



Quantifying species richness at multiple spatial scales in a Natura 2000 network

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Abstract: Even if the establishment of nature reserves is to date a reality and the increase of protected areas is going to grow year after year, monitoring programs aiming to assess the effectiveness of the established protected areas for biodiversity conservation are still needed. That is the case for the Natura 2000 network in Europe, for which monitoring methods and programs are not yet well-established. A probabilistic sampling procedure is proposed and tested for quantifying and monitoring plant species diversity within a local network of protected areas, namely the Natura 2000 network in the Siena Province, Italy. On the basis of a sampling strategy of one 100 m² plot randomly located in each 1 km × 1 km cell, four Sites of Community Importance (SCIs) were investigated in 2005. The gradients in species composition at the plot scale were largely related to elevation and forest cover. The species richness values of the four SCIs were compared by means of sample-based rarefaction curves. Then, additive partitioning of species richness was applied to determine the most important spatial components in determining the total species richness of the network. Compositional differences among the plots within each SCI were the most responsible of the total species richness. These methodologies can be adopted for assessing plant species richness within a large region or within a reserve network and, if combined with additive partitioning, they can be used as a set of large scale indicators of species diversity.

Abbreviation: NMS – Nonmetric Multidimensional Scaling, SCI – Site of Community Importance.

Introduction

Natural ecosystems are experiencing a rapid loss of biodiversity caused by the human activities. The estimates provided by Reid (1992), suggesting a 13% of biodiversity loss from 1990 to 2015, are likely to be even conservative (Nagendra and Gadgil 1999). Within this frame, the EU 92/43/EEC (Habitats Directive) and 79/409/EEC (Birds Directive) directives established the Natura 2000 network as the major tool of the European strategy for nature conservation, with the Sites of Community Importance (SCIs) being the main components of this network (Bock et al. 2005).

The main topic of the Natura 2000 network is to preserve natural and semi-natural habitats and it is urgent to develop quantitative methods to assess if this goals are achieved or not. Advancements have been done to develop indicators about the spatial structure of habitats (Bock et al. 2005), but a good monitoring approach for assessing the effectiveness of biodiversity conservation should not disregard species diversity. In fact, species richness and complementarity are considered the most straightforward components of biodiversity (Colwell and Coddington 1994) and attempts have been made in testing the effectiveness of existing reserve networks to preserve species diversity (e.g., Rodrigues et al. 2004). In this view, vascular plants certainly represent one of

the most important *taxa* for assessing biodiversity in terrestrial ecosystems, given their functional and structural role.

Standard sampling designs are available for assessing species diversity at local scales, such as within plots (see e.g., Stohlgren 2007), but there are no standards for the collection of quantitative data on plant species richness and composition at larger spatial scales (Palmer 1995, Palmer et al. 2002), also because of the lack of reliable species richness estimators (Chiarucci et al. 2003, Melo 2004). At larger spatial scales, floristic data are often collected subjectively. In this way it is impossible to achieve complete species list or to know the degree of completeness of such lists (Robinson et al. 1994, McCollin et al. 2000). Consequently, these lists of species can be used for descriptive aims but not for quantitative ones such as assessing the effects of management on the flora at larger spatial scales (Palmer et al. 2002, Chiarucci and Bonini 2005). The need to develop affordable methods for assessing species richness at larger spatial scales is thus pressing, in particular for monitoring the effectiveness of conservation policies. The results of inadequate biodiversity assessments can lead to wrong conclusions and even to potentially dangerous management actions (Legg and Nagy 2006).

The results of a probabilistic sampling approach developed for assessing and monitoring plant species diversity within a regional network of SCIs are presented here. In par-

ticular, this paper aims to discuss the following methodological and ecological issues: *i*) describe a simple sampling design for performing repeatable measurements of species richness and composition over multiple spatial scales; *ii*) investigate at which scale the record of species diversity should be maximised, to effectively assess and monitor plant species diversity through time.

Survey sites

Seventeen SCIs have been established in the Siena Province, Tuscany (Italy), ranging in size from 483 ha (Lago di Montepulciano) to 13,744 ha (Montagnola Senese), for a total of 58,969 ha. They spread over a wide gradient of ecological conditions, from low elevation (65 m a.s.l.) to high mountain (1685 m a.s.l.); from open habitats, such as cultivated fields and pastures, to almost unmanaged forests. This network of SCIs is expected to host a high plant species diversity, but uneven floristic data are available.

