TERRESTRIAL ISOPODS BIODIVERSITY IN MAJERDA AGROECOSYSTEMS (TUNISIA)

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Résumé.— *Biodiversité des Isopodes terrestres dans les agroécosystèmes de la Majerda (Tunisie)*.— Nous avons entrepris l'étude de la variation saisonnière de la diversité et les effets des types de cultures sur les Isopodes dans douze parcelles de l'agroécosystème de la Majerda. Trois types de cultures ont été comparés (culture maraîchère, culture vivrière et arboriculture) durant quatre saisons. À chacune de ces saisons les Isopodes ont été collectés à l'aide de pièges-fosses ainsi que par collecte à la main afin de compléter la liste des espèces de chaque site. Huit espèces d'Isopodes ont été trouvées. Les Porcellionidés, avec cinq espèces, étaient la famille la mieux représentée, suivie par les Armadillidiidés et Philosciidés, avec respectivement deux et une espèces. *Porcellio laevis* et *Porcellio variabilis* furent les plus observés en fonction des saisons et des types de cultures. Des différences significatives d'activité et de densité des Isopodes sont apparues entre l'automne et le printemps selon les types de cultures. Quelle que soit la saison, les Isopodes s'avérèrent plus divers dans les cultures vivrières et les vergers que dans les maraîchages. Des analyses multivariée ont montré une différence de l'occurrence des espèces en fonction des paramètres physiques et chimiques du sol.

Summary.— We studied the seasonal variation of isopods diversity and the effects of cultivation types on the isopods communities. The study consisted of twelve plots in the Majerda agroecosystems. We compared three types of cultivation : market gardening, vegetable crops and orchards at four seasons. For each season, isopods were collected by pitfalls traps and also by hand (handsearch) to complete the list of species in the studied plot. Overall, eight isopod species were found. Porcellionidae was the best represented family with five species, followed by Armadillidiidae and Philosciidae, represented by two and one species, respectively. *Porcellio laevis* and *Porcellio variabilis* were the most observed in all seasons and types of cultivation. According to the cultivation types, significant differences of activity-density of isopods were seen between autumn and spring. Whatever the season, isopods were more diverse in vegetable crops and orchards than in market gardening cultivation. Multivariate analyses showed a difference of species occurrence related to physical and chemical soil characteristics.

Arthropods play a major role in agriculture as they are an essential component of the biodiversity of cultivated ecosystems (Fuller *et al.,* 1995). Among these organisms, terrestrial isopods (woodlice) play a major role among the soil fauna because of their important function in decomposing leaf litter and mineralizing organic matter in ecosystems (Sutton, 1980 ; Hassall & Sutton, 1978), and this is also true for agricultural lands. However, agricultural practices, such as drainage, conventional tillage and grazing lead to reduction or total destruction of litter and consequently, decrease biomass of isopods (Paoletti, 1987 ; Stinner & House,

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1990 ; Paoletti & Hassall, 1999). The type of agroecosystems also affects terrestrial isopods. It has been observed that isopods tend to be more abundant in semi natural grasslands than in woodlands (Davis & Sutton, 1978 ; Souty-Grosset *et al.* 2005a&b ; 2008) and more abundant in woodlands than in cultivated land. In general, a consistent difference in species composition is observed between deciduous woodlands and cultivated areas (Paoletti & Hassall, 1999). For example, in Italy, *Trachelipus razzautii* (Arcangeli, 1913), which is commonly present near hedgerows, is absent from crop fields (Paoletti, 1988). In Tunisia, only a few studies have been conducted on isopods biodiversity in agricultural area (Fraj, 2008 ; Fraj *et al.,* 2010). Thus, the aim of this study are to produce a list of the isopods collected in the Majerda agroecosystems and to study the seasonal variation of terrestrial isopods diversity related to types of cultivation. Additionally, the effects of soil characteristics on the isopods communities and species distribution are investigated. We hypothesized that community composition of isopods is affected by the physico-chemical characters of soil.

MATERIAL AND METHODS

STUDY AREA

The study area is the Majerda low plain (North-eastern Tunisia), located between Bizerte and Tunis cities. The site area (250, 000 ha) belongs to the upper semi-arid region. The climate is Mediterranean-type. The daily mean air temperature varies between 11 (January) and 27° C (August) and the relative air humidity is between 65 and 80 %. The annual average of precipitation is 433 mm. The landscape is dominated by agricultural land use and the main economic activity is cultivation for market gardening, vegetable crops, orchards and cereals. In this region, the operating system is based on water and irrigation is an important activity. The surface system of irrigation is the most commonly used (Abbes *et al.,* 2005).

