Impact of habitat loss on the diversity and structure of ecological networks between endosymbiont nematodes and spur-thighed tortoises (*Testudo graeca* L.)

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Habitat loss and fragmentation are recognized as affecting the nature of biotic interactions, although we still know little about such changes for reptilian herbivores and their hindgut nematodes, in which endosymbiont interactions could range from mutualistic to commensal and parasitic. We investigated the cost and benefit of endosymbiont interactions between the spur-thighed tortoise (*Testudo graeca* L.) and nematodes in scrublands of southern Spain. For this, we assessed the effect of nematode species richness and abundance on tortoises´ growth rates across levels of habitat loss (low, intermediate and high). Furthermore, by using ecological networks, we evaluated the effect of habitat loss on the diversity and structure of interactions. Nematode richness and abundance showed contrasting relationships with growth rates across levels of habitat loss. At low habitat loss, nematode infestation was positively associated with growth rates (suggesting a mutualistic nematode-tortoise relationship), but the association became negative at high habitat loss (suggesting a parasitic relationship). In addition no relationship was observed when habitat loss was intermediate (suggesting a commensal relationship). The network analysis showed that the nematode community was not randomly assembled but significantly nested, revealing a structured pattern for all levels of habitat loss. The diversity of interactions was lowest at low habitat loss, but the intermediate level showed the greatest specialization, which indicates that individuals were infested by less abundant nematodes in this landscape, whereas at high habitat loss individuals were the most generalized hosts. Related to the latter, connectance was greater at high habitat loss, reflecting a more uniform spread of interactions among nematode species. We conclude that habitat loss affects the complex interactions between tortoises and their nematode endosymbiont species with negative, neutral and positive consequences for tortoise growth rates. Ecological network analysis can help in the understanding of the nature of such changes in tortoise-nematode interactions by showing how generalized or specialized such interactions are under different environmental conditions and how vulnerable endosymbiont interactions might be to further habitat loss.

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ABSTRACT

Habitat loss and fragmentation are recognized as affecting the nature of biotic interactions, although we still know little about such changes for reptilian herbivores and their hindgut nematodes, in which endosymbiont interactions could range from mutualistic to commensal and parasitic. We investigated the cost and benefit of endosymbiont interactions between the spur-thighed tortoise (*Testudo graeca* L.) and nematodes in scrublands of southern Spain. For this, we assessed the effect of nematode species richness and abundance on tortoises´ growth rates across levels of habitat loss (low, intermediate and high). Furthermore, by using ecological networks, we evaluated the effect of habitat loss on the diversity and structure of interactions. Nematode richness and abundance showed contrasting relationships with growth rates across levels of habitat loss. At low habitat loss, nematode infestation was positively associated with growth rates (suggesting a mutualistic nematode-tortoise relationship), but the association became negative at high habitat loss (suggesting a parasitic relationship). In addition no relationship was observed when habitat loss was intermediate (suggesting a commensal relationship). The network analysis showed that the nematode community was not randomly assembled but significantly nested, revealing a structured pattern for all levels of habitat loss. The diversity of interactions was lowest at low habitat loss, but the intermediate level showed the greatest specialization, which indicates that individuals were infested by less abundant nematodes in this landscape, whereas at high habitat loss individuals were the most generalized hosts. Related to the latter, connectance was greater at high habitat loss, reflecting a more uniform spread of interactions among nematode species. We conclude that habitat loss affects the complex interactions between tortoises and their nematode endosymbiont species with negative, neutral and positive consequences for tortoise growth rates. Ecological network analysis can help in the

understanding of the nature of such changes in tortoise-nematode interactions by showing how generalized or specialized such interactions are under different environmental conditions and how vulnerable endosymbiont interactions might be to further habitat loss.

Key words: Antagonism, biotic interactions, commensalism, habitat degradation, mutualism, parasitism.

Introduction

As a consequence of diverse human activities, habitat loss and fragmentation in terrestrial ecosystems have been considered as the main threats to the maintenance of biodiversity worldwide (Brooks et al., 2002; Faharig, 2003). This is because native species are susceptible to habitat degradation through local extinctions and the reduction in the size of their populations. One aspect of land use change that could have an important impact on wild populations and biodiversity is the local extinction of native species caused by changes in the nature of biotic interactions, including both mutualistic (e.g., pollination and seed dispersal) and antagonistic (e.g., pests and pathogens) interactions (Harvell et al., 2002; Patz et al., 2004; Aguilar et al., 2006; Aguirre & Tabor, 2008; Trumbore et al., 2015; Pringle, 2016). The ecological costs of these changes constitute the loss of species and populations and ecosystem degradation (Harvell et al., 2002; Rizzo et al., 2002; Patz et al., 2004; Aguilar et al., 2006; Aguirre & Tabor, 2008; Vurro et al., 2010; Trumbore et al., 2015; Pringle, 2016). There are other biotic interactions, however, that have been little explored in the context of habitat loss, such as commensal interactions in which one species benefits whilst the other receives neither benefit nor damage. In some reptiles, for instance, the symbiotic relationships with hindgut nematodes (endosymbionts) have been categorized as commensal, mutualistic and/or parasitic (O´Grady et al., 2005; O´Grady & Dearing, 2006). In fact, for herbivorous reptiles, hindgut nematodes might be parasitic but might also play a positive role in the digestion and assimilation of plant matter (O´Grady & Dearing, 2006).

Habitat loss and fragmentation influence resource availability and also the distribution, abundance and behaviour of wildlife species and could therefore change the ecology of parasite and/or endosymbiont transmission (Kareiva, 1987; Patz et al., 2004; Aguirre & Tabor, 2008; Fuentes-Montemayor et al., 2009; Suzán et al., 2012; Benítez-Malvido et al., 2016; Pringle, 2016). Furthermore, there is evidence that habitat loss and fragmentation reduce individual fitness for several animal species through changes in individual body size, as well as decreased growth and reproduction, for example (Fuentes-Montemayor et al., 2009). In disturbed habitats, less vigorous and stressed individuals are more vulnerable to parasite infection, whereas an infected individual is more prone to be infected by secondary pathogen species concurrently (Beldomenico & Begon, 2009).

In this study, we used the nematodes present in the hindguts of spur-thighed tortoises, *Testudo graeca* L. (F. Testudinidae) in scrublands of southeastern Spain as a research model to determine whether the level of habitat loss (low, intermediate and high) is associated with the incidence of nematodes and individual body traits as indicated by growth rates. The available information has shown that the abundance of *T. graeca* within its original distribution range is strongly associated to environmental factors, such as rainfall and temperature, because as ectotherms, terrestrial tortoises tend to react strongly to environmental factors (Kaspari & Valone, 2002; Anadón et al., 2006, 2012 a, b). Furthermore, such factors have shown strong shifts in fragmented landscapes when compared to continuous natural habitats in different ecosystems elsewhere (Anadón et al., 2006, 2012 a, b; Fuentes-Montemayor et al., 2009; Laurance et al., 2017).

