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ENVIRONMENTAL RESPONSES OF SOIL ARTHROPOD COMMUNITIES TO LOW INPUT CULTIVATION OF *CYNARA CARDUNCULUS* IN THE MEDITERRANEAN REGION

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ABSTRACT

The qualitative and quantitative changes in arthropod communities are frequently associated with habitat modifications, especially the ones connected with agriculture. During 2012 and 2013, we investigated in low-input cardoon fields (Thessaly plain, central Greece) the role of soil properties (soil organic matter, pH, nitrogen (N), phosphorous (P), potassium (K) and soil temperature), plant diversity (Shannon diversity index H') and ambient humidity and temperature, as well as inter-farm distance on arthropod density. The analysis showed that the low-input farming system applied in the cardoon cultivation supports high arthropod taxonomic richness and enhances their density. The increase in taxonomic richness and density in invertebrates might be in causal relationship through the food chain. In our experimental plots, the density of all arthropod taxa displayed positive correlations, analyzed by means of the Principal Component Analysis, with the concentration of soil organic matter, pH, P, K and plant diversity (Shannon diversity index H'), and the geographic distance from the neighbouring cultivated farm. However, the Redundancy analysis uncovered that the concentration of K, air humidity and geographic distance from uncultivated crops have been positively correlated with the density in Isopoda. Moreover, the Coleoptera (the most abundant taxon together with Isopoda) density was mainly related with soil organic matter and plant diversity (Shannon diversity index H'). We can possibly to conclude that arthropod density is a sensitive bioindicator (parameter) of the environmental change in cardoon fields and could be employed for conservation monitoring and for planning purposes in cardoon crops in all the Mediterranean basin.

Keywords: Biodiversity, Environmental factors, Soil invertebrates, Sustainability, Cardoon, Mediterranean, Greece.

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Introduction

Sustainable agriculture aims to manage and utilize the agricultural ecosystem in a way that it maintains biological diversity, productivity, regeneration capacity, vitality, and ability to function, so that it can fulfill – today and in the future – significant ecological, economic and social functions at the local, national and global levels and does not harm other ecosystems' [1]. Regarding biodiversity, it is certain that "low-input farming" systems, aiming at minimizing external inputs mainly understood as fertilizers, pesticides and energy [2] and maximizing the use of internal resources, provide habitats for wildlife on farmland and contribute to relatively stable biodiversity. The passage of low-input farmlands to more intensive and economically more efficient farming systems is accompanied by a decline in farmland biodiversity [3].

The design management strategy to keep low-input farmlands sustainable is to grow low-input (that means not demanding external fertilizers, pesticides and energy) plants of high economic value such as cardoon (*Cynara cardunculus* L.) [4]. Cardoon, also known as cynara or artichoke, is a C_3 perennial diploid (2n=34) outcrossing plant species. Recognized have been the western *C. cardunculus* subsp. *flavescens* and the eastern *C. cardunculus* subsp. *cardunculus* [5] and varieties as *C. cardunculus* var. *scolymus* (L.) Fiori (cultivated artichoke), *C. cardunculus* var. *sylvestris* (Lam.) Fiori (wild artichoke) and *C. cardunculus* var. *altilis* DC (cultivated artichoke) [6]. Currently, about 90% of cardoon cultivation takes place in the countries bordering the Mediterranean Sea.

We could define cardoon as a low–input plant because of the small typeface C which means a minimization of the risk of environmental pollution due to nitrogen leaching in addition to its deep rooting system trapping salts while maintaining similar crop yields and providing many environmental and social benefits [7,8]. Its cultivation provides a material to produce: a) energy (agropellets, electricity) and paper pulps from the lignocellulosic biomass [7,8,9] b) animal fodder, food and biodiesel [10] from seeds containing oil and proteins [11] and c) medical products, for example, by utilizing the high inulin content in cardoon heads [12] and in roots [13].

