Original Articles

Nematode community indices for microhabitat type and large scale landscape properties

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ABSTRACT

Nematode community indices have been broadly used to assess and monitor soil conditions. Most studies focused on agricultural or other disturbed soils and were usually applied at local-field scale. In this study, we focused on the variation of nematode community indices across fine (among microhabitats) and landscape level scales with the aim to identify indices or combinations of indices that are efficient at distinguishing microhabitats and landscape properties. We calculated nematode community indices in five microhabitats to account for variability in basal resources/organic input (soils, mosses on soil, mosses on rocks, as well as mosses on the lower and the upper part of trees trunks). The different microhabitats were located across the landscape in sites that differed in vegetation cover type (forested and non-forested), altitude (low, high) and aspect (North, East, South).

Results of our models showed that microhabitat type could be predicted by all indices except the Maturity Index (MI) and the Fungivore/Bacterivore (F/B) ratio. Soil could be distinguished from mosses by the higher Plant Parasitic Index (PPI) and Enrichment Index (EI) and the lower Channel Index (CI). The different moss microhabitats could be distinguished from each other by the Summed Maturity Index (SMI) the Structure Index (SI) as well as the Basal Index (BI). At the larger scale, among landscape properties, variation by altitude was more pronounced compared to vegetation cover type and aspect. Samples originated from low altitudes were characterized by higher EI and F/B ratio. Indices for predicting landscape properties of the site a sample was taken from were more efficient when analyzed separately within each microhabitat. The interaction of the BI and the F/B ratio was able to predict vegetation cover type in soils while the same holds for BI, SI and their interaction in mosses. We conclude that nematode community structure is strongly shaped by variation in basal resources in the different microhabitats and microhabitats can be well predicted by most nematode community indices. Landscape properties constitute gradients of exposure of basal resources to ambient conditions, which affects basal resource transformations and availability. This variation across the landscape can also be predicted by some nematode community indices.

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1. Introduction

Soil biodiversity is crucial in determining the ecological and evolutionary responses of terrestrial ecosystems to current and future environmental change (Bardgett and van der Putten, 2014) as well as in regulating functions that support important ecosystem services (De Vries et al., 2013). Soil biodiversity is severely threatened (Orgiazzi et al., 2016 and references therein) and its decline is one major issue to deal with in the upcoming years (McBratney et al., 2014). The diversity and composition of soil communities has been shown to determine ecosystem multifunctionality (Wagg et al., 2014), while also help mitigating climate change through carbon sequestration (e.g. Lal et al., 2015). As soil communities are extremely complex and diverse it is hard to disentangle among driving forces, ecological processes and community characteristics. Thus it is essential to have indices that are efficient and informative at different scales.

Soil nematodes (Nematoda) have been described in many studies as efficient indicators of soil conditions (e.g. Bongers and Ferris, 1999; Zhao and Neher, 2013; Griffiths Römbke et al., 2016). Nematodes play an important role in nutrient cycling and combine a set of different characteristics, e.g. are abundant, include species with different life strategies and sensitivities to disturbance, and have diverse trophic preferences covering all the three energy pathways

(root, bacterial and fungal) through the soil food web (Ferris et al., 2001; Yeates et al., 1993; Zhao and Neher, 2014). Among the many nematode community indices that have been developed the most cited (Scopus- 4.5.2016) are the Maturity and Plant Parasitic Index (Bongers, 1990), as well the Structure, Enrichment and the Channel Index (Ferris et al., 2001). Lately Ferris (2010) developed the Nematode Metabolic footprint indices, which are calculated on the base of nematode biomass. The use of biomass has been found to add complementary information of the soil status (Zhao et al., 2016).

As nematode community indices were developed to monitor changes in the soil status they have been mostly considered for soils more frequently disturbed by human activities. Thus, they have been applied in studies for monitoring the effects of different management regimes in agriculture, land use change, environmental disturbance and pollution (e.g. Briar et al., 2007; Tsiafouli et al., 2007; Salamun et al., 2012; Vonk et al., 2013; Zhao and Neher, 2013). In general we know how different types of management affect the values of indices in soil samples at local scale (e.g. nutrient enrichment increases the Enrichment Index, disturbance decreases the Maturity Index etc.). We know less how values of indices behave in natural ecosystems (but see Zhao and Neher, 2014; Zhao et al., 2016) or habitats different than soils, such as mosses that differ in basal resources/organic input compared to soils.