SAMPLING AND LABORATORY PROCEDURE

Twelve plots in Kalaat El Andalouss, Sidi Thabet, Pont Bizerte and Sidi Othman sectors (Tab. I) and 3 cultivation types (market gardening, orchards and vegetable crops) were studied. Isopods were collected using pitfall traps (plastic cups, 7 cm height, 5.5 cm diameter, 1/4 filled with ethylene glycol 70 %). Five traps were installed, and replaced weekly during 4 seasons (September-December 2008 and March-June 2009). In each season and plot, sample period consisted of 3 consecutive weeks. Isopods were also collected by hand (under rocks and leaves). Individuals were preserved in 70 % ethanol, counted and species identified under Leica MS 5 binocular microscope according to the keys of Vandel (1962) and Hamaïed-Melki (2008).

In each season, 3 soil samples were removed from each plot. In the laboratory, soil fauna, plant parts or stones were discarded from the soil and the soil moisture measured (110° C oven for 24 h). In each season and at the end of isopod sampling, the 3 soil samples were pooled and regarded as one composite sample for soil analysis. Soil pH and conductivity were measured in a soil-water suspension. To analyse the carbon (C) and nitrogen (N) contents and after decalcification, the solution (soil-water) was centrifugalized and the pellet analysed by Loss On Ignition (LOI) 1100° C set. Moreover, the cation exchange capacity (CEC) was estimated by the analysing exchangeable bases ($Ca2^+$, $Mg2^+$, Na^+ and K^+) contents in soil samples. In this case, distilled water was added to fine soil. The solution was filtrated and samples cleared after centrifugation. The supernatant was recovered and analysed by atomic absorption (PERKIN ELMER A ANALYST 200) in the laboratory of LACCO (Laboratoire de Catalyse en Chimie Organique) at Poitiers University.

DATA AND STATISTICAL ANALYSES

Seasonal activity-density (AD ± SE), species richness (S ± SE), Shannon-Wiener index (H' ($\log_2$) ± SE) and Pielou's evenness (J ± SE) were calculated. Multivariate analyses were performed (Principal Component Analysis and Multiple Correspondence Analysis). All data are analysed for normality. Differences in isopods activity-density, richness and diversity among plots, season and cultivation types are compared by Friedman test (two-way non parametric test). The above statistical calculations are performed in R software (R Development Core Team, 2009).

TABLE I

Plots studied in Kalaat El Andalouss, Sidi Thabet, Sidi Othmen and Pont Bizerte

Locations	Code plots	Latitude/Longitude	Cultivation S1 (September 2008)	Cultivation S2 (December 2008)	Cultivation S3 (March 2009)	Cultivation S4 (June 2009)	Type of cultivation	Treatment	Area (ha)
Kalaat El Andalouss	Art-KA1	N37º06'4.26''/ E010º09'16.9''	Artichoke	Artichoke	Artichoke	Artichoke*	Market gardening	++	0,75
Kalaat El Andalouss	Art-KA2		Artichoke	Artichoke	Artichoke	Artichoke*	Market gardening	++	1
Kalaat El Andalouss	Pea-KA3	N37º04'42.4''/ E010º07'50.4''	Pear	Pear	Pear	Pear	Orchards	+	2,5
Kalaat El Andalouss	App-KA4		Apple	Apple	Apple	Apple	Orchards	+	2,5
Sidi Thabet	Alf-ST1	N36º56'07.6''/ E010º02'35.1''	Alfalfa	Alfalfa	Alfalfa	Alfalfa	Vegetable crops	-	1
Sidi Thabet	Alf-ST2	N36º57'07.7''/ E010º01'46.6''	Artichoke	Artichoke	Artichoke	Artichoke*	Market gardening	++	1,5
Sidi Othmen	App-SO1		Apple	Apple	Apple	Apple	Orchards	+	5
Sidi Othmen	App-SO2	N36º56'04.0''/ E009º53'59.3''	Olive	Olive	Olive	Olive	Orchards	+	6
Sidi Othmen	App-SO3		Pear	Pear	Pear	Pear	Orchards	+	3
Sidi Othmen	Tom-SO4/Bar-SO4		Tomato	Barely	Barely	Barely	Vegetable crops	-	7
Pont Bizerte	Sor-PB1/Ber-PB1	N36º58'58.4''/ E 10º03'53.6''	Sorghum	Berseem	Berseem	Berseem	Vegetable crops	-	3
Pont Bizerte	Mai-PB2/Bar-PB2		Maize	Barley	Barley	Barley	Vegetable crops	-	1

S1 : Autumn ; S2 : Winter ; S3 : Spring ; S4 : Summer. * : Artichoke harvested (no irrigation). Treatment : Use of chemical treatment : ++ : high quantity of treatment ; + : low quantity of treatment and - : Absence of treatment.

