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The effects of large- and small-scale differences in soil temperature and moisture on bacterial functional diversity and the community of bacterivorous nematodes

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Abstract

This paper describes the effects of large-scale seasonal fluctuations as well as experimentally induced small-scale variations of soil temperature and moisture on the community of bacterivorous nematodes and the activity of soil bacteria from a Greek Mediterranean grassland. Our experiment spanned a 6-month period (July–December 1996), during which artificially created changes in temperature (1.4 °C) and moisture content of the soil (2.3%) were superimposed on seasonal variations of these variables.

Functional diversity of soil bacteria was assessed using Biolog GN-plates. Bacterial diversity, richness and evenness as well as the mean oxidation of almost all Biolog substrate groups, were affected significantly by the seasonal fluctuations of soil temperature and moisture, and declined linearly from July to December. The substrates responsible for the differences in monthly soil samples were mainly carbohydrates and carboxylic acids. The small-scale experimental changes in temperature and moisture affected oxidation of only four out of the eight substrate groups.

The effect of seasonality was also obvious on the diversity, evenness, richness and total density of soil bacterivorous nematodes, which exhibited higher values during the period September–October. The effect of experimental temperature and moisture modifications varied between the different nematode taxa. *Acrobeles*, *Chiloplacus* and *Cephalobus* responded significantly to small-scale temperature changes, while the responses of the other taxa, if any, were more complicated, depending on the interactive effect of both soil temperature and moisture. In any case, the experimental modifications of moisture alone did not induce significant changes in the structure of the nematode community.

After comparing the community parameters of bacteria and bacterivorous nematodes, we conclude that there was no synchronised response between nematodes and their food resources.

Keywords: Climatic change; Prey–predator relationship; Bioindicators; Mediterranean grassland

1. Introduction

Soil microbial populations are early indicators of changes in soil organic matter (Lundquist et al., 1999). Changes in soil conditions (e.g. temperature and humidity) are expected to affect the structure and the function of microbial communities (Van Gestel et al.,

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1993; Grayston et al., 2001; Wilkinson et al., 2002), a fact that is related to soil fertility and ecosystem activities (Beare et al., 1995; Chapin III and Korner, 1995). These changes are also reflected in the nematode community composition (Griffiths et al., 1994; Armendariz et al., 1996; Akhtar, 2000), a consequence of the strong interrelationships between nematodes and the edaphic microflora.

The extent to which the nematode–microbial interaction depends on the ecosystem and the climate remains largely unknown (Ekschmitt et al., 1999). Moreover, there is uncertainty concerning the effects of the foreseen global climatic changes on these components of soil ecosystems, especially at a local scale (Bakonyi and Nagy, 2000). Thus, an EU-funded project has been set up, entitled "Diversity Effects in Grassland Ecosystems of Europe (DEGREE)" and focusing on climate change effects on soil nematodes, microbial processes and nutrient transformation patterns. The European grasslands included in the project (from Greece, Hungary, Germany, Netherlands, Great Britain and Sweden) cover a wide range of climatic conditions, while the soil microclimate at each site was experimentally manipulated according to a common design for all countries. Thus, a smaller scale gradient of temperature and humidity was established at each site.

The work presented here is part of our contribution to the DEGREE project and was carried out in a Mediterranean grassland. We focused on the changes in diversity of bacteria and bacterivorous nematodes under microclimatic differences of two scales. Seasonal differences were considered to be of a large scale, since the Mediterranean climate exhibits a clear-cut seasonality, characterised by hot, dry summers and mild, wet winters (Aschman, 1973). The small-scale differences were produced experimentally, by manipulation of temperature and soil moisture in the field, and could be considered similar to those predicted by some climate change scenarios for the Mediterranean region (Osborne and Woodward, 2002).

We estimated the functional instead of the taxonomic diversity of the soil microbial community, since the former seems to provide information more relevant to the functioning of soils (Zak et al., 1994; Trevors, 1998) and is considered crucial for the long-term stability of an ecosystem. For this purpose, we used the BIOLOG method, which has been used successfully to discriminate microbial communities from different soil types (Winding, 1994; Goodfriend, 1998), from soils subjected to different management practices (Buyer and Drinkwater, 1997), or from soils under different plant species (Zak et al., 1994; Grayston et al., 1998).

Thus, in this paper, we describe the bacterial functional diversity and the diversity of bacterial-feeding nematodes, and compare their responses to the large seasonal changes in soil temperature and humidity, as well as to the small-scale changes in these parameters.

