
396 contributed to nestedness, because rarer species with small distributional ranges (often
397 specialists or poor-dispersers) were not generally sampled by the least diverse, i.e.
398 poorest communities, while the more common taxa exhibited a wide distributional
399 range [61]. In our study, the least diverse communities were more nested than diverse
400 ant communities, but nestedness among species was less important than among sites.
401 We conclude the sampling mechanism did not fully explain the nested patterns in the
402 ant communities we identified and habitat and abiotic filtering provided more plausible
403 explanations for the patterns. However, we carefully controlled by design the habitat
404 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. Therefore, we assume all sites 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 [78], or metapopulation dynamics involving local extinctions and recolonizations in periods beyond this two-year study [79].

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.

Acknowledgements

This study could not have been carried out without the enthusiastic support of students at Department of Ecology UAM (Cristina Rota, Víctor Pascual, Jesús Miranda, Celia Santos, Rubén Altozano, Inés Cueva) and friends (Montse Reina, Esperanza Iranzo,

Aimara Planillo, Carlos Pérez Carmona, Xavier Espadaler) who helped with field and laboratory work. The study received partial support by the Madrid's Government research group network REMEDINAL3-CM (S-2013/MAE-2719) and is a contribution to project CGL2014-53789-R.

References

[1] Gilman SE, Urban MC, Tewksbury J, Gilchrist GW, Holt RD. A framework for community interactions under climate change. Trends in Ecology & Evolution. 2010;25(6):325-331.

[2] Bellard C, Bertelsmeier C, Leadley P, Thuiller W, Courchamp F. Impacts of climate change on the future of biodiversity. Ecology Letters. 2012;15(4):365-377.

[3] Oliver TH, Morecroft MD. Interactions between climate change and land use change on biodiversity: attribution problems, risks, and opportunities. Wiley Interdisciplinary Reviews: Climate Change. 2014;5(3):317-335.

[4] Wilson RJ, Gutiérrez D, Gutiérrez J, Montserrat VJ. An elevational shift in butterfly species richness and composition accompanying recent climate change. Global Change Biology. 2007;13:1873-1887.

[5] Devictor V, Julliard R, Couvet D, Jiguet F. Birds are tracking climate warming, but not fast enough. Proceedings of the Royal Society B: Biological Sciences. 2008;275:2743-2748.

[6] Walter H, Breckle SW. Walter's vegetation of the earth: the ecological systems of the geo-biosphere. 4th ed. Berlin. Springer; 2002.

450 [7] Schöb C, Armas C, Guler M, Prieto I, Pugnaire FI. Variability in functional traits
451 mediates plant interactions along stress gradients. Journal of Ecology. 2013;101(3):753-
452 762.

453 [8] Nogués-Bravo D, Araújo MB, Lasanta T, Moreno JIL. Climate change in
454 Mediterranean mountains during the 21st century. AMBIO: A Journal of the Human
455 Environment. 2008;37(4):280-285.

456 [9] Intergovernmental Panel on Climate Change (IPCC) 2013. Climate Change 2013:
457 The Physical Science Basis, Contribution of Working Group I to the Fifth Assessment
458 Report of the Intergovernmental Panel on Climate Change. In Stocker TF et al., editors.
459 Cambridge, UK. Cambridge University Press; 2013.

460 [10] Benavides R, Escudero A, Coll L, Ferrandis P, Ogaya R, Gouriveau F, Peñuelas J,
461 Valladares F. Recruitment patterns of four tree species along elevation gradients in
462 Mediterranean mountains: Not only climate matters. Forest Ecology and Management.
463 2016;360:287-296.

464 [11] Majer JD, Alonso LE, Schultz TR. 1st ed. Agosti D, Majer JD, Alonso LE,
465 Schultz TR, editors. Ants. Standard methods for measuring and monitoring biodiversity.
466 Washington D.C.: Smithsonian Institution Press; 2000.