Since arthropods have been recognized as bioindicators of habitat quality and habitat differences [14] they could be useful in the comparison of biodiversity between low-input and high-intensity agricultural lands. Nonetheless, studies of the arthropod biodiversity in cardoon crops have been scarce up to now. Hence, our research goals in the low-input cardoon crops were: (1) determining the occurring arthropod taxa and their frequencies; (2) examining the relationship between total arthropod density and designated environmental factors; and (3) investigating the relationship among arthropod taxa densities and environmental factors.

Material & methods

Study area

The study was conducted in 12 experimental low-input cardoon plots (P1 - P12) in the Thessaly plain (experimental area of the University of Thessaly, Velestino-Lat: 39^0 23', Lon: 22^0 45'), Central Greece (Fig. 1). The size of each experimental plot was 30 x 10 m^2 and the geographic distance between the designated plots ranged from 110 to 340 m.

The study area is part of the *Quercion ilicis* and *Oleo-Ceratonion* subzones of the *Quercetalia ilicis* zone of the Mediterranean-type vegetation at altitudes up to 50 m a.s.l. [15]. The soil of the study area is characterized by clay loam with a bulk density between 1 and 1.7 $g.cm^{-3}$ [16]. The regional Mediterranean climate is characterized by relatively cold, wet winters and hot, dry summers. The mean annual temperature is 16,58 °C and the mean annual rainfall reaches 500 mm (Fig. 2).

The cultivated Cynara is an autumn–spring growing robust thistle with a characteristic rosette of large spiny leaves and branched flowering stems. It grows from September (emergence) to July (seed maturity)

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efficiently using rainfall as a water supply; energetic substances accumulate in roots and regrowth occurs after the summer quiescence. The characteristics and management practices applied to the field experiments are the below: a) size/plot= 300 m^2, b) average number of cardoon plants per m^2 /plot=6 (1x0,5 m), c) planting year=2004, fertilizer N-P-K, 34,5-0-0 kg/ha/plot (per year)=60-0-0, weed control= no application.

