Potential drivers of spatial structure of leaf-litter food webs in south-western European beech forests

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ABSTRACT

Spatial heterogeneity – in terms of topography, and macro- and microclimatic conditions, among others – results in habitat diversity, which in turn may promote species diversity. Thus, the spatial structure (i.e. spatial variance partitioning at different scales and spatial arrangement of drivers of abundance) of the different feeding guilds within food webs may reflect relevant differences in the way populations interact and how these interactions affect ecosystem processes. Our study focuses on the spatial distribution of animals living in the leaf litter layer of beech forests in the National Parks of northern Spain, across sites which differ in precipitation. Using Generalised Linear Mixed Models we estimated the spatial variance components at different scales and for three feeding guilds in leaf litter food webs: saprophagous and microphytophagous mesofauna, saprophagous macrofauna and zoophagous macrofauna. We found that the only consistently significant source of variation for the three feeding guilds was that at the level of "among valleys within Parks", which, among other potential explanations, could reflect meso-climatic differences among valleys. We also studied which factors may control spatial variation in these food webs through a model selection approach. Controlling for all other relevant factors, we still found strong differences in abundances among National Parks. Also, invertebrates were more abundant in micro-sites located at the base of slopes – where nutrients and water accumulate – than in micro-sites located on the slopes – which, due to surface runoff, are drier and accumulate fewer nutrients. Also, as found in beech forests of Central Europe, limestone sites have higher abundances of fauna than siliceous sites with lower pH. The macro- and micro-scale dependence of these food webs on water availability could have important consequences for the persistence of these forests under global warming.

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Introduction

Soil biota is not randomly distributed but their distribution is rather under scale-dependent control, exhibiting spatially aggregated patterns over multiple scales (Ettema and Wardle 2002). Diverse factors operating at different spatial scales – from fine (short-range variation in biotic and abiotic factors due to micro-topography) to very coarse (continental-scale variations because of climate and geological history) – are responsible for soil spatial heterogeneity in terms of structure and physico-chemical properties such as litter layer depth, soil pH, water and nutrient content (Vanbergen et al. 2007; Lark and Corstanje 2009). This spatial heterogeneity, by contributing to community structure, may have significant effects in ecosystem stability and functioning.

It seems obvious that multiple factors, operating across a hierarchy of spatial and temporal scales, determine species distributions (Levin 1992) and arthropods provide a great opportunity to study how species distributions are shaped (Hortal et al. 2010). Certainly, how species distributions integrate to determine the distribution of communities, is not a trivial question. Thus, to understand how significant this variation is in determining ecosystem services, the variability in communities must first be documented and main drivers of such variability identified (Nichols et al. 1998a; Legendre et al. 2005).

One of the main drivers of spatial heterogeneity in soil fauna could be water availability, which is itself highly variable even at the micro-scale level (Herbst and Diekkrüger 2003). For instance, it has been demonstrated that desiccation limits the distribution of soil-dwelling arthropods such as springtails and mites (Madge 1964; Verhoef and Witteveen 1980; Wauthy and Vannier

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1988; Hodkinson et al. 1996; Hertzberg and Leinaas 1998), and that drought strongly and negatively affects microarthropod community abundance and diversity (Whitford 1989; Siepel 1996; Whitford and Sobhy 1999; Frampton et al. 2000; Pflug and Wolters 2001; Lindberg et al. 2002). Furthermore, water availability may impact nutrient cycling by affecting food web interactions (Lensing and Wise 2006), and thus, the role that water plays in structuring food webs could be of central importance. Furthermore, since water runoff is more pronounced on steeper slopes than on relatively flat areas, water accumulates in flat areas at the base of slopes, resulting in water availability also being highly heterogeneous at micro-environmental scales (Herbst and Diekkrüger 2003).

In line with these earlier observations, and as a preliminary approach to understand the effect of spatial variability on water availability – at both macro- and micro-scales – on food web structure, we investigated the potential effect of Mean Annual Precipitation (range: 912–1968 mm year^{-1}) and micro-topography on the abundance of the main feeding guilds of soil fauna. To this end, we sampled the leaf-litter invertebrate fauna of beech forests (*Fagus sylvatica*) from three National Parks in North Spain at 4 different scales (from micro-site to regional), and studied the spatial structure of each of three feeding guilds. Invertebrate fauna was assigned to each of the following feeding guilds following Schaefer and Schauermann (2009): saprophagous and microphytophagous mesofauna, saprophagous macrofauna and zoophagous macrofauna. A hierarchical spatial structure may indicate that different drivers and processes determine the abundance of the different food web components at different scales. In order to hierarchically partition the spatial scale of the abundance of the different feeding guilds in beech forest leaf-litter food webs, we first examined the spatial variance components for the total abundance of fauna and then for the abundance of each feeding guild. We then studied some abiotic factors that could control the spatial structure of the abundance of each feeding guild. Specfically, we used a model selection approach (Burnham and Anderson 2002) to test the following predictions:

(I) Macro-scale effect of water: if water availability affects the productivity of the system, an estimate of secondary productivity (invertebrate abundance) will positively depend on mean annual precipitation.