467 [12] Cushman JH, Lawton JH, Manly BFJ. Latitudinal patterns in European ant
468 assemblages: variation in species richness and body size. Oecologia. 1993;95:30-37.

469 [13] Azcárate FM, Arqueros L, Sánchez AM, Peco B. Seed and fruit selection by
470 harvester ants, *Messor barbarus*, in Mediterranean grassland and scrubland. Functional
471 Ecology. 2005;19:273-283.

472 [14] Wolff A, Debussche M. Ants as seed dispersers in a Mediterranean old-field
473 succession. Oikos. 1999;84(3):443-452.

474 [15] Dean WRJ, Milton SJ, Klotz S. The role of ants nest-mounds in maintaining
475 small-scale patchiness in dry grasslands in Central Germany. Biodiversity and
476 Conservation. 1997;6:1293-1307.

477 [16] Philpott SM, Armbrecht I. Biodiversity in tropical agroforest and the ecological
478 role of ants and ant diversity in predatory function. Ecological Entomology.
479 2006;31(4):369-377.

480 [17] Underwood EC, Fisher BL. The role of ants in conservation monitoring: If, when
481 and how. Biological Conservation. 2006;132(2):166-182.

482 [18] Schmidt FA, Ribas CR, Schoereder JH. How predictable is the response of ant
483 assemblages to natural forestry recovery? Implications for their use as bioindicators.
484 Ecological Indicators. 2013;24:158-166.

485 [19] Machac A, Janda M, Dunn RR, Sanders NJ. Elevational gradients in phylogenetic
486 structure of ant communities reveal the interplay of biotic and abiotic constraints on
487 diversity. Ecography. 2011;34:364-371.

488 [20] Reymond A, Purcell J, Cherix D, Guisan A, Pellissier L. Functional diversity
489 decreases with temperature in high elevation ant fauna. Ecological
490 entomology. 2013;38(4):364-373.

491 [21] Kwon TS, Kim SS, Chun JH. Pattern of ant diversity in Korea: An empirical test
492 of Rapoport's altitudinal rule. Journal of Asia-Pacific Entomology. 2014;17:161-167.

[22] Sanders NJ, Moss J, Wagner D. Patterns of ant species richness along elevational gradients in an arid ecosystem. Global ecology and biogeography. 2003;12(2):93-102.

[23] Orabi GM, Semida FM, Abdel-Dayem MS, Sharaf MR, Zalat SM. Diversity patterns of ants along an elevation gradient at St. Catherine Protectorate, South Sinai, Egypt: (Hymenoptera: Formicidae). Zoology in the Middle East. 2011;54(1):101-112.

[24] Longino JT, Colwell RK. Density compensation, species composition, and richness of ants on a neotropical elevational gradient. Ecosphere. 2011;2(3):1-20.

[25] Nowrouzi S, Andersen AN, Macfadyen S, Staunton KM, VanDerWal J, Robson SKA. Ant Diversity and Distribution along Elevation Gradients in the Australian Wet Tropics: The Importance of Seasonal Moisture Stability. PLoS ONE. 2016;11(4): e0153420. doi:10.1371/journal.pone.0153420

[26] Munyai TC, Foord SH. Ants on a mountain: spatial, environmental and habitat associations along an altitudinal transect in a centre of endemism. Journal of Insect Conservation. 2012;16:677-695.

[27] Munyai TC, Foord SH. Temporal Patterns of Ant Diversity across a Mountain with Climatically Contrasting Aspects in the Tropics of Africa. PLoS ONE. 2015;10(3): e0122035. doi:10.1371/journal.pone.0122035

[28] Sanders NJ, Rahbek C. The patterns and causes of elevational diversity gradients. Ecography. 2012;35(1):1-3.

[29] Szewczyk T, McCain CM. A Systematic Review of Global Drivers of Ant Elevational Diversity. PLoS ONE. 2016;11(5): e0155404. doi:10.1371/journal.pone.0155404

[30] Colwell RK, Lees DC. The mid-domain effect: geometric constraints on the geography of species richness. Trends in Ecology & Evolution. 2000;15(2):70-76.

