

Is duration of organic management reflected on nematode communities of cultivated soils?

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ABSTRACT. The aim of this study was (a) to explore long-term responses of soil nematodes to the conversion from conventional to organic cultivation and (b) to differentiate them from the short-term responses to seasonal agricultural practices. Nematode communities were studied in terms of trophic and generic structure, life strategy and diversity, in asparagus cultivations along a gradient from conventional to organic under conversion (1 and 2 years), and certified organic (4 and 5 years). Samplings throughout the year were conducted with respect to seasonal agricultural practices.

Changing management regime from conventional to organic cultivation seemed to initiate successional long-term changes in nematode communities, such as the gradual decline of phytoparasites in favour of bacterivores and fungivores, the gradual decrease of PPI, the increase of diversity. Alterations in the generic structure of the community were also revealed, driven mostly by the opposite trends of changes in abundance of *Helicotylenchus* vs. *Heterocephalobus*. Seasonal agricultural practices appeared to induce short-term responses of functional guilds of low colonizer-persister values (c-p 1 and 2) mostly, and were reflected in nematode indices, such as Maturity Index, Plant Parasitic Index, Enrichment Index, Channel Index but not Structure Index. Nematode responses at the generic level to seasonal agricultural practices seemed less intense than the ones imposed by changing management regime, and in the case of conventional cultivation they were almost entirely masked.

KEY WORDS : asparagus, conventional agriculture, conversion to organic, diversity, *Helicotylenchus*, *Heterocephalobus*, nematode indices

INTRODUCTION

In the past 20 years, considerable attention has been paid to nematodes, demonstrating that these ubiquitous members of the soil community reflect change in ecological structure and function of soils in ways more predictable and efficient than for other soil flora or fauna (FISCUS & NEHER, 2002). The ecological significance of nematodes lies in their high abundance and diversity, variety of trophic types and reproductive strategies (YEATES, 2003). They have been used to evaluate soil conditions under different crops (WIDMER et al., 2002), under various agricultural practices, such as tillage (FU et al., 2000), fallow farming (VILLENAVE et al., 2001), use of pesticides (LIANG et al., 2001), fungicides (VILLENAVE et al., 2004) or herbicides (YEATES et al., 1999), and to distinguish the effects of physical and chemical disturbance of cultivated soils (FISCUS & NEHER, 2002).

Furthermore, nematodes have been used for evaluating soil quality under organic and conventional agriculture (FRECKMAN & ETTEMA, 1993; FERRIS et al., 1996; NEHER, 1999; NEHER & OLSON, 1999; MULDER et al., 2003; GARCÍA-ÁLVAREZ et al., 2004 among others). However, only a few studies compare agroecosystems managed organically for different time periods, and are mostly confined to comparisons of crop yields or to soil parameters other than nematodes (FRIEDEL & GABEL, 2001; MARTINI et al., 2004; MONOKROUSOS et al., 2006). To our knowledge, the only studies relating nematode communities to duration of organic management are those of BERKELMANS et al. (2003) who monitor changes of nematode communities in sites managed organically from 4 to 12

years, and of VAN DIEPENINGEN et al. (2006) who compare conventional to certified organic systems of less and more than five years of organic management. Thus, there is a lack of data regarding possible trends of succession within communities initiated after changing management regime from conventional to organic.

The main goal of this study was, therefore, to explore responses of soil nematodes along a 5 year gradient from conventional to organic cultivation. According to the Regulation 2092/91/EEC, the period of conversion from a conventional to an organic production system is at least two years for annual crops and three for perennial ones. The agricultural systems included in our study were conventional, organic under conversion (1 and 2 years) and certified organic (4 and 5 years) cultivations of *Asparagus officinalis*. We selected them in order to minimize the effect of other factors that are known to affect nematode populations even more than agricultural practice *per se*, such as the cultivated species and duration of cultivation (YEATES et al., 1999) and the soil type (VAN DIEPENINGEN et al., 2006). Moreover, asparagus cultivations are perennial and not additionally stressed by annual plantings, and thus the history of agricultural practices is better reflected on soil conditions. Nematode communities were studied in terms of trophic and generic structure, life strategy and diversity.

Apart from changing management regime from conventional to organic, seasonal agricultural practices, input of fertilizers (conventional or organic), mechanical disturbance of soil, crop harvesting etc, are shared between management types, constituting periodic disturbances to the soil ecosystem. These disturbances are quite impor-

tant for shaping nematode communities. The effect of the one may mask the effect of the other (FISCUS & NEHER, 2002), but most importantly they may mask the progressive effect of the transition from conventional to organic management. Thus, a second goal of our study was to explore short-term nematode responses to seasonal agricultural practices and compare them to the long-term responses to changing management regime. This was achieved by conducting samplings in periods following all steps within the annual crop growing cycle of asparagus.

MATERIALS AND METHODS

Sites and sampling

The study area is a cultivated plain of Northern Greece, at about 60km north west of Thessaloniki (7.5m *a.s.l.*), where asparagus is traditionally one of the main products. Due to higher price of organic agricultural products and financial support by the state, during the last decade a lot of asparagus cultivations were converted to organic. Soils are classified as luvisol (FAO) with a clay-loam texture (37% sand, 33% silt, 30% clay). The climate is transient between mediterranean and continental. Mean annual precipitation for a 10-year period was 485mm and mean annual temperature 14°C. January was the coldest month (4°C) with the highest precipitation (58mm), while the warmest (24°C) and driest (16mm) month was July.

The experimental plots of our study belonged to agroecosystems with different management history, i.e. conventional, organic under conversion and certified organic cultivations of the perennial *Asparagus officinalis*. Conventional sites covered about 9,000m² and were cultivated with asparagus for more than 6 years. Organic ones were conventionally cultivated for several years before conversion to organic farming, and covered in total about 30,000m² of the wider study area. At the start of the study the certified organic sites were managed organically from 4 to 5 years [O4, O5], while the transitional organic sites were managed organically from 1 to 2 years [O1, O2]. Asparagus cultivations, either organic or conventional, are not tilled, but during February, i.e. one month before the beginning of the harvest season, the soil between field rows is mounded up over the asparagus plants, for producing white spears. The soil mounds are smoothed away during June, i.e. after the end of harvest. Thus, all plots are subject to the same physical disturbance and differ only regarding the type of fertilizers and weed control. Fertilization takes place in summer, with manure and licensed organic fertilizers in one case and synthetic fertilizers in the other (Table 1). Regarding weed control, which also takes place in summer, it is done with hand-hoeing in case of organic cultivation, while in conventional cultivation it is done once every summer by means of Linuron for broadleaved grasses (0.2 l /1,000m²) and Fluazifop-p-butyl for grass weeds (0.2 l /1,000m²).