As for most soil ecological data, nematode community indices are calculated at the sample or plot level, in small scale studies. Because though it is important to understand how landscape characteristics affect biodiversity patterns and ecological processes (Tscharntke et al., 2012) as functional importance of biodiversity appears at landscape scales (Swift et al., 2004; Tscharntke et al., 2005; Culman et al., 2010), it is crucial to find ways to upscale information related to soil biodiversity and soil communities from the local to the landscape scale (Bhusal et al., 2015). Neher et al. (1995, 1998, 2012) tested nematode community indices across spatial scales and quantified their linkage to ecosystem functions. According to Neher et al. (2005) nematode indices are cost-effective, and easy to calibrate and interpret for large-scale environmental monitoring programs. However, they can be effective if they show a higher variation across large rather than across small spatial scales and thus it is essential to verify how broadly used nematode indices respond to variations in climate, soil and vegetation types, management and disturbance at landscape scales (Neher, 2001, 2010; Nielsen and Wall, 2013).

At local scales, regardless of ecosystem type, soil animals may respond predictably to changes of environmental properties like moisture, but over larger scales additional factors, such as climate variability, vegetation composition, and soil properties may influence this relationship (Sylvain et al., 2014). A question that rises here is how landscape properties drive nematode community characteristics beyond the sampling plot and how this is reflected by the several indices that are related to the succession and functional status of the soil food web (e.g. flow of carbon trough the different decomposition channels). In other words: are there any differentiations across the landscapes that are reflected in nematode community indices? This knowledge will help to decide for appropriate land uses and design appropriate strategies for monitoring and protecting/managing soil biota and related ecosystem services.

In a previous study (Bhusal et al., 2015) we investigated the response of nematode metabolic footprints to temperature variations and we found that nematode metabolic footprints varied considerably across the landscape (slopes of the Mt. Holomontas, Greece). For the same study area, in this article, we used the most common nematode community indices that are descriptive for the successional [Maturity Index (MI), Plant Parasitic Index (PPI), Summed Maturity Index (SMI)] and the functional status of the soil food web [Basal Index (BI), Enrichment Index (EI), Channel Index

(CI), Structure Index (SI), Fungivore/Bacterivore (F/B) ratio]. We focused: a). within sites on different microhabitats (soil, mosses on soil, rocks and tree trunks) to account for variability in basal resources/organic input, and b). among sites with different landscape properties (aspect, altitude and vegetation cover type) to account for variability at large scale.

Our aim was to find indices or combination of indices able to distinguish the microhabitat and its position across the landscape. We hypothesized that basal resources/organic input linked to the different microhabitats would strongly shape nematode communities but the exposure of these basal resources to different environmental conditions and thus their availability to the soil food web across the landscape would impose additional variation to nematode communities that can be reflected by nematode community indices.

2. Material and methods

2.1. Study area and sampling design

The study area is located at the slopes around the "Vigla" peak (40° 25′ 42.1′′, 23° 9′ 55.89′′) of Mt. Holomontas, Chalkidiki, Greece (see also Bhusal et al., 2014, 2015 for more details). We considered five microhabitats: namely soil (SL), mosses on soil (SM), mosses on rocks (RM), mosses on the lower part (approx. 10–40 cm from the soil surface) of tree trunks (TL), and mosses on the upper part of tree trunks (TU) (0–30 cm down the maximum height of mosses on the trunk).

We sampled in January 2010 at 10 sites that differed by aspect (East, North and South), altitude (High [H] and Low [L]) and vegetation cover type (Non-forested with low vegetation- Open [O] and Forested [F]). The vegetation of the area is a Mediterranean oak (*Quercus pubescens*) forest except for the East-Low sites, where the vegetation is an evergreen shrub dominated by *Quercus coccifera*. At each site, for each microhabitat we took samples from three sampling plots (replicates). From each plot, we took one sample using a soil corer 5 cm in diameter and 15 cm depth. For mosses we sampled the dominant species *Hypnum cupressiforme* Hedw. (Sabovljevic et al., 2008), by cutting pieces of about 20×20 cm in area from the moss carpet. In the case of smaller patches, we collected a number of neighboring patches to get a similar quantity. It is to be noted that the TU and TL microhabitats were sampled only in the forested [F] sites in the high [H] altitudes, because open [O] sites had no trees, while the trees in the [F] sites at the low altitudes [L] had not the same dominant moss species or they totally lacked mosses on their trunks.

