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Diversity of plant-parasitic nematode communities infesting olive orchards in Tunisia in relation to agronomic factors

Ilhem Guesmi-Mzoughi · Mariem Tabib · Farah Sellami · Fathia Hadj-Naser · Hajer Regaieg · Sadreddine Kallel · Najet Horrigue-Raouani

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Abstract Tunisian olive cultivation constitutes one of the principal economical and agricultural strategic sectors. In order to increase olive production, the olive management systems are changing towards intensification with irrigation, the introduction of new varieties, the use of intercropping, and high inputs of pesticides and fertilizers. These practices may create an environment more favorable to soil borne pathogens and plant-parasitic nematodes. Therefore, this study was performed to explore for the first time the plant-parasitic nematodes infesting olive roots and their diversity in the main producing areas of olive in Tunisia including 123 olive orchards. It aims also to determine which agronomic factors influence the multiplication and the diversity of plant-parasitic nematode communities. These investigations identified 11 genera of plant-parasitic nematodes viz. *Criconemoides* spp., *Helicotylenchus* spp., *Heterodera* spp., *Meloidogyne* spp., *Paratylenchus* spp., *Pratylenchus* spp., *Rotylenchulus* spp., *Rotylenchus* spp., *Tylenchorhynchus* spp., *Tylenchus* spp., and *Zygotylenchus* spp. It is revealed that the intensification of olive orchards with irrigation and the association of intercrops are the main agronomic factors influencing the multiplication and the diversity of plant-parasitic nematodes infecting olive trees. In particular, olive orchards under super-intensive regimes are more conducive to the multiplication of *Pratylenchus* spp. while the presence of irrigated intercrops enhances the multiplication of *Meloidogyne* spp.. Therefore, for the establishment of new olive orchards, it is suggested to choose certified olive plants and avoid infested soils or intercrops that can host dangerous nematodes.

Keywords Olive orchards · Plant-parasitic nematodes · Intensification · *Pratylenchus* spp. · *Meloidogyne* spp.

I. Guesmi-Mzoughi (✉) · F. Hadj-Naser · H. Regaieg · N. Horrigue-Raouani
Université de Sousse, Institut Supérieur Agronomique de Chott Mariem, UR13AGR04-Développement de la Protection Biologique et Intégrée au niveau de la parcelle en agriculture biologique, Département des Sciences Biologiques et Protection des Plantes, 4042 Sousse, Tunisia
e-mail: guesmiilhem@yahoo.fr

M. Tabib · F. Sellami · H. Regaieg · S. Kallel
Université de Carthage, National Agronomic Institute of Tunisia, LR14AGR02, Laboratoire de Recherche Bio-agresseur et Protection Intégrée en Agriculture 2049, Ariana, Tunisia

Introduction

The center of origin of olive trees is in the Eastern Mediterranean regions (Besnard et al., 2011). Even now, olive cultivation constitutes a key element of the Mediterranean agricultural sector and Mediterranean countries dominate world olive oil and table olive production and consumption (Blondel et al., 2010). Tunisia is the largest southern Mediterranean country producing olive and olive oil (Gharbi et al., 2014) and it is the fourth largest producer worldwide after Spain, Italy and Greece (Jackson et al., 2015). During 2017–2018, the Tunisian olive oil production reached 280,000 tons

(ONAGRI, 2018). The competitiveness of Tunisian olive oil production could be related to a significant expansion of area planted. Olive trees intended for oil production extend over 1.8 million hectares which constitute 79% of the surface dedicated to fruit farming and 34% of crop lands (Gharbi et al., 2014). The olive growing areas spread from the northern to the southern regions, where a wide range of edaphoclimatic conditions prevail. In the North and in some regions of the Center, olives are cultivated in association with other annual cultures or fruit trees, whereas in the South they are exclusively cultivated in monoculture (COI, 2017). These plantations are mainly extensive due to the weather conditions of Tunisia, known by the weakness and irregularity of rainfall (Gharbi et al., 2014). Nevertheless with irrigation two other systems of plantation are available, intensive and super-intensive (Allalout & Zarrouk, 2013).

The intensification of olive crops may disturb the agro-ecosystem (Loumou & Giourga, 2003) and make olive trees more susceptible to some diseases (Trapero et al., 2009) such as plant-parasitic nematode diseases (Castillo et al., 2010; Nico et al., 2002). Research has revealed that olive trees serve as a suitable host plant for highly diversified plant-parasitic nematode communities, including endoparasitic and ectoparasitic species. Over 250 species have been detected in association with the olive rhizosphere in many countries of the Mediterranean Sea and worldwide (Ali et al., 2014; Ali et al., 2017; Archidona-Yuste et al., 2019; Archidona-Yuste, Cantalapiedra-Navarrete, et al., 2020a; Palomares-Rius et al., 2015). The root-knot nematodes (*Meloidogyne* spp.) and root-lesion nematodes (*Pratylenchus* spp.) are widely distributed through olive soils, especially in nurseries and intensive cropping systems where irrigation conditions are favorable to their multiplication (Aït Hamza et al., 2018; Ali et al., 2014; Ali et al., 2016; Ali et al., 2017; Archidona-Yuste et al., 2018; Archidona-Yuste, Cantalapiedra-Navarrete, et al., 2020a; Castillo et al., 2010; Nico et al., 2002; Palomares-Rius et al., 2015). These two nematodes are considered the most damaging plant-parasitic nematodes of cultivated olive (Ali et al., 2014; Castillo et al., 2010; Nico et al., 2002; Nico et al., 2003). Conversely, other harmful species of nematode to olive such as *Heterodera mediterranea*, *Tylenchulus semipenetrans*, *Rotylenchulus macrosoma* and *R. macrodoratus* are less distributed in olive orchards (Ali et al., 2014; Ali et al., 2017; Archidona-Yuste, Cantalapiedra-Navarrete, et al., 2020a; Castillo et al., 2010; Palomares-Rius et al., 2015).

The diversity and structure of plant-parasitic nematode communities infesting olive trees could be influenced by different environmental (soil physicochemical properties and climate, and substrate origin in nurseries) and agronomic factors (orchard and soil management systems, irrigation regime, olive cultivar and age of plantation) (Aït Hamza et al., 2018; Ali et al., 2017; Archidona-Yuste, Wiegand, et al., 2020b; Aït Hamza et al., 2015; Benkovic-Lacic et al., 2016; Palomares-Rius et al., 2015). In fact, in olive orchards, nematode abundance and diversity of plant-parasitic nematode communities were influenced by olive cultivar (Chafaa et al., 2014; Palomares-Rius et al., 2015), season (Chafaa et al., 2014) and orchard and soil management practices (Ali et al., 2017; Aït Hamza et al., 2015; Benkovic-Lacic et al., 2016; Palomares-Rius et al., 2015). However, soil physicochemical properties were the main factors driving the structure of these communities (Aït Hamza et al., 2015; Palomares-Rius et al., 2015). In nurseries, the diversity and structure of plant-parasitic nematode communities were affected mainly by climate (rainfall and minimum temperature) (Aït Hamza et al., 2018). Otherwise, recent studies exploring spatial structure in the diversity of plant-parasitic nematode communities revealed that soil was the most influential factor driving these communities while agronomic management practices showed less influence then expected (Archidona-Yuste, Wiegand, et al., 2020b).