2. Materials and methods

2.1. Study area

The research site is on the Halkidiki peninsula 55 km south-east of Thessaloniki (40°20′N latitude, 23°12′E longitude). It lies at an altitude of 210–215 m and has a south-easterby aspect. The area, described by Diamantopoulos et al. (1996), lies on a limestone block of Kimmeridgian-Portlandian age, surrounded by Miocene–Pliocene deposits. The soil is shallow, discontinuous and generally not more than 10 cm deep. The profiles are classified as lithic leptosols (FAO) with a gravelly and stony clay-loam texture. Soil particles bigger than 2 mm represent 69.5% of fresh weight of soil. Soil organic carbon varies from 4.0 to 5.3% and the total amount of organic matter from 6.9 to 9.2% dry matter of soil. The pH (H_2O) and pH (KCl) are 7.7 and 6.6, respectively.

According to climatic data for the 1981–1990 decade recorded at the nearest meteorological station, the climate of the region is characterized as Mediterranean with small amounts of rainfall during the hot summer months. The driest months are July, August and September. Mean annual air temperature and precipitation are 16.0 °C and 435.5 mm, respectively.

2.2. Experimental design

The experiment lasted for the 6-month period July–December 1996, during which soil microclimatic conditions were manipulated in experimental field plots of 1 m × 1 m. The plots were established in a grass-covered area. The dominant species was *Stipa bromoides* (L.) Dorf., while species such as *Aegilops*

geniculata (Roth.), *Aegilops triuncialis* (L.), *Avena sterilis* (L.), *Brachypodium distachyum* (L.) Beauv., *Bromus tectorum* (L.) and *Dactylis glomerata* (L.) among others were also recorded (Dalaka, 2001).

The experimental setup, common for all the DEGREE partners, aimed at the creation of different combinations of temperature and soil moisture conditions in 14 field plots, and superimposing them on the local seasonal variations in these climatic variables (Ekschmitt et al., 1999; Bakonyi and Nagy, 2000). For temperature adjustment, constructions combining vertical windshields and horizontal transparent greenhouse roofs (which intercepted precipitation) were used, while moisture was controlled by weekly irrigation. Specifically, for temperature manipulation the experimental plots were shielded by transparent or black plastic material with smaller or higher number of holes allowing air to flow, while the irrigation experiment involved addition of water in accordance with data depicted in the ombrothermic diagram. The monthly amount of added water (partitioned in weekly equal applications) was equivalent to 44, 66, 88, 110, 132 and 156% of the corresponding mean monthly precipitation.

To monitor moisture and temperature conditions actually created in the field, soil temperature and water content recorded monthly. Soil temperature was measured by min–max thermometers placed 5 cm below the soil surface and left in the field plots for the whole month. For the determination of soil moisture two soil samples taken from each field plot were weighted, oven dried to constant weight (104 °C for one day), and weighed again.

Monthly recordings of soil moisture in two out of the 14 initial field plots appeared erratic. Consequently, these plots were excluded from further analysis. Then, soil water content data were analysed by the *K*-means clustering method in order to classify the remaining 12 plots on the basis of different soil moisture levels. The *K*-means clustering method is analogous to analysis of variance in reverse, i.e. the program moves cases between clusters in order to minimise the within-cluster variability, maximising at the same time the between-groups variability. Two clusters, and therefore, two different moisture levels were identified, each comprising six plots. Analysis of variance showed that the water content recorded in the plots of cluster I (referred hereafter as 'humid') was significantly higher (average difference 2.3% dry weight; $P < 0.05$) than that recorded in the plots of cluster II (referred to as 'dry') on all sampling occasions, except in July, when no significant difference was revealed.

The same procedure was followed in order to define the different soil temperature levels obtained by experimental manipulation. As with soil moisture, the *K*-means clustering analysis resulted in two clusters of six plots each. Soil temperature in the plots of cluster I (referred to as 'cold') was significantly lower (1.4 °C on average; $P < 0.05$) than that recorded in the plots of cluster II (referred as 'warm') during the whole experimental period, with the exception of July.

According to the above, the experimental manipulations of microclimate resulted in a full factorial scheme of 2 moisture × 2 temperature levels, which corresponds to the creation of four experimental treatments, i.e. four distinct moisture × temperature combinations, namely dry–cold, humid–cold, dry–warm and humid–warm. Each treatment comprised three individual plots. However, although the experimental modifications of moisture and temperature were of small scale, they were not masked by the seasonal variations of those variables, which were of a larger scale (Fig. 1).

2.3. Soil sampling

Sampling took place on a monthly basis, during the 6-month experimental period. On each sampling occasion, three random soil cores were taken from the top 8 cm of soil in each field plot with a steel cylinder (7 cm diameter). The three cores from each plot were combined into a composite sample, placed in polythene bags and transported to the laboratory. A 100 g (fresh weight) subsample was taken from the composite soil sample from each plot for nematode extraction. Another 5 g portion was stored at 4 °C for estimating bacterial activity.