Our sampling scheme represented a full factorial design of 5 agricultural systems x 4 dates x 4 replicate plots. The latter were randomly dispersed covering 250m² each. At each plot we took a composite sample of 10 soil cores 2cm in diameter and 20cm in depth. Samples were

TABLE 1

Fertilizers in organic and conventional cultivation. Total amounts [in brackets] and time of application are indicated. All fertilizers used in organic cultivation are permitted for organic agriculture.

Organic	
Manure [1T/1,000m ²]	August (once every two years)
Organic fertilizer (Bioazoto, 12% N 14% C) [200kg/1,000m ²]	May (once every year)
Mineral Potassium Sulphate [550Kg/1,000m ²]	
Organic fertilizer (Dermafert, N P K 8-7-7 + 2MgO + 8SO ₃ +17 C) [1,000kg/1,000m ²]	July (once every year)
Conventional	
Synthetic fertilizer (Hydrocomplex supra N P K 6-15-25 + 3MgO + 30SO ₃) [100Kg/1,000m ²]	July (once every year)

taken in the rows of the asparagus plantations, and more specifically close to the edge of them, so that regardless the seasonal soil mounding, all year samples corresponded to the same soil depth relative to the cultivated plants. Soil sampling was conducted four times throughout a year; in March (asparagus spears start growing and the harvest begins), in May (end of harvest), in October (the fields are left with no agricultural activities for 2 – 3 months) and in December (two weeks after cutting of the aboveground parts of asparagus plants which are then left in the field to decompose). The time span between samplings was long in relation to nematode life span, so that nematode population dynamics decouple nematode counts across time, creating sufficiently independent data.

Nematodes were extracted from 150mL of each composite soil sample. Before taking this subsample, the soil was gently mixed by hand and soil aggregates were broken up. For extraction, we used the modified Cobb's sieving and decanting method proposed by s'JACOB & VAN BEZOOIJEN (1984), according to which a cotton-wool filter is used in the last step. After counting total abundance of nematodes, we fixed them with formaldehyde 4%. Later on from each sample we selected randomly at least 150 nematodes and identified them to the genus level in most cases, using the identification key of BONGERS (1994).

Soil bulk density and pH was measured once, at the beginning of samplings. The former was not found to differ among study sites, ranging from 1.06 to 1.24g/cm³. Values of pH decreased gradually and significantly from 7.99 in [C] to 7.94, 7.90, 7.83 and 7.51 in [O1], [O2], [O4] and [O5] respectively. On each sampling occasion, soil water content (% dry weight) was also estimated from each soil sample taken. It was found to differ seasonally, being higher in December (24.22%) and lower in May (20.36%).

Nematode indices

Nematode taxa were assigned to trophic groups according to YEATES et al. (1993), classified along the colonisation-persistence gradient (c-p values) following BONGERS

(1990) and BONGERS & BONGERS (1998), and arranged to functional guilds (portions of particular trophic groups exhibiting the same c-p value) according to BONGERS & BONGERS (1998) and FERRIS et al. (2001).

The maturity index (MI) for free living nematodes (c-p from 1 to 5) and the plant parasitic index (PPI) for plant feeding nematodes, both indicating the successional stage of communities, were calculated according to BONGERS (1990) as $\sum v_i p_i$, where v_i is the c-p value of taxon i and p_i the proportion of the taxon in the nematode community. The Enrichment index (EI), the Structure index (SI) and the Channel Index (CI), were calculated according to FERRIS et al., (2001). EI and SI, which provide location of the food web along the enrichment and the structure trajectory, were calculated according to the formulas $EI = 100 \times (e/(e+b))$ and $SI = 100 \times (s/(s+b))$. The b component refers to the nematode functional guilds that indicate basal characteristics of the food web, namely the bacterivores with c-p 2 value (Ba2) and the fungivores with c-p 2 value (Fu2), and was calculated as $\sum k_b n_b$, where k_b are the weightings assigned to the Ba2 and Fu2 functional guilds and n_b are the abundances of nematodes in those guilds. The e and s components were calculated similarly, using those guilds indicating enrichment (Ba1, Fu2) and structure (Ba3–Ba5, Fu3–Fu5, Om3–Om5, Pr2–Pr5), respectively. Finally CI which indicates the predominant decomposition pathway, was calculated as $CI = 100 \times (0.8Fu2/(3.2Ba1+0.8Fu2))$.

Data analysis

All analyses aim to estimate and compare the nematode responses to changing management regime and to seasonal agricultural practices, in terms of trophic structure, nematode indices, generic structure and composition and diversity.

For analyzing frequencies of the different nematode trophic groups at the different agricultural systems, we used the log-linear analysis of count data of categorical variables. This method fits log-linear models to the combinations of categorical variables, herein trophic group and site, to find out possible associations between them. More specifically, it estimates the randomly expected frequency of every combination of trophic group and site and compares it to the observed frequency.

For testing differences of nematode indices due to the long-term effect of changing management regime as well as the short-term effect of seasonal agricultural practices, we used two-way ANOVA (site \times date). LSD post hoc comparisons were performed, when significant differences were revealed. Prior to analyses, data were examined for normality, homogeneity of variance (Levene's test) and independence between variance and mean. Since values of nematode indices are bounded, an arc-sinus transformation was used. Kruskal-Wallis test was used in the case where the prerequisites of normality were not met even after transformation.

In order to compare the progressive effects of changing management regime to those of seasonal agricultural practices on the generic structure of nematode communities, all samples and nematode genera abundances were ordinated by means of correspondence analysis (CA), while sample ordination scores for Axis 1 and Axis 2 were analyzed by MANOVA (site \times date). When signifi-

cant differences were revealed, post hoc comparisons were performed by means of LSD-test. Abundances of nematode genera were log+1 transformed prior to CA, while prior to MANOVA data were examined for normality, homogeneity of variance (Levene's test) and independence between variance and mean.