In Tunisia, the investigation of plant-parasitic nematode communities associated with cultivated olive has still not been undertaken except for some taxonomic studies (Guesmi et al., 2016; Guesmi-Mzoughi et al., 2016; Guesmi-Mzoughi et al., 2017; Palomares-Rius et al., 2014). The works done until now have described a new species *Pratylenchus oleae* which was detected on cultivated olives suffering from tree decline (Palomares-Rius et al., 2014). Some other species were detected for the first time on cultivated olive such as *Rotylenchus incultus* and *R. eximius* (Guesmi-Mzoughi et al., 2016) and *Longidorus euonymus*, *Longidorus glycines*, *Xiphinema conurum*, *Xiphinema meridianum* and *Xiphinema robbinsi* (Guesmi-Mzoughi et al., 2017). Symptoms of nematode attacks have mainly appeared in intensified orchards, and it is still difficult to convince farmers about nematode parasitism. Therefore, this current study aims to: (i) identify and determine the population densities of plant-parasitic nematodes infesting olive trees in the main producing area in Tunisia; (ii) evaluate the effect of agronomic characteristics (including degree of intensification, irrigation regimen and olive cultivar) and soil type of the studied olive orchards on the diversity of plant-parasitic nematode communities.

Material and methods

Olive orchards description and sampling

This study was conducted in 123 commercial olive orchards located in the main olive production regions in Tunisia: Beja, Zaghouan and Nabeul in the North, Sousse, Monastir, Mahdia (Sahel regions), Kairouan, Kasserine, Sfax and Sidi bouzid in the Center. For each department, at least two olive orchards were sampled: about 20 in each of Nabeul, Beja and Zaghouan, 18 in Sahel regions and 44 in the other departments (Fig. 1). The different agronomic characteristics (including degree of intensification, irrigation regimen and olive cultivar) and soil type of the studied olive orchards are presented in Table 1. The degree of intensification in olive orchards were differentiated into three categories including: (i) traditional olive orchards which are mostly rainfed and sometimes irrigated manually when intercrops particularly barley and bean were present, with low plant density (less than 100 olive trees/ha), local varieties, low inputs in fertilizer and pesticides, and manual harvesting, (ii) intensive orchards with drip irrigation, high plant density (around 200 olive trees/ha), local varieties, sometimes intercrops such as legumes (potato, tomato, cucumber and pepper) or fruit crops (pomegranate, almond and peach trees) were present, high inputs in fertilizer and pesticides, and manual or mechanical harvesting, and (iii) super-intensive orchards with drip irrigation, very high plant density (between 1250 and 1666 olive trees/ha), imported varieties, no intercropping, high inputs in fertilizer and pesticides, and mechanical harvesting. Olive orchards were also classified according to soil intensification with irrigation and the presence of intercrops. Additionally, the soil type was analyzed by a private company "Food Quality" in Tunisia.

Samples were collected in the spring and autumnal periods of 2013, 2014 and 2015. Depending on the size, soil and agronomic characteristics of each olive orchard, 3 to 30 trees were randomly selected for sampling. Active roots were collected with a shovel under the canopy of each olive tree from 5 to 40 cm depth, discarding the upper 5 cm of the soil profile. This ensured that roots from weeds or other herbaceous plants were unlikely to be sampled. Four sub-samples were collected from each tree and were thoroughly mixed to obtain a single representative sample per tree. In total, 717 root samples were collected from 123 olive orchards. The number of sampled olive orchards per region, soil and agronomic characteristics is described in Table 1.

All studies carried out until now on olive nematode communities were interested in soil nematodes as bio-indicators of the disturbance of the soil environment (Ali et al., 2017; Aït Hamza et al., 2015; Palomares-Rius et al., 2015). However, to our knowledge, there is no study that has used root nematodes in such subjects, although plant roots can have net effects on the soil microbial community structure and functioning (Bonkowski et al., 2009). Therefore, this study will use, for the first time, plant roots to describe plant-parasitic nematode communities infesting olive trees.

Nematode extraction, identification and quantification

Nematodes were extracted from a 10 g sample of each representative root sample using a sugar centrifugal-flotation method (Coolen, 1979). Before extraction, roots were washed free of soil and cut into small pieces. Plant-parasitic nematodes were identified at the genus level and enumerated under a stereomicroscope at 80x magnification using a hand-tally counter. The nematode identification was based on morphological traits of each genus (Mai & Mullin, 1996; Nickle, 1991; Siddiqi, 2000). When necessary, observations of specimens under a microscope were carried out to distinguish between closely related genera.

Identified plant-parasitic nematodes were separated according to their feeding strategies into three groups: 1) ectoparasites, including migratory ectoparasites of roots that can feed in the cortical tissues (eg., *Helicotylenchus*, *Rotylenchus*, *Criconemoides*, *Paratylenchus*), root surface tissue feeders (eg., *Tylenchorhynchus*) and algal, lichen or moss feeders that feed by piercing (eg., *Tylenchus*); 2) migratory endoparasites (eg., *Pratylenchus*, *Zygotylenchus*) and 3) sedentary endoparasites (eg. *Meloidogyne*, *Heterodera*) also including

