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FACULDADE DE CIÊNCIAS E TECNOLOGIA  
UNIVERSIDADE DE COIMBRA

### Collembola diversity in Portuguese forest stands: deriving surrogates for species richness and evaluating the effects of forest type and grazing

Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, realizada sob a orientação científica do Professor Doutor José Paulo Sousa (Universidade de Coimbra).

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## **Abstract**

Forests are very important systems, not only for the diversity of life forms they shelter, but also for the services and resources they provide. Soil organisms are an integral part of these systems, intervening in the various processes that occur in the soil and thus contributing for the provision of several ecosystem services. Over the past decades, soil biodiversity has suffered a decline due to various threats resulting from human activities. Faced with this problem, an effort has been made to monitor soil biodiversity, often using indicators such as the diversity and abundance of Collembola. However, the difficulties in the taxonomical identification of these organisms have led to the search for alternative approaches, including the use of surrogates and functional traits. Taking this into account, this study is divided in two parts: in a first phase, deriving surrogates and sampling effort for biodiversity assessment (chapter II) and, in a second phase, the evaluation of the effects of forest type and grazing on Collembola communities (chapter III).

Aiming to discover if higher taxonomic levels (genera richness) and a classification based on eco-morphological traits (morphotypes richness) would be good surrogates for Collembola species richness, generalized linear models (GLM) were developed using data from previous projects. The required sampling effort to estimate Collembola species richness in forest areas was calculated with the same data. It was possible to conclude that both morphotypes and genera are reliable surrogates for species richness and that 7 to 10 samples should be enough to estimate about 80 to 90% of a site's species



richness. Still, the developed models can be improved by incorporating more data from other forest stands and by their validation.

Afterwards, the classification in morphotypes was used to evaluate the effects of different types of forest and the practice of grazing on Collembola communities, a study integrated in a project that aims the extensive monitoring of Portuguese forests. To achieve this, 20 sites from the project were used, all of them located in the south of Portugal and belonging to two different forest types: *Quercus suber* L. and *Quercus rotundifolia* Lam. In each, 8 samples were collected and the collembolans found, classified into morphotypes. Differences between abundance and morphotypes richness among sites and forest types were assessed with a nested ANOVA. Diversity indices were calculated. Differences in communities between sites were assessed through multivariate analysis. A t-test was used to verify if there were differences between sites with and without grazing. Lastly, through a correlation, the relation between the percentage of vegetation cover and Collembola richness and abundance was studied. Despite the variation in abundance and diversity over the study sites, no significant differences were found neither between the two types of forest nor between sites with and without grazing. The correlation, although significant, was surprisingly weak; suggesting that perhaps more cover variables should be included to study this relation. In the future, it will be important to incorporate more explanatory variables that will help to understand better the patterns of diversity found.

## Resumo

As florestas são sistemas de grande importância, não só pela diversidade de formas de vida que albergam, mas também pelos serviços e recursos que provisionam. Os organismos do solo são parte integrante destes sistemas, intervindo nos diversos processos que ocorrem no solo e assim contribuindo para a provisão de diversos serviços dos ecossistemas. Ao longo das últimas décadas, a biodiversidade do solo tem vindo a sofrer um declínio devido a várias ameaças resultantes de atividades humanas. Face a este problema, tem sido feito um esforço no sentido de monitorizar a biodiversidade dos solos, recorrendo muitas vezes a indicadores como a diversidade e abundância de colêmbolos. No entanto, as dificuldades na identificação taxonómica destes organismos levaram à procura de abordagens alternativas, entre as quais, o uso de *surrogates* e de características funcionais. Tendo isto em conta, este estudo está dividido em duas partes: numa primeira fase, a derivação de *surrogates* e do esforço de amostragem (capítulo II) e, numa segunda fase, a avaliação dos efeitos do tipo florestal e do pastoreio nas comunidades de colêmbolos (capítulo III).

Com vista a perceber se níveis taxonómicos mais elevados (riqueza de géneros) e se uma classificação usando características eco morfológicas (riqueza de morfotipos) seriam bons substitutos para a riqueza em espécies de colêmbolos, foram desenvolvidos modelos lineares generalizados (GLM) com base em dados de projetos anteriores. A partir destes mesmos dados foi calculado o esforço de amostragem necessário para estimar a riqueza de

espécies de colêmbolos em áreas florestais. Chegou-se à conclusão de que tanto morfotipos como géneros são bons *surrogates* de espécies e que 7 a 10 amostras são suficientes para estimar 80 a 90% da riqueza de espécies de um local. Ainda assim, os modelos podem ser melhorados através da incorporação de mais dados de outras parcelas florestais e da validação dos mesmos.

Posteriormente, a classificação em morfotipos foi usada com vista a avaliar o efeito que os diferentes tipos florestais e o pastoreio têm nas comunidades de colêmbolos, estudo integrado num projeto que visa a monitorização extensiva de florestas em Portugal. Para tal, foram utilizados 20 pontos do projeto, todos eles localizados no sul de Portugal e pertencentes a dois tipos florestais: *Quercus suber* L. e *Quercus rotundifolia* Lam. Por local, foram colhidas 8 amostras de solo e os colêmbolos encontrados, separados por morfotipos. Diferenças entre abundância e riqueza de morfotipos entre locais e tipos de floresta foram avaliadas com uma ANOVA hierárquica. Índices de diversidade foram calculados. Diferenças nas comunidades entre locais foram avaliadas por meio de análise multivariável. Com um teste-t verificou-se se existiam diferenças entre sítios com e sem pastoreio. Por último, através de uma correlação, estudou-se a relação entre a percentagem de cobertura vegetativa e a abundância e diversidade de colêmbolos. Apesar da abundância e diversidade de colêmbolos terem variado ao longo dos sítios amostrados, não foram encontradas diferenças significativas nem entre os dois tipos de florestas, nem entre locais com e sem prática de pastoreio. A correlação efetuada, embora significativa, revelou-se fraca, o que sugere que talvez mais variáveis de cobertura devam ser incluídas para estudar esta relação. No futuro

será importante a incorporação de mais variáveis explicativas que ajudem a perceber melhor os padrões de diversidade encontrados.

## **CHAPTER I**

## **General Introduction**

## I.1. Soil Biodiversity and Ecosystem Services

Soils are very complex systems, providing several ecosystem services and harbouring many life forms. Physically, they outline the boundary between atmosphere and lithosphere, comprising elements from both: water, minerals, and a gaseous phase, along with a wide range of organisms and biological materials (Lavelle and Spain, 2001).

Ecosystem services - the benefits that humans get from the ecosystems, supporting their survival and life quality - can be categorized in four different classes: provisioning, regulatory, cultural, and supporting services (Vandewalle et al., 2008). From these, soils are particularly important in regulatory and supporting services, such as water and nutrient cycling, primary production, soil formation and retention, water and erosion regulation, waste treatment, and climate regulation (Lavelle et al., 2006; Vandewalle et al., 2008).

Soil organisms have a strong impact on soil biological processes, either by controlling or performing them, which in turn are responsible for the delivery of ecosystem services (Beck et al., 2005).

According to their role in those processes, soil organisms can be separated into *chemical engineers*, composed by bacteria and fungi (microflora), responsible for the decomposition of organic matter into nutrients available to plants and animals, *biological regulators*, which includes nematodes, enchytraeids, mites, and springtails (microfauna and mesofauna), that predate on plants, microorganisms, and other invertebrates, and *ecosystem engineers*, including earthworms, termites, and ants (macrofauna), who modify and create habitats for smaller soil organisms (Turbé et al., 2010). Nevertheless, just

because certain organisms belong to a functional group, it does not mean they won't intervene in other processes (Table I.1).

**Table I.1: Ecosystem services provided by soil and the contribution of soil organisms for their delivery (Lavelle et al., 2006; Vandewalle et al., 2008).**

<b>Ecosystem services</b>	<b>Organisms Contribution</b>	<b>Functional groups involved</b>
<b>Climate regulation</b>	OM storage in stable biogenic macroaggregates through burrowing and casting activities, allowing carbon sequestration	Ecosystem engineers
<b>Water and erosion regulation</b>	Creation of surface roughness and maintenance of stable porosity	Ecosystem engineers, biological regulators
<b>Waste treatment</b>	Use of carbon from organic compounds in microbial activity	Chemical engineers
<b>Primary production and pest control</b>	Enhancement of plant production	Chemical engineers, biological regulators, and ecosystem engineers
<b>Soil formation and retention</b>	Conversion of nutrients and energy from OM into solid and stable aggregates	Ecosystem engineers
<b>Nutrient cycling</b>	Decomposition of OM and nutrient transformation; OM breakdown by invertebrate feeding activities	Chemical engineers, biological regulators, and ecosystem engineers
<b>Water cycling</b>	Improvement of soil porosity through bioturbation and burrowing activities	Ecosystem engineers

The role of soil organisms in the various processes that lead to the delivery of ecosystem services clearly shows how important it is to have a high soil biodiversity. However, currently, soil degradation, land use intensive management, climate change, chemical pollution, and invasive species pose serious threats to soil organisms (Decaëns et al., 2006; Turbé et al., 2010). In the presence of such pressures, conservation of soil biodiversity is vital and its monitoring, an important step to achieve it.

## **I.2. Monitoring Soil Biodiversity**

Monitoring allows assessing trends in biodiversity, namely, to understand how does biodiversity change and how do soil quality and processes change with it, and for that matter, indicators are necessary to help establishing baseline conditions and trends (Turbé et al., 2010). The combination of indicators defines the normal operating range (NOR) of a system and deviations from NOR are a sign that the system is in a condition of stress (Van Straalen, 2002).

An increasing concern with soil and its biodiversity well-being has led to the development of a few monitoring programs in Europe, namely the Biological Indicator System for Soil Quality (BISK), from the Netherlands (Schouten et al., 1997), and the Biological Soil Classification Scheme (BBSK), from Germany (Römbke et al., 1997).

BISK measures soil organisms and processes, by making use of both structural parameters - abundance and community composition of nematodes,



earthworms, enchytraeids, and soil micro-arthropods – and functional parameters – microbial biomass and respiration, carbon and nitrogen cycles, and microbial diversity (Breure et al., 2005; Rutgers et al., 2008; Sousa et al., 2009). BBSK, on the other hand, is based on the principle that similar soils under a similar climatic influence will have similar soil fauna, allowing the establishment of reference biotic communities and this way measure the deviation between reference and sampled communities for a given site (Breure et al., 2005; Römbke et al., 2005; Sousa et al., 2009). Both of them are based in the comparison between the sampled community and the expected community for a given site: as higher the deviance, higher the disturbance (Breure et al., 2005; Sousa et al., 2009).

Despite the clear utility of soil organisms as indicators in monitoring schemes, identifying them is not an easy task, as it requires specialized personnel and it is time consuming. Because of this, alternatives to species-level identification have been pursued.

The use of surrogates, in particular, higher taxa surrogates and surrogate groups of overall richness, can be useful as a resources-saving method to estimate species richness, having the potential to be implemented in extensive monitoring programs (Forest Focus, 2007).

A different approach is using organisms' functional traits, that is, the characteristics of organisms related with their fitness (Vandewalle et al., 2010).

QBS (*Qualità Biologica del Suolo*) (Parisi et al., 2001) is an example of an index to monitor soil quality based on the morphological traits of organisms. Grounded on the idea that soils with higher quality will have a higher number of microarthropod groups well adapted to soil habitats, it evaluates

microarthropods' level of adaptation to the soil, not requiring an identification to the species level (Parisi et al., 2005). Moreover, recent studies using adaptations of this index for springtails (Collembola) (Vandewalle et al., 2010; Carvalho, 2012) have shown the potential of using this eco-morphological trait approach to discriminate between different forest types and different types of land use management.

### **I.3. Objectives**

This work is part of a larger national project, *Biodiversidade florestal e serviços do ecossistema*, whose prime goal is to launch a program for extensive monitoring of soil biodiversity in Portuguese forests, allowing an evaluation of the changes in biodiversity and soil quality in these systems and their impact in ecosystem services provision, originated by local pressures. Specifically, this thesis focuses on the southern part of Portugal, typically characterized by *Quercus suber* L. and *Quercus rotundifolia* Lam. forests.

