



Concordance between plant species, oribatid mites and soil in a Mediterranean stone pine forest

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(Article begins on next page)

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- 19 Abstract
- 20 Biological interactions between above-ground and below-ground organisms are not clearly defined among communities
- 21 with regard to compositional patterns. The study investigates the concordance of species assemblages between vascular
- 22 plants and oribatid mites and soil chemical properties with special attention to the role of vegetation structure, i.e. tree,
- 23 shrub and herbaceous cover, for biological components. Data was collected in a Mediterranean coastal Nature Reserve
- using sampling design based on random selection of plots with cover of stone pine (Pinus pinea L.) exceeding 15%.
- 25 Agreement of distribution patterns was verified by Spearman's rank correlation coefficient applied to pairs of matrices
- 26 of plot scores by Principal Component Analysis (plants, mites and soil) and the Mantel test. The feasible role of
- 27 vegetation cover on plant and mite assemblages was tested by Redundancy analysis (RDA). Significant correlations
- were found for biological assemblages, indicating congruent plant mite compositional patterns. On the other hand, the
- 29 hypothesis of concordance between biological communities and soil was rejected. Moreover, RDA showed that

vegetation cover was a driver of both plant and oribatid mite assemblages. In particular, herbaceous cover proved to be a good proxy for the two biological communities investigated, with different taxa linked to forest clearings and to areas with denser tree cover. Our results indicate that soil features were not of primary importance for below-ground and above-ground community assemblages in the study area. In the light of our findings and on-going threats in coastal areas, we recommend that management measures be directed at maintenance of diversified vegetation structure which may ensure above-ground and below-ground biodiversity with diverse biological community assemblages.

Keywords: coastal areas; community composition; congruence; oribatid mites; Pinus pinea; vegetation structure.

Introduction

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Understanding of biotic interactions between above-ground and below-ground soil communities is an important target (Hooper et al. 2000), since ecologists regard species interactions as major processes controlling species composition and their richness. The surge of interest in soil biological diversity springs largely from recognition that organisms sharing this physical environment regulate major ecosystem processes and from the likelihood that feedbacks between aboveground and below-ground communities have a key role in governing ecosystem function (Wardle 2002). Plants are the key component for life: they entertain a series of exchanges with the surrounding world, including soil fauna, and influence below-ground environmental conditions by driving changes in litter state, pH and moisture (Wardle et al. 2005; Bezemer et al. 2006). Among micro-arthropods in the soil system, oribatid mites (Arachnida) in the organic horizons are one of the richest groups. They are actively involved in decomposition of organic matter, circulation of nutrients, and formation and maintenance of soil structure (Bardgett and Wardle 2010). Their abundance, species distribution and community structure depend on their reactions to biotic and abiotic environmental conditions, which show spatial variation with latitude and also at fine-scale level within sites, although deterministic and stochastic processes may both play a role (Lindo and Winchester 2009; Nielsen et al. 2012; Minor, 2011; Caruso et al. 2012). They are sensitive to habitat evolution and to several types of disturbance, including pollution, land use, management and fire (Henig-Sever et al. 2001; Lindo and Visser 2004; Migliorini et al. 2005a, b). The intricate relationships between these edaphic invertebrates and their ecological niches in the soil matrix, the fact that many of them live a rather sedentary life, and the stability of community composition at a specific site (Maaß et al. 2014) provide good starting points for bioindication of changes in soil properties (Behan-Pellettier 1999; Feketeová et al. 2015; Tsiafouli et al. 2015).

Concordance among different taxonomic groups can derive from similar but independent responses by these groups to the main environmental gradients (Paavola et al. 2003). Evidence of cross-taxon concordance across spatial and ecological gradients is increasing and it can form a basis for reliable use of surrogates to monitor trends in biodiversity (Rooney and Azeria 2015). Many studies have dealt with congruence, analysing animals, lower plants or fungi interacting with vascular plants (e.g. Chiarucci et al. 2007; Maccherini et al. 2009; Landi et al. 2015), while others relate above-and below-ground communities (e.g. Mitchell et al. 2010; Santi et al. 2010; Rooney and Azeria 2015). Patterns of oribatid mite diversity have been studied in pine plantations composed of *Pinus radiata* (Minor, 2011), whereas to our knowledge congruence between plants, oribatid mites and soil parameters has never been analysed in a stone pine forest. Moreover, in wooded areas, it is generally assumed that vegetation cover can be used as a proxy for light, a major environmental variable for plant communities (González-Alday et al. 2009) and soil organisms (Rutigliano et al.