(II) Micro-scale effect of water (micro-topography): we also predicted that invertebrate fauna would be more abundant in micro-sites located at the base of slopes than in micro-sites located on slopes, where water availability, leaf-litter and nutrients accumulate.

(III) Bedrock type may determine the pH and nutrient availability of the soil, which in turn may affect microbial and invertebrate densities. Thus, as found in beech forests of Central Europe (Schaefer and Schauermann 2009), we predicted that, relative to forests growing on nutrient-poor, acidic soils derived from siliceous bedrock, the abundance of soil fauna would be higher in base-rich forests growing on limestone.

Materials and methods

Study area

We chose to study he European Beech (*F. sylvatica*) system because in Spain, it is at the Southern-most limit of its distribution range and is likely declining due to decreasing precipitation attributed to global warming (Peñuelas and Boada 2003). Beech is a deciduous tree that forms mostly monoculture forests across Europe. In the Iberian Peninsula, these forests are at relatively high altitude and in locations that receive sufficiently high rainfall.

Thus, these trees are limited to the northern half of the Peninsula (Blanco Castro et al. 1997). Beech leaves fall every autumn and build up a continuous, slowly-decomposing organic litter layer on the soil (Wise and Schaefer 1994), establishing a relatively isolated environment with relatively constant conditions favouring the activity of fauna. Although beech trees are dominant in beech forests, other accompanying species can be present, such as holy tree (*Ilex aquifolium*), silver fir (*Abies alba*) and Scots pine (*Pinus sylvestris*) (Blanco Castro et al. 1997).

The study area extends across three National Parks in North Spain – Aigüestortes i Estany de Sant Maurici (1°0′E, 42°35′N), Ordesa y Monte Perdido (0°3′E, 42°40′N) and Picos de Europa (4°55′W, 43°30′N) – which are reasonably close to the south-westernmost limit for the distribution of *F. sylvatica* (the Beseit Ports, Catalonia, Spain at 40°48′N). The Aigüestortes i Estany De Sant Maurici National Park (hereafter referred as Aigüestortes), located in the Catalan Pyrenees with a surface area of 14,119 ha, is the easternmost park within the study, markedly dominated by Mediterranean climate and thus characterised by warm and dry summers, and peak rainfall periods in Spring and Autumn. Beech forests occupy less than 0.5% of the park range (Red Natura 2000 and personal observations). Therefore, we established some sites in the non-park surrounding area, at elevations ranging from 1445 to 1758 m.a.s.l. and mean annual rainfall ranging from 913 to 1250 mm year^{-1} (Ninyerola et al. 2005). Located between the two other parks, the Ordesa National Park (hereafter referred as Ordesa), within the Aragonese Pyrenees, extends across 15,608 ha and exhibits a mixture of climates, with both Mediterranean and Oceanic influences. As a consequence, mean annual rainfall is higher than in Aigüestortes (range: 1129–1690 mm year^{-1}). Beech forests occupy about 6% of the park range (Red Natura 2000), and our sites were located at elevations from 1005 to 1650 m.a.s.l. The third park, Picos de Europa, extends across Asturias, Leon and Cantabria provinces, in the Cantabrian mountain range. It has a surface area of 64,660 ha and is the western-most park, with a pronounced influence of Oceanic climate (Atlantic climate), with cool summers and comparatively warm winters (Felicísimo 1994). It is characterised by a narrower range of annual temperatures than those encountered in the other parks at comparable latitudes, lacking, for instance, the extremely dry summers typical of the other parks, which are more influenced by Mediterranean climate. Beech forests comprise less than 5% of the park area, and our sites were established at elevations ranging from 818 to 1409 m a.s.l. and from 1109 to 1968 mm year^{-1} of mean annual precipitation. Unlike the other parks, most rainfall on Picos de Europa comes as drizzle, and mountain fogs are very frequent due to the Oceanic influence (Felicísimo 1994). Both effects likely contribute to increase the capacity for the soils to retain water. On the other hand, due to the relative closeness to the Mediterranean sea of the other two parks, storm-like precipitations should be more common in Ordesa and Aigüestortes. Storms lead to frequent water surface runoff, which prevents the soil retention of the rainwater. Therefore, rainfall rate, better than total rainfall, could be an important factor determining the spatial patterns in the distribution of soil fauna abundance by affecting soil productivity. Unfortunately, however, accurate rainfall rates were unavailable for our study sites.

Sampling

Each sampling unit consisted of selecting and sifting a portion of leaf litter "in situ" (e.g. Lensing and Wise 2006). We studied the soil largest mesofauna (>0.5 mm in length) and the macrofauna (>2 mm) of the leaf litter layer. To this end, from May to July 2009 a total of 240 sifted samples of leaf litter were collected in the 3 National Parks. Within each park, we collected four samples (separated from each other by approximately 10–20 m) in each of 20

sites, which were in turn 1056 m apart on average (range of nearest-neighbour distances: 215–5044 m) and scattered across a variable number of localities (valleys): 5 valleys in Ordesa, 5 in Aigüestortes and 8 in Picos de Europa.