Ecological networks are increasingly being used as a valuable tool to study the complexity of biotic interactions at a community level, including both mutualistic and antagonistic interactions (Bascompte et al., 2003; Bascompte & Jordano, 2007; Benítez-Malvido & Dáttilo, 2015; Zarazúa-Carbajal et al., 2016; Hernández-Martínez et al., 2018). Within a given community, different organisms and animal species can interact with each other generating complex ecological networks (Bascompte & Jordano, 2007). A large number of studies have found no-random structures in ecological networks, including nestedness (Bascompte et al., 2003; Bascompte & Jordano, 2007). A network is considered nested if species with fewer interactions (specialists) are connected with species with the most interactions (generalists) in cohesive subgroups (i.e., more generalized networks) (Guimarães et al., 2006; Bascompte & Stouffer, 2009). Despite the importance and increased knowledge of ecological networks in the literature, most studies have focused on mutualistic interactions (e.g., pollination and seed dispersal; Bascompte & Jordano, 2013), whereas antagonistic and commensal interactions involving animals and their pathogens and endosymbionts have received less attention (however, see Kareiva, 1987; Benítez-Malvido & Dáttilo, 2015; Zarazúa et al., 2016; Hernández-Martínez et al., 2018).

The southeastern Iberian Peninsula, as well as other Mediterranean peninsulas, has been an important speciation hot spot and refuge for the survival of several plant and animal species (Hewitt, 2011). Nevertheless, these peninsulas have historically been subjected to different human pressures for centuries so that several native species are greatly threatened at present because of increased conversion of natural habitats to agricultural and other land uses (Sánchez-

Zapata & Calvo, 1999; Anadón et al., 2006, 2012 a, b; Hewitt, 2011; Rodríguez-Caro et al., 2017). The general aim of this study was the following: (1) to assess if the level of habitat loss alters the local abundance, species composition, richness, and the diversity and structure of ecological networks of nematodes associated with *T. graeca*, as well as (2) to determine the interaction type between tortoises and nematodes (i.e., mutualistic, commensal and/or parasitic) as indicated by the tortoises´ growth rates. We expected that the growth rates, nematode communities and host-nematode interactions to diverge with the level of habitat loss, with landscapes of high habitat loss showing the greatest divergence (Patz et al., 2004; Qian & Ricklefs, 2006; Fuentes-Montemayor et al., 2009; Suzán et al., 2012). It is clear that habitat degradation affects the nature of biotic interactions and ecosystems´ function; for herbivore reptiles, however, there is a limited understanding of the role that hindgut nematodes have on them. Furthermore, conventional community descriptors have not always been successful in distinguishing among habitats with different levels of degradation, which indicates that alterations of the structure and function of ecological communities might be unnoticed in conservation research that does not document and quantify species interactions through an ecological network approach (Kareiva, 1987; Tylianakis et al., 2007; Kaiser-Bunbury & Blüthgen, 2015).

Material & Methods

Study area

The study region covering the southeastern *T. graeca* Spanish population comprises approximately 2600 km^2 of semiarid coastal mountains between the Almeria and Murcia provinces (longitude N38° 23 to N36° 20´and latitude W0° 30 to W2° 20´; Fig. 1). The climate of the region is semiarid with mild winters, hot summers and low rainfall (200 to 350 mm/year) during the cooler months of the year. This region has been subjected to human intervention for centuries (Hewitt, 2011). Land-use practices are among the most important factors, together with climate, relief and lithology, for determining *T. graeca* presence within its distribution range in southeastern Spain (Anadón et al., 2006). Such practices include traditional agricultural crops (dry crops known as secanos), heavily intensified irrigated agricultural lands, and highways (Anadón et al., 2006). Intensified agricultural practices and fires, as well as habitat reforestation with pines, have been shown to negatively affect *T. graeca* growth and survival (Anadón et al., 2006; Sanz-Aguilar et al., 2011; Rodríguez-Caro et al., 2013, 2017). Furthermore, in this mosaic of land use practices, tortoise populations require scrubland patches larger than ≥ 1 km^2 or 75 % scrubland cover at the landscape level to remain viable and prevent local extinctions (Anadón et al., 2006). The study sites were located in landscapes with different levels of land use intensification, habitat loss and fragmentation. Nevertheless, traditional agricultural crops (secanos), when not extensive, are recognized as suitable habitats for *T. graeca* populations, and therefore the level of habitat loss was established according to the proportion of suitable habitat for tortoises in the landscape. In this sense, the landscapes with low habitat loss (sampling sites in Villaltas, Galera and Marinica) comprised on average 95 % of suitable habitat, including 84.59 % scrubland and 10.75 % of traditional agriculture. Landscapes with intermediate habitat loss (Bas Norte, Sierrecica and Chinas) comprised 81.60 % of suitable habitat, including 62.21 % scrubland and 19.39 % traditional agriculture. Finally, landscapes with high habitat loss (Palas, Bas Sur and Misiripalme) encompassed 76.26 % of suitable habitat, including 40.71 % scrubland and 35.54 % of traditional agriculture (Fig. 1).

The spur-thighed tortoises and the prevalence of nematodes

The Dirección General de Gestión del Medio Natural de la Junta de Andalucía (SGB/FOA/AFR) and the Delegación General de Medio Natural de la Comunidad Autónoma de la Región de Murcia (AUT/ET/UND/48/2010) granted the permission to sample the tortoises. The spur-thighed tortoise (*T. graeca)* is a midsized (up to 30 cm in length) terrestrial tortoise and one of the most widely distributed species of terrestrial tortoises. Although most of its distribution area is principally North Africa, the Middle East and part of Eastern Europe, there are some small isolated populations in Western Europe (Graciá et al., 2017; Fig. 1). This tortoise species is typically present in arid and semiarid scrublands. The diet of *T. graeca* is based on several parts (i.e., leaves, flowers, fruits and twigs) of wild plants such as alfalfa (*Medicago sativa*), thistles (e.g., *Carthamus* and *Carlina*), dandelion (*Taraxacum*), rosemary (*Rosmarinus officinalis*), etc. (Andreu et al., 2000; El Mouden et al., 2006). Furthermore, tortoises may disperse the seeds of the consumed plant species (Cobo & Andreu, 1988; Andreu et al., 2000). Tortoises' diet also includes animal carcasses, slugs and insects, mainly at early life stages. Currently, wild populations of *T. graeca* are severely threatened throughout their whole distribution range because of habitat loss and fragmentation as well as over-collection, principally in southeastern Spain (Anadón et al., 2006, 2012 a, b; Pérez et al., 2012).

From mid March to mid June of 2010, at nine different localities (three for each level of habitat loss), the wild tortoises sighted were captured within standardized transects. Each of the nine sites was visited five times; the sampling time lasted for approximately 2 hours 30 minutes along ca. 1750 m individual transects. After sampling, specimens were marked, and the following individual traits were recorded without harming the tortoises: sex, age estimated from the carapace growth ring (Rodríguez-Caro et al., 2015), body weight (g) and carapace length (mm). Thereafter, a growth rate score per individual was calculated (Rodríguez-Caro et al., 2013). Growth rate scores (mm year^{-1}, k) were calculated using recapture data analysed through the von Bertalanffy model, the most used to describe chelonians´ growth (Zivkov et al., 2007; Macale et al., 2009). For further details, see Rodríguez-Caro et al. (2013).

For the study of nematode communities, individual freshly voided faecal samples were obtained immediately following the handling of the animal or from the cloth bag where animals were placed until they defecated, for details on nematode sampling see Chávarri et al. (2012). Thereafter, individuals were released in their capture sites. We sampled a total of 66 wild tortoises from areas with low (26 individuals), intermediate (20 individuals), and high (20 individuals) habitat loss. In landscapes with low habitat loss, we recorded the following: 14 adult females, seven adult males, four subadults and two juveniles. In landscapes with intermediate habitat loss, we recorded: three adult females, six adult males, seven subadults and three juveniles. Finally, for the landscapes with high habitat loss we recorded: nine adult females, four adult males, five subadults and two juveniles. Adults were considered as those individuals > 8 years old, and subadults were those individuals 5-8 years old. In addition juveniles were all those tortoises 1-4 years old (Sanz-Aguilar et al., 2011).

