Shape matters in sampling plant diversity: evidence from the field

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Abstract

The identification of shape and size of sampling units that maximises the number of plant species recorded in multiscale sampling designs has major implications in conservation planning and monitoring actions. In this paper we tested the effect of three sampling shapes (rectangles, squared, and randomly shaped sampling units) on the number of recorded species. We used a large dataset derived from the network of protected areas in the Siena Province, Italy. This dataset is composed of plant species occurrence data recorded from 604 plots (10 m x 10 m), each divided in a grid of 16 contiguous subplot units (2.5 m x 2.5 m). Moreover, we evaluated the effect of plot orientation along the main environmental gradient, to examine how the selection of plot orientation (when elongated plots are used) influences the number of species collected. In total, 1041 plant species were recorded from the study plots. A significantly higher species richness was recorded by the random arrangement of 4 subplots within each plot in comparison to the 'rectangle' and 'square' shapes. Although the rectangular shape captured a significant larger number of species than squared ones, plot orientation along the main environmental gradient did not show a systematic effect on the number of recorded species. We concluded that the choice of whether or not using elongated (rectangular) *versus* squared plots should dependent upon the objectives of the specific survey with squared plots being more suitable for assessing species composition of more homogeneous vegetation units and rectangular plots being more suited for recording more species in the pooled sample of a large area.

Keywords: biodiversity monitoring, environmental gradient, rarefaction, sampling design, shape, species richness

Acronyms: AC – Accumulation Curve, RC – Rarefaction Curve

1. Introduction

Species richness and complementarity are among the most straightforward indicators of biodiversity (Colwell et al. 2004, Bacaro et al. 2013). Therefore, many efforts have been made to develop tools for maximizing species inventory for biodiversity assessment and long-term monitoring programmes (Rocchini et al. 2005, Bacaro et al., 2009, Chiarucci et al. 2011). Planning long-term monitoring surveys requires a clear identification of the statistical population of concern as well as an operational definition of plant community attribute (e.g., species richness and/or composition, see Chiarucci, 2007). The number of species recorded in a survey is strongly dependent upon: 1) the method of selection of the sampling unit, 2) the sampling effort, 3) the spatial arrangement of the sampling units, and 4) the frequency, precision and accuracy of the measurements (Stohlgren, 2007). Ultimately, the effectiveness of a sampling design depends on the objectives of a study (Kenkel et al., 1989; Yoccoz et al., 2001). An ideal sampling strategy for plants should also provide information on each component of biodiversity, including local diversity (alpha), total/regional diversity (gamma) and species compositional turnover (beta-diversity; Whittaker, 1972; Yoccoz et al., 2001; Baffetta et al., 2007). Since the financial and temporal resources that can be allocated to the evaluation of changes in biodiversity and ecosystem functioning are limited (Gaston and Williams, 1996), long-term monitoring surveys should aim at maximising the probability of detecting the largest number of species in a limited amount of sampling effort to be done in the field (Abella and Covington, 2004).

Sampling methods that provide for the use of multi-scale sampling units have often been recommended to increase the number of species detected in a survey. These methods differ in the size of the sampling unit and in the design of vegetation surveys (e.g., Shmida, 1984; Stohlgren et al., 1995; Peet et al., 1998; Chiarucci et al., 2001, 2008a; Palmer et al., 2002; Keeley and Fotheringham, 2005; Baffetta et al., 2007; Stohlgren, 2007; Marcantonio et al., 2010), and, so far, comparisons among different sampling designs have been made mostly at the scale of 0.1 ha (e.g., Peet, 1974; Whittaker, 1977; Whittaker et al., 1979, 2001; Keeley and Fotheringham, 2005).