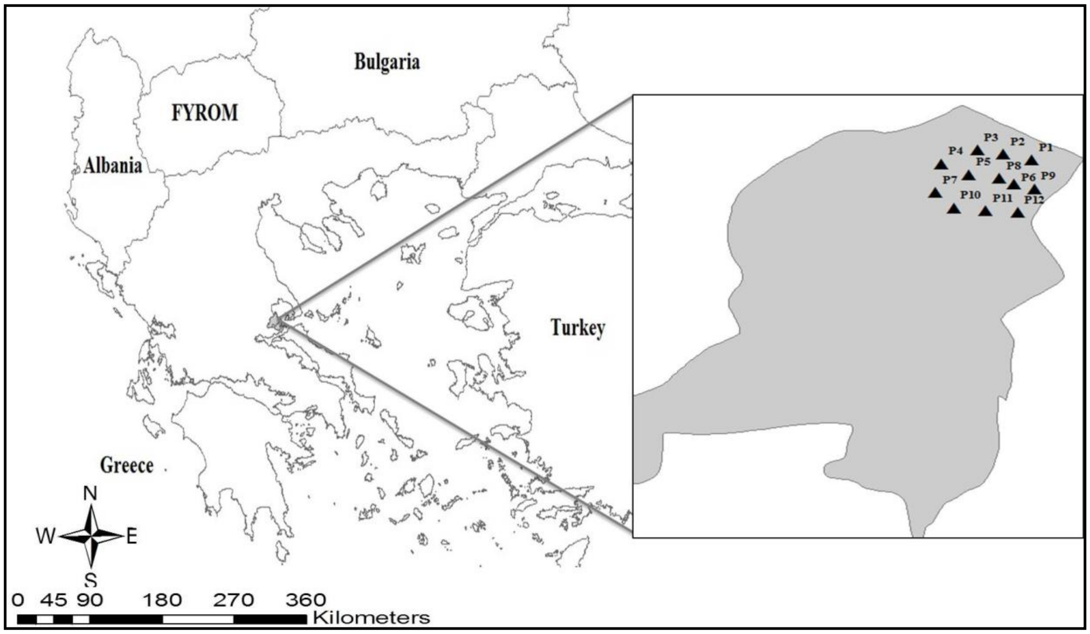

Figure 1. Study area

Sampling

Insects were sampled by using pitfall traps for seven days at the end of each month from September till December in 2012 and again in 2013. In total, 36 (3 traps in each experimental plot) pitfall traps (plastic cups 13 cm in height, 9 cm in diameter), were placed into the ground so that the lip of each cup was slightly below the ground surface. In the traps the fallen specimens were preserved in a water solution of 75% ethanol and 5% glycerin. The geographical position of the traps was measured by means of GPS (Garmin eTrex Vista HCx).

Soil temperature was recorded with a soil Digital Thermometer-TFA close to the traps, every time they were emptied. Besides, soil samples were taken (3 soil samples x 12 plots x 2 years = 72) for laboratory analysis, by using a core sampler (6 cm diameter, 30 cm deep). Soil texture, pH, organic matter (in %), nitrogen, phosphor and potassium were assessed in each experimental plot, according to Bouyoukos (1951), McLean (1982), Nelson and Sommers (1982), Bremner and Mulvaney (1982), Olsen and Sommers (1982) and Thomas (1982) [17,18,19, 20,21,22].

In parallel to the sampling of invertebrates, the sampling of herbaceous vegetation was carried out in the experimental plots in order to estimate plant diversity (Shannon diversity index H') [23,24]. In each plot and in each collection month (September – December) three quadrates of 0.25 m^2 (0.5 m × 0.5 m) were sampled. Air humidity (%) and ambient temperature (°C) were measured by means of a TFA Digital Thermo-Hygrometer. The physically measured geographic distances from the experimental plots to the nearest farms with crops and to the roads ranged from 90 to 450 m. The sampled insect specimens were counted and identified to the level of orders under a Zeiss Stemi 2000-C stereomicroscope.

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Data analysis

The obtained activity densities estimated as the number of individuals/100 trap-days and the collected arthropod datasets were tested for deviations from the normal distribution and for differences in homogeneity of variance by means of the Kolmogorov-Smirnov and Shapiro-Wilk tests. The datasets that failed to meet normality assumption were transformed by using log(x+1). For the analysis of arthropod data, One-way Analysis of variance (ANOVA) was used. Tukey's HSD pairwise comparison tests were applied with $p<0.05$. All the above analyses were performed using IBM SPSS for Windows, v. 19.0 [25].

The inter-farm classification of the arthropod taxa communities was established by employing the Ward΄s clustering method and the squared Euclidean distance. Cluster analysis is an analytic technique used to classify observations into a finite and, ideally, small number of groups based upon two or more variables [26]. Principal component analysis (PCA in CANOCO 4.5 for Windows) [27] was also performed to account for correlations between variables when determining which of the factors [soil organic matter, pH, nitrogen (N), phosphor (P), potassium (K) and temperature, air humidity and temperature, plant diversity (Shannon diversity index H'), distance from neighbouring uncultivated and cultivated farm] would best correlate with the estimated total arthropod taxa density.

The statistical program CANOCO 4.5 for Windows was also used for the ordination of sites based on the response of each arthropod taxa density to several environmental variables namely soil organic matter, pH, nitrogen, phosphor, potassium and temperature, air humidity and temperature, plant diversity (Shannon diversity index H'), distance from neighbouring uncultivated and cultivated farm. In the present study, Redundancy Analysis (RDA, in CANNOCO) [27] was successfully performed using the data concerning the density of taxa and the environmental factors mentioned above.