Details on parasite isolation and identification can be found in Chávarri et al. (2012). Previous coprological analysis in these tortoise populations revealed the presence of oxyurid and ascarid nematodes (adults, larvae and eggs). Overall, 14 adult nematode species (Pharyngodonidae) have been isolated in wild populations of *T. graeca*, including the following: *Tachygonetria dentata, T. longicollis, T. macrolaimus T. conica, T. pusilla, T. numidica, T. robusta, T. setosa, T. palearticus, T. seurati, Alaeuris numídica, Mehdiella stylosa, M. uncinata* and *M. microstoma*. The number of Ascarididae and Oxyuridae eggs and the number of nematode larvae present in faeces were not considered in this study because the lack of taxonomical identification at these immature stages. Previous studies have shown that for captive *T. graeca* individuals, ascarid infections are associated with carapace deformities and symptoms of upper respiratory tract disease, and that oxyurid infections and prevalence increase with age; by contrast, for wild populations, the prevalence of nematodes has shown no association with negative health symptoms so far (Chávarri et al., 2012). The role of nematodes as a limiting factor of tortoise populations is not well understood. Nematodes are the main helminths infecting terrestrial chelonians, and most of them belong to the orders Oxyurida and Ascaridida, which are transmitted by the orofaecal route and sexual contact (Chávarri et al., 2012). There are few reports of tortoise mortality associated with ascarid infestations (Rideout et al., 1987); oxyurids, on the other hand, can be highly prevalent and are considered to have an almost commensal relationship with their hosts (Roca, 1999).

Statistical analysis

Because the levels of nematode infection and prevalence increase with age (Chávarri et al., 2012), we performed the statistical tests by pooling all sampled individuals and, thereafter, we concentrated on adult tortoises exclusively. Firstly, to test if the level of habitat loss affected tortoise body traits and nematode species number and abundance, we used a one-way ANOVA. Body weight and carapace length are strongly correlated to tortoise´s age (Rodríguez-Caro et al. 2013), whereas age classes were not homogeneously distributed across landscapes; therefore, weight and carapace length were not used further in the analyses related to the impact of nematode infestation on body traits across habitat types.

Secondly, to test if tortoise growth rates were related to nematode species richness and abundance across habitat loss categories (categorical predictor variable with three fixed factors), we compared the linear regression slopes among habitats by using one-way ANCOVAs, with tortoise growth rates as the dependent variable and nematode species richness and abundance as the independent variables (covariates). In total, we performed four one-way ANCOVAs, one for each of the two covariables (nematode species richness and abundance), again initially pooling all life stages and then for adults only. For all analyses, the number of nematode species and abundance were log (x + 1) transformed, as well as body traits values, except for growth rates. All statistical tests were performed with the program Statistica 7, and significance was set at the $\alpha = 0.05$ level.

Parasite species dominance

To test whether the level of habitat loss affected nematode species relative abundance and/or dominance, we constructed rank-abundance plots for the nematode communities present in tortoises at each of the scrubland landscapes (low, intermediate and high habitat loss). For each level of habitat loss, we plotted the relative abundance of each nematode species on a logarithmic scale against the species´ rank, ordered from the most to the least abundant species (Magurran 2004). To assess whether nematode species were evenly distributed (ecological evenness) across the levels of habitat loss, we compared the slopes of the rank/abundance plots by means of an ANCOVA. The species evenness of a given community is reflected in the slope of the line that fits the rank-abundance plot (i.e., logarithmic series relationship). A steep slope indicates low evenness since the high-ranking species have greater abundances than the low-ranking species. A gentle slope indicates higher evenness because species abundances are more similar (Magurran 2004).

Patterns of species interactions by using a network approach

Not all sampled tortoises presented adult nematodes in their faeces. Therefore, only individuals with nematodes were considered in the interaction network analysis, mainly adults and subadults. Firstly, we included the presence of different nematode species infecting individuals of *T. graeca* in each of the landscapes (low, intermediate and high habitat loss) as independent interaction networks. We thus constructed three networks, one for each of the habitat loss conditions. Each individual-based tortoise-nematode network was built by an adjacency matrix $\boldsymbol{A}$, where a_{ij} = number of interactions from an individual tortoise j by the nematode species i, or otherwise zero (i.e. absence of interaction). Thus in these networks, nematode species and tortoises are depicted as nodes, and their interactions are represented by links describing the use of tortoise individuals by nematode species.

Firstly, we tested if such networks were nested, a pattern in which nematode species with fewer interactions (specialists) are connected with tortoise individuals with the most interactions (generalists) in cohesive subgroups. We used the *NODF* metric (Almeida-Neto et al., 2008) to estimate the nestedness value of the networks by using the ANINHADO software (Guimarães & Guimarães, 2006). This metric ranges from zero (no nestedness) to 100 (perfect nestedness). The *NODF* metric reduces the chance of overestimating the degrees of nestedness in ecological networks and is less prone to Type I statistical error (Bascompte & Jordano, 2013). To assess if the nestedness value observed was different from that expected by random interaction patterns, we tested the nestedness of each network with 1000 networks generated by Null Model II. In this null model the probability of an occurrence of a new interaction is proportional to the number of interactions of a given species (Bascompte et al., 2003).

Secondly, we considered other network parameters in the analysis as follows: (i) connectance (the proportion of realized links of the total possible in each network, defined as the sum of links divided by the number of cells in the matrix); and (ii) interaction diversity (based on the Shannon diversity index) (*DI*) for each of the three networks. This metric is derived from the Shannon diversity index and ranges from 0 (no diversity) to infinity (Blüthgen et al., 2006). Finally, we considered (iii) specialization index or resource selectivity at the network level (H_2'). This selectivity index ranges from 0 (extreme generalization) to 1 (extreme specialization) and is highly robust regarding changes in sampling intensity and the number of interacting species (Blüthgen et al., 2006). Network features were estimated with the Bipartite package (Dormann, Gruber & Group, 2011). Network plots were obtained by using Bipartite in 'R' (Dormann, Gruber & Group, 2011; R Development Core Team, 2014).

Results

Overall, we found that the level of habitat loss affected some aspects of the complex tortoise-nematode interactions with consequences for tortoise growth rates. Not all sampled individuals, however, where infested with adult nematodes, but the majority of tortoises presented nematodes in their faeces (72 %). The frequency of nematode species varied across tortoises, varying between 1 to10 species, whereas nematode abundance per tortoise varied between 0 to 5245 individuals.