The general objectives of this work are to derive sampling effort and surrogates as tools for biodiversity assessment in extensive monitoring programs and, in a second phase, to evaluate the effects of forest type and land use practices (grazing) on Collembola communities in southern Portugal forest stands, using an eco-morphological trait approach.

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**CHAPTER II**

**Deriving sampling effort and surrogates  
for biodiversity assessment**

## II.1. Introduction

Decline in soil biodiversity has been identified as one of the main threats on the soil (Kibblewhite et al., 2008). Currently, some extensive monitoring schemes are being used in Europe for assessing soil quality and biodiversity. However, because these monitoring programs often involve the identification of the soil organisms to the species level, they are too expensive to use on a broad scale.

To overcome this caveat, several alternative approaches have been suggested to predict species richness and to monitor the spatial and temporal distribution of biodiversity (Cardoso et al., 2004), from which the use of surrogates has been widely discussed (Biaggini et al., 2007; Brennan et al., 2006; Derraik et al., 2002; Duelli and Obrist, 1998; Lindenmayer et al., 2000; Lovell et al., 2007; Sauberer et al., 2004).

Surrogacy can use different approaches: higher taxa richness, the state of environmental variables, and the species richness of indicator taxa (Gaston and Williams, 1993; Gaston and Blackburn, 1995). From all of these, the higher taxonomic level approach (i.e., using genus or families) is particularly advantageous, since it delivers information on a large number of taxa, while not requiring as many time and resources as a species-level identification would (Cardoso et al., 2004), as there is a smaller number of higher taxa and they are more easily identified than species (Moreno et al., 2008).

Despite the advantages of this approach, there are certain factors influencing the relationship between species richness and higher taxa richness that should not be disregarded. Andersen (1995) noted that this relationship is

influenced by sampling intensity, since when the number of samples increases, more species belonging to higher level taxa already registered can keep being recorded. Therefore, when doing surrogacy studies, sampling effort is an important factor to take into account.

In spite of the amount of work done in this area, little or none information is available regarding surrogates of soil mesofauna and, in particular, Collembola.

These organisms have a great potential as indicators of soil quality and are used in monitoring schemes (Breure et al., 2005; Gardi et al., 2009). Therefore there is great interest in learning more about the relation between Collembola species richness and higher taxa richness, and whether this type of surrogacy can be used with some reliability to predict species richness. In addition, given the existence of an alternative approach for classifying collembolans based on their eco-morphological traits (Carvalho, 2012), it would be interesting to find out if this classification would be a good surrogate to the traditional taxonomic classification, since it is a method much easier to apply.

Considering this, the main goal for this phase of the project is to find out if both genera (higher taxa) richness and a classification based on morphological traits (morphotypes richness) can be used as surrogates of Collembola species richness, by developing predictive models based on existing data from previous projects. Furthermore, given the relationship between surrogacy and sampling effort, and considering that knowing how many samples are necessary to have a good estimation of the species richness of a site is very important when planning extensive monitoring programs, it is intended to evaluate sampling effort and its relation with richness estimations in forest areas.



## **II.2. Materials and Methods**

### **II.2.1. Study sites**

In order to derive sampling effort and surrogates, abundance data for Collembola species concerning 18 sites from different forest types was used. For simplification, the sites were named using letters (A to R).

The study sites were collected from different research projects, therefore presenting differences concerning sampling design, but most of them having the same sampling effort: while in A to M the sampling was done at the parcel level, in the remaining 5 (N to R), sampling was performed at a landscape level, covering a larger area.

More details about the number of samples and the forest type for each site can be found in Table II.1. Details about the sampling sites can be found in the respective articles cited also in Table II.1.

**Table II.1: Information regarding the sites used for the derivation of sampling effort and surrogates.**

Site	Number of samples	Number of species	Forest type	Reference
A	9	40	<i>Quercus suber</i>	Barrocas et al., 1998
B	16	19	<i>Eucalyptus globulus</i>	Sousa et al., 1997
C	16	15	<i>Quercus suber</i>	
D	16	26	<i>Eucalyptus globulus</i>	Sousa et al., 2000
E	16	30	<i>Quercus pyrenaica</i>	
F	16	19	<i>Eucalyptus globulus</i>	Sousa et al., 1994
G	16	38	<i>Quercus ilex</i>	
H	16	38	<i>Eucalyptus globulus</i>	Vasconcelos et al., 1994
I	16	33	<i>Pinus pinaster</i>	
J	12	30	<i>Eucalyptus globulus</i>	Barrocas et al., 1998
K	8	38	<i>Quercus canariensis</i>	
L	16	31	<i>Eucalyptus globulus</i>	Gama et al., 1995
M	16	36	<i>Pinus pinaster</i>	
N	16	34	<i>Quercus suber</i>	Sousa et al., 2006
O	16	10	<i>Eucalyptus globulus</i>	
P	16	23	<i>Quercus suber</i> *	
Q	16	25	<i>Quercus suber</i> *	
R	16	27	<i>Quercus suber</i> *	
<b>*gradient of forest fragmentation and land-use intensification (from P to R)</b>				

### II.2.2. Attribution of morphotypes

For the evaluation of the use of morphotype richness as surrogate for Collembola species richness, a morphotype was attributed to each species present in the data, according with Carvalho (2012). This type of classification is detailed on Table II.2.

**Table II.2: Morphological traits of Collembola and the values attributed for the establishment of morphotypes.**

Morphological traits		Value
<b>Ocelli</b>	Present	0
	Absent	4
<b>Antennae</b>	Antennae length > body length	0
	Antennae length > 0.5 body length	2
	Antennae length < 0.5 body length	4
<b>Furca</b>	Present (fully developed)	0
	Present (reduced)	2
	Absent	4
<b>Hairs/scales</b>	Present	0
	Absent	4
<b>Pigmentation</b>	Present with patterns	0
	Present without patterns	2
	Absent	4

For each considered morphological trait, a score is attributed, with higher scores corresponding to characteristic morphological features more adapted to deeper soil layers. Each specimen is characterized by a combination of five scores, with each different combination being considered a morphotype.

### **II.2.3. Processing species data**

In order to derive sampling effort and surrogates, some rules had to be taken into account to avoid problems concerning species overestimation. Non identified juveniles were excluded from the analysis. A few other individuals, identified only to the genus level, were also excluded if following two conditions: (i) if a morphotype could not be attributed and (ii) if there were already individuals from the same genus present in the same site.

### **II.2.4. Sampling effort derivation**

For the derivation of sampling effort, species abundance matrices were first imported to EstimateS 9 (Colwell, 2013), with the purpose of calculating rarefaction curves. This way, it is possible to estimate the number of species expected per number of samples, based on the total number of species found in all the samples. Empty samples were not considered for the analysis. The obtained results were then imported to STATISTICA 7 (Statsoft Inc., 2004), where a species accumulation function - in this case, the linear dependence

model - was applied to all the sites for the estimation of the parameters  $a$  and  $b$ , as given by the equation  $S(t) = a/b * [1-EXP(-b*t)]$ , where  $t$  is a measure of sampling effort,  $S(t)$  is the predicted number of species at  $t$ ,  $a$  is the rate of species increase,  $b$ , a parameter related to the shape of the accumulation of new species with increasing sampling, and  $a/b$  is the asymptote (estimated maximum richness) (Shiu and Lee, 2003).

The remaining procedures to estimate sampling effort were completed in Excel, by applying the linear dependence model derived equation  $t_q = -1/b \ln(1 - q)$ , where  $t_q$  is the sampling effort and  $q$ , a proportion of the asymptote (Shiu and Lee, 2003), to obtain the number of samples required for estimating 60%, 70%, 80% and 90% of diversity for each site. The average number of samples for all the sites was then calculated, as well as the mean for each forest type (*Quercus*, *Eucalyptus*, and *Pinus* stands).

### II.2.5. Surrogates derivation

Aiming to find if genera and morphotypes can be used as surrogates for species, matrices with the number of species, genera, and morphotypes per site were built.

STATISTICA 7 (Statsoft Inc., 2004) was utilized to develop generalized linear models to predict species richness based on genera and morphotypes, using the number of species as dependent variable and the number of genera/morphotypes as independent variables. Poisson distribution with Log

link function was used both for the prediction based on genera and morphotypes.

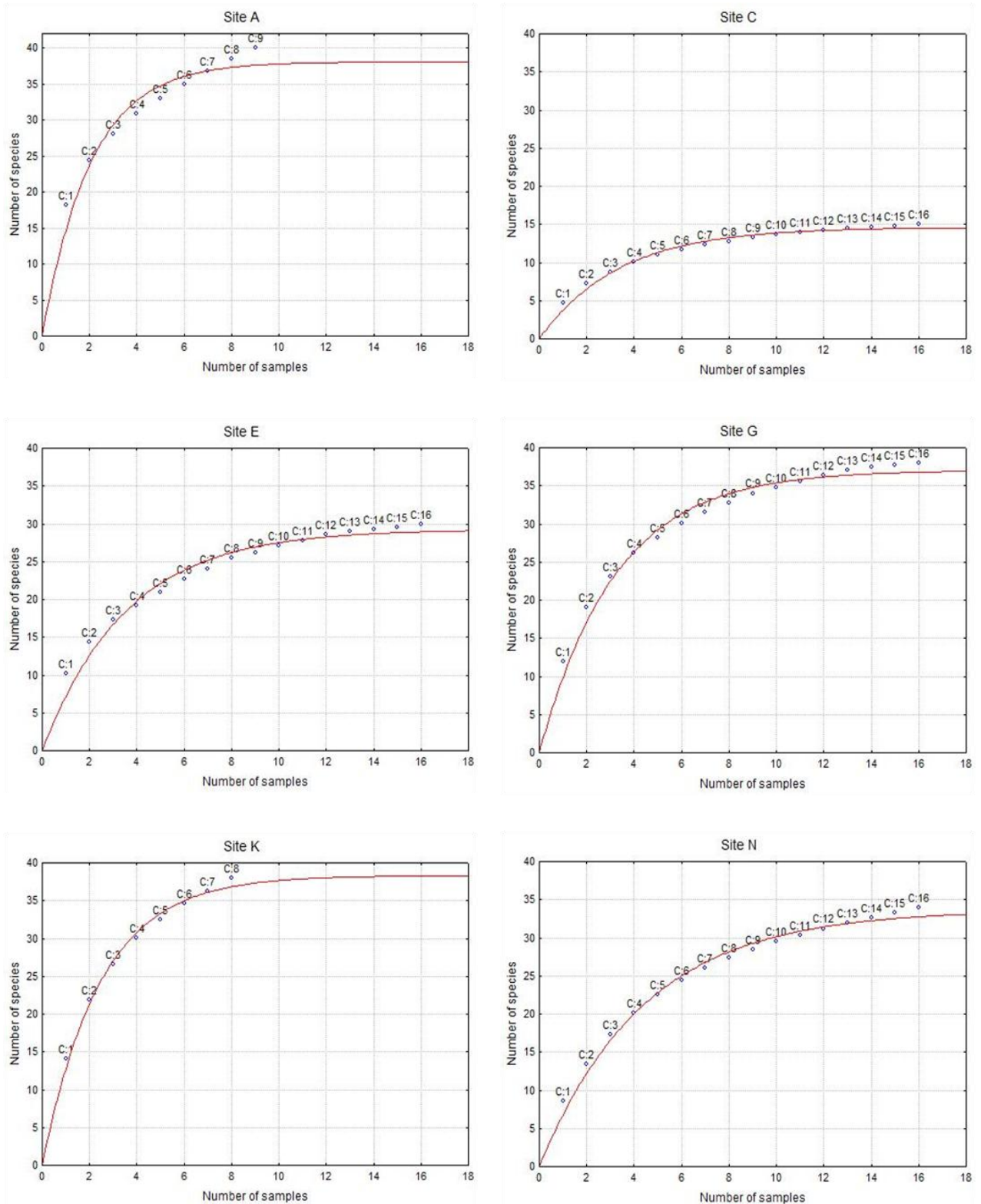
Afterwards, to prove that in sites where there are several species that belong to the same genus the models may not work out as well, the residuals of the models (difference between observed and predicted values) were plotted against the proportion of species of the same genus in relation with the total for each site, through a Pearson correlation in STATISTICA 7.