2.2. Extraction and identification of nematodes

Nematodes were extracted from the soil samples by the modified Cobb's sieving and decanting method, as proposed by S'jacob and van Bezooijen, (1984) and for the moss samples by the modified Baermann funnel method. For extraction we used a subsample of 100 gr (wet weight). Nematodes were collected after 72 h. Total numbers of nematodes were immediately counted using a stereo microscope at $40 \times$ magnification. For further analysis, nematodes were heat killed, and fixed with triethanolamine formaldehyde (TAF) 4% Solution (Shepherd, 1970). From each sample at least 150 randomly selected nematodes were identified to genus level by using the identification key of Bongers (1994). The selected specimens were subsequently allocated to trophic groups (Yeates et al., 1993) and classified along the colonizer-persister gradient (c-p values) following Bongers (1990) and Bongers and Bongers (1998).

2.3. Calculation of nematode community indices

The Maturity Index for free living nematodes (MI) and the Plant Parasitic Index (PPI) for plant feeding nematodes, both indicating the successional status of the nematode community, were calculated according to Bongers (1990). Similarly, as proposed by Yeates (1994), the Summed Maturity Index (SMI) was calculated, by considering free living and plant parasitic nematodes together. The following indices were calculated according to Ferris et al. (2001): a). the Enrichment Index (EI), an indicator of enrichment opportunistic nematodes; b). the Structure Index (SI), an indicator of nematodes with high longevity, body size and disruption sensitivity; c). the Channel Index (CI), an indicator of the degree of fungal participation in the decomposition channels of soil food webs; and d). the Basal Index (BI), an indicator of the prevalence of nematodes that are tolerant to stress. Finally, we calculated the ratio of Fungivorous to Bacteriovorous nematodes (F/B ratio) as proposed by Sohlenius and Boström (1984).

2.4. Data analysis

First, we evaluated differences in nematode community indices according to microhabitat, altitude, vegetation cover and aspect. For this, we used a multivariate linear modeling approach (mvabund package in R) (Warton, 2008; Wang et al., 2012). Linear models were fitted to multivariate response data. The overall as well as the univariate significance of the predictors were evaluated by a permutational procedure. In our case the predictors were factors like microhabitat, altitude, vegetation cover and aspect, thus our analysis is similar to a permutational multivariate anova.

Second, to provide a more direct answer to our main question, that is to select indices or combination of indices that are able to separate samples into classes according to the microhabitat or the site the samples were taken from, we used: a). classification trees when the number of classes were more than two (i.e. for microhabitats), and b). binomial logit models in case the number of classes were only two (high vs low for altitude and forested vs open for vegetation cover).

We used in R (R version 3.0.1, R Core Team, 2013) the manylm function of the mvabund package (Wang et al., 2012) for the multivariate linear modeling, the glm function with a binomial error distribution for fitting the binomial logit models and the rpart package for building classification trees.

3. Results

3.1. Relative contribution of trophic groups by microhabitat

The relative contribution (%) of the various trophic groups to the nematode communities according to microhabitat is depicted in Fig. 1. Bacteriovorous nematodes dominated in all microhabitats but their contribution was lower in soil (SL) and mosses on soil (SM) and became higher in mosses on rocks (RM) and trunks (TL, TU). Plant associated nematodes (root hair feeders) were the second most abundant trophic group, had a relatively higher contribution in SL and SM compared to RM and TL, and were almost absent from TU. The plant parasitic nematodes behaved similarly but had much lower contributions. In general TU differed from all other microhabitats having the highest relative contribution of predatory nematodes.

3.2. Nematode community indices

Average values of nematode indices and major trends of variation of indices by microhabitat and landscape properties are presented in Fig. 2. The summary statistics and the coefficients
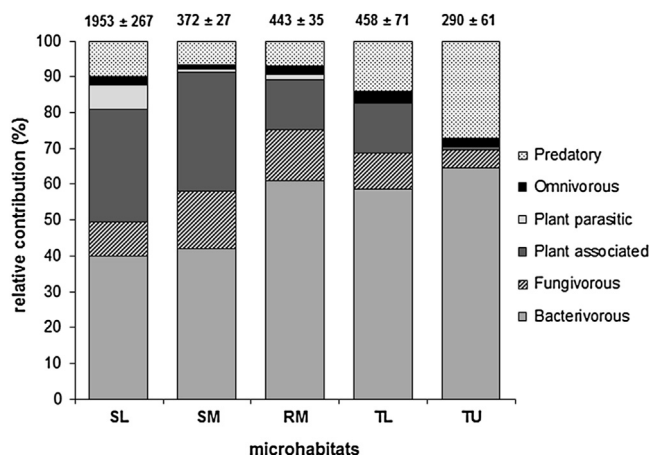

Fig. 1. Relative contribution (%) of nematode trophic groups by microhabitat. The numbers above the bars refer to the average total abundance (±st. er.). Codes: SL: soil, SM: mosses on soil, RM: mosses on rocks, TL: mosses on low part of tree trunk, TU: mosses on upper part of tree trunk.