To test the applicability of the developed sampling design in different ecological situations, a subset composed of four SCIs, with a wide combination of ecological conditions (in terms of topography and plant communities), was chosen for the first test phase of the project. The main features of these four SCIs are reported in Table 1.

Methods

Sampling design

The sampling design was based on the sample points used for the Italian National Inventory of Forests and Carbon Sinks (INFC, Fattorini and Tabacchi 2004). These points were chosen by a restricted random selection: the whole national territory was divided into a grid with cells of 1 km × 1 km and one random point was selected within each cell. All the points falling within the four SCIs previously described were selected as sampling points. By this approach, a sample with a nominal density of 1 point per km² was obtained and can be used for spatial inference (Table 1).

In each point, once located with a high-precision GPS (Trimble GeoXT), a 10 m × 10 m plot was installed and divided into 16 smaller (2.5 m × 2.5 m) subplots. The plot was centred on the sampling point. The presence of each plant species was recorded in each plot/subplot, while the percentage cover of each species was visually estimated for the whole plot area. The time needed to reach the point, install the plot and record plant species presence was also recorded. Only presence/absence data were used in the present paper.

Formally, this sampling method is based on a hierarchical nested sampling design organised on four hierarchical levels: micro-scale level (α_{Subplot}), stand level (α_{Plot}), site level (α_{SCI}) and regional level (α_{Network} or γ). This hierarchical structure was thought to allow the additive partitioning of species diversity into different spatial components, from the local to the regional scale (Allan 1975, Lande 1996, Veech et al. 2002, Crist et al. 2003).

Data analysis

Although species richness was analysed at multiple spatial scale, the plot scale was the closest to the scale often adopted for plant community description and was considered central for analyses. Plot scale species richness was compared among SCIs by using a non-parametric Kruskal-Wallis one-way ANOVA, with Bonferroni correction for multiple comparisons.

Compositional gradients at the plot scale were investigated by non-metric multidimensional scaling (NMS). As other ordination techniques, NMS reduces the multi-dimensional gradients in species composition to a simpler set of derived variables to be used as ordination axes. A peculiarity of NMS is that it differentiates among sampling units by ranking them according to their pair-wise dissimilarity; thus it is suitable for data that deviate from normality or contain non metric measure units (Clarke 1993). NMS ordination was performed by using SYN-TAX 5.0 (Podani 1994). The Sørensen similarity index was used as a measure of resemblance among plots. Multiple runs of the NMS ordination (100 total runs) were applied to the real data to avoid local stress minima, a problem that prevents the NMS algorithm from converging on the lowest possible stress solution. 999 Monte Carlo permutations were used to evaluate the significance of the ordination axes.

To compare species richness of the four SCIs, plot-based rarefaction curves (Sanders 1968, Gotelli and Colwell 2001) were used. These can be calculated as mean value of a number of accumulation curves produced by random sequences of plots (Gotelli and Colwell 2001), or by using the analytical formula (Kobyashi 1974, Chiarucci et al. 2008) as it was done in the present paper. Some authors referred to species richness as the number of species obtained with a sample of individuals and to species density as the number of species obtained with a sample of space units, such as plots (Gotelli and Colwell 2001). Since a general agreement is missing, the term species richness was used here. Plot based rarefaction curves were calculated separately for each SCI and for the pooled sample composed by the 48 plots from the four SCIs.

The additive partitioning of α , β and γ diversity (Lande 1996, Gering et al. 2003, Crist and Veech 2006) was adopted to quantify the contribution of each spatial scale (subplot, plot, SCI) to the species richness of the network. In the present paper, the inventory diversity represents the classic α -diversity, i.e., the number of species recorded in a given unit (subplot, plot, SCI or the whole network), while differentiation diversity represents the classic β -diversity; this latter was calculated as the number of species of the given level that are, on average, absent from each units of the lower level (Crist and Veech 2006). β_{Plot} , β_{SCI} and β_{Network} were calculated in this way to represent the β -diversity within each plot, within each SCI and within the network (for example, β_{Plot} indicated the number of species of the plot that were, on average, absent from each subplot of the same plot).

Table 1. Descriptive data for the four investigated SCIs.