[31] Sanders NJ. Elevational gradients in ant species richness: area, geometry, and Rapoport's rule. Ecography. 2002;25(1):25-32.

[32] Stevens GC. The elevational gradient in altitudinal range: an extension of Rapoport's latitudinal rule to altitude. The American Naturalist. 1992;140(6):893-911.

[33] Botes A, McGeoch MA, Robertson HG, van Niekerk A, Davids HP, Chown SL. Ants, altitude and change in the northern Cape Floristic Region. Journal of Biogeography. 2006;33:71-90.

[34] Smith MA, Hallwachs W, Janzen DH. Diversity and phylogenetic community structure of ants along a Costa Rican elevational gradient. Ecography. 2014;37(8):720-731.

[35] Brühl CA, Mohamed M, Linsenmair KE. Altitudinal distribution of leaf litter ants along a transect in primary forests on Mount Kinabalu, Sabah, Malaysia. Journal of Tropical Ecology. 1999;15:265-277.

[36] Kaspari M, O'Donnell S, Kercher JR. Energy, Density, and Constraints to Species Richness: Ant Assemblages along a Productivity Gradient. The American Naturalist. 2000;155(2):280-293.

[37] Gotelli NJ, Ellison AM. Biogeography at a regional scale: determinants of ant species density in New England bogs and forests. Ecology. 2002;83(6):1604-1609.

535 [38] Lavergne S, Mouquet N, Thuiller W, Ronce O. Biodiversity and climate change:
536 integrating evolutionary and ecological responses of species and communities. Annual
537 Review of Ecology, Evolution and Systematics. 2010;41: 321-350.

538 [39] Laiolo P, Seoane J, Illera JC, Bastianelli G, Carrascal LM, Obeso JR. The
539 evolutionary convergence of avian lifestyles and their constrained coevolution with
540 species' ecological niche. Proceedings of the Royal Society B: Biological Sciences.
541 2015;282(1821):20151808.

542 [40] Laiolo P, Seoane J, Obeso JR, Illera JC. Ecological divergence among young
543 lineages favours sympatry, but convergence among old ones allows coexistence in
544 syntopy. Global Ecology and Biogeography. 2017;26(5):601-608.

545 [41] Kodric-Brown A, Brown JH. Highly structured fish communities in Australian
546 desert springs. Ecology. 1993;74:1847-1855.

547 [42] Baselga A. Partitioning the turnover and nestedness components of beta diversity.
548 Global Ecology and Biogeography. 2010;19:134-143.

549 [43] Wright DH, Reeves JH. On the meaning and measurement of nestedness of
550 species assemblages. Oecologia. 1992;92:416-428.

551 [44] Ulrich W, Gotelli NJ. Null model analysis of species nestedness patterns.
552 Ecology. 2007;88(7):1824-1831.

553 [45] Gaston KJ, Davies RG, Orme CDL, Olson VA, Thomas GH, Ding TS,
554 Rasmussen PC, Lennon JJ, Bennett PM, Owens IPF, Blackburn TM. Spatial turnover in
555 the global avifauna. Proceedings of the Royal Society B: Biological Sciences.
556 2007;274:1567-1574.

557 [46] Fielding CA, Whittaker JB, Butterfield JEL, Coulson JC. Predicting responses to
558 climate change: the effect of altitude and latitude on the phenology of the Spittlebug
559 *Neophilaenus lineatus*. Functional Ecology. 1999;13(1):65-73.

560 [47] Maguire KC, Nieto-Lugilde D, Fitzpatrick MC, Williams JW, Blois JL. Modeling
561 species and community responses to past, present and future episodes of climatic and
562 ecological change. Annual Review of Ecology, Evolution and Systematics.
563 2015;46:343-368.

564 [48] Kleinwächter M, Rickfelder T. Habitat models for a riparian carabid beetle: their
565 validity and applicability in the evaluation of river bank management. Biodiversity and
566 Conservation. 2007;16(11):3067-3081.