RESULTS

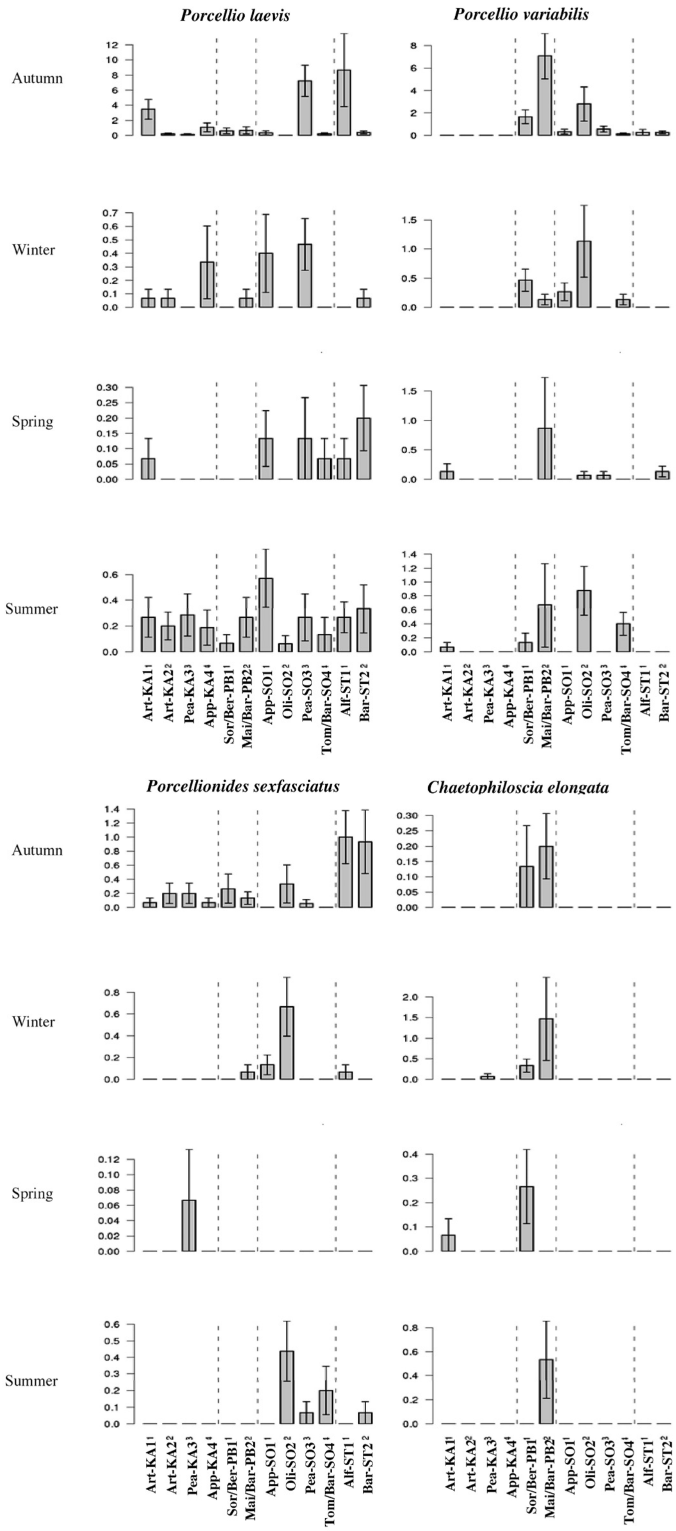
ACTIVITY-DENSITY, SPECIES RICHNESS AND DIVERSITY

A total of 889 isopods belonging to 8 species and 3 families (Armadillidiidae, Philosciidae and Porcellionidae) were trapped (Fig. 1). The Porcellionidae were the most numerous family (62.5 %) with five species, followed by the Armadillidiidae (25 %) and the Philosciidae (12.5 %), represented by two and one species, respectively. Some species were found in one or two plots only, e.g. *Armadillidium sulcatum* in Alfalfa (Alf-ST1), while others (*Porcellio laevis*, *Porcellio variabilis*, *Porcellionides sexfasciatus* and *Chaetophiloscia elongata*) were ubiquitous, recorded in all plots, sectors and seasons (Fig. 1). *Armadillidium tunisiense* was observed in the Sidi Othmen sector only. Isopod communities were markedly depleted in the market gardening system : half of species (*A. tunisiense, A. sulcatum, Lepthotrichus panzeri* and *Porcellionides pruinosus*) were not recorded in this type of cultivation. The plots of vegetable crops contained the richest diversity when compared with the other plots. Statistically significant difference between cultivation types was found only for *P. laevis* ($\chi^2 = 8.2$; $P = 0.01$). The total AD of Oniscidea (Fig. 2) fluctuated substantially, in autumn from [0.3 ± 0.8 in tomato plot (Tom-SO4) and 0.3 ± 0.6 in pear plot : (Pea-KA3)] to 10.4 ± 19.2 in alfalfa plot (Alf-ST1), whereas in winter, more than one-third of the plots had the lowest values of AD. In spring, the AD did not exceed 0.3. In summer, the olive orchard (Oli-SO2) plot had the highest value of AD. According to the plots and the cultivation, differences between seasons were statistically significant ($P_{plots} = 0.01$, $P_{cultivation} = 0.04$). The highest value of species richness (1.47 ± 1.06) was observed in autumn in the alfalfa (Alf-ST1) and maize (Mai-PB2) plot. The Friedman test (P = 0.0002, statistically significant) indicated that season affected species richness. Oniscidea diversity (Fig. 2) was quite high in autumn in maize plot (Mai-PB2) example of vegetables crops cultivation. However, diversity parameters decreased in the others seasons.