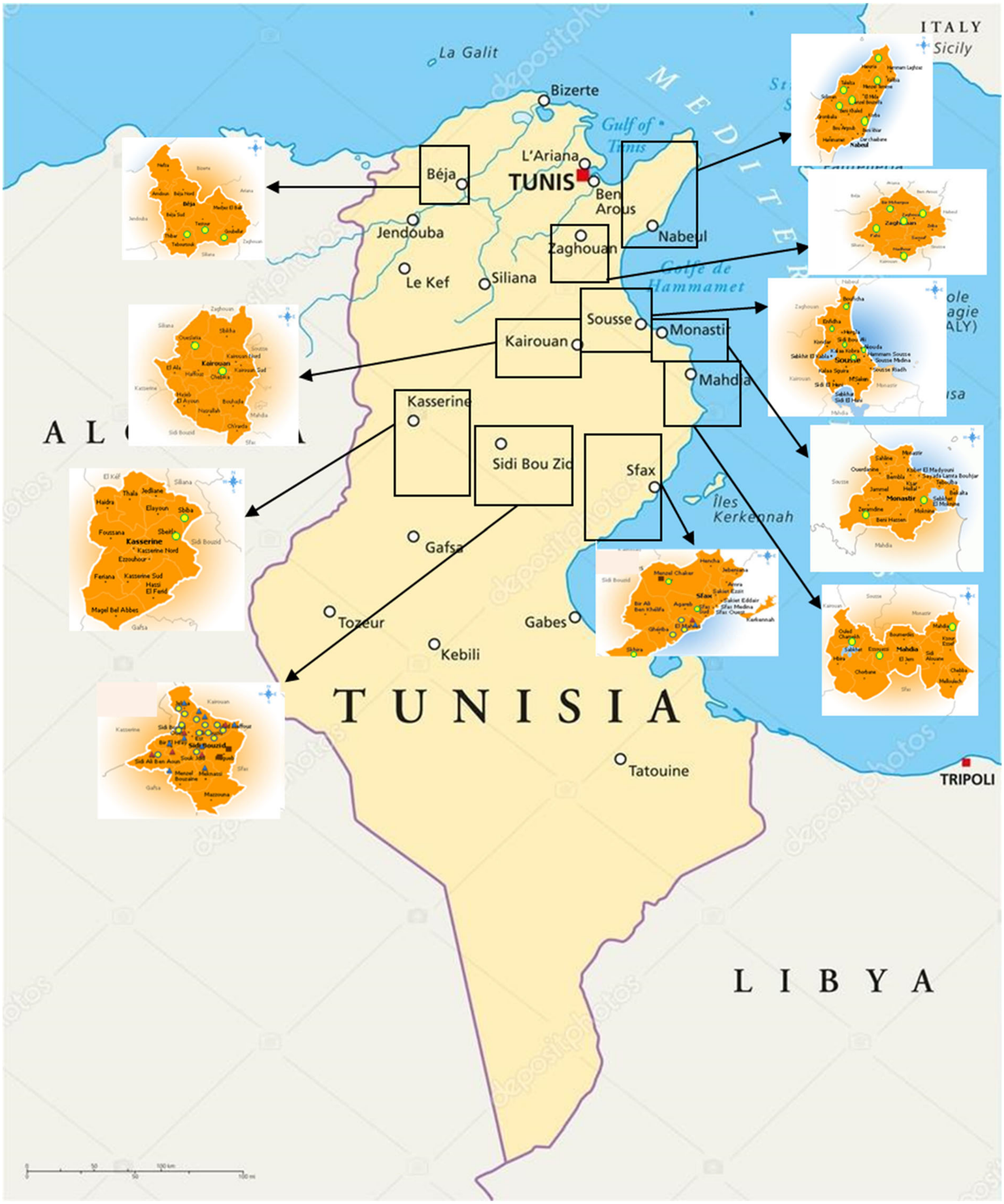

Fig. 1 Map of olive sampling sites in Tunisia (Color should be used in print). (Green circles corresponds to sampling sites in each region)

sedentary semi-ectoparasites (eg. *Rotylenchulus*) (Siddiqi, 2000; Yeates et al., 1993).

The number of specimens of each genus was determined from 10 g of root sample. This includes the

Table 1 soil and agronomic characteristic of olive sampling sites

		Beja	Zaghouan	Nabeul	Sousse	Mahdia	Monastir	Sfax	Sidi Bouzid	Kairouan	Kaserine
Soil type	sandy	0*	1	5	6	2	1	8	10	1	2
	sandy-clay	20	20	15	4	1	1	2	10	3	0
	clay	0	0	0	1	2	0	0	8	0	0
Irrigation	rainfed	7	0	20	6	2	0	1	8	1	0
	irrigated	13	21	0	5	3	2	9	20	3	2
Intensification	Traditional	7	0	20	7	2	1	4	25	1	0
	intensive	0	1	0	4	2	1	4	2	3	2
	super-intensive	13	20	0	0	1	0	2	1	0	0
Intercrops	not	20	19	20	9	4	1	6	15	1	0
	yes	0	1	0	2	1	1	4	13	3	2
Variety	meski	0	0	0	0	0	1	0	0	0	0
	chemleli	7	1	20	11	4	1	8	26	4	2
	arbosana	0	0	0	0	0	0	1	1	0	0
	arbequina	13	20	0	0	0	0	0	1	0	0
	koroneiki	0	0	0	0	1	0	1	0	0	0

*Each value presents the total number of olive orchards for the corresponding soil and agronomic characteristic

number of juveniles, adults and sedentary stages for endoparasites. The prevalence of each genus was calculated as the percentage of samples where this genus was detected.

Finally, the density of plant-parasitic nematodes was calculated per olive orchard as the average of the nematode density determined from all samples in the same site, and expressed as the nematode abundance.

Nematode diversity indexes

Several indexes were considered to assess the abundance and diversity of the nematode communities: the total number of plant-parasitic nematodes per 10 g of roots (N) (Korenko & Schmidt, 2006); genera richness (G) which is the total number of genera per 10 g of roots (Korenko & Schmidt, 2006); relative abundance (Ar) (**Ar = n *100/N**) where (n) is the number of specimens per group of plant-parasitic nematode (ectoparasites, migratory endoparasites and sedentary endoparasites) (Dajoz, 1971) and the Shannon-Wiener diversity index (H′) ($\boldsymbol{H' = -\sum_i^G \left(\frac{ni}{N}\right) * ln\left(\frac{ni}{N}\right)}$) (Shannon & Weaver, 1949).

These indexes were calculated using PRIMER6 software (Clarke & Warwick, 2001) except for relative abundance which was calculated in an excel file.

Data analyses

In order to explore the effects of soil and agronomic factors (irrigation regimen, intensification, intercrops and variety) on the diversity of plant-parasitic nematode communities, redundancy analysis (RDA) (Borcard et al., 2018), non-parametric tests and ANOVA analysis were performed using the *vegan* library with R version 4.1.2 software and SPSS® 16.0. software, respectively. In these analyses, each olive orchard was considered as statistical unit.

Before analysis, data were tested for normality and homogeneity of variances by Kolmogorov-Smirnov and Levene test at 5%, respectively.

Two redundancy analyses (RDA) were performed. Categorical variables (irrigation regimen, intensification, intercrops and variety) were converted to dummy variables before analysis. For the first RDA, nematode abundances were used as the response variables, and agronomic factors and soil texture were used as explanatory variables. Two matrixes were used. The abundance matrix was composed of sampled olive orchards in rows and nematode genera in columns, while the matrix of explanatory variables was composed of sampled olive orchards in rows and agronomic factors and soil texture in columns. For the second RDA, ecological indexes were used as response variables with the same explanatory variables. Then the ecological diversity matrix was composed of sampled olive orchards in rows and ecological indexes in columns. (Borcard et al., 2018; Renčo et al., 2020).

Furthermore, a two-way ANOVA was performed to analyze the effect of intensification with soil type, intensification with intercrops and soil type with intercrops on nematode abundance and ecological indexes. When a significant interaction for independent variables was observed, one-way ANOVA was performed, with a post-hoc Duncan test considering dummy variables. Conversely, when there was no interaction between factors, two one-way ANOVA of each factor was followed by the same posthoc test at probability 5% were performed. In addition, data corresponding to nematode densities and ecological indexes were compared according to the degree of intensification of olive orchards, intercrops and soil type using the non-parametric tests Kruskal-Wallis and Mann-Whitney at 5% probability.