Results & discussion

In total, specimens recorded in the gathered samples represented eight arthropod orders (Coleoptera, Isopoda, Hymenoptera, Diptera, Aranaea, Orthoptera, Heteroptera and Chilopoda). In all samples and in overall the most dominant orders were Coleoptera and Isopoda (Fig. 3, Fig. 4, Table 1), as expected in the Mediterranean area [28]. The relative frequencies of the remaining taxa were fluctuating from sample to sample and were probably influence by the local and regional geographic and climate situations, by the agricultural management regime and by the surrounding vegetation [29,30].

Table 1. Abundance of the studied arthropod taxa in 12 experimental plots.

	Coleoptera	Isopoda	Hymenoptera	Diptera	Aranaea	Orthoptera	Heteroptera	Chilopoda
P1	19.05	57.14	17.46	7.94	4.76	0.00	4.76	0.00
P2	46.03	12.70	15.87	11.11	0.00	1.59	3.17	0.00
P3	63.49	61.90	4.76	17.46	0.00	0.00	0.00	6.35
P4	30.16	11.11	0.00	0.00	0.00	3.17	15.87	0.00
P5	30.16	22.22	12.70	0.00	0.00	3.17	0.00	4.76
P6	50.79	39.68	12.70	0.00	0.00	1.59	7.94	6.35
P7	15.87	9.52	0.00	4.76	0.00	0.00	6.35	7.94
P8	9.52	11.11	0.00	15.87	0.00	3.17	0.00	0.00
P9	20.63	109.52	0.00	7.94	0.00	0.00	0.00	9.52
P10	47.62	34.92	6.35	6.35	0.00	1.59	4.76	0.00
P11	63.49	125.40	0.00	3.17	0.00	0.00	0.00	0.00
P12	52.38	0.00	12.70	0.00	0.00	0.00	0.00	14.29

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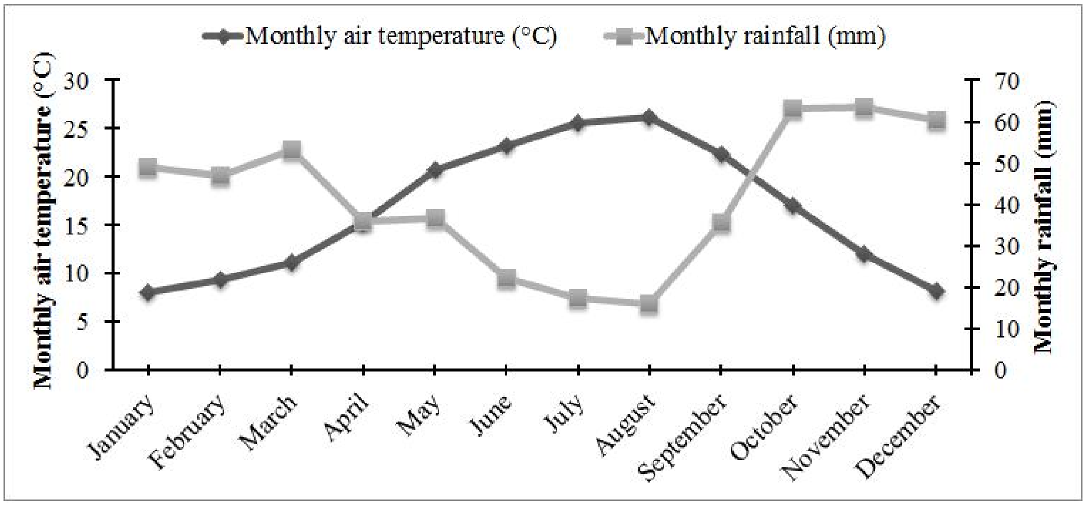
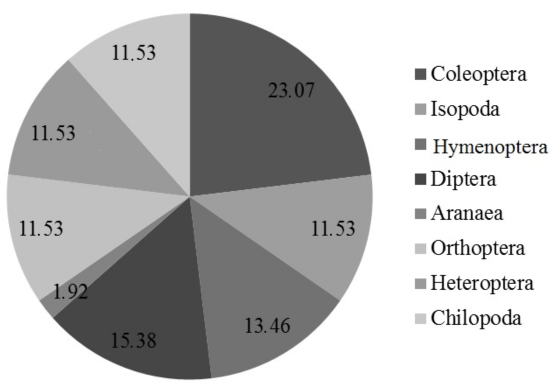


Figure 2. Ombrothermic diagrams of the study area (Source: National Meteorological Service of Greece, 2013).