of the multivariate linear model (manylm) fitting the effects of microhabitat and landscape properties on indices are presented in Table 1. Microhabitat and altitude were the most significant factors and indices showed significant variation either by microhabitat (SI, BI, CI, PPI, SMI) or altitude (F/B) or by both factors (EI). The CI and EI indices are negatively correlated and their values differed significantly by habitat (significantly lower values of EI and higher values of CI in mosses compared to soil) and in the case of EI there was a significant effect of altitude (higher values in the low altitude) (Fig. 2, Table 1). The BI and SI indices are also negatively correlated since they account for the proportional contributions of nematodes connected with the lower and the higher trophic levels of the food web respectively. The BI was higher and the SI was lower in mosses on rocks and soil compared to other microhabitats (Fig. 2). PPI showed significantly lower values in mosses on rocks and trunks compared to soil except in mosses on soil, where the PPI was similar. Significant differences of SMI were observed between TU and all other microhabitats with the latter having higher values. The F/B ratio was the only index that, although not significantly affected by microhabitat, was significantly affected by altitude presenting higher values in low altitudes (Table 1).

3.3. Indices of microhabitats

The classification tree is shown in Fig. 3. SL samples were separated from moss samples mostly by the higher PPI (>2.01) and lower CI values (<78.74). Among mosses TU samples were separated by high SMI values (>2.5), while the SM samples were separated from RT samples (the combined RM and TL samples) again by SMI values (<2.12). Finally RT samples were separated from SL samples by higher BI values (>36.46) and lower EI values (<17.02). The prediction was very good for TU (90%) and quite good for SL (76%) and RT (73%), but not as good for SM (59%).

3.4. Indices of large scale landscape properties (altitude and cover)

In Table 2 we provide the coefficients and summary statistics of the binomial models used to predict the altitude or the cover type of the site a sample was taken from. For aspect the models failed to provide predictions, so the results are not shown. Only TU samples showed variation of some indices among the different aspects. BI values were higher and SI values were lower in South compared to
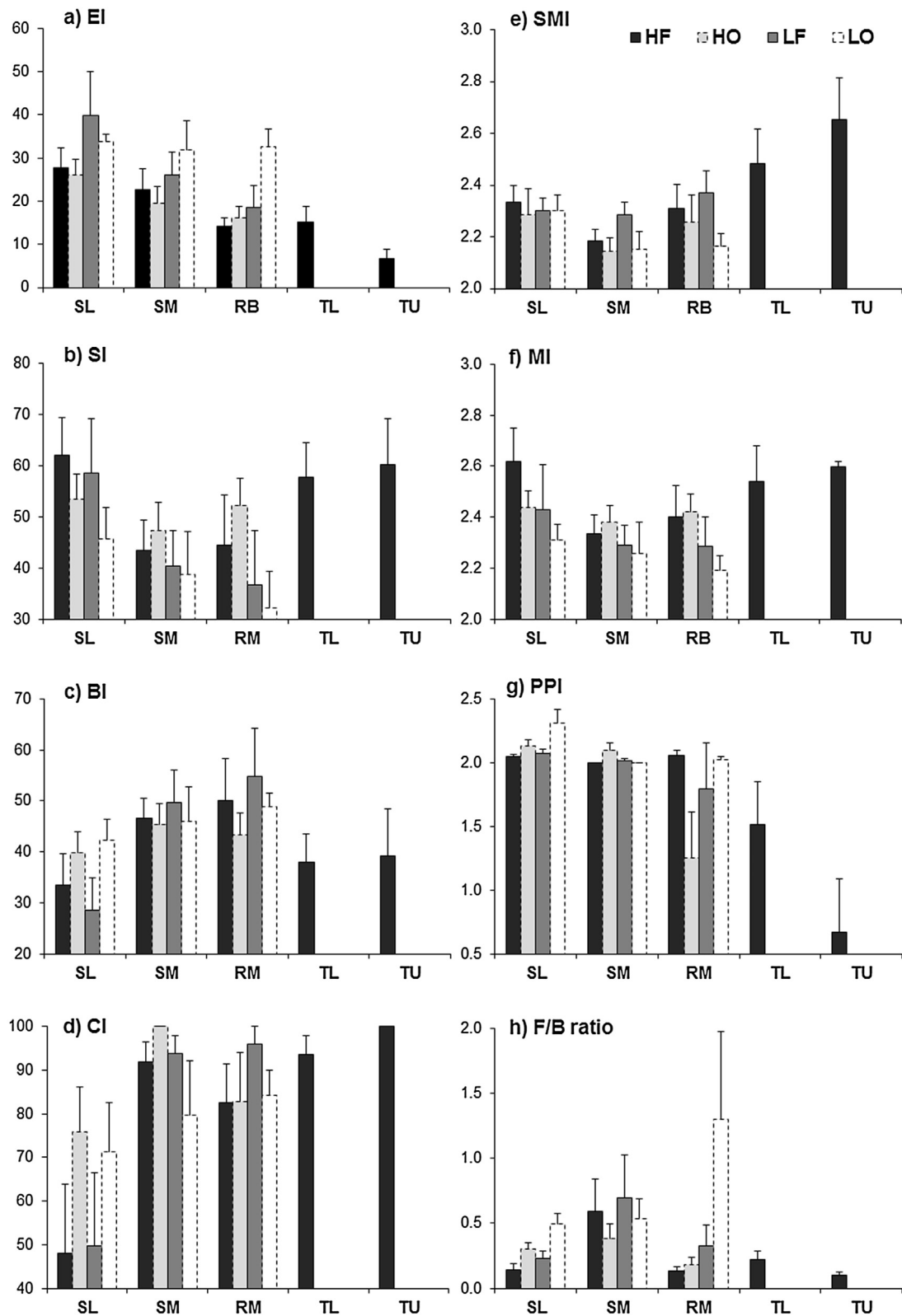
Fig. 2. Mean values (±std. er.) of nematode community indices by microhabitat, altitude and cover. Data for the different aspects were pooled together. Codes: SL: soil, SM: mosses on soil, RM: mosses on rocks, TL: mosses on low part of tree trunk, TU: mosses on upper part of tree trunk. HF: High-forested sites, HO: High Open sites, LF: Low Forested sites, LO: Low open sites.