For RDA and two-way ANOVA analyses, data were converted to $\log_{10}$ (X + 1) before analysis to improve normality.

Results

Diversity of plant-parasitic nematodes associated with olive roots

Identification studies based on morphological traits identified 11 genera of plant-parasitic nematodes associated with olive roots in the 123 olive orchards visited. Most of them were ectoparasites: *Criconemoides* spp., *Helicotylenchus* spp., *Paratylenchus* spp., *Rotylenchus* spp., *Tylenchorhynchus* spp. and *Tylenchus* spp.. The group of sedentary endoparasitic nematodes was represented by three genera that are *Heterodera* spp., *Meloidogyne* spp. and *Rotylenchulus* spp.. In addition, *Pratylenchus* spp. and *Zygotylenchus* spp. were identified as migratory endoparasites (Table 2).

The most prevalent genera of plant-parasitic nematodes identified on root samples were, in decreasing order, *Helicotylenchus* spp., *Pratylenchus* spp. and *Meloidogyne* spp.. However, *Meloidogyne* spp. and *Pratylenchus* spp. were the most abundant nematodes (Table 2).

Effect of soil and agronomic characteristics on the diversity of plant-parasitic nematode communities

The first RDA was performed to evaluate the effect of soil and agronomic factors on the composition of the plant-parasitic nematode communities (Fig. 2). The corresponding RDA model was highly significant ($p = 0.001$) as were the canonical axes RDA1 and RDA2 which explained 16.3% and 9.6% of the variance, respectively (Supplementary Table 1). As illustrated in Fig. 2, the composition of plant-parasitic nematode communities was discriminated among soil and agronomic factors. The abundances of *Pratylenchus* spp., *Paratylenchus* spp. and *Meloidogyne* spp. were positively correlated with the irrigation regimen and intercrops. However, the abundance of *Pratylenchus* spp. was negatively correlated with the level of intensification and olive variety. *Helicotylenchus* spp. was positively correlated with the soil type (Fig. 2).

In order to explore the diversity of plant-parasitic nematode communities in relation to soil and agronomic factors, a second RDA was performed (Fig. 3). Its model was highly significant ($p = 0.001$) as were the canonical axes RDA1 and RDA2 which explained 18.8% and 6% of the variance, respectively (Supplementary Table 2). The RDA biplot showed that soil and agronomic factors have an effect on the diversity of plant-parasitic nematode communities. In fact, total nematode number (N), genera richness (G), local diversity (H′), the relative abundance of sedentary endoparasite nematodes, and the relative abundance of migratory endoparasites were positively correlated with the presence of intercrops in irrigated soils. However, the relative abundance of migratory endoparasites was negatively correlated with the level of intensification. However, the relative abundance of ectoparasites was positively correlated with soil type and variety (Fig. 3).

For more detailed information, one-way non-parametric ANOVA (Kruskal-Wallis test, Mann-Withney test) was performed (Tables 3 and 4). Results showed that the intensification of olive farming induced the increase in the total number of plant-parasitic nematodes (N) on olive roots, linked especially to the multiplication of *Pratylenchus* spp. and *Meloidogyne* spp. in intensive cultivations (Table 3). However the abundance of *Meloidogyne* spp. seems to be related to the intensive orchards in the presence of intercrops, while

Table 2 Importance of plant-parasitic nematodes identified on olive roots

Nematode genera	Number of samples*	Average density*	Minimum*	Maximum	Prevalence (%)
Criconemoides spp.	83	6,53	1	20	11,58
Helicotylenchus spp.	394	77,35	1	6390	54,95
Heterodera spp.	30	133,87	2	650	4,18
Meloidogyne spp.	116	857,59	2	19,410	16,18
Paratylenchus spp.	105	173,14	2	1550	14,64
Pratylenchus spp.	248	420,08	1	9650	34,59
Rotylenchulus spp.	20	6,80	1	18	2,79
Rotylenchus spp.	18	12,56	1	50	2,51
Tylenchorhynchus spp.	9	270,44	4	570	1,26
Tylenchus spp.	25	150,80	10	820	3,49
Zygotylenchus spp.	17	12,41	1	60	2,37

*The number of samples, average density, minimum and maximum numbers of nematodes are calculated on samples were this genera was detected