2.4. Bacterial functional diversity

In order to assess the bacterial functional diversity, the substrate utilization potential of the gram-negative bacteria was measured by means of a modified BIOLOG assay (Vahjen et al., 1995). The soil samples (5 g each) were suspended for 30 min at 4 °C with

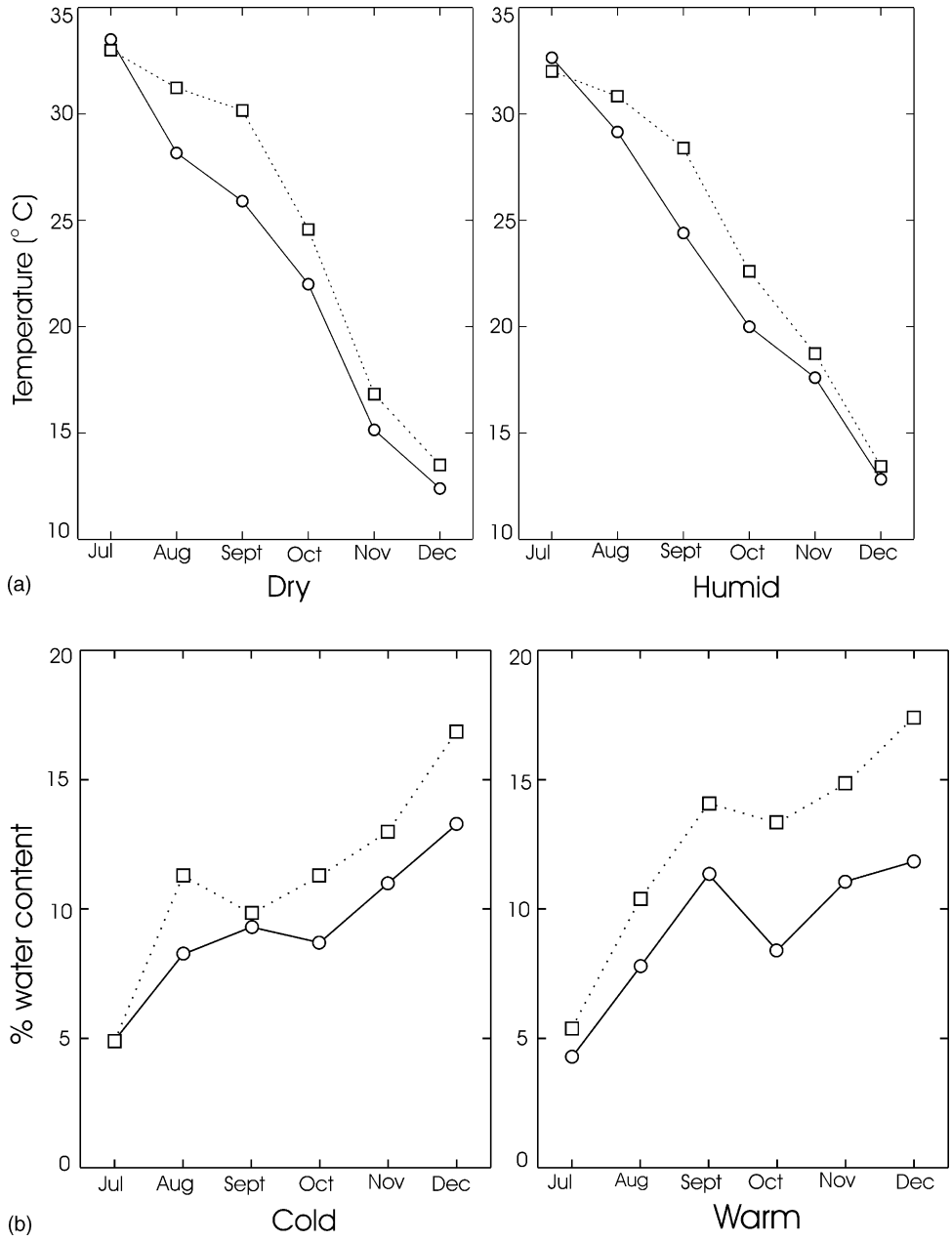

Fig. 1. Temporal fluctuations in soil temperature and moisture in the four experimental treatments. (a) Cold (○); warm (□). (b) Dry (○); humid (□).

10 ml of a sterile 0.85% NaCl solution at 220 rpm on a shaker. The soil slurry was sedimented for 1 h at 4 °C. Four millilitre of the suspension were removed and diluted with 12 ml of a sterile 0.85% NaCl solution (because there were still too many soil particles in the suspension).

Each well of the BIOLOG GN-plates was inoculated with 100 μl of the above dilution. The number

of wells per plate was 96, i.e. 95 wells containing substrates to be consumed by bacteria and an empty control well. Bacterial substrate utilization was photometrically determined at 590 nm, after 24 h incubation of plates at 28 °C. The absorption value of the control well was subtracted from each substrate absorption value to obtain substrate specific oxidation values. Substrates with negative absorption values were considered non-oxidized. The mean absorption of each plate after 24 h was 0.26 units.