North and East aspects. CI values were higher in North compared to South and East aspects.

Altitude: For soil samples the interaction of the BI and EI was used for predicting the altitude a sample was taken from (Table 2a).

A soil sample was predicted as taken from a low altitude if BI*EI was high. Soil samples were correctly predicted as originated from a high altitude in 88% of cases. The corresponding correct classification of low altitude samples was 67%. For moss samples, EI As

Table 1

Summary statistics and coefficients of the multivariate linear model fitted to nematode indices multivariate data. Significant coefficients are given in bold. Reference classes were 'soil' for microhabitat and "high" for altitude. Codes for Microhabitats: SM: mosses on soil, RM: mosses on rocks, TL: mosses on the lower part of tree trunks, and TU: mosses on the upper part of tree trunks; Indices: EI: Enrichment Index, CI: Channel Index, BI: Basal Index, SI: Structure Index, PPI: Plant Parasitic Index, SMI: Summed Maturity Index, and F/B: Fungivore/bacterivore ratio.

Summary statistics			Coefficients						
Factor	F	p	EI	CI	BI	SI	PPI	SMI	F/B
Intercept	3086.3	<0.001	**26.43**	**62.38**	**36.52**	**57.30**	**2.06**	**2.33**	0.15
Microhabitat	32.9	0.001							
SM	32.4	0.001	**−6.66**	**30.79**	**10.53**	**−12.28**	−0.09	−0.11	0.27
RM	37.7	<0.001	**−11.51**	**23.90**	**12.64**	**−12.78**	**−0.36**	−0.02	0.15
TL	19.9	0.01	**−9.36**	**19.19**	−0.21	2.90	**−0.59**	0.16	0.07
TU	67.5	<0.001	**−17.72**	13.87	−1.96	8.38	**−1.28**	**0.40**	−0.08
Altitude	27.2	0.002							
Low	27.7	<0.001	**11.01**	−1.24	−0.63	−5.01	0.16	−0.05	**0.32**