## **II.3. Results**

### **II.3.1. Sampling effort**

The applied linear dependence model resulted in the following species saturation curves (Figures II.1 to II.4). Generally, the number of samples collected in each site was enough to estimate the complete species richness of that particular forest stand, as it is possible to verify a stabilization of the curve (the asymptote was reached) in most sites.

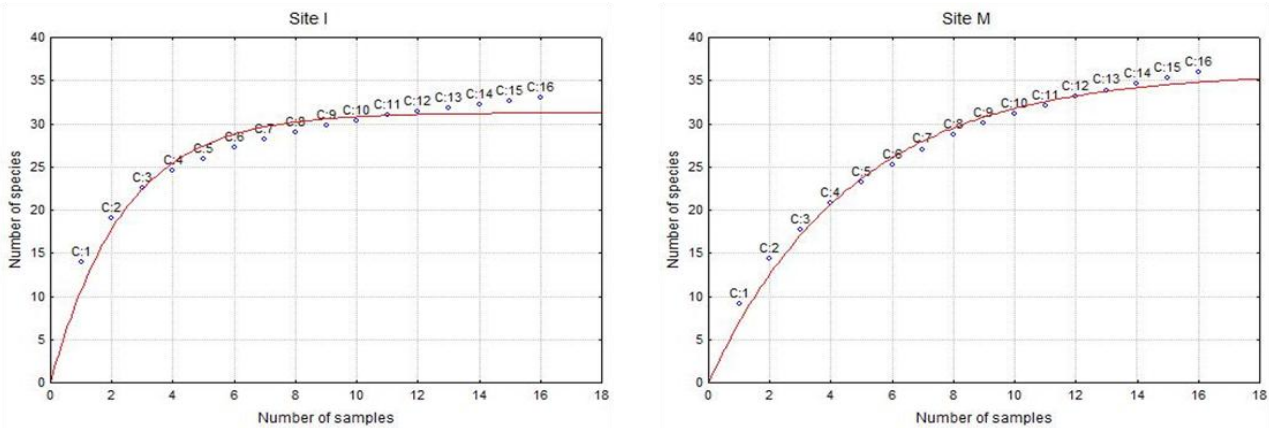
The obtained curves for *Quercus* spp. forests (Figure II.1) showed that the number of samples was sufficient. Surprisingly, for sites A and K, the asymptote was reached with fewer samples.



**Figure II.1: Saturation curves for *Quercus* spp. forests.**

Collembola diversity in Portuguese forest stands: deriving surrogates for species richness and evaluating the effects of forest type and grazing

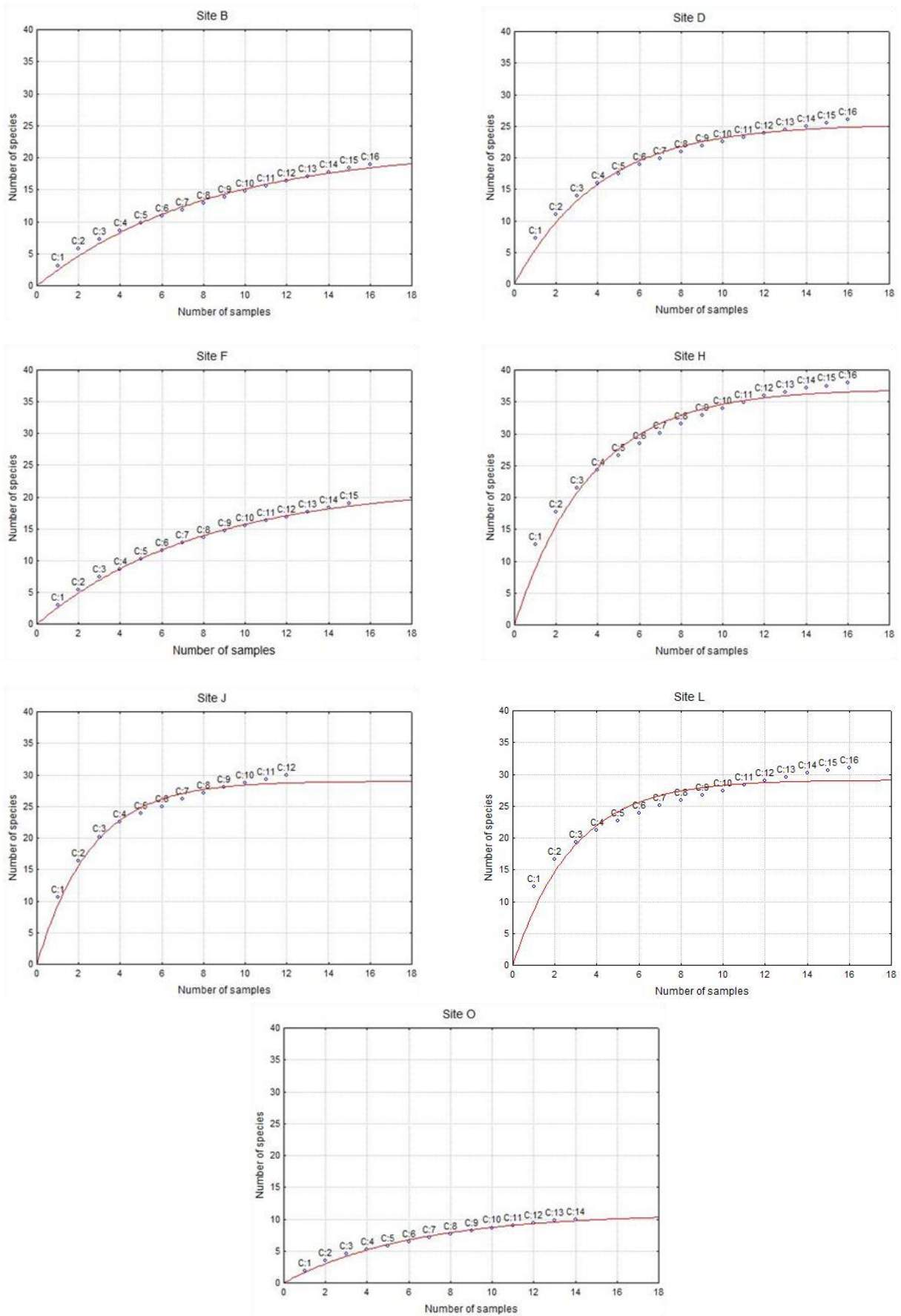
For *Pinus pinaster* forests (Figure II.2), although the asymptote was reached, for one of the places (site I) this happens even with a lesser number of samples.



**Figure II.2: Saturation curves for *Pinus pinaster* forests.**

Concerning *Eucalyptus globulus* stands (Figure II.3), a larger number of samples seems to be necessary in order to reach an asymptote in sites B, F, and O, while in others the collected samples were enough.

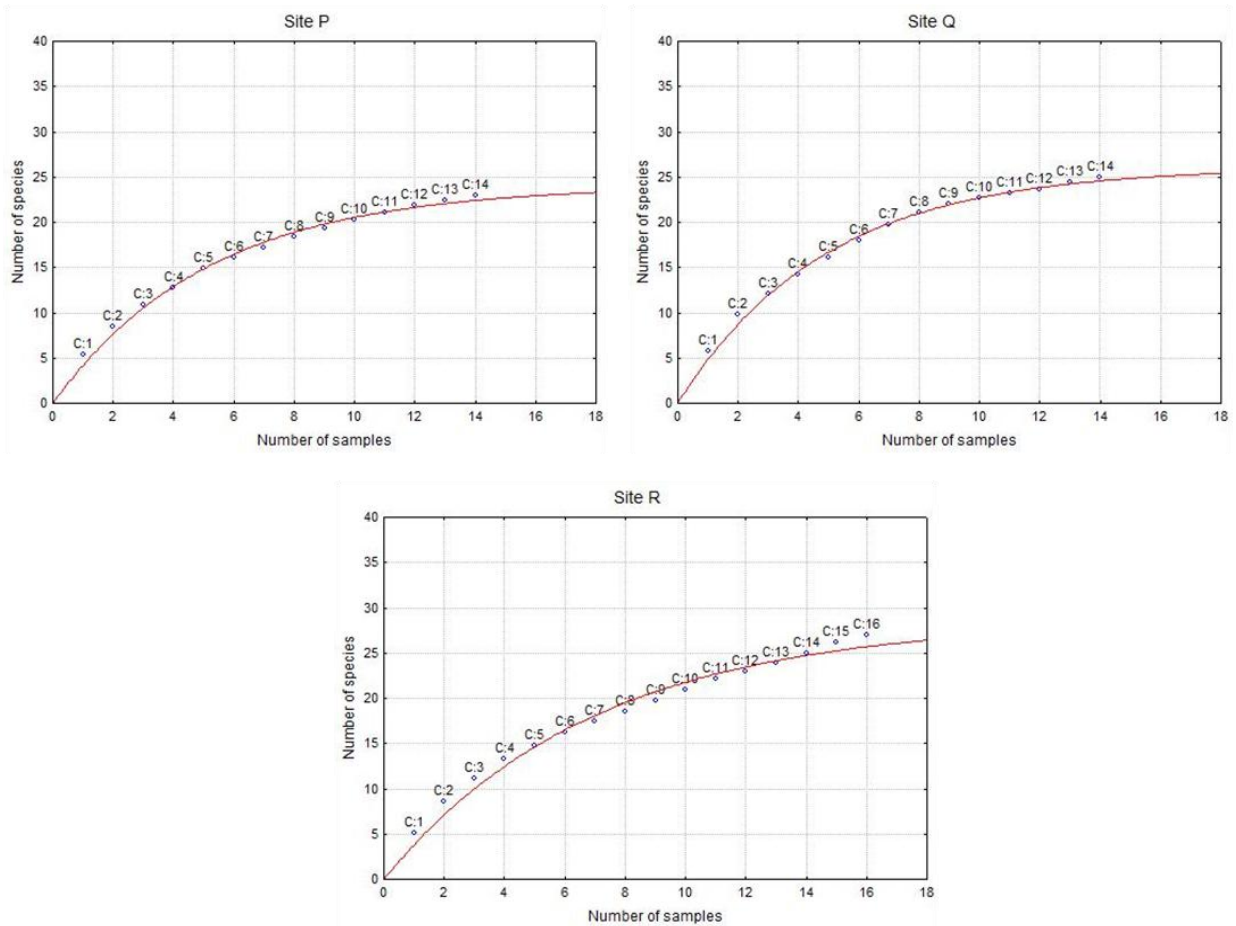




**Figure II.3: Saturation curves for *Eucalyptus globulus* stands.**

Collembola diversity in Portuguese forest stands: deriving surrogates for species richness and evaluating the effects of forest type and grazing

Finally, *Quercus suber* forests sampled over a land use intensification gradient and forest fragmentation (Figure II.4) need a larger number of samples to obtain a stabilization of the curve, since the asymptote was not reached in none of the sites.



**Figure II.4: Saturation curves for *Quercus suber* forests over a land use intensification gradient.**

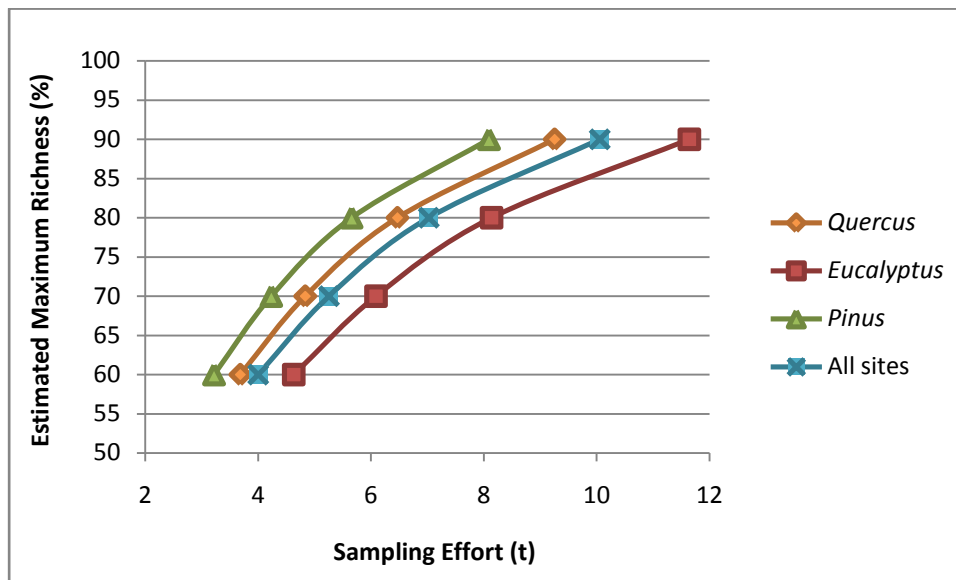
All the parameters associated with the calculation of the sampling effort are presented on Table II.3.