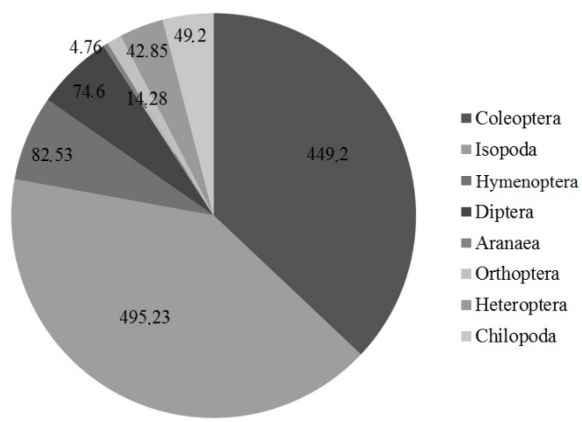


Fig. 3. A circular graph showing the relative frequencies of occurrence of arthropod taxa in the 12 experimental plots.

Fig. 4. A circular graph showing the total number of abundance of arthropod taxa in the 12 experimental plots.

The potential of the land to cultivate energy crops is determined by an array of biophysical and economic variables and among them climate, soil and geomorphological environmental components are the most important agro-ecological ones [31].

Significant inter-plot difference (One way ANOVA, F= 5409.86, $p<0.001$) was observed in the arthropod taxa activity density (individuals/100 trap-days). In pair-wise comparisons, the “activity density” was highest in F11. The cluster analysis indicates the occurrence of the following four plot groups according to similarities in arthropod taxa community composition (F2-F10, F8-F11, F3-F9, F5-F6) (Fig. 6). The occurrence of diverse arthropod

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populations in the low-input plots could result from the availability of different food types of plants in the plant cover. This factor is important to establish and maintain soil arthropod communities [32,33].

Based on the Principal Component Analysis (PCA), the eigenvalues for PCA axes 1 and 2 were 0.63 and 0.32, respectively, thus capturing 95.0% of the total variance in the data (Table 2). Arthropod taxa density was positively correlated with soil organic matter, pH, P, K and plant diversity (Shannon diversity index H'), and with the geographic distance from neighbouring cultivated plots and negatively correlated with the distance from the neighbouring uncultivated plots (Fig. 7).

Table 2. Results of the principal component analysis for the total arthropod taxa density and environmental factors.

Axis variable	1	2	Total inertia
Eigenvalues	0.63	0.32	1.00
Cumulative percentage variance of species data	63.5	95.5	
Sum of all eigenvalues			1.00

It is well known and documented [34,35] that the organic matter contained in soil is beneficial to the majority of soil animal groups and that species richness and abundance are strongly linked to the availability of energy resources and essential nutrients [36]. Specifically, nutrients have long been shown to directly affect arthropod community structure and density [37,38] as limiting factors.

Moreover, species richness and abundance of the organismal groups in agricultural habitats are influenced by interactions with the surrounding landscape. For example, the use of pesticides in the fields surrounding the studied plots could possibly explain the somewhat reduced arthropod density recorded in the present study. According to Aktar *et al.* (2009) [39] and Isenring (2010) [40], pesticides are an important factor that can be directly toxic to organisms, or affect their habitats and the food chains. On the other hand, uncultivated plots may offer more complex habitat composition supporting more taxa because they possibly provide a greater access to a wider range of alternative food resources [41].