Hooper's $R^2 = 0.21$, Lawley-Hotelling trace statistic = 33.34, $p < 0.001$. Numbers in bold indicate significance ($p < 0.05$).
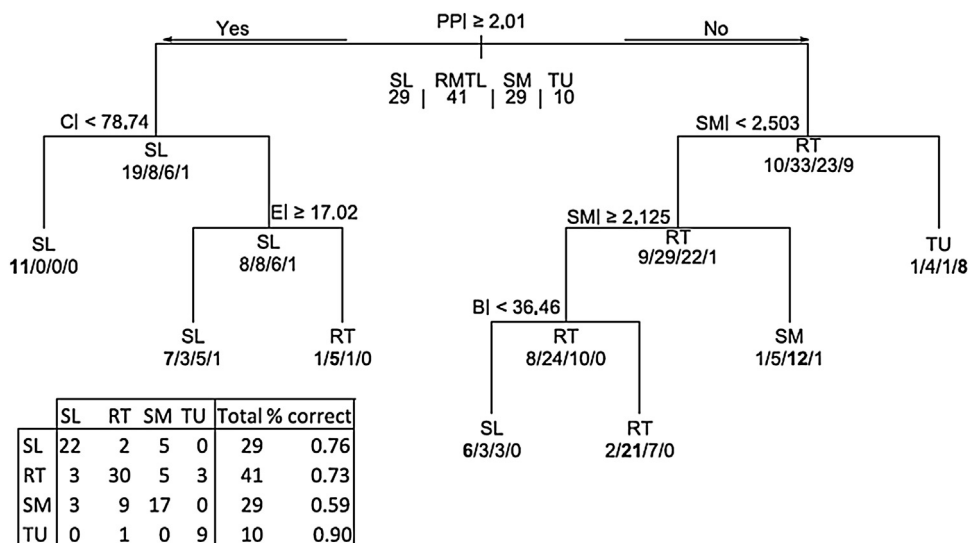

Fig. 3. Pruned classification tree for the classification of microhabitats on the basis of nematode community indices. Codes: SL: soil, SM: mosses on soil, RM: mosses on rocks, TL: mosses on low part of tree trunk, TU: mosses on upper part of tree trunk. RT is the combined group of RM and TL samples. The confusion matrix is embedded.

Table 2

Binomial logit coefficients and confusion table for the prediction of altitude and cover type a sample was taken from, based on nematode indices of soil and mosses (SM and RM samples are fused, TU and TL are not used in the analysis since those where only present in the high forested areas). Reference classes were "high" for altitude and "forested" for cover. Codes: H: High, and L: Low; Indices: EI: Enrichment Index, BI: Basal Index, SI: Structure Index, SMI: Summed Maturity Index, and F/B: Fungivore/bacterivore ratio.

Variables	Binomial model coefficients and summary statistics					confusion table			
a. Altitude (Low)									
soil	Estimate	Std. Er.	z value	p	S level			Predicted class	
Intercept	−1.453	0.914	−1.590	0.112				H	L
BI	−0.052	0.030	−1.748	0.080		H		15	2
BI:EI	0.002	0.001	2.634	0.008	**	L		4	8
mosses	Estimate	Std. Er.	z value	p	S level			Predicted class	
Intercept	−1.798	0.503	−3.576	0.000	***			H	L
EI	0.048	0.017	2.762	0.006	**	H		30	5
						L		12	11
b. Cover (Open)									
soil	Estimate	Std. Er.	z value	p	S level			Predicted class	
Intercept	−2.121	0.754	−2.814	0.005	**			F	O
BI:F/B	0.190	0.071	2.685	0.007	**	F		13	1
						O		4	11
mosses	Estimate	Std. Er.	z value	p	S level			Predicted class	
Intercept	−0.080	4.409	−0.018	0.985				F	O
BI	−0.121	0.053	−2.269	0.023	*	F		19	11
SI	−0.139	0.058	−2.391	0.017	*	O		9	19
SMI	3.441	2.217	1.552	0.121					
BI:SI	0.002	0.001	2.057	0.040	*				

fungal-based food webs are more resistant was the only variable used to predict the altitude a sample was taken from. The prediction in this case was rather poor (86% correct for high, but only 48% correct for low).

Cover: For soil samples the binomial model is based on the interaction of BI with F/B (Table 2b), predicting that a sample was taken from an open site if BI*F/B is high (93% correct for forested and 73% for open sites). For mosses the binomial model is based on BI, SI, their interaction and SMI (63% correct for forested and 68% for open sites).

4. Discussion

Nematode community indices have been widely utilized to evaluate soil conditions; mostly in stressed and disturbed soils, like for example agricultural soils, that are characterized by low values of the SI and a high values of the EI. Our soil and moss samples on the contrary showed relatively high SI and low EI values and are classified as undisturbed and stable (Ferris et al., 2001; Hohberg, 2003; Tomar and Ahmad, 2009; Austin et al., 2009). On the base of nematode community indices, community structure was strongly differentiated at fine scale according to microhabitat types (soil and mosses on soil, rock or trunk). At a larger scale, sites were also distinguished by specific indices according to landscape properties.

4.1. Fine scale patterns: indices distinguishing microhabitats

Microhabitat type exerts strong effects on nematode communities that are even obvious at higher taxon biodiversity surrogates (Bhusal et al., 2014). In this study the microhabitat could be predicted by the value of one or combination of more indices. Soil was well separated from mosses and among mosses TU was separated from all other.