The shape of the sampling unit may play a central role in determining the number of species recorded in multi-scale species inventories (Clapham, 1932; Stohlgren et al., 1995; Condit et al., 1996; Kunin, 1997; Laurance et al., 1998; Keeley and Fotheringham, 2005; Nascimbene et al., 2010). In particular, there has been an increasing interest in assessing the capacity of elongated (rectangular) sampling units to collect more species compared to squared sampling units of the same area (see Keeley and Fotheringham, 2005).

A number of studies have shown that elongated sampling units tend to capture more species compared to squared ones due to a tendency of non-square units to encompass higher environmental heterogeneity (e.g., Stohlgren et al., 1995; Stohlgren, 2007; Dengler, 2008). However, Keeley and Fotheringham (2005) in a study of different plant community types in the Sierra Nevada Mountain Range, California, found that, at the 0.1 ha or finer scales, the effect of plot shape on the overall number of species recorded in a plot was minimal or not predictable. In particular, these authors showed that, within the investigated Mediterranean vegetation types, the shape of the sampling units (1:4 rectangles *versus* squares) did not have any substantial effect on the recorded species richness; the observed pattern was likely due to the fact that beta diversity tends to vary along complex environmental gradients that are both parallel and perpendicular to the long axis of rectangular plots.

Despite the importance of the potential implications on the shape of the sampling unit on the overall number of species recorded in large-scale surveys, and, ultimately, in conservation and management planning (Keeley and Fotheringham, 2005), few studies, mostly over small spatial scales (Whittaker, 1977; Keeley and Fotheringham, 2005; Stolghren, 2007), have assessed the effect of plot shape on the number of plant species recorded in such studies, and consistent findings are yet to be found (Dengler, 2008).

The general aim of this study was to address the above-mentioned issues by evaluating the effect of the shape of sampling units using a large dataset derived from a plant diversity survey based on a probabilistic sampling design. Specifically, we 1) evaluated the effect of the shape of the sampling unit on sampling effectiveness, as defined by the cumulative number of recorded species and 2) tested the hypothesis put forward by Keeley and Fotheringham (2005), to examine whether the orientation of elongated sampling units according to the main environmental gradient (given by slope and aspect at local scale) significantly affects the cumulative number of recorded species.

2. Material and methods

2.1 Study area

The Natura 2000 Network (Habitat Directive 92/43/EEC) in the Siena Province (Tuscany, Central Italy, centroid: longitude 11° 26′ 54″ E, latitude 43° 10′ 12″ N, datum WGS84) consists of 17 Sites of Community Importance (SCIs), with size varying between 483 ha (Lago di Montepulciano) and

13,744 ha (Montagnola Senese), making a total surface of 58,969 ha, and ranging from low elevations (65 m a.s.l.) to mountains (1,685 m a.s.l.).

The Siena Province also encompasses a set of 14 nature reserves (established in 1996 and 2008 by the Province Administration of Siena) covering approximately 9,661 ha (see Marcantonio et al., 2010). Most of the area of these reserves is included in the SCI network, and only a small portion is to be considered as a newly protected area. Thus, the area included in the nature reserves and which is not part of the SCI network was also included in the sampling protocol.

The total protected area sampled in this study includes 21 sites and covers a surface of 61945 ha. These sites spans over a range of habitats including open areas and unmanaged forests, from the plains to the mountain belts. The complexity of this network is reflected in the presence of a variety of vegetation types, from thermophilous communities dominated by *Quercus ilex* L. and *Q. cerris* L., to mesic ones dominated by *Fagus sylvatica* L. and *Castanea sativa* Mill.. The network also encompasses croplands, semi-natural grasslands, and shrublands. Due to the complexity of such a mosaic of habitat, plant community, and land use types, this network supports a high richness of plant species (for a more thorough description see Chiarucci et al., 2008b, 2012).