SCI	Area (km ² in Siena Province)	Altitudinal Range (min-max)	Number of plots	Number of species per subplot (mean and range)	Number of species per plot (mean and range)	Pooled Number of species
Cono Vulcanico del Monte Amiata	17.68	782 - 1685 m	16	4.8 (1 - 26)	13.5 (4 - 40)	91
Lucciolabella	14.17	315 - 668 m	13	10.0 (0 - 28)	25.8 (0/10 - 58)	153
Foreste del Siele e Pigelleto di Piancastagnaio	11.72	494 - 968 m	11	15.2 (6 - 33)	39.3 (16 - 59)	175
Ripa d'Orcia	8.31	205 - 522 m	8	11.6 (1 - 37)	30.0 (1 - 81)	132
Set of four SCIs	51.88	205 - 1685 m	48	9.7 (0 - 37)	25.4 (0 - 81)	362

The software PARTITION (Veech and Crist 2007b) was used to test for departure from random expectations of the species richness values obtained at each spatial scale. Since these data were not individual based, a sample-based randomization was used to test the significance of observed values with respect to the random expectation (Crist et al. 2003). Within each SCI, randomization was performed to test the significance of the α_{subplot} and β_{plot} values. The same procedure was applied at the network scale for testing the significance of the α_{plot} and β_{SCI} values and of α_{SCI} and β_{Network} values; these were performed separately, because of the non additive properties of random expectations when based on sample data (Crist et al. 2003). For each level, a null statistical distribution of expected values was created by using 10,000 iterations of the randomization routine. The statistical significance of each diversity component was assessed as the proportion of null values greater than (or less than) the observed diversity value. This proportion was given as a *P*-value indicating the probability of obtaining a diversity value greater (or smaller) than that observed one by chance.

Results

General features of communities

A pooled species list of 362 species was obtained by the 48 sampled plots. Species richness per plot (100 m²) ranged from 0 (in a pond, without aquatic vegetation, at Lucciolabella) to 81 (in a sparse evergreen shrubland colonising a xeric grassland, at Ripa d'Orcia). The variability of species richness at the plot-scale among the four SCIs was high (Table 1). The Kruskal-Wallis statistic equalled 17.03 ($p < 0.001$), indicating that significant differences in plot scale species richness existed among the four SCIs. When tested for all the contrasts, only the SCIs Amiata and Pigelleto resulted significantly different in plot scale species richness (with mean species richness of 13.5 and 39.3, respectively). Differences in the species richness mean values were also found at the subplot and the SCI scale (Table 1).

Gradients in community composition

Preliminary runs of the NMS algorithm applied to 47 plots (one plot had no species) indicated that a two-dimensional ordination was optimal. The first axis (mean stress 52.42, $p < 0.001$) well differentiated between Lucciolabella, the unique non-forest SCI, with respect to the other SCIs, that were all prevalently covered by forests (Figure 1). The second axis (mean stress 26.54, $p < 0.001$) well separated Ripa d'Orcia and Pigelleto, i.e., those SCIs located on relatively low elevation (they showed a similar score along the first axis of Lucciolabella, that also was a low-elevation SCI) from Amiata, a SCI located on higher mountain slopes (Table 1). Therefore, the NMS gradient analysis revealed compositional differences at the plot scale among the four SCIs which were largely related to the forest cover and the elevation gradient.

Species rarefaction curves

The plot-based rarefaction curves showed different patterns of species richness recorded in the four sampled SCIs as a function of the number of sampled plots (Figure 2): the curve of Pigelleto was the highest, those of Ripa d'Orcia and Lucciolabella were intermediate, while that of Amiata was the lowest. The rarefaction curve obtained by the pooled sample of 48 plots was high, but in its first part lower than that of Pigelleto, indicating the high species richness and compositional heterogeneity for this latter SCI. No asymptotic trend was found in any curve of the SCIs (except maybe for Amiata), and in the pooled sample, suggesting that the species richness of each SCI and the whole network is expected to be much higher.

Partitioning of species diversity

Within each SCI, subplot species richness (α_{subplot}) contributed to only 5.3-8.8% of the total species richness, while plot species richness (α_{plot}) contributed to 14.8-22.7% of the total species richness (Figure 3). Thus, a very high proportion of species diversity (77.3-85.2%) was due to the composi-

Figure 1. Nonmetric multidimensional scaling (NMS) ordination of 47 plots sampled in the four SCIs (one plot with zero species was excluded from the analysis). Abbreviations: AM: Cono Vulcanico del Monte Amiata; PIG: Foreste del Siete e Pigelleto di Piancastagnaio; LUC: Lucciolabella; RIPA: Ripa d'Orcia.