By the handsearch, seven species were collected : *Ch. elongata, P. laevis, P. variabilis, Ps. pruinosus, Ps. sexfasciatus, Ar. sulcatum* and *Ar. tunisiense*.

IMPACT OF SEASON, CULTIVATION TYPES AND SOIL CHARACTERISTICS ON ISOPOD DIVERSITY

Results of MCA analysis showed that the isopod diversity in autumn was different compared to the other seasons (Fig. 3a). According to the type of cultivation, MCA analysis revealed that vegetable crop (A) had similar isopods diversity as orchards (B) whilst market gardening (C) was clearly distinct and always characterized by the lowest levels of isopod diversity (Fig. 3b). This difference is statistically significant ($\chi^2 = 6.5$; $P = 0.04$) and the post-hoc test confirms the significant difference in the activity-density of isopods between vegetable crops and market gardening ($P = 0.03$). Soil moisture content was higher in the vegetable crop plots compared to orchards and market gardening (Tab. II). The Ca^{2+} content was higher in the alfalfa plot characterized by important number of species. Soil conductivity was higher in orchards than in other cultivation types. Furthermore, both the soil pH water and pH KCl did not differ between the studied plots. Carbon and nitrogen contents varied between all plots and within plots of the same type of cultivation. The PCA analysis summarized in Figure 4 showed that only *Armadillidium tunisiense* is related to the majority of soil parameters (Ca^{2+}, pH KCl, conductivity and soil moisture). However, *L. panzeri*, *C. elongata*, *P. variabilis* and *P. pruinosus* were correlated only with the Na^+ content.



Porcellio laevis
Porcellio variabilis
Porcellionides sexfasciatus
Chaetophiloscia elongata
Autumn
Winter
Spring
Summer
Art-KA1[1]
Art-KA2[2]
Pea-KA3[3]
App-KA4[4]
Sor/Ber-PB1[1]
Mai/Bar-PB2[2]
App-SO1[1]
Oli-SO2[2]
Pea-SO3[3]
Tom/Bar-SO4[4]
Alf-ST1[1]
Bar-ST2[2]