**Table II.3: Parameters related with sampling effort and required sampling effort (t) to sample 60, 70, 80, and 90% of the total richness of each site.**

Sites	Sobs	T	A	B	R <sup>2</sup>	As.	% As.	t(60%)	t(70%)	t(80%)	t(90%)
A	40	9	18,62656	0,489248	0,929	38	105,065	2	2	3	5
B	19	16	2,620568	0,122127	0,988	21	88,546	8	10	13	19
C	15	16	4,332910	0,296071	0,979	15	102,496	3	4	5	8
D	26	16	6,204442	0,244901	0,977	25	102,627	4	5	7	9
E	30	16	8,258565	0,282280	0,961	29	102,541	3	4	6	8
F	19	15	2,776470	0,127412	0,997	22	87,191	7	9	13	18
G	38	16	11,52803	0,311195	0,978	37	102,580	3	4	5	7
H	38	16	10,13915	0,273840	0,962	37	102,631	3	4	6	8
I	33	16	13,16643	0,420844	0,933	31	105,479	2	3	4	5
J	30	12	11,22767	0,387181	0,979	29	103,454	2	3	4	6
K	38	8	15,60187	0,407153	0,989	38	99,166	2	3	4	6
L	31	16	10,25475	0,352001	0,910	29	106,410	3	3	5	7
M	36	16	7,727267	0,214815	0,986	36	100,078	4	6	7	11
N	34	16	7,611426	0,226047	0,988	34	100,975	4	5	7	10
O	10	14	1,756430	0,161241	0,989	11	91,800	6	7	10	14
P	23	14	4,620479	0,192050	0,991	24	95,599	5	6	8	12
Q	25	14	5,329290	0,204455	0,994	26	95,911	4	6	8	11
R	27	16	4,095668	0,143296	0,981	29	94,465	6	8	11	16
<b>Mean (t)</b>								<b>4</b>	<b>5</b>	<b>7</b>	<b>10</b>

The calculations of the asymptote (a/b) - As. - and its percentage - % As. - allowed confirming what was concluded from the analysis of the saturation curves: in 6 of the sites (B, F, O, P, Q, and R), the number of samples was not enough to reach an asymptote. For every other site, the model predicted a lower (or equal) asymptote compared with the observed data, indicating that, theoretically, no more species are to be recorded.

The obtained results concerning the average number of samples required to sample 60, 70, 80, and 90% of the total richness are presented on Figure II.5, as well as the average of samples required for the different forest types.



**Figure II.5: Average sampling effort to sample 60, 70, 80, and 90% of the total richness for all the sites and for different forest types.**

On average, when compared with other sites, *Eucalyptus globulus* forests need more samples (8 to 12) so that 80 to 90% of species richness can be obtained. On the other hand, *Pinus pinaster* forests require a fewer number of samples (6 to 8) to attain the same percentage of richness. However, these results may be biased, since only 2 *Pinus pinaster* sites were used in the analysis. *Quercus* spp. forests are somewhat in the middle, requiring about 7 to 9 samples.

## II.3.2. Surrogates of Collembola species richness

### II.3.2.1. Predictive models

The developed generalized linear models resulted in the two following equations, for prediction of species richness ( $S\_Coll\_sp$ ) based on morphotypes richness ( $S\_Coll\_morph$ ) and number of genera ( $S\_Coll\_gen$ ), respectively:

$$S\_Coll\_sp = \exp(1,906 + 0,108 * S\_Coll\_morph)$$

$$\text{Pseudo } R^2 = 0.7674 = 76.7\%$$

$$S\_Coll\_sp = \exp(2,242 + 0,043 * S\_Coll\_gen)$$

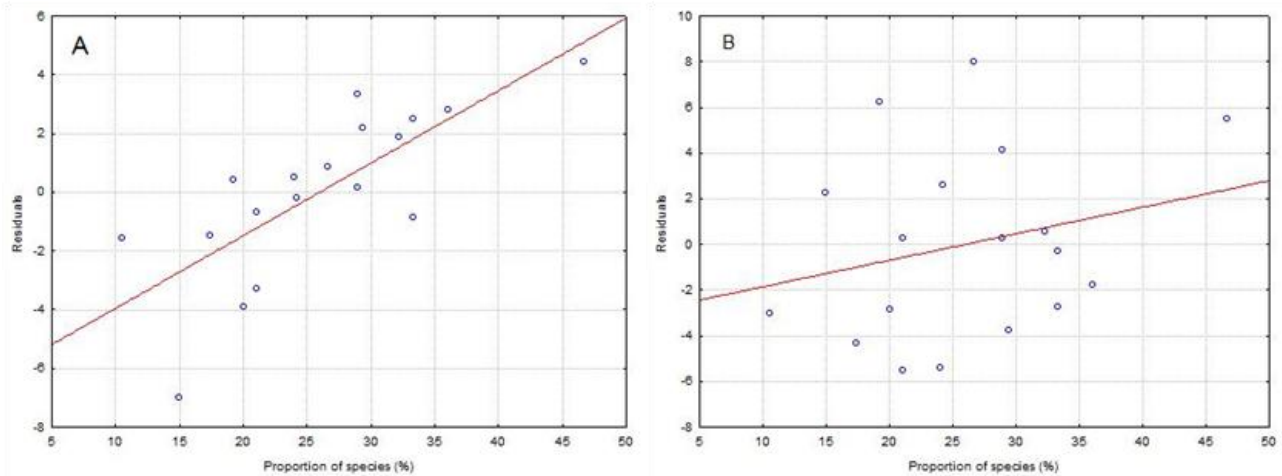
$$\text{Pseudo } R^2 = 0.9049 = 90.5\%$$

For both the equations, independent variables were highly significant ( $p < 0.0001$ ) having originated very high pseudo  $R^2$  values.

### II.3.2.2. Correlation between models' residuals and proportion of species

The relation found between species richness and genera richness, and between species richness and morphotype richness, may not always work out so well, namely, in sites with several species belonging to the same genus. Evidence of this is shown in the plotted graphics for the correlation between the

residuals of the developed models and the proportion of species from the same genus in relation with the total number of species (Figure II.6).



**Figure II.6: Correlation between the proportion of species from the same genus in relation with the total number of species and the residuals of the developed models for prediction of species richness based on genera (A) and morphotype richness (B). The line helps to visualize the trend.**

As expected, for genera, the correlation between the residuals and the proportion of species from the same genus (Figure II.6A) was strong ( $R=0.76$ ) and significant ( $p<0.0003$ ), indicating that a bias when estimating species richness can occur. As expected, in the case of morphotypes (Figure II.6B), the correlation was weak ( $R=0.25$ ) and not significant ( $p=0.3$ ).

## II.4. Discussion

Sampling effort results showed that, on average, 7 to 10 samples are enough to have a good estimate of a site's species richness, corresponding to 80 and 90% of the total richness. Even though, some of the studied sites clearly needed a larger number of samples to attain this percentage of species richness. This was noticeable in some *Eucalyptus* stands (B, F, and O), but also in some *Quercus suber* forests (P, Q, and R). Although this may seem strange particularly in the eucalyptus sites, known by having a general lower species richness than other forest stands from the same region, this finding is most probably associated with the structure of the habitat and heterogeneity (Boulinier et al., 1998; Gotelli and Colwell, 2001), since more heterogeneous sites will naturally require a larger number of samples to attain the same relative level of species richness (Cooperrider et al., 1986). Several of the eucalyptus sites sampled presented a spatially heterogeneous habitat configuration, with tree rows having medium to high accumulation of plant residues, whereas between row spaces being depleted of soil cover (Sousa et al., 2000). However, this aspect of the spatial heterogeneity is particularly evident in the case of the mentioned *Quercus suber* sites, since the sampling was done over larger areas along a gradient of forest fragmentation. This is an important information that should be retained when planning monitoring schemes, more specifically when applying these type of models especially at larger spatial scales that a forest patch.

Another interesting point to discuss and to work on in the future is using alternative models to estimate sampling effort. In the case of species-area

relationships there are several models that can be applied and there is a lot of discussion about which model would be the best (Tjørve, 2009). Knowing this and considering that the linear dependence model predicted a lower asymptote for almost every site, it would be interesting to compare these results with the results obtained from applying the same data to different models.

On the subject of surrogates, number of genera seems to be a very reliable substitute of species richness, as expected, given the high correlation found between species richness and higher taxa richness in past surrogacy studies related with soil arthropods (András, 2003; Biaggini et al., 2007; Cardoso et al., 2004; Forest Focus, 2007).

Regarding morphotype richness, it could be verified that they can be used with some degree of confidence to replace species richness, with the great advantage of being very simple to classify and separate individuals using this type of classification, saving hours of work and not requiring specialized technicians. Moreover, since morphotypes are linked to the adaptation level of collembolans to the soil and their dispersal ability (Carvalho, 2012), they can give us additional information related with the communities' response to factors of stress, since this response is sometimes noticeable in the composition of the community (Vandewalle et al., 2010), and not necessarily in the number of species present.

However, in sites with many species belonging to the same genus, the relation between genus and species may not work out as well, as the model will underestimate the number of species present. This was corroborated by the strong positive correlation between the residuals of the model for prediction of species richness based on genera and the proportion of species from the same



genus: the model tends to predict much lower values for number of species than those observed in sites with a higher proportion of species from the same genus. In relation to morphotypes, since it is a classification based on morphological traits, the problem of underestimation is not constricted to species and can also be applied to genera; hence there is not a strong correlation between the residuals of the model and the proportion of species.

Although it was possible to prove the existence of a very good relationship between number of genera and species richness, and between morphotype richness and species richness, this relation should be further investigated. The models can be further improved by adding more data from other forest stands. Another important step that still needs to be taken is the validation of the developed models to verify if they work out as well for other forest stands and, therefore, if they can be generally applied across sites.

Later, it would also be interesting to do more research about the possibility of using morphotypes as surrogates in other groups of soil organisms.

Concerning the relationship between sampling effort and surrogates, as for the sites used in this study, since the results showed that the number of samples taken was generally enough for estimating high levels of the total diversity, morphotypes and genera would still be considered good surrogates for Collembola species richness, seeing it is unlikely that more species would be recorded.

In conclusion, while surrogates cannot assess all species within a site, they are certainly very useful in rapid biodiversity assessments, as it is the purpose of extensive monitoring programs.

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**CHAPTER III**

**Evaluating the effects of forest type and  
grazing on Collembola communities**

### III.1. Introduction

Soil organisms have a major role in the delivery of important ecosystem services like nutrient cycling and climate regulation (Lavelle et al., 2006). The fact that the level of biodiversity within an ecosystem allows it to deliver a certain range of services (Vandewalle et al., 2008) illustrates how important it is to maintain a high soil biodiversity.

However, due to human activities, soils and soil biodiversity have long been exposed to several threats. Monitoring soil biodiversity assumes then an important role in detecting changes in biodiversity and assessing soil quality. For this purpose, it is important to establish widely applicable indicators (Vandewalle et al., 2010).

An indicator is, *per* definition, a characteristic that should be simple, measurable and quantifiable, while at the same time, able to respond to changes in environmental conditions and to ecological processes and functions (Harrington et al., 2010). Regarding soil biodiversity monitoring, a number of indicators has been purposed, among them, collembolan diversity and abundance (Bispo et al., 2009; Gardi et al., 2009).

Collembola are small hexapods that can be found in the litter or in the pore space of the upper 10 to 15 cm of soil (Lavelle and Spain, 2001). They are very diverse and abundant, being quite important for nutrient cycling, since they feed mainly on fungi and bacteria, affecting decomposition rates (Jeffery et al., 2010). In addition, collembolans are affected by agricultural practices and changes in land use (Hopkin, 1997). Altogether, these characteristics make them suitable indicators.