In order to account for the potential effect of micro-topography, two of the four samples collected within each site were from dry-poor (DP) micro-sites located on steep terrain, in which surface runoff washes away the leaf litter layer and both nutrients and water are subsequently depleted here compared to wet, nutrient-rich (WR) micro-sites, located on flat areas at the base of slopes or where slopes were intercepted by tree trunk (whether dead or alive) or rocks, and from which we collected the other two samples. Decisions on where to collect and whether to assign each micro-site to WR or DP were reached after discussion and the consent of at least two observers. In June 2010, we opportunistically measured the temperature and relative humidity (RH) of the leaf-litter in 14 micro-sites within Picos de Europa (7 in DP and 7 in WR). We used a DeltaOHM HD2301.0 thermo-hygrometer and found that DP micro-sites were warmer (T, mean $\pm$ SD, 19.1 °C $\pm$ 1.0 vs. 16.7 °C $\pm$ 1.4, $p = 0.003$) and drier (HR, 76.3% $\pm$ 5.2 vs. 88.6% $\pm$ 3.3, $p < 0.001$) than WP micro-sites. In addition, within the micro-site of this study, DP micro-sites had shallower leaf-litter (depth = 4.6 cm $\pm$ 1.6) than WR micro-sites (depth = 8 cm $\pm$ 2.8; $N = 216$, $p < 0.0001$). Unfortunately, the first five samples collected from Aigüestortes did not follow the above DP-WR protocol and had to be removed from the analyses.

Furthermore, in order to minimise temporal effects in our sampling – which could have been increasingly confounding as the dry Mediterranean summer approached, we conducted two rounds of collection in each park. We first collected samples from 10 sites in Aigüestortes, then travelled to Ordesa to collect samples from 10 sites, then an additional 10 samples from sites in Picos de Europa. We repeated this sampling scheme once more for a total of 20 samples from each site in each park.

The basic sample unit consisted in rapidly collecting 28.5 L of leaf litter in a field container and then carefully transferring the litter to a plastic bag. Thus, the area of the surface collected was dependent on the depth of the litter in each micro-site (surface range: 0.17–1.58 m^2). Although we were careful to handle bags gently, some soft-bodied arthropods could have been damaged during handling. However, careful inspection during sorting showed that the number of dead animals due to handling was rather small. We also systematically took four depth measures of the surrounding litter layer (<50 cm from the actual collecting site) by means of a long knitting needle, which was gently introduced in the litter until it stopped. A mark was drawn in the needle to signal the depth at which the needle penetrated the litter. Depth was then estimated by tape-measuring the length of the segment of the needle which was buried in the leaf litter. In order to minimise disturbance and prevent the escape of soil fauna back into the ground, these measures were taken after the collection of the leaf litter. The mean among the four measures was used as an estimate of the leaf litter accumulated in the micro-site. Location of sampling sites (GPS), and both date and hour of sampling were recorded. Leaf-litter was then gently sifted (Wise and Chen 1999; Lawrence and Wise 2000, 2004; Lensing and Wise 2004) through a 1.5 cm mesh screen. The resulting debris was then scattered on a white tray and all invertebrates picked with soft forceps and preserved in 100% ETOH for future DNA barcoding analysis. We scattered the debris as many times as necessary until we were sure that all the animals of length above 0.5 mm had been collected. Each sample was processed by one to five people at a time.

Counting and identification of fauna

By visual inspection under a dissection microscope specimens were identified to the lowest taxonomic affiliation possible as follows: myriapods were identified to order, annelids and beetles to family, and the remaining arthropods to class. Within taxonomical categories, a broad functional or feeding classification of three groups was performed based on body size and feeding habits (following Schaefer and Schauermann 2009): enchytraeids annelids and collembolans were grouped as saprophagous and microphytophagous mesofauna; lumbricid annelids, isopods, diplopods (millipedes) and elaterid beetles as saprophagous macrofauna and, finally, araneids, pseudoscorpions, opilionids, chilopods (centipedes) and predatory beetles (e.g. carabids, staphylinids) as zoophagous macrofauna (Appendix Table 2). Because the classification of mites (acari) is still awaiting deeper taxonomic identification, they could not be assigned to feeding guilds as they were poorly identified, and were thus not considered for further analyses. In addition, other minor groups that could not be identified with enough accuracy to determine their feeding habits (e.g. some beetle families) or that were not typical of soil fauna (e.g. Curculionidae which feed on live leaves) were also excluded. In this way, abundance data for three feeding guilds were available for statistical analyses.

Geographic and climatic variables

To examine the distribution of soil biota abundance at different spatial scales, we distinguished the following spatial scales: micro-site (DP vs. WR micro-sites, no more than 20 m apart); site, at the sampling site level within each valley (1–7 sites/valley); local, across different valleys within each park (5–8 valleys/park) and regional, across the 3 National Parks. Mean annual precipitation (MAP) and mean annual temperatures for each site were obtained from the Digital Climatic Atlas of the Iberian Peninsula (Ninyerola et al. 2005). Using a GPS device we also calculated altitude, latitude and longitude. Although we were primarily interested in the effect of precipitation on the distribution of invertebrate abundance, we considered including temperature, as this could be a potential factor explaining the abundance of fauna (e.g. once controlled for MAP, sites with lower temperatures, should be less productive). However, as the range of annual mean temperatures among the sites was relatively small (6–10 °C), and temperature was highly and negatively correlated with altitude ($r = -0.85$; $p < 0.0001$) and was never entered into the best model in preliminary tests, we decided not to include this factor for analysis. Moreover, latitude was not included in the statistical models because the range was also very narrow; i.e. the three national parks were located at similar latitudes, differing by less than one degree (from 42°33′46.05″ to 43°15′53.87″), and we thus anticipated no effects.