67 2004; Iovieno et al. 2010).

Considering the strong interactions of vascular plant and oribatid mite communities with the soil system, we determined to verify: i) the hypothesis of concordance between biological communities (vascular plants and oribatid mites) and the chemical features of soil; ii) the role of vegetation cover as a driver of vascular plant and oribatid mite communities in a wooded protected area in the Mediterranean basin. The main aim of our research was to gain insights into interactions between the two biotic communities and soil features to use as a basis for forest management in protected areas.

Materials and methods

74 Study area

The study area was the "Nature Reserve of Duna Feniglia" on the Tyrrhenian coast of southern Tuscany (Italy) (UTM 32T 684506.73 E; 4699193.80 N). The Feniglia Dune was declared a protected area with State Nature Reserve status in 1971, and acquired Special Protection Area status in 1979 to protect the lagoon of Orbetello. The protected area is a sandy strip (maximum altitude 14 m) connecting the promontory of Mt Argentario with the coast. It is largely vegetated with stone pines (*Pinus pinea*) managed for conservation and not subject to cutting. The area experienced human-induced degradation (between 1700 and 1900) and subsequent rehabilitation, including dune consolidation and sowing of stone pines in the early 1900s (see Landi et al. 2012; Angiolini et al. 2013).

Stone pine stands have been a frequent planting choice in the Mediterranean area over the last century, characterizing large stretches of coast in the western part of Italy and becoming a widespread semi-natural ecosystem. These areas are currently considered a priority habitat for conservation in Europe under the Habitats Directive 92/43/CEE (European Commission 2003). Although the autochthony of this species in Italy is still unclear, these forests, traditionally cultivated for timber and pine nuts, have the important function of stabilizing sand dunes and defending back-dune crops from sea spray (Antonellini and Mollema 2010; Cutini et al. 2015).

The geology of the area features quaternary deposits of carbonate-rich marine sandy and sandy-silty sediments forming coastal and inner dunes. According to Barazzuoli et al. (1993), climate is sub-arid Mediterranean with mild winters and very hot dry summers. The mean annual precipitation is approximately 677 mm with a Mediterranean rainfall regime (Regione Toscana 2014).

Field sampling design and laboratory procedures

The study focused on wooded dunes with *Pinus pinea* belonging to priority habitat 2270*. The area of Feniglia Dune Nature Reserve with stone pine forest was selected from ortho-photographs (scale 1:5000), interpreted visually on a video screen with the aid of Geographic Information System. Twenty plots (10 × 10 m) in the inner dunes were randomly allocated and sampled. When tree cover proved to be less than 15%, another plot was chosen. The study did not involve any experimental manipulations or disturbance of naturally developed relationships. During field surveys (conducted in spring), all vascular plant species occurring in each plot were recorded and their individual covers estimated (vertical projection of plants) as percentages between 0 and 100. A field estimate of cover was also recorded for each layer (tree, shrub and grass layer). Plant species nomenclature was according to Conti et al. (2005).

At the same time as the vegetation survey, litter was removed at the centre of each plot in order to sample the surface layer of the soil profile (top 20 cm). A cubic core (20 × 20 × 20 cm) was sampled to study soil. Three cubic cores (10 × 10 × 10 cm repetitions) were also obtained nearby to study oribatid mites. The cores were sampled as close as possible to each other. Oribatid mites were subsequently extracted with a modified Berlese-Tullgren apparatus and preserved in 75% ethyl alcohol. Adult individuals were identified and counted at species level using a stereoscopic microscope. The species were listed according to Weigmann (2006). Soil samples were taken using decontaminated polypropylene tools and kept in hermetically sealed decontaminated polypropylene containers. In order to preserve soil integrity, the samples were immediately transported under gel packs and refrigerated (4°C) on arrival at the laboratory. In the laboratory, soil samples were air-dried and sieved using a 2 mm mesh sieve. The soil fraction <2 mm (fine-earth fraction) was homogenized by quartering and pulverization and analysed to determine the following properties: pH, salinity (as NaCl; g/kg), carbonate content (as CaCO3; g/kg), soil organic matter (SOM; g/kg), total nitrogen content (TN; g/kg), field capacity (FC; g/kg) and density (D; kg/L). Measurements of soil properties were according to the USDA/NRCS (2004) methods manual. Total nitrogen was determined by direct total flash combustion using an element analyser with thermal conductivity detector (CHN/O 200, Perkin Elmer). Recoveries and reproducibility were checked by analysing procedural blanks and standard reference materials (SRM1944 and Tibet Soil); the reproducibility test indicated a coefficient of variation of >95% and the analytical limit of quantification (LOQ) was 0.01% for TN. All laboratory analyses were performed in triplicate.