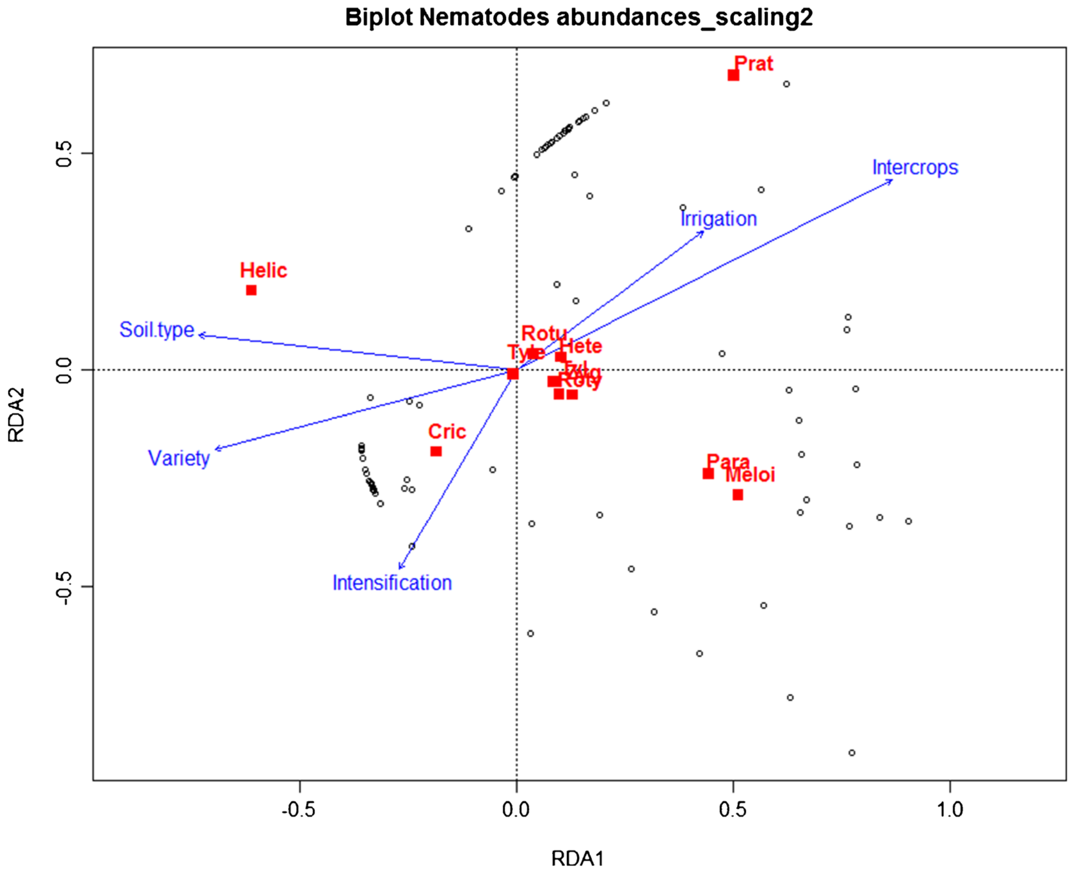

Fig. 2 RDA biplots of the relationship between nematode abundances and soil and agronomic factors (Color should be used in print). (Cric:*Criconemoides*; Helic:*Helicotylenchus*; Hete:*Heterodera*; Meloi:*Meloidogyne*; Para:*Paratylenchus*; Prat:*Pratylenchus*; Rotu:*Rotylenchulus*; Roty:*Rotylenchus*; Tyle:*Tylenchorhynchus*; Tyl:*Tylenchus*; Zyg:*Zygotylenchus*)

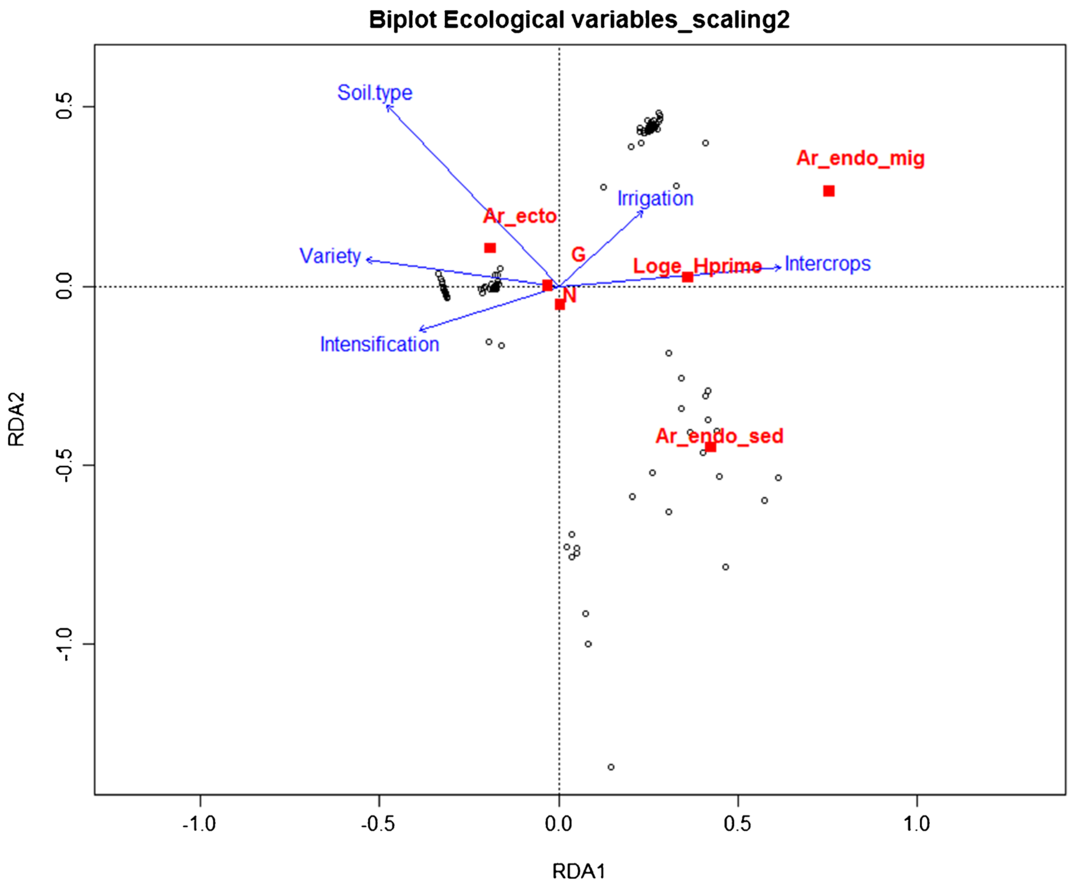

Fig. 3 RDA biplots of the relationship between nematode ecological indexes and soil and agronomic factors (Color should be used in print). (N:total number of plant-parasitic nematodes; G:genera richness; Loge_H_prime: Shannon-wiener diversity index; Ar-ecto:relative abundance of ectoparasites; Ar-endo-mig:relative abundance of migratory endoparasites; Ar-end-sed:relative abundance of sedentary endoparasites)

Pratylenchus spp. is more related to the super-intensive orchards without intercrops (Tables 3 and 4).

In fact, the intensification of soil in association with irrigated intercrops such as legumes (potato, tomato, cucumber and pepper) or fruit crops (pomegranate, almond and peach trees) which are suitable hosts for *Pratylenchus* spp. and *Meloidogyne* spp., enhances the multiplication of plant-parasitic nematodes (N), genera richness (G) and the relative abundance of endoparasite nematodes (Ar endo-sed, Ar endo-mig,) (Figs. 2, 3 and Tables 3 and 4). However the abundance of ectoparasites, mainly *Helicotylenchus* spp., was more important in traditional olive orchards (Tables 3 and 4).

In addition, the one-way ANOVA analysis demonstrated that sandy soils are more suitable for multiplication of *Rotylenchulus* spp., *Meloidogyne* spp., *Heterodera* spp. and *Rotylenchus* spp.. In contrast, *Pratylenchus* spp. are more abundant in clay soils (Table 4).

Two-way ANOVAs revealed that there was a statistically significant interaction between the effects of intensification and soil type on the abundance of *Rotylenchulus* spp. ($F(3)$ = 4.319, p = 0.006), *Pratylenchus* spp., ($F(3)$ = 59.174, p = 0.000), *Zygotylenchus* spp. ($F(3)$ = 3.331, p = 0.022), the total number of plant-parasitic nematodes (N) ($F(3)$ = 29.202, p = 0.000), the relative abundance of ectoparasites ($F(3)$ = 6.426, p = 0.000) and the relative abundance of migratory endoparasites ($F(3)$ = 10.453, p = 0.000). Comparisons with the Duncan test showed that *Rotylenchulus* spp. were more abundant in sandy soils in traditional olive orchards, *Pratylenchus* spp. were more abundant in clay soils in super-intensive systems, while *Zygotylenchus* spp. were more abundant in sandy-clay

Table 3 Impact of intensification of olive orchards on the plant-parasitic nematode densities and ecological indexes