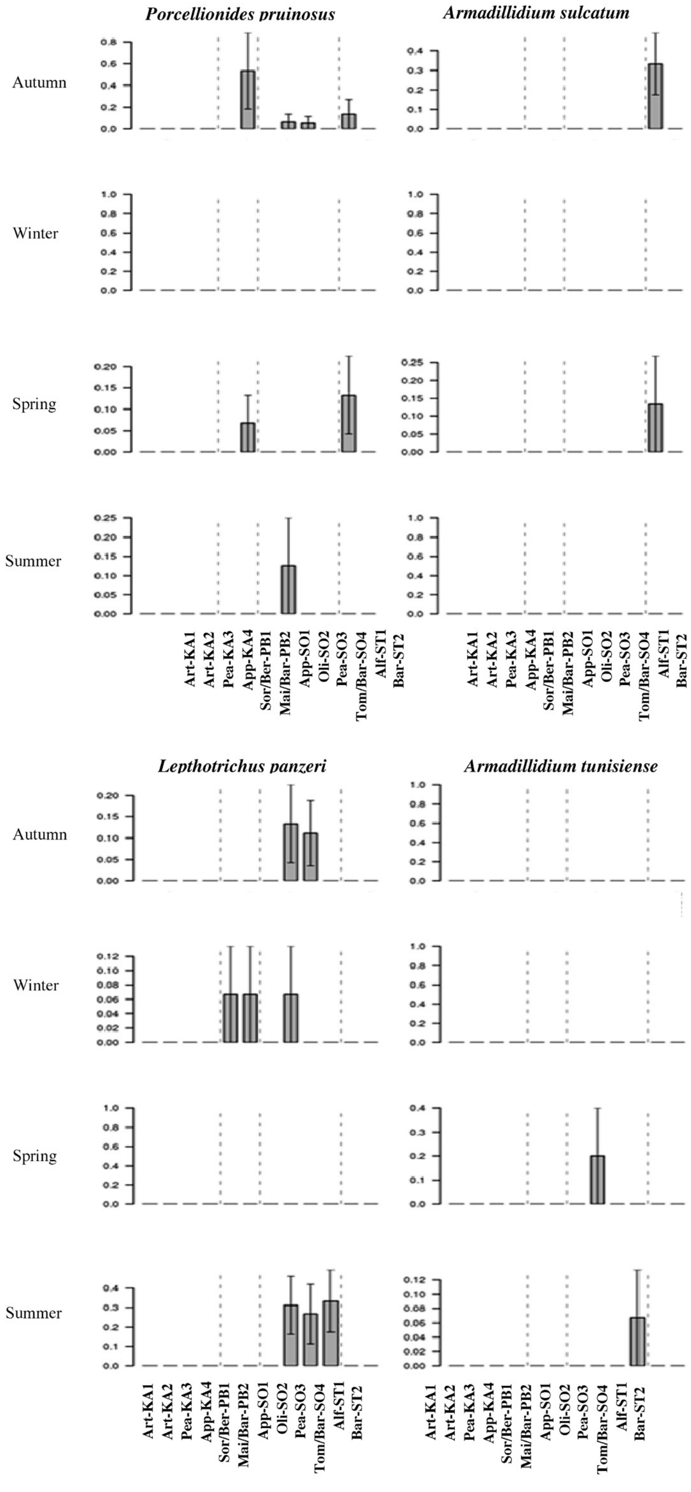


Figure 1.— Seasonal variation of activity-density (AD±SE) of species per trap in each studied plot.

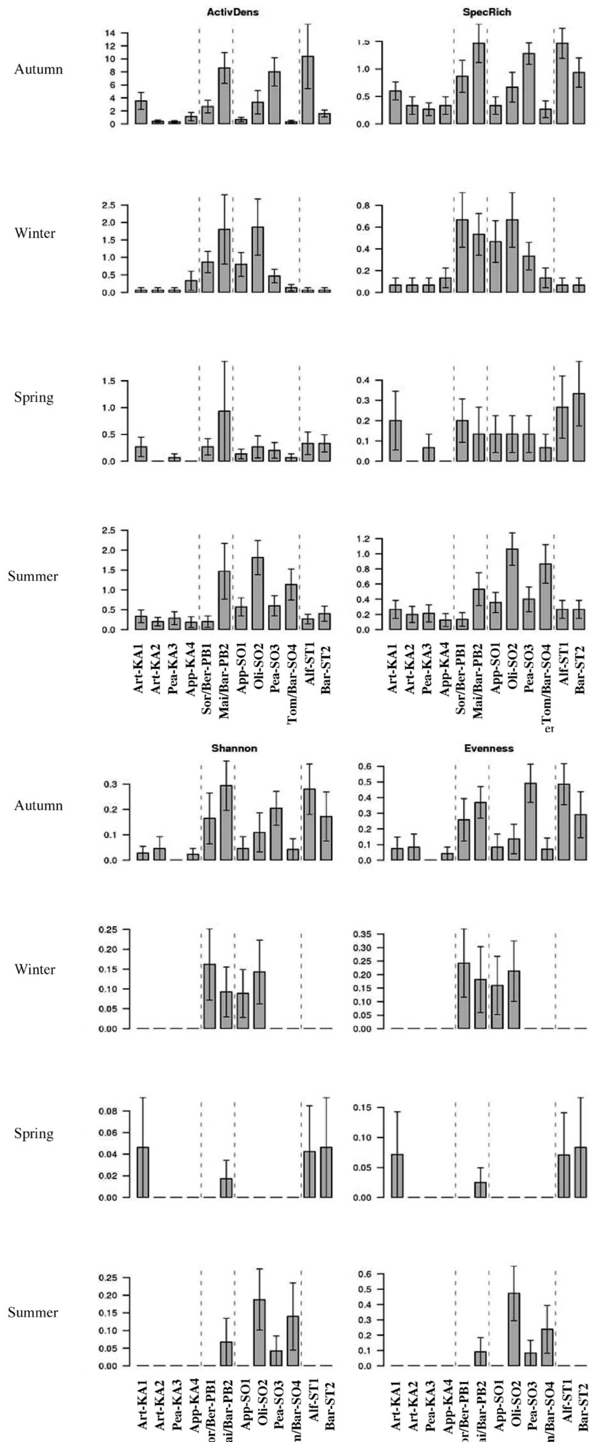


Figure 2.— Seasonal variation of average (±SE) of activity-density (Activ-Dens), richness (SpecRich), diversity (Shannon) and evenness of isopods per trap.