Habitat loss, body traits and prevalence of nematodes

On the one hand, the one-way ANOVAs showed that the level of habitat loss had no significant effect on tortoise body traits, nor on nematode richness and abundance, by pooling all life stages and comparing adults only (Table 1). On the other hand, when used as covariables, both the number of species and nematode abundance significantly affected growth rates according to the level of habitat loss, both by pooling all life stages and then focusing on adults. The ANCOVA tests showed a significant, positive effect of nematode species richness on growth rates, but only in landscapes with low habitat loss, as shown by the *habitat loss level x nematode species richness* interaction term (richness, $F_{2, 58} = 3.46$, $P = 0.038$) (Fig. 2 a). Considering adult tortoises only, contrasting effects of nematode infestation on growth rates across habitat types were detected. In landscapes with high habitat loss, tortoises sustained greater growth rates than those with low and intermediate habitat loss (richness, $F_{1, 2} = 6.39$, $P = 0.0042$; abundance, $F_{1, 2} = 4.42$, $P = 0.019$), whereas the *habitat loss level x nematode infestation* interaction term was significant for both species richness ($F_{2, 35} = 6.17$, $P = 0.0051$) and nematode abundance ($F_{2, 35} = 4.24$, $P = 0.022$) (Fig. 2 b). While growth rates were positively related to nematode infestation in landscapes with low habitat loss, the opposite was found in landscapes with high habitat loss; at intermediate habitat loss, no relationship existed between the two variables (Fig. 2 b).

Nematode species dominance in fragmented landscapes

The nematode community for the three levels of habitat loss followed a log series model of a small number of abundant species and a large proportion of rare species. The dominant nematodes infecting tortoises throughout the landscapes were *T. dentata* and *T. longicollis* (Fig. 3). The following patterns emerged in the rank-abundance plots: (1) all levels of habitat loss showed the same dominant nematode species; (2) although rare species changed ranks across the three levels of habitat loss, rare species were the same elsewhere; and (3) the landscapes with high habitat loss presented one exclusive species, *T. seurati*, whereas *T. palearticus* was absent from this landscape type (Fig. 4). The slope analysis in the ANCOVA showed that nematode relative abundance differed significantly across habitats with more rare species at low habitat loss ($F_{2, 33} = 28.28$, $P < 0.001$). Furthermore, the slope at low habitat loss is significantly steeper than the slopes at intermediate and high habitat loss, as shown by the *habitat loss level x species rank* interaction term ($F_{2, 33} = 13.93$, $P < 0.001$), indicating that nematode species are more evenly distributed in the landscapes with intermediate and high habitat loss than in the landscapes at low habitat loss (Fig. 3).

Network structure

The size of our networks (hereafter referred to as tortoise-nematode networks) was similar in the number of nematode species among landscapes, suggesting that *T. graeca* maintains a stable community of nematodes at the three levels of habitat loss (Table 2 and Fig. 4). Network metrics showed the lowest value of diversity of interactions (*DI*) at low habitat loss, indicating lower community stability. In contrast, connectance was greater in the landscapes with high habitat loss, reflecting a more uniform spread of interactions among nematode species in the community (Table 2). Finally, the intermediate level of habitat loss showed the greatest specialization level (H_2'), indicating that individuals were infested by less abundant nematodes in these landscapes, whereas at high habitat loss individuals were the most generalized (higher functional redundancy), which agrees with the higher connectance in them. The three levels of habitat loss showed a significant nested pattern of interactions, though the landscapes with high habitat loss exhibited the greatest nestedness values (Table 2). Nevertheless, for the three levels of habitat loss, low (*NODF* = 54.04, $P < 0.0001$), intermediate (*NODF* = 62.80, $P < 0.0001$) and high (*NODF* = 64.43, $P < 0.0001$), nestedness values were lower than those expected by random interaction patterns, as is typical for antagonistic interactions (Table 2).

Discussion

Overall, the level of habitat loss was found to affect some aspects of the ecological interactions between the spur-thighed tortoises and their nematodes, with differential impacts on the hosts´ growth rates. These changes were evident in spite of the similar nematode communities in terms of species richness and composition across landscapes. In general, the impact of nematode infestation on growth rates was related to the level of habitat loss, ranging from positive to neutral, to negative, for landscapes encompassing low, intermediate and high habitat loss, respectively.

Habitat loss and the costs and benefits of nematode infestation

The modification of the original vegetation by anthropogenic activities produces strong shifts in the spatial configuration of the natural landscapes, as well as on their physical (e.g., rainfall and temperature) and biological environments (e.g., vegetation structure, species diversity and resources availability) (Patz et al., 2004; Qian & Ricklefs, 2006; Suzán et al., 2012). These environmental shifts may occur at multiple spatial and temporal scales, which affect complex species interactions, including host-nematode interactions of any kind (parasitic, commensal and/or mutualistic) in the case of herbivorous reptiles (Harvell et al., 2002; Tylianakis et al., 2007; Benítez-Malvido et al., 2016, 2018; Hernández-Martínez, et al., 2018).

Although our findings represent the standing incidence of nematodes in *T. graeca* tortoises (i.e. a single observation in time), we detected that changes in landscape configuration, exemplified by the amount of suitable habitat, influenced some aspects of host-nematode interactions as revealed by differences in nematode infestation levels, nematode species ecological evenness, tortoises´ growth rates and by differences in the networks´ structure. There is evidence showing that the most disturbed and homogenized landscapes (e.g., highly degraded landscapes) are more susceptible to pest and pathogen invasions because human activities facilitate the movement of exotic/invasive species to new areas and provide the conditions for their establishment. An additional reason is hosts might change their behavioural ecology (Patz et al., 2004; Qian & Ricklefs, 2006; Suzán et al., 2012). Therefore, the type of endosymbiont interaction (mutualistic, antagonistic and/or commensal) between tortoises and hindgut nematodes seems to depend on the environmental context, in which differences in resource availability across habitats could be driving the observed variations among them (Pringle, 2016).

Large nematode infestation negatively affected growth rates for adult tortoises in landscapes at high habitat loss, suggesting a more parasitic interaction (Fig. 3). Our results also showed, however, that high nematode species richness and abundance in tortoises is positively related to growth rates at low habitat loss, which suggests that nematode infestation could play a positive role in the digestion and thus assimilation of plant matter suggesting a mutualistic interaction (O´Grady et al., 2005; O´Grady & Dearing 2006). Finally, tortoises within landscapes at intermediate habitat loss showed no significant relationship between nematode infestation and body traits, suggesting a commensal interaction in which nematodes had no apparent influence on tortoises´ growth rates.

Nevertheless, overall growth rates were greater at high habitat loss for adult tortoises than at low habitat loss. In this context, some native animal species are able to persist in degraded lands through morphological, physiological and behavioural adjustments (Isabirye-Basuta & Lwanga, 2008). Terrestrial tortoises are ectotherms and therefore may strongly react to environmental changes (Kaspari & Valone, 2002; Anadón et al., 2006). It is possible that reduced scrubland vegetation cover (greater canopy openness) in landscapes with high habitat loss aid tortoises in more effectively regulating their body temperatures (Row & Blouin-Demers, 2006; Sato et al., 2014). In addition, in some reptile species, an adequate thermoregulatory behaviour has been found to optimize the digestive performance, which in turn can affect growth rates (Avery et al., 1993; Anguilletta et al., 2002). Further studies should consider other indicators of habitat loss on resource availability for tortoises and impact on their populations, such as survival and reproductive success.

There is evidence showing that any habitat fragment, regardless of its size, is likely to support long-term viable *T. graeca* populations (Hailey & Willemsem 2003). For the present study region, however, habitat loss and fragmentation have been found to negatively affect the persistence of tortoise populations, but also that the presence of scrubland habitat in the vegetation matrix enables *T. graeca* to persist in anthropogenic landscapes (Anadón et al., 2006). There are no base-line studies, however, that help us to precisely determine the overall impact of land-use change in *T. graeca* populations because the study region has been historically modified for centuries by anthropogenic activities of distinct magnitude and intensity (Hewitt 2011). Therefore, long-term studies on *T. graeca* population dynamics and nematode infestation dynamics and their consequences (in survival, growth rates and reproduction) are crucial for understanding habitat loss processes in population´s viability, as well as the wide-ranging role of nematode endosymbiotic interactions on tortoises.