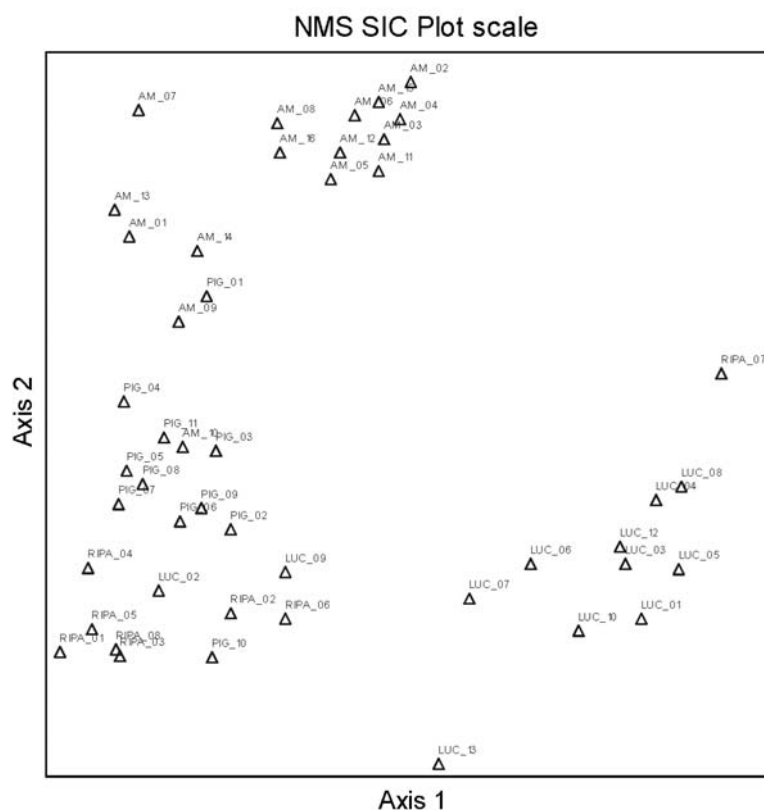
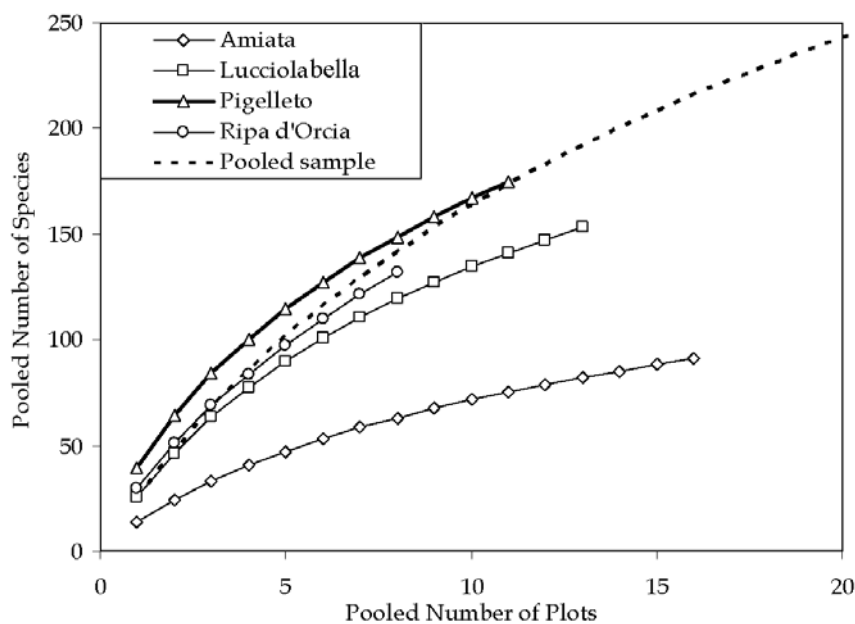


Figure 2. Plot-based rarefaction curves for the four sampled SCIs. The first part (up to 20 plots) of the plot-based rarefaction curve for the pooled sample of 48 plots from the four SCIs is also shown.



tional differences among the plots within the same SCI (β_{SCI}). The randomization indicated that the four values of α_{Plot} were significantly lower than expected by the randomization tests ($p < 0.001$), while the four β_{SCI} values were significantly higher. This indicated that important differences in species composition existed across the plots of the same SCI. Therefore, the local differences among habitats were the main determinants of the species richness at the SCI scale.