2.2 Sampling Design

To evaluate whether the shape of the sampling unit in a vegetation survey affects the number of recorded species and whether main environmental gradients (such as those reflected by slope and aspect) interact with the shape and orientation of the sampling unit, we used a dataset based on the results of a large-scale monitoring program for the protected areas above described (Chiarucci et al., 2012). The adopted sampling design was based on a restricted random selection of plots. In particular, the whole area of the Siena Province was covered by a grid of 1 km × 1 km cells and one random point was selected within each cell (Fig. 1). All those points falling within the perimeter of nature reserves or SCI of the Siena Province were included in the sample (see Chiarucci et al., 2008b). These points, with a nominal density of one point per km^2, were used to locate sampling plots. Each plot, 10 m x 10 m in size, was located using a high precision GPS and was then divided into four quadrants and sixteen 2.5 m x 2.5 m subplots (four per quadrant). In total, we recorded plant occurrence data within 604 plots and 9,664 subplots (see Table 1). Field sampling was performed during years 2005 - 2008.

Within each plot, data were collected using a standardized technique, consisting in sampling the first subplot within the North-West quadrant, following a clockwise order for the first four units (subplots 1, 2, 3 and 4), and then moving to the second North-East quadrant (subplots 5, 6, 7 and 8), and so on until the 16 subplots were sampled. In this way, it was possible to assign each quadrant and each subplot to a specific orientation (North-South *versus* West-East) along an elevation gradient. The list of all vascular plant species occurring in each of the 16 subplots was compiled, while data about slope, aspect and vegetation structure were recorded at the plot scale (see Chiarucci et al., 2008b, 2012, for further details on the field data collection).

2.3 Data Analyses

Data analyses were designed to evaluate the effect of the shape of the sampling unit, here defined as the spatial arrangement of a combined set of subplots, on the total number of plant species recorded at a site. To achieve this task, the complete data set of 9,664 subplots was re-sampled using three designs: specifically, the re-sampling procedure was designed to achieve species richness values recorded at each plot by different subplot combinations, of the same size (25 m^2, 25% of the total plot size) but with different shapes: 1) 1:4 rectangles = 1 × 4 subplots; 2) squares = 2 × 2 subplots; and 3) random = four randomly located subplots within each plot (Fig. 2). Following this approach, the same total plot area was sampled by four subplots, but the different shape allowed comparing directly the capacity of each design to capture plant species richness.

More in detail, within each plot it was possible to enumerate and identify eight separate combinations of rectangles and nine different combination of squares (each composed by four subplots) as well as factorial of 16 random combinations of four subplots (Fig. 2). In this latter case, the random sampling of subplots was performed without replacement; furthermore, no spatial constrains was imposed in the selection of the four subplots while, on the other side, contiguity was not avoided (as showed in Fig. 2, third line).

To compare cumulative species richness recorded by using the different shapes, we operated as follows: for each shape (eight possible rectangles and nine squares in each plot) a rarefaction curve (hereafter RC) was calculated as the average of 1000 accumulation curves (hereafter AC) obtained by randomly ordering the set of 604 plots and selecting from each plot that specific shape: this process resulted in eight RCs for rectangles and nine RCs for quadrats. One “average” RC for rectangles and one “average” RC for squares were then calculated as the mean of all the RCs for that specific shape.

These two RCs were then compared to the RC calculated based on the species recorded in a subset of 4 subplots randomly selected within the plot: using the same procedure as described above, an “average” RC was calculated by averaging 1000 ACs obtained by randomly selecting four subplots within each plot. For a mathematical review of the rarefaction methods, see Kobayashi, 1974; Olszewski, 2004; Colwell et al., 2004; Ulrich and Buszko, 2007; Chiarucci et al., 2008d; Oksanen et al., 2010.

A one-way analysis of variance (ANOVA) was performed to test effects of shapes on the total number of species calculated for all the 604 plots for each sampling design (factor shape, fixed, three levels: rectangles, squares, and random; see Gotelli and Ellison, 2004; Keeley and Fotheringham, 2005). A posteriori Tukey's honest significant difference tests were applied when analysis of variance resulted significant.