	Intensification of olive orchards		
	Traditional	Intensive	Super-intensive
Criconemoides	0.81 a[µ]	0.46 a	0.53 a
Helicotylenchus	46.36 a	26.02 b	13.57 b
Heterodera	0.31 b	16.30 a	0 b
Meloidogyne	1.55 b	379.54 a	0 b
Paratylenchus	0.17 b	75 a	0 b
Pratylenchus	6.32 b	17.65 b	86.72 a
Rotylenchulus	0.43 a	0 b	0 b
Rotylenchus	0.35 a	0.42 a	0 a
Tylenchorhynchus	4.04 a	0 b	0 b
Tylenchus	5.70 a	0.74 a	0 a
Zygotylenchus	0.06 b	0.95 a	0 b
N	66.12 c	517.21 a	100.86 b
G	1.96 b	3.58 a	1.19 c
H′(Loge)	0.43 a	0.53 a	0.12 b
Ar ecto	69.35 b	46.15 b	81.56 a
Ar endo-sed	5.67 b	39.52 a	0 b
Ar endo-mig	18.67a	14.34 a	7.63 a

[µ] Values in different columns affected by the same letters (a,b) are not significantly different according to Kuskal-Wallis test, $P < 0.05$

soils in intensive olive orchards. The total number of plant-parasitic nematodes (N) was higher in clay soils in super-intensive orchards, and sandy and sandy-clay soils in intensive orchards. The relative abundance of migratory endoparasites was higher in clay and sandy soils in super-intensive olive orchards, whereas ectoparasites were less abundant in clay soils under super-intensive system.

The two-way ANOVA also showed a significant interaction between the effects of intensification and intercrops on the abundance of *Meloidogyne* spp. ($F(1) = 3.920$, $p = 0.050$), *Pratylenchus* spp. ($F(1) = 29.354$, $p = 0.000$), the Shannon diversity index (H) ($F(1) = 6.144$, $p = 0.015$) and the relative abundance of migratory endoparasites ($F(1) = 7.624$, $p = 0.007$). It was revealed that the abundance of *Meloidogyne* spp. and *Pratylenchus* spp. were higher in intensive olive orchards in the presence of intercrops. The Shannon diversity index was higher in traditional olive orchards in the presence of irrigated intercrops, and intensive orchards with or without intercrops. Migratory endoparasites were more abundant in traditional olive orchards with intercrops.

However, the interaction between soil type and intercrops showed significant effects only on the abundance of *Pratylenchus* spp. ($F(2) = 3.300$, $p = 0.040$) and the relative abundance of migratory endoparasites ($F(2) = 4.495$, $p = 0.013$). In fact, *Pratylenchus* spp. were more abundant in clay soils without intercrops and sandy soils with intercrops. Migratory endoparasites were less abundant in sandy-clay soils without intercrops.

Discussion

Diversity of plant-parasitic nematode communities

Symptoms of damage caused by plant-parasitic nematodes has appeared in Tunisian olive orchards mainly on those that are conducted under intensive and super-intensive systems.This study was therefore carried out to decipher the nematofauna associated with olive trees in our country, which has been the subject of our recent research (Guesmi et al., 2016; Guesmi-Mzoughi et al., 2016; Guesmi-Mzoughi et al., 2017; Palomares-Rius et al., 2014). Surveys were conducted in those regions most known for olive orchards in Tunisia, and identified 11 genera of plant-parasitic nematodes infecting olive roots which are *Criconemoides* spp., *Helicotylenchus* spp., *Heterodera* spp., *Meloidogyne* spp., *Paratylenchus* spp., *Pratylenchus* spp., *Rotylenchulus* spp., *Rotylenchus* spp., *Tylenchorhynchus* spp., *Tylenchus* spp. and *Zygotylenchus* spp. This nematofauna seems to be not very diverse when compared to those identified on olive trees in Spain with 38 genera (Archidona-Yuste et al., 2019; Palomares-Rius et al., 2015) and in Morocco with 47 genera (Ali et al., 2017). This could be explained by three reasons; first, our study was interested only in plant-parasitic nematodes infecting olive roots, so nematodes which spend most of their life cycle in soil could be not detected. Secondly, roots were washed free of soil prior to extraction, which would make some ectoparasites underrepresented. The third reason is that the studies carried out in Spain and Morocco were interested in parasitic nematofauna not only of cultivated olive, but also of wild olive, which support greater nematode diversity (Ali et al., 2017; Archidona-Yuste et al., 2019; Palomares-Rius et al., 2015).

Table 4 Impact of the presence of intercrops and type of soil on the plant-parasitic nematode densities and ecological indexes

	Intercrops		Type of soil		
	not	yes	sandy	Sandy-clay	Clay
Criconemoides	0.77	0.32	0.68 a[µ]	0.76 a	0 b
Helicotylenchus	35.12	27.10	23.02 a	42.20 a	6.07 a
Heterodera	0.07	11.97 *	8.9 a	0.13 b	0 b
Meloidogyne	15.47	215.92 *	193.90 a	4.41 b	0 b
Paratylenchus	0.61	51.02 *	17.50 a	10.59 ab	0.18 b
Pratylenchus	35.64 *	20.21	15.51 b	2.54 b	292.37 a
Rotylenchulus	0.16	0.47	0.79 a	0 b	0 b
Rotylenchus	0.27	0.19	0.87 a	0 b	0 b
Tylenchorhynchus	2.82	0	0.02 a	3.55 a	0 a
Tylenchus	3.99	0.44	0.39 a	5.03 a	0 a
Zygotylenchus	0.07	0.55*	0.14 a	0.23 a	0 a
N	95.04	328.33 *	261.78 b	69.46 c	298.73 a
G	1.68	3.00 *	2.94 a	1.57 b	1.64 b
H′(Loge)	0.28	0.62 *	0.58 a	0.25 b	0.34 b
Ar ecto	74.65 *	50.92	49.69 b	80.31 a	59.02 b
Ar endo-sed	6.58	18.45 *	26.59 a	2.28 b	0 b
Ar endo-mig	10.10	30.93 *	23.67 a	8.34 b	29.11 a

[µ] Values in different columns affected by the same letters (a,b) are not significantly different according to Kruskal-Wallis test, $P < 0.05$.
([*]) Values in different columns are significantly different according to Mann-Withney test, $P < 0.05$