Statistical analyses

We used the freely available statistical software R, version 2.13.1 (R Development Core Team 2011). For all analyses, we used Generalised Linear Mixed Models (GLMM), using the "lmer" function in the "lme4" package, with Gaussian (Normal) errors and identity link functions. First, we analysed the hierarchical structure of the abundance of each feeding guild in space by estimating the variance components for each nested level in space. To accomplish this, we employed a nested GLMM without fixed factors as well as a nested random structure representing the spatial hierarchy; i.e. micro-site nested within site nested within valley nested within park. We then calculated the estimates for the variance components as well as their confidence intervals using the MCMC Bayesian procedure (function "pvals.fnc") from the R library "language R" (Hadfield 2010).

To find a model with a set of hypotheses that best fit our data, we used a model selection approach (Burnham and Anderson 2002). From this, we generated a set of models including
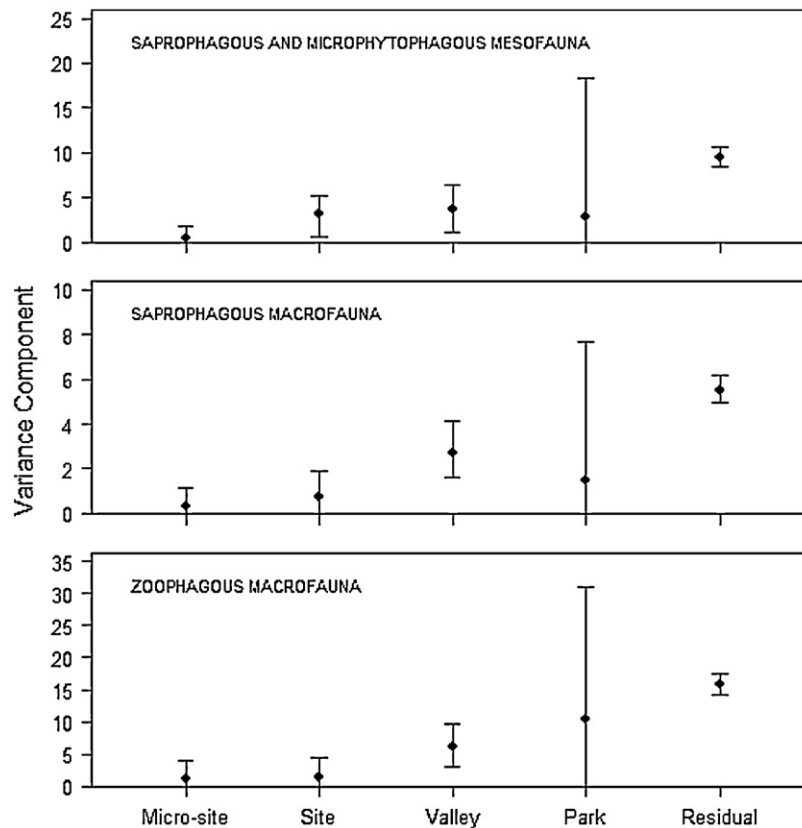
Fig. 1. Results of the spatial variance components analyses of the abundance of 3 functional groups in the leaf-litter food web. The scales in descending order are continental (Park nested within Valley within Site within Micro-site), regional (Valley nested within Site within Micro-site), local (Site nested within Micro-site) and micro-local scale (Micro-site, WR vs. DP). Error bars represent MCMC 95% confidence intervals. A significant variance component occurs when these error bars do not intercept with 0. Note that these components should not add up to 100 because they are not percentages.

hypotheses which concerned the biological processes underlying the data (see the Introduction) and used the Akaike's Information Criterion (AIC) and AIC weights to compare GLMM models. A model whose Akaike weight approximates 1 indicates the best fit with the data (Burnham and Anderson 2002). We used GLMM models including only "site" as a random factor to control for pseudoreplication. However, models using the entire nested hierarchy shown in the variance components analyses reported very similar results (not shown). Because we wanted to know if each park had its own idiosyncratic nature (i.e. abundances of the different feeding guilds that could not be explained by any of the predictors), we also included park as a fixed factor. *p*-Values for each effect were reported as calculated in the MCMC procedure described above. For all models we needed to apply the Box–Cox transformation ("boxcox" function in the MASS package of R) to meet the assumption of normality in the residuals. However, the results of the analyses were similar regardless of data transformation. We also obtained the same qualitative results with or without using GLMM models with a Poisson distribution and individual sample as a random factor to control for overdispersion (function "lmer" in library "lme4") (not shown). Partial effects of significant variables in models were calculated and plotted using the R library "effects".