The hypothesised effect of orientation of elongated (rectangular) sampling units placed according to environmental gradients on the overall number of recorded species was then assessed as follows: from the whole data set (604 plots) we extracted two data sets with a certain slope and aspect value. First we selected all the plots with a slope > 5° from which we extracted two data sets on the basis of the main aspect characterizing each plot: the first set included 93 plots with a north or south aspect (in detail, we included those plots with aspect 0° ± 22.5 and 180° ± 22.5); ii) in the same way, the second set included 98 plots with west or east aspect (90°± 22.5 and 270° ± 22.5). For the first set of plots, we calculated rarefaction curves and mean species richness for the four rectangles parallel to the main environmental gradient (corresponding to the very local elevation gradient) and we compared these values with those obtained by considering the four rectangles perpendicular to the same gradient. The same procedure was repeated for the set of west-east oriented plots, where, of course, perpendicular and parallel rectangles were the opposite with respect to the north-south oriented plots. Differences among rarefaction curves were calculated as the absolute difference in the total number of species for each orientation (North-South and East-West and parallel vs perpendicular to the selected gradient). Moreover, statistical differences in the number of collected species among parallel and perpendicular rectangles (for North-South and East-West datasets separately) were assessed by one-way ANOVA .

3. Results

In total, 1,041 species were recorded in the whole set of plots. Mean species richness per plot was 31.5, while 12.3 species were found on average at the subplot level (Table 1). The mean number of species recorded using the different sampling strategies was highest for the 'random' design (924 species, 88.4% of the total number of species recorded in the whole set of 604 plots). Rectangles captured more species (912, 87.3%) compared to squares (899, 86.1%). RCs for rectangles, squares, and random arrangement showed similar trends (Fig. 3a), but the cumulative number of species recorded in the whole survey was rather different (Fig. 4). When only squares and rectangles were considered, the differences in species richness between the two shapes behaved linearly as a function of the sampling effort: average differences ranged from 0.82 species (for one plot) to 10 species (after about 200 plots). However, an almost constant difference of 13 species was reached after about 350 sampled plots (Fig. 3b).

ANOVA showed that the spatial arrangement of four subplots within each sampling plot (i.e., the shape of the sampling unit) significantly affected the overall number of recorded species (Table 2). Post-Hoc tests showed that differences in species richness were significant between all shapes (rectangles-squares t=-117.94 $P \leq 0.001$; rectangles-random t=-49.56 $P \leq 0.001$; squares-random t=-104.24 $P \leq 0.001$, Fig. 4).

Interestingly, we observed that the orientation of rectangular sampling units with respect to the environmental gradient related to slope (parallel *versus* perpendicular) only marginally influenced the number of recorded species in North- and South- facing sites (Fig. 5a): in this case, a higher number of species was recorded for rectangles parallel to the local gradient compared to those oriented perpendicular to this gradient. Conversely, the sites with East-West aspect showed an opposite pattern: a higher number of species was recorded in rectangles perpendicularly oriented with respect to the main environmental gradient (Fig. 5c). The maximum calculated difference (two species) for North-South oriented sites was recorded when approximately half of the rectangles were accumulated (Fig. 5b). In the East-West oriented sites, we observed an opposite pattern: rectangles perpendicularly placed collected, on average, more species than the parallels ones: this difference increased monotonically with increasing sampling effort and reached the maximum value of eight species (Fig. 5d). However, these small differences did not exceed the conventional level of statistical significance when analysed by ANOVA (Table 3).

4. Discussion

Characterising the effects of the shape of the sampling units used in vegetation surveys over different spatial scales is central to developing cost-effective programmes aimed at evaluating and monitoring biodiversity patterns. In this study we showed that shape and spatial arrangement of the sampling unit (provided that area remains constant) affect the cumulative number of plant species recorded in a vegetation survey, although the difference was rather small compared to the overall number of species recorded in the study area.