Furthermore, it has been pointed out that long-term studies are crucial for understanding the impact of sporadic natural disturbances and/or catastrophic events (e.g., flooding, fire and drought) on disease emergence, virulence and transmission (e.g., infection cycles) and on overall host health (Baldemonico & Begon, 2009; Zarazúa-Carbajal et al., 2016; Hernández-Martínez et al., 2018). The latter might apply as well for nematode endosymbionts. This information is relevant for the management of wild *T. graeca* populations and cannot be obtained in short term studies, such as the present one. For instance, the information gathered in the present study was obtained during the spring months when resource availability is high (i.e., several plant species are blooming and fruiting). There is no information, however, on the prevalence and intensity of nematode infestation during seasons when food sources are scarce, nor between years, nor on the consequences for tortoise populations.

Ecological networks in the management of spur-thighed tortoise populations

The nematode community infesting *T. graeca* populations was not randomly assembled but nested, revealing a structured pattern for all levels of habitat loss. Tortoise–nematode network properties appear to be maintained in landscapes with contrasting levels of habitat loss, despite differences in species dominance as well as host number and quality (tortoises´ sex, age and size). Our study contributes to the understanding of the structure of ecological interactions in contrasting landscapes. Within the same host population, we can often find both more selective (those individuals colonized by a few nematode species) or more opportunistic (those individuals colonized by many nematode species) individuals. Some network distribution metrics, such as *DI* and H_2’, may be suitable indicators for detecting human-induced changes in some endosymbiotic interactions (e.g., mutualistic, commensal and antagonistic), including tortoise-nematode interactions (Kareiva, 1987; Tylianakis, et al., 2007; Kaiser-Bunbury & Blüthgen, 2015). We found that as habitat loss increases, host specificity decreases, in other words hosts are infested by a wider array of nematodes, making tortoises more vulnerable in an already resource-limited environment in the case the nematode infestation turns out to be parasitic. The greater nested pattern in landscapes with intermediate and high habitat loss may also imply that nematodes can be more easily transmitted through the entire network, something that would be less likely if the network had a more modular structure (i.e. consisting of very specialized host-nematode interactions).

Conclusions

We aimed to understand the ways habitat loss affected spur-thighed tortoise populations and to link habitat loss and tortoise-nematode interactions by using a network approach. Our findings indicate that the level of habitat loss affected growth rates and host-endosymbiont interactions; in this sense, this study is the first to provide the structure of such networks. Animals, at any stage of their life cycles, are subject to infestation by many different organisms that may modify, improve or interrupt their vital functions that affect their fitness (Patz et al., 2004; Aguirre & Tabor, 2008; Suzán, et al., 2012; Pringle, 2016). On the one hand, the rapid degradation of natural habitats might cause a large proportion of species and populations to be vulnerable to disease, and native and exotic pathogens could become a threat for both plant and animal communities (Anderson et al., 2004; Patz et al., 2004; Vurro et al., 2010; Benítez-Malvido et al., 2018). On the other hand, habitat degradation might also cause the loss of mutualistic and commensal interactions. Therefore, it is essential to understand the nature and dynamics of symbiotic interactions, the life histories of the animal hosts and the effects of the local environmental conditions on all of them.

Acknowledgements

We warmly thank J.M. Lobato for careful technical support and P. M. Mojica and M. Fernández for their help with sampling.

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Figure legends

Figure 1. The map shows the distribution of the spur-thighed tortoise (*Testudo graeca*) in the Mediterranean basin. The wild populations sampled for the present study and their localities are indicated with dots, where: Villaltas, Galera and Marinica, correspond to landscapes with low habitat loss for tortoises; Bas Norte, Sierrecica and Chinas, landscapes with intermediate habitat loss; and Palas, Bas Sur and Misiripalme, landscapes with high habitat loss. These wild Spanish populations of *T. graeca* cover approximately 2600 km^2 of semiarid coastal mountains between Almeria and Murcia provinces.

Figure 2. Best-fit linear regressions between nematode infestation (i.e., species richness and abundance) and tortoise´s (*Testudo graeca*) growth rates in scrubland landscapes, Southern Spain. Tortoises were present in landscapes sustaining different levels of habitat loss (low, intermediate and high), where: (a) refers to tortoise populations at all life stages, and (b) refers to adult tortoises only. Significant relationships are indicated by, $^*P < 0.01$ an/or $^{**}P < 0.001$.

Figure 3. Rank/abundance plots for the nematode community infesting tortoises (*Testudo graeca*) in scrubland landscapes with different levels of habitat loss (low, intermediate and high) in Southern Spain. For each landscape, the relative abundance of each nematode species is plotted on a logarithmic scale against the species' rank, from the most to the least abundant species. Nematode species: *Alaeuris numidica* (A_num), *Mehdiella stylosa* (M_styl), *M. uncinata* (M_unci), *M. microstoma* (M_micro), *Tachygonetria longicollis* (T_long), *T. dentata* (T_dent), *T. conica* (T_coni), *T. robusta* (T_rob), *T. macrolaimus* (T_mac), *T. numidica* (T_numi), *T. setosa* (T_set), *T. palearticus* (T_pale), *T. pusilla* (T_pusi), and *T. seurati* (T_seur).

Nematode species not found under all habitat loss conditions are in **bold**.