Data analysis

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calculated using CANOCO v. 5.03 (ter Braak and Šmilauer 2012).

Four datasets were obtained: i) percentage cover for each plant species (plant community composition); ii) oribatid mite species mean abundance (mite community composition); iii) physical and chemical soil properties; iv) percentage cover of each vegetation layer, i.e. tree, shrub and herbaceous layer (vegetation structure). The plant dataset was subject to generalized log transformation [log generalized = log(x + x min) - log(x min)], because there were values less than 1.0 in the matrix (McCune and Grace, 2002; Peck, 2010). With regard to mites, the mean abundance of the three repetitions in each plot was calculated and log(x + 1) transformation was used. Because soil data was expressed in incompatible units, it was reduced to a common scale (interval 0 - 1) by subtracting the minimum observed for each variable and dividing by the range, namely applying the method of ranging proposed by Sneath and Sokal (1973). To prevent excessive noise in the data matrix from obscuring certain data features, plant species with cover less than or equal 0.1 occurring only once (29 species) and oribatid mite singletons (37 species) were excluded from the analysis. In initial analysis with all the data, it was confirmed that these two categories do not improve the analyses. The concordance among plant communities, mite communities and soil properties was tested using two techniques with the intention of determining whether the results remained consistent under different procedures. In order to decide if it was better to use ordination methods based on a model of linear species response to the underlying environmental gradient or weighted averaging ordination methods, corresponding to a model of unimodal species response (Leps and Smilauer 2003), explorative Detrended Correspondence Analysis (DCA) was performed. Since the first axis gradient was always <4.00 SD, three Principal Component Analyses (PCA) were performed. Coordinates (scores) of plots in each PCA (mites, plants and soil) were used to create pairs of matrices, tested with Spearman's rank correlation coefficient (SRCC) to evaluate the concordance in distribution patterns between plant community, mite community and soil properties. A Mantel test (MT) was also performed to assess concordance of community-level patterns between plants, mites and soil properties from the same set of sample units. MT evaluates the null hypothesis of no relationship between two dissimilarity (distance) or similarity matrices. Finally, two separate Redundancy analyses (RDAs) were performed with mites and plants, using species and tree, shrub and herbaceous cover data as explanatory variables. The significance of the first ordination axis and all canonical axes together was tested by a Monte Carlo randomization method, using 999 permutations. DCA, PCA and MT analyses were performed using the software package PCORD 6.0 (McCune and Mefford 2011). SRCC was performed using the STATISTICA v. 7.1 software package (StatSoft Italia S.r.l. 2005). RDAs were

Results

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148 Biological components

A total of 95 vascular plants was recorded in 20 plots (for details see Online Resource 1) with an average number of species per plot of 13.7 ± 7.4 (mean ± SD). The highest number of species per plot was 32, the lowest 3. Pinus pinea was the only species present in all plots, followed by Pistacia lentiscus (17 out of 20) and Inula conyzae (12 out of 20 plots). Mean tree cover was 54.65% (range 15-98%) with dominant species such as Fraxinus angustifolia subsp. oxycarpa, Pinus pinea, Ouercus ilex, O. suber, Robinia pseudacacia and Ulmus minor. Mean shrub cover was 33.15% (range 0-100%) with dominant species such as Phillyrea angustifolia, Pistacia lentiscus and Juniperus oxycedrus subsp. macrocarpa. Mean herbaceous cover was 14.65% (range 0.5-85%) with frequent species such as Anagallis arvensis, Carex distachya, Catapodium rigidum and Brachypodium rupestre. Vines such as Hedera helix and Tamus communis were also present. A total of 100 oribatid mite species were recorded in 20 plots (for details see Online Resource 2) with a mean number of species per plot of 20.3 ± 9.09 (mean ± SD). The highest number of species per plot was 40, the lowest 4. The species with the greatest number of individuals was Sphaerochthonius splendidus. The highest number of individuals per plot was 508, the lowest 16. The average number of individuals per plot (density) was 181 ± 144.01 per cubic core. Species occurring more frequently were Microppia minus (17 out of 20) and Chamobates borealis (15 out of 20 plots). Soil properties Table 1 shows the main statistical indices of physical and chemical properties measured in soil samples (for details see Online Resource 3). The analytical data indicated low to moderate variability in most properties (except a few anomalous values of pH). Although pH values were highly variable, most soils were moderately to slightly acid or neutral. NaCl content was distinctive of non-saline soils. Higher NaCl concentrations were measured in some slightly to moderately saline soils collected closer to coastal dune zone. In line with the geolithological characteristics of the parent rock (carbonate-rich sandy and sandy-silty sediments), soils were moderate to high calcareous. Assuming that SOM typically contains 58% carbon, the soils of Feniglia Dune had high to very high organic carbon content (Costantini 2006) as well as medium to high nitrogen content. The high levels of field capacity (FC) were typical of clay-loam to