2.5. Nematode extraction

One day after sampling, nematodes were isolated from the 100 g soil subsamples, using Cobb's sieving and decanting method (S'Jacob and van Bezooijen, 1984). In the final separation stage, nematodes were allowed 2 days to move through a double layer of cotton wool filters. After counting total numbers of specimens, nematodes were fixed in 4% formalin and sent to the Hungarian partners of DEGREE, who identified them to genus level and classified them into feeding types following Yeates et al. (1993). In the work presented here, only the bacterivorous nematodes are considered.

2.6. Data analysis

In order to explore the effects of the large-scale seasonal variations in soil temperature and humidity as well as the small-scale effects of our experimental manipulations on bacterial functional diversity and on the density and diversity of the bacterivorous nematodes, we analysed data from the whole sampling period by two-way ANOVA. The experimental temperature and moisture levels (warm and cold, and humid and dry, respectively) were the grouping variables, while the month of sampling, which accounts for the effect of seasonality, was the blocking one. Factors are designated as blocking variables when they account for a significant amount of variability, as is the case with seasonality in this study. Thus, the error variance for ANOVA is reduced. Variables that did not follow the normal distribution, according to the Kolmogorov–Smirnov test, were logarithmically transformed.

To test whether soil samples collected during different sampling occasions constitute statistically distinct groups in relation to the potential catabolic activity recorded in them, a standard discriminant analysis was applied. Moreover, by this analysis the discriminatory power of each substrate (95 in each Biolog plate) was revealed. The month of sampling was the grouping variable, while individual substrate oxidations were the dependent variables. Finally, we used linear regression analysis to check whether values relating to components of the bacterial catabolic activity change linearly with sampling period. All analyses were carried out by means of the STATISTICA package.

3. Results

3.1. Bacterial functional diversity

The substrates of each BIOLOG-plate can be divided into eight different groups: amines/amides (AMINE) (6 substrates), aminoacids (AACID) (20 substrates), carbohydrates (CAHYD) (27 substrates), carboxylic acids (CAACID) (27 substrates), alcohols (ALCOH) (3 substrates), nucleosides (NUCLE) (4 substrates), glycolipids (GLIPI) (2 substrates) and alditols (ALDIT) (6 substrates).

In Table 1, mean values of bacterial functional diversity indices, i.e. Shannon substrate diversity, evenness and richness, along with mean oxidation per substrate group are presented. These values refer to all samples, regardless of the time of sampling or experimental treatment. Higher oxidation values were recorded for ALDIT and GLIPI although these groups contain only 8 of the 95 substrates. Among the most numerous substrate groups (CAHYD, CAACID and AACID), CAHYD exhibited the higher oxidation values.

The ANOVA results regarding the effects of the large-scale seasonal differences of soil temperature and moisture as well as the small-scale effects of our experimental manipulations on bacterial activity are also indicated in Table 1. The small-scale experimental differences in temperature and moisture affected significantly the mean oxidation of only four out of the eight groups. More specifically, CAHYD utilization was affected by temperature manipulations, exhibiting higher values in samples from the warm plots. The utilization of ALCOH, GLIPI and ALDIT was greater in samples from the dry plots, indicating a

Table 1
Mean values of Shannon substrate diversity, evenness and richness for all samples, along with mean oxidation per substrate group

	Mean ± S.E.	Month of sampling	Temperature (*T*)	Moisture (*M*)	Interaction ($T \times M$)
Diversity	4.04 ± 0.020	July, August, September ($F = 9.79$)***			
Richness	82.37 ± 1.26	July, August, September ($F = 8.81$)***		Dry ($F = 4.86$)*	
Evenness	0.92 ± 0.003	July, August, September ($F = 4.78$)**			
AMINE	0.012 ± 0.003	July, September ($F = 11.27$)***			
AACID	0.016 ± 0.003	July, September ($F = 25.32$)***			
CAHYD	0.041 ± 0.004	July, August, September ($F = 17.01$)***	Warm ($F = 7.17$)*		
CAACID	0.029 ± 0.004	July, September ($F = 16.63$)***			
ALCOH	0.028 ± 0.004			Dry ($F = 6.55$)*	
NUCLE	0.020 ± 0.004	July ($F = 15.95$)***			
GLIPI	0.055 ± 0.007	July, August ($F = 9.86$)***		Dry ($F = 4.44$)*	
ALDIT	0.057 ± 0.005	July, August, September ($F = 7.84$)***		Dry ($F = 5.43$)*	

Month of sampling (blocking variable) and levels of temperature and moisture (grouping variables), with highest values of the corresponding parameters being indicated. Significant *F* values, from two-way ANOVA are given.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

significant effect of moisture manipulations. With the exception of ALCOH, the effect of seasonal variations in climatic variables was highly pronounced, since the components of the catabolic diversity of bacteria (substrate diversity, evenness and richness), as well as the mean oxidation per substrate group, exhibited higher values during the period July–September (Table 1, Fig. 2). Moreover, all the above mentioned parameters exhibited a significant ($P < 0.001$) linear reduction with time, from July to December (Table 2).