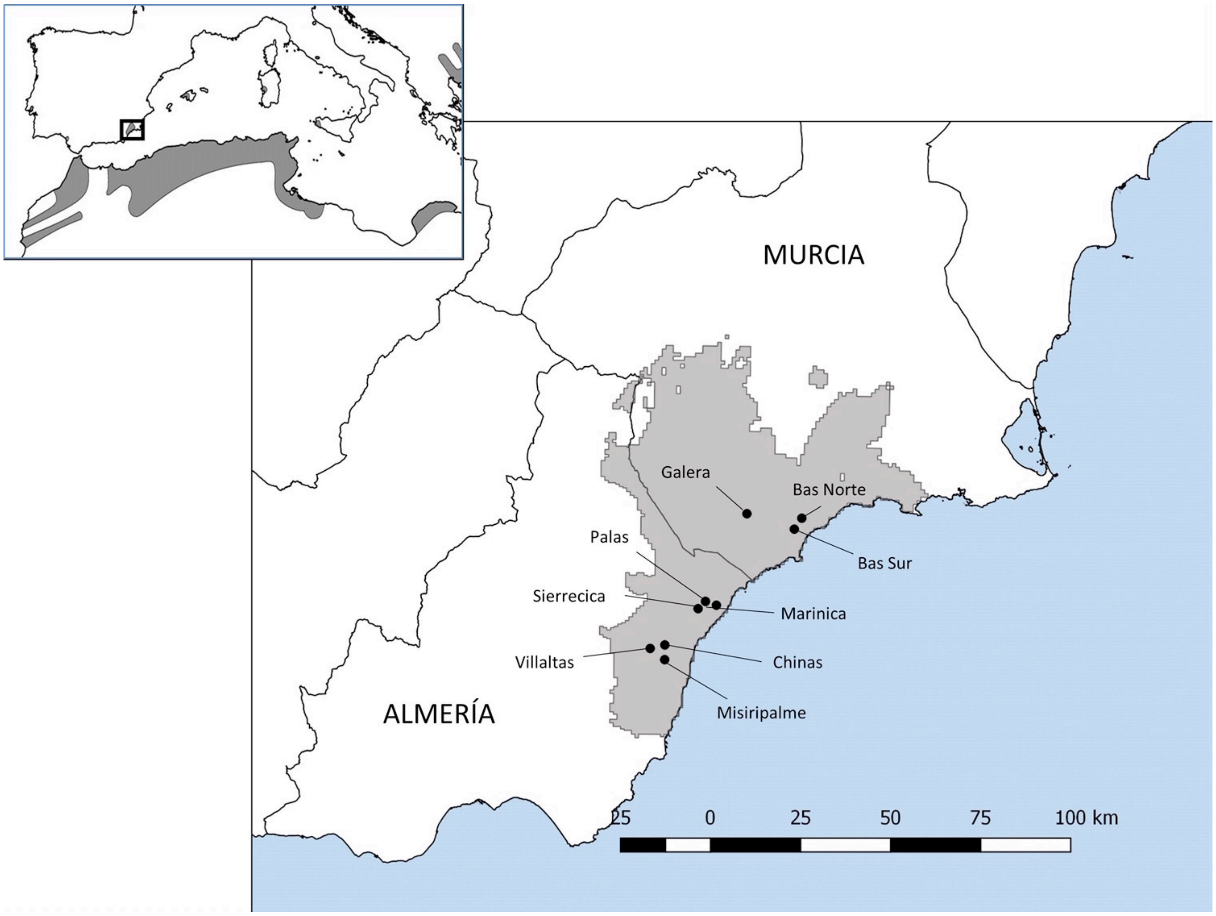
Fig. 4. Tortoise–nematode interaction networks including the interactions observed in landscapes encompassing different levels of habitat loss (low, intermediate and high) in Southern Spain. For each landscape, only tortoises (*Testudo graeca*) with nematode infestation were included in the networks. The bar size represents the number of interactions each nematode species (black) has with each individual tortoise in the population (grey), and the linkage width represents the proportion total number of nematode individuals involved in such interactions.

Figure 1(on next page)

Map of the study region

The map shows the distribution of the spur-thighed tortoise (*Testudo graeca*) in the Mediterranean basin. The wild populations sampled for the present study and their localities are indicated with dots, where: Villaltas, Galera and Marinica, correspond to landscapes with low habitat loss for tortoises; Bas Norte, Sierrecica and Chinas, landscapes with intermediate habitat loss; and Palas, Bas Sur and Misiripalme, landscapes with high habitat loss. These wild Spanish populations of *T. graeca* cover approximately 2600 km^2 of semiarid coastal mountains between Almeria and Murcia provinces.

Figure 1.


MURCIA
ALMERÍA
Galera
Bas Norte
Bas Sur
Palas
Sierrecica
Marinica
Villaltas
Chinas
Misiripalme
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25
50
75
100 km

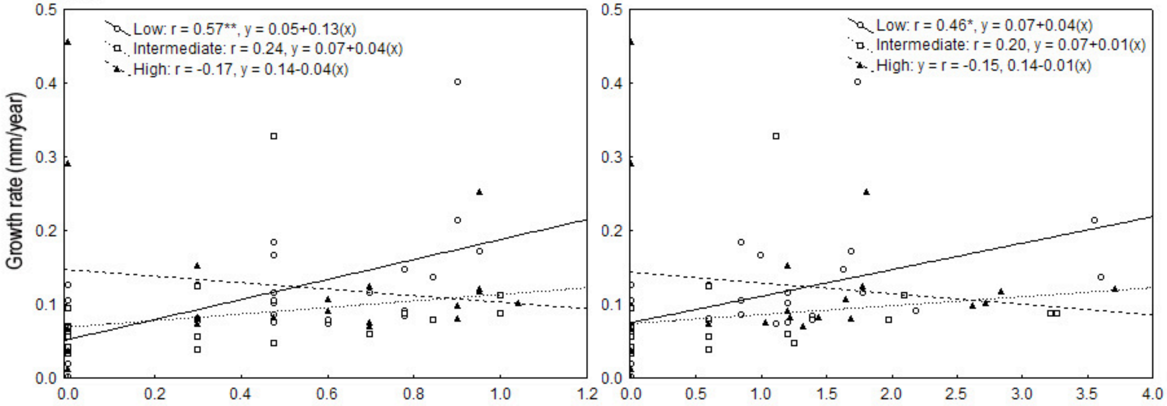
Figure 2(on next page)

Tortoises´growth rates and nematode infestation

Best-fit linear regressions between nematode infestation (i.e., species richness and abundance) and tortoise´s (*Testudo graeca*) growth rates in scrubland landscapes, Southern Spain. Tortoises were present in landscapes sustaining different levels of habitat loss (low, intermediate and high), where: (a) refers to tortoise populations at all life stages, and (b) refers to adult tortoises only. Significant relationships are indicated by, *P < 0.01 an/or **P < 0.001.

Figure 2.