Results

We found a strong spatial structure in abundance showing significant and consistent variation at the level of valleys/localities nested within parks. In addition, we detected important differences among parks and that micro-topography, bedrock type and precipitation affected the distribution of abundance of the different components of the leaf-litter food web.

Spatial structure: spatial variance components of abundance

The only consistent source of spatial variation in abundance across the three feeding guilds appeared at the local scale; that is, among valleys within parks (Fig. 1). However, the variance component for site was also significant for the saprophagous and microphytophagous mesofauna, likely indicating that this group is more sensitive to environmental variability at smaller spatial scales. We then further analysed which of the measured environmental variables could be responsible for this structure.

Model selection approach: determining the drivers of spatial structure

The 'most parsimonious model' for the total abundance of fauna was number 8 and included as explanatory variables park, bedrock type, micro-site type (WR vs. DP), date and hour of sampling, and MAP as fixed variables. We then tested how this model fit the data for each feeding guild. Note that all the effects described from GLMMs were partial, meaning that the effects reported for one variable were estimated as if all other variables in the model were held constant. Below we discuss the effect of each variable in more detail.

Total abundance – We found significant differences among National Parks (Fig. 2a), with greater abundance of fauna in the easternmost park, Aigüestortes (see Table 1 and Appendix Table 2). We found an important effect of bedrock type (Fig. 2b), with significantly greater abundance of fauna in base-rich soils (limestone bedrocks) than in acidic ones (siliceous bedrocks). There was a significant effect of the micro-site type as well (Fig. 2c), with greater abundance of fauna in WR areas than in DP ones. Finally, we found

Table 1
Factors potentially controlling the abundance of fauna living in the leaf litter layer of beech forests according to model selection and GLMM. The "total community size" refers to the total abundance of fauna.

Total community size

Variable	Group	Fixed effects					
		Estimate	MCMC mean	HPD95lower	HPD95upper	pMCMC	p value
(Intercept)		114.5907	105.3758	52.1042	161.8123	0.0001	
Parks[*]	Ordesa	−77.3869	−77.8645	−106.3935	−49.5765	0.0001	0.0001
	Picos de Europa	−60.4628	−62.8193	−91.0052	−33.8203	0.0002	
Rock type[*]		−34.5657	−35.0984	−53.6705	−17.2273	0.0001	
Type of micro-site[*]		−24.1393	−24.2287	−34.4026	−13.7468	0.0001	
Date	Date_late spring	−5.6155	−4.8096	−20.4921	11.8484	0.5552	0.2407
	Date_summer	−20.6140	−18.1483	−38.2221	1.8542	0.0726	
Hour	Hour_noon	3.1413	8.9862	−8.5626	27.1529	0.3270	0.3748
	Hour_afternoon	−7.8559	−8.8070	−27.5917	9.7443	0.3596	
Annual precipitation[*]		0.0736	0.0792	0.0384	0.1197	0.0008	

		Random effects				
		Std. dev.	MCMC median	MCMC mean	HPD95lower	HPD95upper
Site[*]	(Intercept)	23.7649	13.1119	12.7058	3.7822	21.0682
Residual		35.2038	38.7377	38.8161	34.5239	43.3818

Saprophagous and microphytophagous mesofauna

Variable	Group	Fixed effects					
		Estimate	MCMC mean	HPD95lower	HPD95upper	pMCMC	p value
(Intercept)		1.6448	1.1963	−11.0715	13.9348	0.8492	
Parks[*]	Ordesa	−16.8072	−16.9106	−23.4359	−10.2575	0.0001	0.0001
	Picos de Europa	−16.1190	−16.1259	−22.6351	−9.2047	0.0001	
Rock type[*]		−4.5245	−4.5788	−8.8086	−0.6208	0.0288	
Type of micro-site		−1.0470	−1.0071	−3.6435	1.6070	0.4330	
Date[*]	Date_late spring	−3.6648	−3.5997	−7.2575	0.2225	0.0598	0.0108
	Date_summer	−8.1218	−8.3505	−12.9132	−3.8283	0.0010	
Hour	Hour_noon	0.2701	0.9698	−3.2921	5.0294	0.6436	0.4895
	Hour_afternoon	−1.8700	−2.0010	−6.5020	2.0675	0.3552	
Annual precipitation[*]		0.0270	0.0273	0.0178	0.0366	0.0001	

		Random effects				
		Std. dev.	MCMC median	MCMC mean	HPD95lower	HPD95upper
Site	(Intercept)	4.5117	2.1137	2.0044	0.0000	3.7895
Residual		9.1131	9.7572	9.7719	8.7112	10.8000

Saprophagous macrofauna

Variable	Group	Fixed effects					
		Estimate	MCMC mean	HPD95lower	HPD95upper	pMCMC	p value
(Intercept)		18.2448	17.8053	10.0897	25.9407	0.0001	
Parks[*]	Ordesa	−7.4400	−7.5860	−11.9379	−3.4141	0.0012	0.0177
	Picos de Europa	−6.1782	−6.4689	−10.6873	−2.1998	0.0038	
Rock type[*]		−3.6971	−3.8093	−6.5038	−1.1099	0.0074	
Type of micro-site[*]		−3.4682	−3.4831	−4.9624	−2.1332	0.0001	
Date	Date_late spring	−1.2987	−1.5461	−3.9453	0.8694	0.2102	0.6601
	Date_summer	−0.3980	−0.0356	−2.9600	2.9853	0.9880	
Hour	Hour_noon	−1.6626	−1.1074	−3.5618	1.5091	0.3916	0.6200
	Hour_afternoon	−0.6616	−1.2930	−3.8921	1.5921	0.3488	
Annual precipitation		0.0022	0.0026	−0.0032	0.0087	0.3698	