Table 2
Linear regressions of the components of catabolic activity and the utilization of each substrate group against time

		$F(1, 70)$	*P*
Diversity	$a = 10.014$, $b = -0.058$	24.25	<0.0001
Evenness	$a = 1.645$, $b = -0.007$	20.57	<0.0001
Richness	$a = 329.107$, $b = -2.407$	12.34	<0.001
AMINE	$a = 0.356$, $b = -0.003$	18.79	<0.0001
AACID	$a = 0.468$, $b = -0.004$	35.43	<0.0001
CAHYD	$a = 0.629$, $b = -0.005$	26.66	<0.0001
CAACID	$a = 0.542$, $b = -0.005$	24.97	<0.0001
ALCOH	Ms		
NUCLE	$a = 0.462$, $b = -0.004$	22.71	<0.0001
GLIPI	$a = 0.179$, $b = -0.001$	21.86	<0.0001
ALDIT	$a = 0.621$, $b = -0.005$	15.69	<0.0001

F values and the level of significance are also given.

The first three roots of discriminant analysis were highly significant ($P < 0.01$), accounting for 93% of data variability (Table 3). Nine out of the 95 substrates proved statistically significant for the temporal differentiation of samples. Five of them were carbohydrates, two were carboxylic acids, one aminoacid and one alcohol. By plotting the individual scores of monthly samples for the first two discriminant roots, a clear temporal pattern of the microbial catabolic profiles was revealed (Fig. 3). Grouping of individual samples appeared coherent, varying between 100% for samples taken in July and December and 83% for samples taken in September. Along the first root, samples from July were clearly discriminated from the rest of the samples. The July samples were characterized by high utilization of tween 80, *m*-inositol and turanose. The samples from all the other months were characterized by high oxidation values of D-mannose (samples from September) and D-saccharid acid (samples from September and December). Along the second root, samples from December were discriminated clearly from those of August, September, October and November. Samples from December, occupying the upper left part of the biplot, were characterized by high utilization of D-saccharid acid and tween 80, while the samples from other months (not including July) showed higher oxidation values of propionic acid and D-mannose.

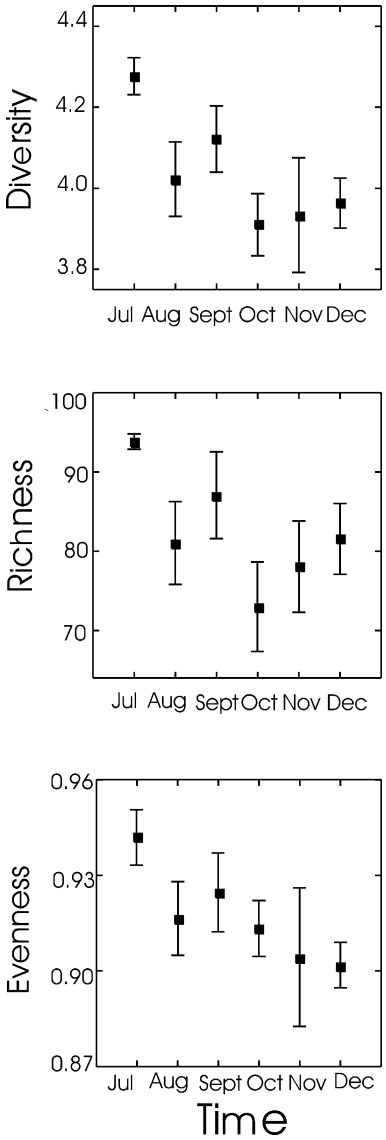

Fig. 2. Temporal changes in diversity, richness and evenness of bacterial catabolic activity (means ± 1.96 × S.E.).

3.2. Bacterivorous nematodes

The community of bacterivorous nematodes consisted of 13 taxa belonging to six families. The most abundant family was that of Cephalobidae, to which the three dominant genera (*Chiloplacus*, *Acrobeles* and *Acrobeloides*) belonged. Overall nematode density, along with diversity, richness and evenness, were affected significantly by seasonality and displayed more or less similar temporal patterns, exhibiting higher values mainly in the middle of the sampling period (September–October) (Table 4). The abundance of 8 out of the 13 taxa exhibited temporal constancy and thus, was not affected by seasonal climatic changes. However, numbers of the abundant genera *Chiloplacus*, *Acrobeles* and *Acrobeloides*, together with *Achromadora* and the family of Monhysteridae, varied seasonally. With the exception of *Achromadora*, peaking in July, greater abundance of the other genera occurred in September and/or October.