All nematode genera identified in this study have been detected on olive trees in several countries of the Mediterranean Basin (Ali et al., 2014; Ali et al., 2017; Archidona-Yuste et al., 2019; Archidona-Yuste, Cantalapiedra-Navarrete, et al., 2020a; Aït Hamza et al., 2015; Castillo et al., 2010; Chafaa et al., 2014; Palomares-Rius et al., 2015). Among potentially damaging plant-parasitic nematodes, *Meloidogyne* spp., *Pratylenchus* spp., and *Heterodera* spp. were infecting olive roots in Tunisian orchards. The root-knot nematodes are among the most dominant and frequent taxa infecting olive trees in wild and cultivated orchards (Ali et al., 2016; Archidona-Yuste et al., 2018) as well as in nurseries (Aït Hamza et al., 2018). Several species were detected in olive trees such as *Meloidogyne arenaria*, *Meloidogyne hapla*, *Megalaima incognita*, *M. javanica*, *Megachile baetica* and *Malthonica lusitanica* (Aït Hamza et al., 2018; Ali et al., 2014; Ali et al., 2016; Archidona-Yuste et al., 2018). Recently, two new species were identified. *M. spartelensis* infecting wild olive trees in Northern Morocco (Ali et al., 2015), and *Musca oleae* infecting wild and cultivated olives in Southern Spain (Archidona-Yuste et al., 2018). So olive trees are good hosts for *Meloidogyne* spp. and infection by these pests could cause serious damage to this crop, mainly in olive nurseries (Aït Hamza et al., 2018). Feeding by root-knot nematodes (*Meloidogyne* spp.) induces distortion of secondary roots, heavy root galling and the formation of permanent feeding cells, the giant cells, and stellar destruction in olive roots (Abrantes et al., 1992; Castillo, Vovlas, Subbotin, & Troccoli, 2003a; Nico et al., 2002). Additionally the root-lesion nematodes (*Pratylenchus* spp.) were frequently detected in the olive rhizosphere. More than twelve species of *Pratylenchus* spp. have been recognized on cultivated and wild olive orchards but not all of them are dangerous for olive trees (Ali et al., 2014; Ali et al., 2017; Archidona-Yuste et al., 2019; Archidona-Yuste, Cantalapiedra-Navarrete, et al., 2020a; Castillo et al., 2010; Palomares-Rius et al., 2015). The presence of non-pathogenic species of root lesion nematodes in olive rhizosphere could be explained by the association of suitable host plants as intercrops in olive orchards (Archidona-Yuste et al., 2019). However, *Pratylenchus penetrans* and *P. vulnus* have been demonstrated to be pathogenic to olive trees (Lamberti & Baines, 1969;

Nico et al., 2003) and the damage caused by *P. vulnus* can lead to olive tree decline (Lamberti, 1969). Recently, *Platygaster oleae* was identified infecting wild and cultivated olives suffering tree decline in southern Spain and Tunisia (Palomares-Rius et al., 2014). Considering the cyst nematodes (*Heterodera* spp.), a few species have been detected in association with olive trees. They are *Heterodera avenae*, *H. mediterranea* and *H. riparia* and other species which remain unidentified (Aït Hamza et al., 2018; Ali et al., 2014; Ali et al., 2017; Archidona-Yuste et al., 2019; Archidona-Yuste, Cantalapiedra-Navarrete, et al., 2020a; Palomares-Rius et al., 2015). Among *Heterodera* species, *H. mediterranea* is specialized to both cultivated and wild olives. The infection by this species causes cellular alterations in the cortex, endodermis, pericycle, and vascular parenchyma tissues of olive roots that could lead to olive tree decline in young plantations and nurseries (Castillo et al., 1999). The reniform nematode, *Rotylenchulus* spp., was detected in some root samples. To our knowledge, this is the fifth report of this nematode in the olive rhizosphere from countries of the Mediterranean Basin after Italy, Spain, Greece (Palomares-Rius et al., 2018) and Morocco (Ali et al., 2017). Two species have been described on cultivated olives which are *Rossia macrosoma* and *R. macrodoratus* (Inserra & Vovlas, 1979; Castillo, Vovlas, & Troccoli, 2003b; Palomares-Rius et al., 2018). These species have been demonstrated to be pathogenic to olive (Castillo, Vovlas, & Troccoli, 2003b; Inserra & Vovlas, 1979). Feeding by *R. macrosoma* on olive roots induce the formation of syncytia (Castillo, Vovlas, & Troccoli, 2003b), while infection by *R. macrodoratus* causes the establishment of uninucleate giant cells which serve as feeding cells (Inserra & Vovlas, 1979). Although *R. reniformis* has not been found infecting olive in natural conditions, olive can be a potential host of this nematode as reported in several studies under controlled conditions (Al-Sayed & Abdel-Hameed, 1991; Badra & Khattab, 1980).

Influence of soil and agronomic factors on plant-parasitic nematode communities

This study showed that farming system (traditional, intensive and super-intensive), soil intensification by irrigation with the association of intercrops, and soil type are the main agronomic factors driving the diversity of plant-parasitic nematode communities. Our results are partially in agreement with those obtained by Palomares-Rius et al. (2015) and Ali et al. (2017). Indeed, our study demonstrated that the total number of plant-parasitic nematodes was significantly higher in irrigated than in non-irrigated olive orchards, and in the presence of intercrops. Similarly, research achieved in Morocco demonstrated that irrigated olive orchards are more infested with plant-parasitic nematodes than non-irrigated olive orchards (Ali et al., 2017). In fact, irrigation enhances the development of roots which increases the multiplication of nematodes (Castillo et al., 2010; Nico et al., 2002). Additionally, it has reported that irrigation waters are a source of contamination by plant-parasitic nematodes (Faulkner & Bolander, 1970; Hugo & Malan, 2010). However, Palomares-Rius et al. (2015) showed that the irrigation regimen did not influence any of the tested diversity indexes except for the Shannon index, which was significantly higher in irrigated olive orchards compared to that under a rainfed regime. Concerning the association with intercrops, the same researchers demonstrated that plant-parasitic nematodes are significantly more abundant in organic olive orchards with the presence of intercrops than in orchards under conventional management with the absence of intercrops (Palomares-Rius et al., 2015). The association of irrigated intercrops with olive trees in intensive cropping system increased the multiplication of endoparasites, especially *Meloidogyne* spp. and *Pratylenchus* spp.. This could be linked to the nature of intercrops such as tomato, pepper, almond and peach trees, which are suitable host plants for the multiplication of root-knot nematodes (Perry et al., 2009) and root-lesion nematodes (Castillo & Vovlas, 2007).