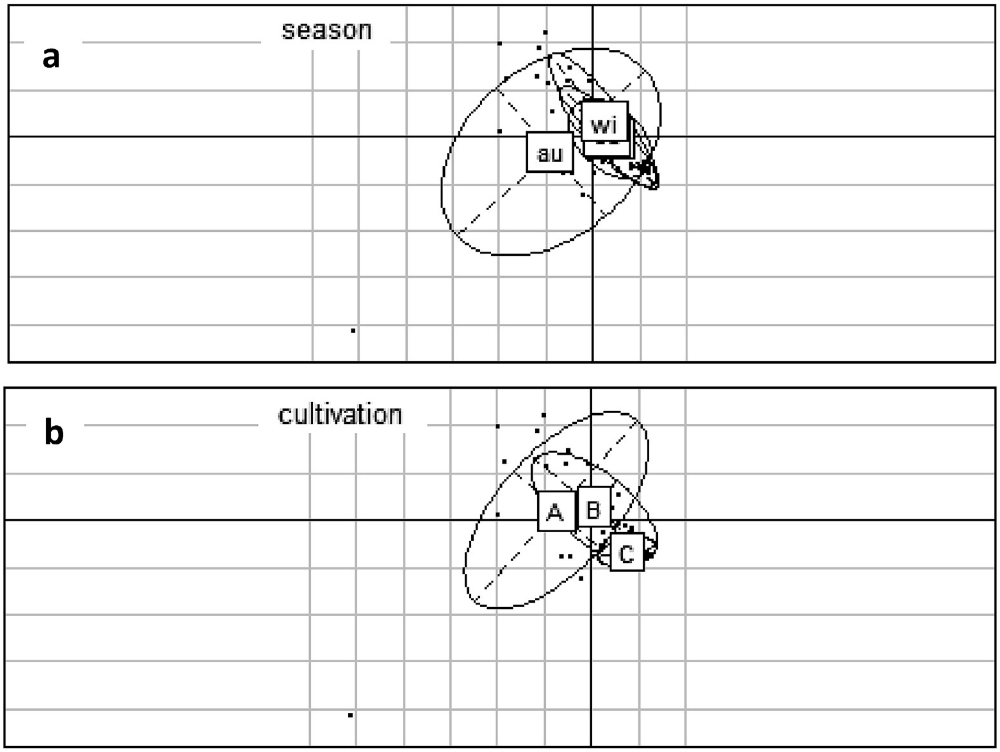


Figure 3.— Multiple component analysis (MCA) between (a) seasons : au : autumn, wi : winter, sp : spring, su : summer ; and (b) cultivation types : A : vegetable crops, B : orchards, C : market gardening.

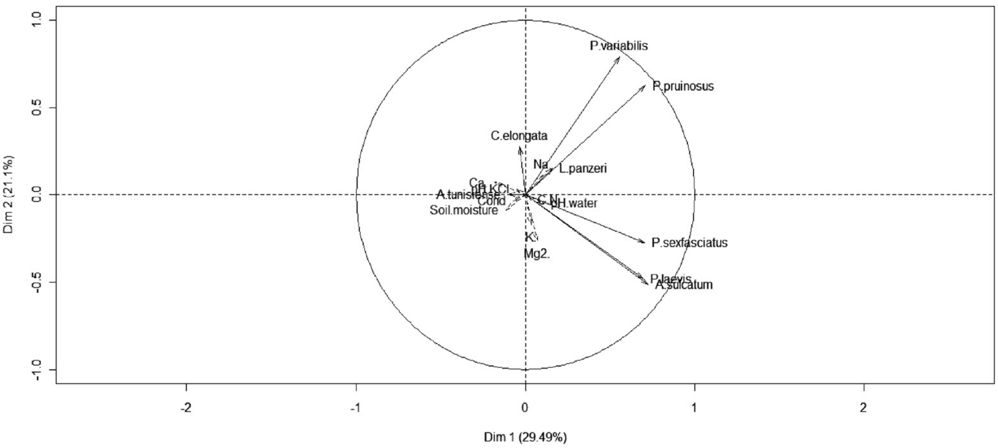


Figure 4.— Principal component analysis (PCA) between species and soil parameters.