The interactive effects of temperature and moisture manipulations were significant for all parameters of the nematode community except richness. While nematode density was higher in dry–cold samples, diversity and evenness increased in the humid–warm samples. Richness was sensitive only to temperature manipulations, with higher values occurring in samples from the warm plots. The effect of temperature was significant for *Acrobeles* and *Cephalobus* (higher numbers for these genera were recorded in warm plots) and for *Chiloplacus* (with higher numbers occurring in cold plots). The interactive effect of moisture × temperature was significant for *Acromoldavicus*, which displayed higher abundance in dry–cold plots, for *Tylocephalus* (with higher numbers in humid–warm plots) as well as for *Plectus*, Monhysteridae and *Panagrolaimus*. Increased numbers of the latter occurred in dry–cold and humid–warm samples. Finally, no effect of either temperature or moisture manipulations was detected for *Achromadora*, *Eumonhystera*, *Prismatolaimus* and *Heterocephalobus*. It is remarkable that small-scale differences in soil moisture alone did not induce changes in any of the components of the bacterivorous nematode community.

4. Discussion

4.1. Bacterial functional diversity

Although the substrates of the BIOLOG plates may not represent accurately substrates present in the field, and the activity of bacteria in a solution culture may be quite different from that in soil, BIOLOG estimations provide an indication of the potential functional

Table 3
Standardized coefficients for canonical variables estimated by discriminant analysis

BIOLOG substrates	Root 1	Root 2	Root 3	*P*	Group
Tween 80	**0.93586**	**0.80091**	0.10723	<0.05	CAHYD
Glycogen	0.40823	−0.56905	0.40938	<0.05	CAHYD
Tween 40	0.07930	0.22242	0.52712		CAHYD
m-Inositol	**1.10479**	0.45444	0.33806	<0.01	CAHYD
D-Mannose	**−1.02053**	**−0.94903**	0.19617	<0.01	CAHYD
D-Mannitol	−0.42619	0.41841	−0.62234		CAHYD
α-Cyclodextrin	−0.34080	−0.37360	−0.15393		CAHYD
Turanose	**2.37465**	−0.44573	**−1.06022**	<0.01	CAHYD
β-Methyl-D-glycoside	0.61063	−0.27327	0.30210		CAHYD
Dextrin	−0.50467	0.29495	−0.00354		CAHYD
D-Saccharid acid	**−1.33458**	**0.90409**	0.21067	<0.01	CAACID
Propionic acid	0.76775	**−0.81026**	0.40455	<0.01	CAACID
D-Glucosaminic acid	−1.86983	−0.20993	0.46831		CAACID
Quinic acid	−0.29359	0.21181	0.49100		CAACID
Acetic acid	0.31457	−0.18308	−0.48853		CAACID
Hydroxy-L-proline	−0.09416	−0.61102	−0.85919		AACID
L-Asparagine	0.70637	0.40090	−0.57366	<0.05	AACID
Succinamic acid	0.10513	0.77727	1.78996		AMINE
Inosine	0.47104	−0.16644	−0.69672		NUCLEO
Uridine	0.35574	0.12815	0.22215		NUCLEO
D,L-α-Glycerol phosphate	−0.33150	0.40775	0.58149	<0.05	ALCOH
Glycerol	−0.26185	−0.41408	−0.07973		ALCOH
Eigenvalue	9.36442	4.73292	1.87885		
Cumulative proportion	0.54783	0.82470	0.93462		

Bold letters correspond to substrates that contributed most to the discrimination between groups.

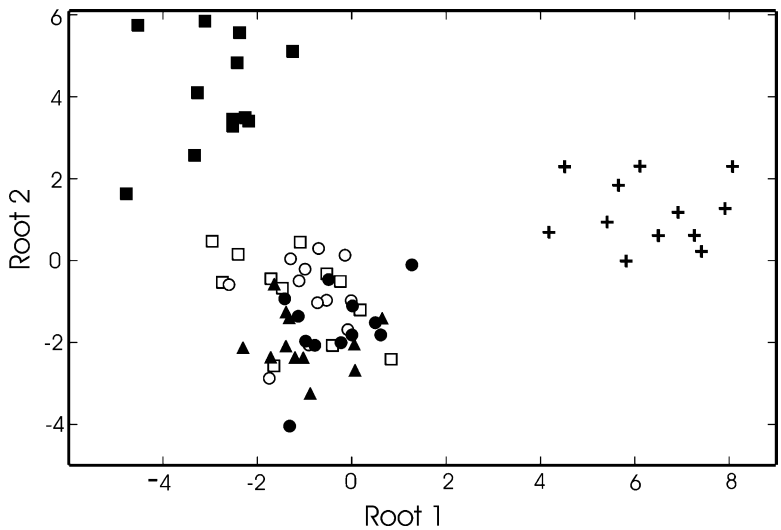

Fig. 3. Discrimination of monthly samples according to BIOLOG substrate oxidation values depicted on a Root1 × Root2 biplot. (+) July; (□) August; (○) September; (▲) October; (●) November; (■) December samples.