567 [49] Duncan RP, Cassey P, Blackburn TM. Do climatic envelop models transfer? A
568 manipulative test using dung beetle introductions. Proceedings of the Royal Society B:
569 Biological Sciences. 2009;267:1449-1457.

570 [50] Torres LG, Sutton PJH, Thompson DR, Delord K, Weimerskirch H, Sagar PM, et
571 al. Poor transferability of species distribution models for a pelagic predator, the grey
572 petrel, indicates constrasting habitat preferences across ocean basins. PLoS ONE.
573 2015;10(3): e0120014. doi:10.1371/journal.pone.0120014.

574 [51] i Casals MN, Pons X, i Nolla JMR. [Digital Climatic Atlas of the Iberian
575 Peninsula. Methodology and applications in bioclimatology and geobotany] Bellaterra
576 UAB; 2005. Spanish.

577 [52] Azcárate FM, Peco B. Abandonment of grazing in a mediterranean grassland area:
578 consequences for ant assemblages. Insect Conservation and Diversity. 2012;5:279-288.

[53] Andersen AN. Sampling communities of ground-foraging ants: Pitfall catches compared with quadrat counts in an Australian tropical savanna. Australian Journal of Ecology. 1991;16:273-279.

[54] Azcárate FM, Seoane J, Castro S, Peco B. Drove roads: Keystone structures that promote ant diversity in Mediterranean forest landscapes. Acta Oecologica. 2013;49:107-115.

[55] Hevia V, Azcárate FM, Oteros-Rozas E, González JA. Exploring the role of transhumance drove roads on the conservation of ant diversity in Mediterranean agroecosystems. Biodiversity and Conservation. 2013;22:2567-2581.

[56] Wang YI, Naumann U, Wright ST, Warton DI. mvabund–an R package for model-based analysis of multivariate abundance data. Methods in Ecology and Evolution. 2012;3(3):471-474.

[57] Rodríguez-Gironés MA, Santamaría L. A new algorithm to calculate the nestedness temperature of presence–absence matrices. Journal of Biogeography. 2006;33(5):924-935.

[58] Almeida-Neto M, Guimarães P, Guimarães Jr PR, Loyola RD, Ulrich W. A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. Oikos. 2008;117:1227-1239.

[59] Ulrich W, Almeida-Neto M, Gotelli NJ. A consumer's guide to nestedness analysis. Oikos. 2009;118(1):3-17.

[60] Ulrich W, Gotelli NJ. Pattern detection in null model analysis. Oikos. 2013;122(1):2-18.

601 [61] Nolte D, Schuldt A, Gossner MM, Ulrich W, Assmann T. Functional traits drive
602 ground beetle community structures in Central European forests: Implications for
603 conservation. Biological Conservation. 2017;213:5-12.

604 [62] Moore JE, Swihart RK. Toward ecologically explicit null models of
605 nestedness. Oecologia. 2007;152(4):763-777.

606 [63] Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al.
607 Vegan: community ecology package R. Version 24-3 [software]. 2017 [cited 21 July
608 2018]. Available from: https://github.com/vegandevs/vegan.

609 [64] Baselga A, Orme CDL. betapart: an R package for the study of beta
610 diversity. Methods in Ecology and Evolution. 2012;3(5):808-812.

611 [65] Joffre R, Rambal S, Ratte JP. The dehesa system of southern Spain and Portugal
612 as a natural ecosystem mimic. Agroforestry Systems. 1999;45:57-79.

613 [66] Bergmeier E, Petermann J, Schröder E. Geobotanical survey of wood-pasture
614 habitats in Europe: diversity, threats and conservation. Biodiversity and Conservation.
615 2010;19:2995-3014.

616 [67] Sanders NJ, Lessard JP, Fitzpatrick MC, Dunn RR. Temperature, but not
617 productivity or geometry, predicts elevational diversity gradients in ants across spatial
618 grains. Global Ecology and Biogeography. 2007;16(5):640-649.