For assessing the diversity of nematode communities, we used the method of diversity ordering proposed by RENYI (1961). Renyi's parametric index of order a shows varying sensitivity to the rare and abundant species of a community, as the scale parameter a changes (RICOTTA, 2000). For each community it provides a profile of the most widely used diversity indices. For $a=0$, the index equals log species number, for $a=1$, it equals Shannon's index, for $a=2$, it equals Simpson's index. For a tending to infinite, the index is most sensitive to the abundant species of a community. Thus, when diversity profiles differ in the range of low a values, this is due to the number of species. In the range of high a values, differences between communities are due to presence of abundant species. When diversity profiles intersect, the communities may be ordered differently by different diversity indices.

In order to study changes of generic composition, we used the IndVal (Indicator Value) method of DUFRENE & LEGENDRE (1997). This method assigns indicator (characteristic) species to a site or a group of sites, on the basis of species relative abundance and relative frequency of occurrence in the samples. For the classification of sample units required by IndVal, we used a complete linkage hierarchical classification tree based on 1-Pearson r distance measure. The statistical significance of the species indicator values was also evaluated by the IndVal program by means of a randomization procedure.

For log-linear analysis, ANOVA, MANOVA, Kruskal-Wallis and tree clustering we used the SPSS software package (version 11). For Correspondence analysis we used CANOCO (version 3.10) software package (TER BRAAK, 1988), while diversity ordering was performed by means of DivOrd (TOTHMERESZ, 1995).

RESULTS

Trophic structure

Bacterivores were the dominant trophic group in all organic plots comprising over 48% of the nematode communities (Table 2). In the case of conventional plots the dominant trophic group was that of phytoparasitic nematodes (50%), followed by bacterial feeders (30%). Predators and omnivores accounted for less than 2% in all cases. Total abundance ranged from 425 to 1345 ind./100ml soil. Abundance changes did not exhibit a specific trend, masking possible interactions between trophic groups and sites, which are apparent from the % contribution of each group. Therefore, in order to test statistically the frequency of appearance of each group at each site we proceeded to log-linear analysis.

Associations between a trophic group and a site, explored by log-linear analysis, are presented in Fig. 1. Bacterivores and non parasitic plant feeders seemed to appear less frequently at [C] and [O1], being more frequent at older organic cultivations [O4, O5]. An exactly opposite trend

was exhibited by phytoparasites. Fungivores appeared more frequently at organic plots instead of [C], not exhibiting though gradual changes from recent to older organic cultivations. Predators were more frequent at [O4] and [O5],

while omnivores at [C] and [O1]. However, contribution of these trophic groups was very low and therefore sampling errors as indicated by confidence intervals were very high to arrive at a secure conclusion about their response.

TABLE 2

The contribution of individual trophic groups (%) and the total nematode abundance (\pm SE) in the studied agricultural systems. [C]: conventional, [O1], [O2], [O4], [O5]: managed organically for 1, 2, 4 and 5 years respectively.

	[C]	[O1]	[O2]	[O4]	[O5]
Bacterivores (%)	29.78 (5.31)	48.23 (5.91)	50.61 (4.25)	51.66 (3.57)	61.01 (2.86)
Fungivores (%)	10.48 (1.59)	23.69 (4.66)	19.41 (2.55)	25.59 (2.16)	17.40 (2.09)
Phytoparasites (%)	49.98 (5.30)	19.06 (3.78)	16.29 (4.46)	7.63 (2.00)	9.85 (2.46)
Non parasitic plant feeders (root hair & algal feeders) (%)	7.17 (1.91)	7.52 (0.87)	12.11 (1.98)	13.41 (1.97)	10.33 (1.69)
Predators (%)	1.13 (0.38)	1.19 (0.35)	0.72 (0.29)	1.63 (0.45)	1.34 (0.28)
Omnivores (%)	1.46 (0.55)	0.30 (0.17)	0.86 (0.3)	0.08 (0.08)	0.07 (0.07)
Total abundance (ind./100ml soil)	1068 (180)	1345 (244)	1018 (193)	425 (90)	778 (128)

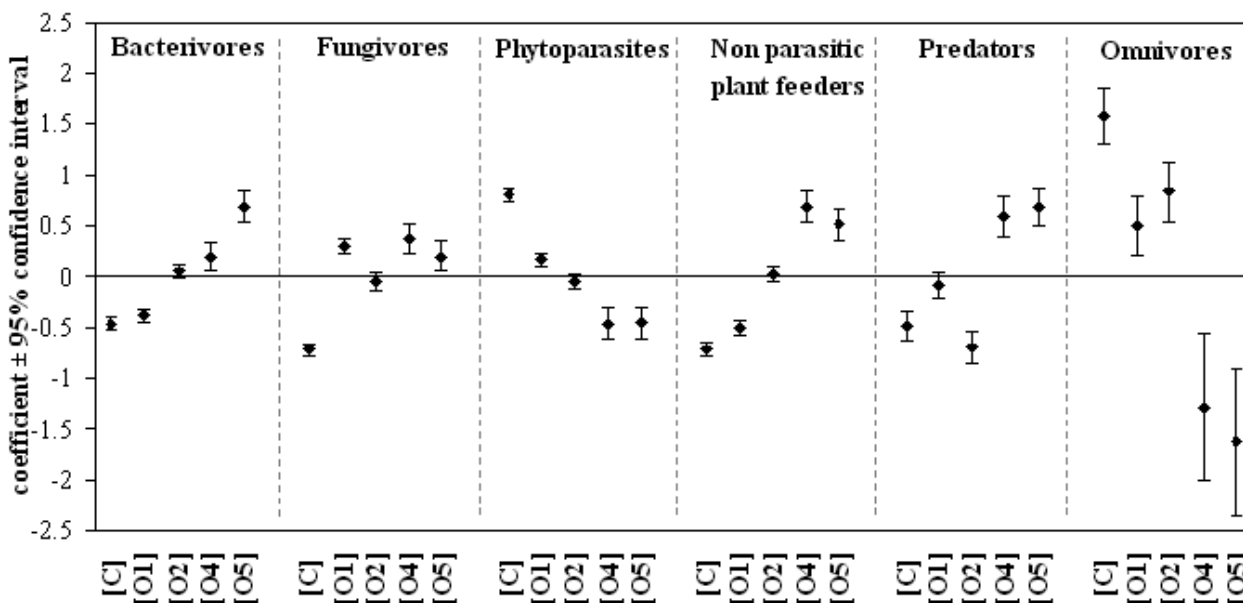


Fig. 1. – Coefficients of the Log-Linear model for each combination of trophic group and site. When the confidence intervals of the coefficient include 0 the nematode population of the specific trophic group at the specific site does not differ from the overall average. Positive values of the coefficient indicate that the specific trophic group appears at the specific site more frequently than randomly expected, while the inverse happens for negative values. Codes as in Table 2.