Soil was distinguished from mosses mainly by the presence of herbivorous nematodes and especially parasitic ones which was reflected in the higher values of the PPI. A value even slightly higher of 2, which is the lowest value this index can get (see also Bongers, 1990, Bongers and Ferris, 1999) was separating soil from moss samples. The PPI has been found to significantly interact with plant composition (Viketoft et al., 2011) and is in general higher in areas with higher vegetation diversity (Verschoor et al., 2001). Plant parasitic nematodes were absent from mosses, which is expected as mosses don't have a real root system, though the presence of plant associated nematodes (root hair feeders) was very characteristic especially in soil mosses. The moss carpet is in tight contact with soil and it is inevitable that soil particles with the associated fauna are included in moss samples. It has been reported however (Elgmork and Saether, 1970; Yeates et al., 1993), that species of the genus *Tylenchus* actually feed on parts of mosses. Furthermore, some plant associated nematodes can also feed on fungi (e.g. Okada et al., 2005) and thus are not much dependent on root hairs.

Mosses on rocks and soil were further distinguished from soil samples by the CI, by having a value higher than 78.7 indicating a strong dominance of the fungal decomposition pathway in the mosses. The preference of fungivorous nematodes to these habitats might be related to the growth of fungi in the dead parts of mosses (Lindo and Gonzalez, 2010). As fungal-based food webs are more resistant to drought than bacterial-based ones (De Vries et al., 2006; Six, 2012) fungivorous nematodes are less affected by drought than bacterial ones (Bakonyi and Nagy, 2000: Bakonyi et al., 2007; Brair et al., 2012). Thus, they appear to be better adapted to the moisture regime of mosses, especially those on rocks.

The contribution of omnivorous and predatory nematodes with K-selected life history strategies (c-p values mostly of 4 or 5) was high in mosses on tree trunks and especially in the upper part of them (TU). This thereby led to higher values of MI and SI in the latter. Eventually TU is separated from other microhabitats by higher SMI values (>2.5). Our results are in contrast with the study of Lazarova et al. (2004), where predators in mosses were almost absent, but a high proportion of them, even higher than that recorded in our study, was reported by Barbuto and Zullini (2006). Mosses in the upper trunk parts might provide shelter to several animals which serve as a food resources for predator and omnivorous nematodes (Zullini and Peretti, 1986).

4.2. Large scale patterns: indices distinguishing sites with different landscape properties

We found that among the properties characterizing the landscape and hence the position of the site a microhabitat belongs to, altitude was the most important one in shaping the functional composition and structure of nematode communities and thus affecting the nematode community indices. The effects of vegetation cover type were also significant but were differentiated among microhabitats. The effect of aspect was not significant. Only in TU the indices BI, SI and CI showed some differentiation among aspects (results not shown). Mainly the South aspect was different from the North and East aspect. This differentiation might be explained by the effect the aspect has on moss vitality (Hazell and Gustafsson, 1999) and growth (Hylander, 2005) with mosses in South facing slopes being less favored than in North. Pen-Mouratov et al. (2009) found significant differences in the generic composition of nematodes communities and in the abundance of some trophic groups between South and North aspects but not in nematode indices such as the MI and the SMI.

4.2.1. Altitude

For soil nematodes altitude is important in shaping their communities, regarding characteristics like their distribution (Zhang et al., 2012), species richness (Háněl and Čerevková, 2010) and abundance (Porazinska et al., 2012). In our study, we found considerable changes in the decomposition channel ratio by altitude as indicated by the F/B ratio index. The F/B ratio increased in sites located at lower altitudes. This indicates that towards lower altitudes the fungal decomposition pathway becomes stronger and hence decomposition rate becomes slower, as the fungal-mediated decomposition is slower that the bacterial mediated one (e.g. De Vries et al., 2006). Ferris et al. (2001) and Hohberg (2003) suggested that a fungal-dominated decomposition pathway occurs where cellulose and lignin rich litter material is the main source of nutrient input to the soil food web. Furthermore Ruess (2003) found that the F/B ratio was more affected by soil and climate factors than by vegetation type. In our study the enhancement of the fungivore decomposition channel at the lower altitudes cannot be attributed to the nature of litter material as it was observed in both vegetation cover types and was not depended on the microhabitat type but rather by environmental factors such as climate. Moreover the results of the multivariate linear model showed that this index although able to predict the relative altitude was not able to predict the microhabitat a sample was taken from. Thus, there is evidence that the change of the F/B ratio is not related to basal inputs *per se* but to ambient environmental factors these inputs are subjected to. This in turn means that the flow of carbon through the different decomposition channels varies considerably across the landscape. Indeed in a previous study (Bhusal et al., 2015) we found higher fungivorous and lower bacterivorous nematode metabolic footprints (i.e. channels of carbon flows) within sites positioned at lower altitudes in the landscape, characterized by high seasonality and low isothermallity.