619 [68] Malsch AKF, Fiala B, Maschwitz U, Mohamed M, Nais J, Linsenmair KE. An
620 analysis of declining ant species richness with increasing elevation at Mount Kinabalu,
621 Sabah, Borneo. Asian Myrmecology. 2008;2:33-49.

622 [69] Jenkins CN, Sanders NJ, Andersen AN, Arnan X, Brühl CA, Cerda X, et al.
623 Global diversity in light of climate change: the case of ants. Diversity and Distributions.
624 2011;17:652-662.

625 [70] Azcárate FM, Kovacs E, Peco B. Microclimatic Conditions Regulate Surface
626 Activity in Harvester Ants *Messor barbarus*. Journal of Insect Behavior.
627 2007;20(3):315-329.

628 [71] Seoane J, Laiolo P, Obeso JR. Abundance leads to more species, particularly in
629 complex habitats: a test of the increased population size hypotheses in bird
630 communities. Journal of Biogeography. 2017;44:556-566.

631 [72] Storch D, Bohdalková E, Okie J. The more-individuals hypothesis revisited: the
632 role of community abundance in species richness regulation and the productivity–
633 diversity relationship. Ecology Letters. 2018;21:920-937.

634 [73] Flexas J, Diaz-Espejo A, Gago J, Gallé A, Galmés J, Gulías J, et al.
635 Photosynthetic limitations in Mediterranean plants: A review. Environmental and
636 Experimental Botany. 2014;103:12-23.

637 [74] Médail F, Quézel P. Biodiversity Hotspots in the Mediterranean Basin: Setting
638 Global Conservation Priorities. Conservation Biology. 1999;13(6):1510-1513.

639 [75] Sfenthourakis S, Legakis A. Hotspots of endemic terrestrial invertebrates in
640 southern Greece. Biodiversity & Conservation. 2001;10(8):1387-1417.

641 [76] Baselga A. Determinants of species richness, endemism and turnover in European
642 longhorn beetles. Ecography. 2008;31:263–271.

[77] Evans KL, Warren PH, Gaston KJ. Species-energy relationships at the macroecological scale: a review of the mechanisms. Biological Reviews. 2005;80:1-25.

[78] Wilbur HM, Alford RA. Priority effects in experimental pond communities: responses of Hyla to Bufo and Rana. Ecology. 1985;66(4):1106-1114.

[79] Hanski I. Metapopulation dynamics. Nature. 1998;396:41-49.