TABLE II

Average values (±SE) of the physical and chemical soil characteristics in the studied plots (Cond : conductivity)

	Soil moisture (%)	pH water	pH KCl	Cond (µS/cm)	C/N	Na+	K+	Mg2+	Ca2+
Art-KA1	9.86±5.21	8.20±0.18	7.63±0.12	237±0.14	0.00±0.0	0.05±0.03	0.29±0.42	0.01±0.01	5.65±1.98
Art-KA2	8.77±5.49	8.12±0.13	7.60±0.07	236±0.09	15.10±24.34	0.16±0.08	0.08±0.03	0.03±0.01	2.97±1.87
Art-ST2	7.24±8.29	8.15±0.21	7.63±0.09	238±0.08	16.22±16.97	0.10±0.05	0.04±0.02	0.02±0.00	2.01±0.50
Alf-ST1	11.51±7.17	8.08±0.27	7.48±0.18	228±0.09	9.77±6.98	0.17±0.13	0.06±0.01	0.02±0.01	6.40±2.97
Ber-PB1	11.86±7.58	8.18±3.7	7.57±0.07	233±0.05	11.42±13.54	0.13±0.01	0.10±0.03	0.03±0.00	3.62±1.93
Bar-PB2	13.88±7.73	8.26±0.22	7.61±0.16	246±0.11	11.01±17.09	0.09±0.09	0.04±0.01	0.02±0.00	4.80±4.26
Bar-SO4	12.23±5.17	8.07±0.18	7.67±0.09	242±0.11	1.93±3.87	0.12±0.07	0.04±0.01	0.01±0.00	2.69±2.17
Pea-KA3	7.21±2.04	8.14±0.17	7.67±0.09	625±12.3	7.98±7.50	0.08±0.05	0.05±0.01	0.02±0.01	3.75±3.51
App-KA4	8.14±4.07	8.02±0.27	7.66±0.12	804±1.31	10.23±15.55	0.15±0.08	0.08±0.01	0.02±0.01	2.94±2.72
App-SO1	8.26±5.05	8.27±0.38	7.51±0.10	237±0.07	2.28±4.56	0.05±0.01	0.05±0.03	0.01±0.00	3.55±4.01
Oli-SO2	7.58±3.97	7.96±0.20	7.67±0.07	915±2.4	4.93±9.85	0.15±0.07	0.05±0.01	0.03±0.01	4.22±0.79
Pea-SO3	6.95±2.65	8.19±0.22	7.57±0.05	239±4.7	2.03±4.07	0.18±0.16	0.05±0.03	0.02±0.01	3.51±1.92

DISCUSSION

In Majerda agroecosystems, a relatively moderate number of isopods (8 species) was recorded, compared to other natural habitats. Recently, Achouri *et al.* (2008a) collected 12 terrestrial isopod species in natural and uncultivated habitats in the Berkoukech catchment area (Kroumirie), in the North-West of Tunisia. The investigation of Hamaïed-Melki *et al.* (2011) showed 11 species recorded in the North Western Wadi Moula-Bouterfess catchment area (Kroumirie). Nevertheless, differences in species richness between the cultivated and natural regions are mainly attributable to the fact that isopods are particularly sensitive to anthropogenic changes in habitat quality (irrigation system, rotation and cultivation types) caused by agricultural activities. Compared with previous studies on the isopod diversity in cultivated area in different region of the world, species richness is moderately high in the Majerda low plain. Indeed, 3 species are identified in a cultivated field in the Netherlands (Paoletti & Hassall, 1999), 7 species in the grasslands of Poitou-Charentes (France) (Souty-Grosset *et al.,* 2005a) and 13 species on seven plots of maize monocultures in North-eastern Italy (Paoletti & Hassall, 1999). The most encountered species in the present study were *P. laevis* and *P. variabilis* as they were sampled in all plots and seasons. These species were already considered by Medini & Charfi-Cheikhrouha (1998) and Medini-Bouaziz (2002) as widespread species in Tunisia (from North to South), colonizing different types of habitats. In natural ecosystem in North Tunisia, *P. variabilis* is the most abundant species whereas *P. laevis* is absent (Achouri *et al.,* 2008a ; Hamaïed-Melki *et al.,* 2011). *P. sexfasciatus* and *C. elongata* are captured in all seasons but with low numbers of individuals. In the natural environment in Tunisia, *C. elongata* is found in many habitats. Further, *P. sexfasciatus* is abundant in a grazed meadow (Achouri *et al.,* 2008a ; Hamaïed-Melki et *al.,* 2011). All other species (*P. pruinosus*, *L. panzeri*, *A. tunisiense* and *A. sulcatum*) are poorly represented and their presence varied with seasons. It has been demonstrated that the dominant woodlice species changed according to the time of the year (Souty-Grosset *et al.,* 2005b). Compost heaps sometimes host high numbers of *P. pruinosus*. This species occurs in all types of habitats, except too cold or too humid ones (Vandel, 1962). However, cultivated areas do not provide favourable conditions (too high humidity). Compost provides optimal temperature and humidity and allows high reproductive activity for isopods (Juchault *et al.,* 1985). The presence of *A. tunisiense* and *A. sulcatum* during the spring and in the moist soil confirms that these species prefer habitats with a high humidity (Hamaïed-Melki, 2008).