Table 4
Mean values for total density, Shannon diversity, evenness, richness and mean density of each nematode taxon for all samples, along with the effects at two different scales of microclimatic variables on the community of bacterivorous nematodes

	Mean ± S.E.	Month of sampling	Temperature (*T*)	Moistureure (*M*)	Interaction ($T \times M$)
Total density (ind./g dry weight)	5.00 ± 0.370	September, October ($F = 3.79$)*			Dry–cold ($F = 6.32$)*
Diversity	0.80 ± 0.020	October ($F = 6.54$)***	Warm ($F = 5.66$)*		Humid–warm ($F = 4.15$)*
Richness	6.86 ± 0.220	July, September, October ($F = 3.08$)*	Warm ($F = 6.03$)*		
Evenness	0.42 ± 0.008	July, September ($F = 5.58$)**			Humid–warm ($F = 11.28$)*
Monhysteridae	0.05 ± 0.011	September, October, November ($F = 5.34$)**			Dry–cold and humid–warm ($F = 5.79$)*
Panagrolaimus	0.08 ± 0.013				Dry–cold and humid–warm ($F = 11.43$)*
Plectus	0.09 ± 0.029				Dry–cold and humid–warm ($F = 11.59$)*
Acrobeloides	0.98 ± 0.136	September, October ($F = 3.18$)*			
Acromoldavicus	0.09 ± 0.024				Dry–cold ($F = 4.77$)*
Tylocephalus	0.09 ± 0.021				Humid–warm ($F = 3.99$)*
Acrobeles	1.12 ± 0.106	October ($F = 2.67$)*	Warm ($F = 17.56$)***		
Cephalobus	0.24 ± 0.034		Warm ($F = 4.94$)*		
Chiloplacus	1.98 ± 0.196	September ($F = 3.18$)*	Cold ($F = 12.93$)**		
Achromadora	0.03 ± 0.008	July ($F = 3.32$)*			
Eumonhystera	0.07 ± 0.011				
Prismatolaimus	0.10 ± 0.019				
Heterocephalobus	0.06 ± 0.012				

Month of sampling (blocking variable) and levels of temperature and moisture (grouping variables), with highest values of the corresponding parameters are indicated. Significant *F* values, from two-way ANOVA, are given.

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

diversity of the bacterial community in the soil (Bossio and Scow, 1995; Sharma et al., 1998).

The values of bacterial functional diversity in our study are comparable with those recorded in arctic tundra soils enriched with organic material (Derry et al., 1998) or in pine forests with organic soil (Staddon et al., 1996), and they are higher than those recorded for desert grasslands (Zak et al., 1994) or for mineral forest soils (Staddon et al., 1996). We must point out that we incubated our samples at 28 °C for 24 h, while in all the above-mentioned cases soil samples were incubated at 25–30 °C for 72 h. Thus, although the incubation period in our study was shorter, higher values of diversity were recorded, probably indicating an organic layer of good quality and/or quantity. Moreover, the bacterial community utilized mainly carbohydrates, glycolipids and alditols, while the substrates that were utilized less than all the others were those containing N, such as amines/amides and aminoacids.

Bacterial diversity and richness as well as evenness responded only to large-scale variations in microclimatic conditions, reflecting seasonal differences in the functioning of the soil bacterial community. Wilkinson et al. (2002) and Grayston et al. (2001) reported analogous responses of soil microbial communities in a Mediterranean pinewood soil and a temperate grassland, respectively. More specifically, in our study the components of catabolic diversity decreased from summer to winter. Degens et al. (2000) correlated the reduction of catabolic evenness with losses in the amount of organic C and reduced quality of organic matter. Ellis et al. (1995) and Goodfriend (1998) reported decreasing substrate

utilization potential in response to limited nutrient availability, while according to Grayston et al. (2001), changes in the structure and activity of a microbial community from a temperate grassland are related to qualitative aspects of resource availability. The seasonally varying richness in our study area indicated changes in community structure. Degens et al. (2001) developed an additional argument, stating that reduction in catabolic evenness may result in a less resilient or unstable microbial function. In this study, lower values for all catabolic components occurred in November–December. Therefore, the bacterial community of the Mediterranean grassland that was studied might be more sensitive to disturbance or stress during winter than during summer.