		Random effects				
		Std. dev.	MCMC median	MCMC mean	HPD95lower	HPD95upper
Site[*]	(Intercept)	3.7241	2.1431	2.1269	1.1896	3.0527
Residual		4.8318	5.3742	5.3883	4.8034	6.0343

Table 1 (*Continued*)

Zoophagous macrofauna							
Variable	Group	Fixed effects					
		Estimate	MCMC mean	HPD95lower	HPD95upper	pMCMC	p value
(Intercept)		61.5769	59.9789	39.4274	80.7183	0.0001	
Parks[*]	Ordesa	−29.3099	−29.3592	−40.2049	−18.9197	0.0001	0.0001
	Picos de Europa	−19.3720	−19.6436	−30.6344	−9.1014	0.0004	
Rock type[*]		−8.0878	−8.1984	−15.0067	−1.5749	0.0186	
Type of micro-site[*]		−11.0579	−11.0349	−15.0979	−7.0435	0.0001	
Date	Date_late spring	−0.2548	−0.3629	−6.4620	5.7176	0.9074	0.1751
	Date_summer	−7.2996	−6.8410	−14.7391	0.5543	0.0758	
Hour	Hour_noon	1.3679	2.4783	−4.1775	9.4099	0.4620	0.8881
	Hour_afternoon	0.0408	−0.7971	−7.8350	6.3686	0.8248	
Annual precipitation		0.0085	0.0096	−0.0061	0.0249	0.2288	
		Random effects					
		Std. dev.	MCMC median	MCMC mean	HPD95lower	HPD95upper	
Site	(Intercept)	8.2285	4.4067	4.2095	0.000	6.7188	
Residual		13.9231	15.0997	15.1388	13.487	16.8938	

[*] $p < 0.05$.

a strong positive effect of MAP on the total abundance of fauna (Fig. 2d).

Saprophagous and microphytophagous mesofauna – Among the above-mentioned variables, neither micro-site type nor sampling hour significantly explained the abundance of collembolans and enchytraeids. However, we found significant differences in the abundance of this fauna among parks, with greater abundance in Aigüestortes (Table 1). Bedrock type was also a significant factor, with greater faunal abundance in base-rich soils compared to acidic ones. Sampling date was significant as well, with
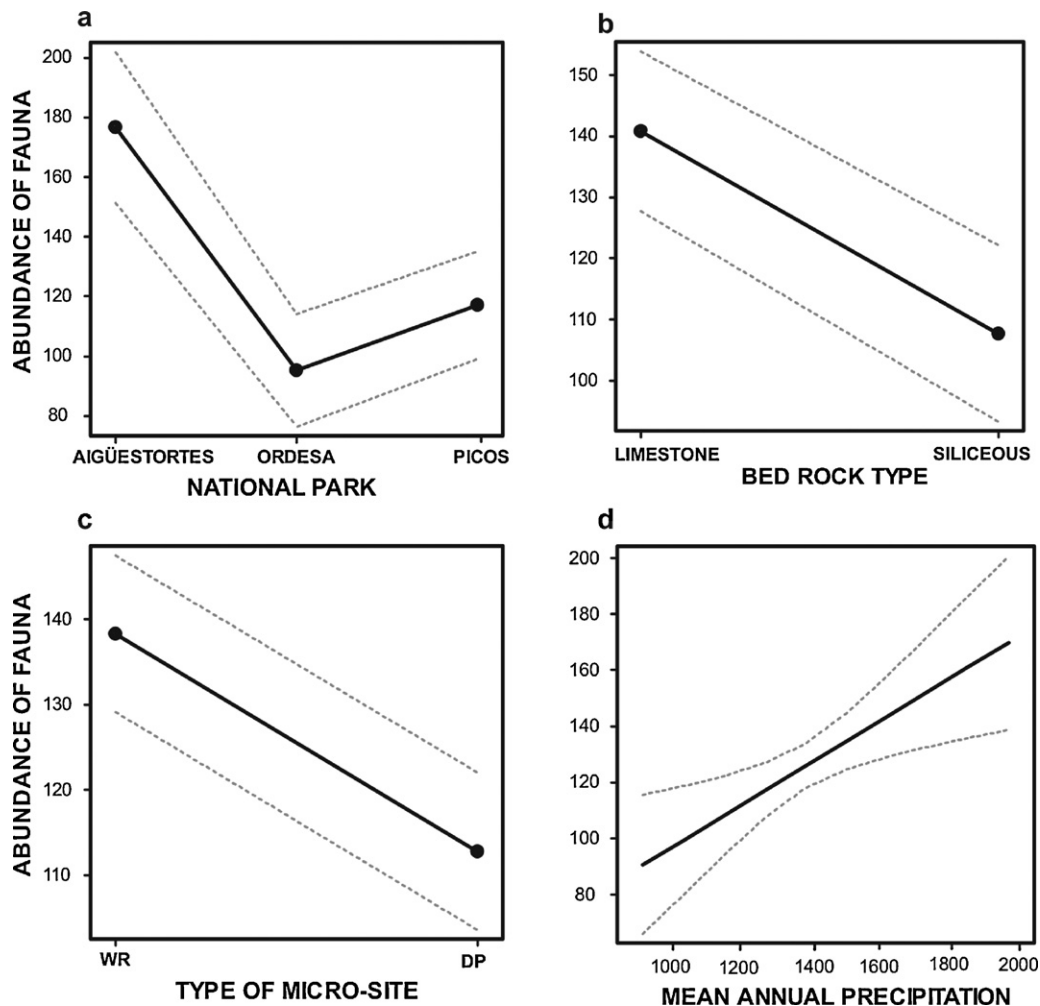