Supporting information

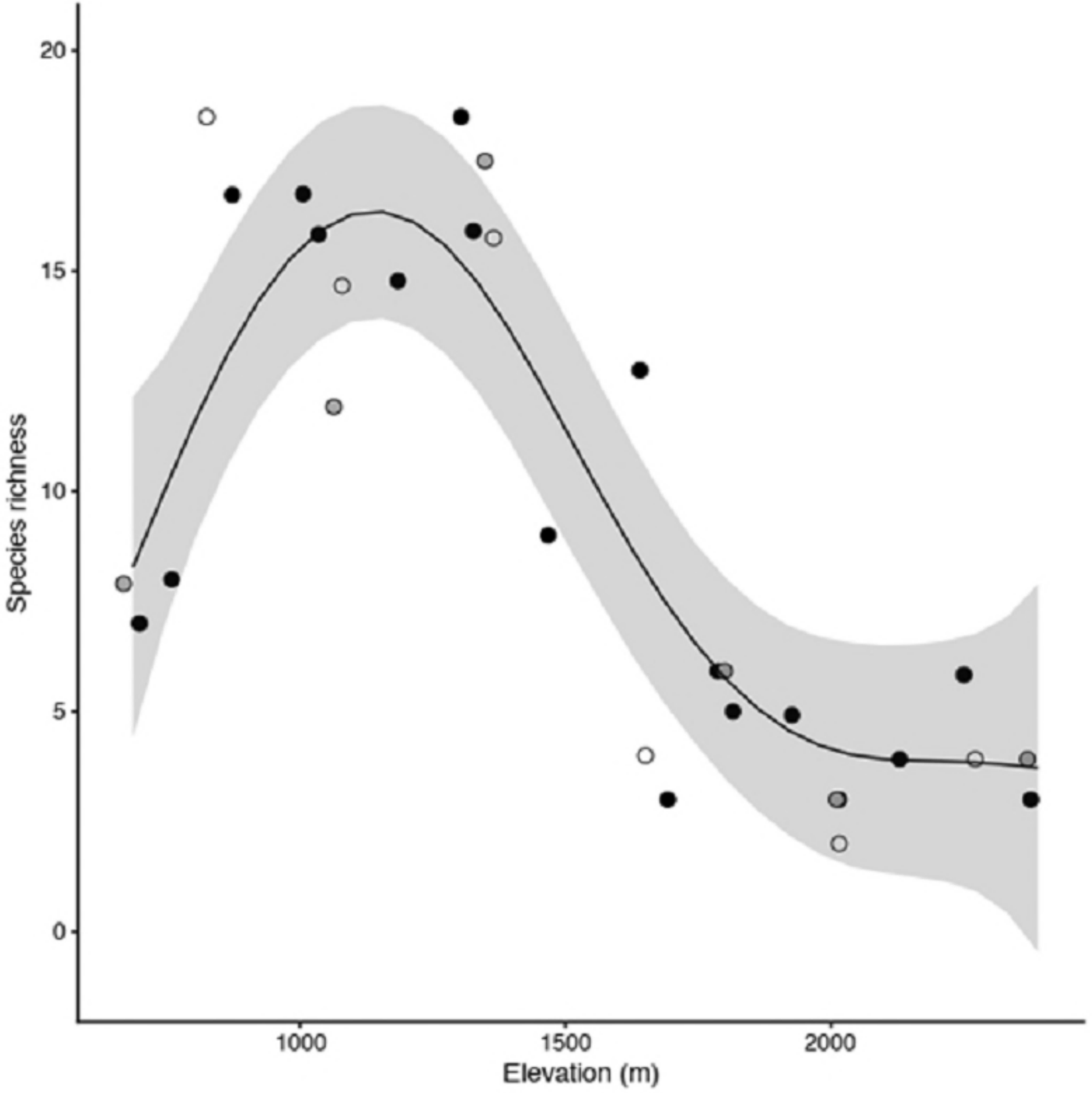
S1 Table. Species presence in each survey.