In the whole network, the sample based gamma diversity of 362 species was obtained by an average α -diversity at the plot scale of 25.5 species (given by mean values of $\alpha_{Subplot}$ and β_{Plot} of 9.7 and 15.9, respectively), summed to a mean beta-diversity within the SCIs (β_{SCI}) of 112.3 species (to give an average α_{SCI} of 137.8 species) and a mean beta-diversity across the SCIs ($\beta_{Network}$) of 224.3 species (Figure 4). Therefore, only a small proportion of the total species richness was

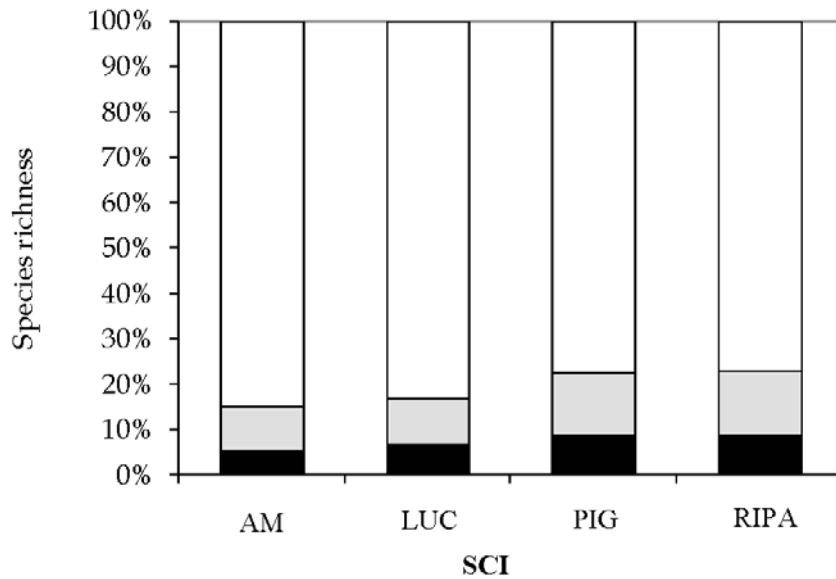


Figure 3. Additive partitioning of the species diversity within the four SCIs. Black: contribution to the total SCI species richness given by species richness at the subplot scale (α_{Subplot}); gray: contribution to the total SCI species richness given by species richness at the plot scale (α_{Plot}); white: contribution to the total SCI species richness given by compositional differences among plots (β_{SCI}). Abbreviations for the SCIs: AM: Cono Vulcanico del Monte Amiata; PIG: Foreste del Siele e Pigelletto di Piancastagnaio; LUC: Lucciolabella; RIPA: Ripa d’Orcia.

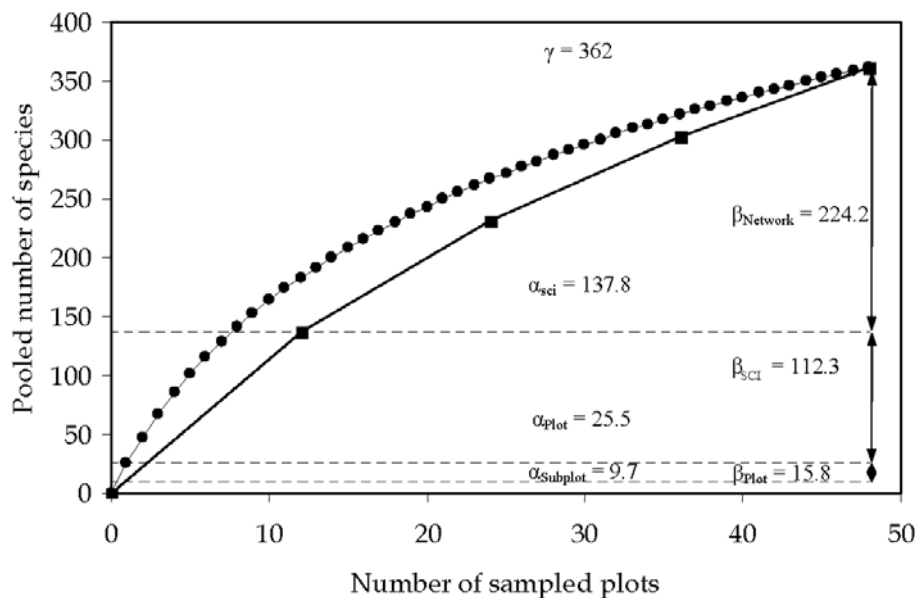


Figure 4. Plot-based and SCI-based rarefaction curves of species richness in the four sampled SCIs. An indication (in terms of number of species) of the different components of the species richness partitioning is also reported on the graph.