(a) All tortoises



(b) Adult tortoises

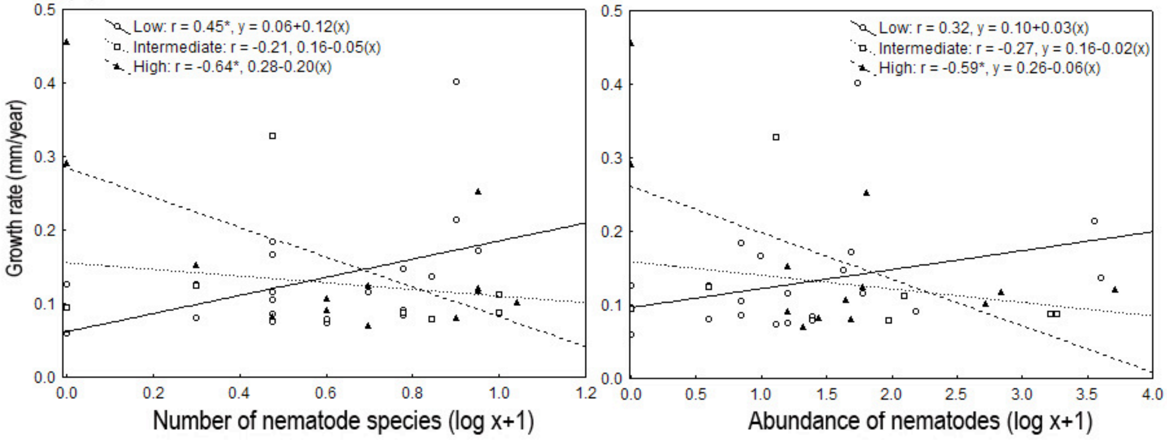
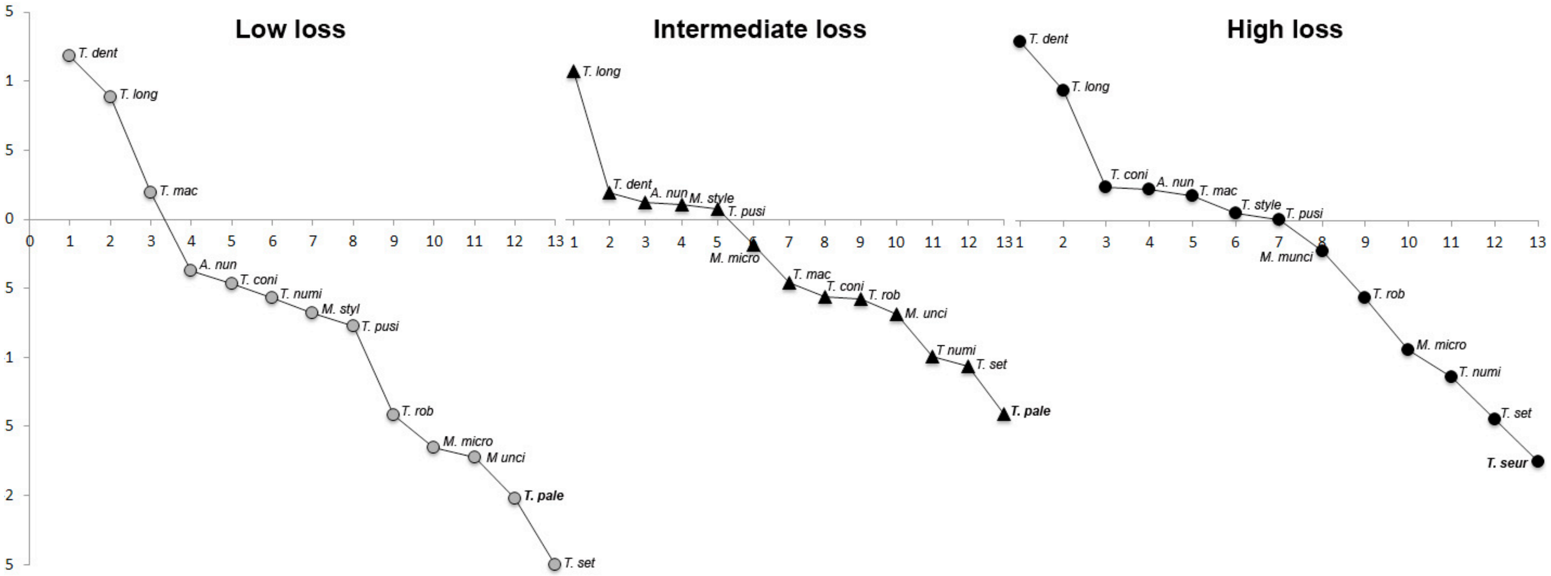


Figure 3(on next page)

Rank-abundance netamatode species curves

Rank-abundance plots for the nematode community infesting tortoises (*Testudo graeca*) in scrubland landscapes with different levels of habitat loss (low, intermediate and high) in Southern Spain. For each landscape, the relative abundance of each nematode species is plotted on a logarithmic scale against the species' rank, from the most to the least abundant species. Nematode species: *Alaeuris numidica* (A_num), *Mehdiella stylosa* (M_styl), *M. uncinata* (M_unci), *M. microstoma* (M_micro), *Tachygonetria longicollis* (T_long), *T. dentata* (T_dent), *T. conica* (T_coni), *T. robusta* (T_rob), *T. macrolaimus* (T_mac), *T. numidica* (T_numi), *T. setosa* (T_set), *T. palearticus* (T_pale), *T. pusilla* (T_pusi), and *T. seurati* (T_seur). Nematode species not found under all habitat loss conditions are in **bold**.

Figure 3.


Low loss
Intermediate loss
High loss
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A. nun
T. coni
T. numi
M. styl
T. pusi
T. rob
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T. set
1 2 3 4 5 6 7 8 9 10 11 12 13
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T. numi
T. set
T. seur

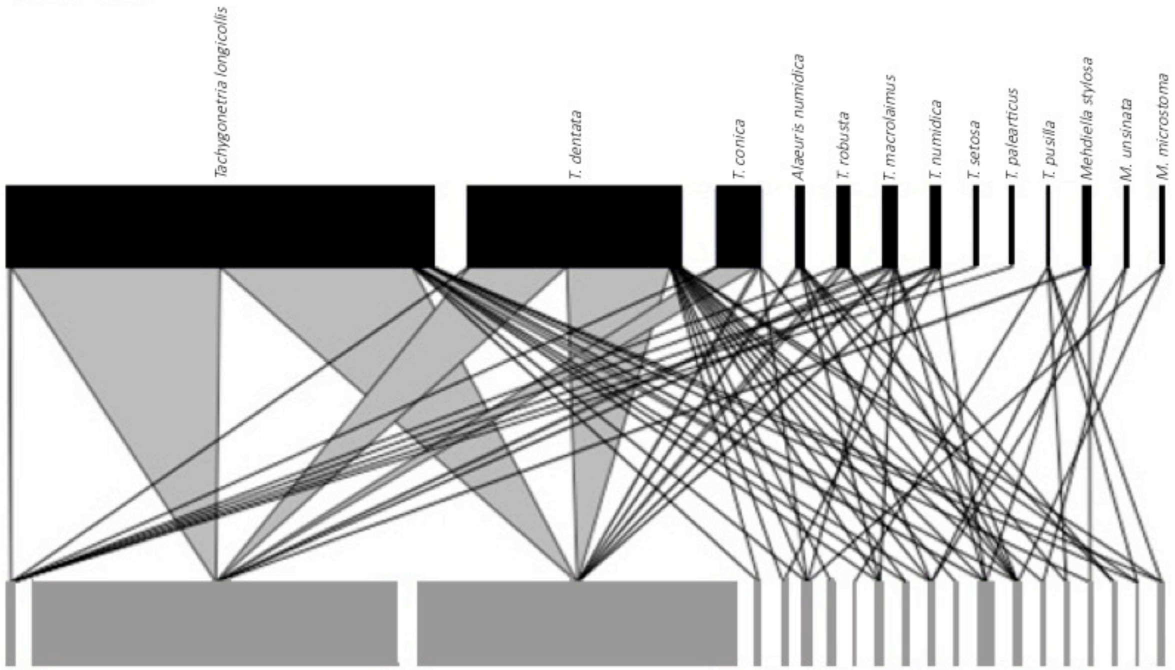
Figure 4(on next page)

Tortoise-nematode ecological networks

Tortoise–nematode interaction networks including the interactions observed in landscapes encompassing different levels of habitat loss (low, intermediate and high) in Southern Spain. For each landscape, only tortoises (*Testudo graeca*) with nematode infestation were included in the networks. The bar size represents the number of interactions each nematode species (black) has with each individual tortoise in the population (grey), and the linkage width represents the proportion total number of nematode individuals involved in such interactions.

Figure 4.