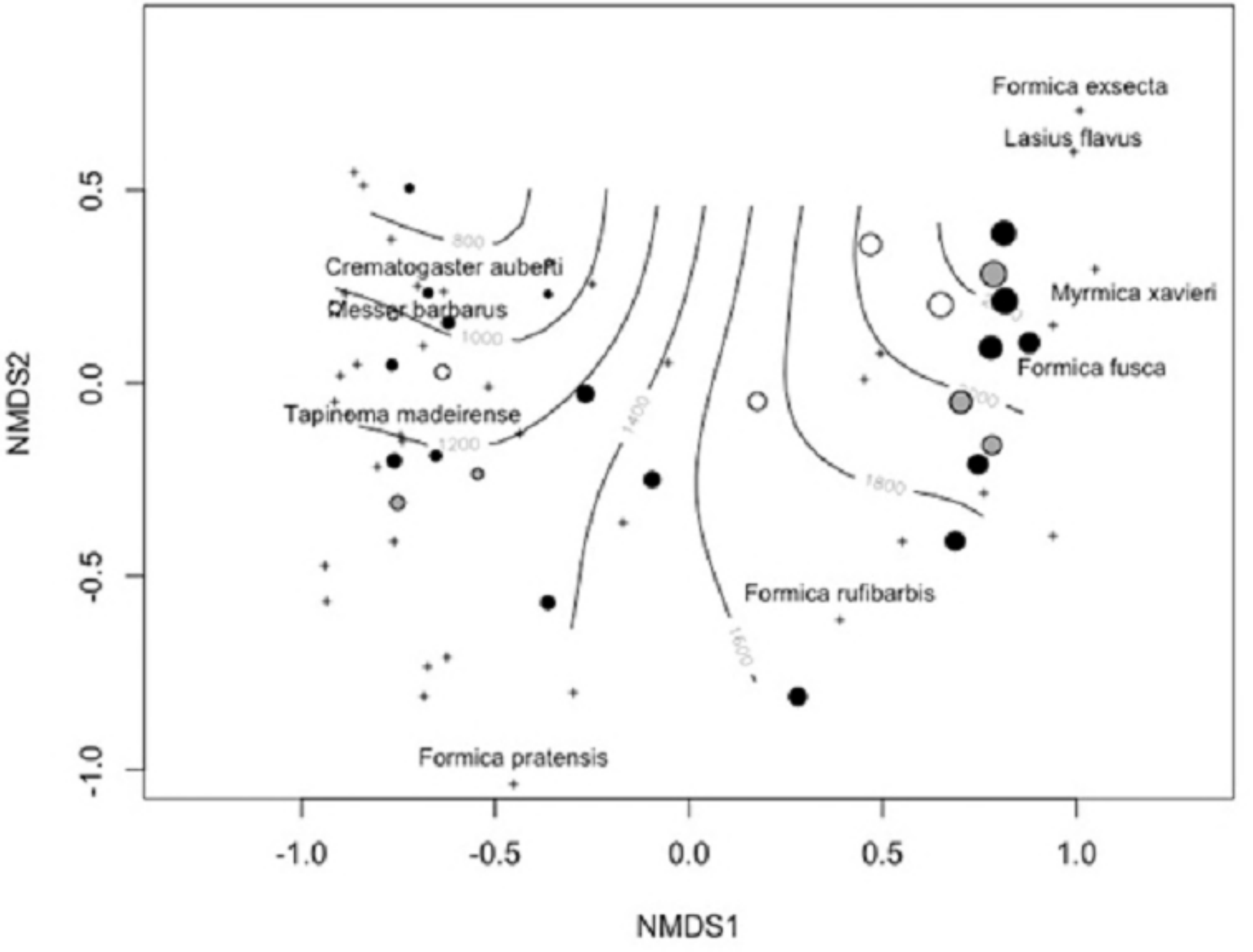
S2 Table. Nestedness indices for dry grassland ant assemblages in surveys from all study areas in central Spain. Guadarrama range (-G2014- year 2014: 18 grasslands, 35 species; -G2015- year 2015: 6 grasslands, 26 species) and Serrota range (-S2015- year 2015: 6 grasslands, 20 species). Indices are Nestedness 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).