due to the subplot (α_{Subplot}) and plot (α_{Plot}) scales: 2.68% and 7.04%, respectively. A much higher proportion of the total species richness was due to the compositional variation across the plots within each SCI (β_{SCI}) and, especially, to the variation among SCIs (β_{Network}): 31.02% and 61.94%, respectively. The randomization tests, performed separately for the two scales, indicated that: *i*) at the plot scale, α_{Plot} was significantly lower ($P = 1.000$) than expected by chance, while β_{SCI} was significantly higher ($P < 0.001$); *ii*) at the SCI level, α_{SCI} was significantly higher than expected ($P < 0.000$), while β_{Network} was not significantly different from random expectations ($P = 0.598$). These results suggest that, also at the network scale, the differences in species composition at the plot scale within each SCI were the main determinant of the total species diversity of the network.

Discussion

In the present study, rarefaction curves provided a global picture of the whole diversity of the four SCIs within a local Natura 2000 network, evidencing different patterns in species accumulation as a function of the number of sampled plots. The rarefaction curve calculated for each SCI or the whole network did not show any asymptotic pattern, suggesting that the pooled species richness of this set of SCIs is expected to be higher than recorded. This would mean the need of a higher sampling density and consequently higher inventory costs. This problem is particularly relevant given the lack of reliable estimators for estimating species richness (D’Alessandro and Fattorini 2002, Chiarucci et al. 2003, Melo 2004). Only in the case of the Amiata SCI, covered al-

most exclusively by *Fagus sylvatica* L. forests, the observed quasi-asymptotic pattern suggests that the adopted sampling intensity was almost adequate to get a picture of the flora (Gotelli and Colwell 2001, Fattorini 2007). In this part of Italy, *Fagus sylvatica* L. forests are known to be characterised by floristic homogeneity and very low species diversity (Chiarucci et al. 2001).

As a consequence of this lack of getting a complete picture of the flora, some concerns may arise about the density of sampling points adopted in this project (one 10 m × 10 m plot within each cell of 1 km × 1 km). However, this sampling density would correspond to 592 and 2824 sampling points for the Natura 2000 network within the Province of Siena and Tuscany Region, respectively, and these numbers are clearly rather high, especially if costs need to be considered. As a comparison, in the Biodiversity Monitoring Program of Switzerland (see Plattner et al. 2004, for details), the sampling points are located with a density of one point in each 5 km × 5 km cell (25 km²). Even if it is not possible to make simulations about the completeness of the data collected in this project, the density of sampling points used in this test is certainly very high for the purposes of installing a permanent monitoring program.

When a network of protected areas covers a large surface, it is impossible to get complete lists of plant species therein and the probability of missing some species increases with size of the investigated area (Palmer 1995, Palmer et al. 2002). Noteworthy the use of statistical tests on data for which the degree of completeness is unknown is very limited. In this view, using statistical samples of the species occurring within a site or the whole network should allow to overwhelm the problem (Chiarucci 2007), particularly when coupled with rarefaction approaches (Sanders 1968, Gotelli and Colwell 2001, Crist and Veech 2006). A similar approach was used to compare beetle species richness in two ecoregions of USA (Gering et al. 2003), plant species diversity across seven biogeographical regions of Switzerland (Koellner et al. 2004) or plant species richness across different forest estates in central Italy (Chiarucci and Bonini 2005).