Moreover, terrestrial isopods are not evenly distributed among the different types of cultivation ; they varied in numbers of species as well as individuals. Our results showed that isopods are more abundant in vegetable crops than in orchards and market gardening cultivations. This could be explained by the lowest use of chemical treatments and agricultural practices in the vegetable crops compared to orchards and market gardening (Personal communication from the farmers). In market gardening there is intensive weeding in the fields. This is one of the harsh environmental conditions that can affect woodlice. Thus, in these continuously weeded plots, the decrease in diversity and quality of food can explain the recorded low diversity of isopods. Sfenthourakis *et al.* (2005) and Zimmer (2002) showed that the diversity of terrestrial isopods varied with food quality (vegetation). Same results were obtained by Fraj *et al.* (2010), that abundance and species richness of woodlice are higher in the alfalfa (vegetable crop) than among artichokes (market gardening).

In this study, *P. laevis*, *P. variabilis*, *P. sexfasciatus* and *C. elongata* are common in all studied cultivation types. These species are capable of colonizing different types of habitats and may be insensitive to agricultural practices. The Philosciidae species *C. elongata*, like *Philoscia muscorum* in France (Souty-Grosset *et al.,* 2005a), is recorded in different types of cultivation. The four other species are differently represented. Indeed, *P. pruinosus* and *L. panzeri* are recorded in the vegetable crops and orchards. These species are colonizing many habitats in Tunisia and may be sensitive to intensive agricultural activity and their abundance declined in market gardening cultivation. In fact, a negative effect of the mode of exploitation of plots on the abundance of terrestrial isopods has been documented (Paoletti & Hassall, 1999). *A. sulcatum* and *A. tunisiense* are present only in vegetable crops and orchards, respectively. *A. sulcatum*, characterized by morphological and ecological variability in Tunisia (Hamaïed-Melki, 2008), is also sensitive to plot disturbance. Achouri *et al.* (2008b) reported that this species is present in many natural habitats in Tunisia, but is absent in a grazed meadow.

In general, some authors such as Altieri (1999) and Cortet *et al.* (2002) showed that the presence and absence of arthropods species could depend on the cultivation types and other cultural practices, mainly the use of chemical fertilizers and pesticides. Woodlice are very influenced by variations in habitat structure and floristic composition which are affected by agricultural practices (Davis, 1984). However, in the Mediterranean region, few studies and published works have discussed the effect of type of cultivation on terrestrial isopod diversity.

The isopod community is affected by many other factors, for example the type and structure of soil (Souty-Grosset *et al.,* 2005a). In this study, the soil parameters considered did not correlate closely with the distribution of woodlice. As our studied plots did not include an acid soil the full preferences of isopod species could not be recorded. However in the literature, many authors (Sastrodiharjdo & Van Straalen, 1993 ; Zimmer & Topp, 1997) mentioned that isopods react to variations in pH and some species such as *A. vulgare* and *A. pelagicum* may be used as indicators of acidification (Sastrodiharjdo & Van Straalen, 1993 ; Souty-Grosset *et al.,* 2005a). The distribution of *Armadillidium* species is further influenced by soil conductivity and water content. *A. sulcatum* is found in soil with high conductivity while *A. tunisiense* is observed only in soil where the conductivity is low. Both species depend on habitat moisture (Hamaïed-Melki, 2008). Otherwise, from this study it is apparent that isopods do not have a preference related to Na^+, K^+ and Mg^{2+} factors. However, the Ca^{2+} content is highest in the alfalfa plot characterized by the higher number of species. This element is important in the constitution of isopods cuticle during the moulting cycle. The C/ N ratio is optimal in the alfalfa cultivation (high number of species and the presence of *A. sulcatum* species), indicating a good activity of soil fauna and degradation of organic matter. Some studies (Frouz *et al.,* 2007 ; Antunes *et al.,* 2008) showed that the C/ N ratio is the abiotic parameters with the greatest influence on the variation of macroarthropod abundance and its presence/ absence among habitats.

In conclusion, from this preliminary study evaluating the seasonal diversity of woodlice related to cultivation types and some soil parameters, our results suggest that isopod diversity can be used to qualify the soil health and its production capacity, as are ants for olive grove agroecosystems (Santos *et al.,* 2007). A clear difference in species richness, composition and abundance is observed. The vegetable crop is the most favourable type of cultivation to isopod diversity. In this cultivation, *A. sulcatum* is found in calcium-rich soil, characterized by a good fauna activity determined by the optimum content of C/ N ratio. This species may be useful to evaluate the impacts of some agricultural practices on the soil fauna. We thus conclude that changes in land-use had a major impact on the isopods communities. The close relationship between soil characteristics and isopods abundance and diversity in these agroecosystems indicate that a powerful management tool for maintaining abundant isopods in agricultural area would be to provide an adequate amount of chemical treatments of the soil. Further work is in progress to verify the most important factors influencing isopod diversity and species preferences in agricultural areas.

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