Low loss



Intermediate loss

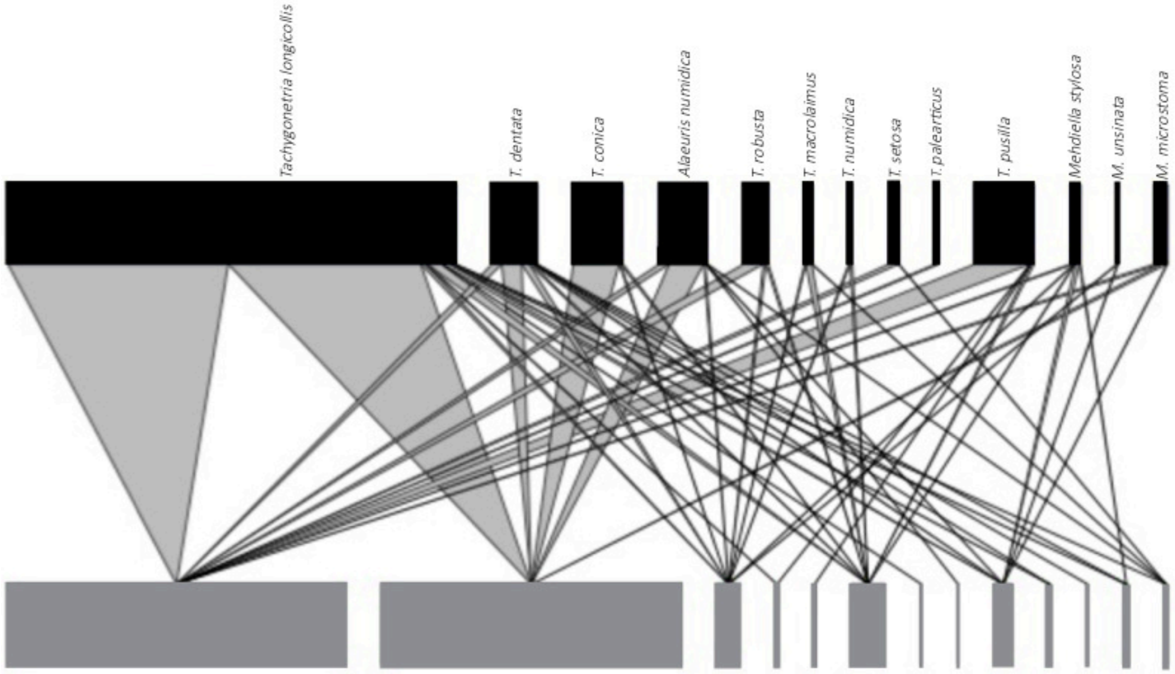


Figure 4. continued

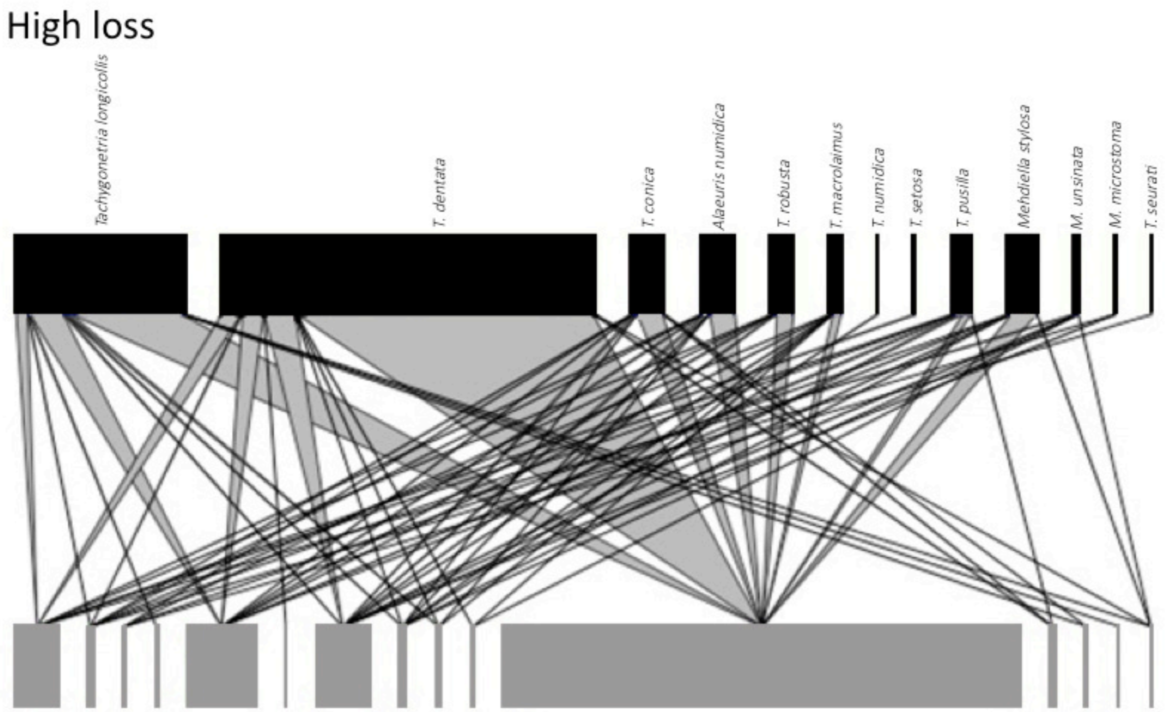


Table 1(on next page)

Body traits and nematode infestation of tortoise populations (*Testudo graeca*) at scrubland landscapes with different levels oh habitat loss, Southern Spain.

Body traits (mean ± SE) and nematode infestation of spur-thighed tortoise populations (*Testudo graeca*) at scrubland landscapes with different levels oh habitat loss, Southern Spain. The values in **bold** are those exclusively from adult tortoises. There were no significant differences among levels of habitat loss on any variable (one-way ANOVA, Tukey HSD test, $P > 0.05$).

Table 1. Body traits (mean ± SE) and nematode infestation of spur-thighed tortoise populations (*Testudo graeca*) at scrubland landscapes with different levels oh habitat loss, Southern Spain. The values in **bold** are those exclusively from adult tortoises. There were no significant differences among levels of habitat loss on any variable (one-way ANOVA, Tukey HSD test, *P* > 0.05).

Body traits and nematode infestation	Low (N = 26) (N = **21**)	Intermediate (N = 20) (N = **9**)	High (N =20) (N = **13**)
Weight (g)	431.79 ± 83.04	267.36 ± 59.41	410.10 ± 91.13
	527.00 ± 47.90	**486.00 ± 75.78**	**534.91 ± 59.95**
Carapace length (mm)	129.95 ± 6.86	123.44 ± 7.45	132.00 ± 7.84
	131.12 ± 4.01	**127.10 ± 6.14**	**128.45 ± 5.45**
Growth rate (mm year^{-1}	0.115 ± 0.016	0.085 ± 0.019	0.124 ± 0.018
	0.131 ± 0.0149	**0.123 ± 0.019**	**0.157 ± 0.025**
No. of nematode species	2.59 ± 0.567	2.60 ± 0.66	3.55 ± 0.66
	3.82 ± 0.54	**5.57 ± 1.31**	**5.27 ± 0.90**
Density of nematodes	303.63 ± 184.20	200.05 ± 214.01	360.30 ± 214.01
	480.82 ± 308.32	**561.00 ± 311.05**	**614.27 ± 468.36**

Table 2(on next page)

Tortoise-nematode ecological network attributes.

Tortoise-nematode ecological network attributes at different levels of habitat loss in scrubland landscapes of Southern Spain. Only individuals infested with nematodes were included.

Table 2. Tortoise-nematode ecological network attributes at different levels of habitat loss in scrubland landscapes of Southern Spain. Only individuals infested with nematodes were included.

Network metrics	Low	Intermediate	High
No. of tortoises	20	13	15
No. of nematode species	13	13	13
Nestedness (*NODF*)	37.23	45.90	43.696
Connectance (*C*)	0.269	0.308	0.364
Shannon diversity (*DI*)	2.034	2.371	2.397
Specialization index (H_2’)	0.125	0.335	0.141