clay soils rich in organic matter. Density (D) values decreased with increasing SOM and confirmed that soils were rich

Community concordance between plant community, mite community and soil properties

Variance explained by the first PCA axis was 21.97% for plant data, 24.04% for oribatid mite data and 49.14% for soil properties. The correlation between scores of each PCA first axis of plants, oribatid mites and soil features only showed significant correspondence between the data of plant and mite communities (r=0.55; p<0.05). The results of MT agreed with those of PCA, only showing a significant correlation between plant and mite communities (r=0.428; p<0.01).

All RDA axes with data constrained by vegetation layer cover were significant for plant and mite community data (p<0.01) and the total explained variation was 26.5% and 30.8%, respectively. The biplots of RDA in Figure 1 and Tables 2 and 3 show the relationships between vegetation covers (vectors) and biological data. In the biplot for plants (Figure 1a) the main correlations were: axis 1 was positively associated with herbaceous cover and *Brachypodium rupestre*; axis 2 was negatively associated with shrub cover and *Pistacia lentiscus*; axis 3 was negatively associated with tree cover and *P. pinea*. In the biplot for oribatid mites (Figure 1b) the main correlations were: axis 1 was positively associated with herbaceous cover and *Scheloribates laevigatus*; axis 2 was positively associated with tree cover and *Metabelbella interlamellaris*; Axis 3 was negatively related to shrub cover and *Scheloribates initialis*.

Discussion

Vegetation composition, variation in soil properties and the spatial configuration of habitat patches shape below-ground communities, affecting composition and species diversity patterns of soil fauna (Widenfalk et al., 2015) which generally prove sensitive to habitat evolution, although sensitivity varies considerably between groups and single species (Maraun and Scheu 2000). In line with this concept, our results suggest a concordant distribution pattern between plant and mite communities in a Mediterranean stone pine forest, with significant correlation in ordinations indicating parallel variation in species composition among taxonomic groups (Santi et al. 2010; Rooney and Azeria 2015). These findings were consistent across all analyses (PCA, MT and RDA). Vegetation composition is affected directly and indirectly by a wide range of drivers, such as climate, land management and soil chemistry, that will also influence soil fauna. Moreover, in some cases vegetation composition can summarize these drivers and proves more related to oribatid mites than to soil chemistry. Mitchell et al. (2010) recorded similar results for soil microbial communities. The common distribution patterns we observed between communities of vascular plants and mites can also be due to factors not investigated here, such as the physical space created by roots (less compact texture), which creates a more suitable habitat for mite communities. This below-ground network can affect soil microclimate, maintaining temperatures constant, providing nourishment (mites can be predators of root pests; Wissuwa et al. 2012) and modifying rhizodeposition, as well as the quality and quantity of litter (Maharning et al. 2009). Conversely, the influence of soil mesofauna on vascular plants is reflected by patterns resulting from feedback involved in community dynamics (Klironomos 2002; De Deyn et al. 2003). It is worth recalling that: i) cross-taxon congruence patterns are highly complex; ii) correlations cannot be causal in nature; iii) correlation of above-ground and below-ground diversity does not necessarily imply mechanistic linkages, but is a first step in assessing whether such linkages exist; iv) above-ground organisms and their ecological mechanisms are better known than below-ground ones (Hooper et al. 2000; Santi et al. 2010).