There is a growing consensus of quantitatively comparing the species diversity of different sites by using sample based rarefaction techniques and this study shows that this method could be used also within a network of protected areas. The number of species found in sample-based rarefaction curves is driven by different factors, such as extent, grain and number of sampling units (Wiens 1989, Palmer and White 1994, Condit et al. 1996, Nekola and White 1999, Dungan et al. 2002). The extent, i.e., the size of the area where sampling units are located, is fundamental in controlling species richness: a larger extent means, on average, larger ecological gradients and thus higher species richness (Palmer and White 1994, Dungan et al. 2002, Chiarucci and Bonini 2005). In this study, the extent was defined by the area covered by the four SCIs and their spatial arrangement. However, it must be noticed that spatial extent influences sample-based rarefaction curves at all spatial scales and consequently

the comparison of sites that largely differ in size should be done with caution (Condit et al. 1996, Hui 2008). On the other hand, the number of sampling units and their size (grain) are important issues to be planned, since they differently affect the species richness observed in the sample. As a general rule, a larger number of smaller sampling units will result in a higher number of species than a smaller number of larger sampling units (Dungan et al. 2002). Different combinations of grain and number of sampling units can be adopted, in any case, keeping in mind that detection errors should be minimized or at least controlled (Yoccoz et al. 2001, Ferretti and Chiarucci 2003) and the use of small grained plots can provide an effort in this direction (Palmer 1995, Palmer et al. 2002, Chiarucci 2007). In this program, a grain of 100 m² was decided *a priori* for the sampling units. Given the problem of listing the number of plant species in larger grain sizes (Palmer 1995, Palmer et al. 2002), such or similar grain sizes should be adopted to reduce the probability of missing species during the field records.

More powerful sampling design can be thought to get statistically correct estimates of species richness at one given scale (see e.g., Baffetta et al. 2007), but if one is interested in getting a more complete picture of the relations between species richness and spatial scales a sampling design as that here adopted is certainly more useful. In fact, the combination of sample-based rarefaction curves with additive partitioning of species richness as proposed by Crist and Veech (2006) allowed to partition the total species richness of the network across different spatial scales. This approach evidenced the importance of the compositional differences across the plots. These were sampled at a scale (10 m × 10 m) comparable to those used for the classic level adopted for the description of vegetation types (see e.g., Chytrý and Otýpková 2003). Most of the total diversity was due to the β -diversity component, i.e., to the differences in species composition among the different plots within each SCI. As known for other biological communities (Veech and Crist 2007a), the landscape level heterogeneity was responsible for most of the species richness in this network of protected areas. Thus, the differences in habitat structure and management practices were extremely important in determining the total species richness contained in this Natura 2000 network and conservation practices should guarantee the maintenance of such heterogeneity.

Given the importance of compositional differences among plots in determining the total species richness, an optimal sample design to quantify and monitor plant species diversity within a network of protected areas should maximise the number of sampled plots and, eventually, limit their grain. This is logical considering that many researches evidenced that compositional heterogeneity can be elevated at each spatial scale (e.g., Nekola and White 1999, Wilson and Chiarucci 2000, Gering et al. 2003, Veech and Crist 2007a) and this needs to be quantified if one wishes to assess the effects of conservation management on species diversity. With the time and cost limitations typical of any monitoring program, several, relatively small-scale plots should provide

the best view of how the species diversity is partitioned among the different spatial components within a network of protected areas and how the management actions affect these components through time. Once the overall diversity was partitioned among spatial scales, monitoring efforts can be focused on more specific objectives, such as, for example, the re-sampling through time of a representative subset of sampling units for monitoring purposes. If costs do not represent a limiting factor, the re-sampling of the whole set of plots could allow the detection of changes in the contribution of the different spatial scales to the total diversity of the system.

Conclusion

Natura 2000 network represents the most important set of areas devoted to nature conservation at the European scale. Starting from this evidence, it is the time for a step forward in the direction of assessing its effectiveness in space and time. Quantifying and monitoring the amount of species diversity present within the network is probably the right way to achieve this aim. The use of a spatially restricted random sample for assessing and monitoring plant species diversity could provide different quantitative results, based on robust but straightforward statistics, such as descriptive summaries, ordination patterns and rarefaction curves. These are nowadays standard descriptive tools and could be profitably used for evaluating the temporal shifts in species diversity. Moreover, other techniques, such as the additive partitioning of species diversity, could be applied to similarly collected data to quantify the contribution of the different spatial scales to the species diversity of the whole system, and these tools could be used for both assessment and monitoring purposes. Of course, when the analysis is performed on the basis of a sample based approach, the results can be compared only using the same sampling design. Therefore, it is extremely important that sampling design issues are properly considered and possibly standardized.

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