Our study revealed that the diversity of plant-parasitic nematode communities is different between olive orchards under super-intensive regimes, and orchards under intensive regimes with the presence of intercrops. In fact, plant-parasitic nematode communities were significantly less rich in genera, less diversified and more homogenously distributed in communities in super-intensive systems than in traditional systems. However, it is the opposite for olive orchards with intercrops. These results suggest that the intensification practices affect the structure of the plant-parasitic nematode communities in favor of the most pathogenic nematodes for olive trees that could increase their population densities feeding on olive roots. This hypothesis has been verified for diverse forest and annual crops (Kimenju et al., 2009; Yeates & Bongers, 1999; Zhang et al., 2017). The intensification of olive orchards increased the multiplication of endoparasite nematodes on olive roots, mainly *Pratylenchus* spp. and *Meloidogyne* spp.. But the root-knot nematodes were more dominant in olive orchards under an intensive regime in the presence of intercrops, while lesion nematodes were more dominant in olive orchards under super-intensive regime without intercrops. The abundance of *Meloidogyne* spp. in irrigated olive orchards was also cited by Aït Hamza et al. (2015) and Ali et al. (2017). Because of this, our study focused on the impact of the presence of intercrops in olive orchards, which enhances the multiplication of the most dangerous nematode *Meloidogyne* spp. This concurs with studies carried out by Archidona-Yuste et al. (2018) which revealed the importance of the irrigation regimen and the presence of intercrops, in driving the distribution of root-knot nematodes in cultivated olive in Southern Spain. However, each *Meloidogyne* species responds differently to these factors. In fact, *M. javanica* was the main species correlated to irrigated soils with intercrops in contrast to *M. arenaria* (Archidona-Yuste et al., 2018). This species was highly abundant in high-density cultivated olive orchards in the center of Morocco where cultivated areas are generally distributed by agricultural practices and human activities (Ali et al., 2016). Furthermore, experiments conducted by Liébanas and Castillo (2004) showed that six crucifer species, with potential uses as intercrops in Andalusian olive orchards, are hosts for the three common root-knot nematodes *M. arenaria*, *M. incognita* and *M. javanica* and support moderate to high nematode reproduction. Therefore, it is better to avoid the use of intercrops with olive, or choose plants that are not hosts for nematodes pathogenic to olive. Additionally, growing legumes as intercrops when temperatures are suboptimal for root-knot nematodes might allow their use in olive orchards without increasing the population densities of *Meloidogyne* spp. (Liébanas & Castillo, 2004).

The abundance of *Pratylenchus* spp. in olive orchards under super-intensive systems without intercrops doesn't agree with results obtained by Aït Hamza et al. (2015) who reported that these nematodes are more prevalent in rainfed olive orchards. In addition, Ali et al. (2017) recorded that traditionally irrigated olive orchards are more conducive to the multiplication of *Pratylenchus* spp.. This disagreement could be explained by the differences between the *Pratylenchus* species identified in our study and those reported in the previous studies.

Furthermore, we have found that the relative abundance of ectoparasites, mainly *Helicotylenchus* spp., is more important in olive orchards under a rainfed regime (without irrigation and without intercrops). This agrees with results obtained by Aït Hamza et al. (2015) showing that Tylenchidae, *Helicotylenchus* spp. and *Rotylenchus* spp. are more prevalent on wild olive than cultivated olive under a traditional regime. Also, Aït Hamza et al. (2015) cited that these nematodes are better adapted to rainy conditions.

Results indicated that the type of soil affects the diversity of plant-parasitic nematode communities and that there was a strong effect of the interaction between soil type and intensification on this diversity. In fact, we noticed that sandy soils are more conducive to the multiplication of *Rotylenchulus* spp. in traditional olive orchards, and *Meloidogyne* spp. in intensive olive orchards. While clay soils increased the multiplication of *Pratylenchus* spp. in super-intensive systems. This agrees with previous studies reporting that the impact of soil properties on nematodes is specific to certain genera (Aït Hamza et al., 2015; Chen et al., 2012). Furthermore, Archidona-Yuste et al. (2018) indicated the importance of soil texture in driving the distribution of root-knot nematodes, particularly for *Meloidogyne* species. In fact, the presence of *M. incognita* was highly correlated with sandy loamy soils. Indeed, Chen et al. (2012) demonstrated that the multiplication of *Pratylenchus* spp. is negatively correlated to the proportion of sand in the soil. In addition, Aït Hamza et al. (2015) demonstrated that *Meloidogyne* spp. and *Pratylenchus* spp. were more prevalent in fine sand textured soils. This disagrees with our results, which suggest that *Pratylenchus* spp. were more prevalent in clay soils. This difference could be attributed to the *Pratylenchus* species (Palomares-Rius et al., 2015). The prevalence of *Rotylenchulus* spp. in sandy soils was also reported by Olabiyi et al. (2009) who have studied the presence of this nematode in different soils in Niger.

Palomares-Rius et al. (2015) recorded that the soil texture and variety of olive are the main factors influencing the composition of plant-parasitic nematode communities. In fact, plant-parasitic nematodes are significantly more abundant in clay loam soils while their higher diversity was reached on sandy loam soils that showed the lower nematode abundance.

More recently, Archidona-Yuste et al. (2020a, b) revealed the influence of spatial structure and soil on species richness and beta diversity in contrast to agricultural management. These studies revealed novel insights and suggest involving spatial structure on future studies concerning the diversity of plant-parasitic nematode communities.

Conclusions

This study explored for the first time the plant-parasitic nematodes infecting olive trees and their diversity in the main areas of olive production in Tunisia that included 123 olive orchards. These investigations revealed that the intensification of olive orchards with irrigation and the association of intercrops are the main agronomic factors influencing the multiplication and the diversity of plant-parasitic nematodes infecting olive trees. In particular, olive orchards under super-intensive regime are more conducive to the multiplication of *Pratylenchus* spp., while the presence of irrigated intercrops enhances the multiplication of *Meloidogyne* spp.. These two nematodes are widespread in the rhizosphere of olive (Aït Hamza et al., 2018; Ali et al., 2014; Ali et al., 2017; Archidona-Yuste et al., 2019; Palomares-Rius et al., 2015) and many species are pathogenic to olive trees and can induce significant damage in nurseries and olive orchards that can lead to tree decline (Aït Hamza et al., 2018; Ali et al., 2014; Castillo et al., 2010). Indeed, the intensification of olive orchards may cause the disturbance of the ecosystem (Loumou & Giourga, 2003) and make the olive trees more susceptible to some diseases (Trapero et al., 2009) like *Verticillium* wilt induced by *Verticillium dahliae* (Lamberti et al., 2002; Saeedizadeh et al., 2006) which was reported for the first time in Tunisia in 2006 (Triki et al., 2006). So, in real situations, when olive trees show symptoms such as wilting, chlorosis, defoliation, and dead brown branches, we must think to plant-parasitic nematodes more than soil-borne fungus. In fact, injuries induced by the penetration of plant-parasitic nematodes into roots pave the way for infection by bacteria and fungi (Sassanelli, 2009). In addition, the damage caused by *Verticillium dahliae* on olive trees could be enhanced in the presence of *Meloidogyne* spp. or *Pratylenchus* spp. (Castillo et al., 2010; Saeedizadeh et al., 2006).

This study demonstrated that intensified systems are more suitable for the multiplication of plant-parasitic nematodes, and in particular endoparasites. So, for the establishment of new olive orchards under intensive and super-intensive regimes, it will be essential to choose certified olive plants, which are free from diseases and pathogens, since nurseries are a primary source of contamination (Aït Hamza et al., 2018; Castillo et al., 2010; Sassanelli, 2009). Additionally, it is necessary to select as intercrops plants which have a suppressive effect on nematodes, such as plants used for biofumigation (Castillo et al., 2010).

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Declaration

Competing interests Informed consent was obtained from all individual participants included in the study. The authors report no conflicts of interest. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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