Fig. 2. Partial effects of the significant factors potentially driving the total abundance of fauna (total community size). (a) National Parks; (b) bed rock type; (c) type of micro-site (WR, wet-rich; DP, dry-poor) and (d) mean annual precipitation.

decreased abundance as the summer progressed. Moreover, there was a strong positive effect of MAP, with greater abundance of saprophagous and microphytophagous mesofauna in rainier areas.

Saprophagous macrofauna – We found significant differences between micro-site types as well as bedrock types (Table 1), with greater abundance in WR areas than in DP ones and in base-rich soils in comparison with acidic ones. Additionally, there were significant differences among parks, with higher abundance of saprophagous macrofauna in Aigüestortes. However, none of the following effects: sampling date, sampling hour or MAP had a significant effect on the abundance of this feeding guild.

Zoophagous macrofauna – Again, among the explanatory variables we found significant differences among parks (Table 1), and between types of micro-sites and bedrock, with greater abundance in WR areas and base-rich soils than in DP ones and acidic soils. Sampling date and hour, and MAP did not have significant effects on abundance.

Although we lacked statistical power to test the responses of each taxon, we could conduct preliminary comparisons to determine potential differential responses among taxa. Appendix Tables 2.a, 2.b, 2.c and 2.d show the means and standard deviations for the more abundant taxa in each park, valley and micro-site (WR vs. DP).

Discussion

Some relevant patterns concerning the horizontal distribution of animals living in the leaf litter layer of beech forests arose from our analyses. We found a consistent effect of scale on the distribution of meso- and macrofauna, with the only significant source of variation for all feeding guilds at the local scale; i.e. among valleys within parks. In addition, by means of model selection we also found substantial differences in abundance among parks, between micro-site and bedrock types, and significant positive effects of mean annual precipitation on abundance.

Variance components analysis

In the first section of our study we examined the variance components of invertebrate abundance at different spatial scales – from micro-site to the regional scale – and found the same spatial patterns for the three studied feeding guilds, suggesting that there is a reliable and persistent effect of scale on the distribution of meso- and macrofauna living in the leaf litter layer of beech forests. Moreover, the only significant spatial variance component in abundance common to the three feeding guilds appeared at the local scale, among valleys within parks; that is, valleys within a park differ among each other sufficiently to significantly contribute to the overall spatial variance. Other sources of variation, for instance park, can contribute to overall variability. However, the relatively small sample sizes (e.g. $n = 3$ for parks) resulted in error terms that were too large to detect a significant contribution if it existed. The significant effect of valleys could be due to the fact that valleys are depressions that extend predominantly in one direction, and thus, mesoclimatic conditions (i.e. the climate of a natural region of small extend that may not be fully representative of the general climate because of subtle differences in elevation and exposure) may have an important effect on the spatial distribution of the soil fauna. Therefore, the orientation of these valleys relative to the general circulation of the atmosphere (from west to east for these latitudes) will dictate how much rainfall they receive, which in turn may affect soil moisture, tree productivity and fungal and bacterial growth (Rousk and Bååth 2011). Other differences such as soil type, nutrient content and the biogeographic history of the forests could also be affecting invertebrate abundance in each of these valleys. However, none of the above variables were measured in this study.

Finally, we also found a significant variance component within sites for the "saprophagous and microphytophagous mesofauna", which may reflect other spatial patterns resulting from microclimatic conditions or at least that there are different predictors at different spatial scales. This result could point to substantial heterogeneity in forest soils at smaller spatial scales, which could be relevant for enchytraeids and springtails. Alternatively, the smaller body sizes of these groups and/or differences in their dispersal ability, could explain the pattern. However, we know that many crucial factors responsible for patterns of faunal distribution, such as soil pH, porosity, water availability, and the depth and structure of the litter layer, among others, can largely depend on topographical differences (Ettema and Wardle 2002; Coulson et al. 2003; Herbst and Diekkrüger 2003; Lavelle et al. 2006). Importantly, residual variation in the spatial component of invertebrate abundance was large (Fig. 1), suggesting that many other factors, not measured in the present study (abiotic or biotic) and that do not necessarily covary with the measured spatial scale, may be important. Therefore, other relevant factors can actually be estimated by indirect methods. For instance, although weather stations were not available in each valley, we could estimate precipitation in each of our sites from interpolated algorithms and temporal series from the closest weather stations (Ninyerola et al. 2005) and use this estimate to test for a positive effect of precipitation on invertebrate abundance in the soil. We also tried to disentangle what other environmental factors, among those available, could explain this spatial pattern.