Redundancy analysis (RDA) was performed with the density of soil arthropod groups treated as dependent variables and the environmental variables mentioned above regarded as independent variables. The results of RDA applied to the soil arthropod density and the environmental variables are shown in Fig. 8 and in Table 3.

Table 3. Results of the redundancy analysis.

Axis	1	2	Total inertia
Eigenvalues:	0.65	0.25	1.00
Species-environment correlations:	0.97	0.98	
Cumulative percentage variance			
Of species data:	65.0	90.5	
of species-environment relation:	81.7	98.0	
Sum of all eigenvalues:			1.00
Sum of all canonical eigenvalues:			0.92

A Monte Carlo permutation test was used to evaluate the relationship between macroarthropod taxa and the whole set of environmental variables (air temperature, air humidity, soil temperature, organic matter, soil pH,

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K, N and P, plant diversity (Shannon diversity index H'), EC, geographic distances of uncultivated and cultivated farms). The most significant relationships were found with air humidity (AH) ($F = 10.85$, $p = 0.0017$), distance of uncultivated crop (DUC) ($F = 2.46$, $p = 0.0592$), concentration of potassium (K) ($F = 4.40$, $p = 0.0342$) and amount of soil organic matter (OM) ($F = 7.77$, $p = 0.0067$).

Axis 1 accounted for 65.3% of the total variance in macroarthropod density and was mainly correlated (positively) with K and air humidity. More specifically, the Isopoda density was positively correlated with K and air humidity.

Soil potassium is vital to many plant processes. It has thus an indirect effect on isopod density as isopods are known to be omnivores feeding on dead or decaying plants or animals. They are also known to eat live plants [42]. Also, high air humidity is a key factor for survival of isopods [43] possibly because isopods have gills and need water to breathe. Therefore, isopods are able to live under a wide variety of conditions with a preference for moist places [44,45].

Also, Axis 2 accounted for 25.1% of the total variance in density data. It was correlated with the soil organic matter and pH. In particular, the Aranaea density was positively correlated with the soil pH and the Coleoptera density was mainly related with the soil organic matter and plant diversity (Shannon diversity index H'). The soil pH may indirectly impact Aranaea density because the distribution of spider populations in the cropland did not correlate with the soil acidity (pH) [46] but was dependent on the prey availability and other factors such as vegetation composition and structure [47]. Specifically, the soil pH plays an important role in plant growth since it determines the availability of nutrients for plants. However, in the field margins lycosid and thomisid spiders were usually found in the soils with higher acidity.

Regarding the Coleoptera density, as organic matter and plant diversity (Shannon diversity index H') increased, several relatively large predatory species (such as *Carabus graecus* and *Carabus coriaceus*) flourished. They were possibly taking advantage of the more suitable conditions and increased prey availability [48,49,50].

Strong relationship between macrofauna, soil organic matter, soil physical properties and plant composition and density [51] implicates that the higher content of C-organic soil, leads not only to a higher index of soil aggregate stability [52] but also to the establishment of microhabitat conditions that support better soil macrofauna [53].