Nematode indices

Mean values of MI, PPI, EI, SI and CI at the different sites and seasonal samplings are presented in Table 3, together with the results of two-way ANOVA (site x date). Significant changes regarding sampling site were observed only in the case of PPI, which increased gradu-

ally from the older to the recent organic and further to conventional cultivation. Regarding sampling date, all indices except SI displayed significant differences. MI was higher in October, i.e. in a period long after any agricultural practice. PPI was higher in December, i.e. when the above ground parts of asparagus plants were cut and left in the field to decompose. EI was higher in March, i.e.

in the beginning of the growing season, shortly after the soil was mounded up. CI was lower in March and May, i.e. during the whole harvest season.

Generic structure

The ordination of all samples along the two first axes of Correspondence Analysis is depicted in Fig. 2, while the results of MANOVA (site x date) on Axis 1 and Axis 2 ordination scores are given in Table 4. The effects of changing management regime on generic structure of the nematode community were significant at both axes, while those of seasonal agricultural practices were significant only along the second axis. Samples from [C] were clearly and significantly distinct from [O4] and [O5] samples, forming a gradient from conventional to recent and further to older organic cultivations along the first axis. Samples taken during the harvest season when the soil was mounded, i.e. March and May, were ordinated on the lower half of the plot, differing significantly from October and December samples, which were ordinated mostly on the upper half corresponding to samplings carried out during the post-harvest season when soil was flat. Because of these seasonal differences in generic structure, we provide separate results for the harvest and the post-harvest season in all subsequent analyses of the data.

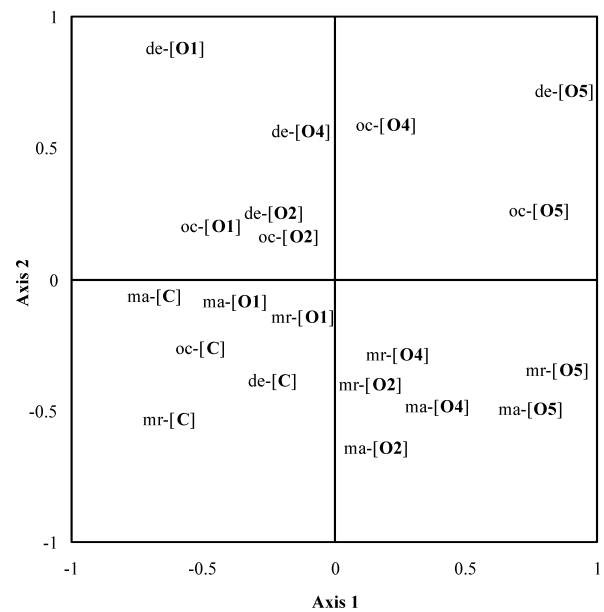


Fig. 2. – Correspondence Analysis two axes plane for samples from different sites and sampling dates. Each point represents mean values of sample scores for each site at each sampling date. Site codes as in Table 2. (mr: March, ma: May, de: December, oc: October).

TABLE 3

Mean values (\pm SE) of Maturity Index, Plant Parasitic Index, Channel Index, Enrichment Index, Structure Index and results of two-way ANOVA (site x date) or Kruskal-Wallis test (for SI). Superscripts a, b, c, d indicate differences revealed by LSD-test, (*: $P < 0.05$, **: $P < 0.001$, MS: Mean square). Codes as in Table 2.

	MI	PPI	CI	EI	SI
SITE (4 d.f)					
[C]	1.92 (0.07)	2.85 (0.04) ^d	31.77 (6.17)	60.77 (4.38)	27.31 (6.25)
[O1]	1.85 (0.04)	2.64 (0.05) ^c	35.51 (7.49)	58.83 (2.87)	11.63 (2.69)
[O2]	1.83 (0.05)	2.48 (0.08) ^c	34.11 (6.50)	58.11 (4.44)	13.68 (3.30)
[O4]	1.86 (0.04)	2.29 (0.06) ^b	35.82 (5.58)	56.24 (3.49)	11.88 (2.51)
[O5]	1.77 (0.04)	2.06 (0.03) ^a	20.69 (3.48)	61.79 (3.95)	11.66 (2.67)
MS	0.07	2.59	0.10	0.02	
F	2.07	35.40	1.31	0.53	H=4.17
P	NS	**	NS	NS	NS
DATE (3 d.f)					
March	1.75 (0.05) ^a	2.42 (0.06) ^a	17.59 (3.08) ^a	70.26 (2.77) ^c	19.93 (4.18)
May	1.84 (0.04) ^a	2.45 (0.08) ^a	22.67 (3.80) ^a	58.23 (3.23) ^b	13.78 (3.48)
October	1.95 (0.02) ^b	2.38 (0.09) ^a	46.75 (5.92) ^b	48.13 (2.87) ^a	14.29 (3.68)
December	1.85 (0.04) ^a	2.60 (0.08) ^b	39.30 (5.60) ^b	59.96 (2.97) ^b	12.93 (2.76)
MS	0.15	0.39	0.60	0.27	
F	4.72	5.34	7.58	8.89	H=1.47
P	*	*	**	**	NS
SITE x DATE (12 d.f)					
MS	0.08	0.01	0.12	0.03	
F	2.65	1.33	1.56	1.05	
P	*	NS	NS	NS	