The one problem with using these organisms, though, is the amount of resources needed for their identification. This problem is common to other organisms, leading to a search for alternative approaches to the traditional taxonomic classification. Parisi (2001) purposed an index of soil quality based on eco-morphological traits of soil arthropods, that has been successfully adapted and used for collembolans to discriminate between different forest types and different types of land use management (Vandewalle et al., 2010; Carvalho, 2012).

In Portugal, this concept of monitoring soil quality and biodiversity is relatively recent. Because of this, there is a lack of information about soil biodiversity patterns across different systems. In the particular case of forest systems, that are acknowledgeable very important for global climate and carbon cycle, water balance, resources provisioning and habitat purveyance (Jeffery et al., 2010), it is very important to learn more about the state of soil biodiversity and how would this affect ecosystem services provision.

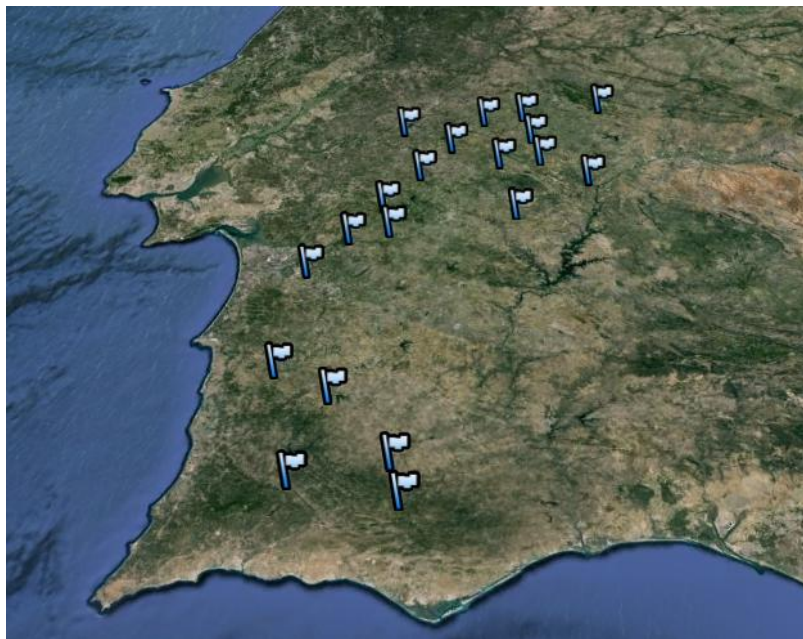
This study is integrated in the project *Biodiversidade florestal e serviços dos ecossistemas*, whose main goal is to launch an extensive monitoring program in Portuguese forests, covering 100 sampling sites from diverse forest types across the whole country.

The major aim for this stage of the work is to evaluate the effects of (i) forest type, in this case, considering two different forest types, *Quercus suber* L. and *Quercus rotundifolia* Lam., (ii) land use practices, namely grazing, and (iii) habitat structure regarding type of cover, on Collembola communities, using a classification based on morphological traits.

## III.2. Materials and Methods

### III.2.1. Study sites

In total, 20 sites from the project were used in this study, all of them located in the south of Portugal, Alentejo and Algarve (Figure III.1). Half of these sites were cork oak (*Quercus suber* L.) forests, while the other half, holm oak (*Quercus rotundifolia* Lam.) forests. The names attributed to the sites correspond to their number in the project (from 1 to 100), followed by a code that indicates the type of forest (in this case, QS for cork oak and AZ for holm oak).

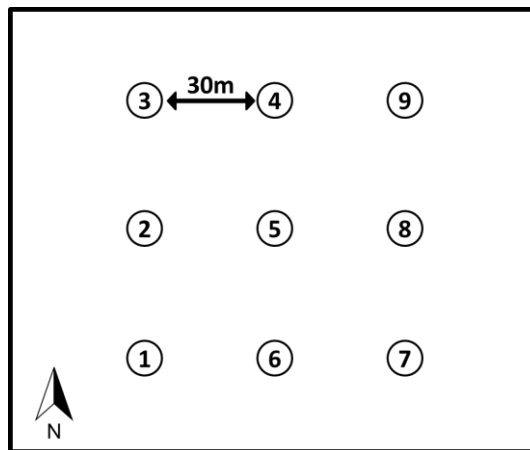


**Figure III.1: Geographical location of the study sites.**

### III.2.2. Sampling

Sampling took place during two different periods: from December 2012 to January 2013 and from November 2013 to February 2014.

In each sampling site, 8 soil samples were collected using a split corer, by removing a cylinder with the first 5 cm of the soil layer (ISO, 2005). The sampling design formed a square with the samples 30 meters apart from each other (Figure III.2).



**Figure III.2: Sampling design used for all the studied sites.**

Soil was collected from all sampling points and from an additional central point (number 5 in Figure III.2) for posterior physicochemical analysis, giving a total of 3 compound samples per site, with soil collected from the 9 points.

Finally, a photographic registry was made in each sampling point for later characterization of the type and percentage of vegetation cover.

### **III.2.3. Extraction and sorting of microarthropods**

All the soil samples were processed under the same conditions. They were taken to the laboratory, where soil microarthropods were extracted following the protocol by ISO (2005), the samples being placed in a MacFadyen extractor for 10 days. After the extraction, samples were sorted, with soil microarthropods being separated according to their Order. In addition, collembolans were classified in morphotypes. Samples without collembolans were not included in the statistical analysis.

### **III.2.4. Collembola classification into morphotypes**

Collembola were classified using morphotypes according with Carvalho (2012). A score was attributed to each individual considering a combination of 5 morphological traits related with their adaptation to soil: presence/absence of ocelli (0-present, 4-absent), antennae size (0-longer than body size, 2-longer than half the body size, 4-shorter than half the body size), furca development (0-fully developed, 2-reduced, 4-absent), presence/absence of scales and hairs (0-present, 4-absent), and pigmentation (0-present with patterns, 2-present without patterns, 4-absent). So, those more adapted to live in the soil and with lower dispersal ability (eu-edaphic life forms) receive a higher score, while those more adapted to live in the soil surface and with higher dispersal ability (epigeous) receive a lower score.

Each different combination of individual scores is a morphotype and the global score of the individual is obtained by adding the values attributed for each characteristic; scores may range from 0 (morphotype 00000) to 20 (morphotype 44444).

In this case, collembolans were considered epigeous (Ep) if the attributed score was between 2 and 8, hemi-edaphic (He), with a score between 10 and 12, and eu-edaphic (Ed), if having a global score between 14 and 20 (Table III.1).

**Table III.1: Identified morphotypes in this study and respective scores.**

<b>Morphotype</b>	<b>Score</b>
<b>Ep1</b> m02000	2
<b>Ep2</b> m02002	4
<b>Ep3</b> m04000	4
<b>Ep4</b> m02040	6
<b>Ep5</b> m04002	6
<b>Ep6</b> m04004	8
<b>Ep7</b> m04040	8
<b>Ep8</b> m02042	8
<b>He1</b> m02044	10
<b>He2</b> m04042	10
<b>He3</b> m04242	12
<b>He4</b> m04044	12
<b>He5</b> m44004	12
<b>Ed1</b> m04244	14
<b>Ed2</b> m04442	14
<b>Ed3</b> m04444	16
<b>Ed4</b> m44044	16
<b>Ed5</b> m44244	18
<b>Ed6</b> m44444	20

### **III.2.5. Explanatory variables**

#### **III.2.5.1. Land use practices**

The only land use practice that could be incorporated in this study at this stage was grazing, an activity common to several of the sampled sites (55AZ, 57QS, 61AZ, 63AZ, 68AZ, 70AZ, 71AZ, 75QS, 77AZ, 79AZ, and 80AZ). At this phase of the project, more detailed information on the frequency of this activity and animal density is being collected so it was not possible to use in this study.

#### **III.2.5.2. Habitat structure**

The variables considered in this category are related to the type and percentage of vegetation cover. The photographs taken to assess this variable were analysed considering 2 variables: green cover (mainly herbaceous plants) and litter (mainly fallen leaves). In each sampling point, 4 photographs were taken using a square (1x1 meters), in order to form a quadrant whose centre is the sampling point. Each photograph was then analysed using a grid composed by 25 smaller squares, so that each one of them would represent 1% of the total area's cover, this way allowing an estimation of the percentage of litter and green cover around the area where the soil core was taken.

### **III.2.5.3. Soil parameters**

The soil samples collected for physicochemical analysis were processed and sent for analysis of texture, pH, water holding capacity (WHC), cation-exchange capacity (CEC), and organic matter content, among others. The analysis is still on progress on a contract laboratory; therefore, soil parameters could not be included in this study.

### **III.2.6. Statistical analysis**

#### **III.2.6.1. Assessing differences between abundance and morphotype richness among sites and forest types**

To check if there were significant differences in Collembola abundance and morphotype richness between different forest types, and between sites within each forest type, a nested ANOVA was carried out, after verifying that there was no violation of normality and homocedasticity using the Kolmogorof-Smirnov and Bartlett tests. Samples were nested within sites; abundance data was log transformed prior to analysis. The differences between sites were assessed using a Tukey's test. These analyses were done in STATISTICA 7 (Statsoft Inc., 2004).

### **III.2.6.2. Diversity descriptors**

The biodiversity indices, Margalef, Shannon-Wiener, and Pielou (Magurran, 2004), were calculated using PRIMER 5.2.6 (PRIMER-E Ltd, 2001). For these calculations each morphotype was considered a “species”.

### **III.2.6.3. Comparison of community composition between sites and forest types**

Prior to the main multivariate analysis, a Detrended Correspondence Analysis (DCA) was performed to test if data had a linear or unimodal distribution. Detrending was done by segments and species data was log transformed. Since the length of the gradient on axis 1 was lower than 3, it was considered that data had a linear response, and thus PCA was chosen for the remaining analysis. For a better visualization and interpretation of the graphics, instead of using all the samples, the total number of individuals and morphotypes for each site were used. Sites were classified according with the forest type, presence/absence of grazing, geographical location, and sampling period. Because there was a visible separation of the sites according to the sampling period, another PCA was executed, this time using both geographical location (coordinates) and sampling period as covariables, to exclude their influence on the data. DCA and PCAs were performed in CANOCO 4.5 (Biometris, 1997-2002).



Subsequently, ANOSIM and SIMPER analysis (using the Bray-Curtis coefficient) was performed to learn more about the morphotypes originating differences in Collembola communities between sites. Both analyses were done using PRIMER 5.2.6 (PRIMER-E Ltd, 2001).

#### **III.2.6.4. Relation with explanatory variables**

To test if there were differences between grazed and non-grazed sites in terms of abundance and morphotypes richness, a t-test was performed in STATISTICA 7 (Statsoft Inc., 2004) considering grazing as the main factor.

Regarding habitat structure, the relationship between the percentage of vegetation cover and the abundance of individuals was measured with a correlation in STATISTICA 7, using log transformed abundance and morphotypes number as dependent variables and the total percentage of cover (litter and green cover) as independent variable. This was done considering data from all the sampling points for all sites.

### III.3. Results

#### III.3.1. Total abundance and morphotypes richness

From the 20 sites used in this study, 6388 individuals were collected and 19 morphotypes were identified. There was a clear dominance of the morphotype He2, followed by He4. Some morphotypes had very low representation, namely, Ep1, Ep2, and He1, neither of which had more than 5 individuals.