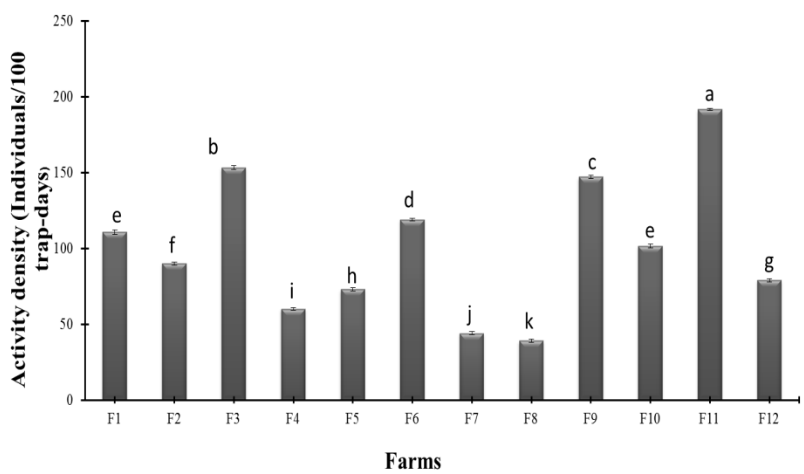

Fig. 5. Comparison of the experimental plots (= Farms) according to mean values (±SD) of arthropod taxa density.

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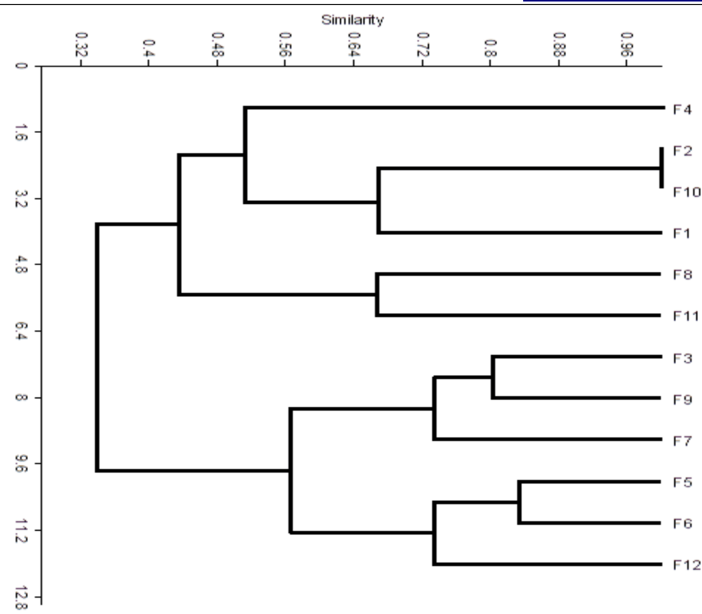

Fig. 6. Dendrogram of arthropod taxa community similarity among the studied plots. The dendrogram was constructed by the Ward's method based on the squared Euclidean distance.