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The present study did not find support for the hypothesis of concordance between biological communities and soil chemical properties, although plants and mites were concordant and shared the soil matrix. This may at first seem surprising, as certain studies have shown that high variability of soil chemistry as well as environmental and/or human disturbance might determine evident gradients that reflect similar patterns in soil and oribatid mites (Migliorini et al. 2005b; Keith et al. 2012) or vascular plants (Bernard-Verdier et al. 2012; Angiolini et al. 2013). The lack of relationships could also be due to: i) scale, that is important to understand above/below-ground interactions; ii) the uniform geolithological background consisting essentially of sand; iii) homogeneous land use, namely conservation of pine forest. In line with Nielsen et al. (2012) we can state that soils support highly diverse communities due to their great spatial complexity at very fine scales and the small size of soil organisms. Oribatid mites have no specialized dispersal stages, and dispersal is very limited, thus they may only travel distances of a few centimetres per day. In any case, the appropriate scale to avoid misleading spatial autocorrelation sampling is still unclear for managed and natural environments alike (Minor, 2011). Furthermore, it is relatively common not to find any correlation or only weak ones. Gradients in soil systems may change at plot scale due to high spatial variability in abiotic factors. In the stone pine forest of the study area only soil pH was highly variable, mainly due to the influence of pine needles and soil mineralogical features, specifically carbonate content, but it was not significantly linked to biotic composition. This agrees with previous findings that soil pH does not directly affect oribatid mite densities and composition, but may control other physico-chemical and biotic factors not investigated in this study (e.g. other saprophagous groups or macro-decomposers such as earthworms). Oribatid mites seem to tolerate much variation in soil chemical and physical parameters and they do not seem directly affected by factors such as soil pH. In fact, scrolling through the literature, we see for example that proventricular glands have been described as multifunctional organs (Alberti and Coons, 1999; Pigino et al., 2006) devoted to the production of digestive enzymes, as well as participation in light reception and pH regulation. However, in other studies conducted in pine forests, pH seemed to influence the occurrence of mites (Minor, 2011) and vascular plants (Angiolini et al. 2013), directly and in a major way. Our results may therefore be affected by the scale of sampling, which is known to affect cross-taxon studies (Hess et al., 2006), also because at finer scales, species distribution is probably less related to soil parameters than at regional scales (Palmer, 1990) and at finer scales, biotic site parameters may be more important than soil conditions (Bruelheide and Udelhoven, 2005). This circumstance may be related to the fact that local mite communities in soil are known to be sensitive to fine-scale environmental gradients, each related to the physical structure of the underground parts of plants or litter quality (Gergócs et al. 2015), even if stochastic factors can dominate oribatid mite assemblages at different scales, as suggested by Caruso et al. (2012). These authors also attribute this source of stochasticity to different environmental conditions typical of certain areas, making it necessary to interpret local soil communities as islands subject to extinction and immigration (Maaß et al. 2014).

The role of vegetation cover, tested as a key factor driving changes in biological communities, proved to be a driver that influences vascular plant and mite community compositions, in line with their congruent distribution patterns, probably related to similar auto-ecology of the species or their spatial covariation. The main driver of plant and mite communities among vegetation cover was herbaceous layer cover. Correspondence between species of communities in relation to vegetation cover was clearly revealed by direct ordinations linked to stone pine canopy heterogeneity. Under conditions of high tree cover, more homogeneous plant data was recorded. On the other hand, in clearings with high herbaceous cover, there was more plant variability, as found in other semi-arid Mediterranean areas (González-Alday et al. 2009; Madrigal-Gonzalez et al. 2010). Interestingly, some commonalities and strong relationships between certain plant and mite species and vegetation cover were identified. Many studies have demonstrated that the distribution of oribatid mites is not random but aggregates according to trophic resources and soil moisture (Usher et al., 1982). For example, the decline in population density during the dry months is less severe in soils with the dense vegetation cover; daily and seasonal variations in temperature are less extreme than in open sites, and supply of organic matter provides food resources and trophic niches for oribatid mite communities throughout the year (Migliorini et al. 2002). In our study, mite communities showed a certain number of euryecious species related to meadows and open areas (Magowski and Niedbala 2013). Scheloribates laevigatus, constantly present in grasslands at these latitudes, was associated with herbaceous cover dominated by Brachypodium rupestre, a species of meso-thermophilic herbaceous edges of pine forests and woods in general, and other graminoids. Relationships between tree cover, Pinus pinea and Metabelbella interlamellaris seemed to confirm the ecological traits of the latter species, typical of Mediterranean thermophilous forests. Similarly, Scheloribates initialis, constantly collected in transition habitats, was related to a shrub species, Pistacia lentiscus; both are typical in the same biogeographical district.