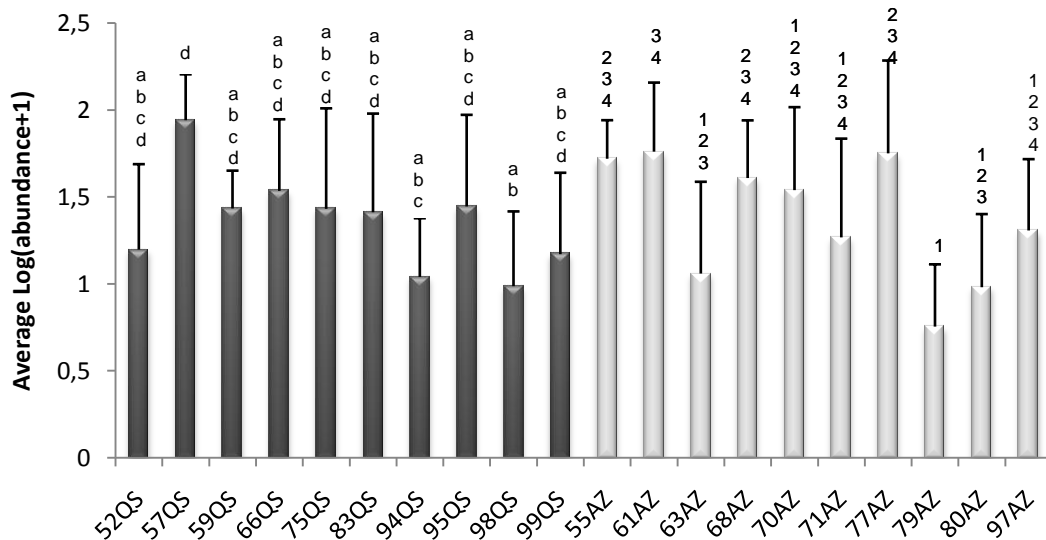
Cork oak forests had a lower number of individuals (2921) than holm oak forests (3467). Information about Collembola abundance and number of morphotypes found in each site is detailed in Table III.2.

**Table III.2: Collembola abundance and number of morphotypes found in each site, for both forest types. Time period when the sites were sampled is marked with asterisks: \*November 2013 to February 2014 and \*\*December 2012 to January 2013.**

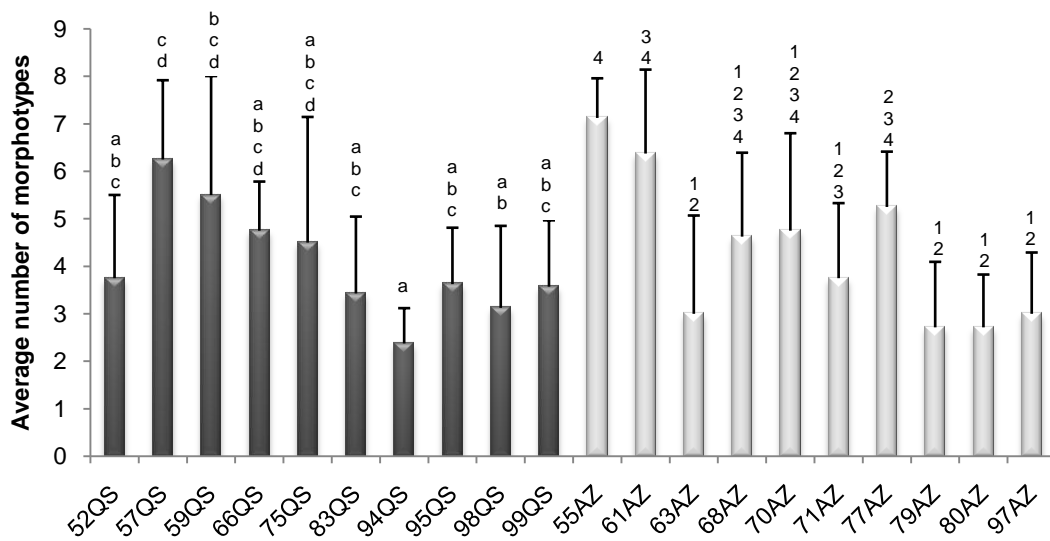
<i>Quercus suber</i>			<i>Quercus rotundifolia</i>		
	Abundance	Morphotypes		Abundance	Morphotypes
<b>52QS*</b>	192	8	<b>55AZ*</b>	463	12
<b>57QS*</b>	802	11	<b>61AZ*</b>	642	14
<b>59QS*</b>	233	11	<b>63AZ*</b>	147	7
<b>66QS*</b>	374	11	<b>68AZ*</b>	385	10
<b>75QS**</b>	228	12	<b>70AZ*</b>	410	12
<b>83QS**</b>	286	8	<b>71AZ*</b>	299	10
<b>94QS**</b>	106	8	<b>77AZ*</b>	796	12
<b>95QS**</b>	407	10	<b>79AZ**</b>	49	6
<b>98QS**</b>	122	8	<b>80AZ**</b>	86	7
<b>99QS**</b>	171	9	<b>97AZ**</b>	190	7

The nested ANOVA did not reveal any significant differences between forest types on the average morphotype richness ( $F= 0.147$ ;  $p=0.36$ ) nor on the average abundance of individuals ( $F= 0.011$ ;  $p=0.83$ ). Nevertheless, there are significant differences between various sites within each forest type, concerning both average abundance ( $F=4.132$ ;  $p<0.001$ , Figure III.3) and average number of morphotypes ( $F=5.708$ ;  $p<0.001$ , Figure III.4).

The average abundance of individuals is higher in sites 57QS, 55AZ, 61AZ, and 77AZ and lower in sites 79AZ, 80AZ, 94QS, and 98QS (Figure III.3). Concerning the number of morphotypes (Figure III.4), even more differences were identified between sites. The average number of morphotypes is higher for sites 57QS, 59QS, 55AZ, 61AZ, and 77AZ. The lowest richness is found in sites 94QS, 98QS, 63AZ, 79Z, 80AZ, and 97AZ.



**Figure III.3: Average (+ standard deviation) log transformed abundance for each site. Dark grey bars represent cork oak forests and light grey bars, holm oak forests. Homogeneous groups within each type of forest are represented with letters and numbers: letters for cork oak forests and numbers for holm oak forests. Sites that do not have at least one letter (or number) in common are significantly different ( $p<0.05$ ).**



**Figure III.4: Average number (+ standard deviation) of morphotypes for each site. Dark grey bars represent cork oak forests and light grey bars, holm oak forests. Homogeneous groups within each type of forest are represented with letters and numbers: letters for cork oak forests and numbers for holm oak forests. Sites that do not have at least one letter (or number) in common are significantly different ( $p < 0.05$ ).**

### III.3.2. Diversity descriptors

Margalef, Shannon-Wiener, and Pielou indices calculated are expressed in Table III.3.

**Table III.3: Values obtained for the calculated indices, Margalef ( $D$ ), Pielou ( $J'$ ), and Shannon-Wiener ( $H'$ ), where  $S$  is the number of morphotypes and  $N$ , the total abundance.**

Sites	S	N	D	J'	H'(log2)
52QS	8	192	1,33	0,78	2,33
57QS	11	802	1,50	0,48	1,66
59QS	11	233	1,83	0,76	2,64
66QS	11	374	1,69	0,74	2,57
75QS	12	228	2,03	0,68	2,43
83QS	8	286	1,24	0,62	1,87
94QS	8	106	1,50	0,54	1,62
95QS	10	407	1,50	0,31	1,02
98QS	8	122	1,46	0,75	2,25
99QS	9	171	1,56	0,63	1,99
55AZ	12	463	1,79	0,67	2,39
61AZ	14	642	2,01	0,60	2,30
63AZ	7	147	1,20	0,67	1,89
68AZ	10	385	1,51	0,62	2,06
70AZ	12	410	1,83	0,61	2,19
71AZ	10	299	1,58	0,49	1,61
77AZ	12	796	1,65	0,54	1,94
79AZ	6	49	1,28	0,85	2,20
80AZ	7	86	1,35	0,66	1,85
97AZ	7	190	1,14	0,64	1,80

There are some discrepancies between the indices. Margalef index considered sites 61AZ and 75QS as having higher richness, being the only sites with a value higher than 2; site 59QS and 70AZ closely follow. The highest value and closest to 1 for Pielou index was found in site 79AZ, even though this

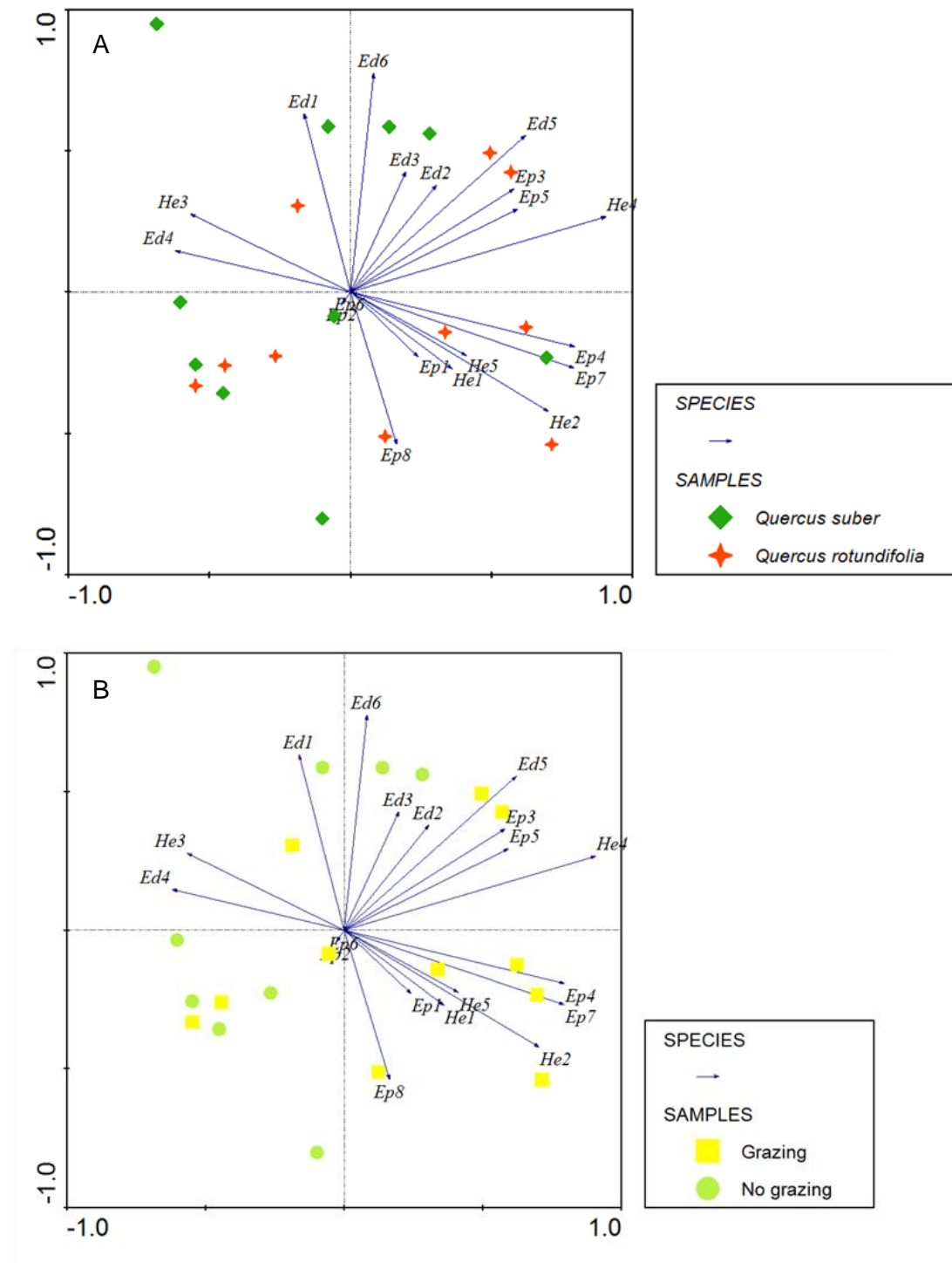
was the site with lowest abundance, an indication that the morphotypes found are similar in abundance. Shannon-Wiener index places sites 59QS and 66QS as having a higher richness. Apparently, differences in diversity concerning different forest types and land use practices are not detectable.