TABLE 4

Results of MANOVA (site x date) for Axis 1 and Axis 2 sample ordination scores. Superscripts a, b, c, d indicate differences revealed by LSD-test, (*: $P < 0.05$, **: $P < 0.001$, ***: $P < 0.0001$, MS: Mean square). Codes as in Table 2.

overall effect					
	Value	Error d.f.	Hypothesis d.f.	F	P
Intercept	0.99	59	2	0.19	NS
SITE	0.21	118	8	17.47	***
DATE	0.51	118	6	7.85	***
SITE x DATE	0.52	118	24	1.93	*

univariate results									
		SITE (4 d.f)				DATE (3 d.f)			
		LSD	MS	F	P	LSD	MS	F	P
Axis 1	[C] ^a [O1] ^a [O2] ^b [O4] ^c [O5] ^d		4.51	39.31	***		0.06	0.56	NS
Axis 2	[C] ^a [O2] ^{ab} [O5] ^{bc} [O4] ^{bc} [O1] ^c		0.72	4.72	*	march ^a may ^a octob ^b decemb ^b	2.90	18.85	***

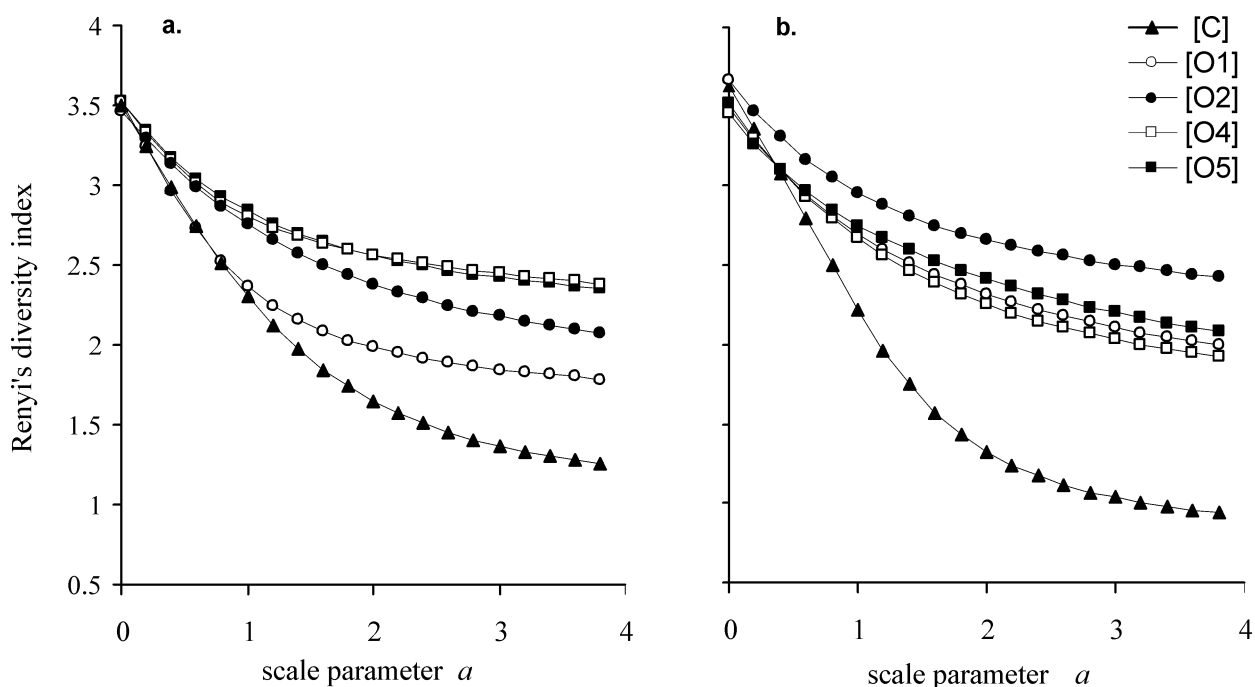
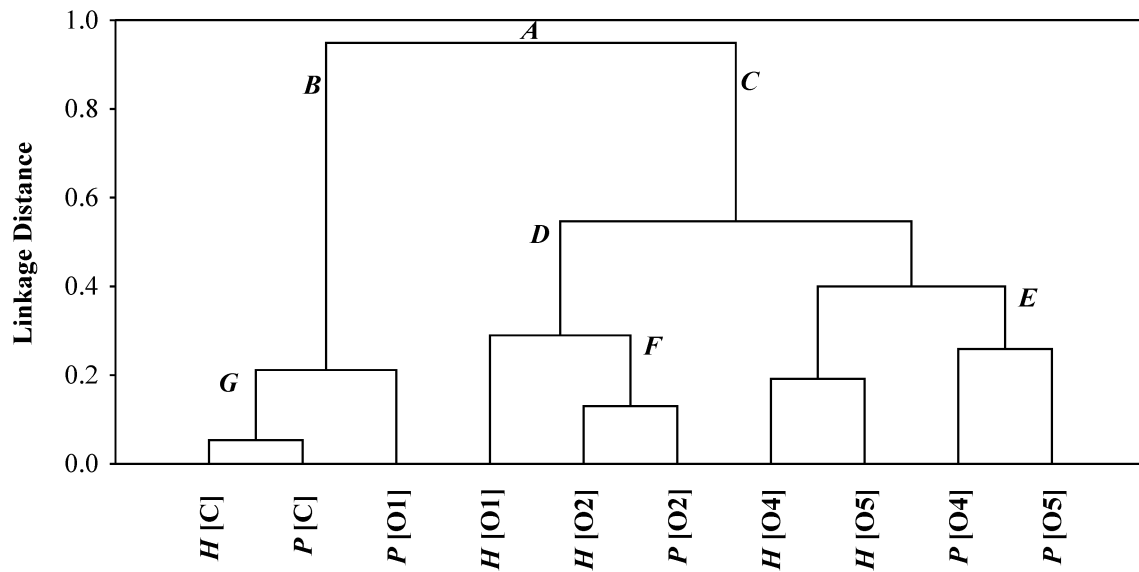


Fig. 3. – Diversity profiles of nematode communities in the studied agricultural systems during (a) the post-harvest season and (b) the harvest season. Codes as in Table 2.