Conclusions

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The community similarity approach, comparing patterns of concordance, is an appropriate protection measure, although no single path of conservation planning can be sufficient for all environmental situations (Su et al. 2004). The link between above- and below-ground diversity in natural environments is certainly convoluted and depends on the taxa

studied and the scale used (Hooper et al. 2000; Ettema and Wardle 2002; Wardle et al. 2004; Mitchell et al. 2010). Our efforts provide insights on a small part of this intricate system. Indeed, even with awareness of the complexity of these types of interactions, interrelationships, factor combinations and their underlying mechanisms, the webs are very hard to study and predict. Although further research is needed to test our results over a wider range of vegetation and soil types, our study demonstrates a clear relationship between vascular plant and oribatid mite communities in a Mediterranean stone pine forest. Vegetation cover can be considered a driver of both mite and plant communities. The data suggests that maintenance of diversified vegetation cover may ensure various biological community assemblages and higher biodiversity in stone pine forest stands. Thus, these results offer some perspectives for practical application in biodiversity conservation management and planning in Mediterranean stone pine forests and possibly for priority habitats in general. With due precaution, they could open the way to joint management considerations for these two biological communities, improving awareness of protection of two (above- and below-ground) communities in one, but also awareness that any wrong management of one of these two communities may also have a negative impact on the other.

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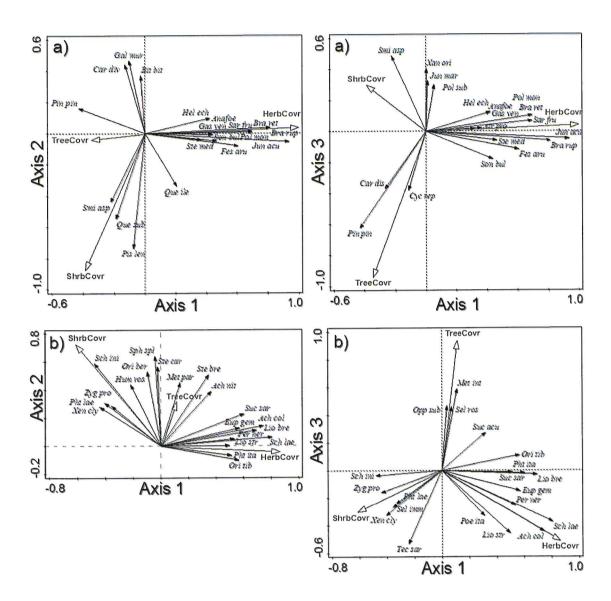


Figure 1 Constrained ordinations (RDA) are reported showing the 20 species of best fit: a) plants; b) mites.

Abbreviations of environmental variables: TreeCovr = Tree cover; ShrbCovr = Shrub cover; HerbCovr = Herbaceous cover. Abbreviations for plants and mites can be found in Online Resource 1 and 2.

Table 1 Main statistical indices (mean, standard deviation, maximum and minimum) of physical and chemical properties of soil samples: pH, salinity (as NaCl; g/kg), carbonate content (as CaCO₃; g/kg), soil organic matter (SOM; g/kg), total nitrogen content (TN; g/kg), field capacity (FC; g/kg) and density (D; kg/L)

	pН	NaCl	CaCO ₃	SOM	TN	FC	D
Mean	6.3	0.6	115.6	61.4	2.1	413.4	1.9
DS	0.96	1.01	75.85	46.31	2.23	115.72	0.17
Max	7.86	4.33	290	215.47	8.10	703.94	2.16
Min	4.36	0.08	32	13.62	0.001	250.67	1.54

Table 2 Correlation matrix of constrained ordination (RDA) of plants and cover

	Ax1	Ax2	Ax3
Variance (%)	(14.17)	(9.65)	(2.71)
Trees cover	-0.3181	-0.0305	-0.5216
Shrubs cover	-0.3541	-0.7093	0.1690
Herbaceous cover	0.9087	0.0319	0.0252

Table 3 Correlation matrix of constrained ordination (RDA) of mites and cover

	Ax1	Ax2	Ax3
Variance (%)	(14.55)	(9.69)	(6.54)
Tree cover	0.1033	0.2320	0.7508
Shrub cover	-0.5669	0.5273	-0.2376
Herbaceous cover	0.7913	-0.0281	-0.4047