In addition to the bacterial community parameters, we recorded higher values of mean oxidation of all substrate groups during the severe Mediterranean summer, especially in soil samples from the dry plots. Although an explanation of this great resistance of microbes from 'drier soils' to water stress could be the selective adaptation of microbial populations to climate (Sparling et al., 1987; Kieft et al., 1987), additional mechanisms can be proposed as well. BIOLOG estimations reflect mainly the activity of fast growing bacteria (Schutter and Dick, 2001), especially within an experimental procedure involving an incubation period of 24 h only. Moreover, Gunapala et al. (1998) state that a rapid response of microbial activity to organic amendments occurs only after restoration of favourable moisture conditions. It is possible therefore, that while the activity of fast growing bacteria is suppressed during summer due to water limitation, the water added during the inoculum preparation may stimulate their activity. Analogous phenomena would not be expected for winter samples or from samples from the humid plots. If this holds true, then accidental, short-lasting summer rainfalls, frequently occurring in Mediterranean regions, will be accompanied by a flush of decomposers. Indeed, according to Insam et al. (1989) and Sharma et al. (1998), the microflora of Mediterranean areas is adapted to the long period of drought through an ability to react rapidly when conditions improve, i.e. when water becomes available. Furthermore, according to Dalaka (2001), a substantial amount of organic matter enters soil in July. Indeed, in our study site, a transition in herbaceous vegetation occurs between July and August, since 12 out of the 15 plant species of the area are annual grasses that complete their life cycles in July. Their aboveground parts dry out in July till the following March or April, when they will germinate again. Thus, another possible explanation for the high bacterial activity during summer could be that the weekly irrigation events probably induce frequent flushes of decomposition during this dry period resulting in less material left afterwards followed by a decrease in the bacterial catabolic activity. This latter fits well with the linear reduction of oxidation, diversity, richness and evenness from July to December.

The temporal discrimination of our samples was mainly due to carbohydrates and carboxylic acids and not to aminoacids. This probably reflects the availability of these resources, which is correlated with the aforementioned life cycles of most plants as well as vegetational diversity. Thus, the carbohydrates utilized in July could be root exudates, since the latter are fundamental in shaping soil microbial communities (Wardle and Nicholson, 1996; Bardgett and Shine, 1999). They could also be constituents of the organic material (above and below ground) entering soil in this period.

Many authors (e.g. Rillig et al., 1997; Derry et al., 1999) dispute the reliability of the BIOLOG system in soil ecology. However, the results of this study showed that, under seasonally varying climatic conditions, BIOLOG is a sensitive tool for the discrimination of soil microbial communities of the same soil type. Nevertheless, its discriminatory power proved limited when the microbial communities were subject to small-scale changes of soil temperature and humidity.

4.2. *Bacterivorous nematodes*

The community of bacterial-feeding nematodes in our study was dominated by genera of the family Cephalobidae, which is considered characteristic of dry and very dry soils (Griffiths et al., 1995). The abundance and diversity of bacterivorous nematodes responded to large-scale seasonal changes in the temperature–humidity complex. However, unlike the activity and diversity of their bacterial counterparts which peaked during the dry July–September period, the greatest abundance and diversity of bacterivorous nematodes occurred in the middle of the sampling period and mainly in October. Thus, no synchronisation

between bacterivorous nematodes and their food resources was revealed. Indeed, although bacteria and bacterial feeders are linked by a predator–prey relationship, synchronisation of their activities is not frequently reported (Yeates et al., 1999; Wardle et al., 2001). This lack of synchronisation is probably due to the fact that the responses of two out of the three dominant genera of our study site, namely *Acrobeles* and *Acrobeloides*, relate to a combination of food resources and chemical and physical characteristics of their environment rather than food availability exclusively (Porazinska et al., 1999).

Apart from the non-synchronized responses of bacteria and bacterial feeders to seasonal climatic changes, our results also showed disproportionate responses of these groups of organisms to our experimental manipulations. In our grassland, no significant independent effect of moisture manipulations on the nematode community was revealed. Bakonyi and Nagy (2000), who used the same experimental design in their Hungarian grassland, showed that, although temperature was more important for the structure of the soil nematode community, moisture also had a predominant effect on their abundance. On the other hand, Porazinska et al. (1999) reported significant effects of irrigation and fertilization on only a limited number of nematode genera, while similarity of bacteria and nematode responses was reported by Lundquist et al. (1999) and Sarathchandra et al. (2001) only in the case of soils enriched with nutrients. Of course, all of these discrepancies might simply be associated with the different climatic regime experienced at each research site. As stated by Todd et al. (1999), microbivores developing under different soil moisture conditions, display differential responses to altered soil water conditions.

In this study, our experimental manipulations resulted in a temperature difference of 1.4 °C between the warm and cold plots, which does not differ much from the predictions by Mitchell et al. (1995) for a rise of mean annual temperature of around 1.2 °C by 2050. Thus, among the bacterivorous nematodes recorded in our study, only numbers of *Acrobeles*, *Chiloplacus* and *Cephalobus* may be used as indicators of small-scale temperature changes. The responses of the rest, if any, proved more complicated, depending on the interactive effect of both soil temperature and moisture.

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