The diversity profiles of the nematode communities for all studied systems are shown in Fig. 3. In the post-harvest season (Fig. 3a), diversity was higher in [O5], [O4] and gradually decreased to [O2], [O1] and further to [C], reflecting a gradient of a long-term management regime. In the harvest season (Fig. 3b), the diversity of recent organic plots increased, while [C] was even less diverse than in the previous season. In both seasons, differences of diversity were not due to the number of genera, since the profiles differed mainly in the range of high values of the scale parameter a . This means that differences of diversity were mainly due to the distribution of nematode numbers among genera. Indeed, the contribution of the most abundant genera of the organic sites did not exceed 22%. In [C] the community displayed a quite different structure due to the strong dominance of a single genus,

namely *Helicotylenchus*, which accounted for 40% of the community in the post-harvest season. The strong dominance of this semiendoparasite in [C] was even higher (50%) in the harvest season, causing the observed decrease of diversity in this case.

A tree clustering of sites is presented in Fig. 4. Each site is represented twice; once for the post-harvest and once for the harvest season, coded as P and H respectively. Moreover, we provide the maximum indicator value of each genus at the site or cluster of sites to which it is assigned. Changing management regime from conventional to organic cultivation caused profound modifications in generic structure and composition, since organic plots were separated from conventional, regardless of season. The most recent organic plots constituted an exception since they were clustered either with the



	IndVal		IndVal		IndVal
A		D		H [O1]	
Acrobeloides (BA-2)	100	Drilocephalobus (BA-2)	30.87**	Buonematidae (BA-1)	18.19**
Aphelenchus (FU-2)	98.75	Ditylenchus (FU-2)	26.12**	H [O2]	
Chiloplacus (BA-2)	98.75			Miculenchus (NP-2)	13.58
Filenchus (NP-2)	90	E		Odontopharynx (BA-1)	12.50
Eucephalobus (BA-2)	85	Thonus (PR-4)	22.66**	Alaimidae (BA-4)	25
Aphelenchoides (FU-2)	75	Diplogasteridae (BA-1)	8.11	P [O2]	
Panagrolaimus (BA-1)	72.50			Lelenchus (NP-2)	12.50
Malenchus (NP-2)	71.25	F			
Mesorhabditis (BA-1)	71.25	Pristionchus (BA-1)	26.53	H [O4]	
Eumonhystera (BA-2)	67.50			Hexatylyus (FU-2)	50**
Protorhabditis (BA-1)	61.25	G		H [O5]	
Rhabditidae 1 (BA-1)	52.50	Zygotylenchus (PA -3)	12.50	Pelodera (BA-1)	27.97**
Boleodorus (NP-2)	42.50			Pelioditis (BA-1)	12.50
Paraphelenchus (FU-2)	42.50	H [C]		Seinura (PR-2)	8.20
Bursilla (BA-1)	41.25	Laimydorus (OM-5)	36.98**	P [O4]	
Tylenchus (NP-2)	40	Plectus (BA-2)	12.50	Neopsilenchus (NP-2)	14.81
Diploscapter (BA-1)	31.25	Epidorylaimus (OM-4)	12.50	Tylencholaimellus (FU-4)	12.50
Coarctadera (BA-1)	30	Discolaimidae (PR-5)	12.50	Mylonchulus (PR-4)	9.98
Cervidellus (BA-2)	28.75	Basiria (NP-2)	10.53	P [O5]	
		Cephalobus (BA-2)	10.51	Paratylenchus (PA -2)	74.39**
B		Acrobelophis (BA-2)	7.66	Acrobeles (BA-2)	50.43**
Helicotylenchus (PA -3)	76.31**	P [C]		Aporcelaimellus (PR-5)	28**
Tylenchorhynchus (PA -3)	50.80**	Criconematidae (PA -3)	25	Aglenchus (NP-2)	12.50
Eudorylaimus (PR-4)	32.28**	Teratocephalus (BA-3)	25	Wilsonema (BA-2)	10.21
Cuticularia (BA-1)	14.13**	Rhabditidae 2 (BA-1)	21.08		
C		P [O1]			
Heterocephalobus (BA-2)	67.30**	Pseudhalenchus (FU-2)	46.81**		
		Mesodorylaimus (OM-5)	22.59**		
		Neothada (NP-2)	12.50		
		Prismatolaimus (BA-3)	12.50		

Fig. 4. – Dendrogram presenting the indicator genera assigned by IndVal to each combination of site and season. *H* and *P* before site codes stand for “harvest season” and “post-harvest season” respectively. The Indicator Value of each genus for each cluster is given, while the trophic group and c-p value are indicated in parentheses. BA: Bacterivore, FU: Fungivore, PA: Phytotparasite, NP: Non parasitic plant feeder, PR: Predator, OM: Omnivore (** $P < 0.01$).

conventional or with the organic plots depending on the season of sampling. At a second level, older organic cultivations [O4] and [O5] were grouped together, without nevertheless masking the effect of seasonal agricultural practices on nematode communities. On the other hand, this seasonal effect was not revealed in the case of conventional plots, since [HC] and [PC] were grouped together with the lowest linkage distance.

According to IndVal method, from the total of 61 genera recorded, 19 could be considered erytopic, since they were found at all sites in both seasons. Among these genera no phytoparasites were found. The highest IndVal values were displayed by *Acrobeloides*, *Aphelenchus* and *Chiloplacus*, which were well presented at all samples constituting together about 28% of the total nematode community. It seems that the major separation of the organic from the conventional cluster was mainly due to

two genera; the bacterivore *Heterocephalobus* characterized the organic cluster, while the phytoparasite *Helicotylenchus* was assigned to the conventional cluster with the highest indicator value. The second most important genus characterizing the conventional cluster was the phytoparasitic *Tylenchorhynchus*, followed by the predator *Eudorylaimus*, which however was of very low abundance. Another genus that may be considered bioindicator, is the phytoparasite *Paratylenchus*, which was recorded only in the older organic cultivations.

Data depicted in Fig. 5, concerning the abundances of the two main indicator genera, namely *Helicotylenchus* and *Heterocephalobus*, revealed a completely opposite trend of abundance change along the gradient from conventional to recent and further to older organic cultivations.