### **III.3.3. Comparison of communities from different sites and forest types**

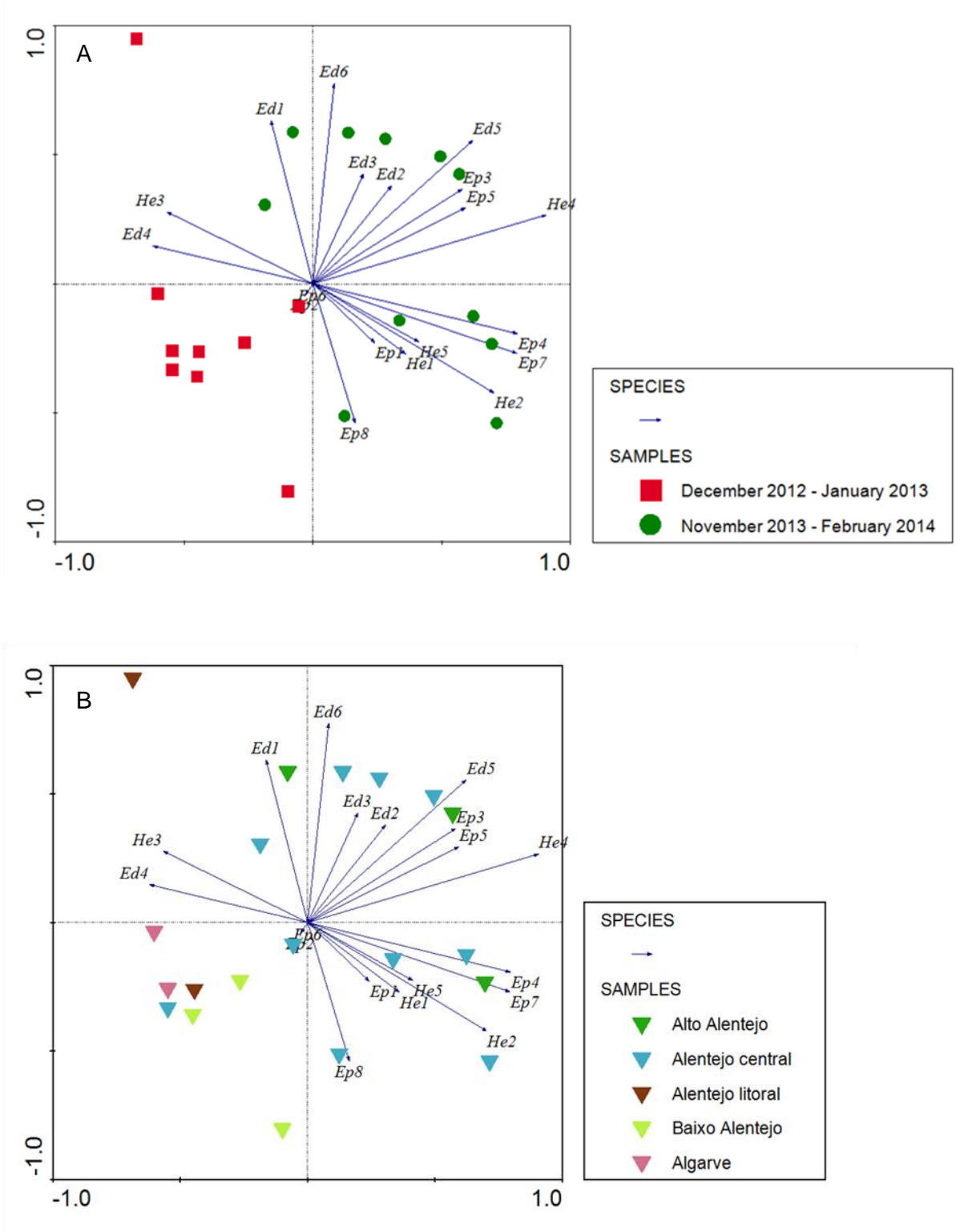
#### **III.3.3.1. Principal Component analysis (PCA)**

The graphics that resulted from the PCA are presented in Figures III.5 and III.6. Sites were classified according with diverse factors: type of forest (Figure III.5A), grazing (Figure III.5B), time period they were sampled (Figure III.6A) and geographical location (Figure III.6B), considering regions of Portugal.

There is no clear separation between sites from different forest types or even with different management concerning grazing. However, there is a clear separation between those sites that were sampled between December 2012 to January 2013 and those that were sampled a year later. Geographical location also appears to exert some kind of influence, since the majority of sites located in Alto Alentejo and central Alentejo regions are placed in the right side of the graphic, while those located more to south and closer to the sea are placed in the left side.



**Figure III.5: PCA results with sites classified according to forest type (A) and absence or presence of grazing (B). PCA was centred by species and data was log transformed. Axis 1 explains 38.3% of total variation, while axis 2 explains 15.7%.**



**Figure III.6: PCA results with sites classified according to sampling period (A) and geographical location (B). PCA was centred by species and data was log transformed. Axis 1 explains 38.3% of total variation, while axis 2 explains 15.7%.**



To rule out the influence of these factors, they were used as covariables in another PCA (Figure III.7).

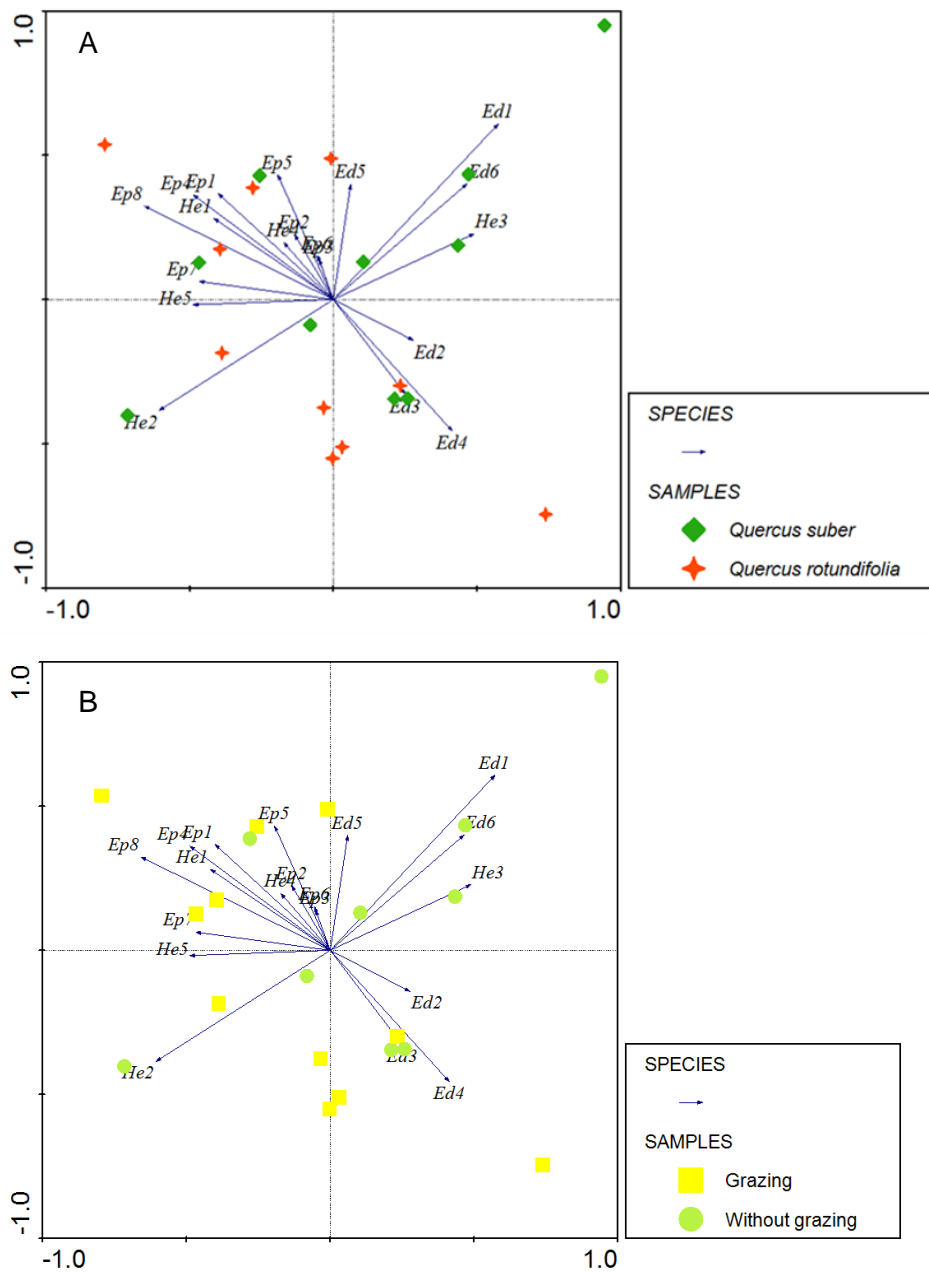


Figure III.7: PCA results with sites classified according to forest type (A) and absence or presence of grazing (B), using sampling period and geographical location (coordinates) as covariables. PCA was centred by species and data was log transformed. Axis 1 explains 28.3% of total variation, while axis 2 explains 17.9%.

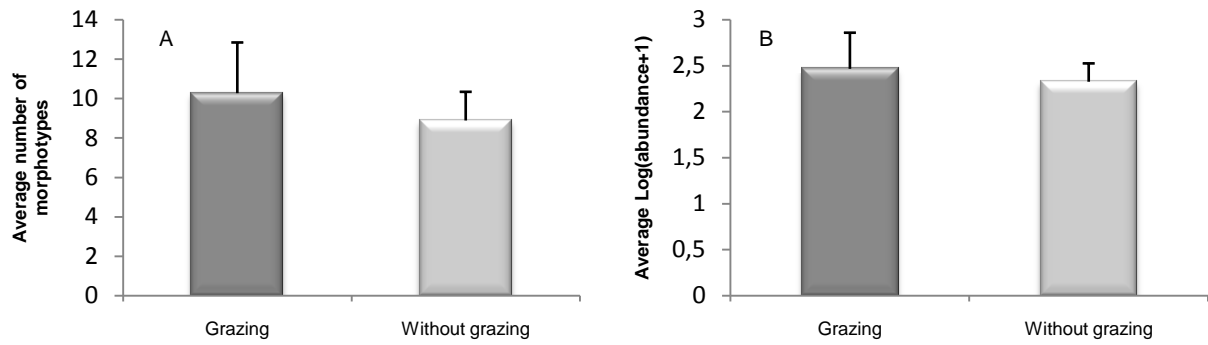
The configuration and distribution of the sites clearly changed with these alterations, although it is still not possible to detect visible differences between forest types or grazed and non-grazed sites.

### **III.3.3.2. ANOSIM and SIMPER**

In accordance with the results from the nested ANOVA and of the PCAs performed, ANOSIM showed no significant differences among forest types, but indicated significant differences between the sites, with a significance level of 0.1%. SIMPER results identified morphotypes He4, He2, and He3 as those contributing the most for the dissimilarity between the sites, with an average contribution of 19.37%, 19.19%, and 18.53%, respectively.

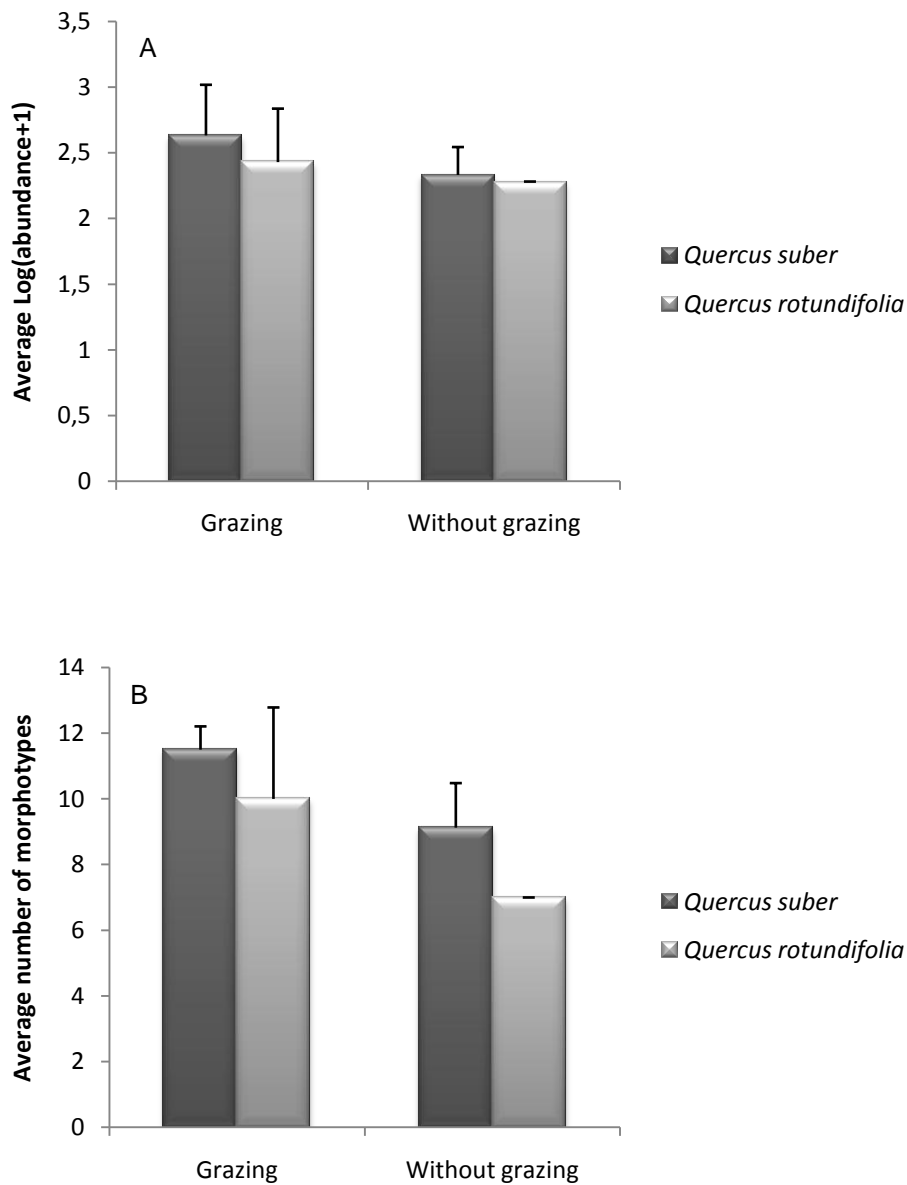
### **III.3.4. Comparison of communities from sites with and without grazing**