Model selection approach

Prediction (I): *Macro-scale effect of water.* MAP affects invertebrate abundance.

We found a substantial effect of rainfall on invertebrate abundance of fauna, with an increase of 7.4 additional individuals per sample for each 100 mm increase in annual rainfall. This increase was also substantial (2.2 individuals/100 mm) for saprophagous and microphytophagous mesofauna, the only feeding guild that showed a significant response to rainfall. This pattern likely indicates that more water may lead to higher microbial biomass available for the saprophagous and microphytophagous mesofauna. Moreover, the fact that sampling date also negatively affected the abundance of mesofauna, may reflect that a decrease in rainfall with the onset of summer (Ninyerola et al. 2005), typical of Mediterranean weather, affected microbial growth and the risk of desiccation. Actually, several studies have reported the extreme vulnerability of soil organisms – particularly collembolans and enchytraeids – to drought stress (Lundkvist 1982; Didden 1993; Briones et al. 1997; Verhoef and Witteveen 1980; Vegter 1983; Beylich and Achazi 1999; Frampton et al. 2000).

Prediction (II): *Micro-scale effect of water (micro-topography).* Greater abundance of fauna in WR areas.

We found a substantial effect of micro-topography, with greater total invertebrate abundance, as well as greater abundances of both saprophagous and zoophagous macrofauna, in WR relative to DP micro-sites. As a result of surface runoff, water, leaf-litter, and likely inorganic nutrients accumulate in WR areas, leading to higher relative humidity, which would enhance microbial growth (Rousk and Bååth 2011) and prevent invertebrate desiccation (see above).

Prediction (III): *Bedrock type may determine the pH and nutrient availability of the soil, which in turn affects microbial and invertebrate densities.* Higher abundance of fauna in base-rich forests growing on limestone.

As reported for other beech forests (Schaefer and Schauermann 2009), we found a greater abundance of invertebrates in base-rich (limestone) than in acidic, nutrient poor (siliceous) beech forests. Acidic soils impede cation exchange and are therefore

poorer in nutrient status, supporting lower microbial biomass and lower invertebrate abundances, especially in the more superficial horizons. In addition, some invertebrates, such as earthworms (Edwards and Bohlen 1996) and animals with calcareous skeletons (e.g. diplopods), are known to be susceptible to low pH values.

Furthermore, the significant and relevant differences in total faunal abundance found among National Parks, as well as for the three feeding guilds are notable. Although park was not a significant component of spatial variance (likely because the small sample size and large error terms), we still found differences in mean abundances among parks, which means that these differences are important. Actually, environmental conditions might differ at the local scale and thus give rise to dissimilar communities with characteristic densities at the regional scale. For instance, we found the highest abundances in Aigüestortes, the easternmost park, where *F. sylvatica* sometimes mixes with other woody species, such as silver fir (*A. alba*) and Scots pine (*P. sylvestris*) and thus, there may be some distinctive factors enhancing the abundance of invertebrates. Unfortunately, we lack the appropriate information to know whether this is the case or not.

Caveats of the study

Hand sifting leaf litter underestimates the abundance of mesofauna, as only mesofauna > 0.5 mm was collected. Litter extraction by means of humidity gradients would be a more suitable technique to estimate meso- and microfauna (Macfadyen 1961). However, time and logistic constraints prevented us from establishing litter extractors. In fact, the aim of the present study was to perform an extensive survey which could provide us with an overview of geographic variability in faunal abundance. Thus, instead of sampling a few sites more intensively, we decided to sample several localites less intensively. Moreover, during handling, some soft-bodied arthropods – specifically springtails – could have been damaged, leading to an underestimation of their abundance. Nevertheless, we found important effects of the factors studied on mesofauna which confers some degree of confidence to our data, which is also supported by previous studies (e.g. Moya-Laraño and Wise 2007).

Conclusions

The spatial distribution of invertebrates in the leaf-litter food web of beech forests shows a heterogeneous structure, likely influenced by mesoclimatic conditions. Since water availability, as well as the related productivity in the soil community, seems to be a key driver of fauna abundance at different spatial scales, such heterogeneity could affect ecosystem functioning. Heterogeneity is an important attribute of most ecosystems and it is well known that spatial and temporal heterogeneity plays a central role in population, community, and ecosystem processes (Tilman and Kareiva 1997; Hutchings et al. 2000; Wardle 2002). Understanding the relationship between water availability, nutrient cycling and food web structure and dynamics may be crucial to know why global warming and the associated decreased precipitation affects the decline of beech forests at the southernmost edge of their distribution (Peñuelas and Boada 2003). However, in order to understand the soil fauna in these forests in greater detail, we need more intensive studies, in which litter extraction of fauna allows accurate estimates of the densities of meso- and microfauna.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.pedobi.2012.06.003.

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