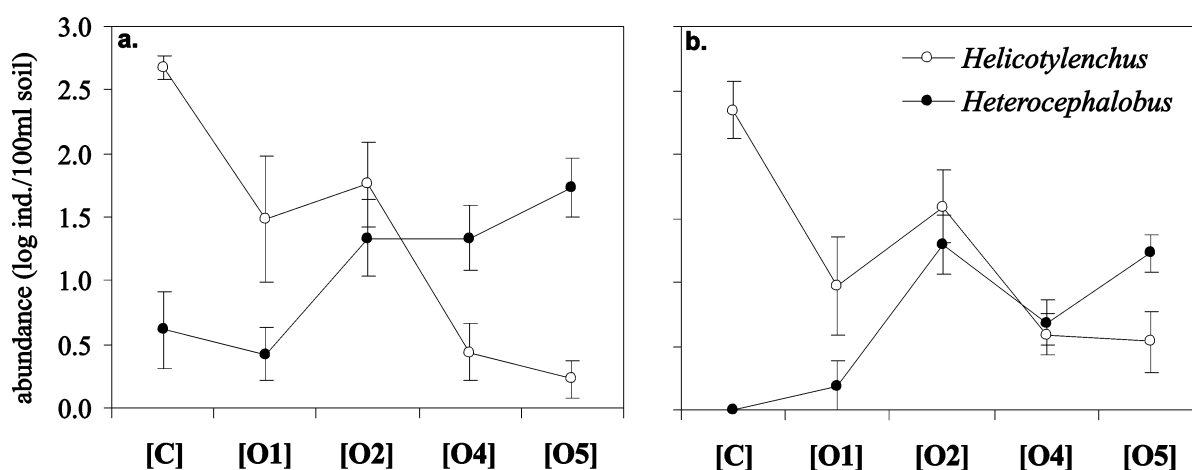


Fig. 5. – Abundance of *Helicotylenchus* and *Heterocephalobus* in the studied agricultural systems in (a) the post-harvest season and (b) the harvest season.

DISCUSSION

This study aims to demonstrate the differential effect of agricultural practices on the soil nematode community under asparagus cultivation. Our experimental design reflected two types of changes in agricultural practices. First, our plots constituted a gradient from conventional asparagus cultivation to recent organic of 1 and 2 years, which are considered transitional, and further to older and certified organic cultivations of 4 and 5 years, reflecting thus long-term effects of changing management regime. Along this gradient, changes regarding the type of nutrient input and weed control are involved. Second, the replication of samplings throughout the year reflected seasonal agricultural practices shared between both organic and conventional management.

Transition from conventional to organic

The investigation of nematode communities in soils with different history of management practices revealed several differences at various levels of community analysis. The most important finding from the analysis of

trophic structure was the gradual reduction of plant feeders from conventional to the older organic cultivation, while the exactly opposite trend was revealed in the case of nematodes that feed on decomposers, i.e. bacteria and fungi. Our results support the hypothesis of VAN DIEPENINGEN et al. (2006) that the differences between the two management types are more gradual than black and white, and are in agreement with the findings of several authors (e.g. NEHER & OLSON, 1999; GARCÍA-ÁLVAREZ et al., 2004), who reported an increase of decomposer feeders and especially bacterivores under organic cultivation.

In our study, the ratio of plant feeders to decomposer feeders was 1:0.7 under conventional cultivation, changed approximately to 1:2.6 in the recent and to 1:3.8 in the older organic plots, reflecting gradual changes between the “grazing food web” and the “detritus food web” of PETERSEN & LUXTON (1982) or the “root-herbivore” and “decomposer” guilds of BRUSSAARD (1998). This shift probably resulted from the different form of nutrient input in the cultivated plots. Organic fertilizers are expected to favour bacterivores and fungivores, since they act via the microbial soil component. Feeding activity of these groups stimulates decomposition and nitrogen

mineralization (FERRIS et al., 2004). Indeed in a parallel experiment carried out in our experimental area, MONOKROUSOS et al. (2006) found that N-mineralization rates were lower in conventionally cultivated plots and increased gradually to older organic plots.

The long-term shift in trophic structure was even more pronounced when only phytoparasites instead of total plant feeders were considered. Non parasitic plant feeders actually appeared more frequently in the older organic plots as revealed by log-linear analysis and also by PPI values, since this trophic group consists of cp-2 nematodes. PPI values were significantly higher under conventional cultivation and declined gradually with increasing time of organic management, following the changes of the fraction of cp-3 parasitic nematodes. A decline of phytoparasitic nematodes in organic agro-ecosystems is reported by many authors (e.g. BOHLEN & EDWARDS, 1994; AKHTAR & MAHMOOD, 1996; WIDMER et al., 2002). MCSORLEY & GALLAHER (1995) as well as AKHTAR & MALIK (2000) have proposed that phytoparasitic nematodes may be suppressed by toxic by-products from decomposition of organic amendments and that the long-term use of the latter may even stimulate the activity of biological antagonists of nematodes. On the other hand, mineral fertilizers are known to be the reason for softer plant tissues as carbohydrates are diverted to protein synthesis instead of cell wall construction (TISDALE & NELSON, 1975), which makes plants more susceptible to phytoparasites. Thus, the gradual decrease of phytoparasites with time of organic management may indicate a long-term recovery of asparagus after ending the use of mineral fertilizers. In contrast to our results, NEHER (1999) found more parasitic nematodes in her organically cultivated plots but since the latter were cultivated by various crops, she attributed her results to host specific relationships. Moreover, FERRIS et al. (1996) claims that phytoparasitic nematodes are strongly influenced by host status of the current and previous crop. We should remind though that all experimental plots of our study were cultivated with the same perennial crop.

Apart from PPI, all other nematode indices did not change significantly with increasing time of organic management. BERKELMANS et al. (2003), who have studied nematode communities in cultivations with different history of organic management, also found that nematode indices which did not include plant feeders were insensitive to residual effects of management. Furthermore, the Maturity Index (MI) and the Structure Index (SI) indicate the successional stage of communities, which are in any case premature in cultivated soils. As NEHER & OLSON (1999) state, all agricultural practices aim at maintaining ecosystems at a more productive, pre-mature successional stage. On the other hand, both MI and SI increase with increasing contribution of predators and omnivores, which are of high c-p values, i.e. their life strategies are closer to the right end of the of the r-K continuum. However, the proportion of these trophic groups is very low in nematode communities of cultivated soils (FERRIS et al., 1996; FRECKMAN & ETTEMA, 1993; LIANG et al., 2001 among others) and in our samples they appeared sparsely.