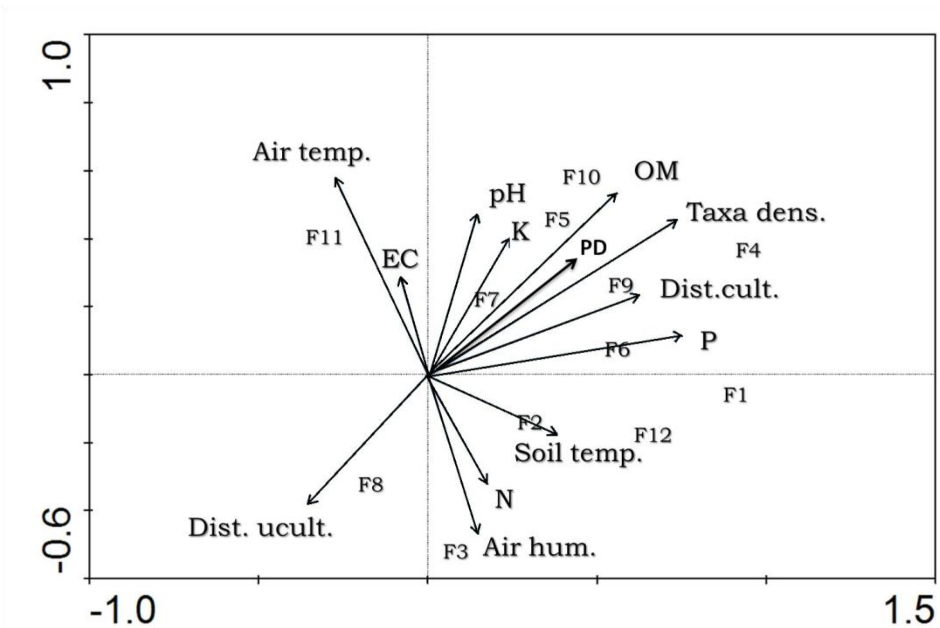

Fig. 7. Principal Component Analysis (PCA) ordination diagram for total arthropod taxa density and environmental factors [Air temp: air temperature, Air hum: air humidity, Soil temp: soil temperature, OM: organic matter, pH: soil pH, K: potassium, N: nitrogen, P: phosphor, PD: Plant diversity (Shannon diversity index H'), EC: electrical conductivity, Dist. ucult: distance of uncultivated farm, Dist. cult: distance of cultivated farm, Taxa dens: taxa density].

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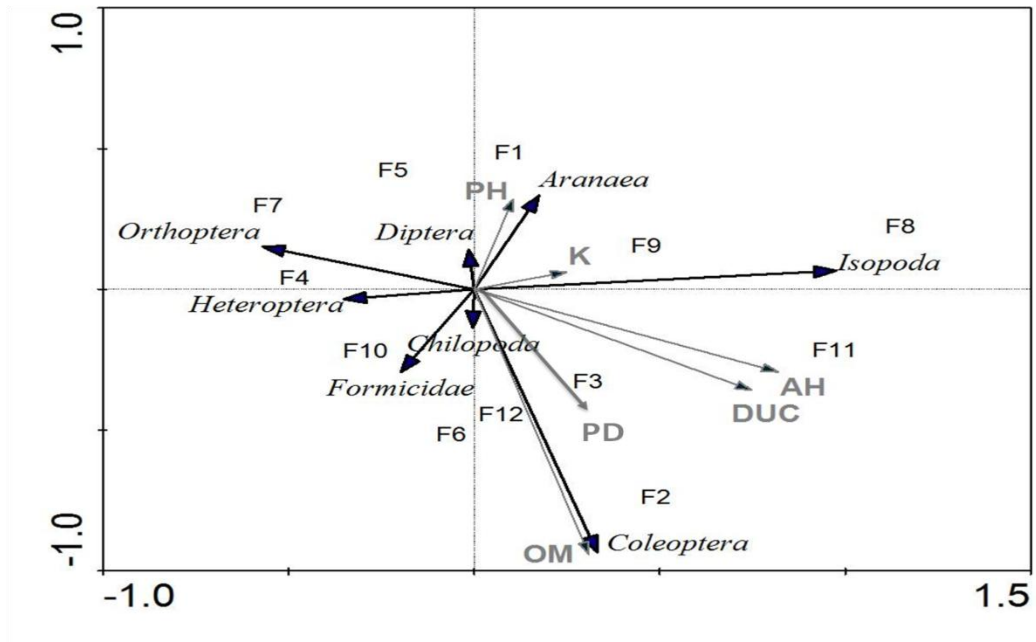

Fig. 8. Redundancy analysis based on arthropod taxa density found in the 12 low-input farms [pH: Soil pH, K: potassium, AH: Air humidity, PD: Plant diversity (Shannon diversity index H'), DUC: Distance of uncultivated crop and OM: Soil organic matter].

Conclusions

Our results are showing that the low-input farming system benefits the arthropod population associated with cardoon crops by providing and maintaining diverse and structurally varied habitats. Our ability to classify sites with different environmental characteristics by means of qualitative and quantitative analysis of the differences among soil arthropod communities is thus confirmed. Therefore, soil arthropods, even at the ordinal level, could be used as an "indicator" of the environmental responses of cardoon crop ecosystems. In general, we conclude that arthropods can be used for conservation monitoring and planning purposes in cardoon crops with low-input farming system in central Greece. Finally, the conclusions obtained here are probably applicable to other areas of the Mediterranean basin characterized by similar climatic conditions.

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