Besides the F/B ratio the EI was also helpful in distinguishing sites according to their relative altitude, but was more efficient

when analyzing the effect of altitude per microhabitat. A sample was predicted to have been taken from a low altitude if EI is high in the case of mosses, and if the interaction of BI by EI is high in the case of soil. As the higher EI values indicate resource enrichment (Ferris et al., 2001), our findings suggest that across the landscape resources are richer at lower, than at higher altitudes. This could be explained as the result of slow erosion processes, which leads to the concentration of nutrients at lower altitudes. The EI*BI interaction indicates furthermore that soil positioned at lower altitudes in the landscape is relatively more enriched but in the case of non-forested open sites are also more depleted. The latter could be attributed due to easier access of grazing animals in the area and relatively more frequent human presence.

4.2.2. Vegetation cover type (tree covered vs. non tree covered sites)

As expected the type of vegetation cover had a significant influence for the soil inhabiting nematode communities. Several studies have shown that basal resources such as leaf litter and rhizodeposits, which are linked to vegetation type or specific plant species, are important in controlling energy channels in the soil systems and structuring soil food webs (e.g. De Deyn et al., 2004; Cesarz et al., 2013; Ciobanu et al., 2015).

In the case of soil the interaction of BI and F/B was able to predict the vegetation cover type a sample was taken from. The higher values of these indices in the non-forested open sites indicate both a degree of depression and also a stronger fungal decomposition pathway (Ferris et al., 2001), as indicated also by the higher CI (Fig. 2). That the soil samples in the open sites are more depressed or stressed compared to the soils under the forest cover is also supported by the lower values of SI and MI, which is in line to the findings of Briar et al. (2007). Although areas under both types of vegetation cover were rather undisturbed, the non-forested open sites can be considered as more exposed to disturbance because of occasional grazing and trampling in the area.

The effect of vegetation cover was clearer for soil, while for mosses it was rather complicated. Mosses are grown as epiphytes and are forming their own substrate which apparently influences the nematode community stronger than the surrounding vegetation cover type. On the other hand mosses are able of capturing particulate organic debris (Chapin et al., 1987; Carleton and Dunham, 2003; Cornelissen et al., 2007) and this difference might be related to different organic inputs between mosses in forested and non-forested sites. Too many indices are required to predict the cover type a sample was taken from (BI, SI, and BI*SI), which is, in the very end, case specific for the different moss types.

Our results point out that the different moss types according to their specific location are experiencing different degrees of exposure to climate variation. For example turnover rates of dead moss parts and thus enrichment with nutrients (Lindo and Gonzalez, 2010) might be higher in the mosses of the non-forested open sites which are more exposed to temperature variation and solar radiation (Suggitt et al., 2011). On the other, as shown by our previous study in the same study area (Bhusal et al., 2015), forest cover reduces diurnal variation and keeps relatively cooler temperatures during summer and warmer temperatures during winter. Indeed in the present study the EI, which indicates enrichment by nutrients as already mentioned, was especially high in the non-forested lower altitudes where the moss carpet was scattered and fragmented.

To sum up, our results regarding the effect of vegetation cover type on nematode community indices imply that beside the effects of vegetation type affecting basal resources per se especially in soil, there are also other effects linked to vegetation cover type: the effect of posing a natural barrier, thus altering the possibility of exposure to disturbance and the effect of shading under the canopy, thus altering how the basal resources in the different microhabitats are exposed to climate variation and solar radiation.

5. Conclusion

Our results indicate great differences in nematode community indices among microhabitats but also within microhabitats located at different positions across the landscape. Overall our findings refer to two phenomena: first, the nematode community structure is more strongly shaped by variation in basal resources in the different microhabitats and the microhabitat a sample was taken from can be well predicted by most nematode community indices either in combination or alone (BI, SI, CI, EI, PPI); second, nematode community indices respond to broad scale properties across the landscape that constitute gradients of exposure to ambient conditions. Variation of conditions affects basal resource transformations and availability. Among the most efficient indices for broad scale properties like altitude, were the EI index and the F/B ratio index, which was though not efficient in separating microhabitats. Our findings can be used in further studies considering different microhabitats but more importantly we recommend their use in studies considering large scale effects on soil biological properties as we found that different indices work differently at the different scales.

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