The long-term effect of changing management regime was also obvious from the analyses at the generic level,

both in terms of diversity and of community composition. Regarding diversity, our approach was based on producing diversity profiles for nematode communities instead of the commonly used diversity indices, because the latter are not fruitful in describing a multidimensional concept, such as a community, reducing it to a single number. Diversity was found to increase gradually with time of organic management. This was not due to an increase of genera richness but due to differences in dominance patterns. Under conventional cultivation, we observed strong dominance of the phytoparasite *Helicotylenchus*. A more even distribution of nematode numbers among genera was found with increasing time of organic management. The high abundance of phytoparasites and especially the strong dominance of few species among them, according to WASILEWSKA (1997), occur in long-term monocultures and are related to environmental degradation. The higher nematode diversity in the organically managed systems might also result from the increased availability of microhabitats due to organic amendments and lack of herbicides. The latter allows to an extent immigration from the weed species pool of the larger area. This is in agreement with the work of DE DEYN et al. (2004) who observed strong dominance patterns of nematodes in experimental plant monocultures and more even and diverse nematode communities in plots with higher plant diversity.

Regarding the composition of the nematode community, nineteen genera belonging mainly to the bacterivore and fungivore group were classified as eurytopic, i.e. with not specialized habitat requirements. The most abundant among them, namely *Aphelenchus*, *Acrobeloides* and *Chiloplacus* have been reported in a vast number of studies to dominate in soils under various agricultural practices, crops, ecosystems, as well as different geographic regions, climate and soil types (LIANG et al., 1999; LIANG et al., 2005a; LIANG et al., 2005b; PAPTHEODOROU et al., 2004; ILIEVA-MAKULEC, 2000; ZOLDA, 2006). Changing management regime caused gradual changes in generic structure and composition, separating the older organic plots from conventional. The relatively vague classification of recent organic plots indicates their transitional character along the gradient from conventional to organic. A striking finding was the combined decrease of *Helicotylenchus* and increase of *Heterocephalobus* with time of organic management. *Helicotylenchus* was also found in high abundance by YEATES et al. (1999) in asparagus cultivations where mineral fertilizers were applied. The bacterivore *Heterocephalobus* on the other hand was proposed by (FISCUS & NEHER, 2002) as an indicator of soil quality due to its sensitivity to disturbance, and was reported by MULDER et al. (2003) to prefer lower values of soil pH. Changes in abundance of *Heterocephalobus* might be related to the gradual pH decline from the conventional to the older organic plots of our study, which probably resulted from the long-term use of organic amendments (WIDMER et al., 2002). We should also note that the phytoparasite *Tylenchorhynchus* exhibited the same trend as *Helicotylenchus* with time of organic management. Indeed, according to VESTERGÅRD (2004) this ectoparasitic plant feeder is stimulated by N-fertilization.

Seasonal agricultural practices

Nematode indices were found useful mostly for distinguishing seasonal agricultural practices. Maturity Index (MI) was higher during October, i.e. the season with least disturbance, since fields were left with no agricultural activities for several months. The growth period of asparagus stops in autumn and during winter nutrients are translocated from the fern to the rhizome from where they will be remobilised into new spear growth in spring (FAVILLE et al., 1999). This nutrient translocation seems to favour phytoparasites leading to a higher Plant Parasitic index (PPI) in December.

Channel Index (CI) was higher in October and December, i.e. during the post-harvest season. This indicates a shift from a more bacterial to a more fungal mediated decomposition pathway (FERRIS et al., 2001). During this period, there were amendments of plant biomass, originating either from dead weed residues after summer control or from aboveground asparagus parts, which were cut in late November and left in the fields to decompose. Plant residues constitute amendments of high C/N ratio, favouring populations of fungivores (BOHLEN & EDWARDS, 1994). Two weeks before the beginning of spear growing the soil was mounded and thus the plant residues were incorporated into the soil. Their advanced decomposition renders this organic source more labile, favouring the enrichment opportunist bacterivores with c-p value 1 (FERRIS & MATUTE, 2003). The increase of the Ba-1 functional guild resulted in significantly higher values of the Enrichment Index (EI) during March.

The distinction between the post-harvest and the harvest season was also evident from the analyses of community structure at the generic level, but mostly in the older organic plots. This was mainly due to changes in abundance of genera which were classified as eurytopic. For example *Panagrolaimus*, *Diploscapter* and *Bursilla*, which all belong to the Ba-1 functional guild, increased in the harvest season, while the Fu-2 *Aphelechooides* and *Paraphelenchus* and the root hair feeder of c-p 2 *Filenchus* displayed higher abundance in the post-harvest season. *Heterocephalobus* (Ba-2) which characterized the organic plots displayed a significantly higher abundance during this season. On the other hand, seasonal patterns were not pronounced in conventional plots. The predominance of *Helicotylenchus* (c-p 3 phytoparasite) in these plots during both seasons diminished the importance of any other changes in the contribution of the remaining species. It seems that a strong pressure as the one imposed by long-term conventional management masks the results of less intense pressures, as the ones imposed by short-term management practices.

CONCLUSION

Changing management regime of asparagus cultivation from conventional to organic as well as seasonal agricultural practices appeared to constitute driving forces altering nematode communities in different ways. More specifically, changing management regime seemed to initiate successional long-term changes in nematode communities, such as the gradual decline of phytoparasites from

conventional to organic cultivation in favour of bacterivores and fungivores, the increase of non parasitic plant feeders, the gradual decrease of PPI, the increase of diversity. Alterations in the generic structure of the community were also revealed, driven mostly by the opposite trends of changes in abundance of *Helicotylenchus* vs. *Heterocephalobus*. Seasonal agricultural practices appeared to induce short-term responses of functional guilds of lower c-p values, and were reflected in all nematodes indices studied except SI. Nematode responses at the generic level to seasonal agricultural practices seemed less intense than the ones imposed by changing management regime, and in the case of conventional cultivation they were almost entirely masked.

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