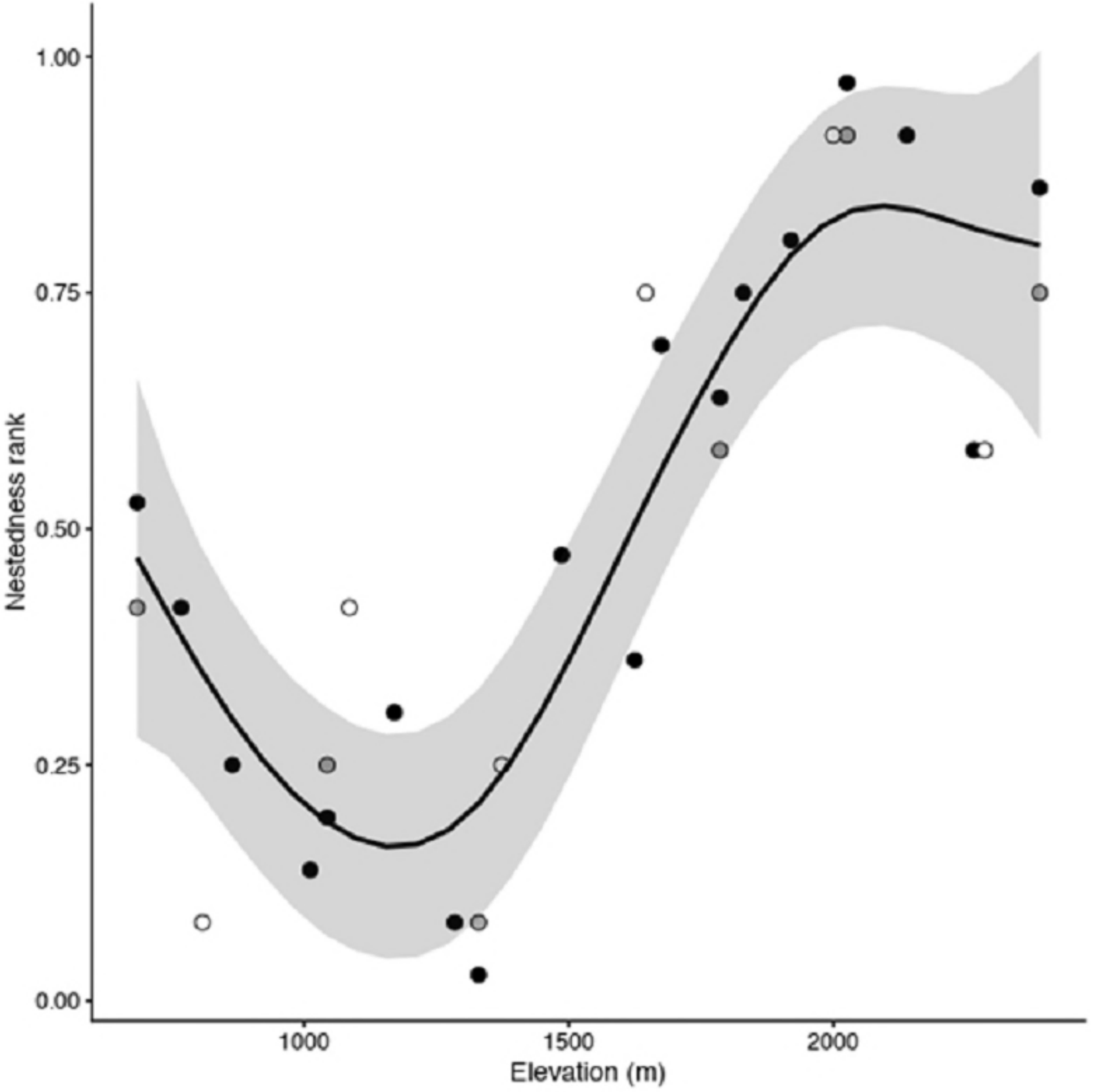
S3 Fig. Temporal validation of the relationship between nestedness and elevation. Generalized additive model (GAM) of nestedness ranks (based on nestedness temperature index) on elevation in ant assemblages from Guadarrama range (central Spain) fit to Guadarrama 2014 data and validated with Guadarrama 2015 data.

S4 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.

S5 Table. Multiple-site dissimilarities of beta diversity. Multiple-site dissimilarities accounting for the spatial turnover (β_{SIM}) and the nestedness components (β_{NES}) of beta diversity, and sum of both values (β_{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.


20
15
10
5
0
Species richness
1000
1500
2000
Elevation (m)


Formica exsecta
Lasius flavus
Crematogaster auberti
Messor barbarus
Myrmica xavieri
Formica fusca
Tapinoma madeirense
Formica rufibarbis
Formica pratensis
800
1000
1200
1400
1600
1800
NMDS2
NMDS1
0.5
0.0
-0.5
-1.0
-1.0
-0.5
0.0
0.5
1.0


1.00
0.75
0.50
0.25
0.00
Nestedness rank
1000
1500
2000
Elevation (m)