Even though grazed sites have a slightly larger number of morphotypes (Figure III.8A) and average abundance of individuals (Figure III.8B), the results from the applied t-test showed no significant differences between sites with and without grazing, neither concerning abundance ( $t=0.97$ ;  $p=0.34$ ) nor morphotypes richness ( $t=1.43$ ;  $p=0.17$ ).



**Figure III.8: Average (+ standard deviation) of the number of morphotypes (A) and log transformed abundance (B), regarding sites with and without grazing.**

The average values for number of morphotypes and abundance, considering a division in both grazed and non grazed sites, and forest types is presented in Figure III.9.

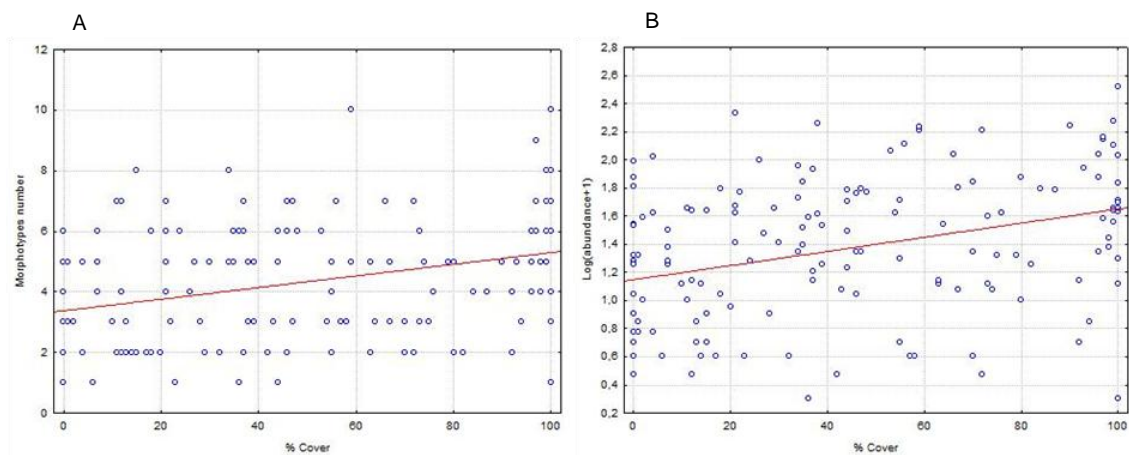


**Figure III.9: Average (+ standard deviation) of log transformed abundance (A) and of number of morphotypes (B), regarding sites with and without grazing, considering both *Quercus suber* and *Quercus rotundifolia* stands. Because there is only one site that is simultaneously non-grazed and a holm oak forest, no standard deviation can be presented in the respective bar.**

Once again, sites with grazing have both a higher abundance of individuals and a higher number of morphotypes. This is valid for both forest types, although most probably not significant (no analysis was done due to the highly unbalanced design).

### III.3.5. Correlation between Collembola richness and abundance and the percentage of vegetation cover

The positive correlation between morphotypes number and percentage of vegetation cover (Figure III.10A), although significant ( $p < 0.001$ ), was weak, with a correlation coefficient lower than 0.5 ( $r \approx 0.34$ ). The same phenomenon was observed for the correlation between abundance and percentage of vegetation cover (Figure III.10B), with a similar correlation coefficient ( $r \approx 0.35$ ;  $p < 0.001$ ).



**Figure III.10: Correlation of morphotype richness (A) and log transformed abundance of individuals (B) with the total percentage of vegetation cover (litter and green cover), considering all the sampling points from all the sites.**

### III.4. Discussion

Collembola diversity and abundance varied across the sites, but the differences found seemingly little to do with forest type or even with the presence or absence of grazing. The morphotypes with the largest contribution for the differences between the sites (the hemi-edaphic types He4 and He2) were also those with the highest abundance.

In spite of the differences found between the sites, community composition from different forest types was not different. This was expected not only due to the intra forest type variation, but also due to the configuration of the different stands. Some of the sites where sampling occurred were not exclusively holm oak or cork oak forests and although an effort was done so that sampling would fall within the corresponding forest type, sometimes the surroundings of the sampled area would have mixed holm oak and cork oak trees or even other tree species.

Grazing, on the other hand, was expected to have a larger influence on the communities present. However, as the results have shown, there are no evident differences between sites where grazing is practiced and those with an absence of this activity. Nevertheless, it was noticeable that grazed sites generally had a larger abundance and number of morphotypes. The increase in abundance can be explained by a more elevated microbial biomass in grazed sites (Bardgett et al., 1997; Dombos, 2001), which in turn will affect the microbial-feeding Collembola; however, it would be expected that the activity would decrease Collembola diversity at the same time (Dombos, 2001), which does not happen. Additional factors that may be influencing these results are the intensity of this

activity and for how long it has been practiced in the sites, since these aspects are proven to have an effect in the microbial communities present (Bardgett et al., 2001). So, a more detailed evaluation of this factor will be possible in the future when this information will be incorporated into the analysis.

Concerning habitat structure, since vegetation is linked with soil animal diversity and it is a source of organic matter inputs (Sylvain and Wall, 2011), it was expected that a stronger correlation would be found between the abundance and diversity of individuals and the percentage of cover (litter and green cover). In addition, the presence of vegetation cover reduces temperature fluctuations in soils (Lavelle and Spain, 2001); this offers some protection to soil organisms and allows them to thrive. However, from the results, it cannot be said that there is an increase in abundance or diversity with higher percentages of cover. Perhaps more variables should be used for the study, instead of just the 2 mentioned above; the percentage of shrub cover may be an interesting variable to add, as some of the sampling points that had no herbaceous or litter cover sometimes had shrubs, whose cover percentage was not included in the analysis.

Another interesting point to discuss is the influence that sampling period exerts on the data. Generally, it appears that sites sampled from November 2013 to February 2014 have a higher abundance of individuals than those sampled from December 2012 to January 2013 (Table III.2). This probably had to do with differences in precipitation and temperature between one year and the other. This evidence prompts a question regarding the selection of sampling periods and how to deal with differences in (more often unpredictable) climate

events in extensive monitoring programmes like this one, where field missions cannot be done in a single period.

The differences between sites can be better explained by adding other land use management practices that were not possible to include in the analysis at this stage. For instance, activities related with agricultural practices, such as tillage and application of pesticides, are known to affect Collembola communities (Loranger et al., 1998). Soil parameters will probably also help to clear out the results, given that collembolans are affected by changes in soil chemistry (Loranger et al., 2001).

Undoubtedly, data obtained from these sites has a lot of potential to be revisited once other explanatory variables (land-use management practices, soil parameters) can be added. Of course, this will take some work in the future, requiring cooperation with the owners and workers from the selected sites. Nevertheless, once having detailed information about the history of the sites, there will be a better understanding of the patterns found.



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## **CHAPTER IV**

## **General Discussion**

#### **IV. 1. Sampling effort: findings and future actions**

Species richness estimates are dependent on sampling effort and the observed richness in a site is related to sampling intensity (Gaston, 1996; Magurran, 2004). Therefore, so that the diversity of a certain area can be assessed, it is essential to know how many samples are necessary to have a good estimation of it. There was a lack of information on sampling effort pertaining to soil mesofauna, hence the importance of tackling this subject.

Sampling effort derivation allowed the estimation of the number of samples (7-10) necessary to assess about 80 to 90% of the species richness in different forest stands. Linking this information with the sampling design used for the study sites in Chapter III, it can be concluded that we have been sampling about 85% of species richness, since 8 samples were collected.

In spite of these results, it cannot be disregarded that habitat heterogeneity clearly had a major influence in the number of samples necessary. In fact, from the sites used for sampling effort derivation, those that had a particularly heterogeneous habitat structure or that were sampled on a broader spatial scale (at landscape scale) would require a larger number of samples to attain the same percentage of richness. Sampling effort is expected to be also positively related with dissimilarity between samples in terms of composition. These two aspects (inter sample heterogeneity and habitat heterogeneity) are related, so the characteristics of the area sampled should always be taken into account in the planning of monitoring schemes, particularly when sampling is to be done at a larger scale (landscape), covering a broader area.

There is still room for more research on sampling effort for soil mesofauna, namely by studying the possibility of applying different models to the data in alternative to the linear dependence model (Shiu and Lee, 2003) used. Also, the inclusion of more sites could allow a more accurate derivation of sampling effort for different forest types.

#### **IV. 2. Surrogates for species richness: potential and models' improvement**

Past studies on surrogacy, more precisely, higher taxa surrogacy, had already concluded that this could be an effective approach for biodiversity assessment in soil arthropods (András, 2003; Biaggini et al., 2007; Cardoso et al., 2004; Forest Focus, 2007).

With this work, it was proven that both genera richness and morphotypes richness are reliable surrogates for Collembola species richness, although in sites with a greater amount of cryptic species or with many species belonging to the same genus, the relation between genus and species may not work out as well, as the model will underestimate the number of species present. In relation to morphotypes, since we are dealing with morphological traits, the problem of underestimation is not constricted to species; however, this approach has the great advantage of including organisms' eco-morphological traits, allowing the inclusion of the functional component of diversity (Vandewalle et al., 2010) and at the same time reducing enormously the amount of work that otherwise would be necessary for a species or even genus level identification.

In conclusion, these results provide evidence that the classification based on morphotypes has a lot of potential to be integrated in extensive monitoring schemes, where they can be used as surrogates for species richness, at least in an initial stage.

On a final note regarding this topic, the improvement of the developed models is an important procedure that can be achieved by adding more data from different forest stands and by their later validation. Initially, it was thought to use data from some of the study sites from chapter III, once the specimens of a few of them had been identified. However, work is now on progress concerning this topic, by exploiting and including data from additional forest stands for their development and validation.

#### **IV. 3. Effects of forest type and land use practices on Collembola communities: further research and future perspectives**

The short information on land use practices was definitely a limitation in this study. The existence of more explanatory variables would aid to better perceive differences in diversity, abundance and community composition of collembolans across the sampled sites.

The practice of grazing was not the main driver for the differences between sites, given that significant differences were found even between sites where this activity is absent and between sites where this activity is present; hence, it is important to know what the additional factors responsible for these results may be.

For this reason, it will be essential to reanalyze data from these sites, once the results of physicochemical analysis on soils are available and more land use variable can be incorporated. Furthermore, it will be important to evaluate if landscape structure has any influence in the biodiversity patterns in these forest areas.

In the long-term, as more data from the other sampling sites covered by the project and information on land use practices are incorporated, the knowledge of soil mesofauna biodiversity patterns found across different types of forest with different levels of human intervention will increase.

In the future, the integration of all this information will allow the establishment of the normal operating range for these forest systems.

#### **IV.4. References**

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