Our results showed that rectangles with a 1:4 length to width ratio accumulated more species (13 species, equals to 1.2% of the average number of species collected by the three different strategies) than squares of comparable area. Although relatively small compared to the overall number of species recorded in this survey, the difference in species richness increased with sample size and is expected to increase even more when the number of sampling units and/or the total surveyed area increase. The tendency of rectangular plots to capture more species compared to squared plots is consistent with the results of previous investigations. Indeed, Stohlgren et al. (1995) showed that, at different spatial scales, the shape of plots significantly affects species richness, with elongated (rectangular) plots (with length to width ratio equal to 1:4) capturing over 30 % species than square subplots, when species richness was compared between plots of comparable areas in forest and prairie vegetation in Colorado and South Dakota (USA). Harte et al. (1999), referring to the self-similarity community theory, suggested that rectangular plots support more species compared to square plots of comparable area (see also Wilson and Chiarucci 2000; Hill 2001). In a comparison of 50 ha plots in three tropical forests, Condit et al. (1996) showed that a significantly higher number of species was predicted in rectangular plots compared to square ones of the same area, although long, narrow rectangles (1000 m × 1 m) were found to underestimate true species richness. Despite the large number of studies showing that a higher species richness in rectangular than square plots, other investigations did not provide any evidence for a higher species richness in rectangular, elongated sampling units compared to squares (Keeley and Fotheringham, 2005).

Inconsistencies in the findings of studies aimed at comparing the efficacy in the number of species recorded from square *versus* rectangular plots are related to a range of factors. These include differences in environmental and habitat heterogeneity (Condit et al., 1996; Dengler, 2008), density-dependence effects (Condit et al., 1996), the spatial arrangement of plots (contiguous *versus* non-contiguous plots), as well as mechanism of species dispersal and lateral spread (Dengler, 2008). Species richness is expected to increase with a decrease in the degree of compactness or adjacency of sampling units (from squares to long, elongated plots), due to expected increases in the probability to include a larger number of habitats and/or a higher heterogeneity in the environmental conditions (Kunin, 1999; Dengler, 2008, 2009; Bacaro and Ricotta, 2007; Stohlgren, 2007; Rocchini et al., 2009; Chiarucci et al., 2009). In particular, species richness tends to decrease with increasing compactness (Kunin, 1997; Stohlgren, 2007; Dengler, 2008,

2009) or with increasing dispersal limitation, as suggested by Hubbell's (2000) neutral theory. However, a capacity to encompass more environmental variability likely depends upon the orientation of the long side of rectangles or elongated plots in relation to the direction on one or more environmental gradients.

The scale at which a study or survey is conducted may also substantially affect the differences in the number of species captured by squared and rectangular plots. Using data on plants from floral atlases at the European, British, and county level, Kunin (1997) showed that plot shape (composed by 16 cells 1 m^2 each) significantly affected the number of species recorded over the same surface area at larger spatial scales (50 km and 10 km sample). While elongated (1 × 16 cells) and rectangular (2 × 8 cells) plot shapes contained a significantly higher number of species compared to square plots (4 × 4 cells) at regional scale, the difference between rectangular and square plots was not significant at the 2 km sample scale: the absence of significant effects at this finest spatial scale was explained by Kunin (1997) as a consequence of the relatively small number of species involved in that survey (altogether, the 13 fine-scale sample areas included only 387 total species, whereas the broader scale samples generally considered a pool of 500 or more species each.

In this paper, we provide evidence that, in general, square and rectangle shaped plots collect species richness in a slightly different way, even though the presence of complex environmental gradients can hinder their performance.

In comparison with other studies, our analyses have the advantage to address a number of criticisms that have raised with respect to previous investigations on the same subject: firstly, we sampled all the possible combinations of squares and rectangles nested in 10 m × 10 m plots, so that the differences in species richness were not dependent upon the subjective spatial arrangements of the subplots within each plot. Second, the species pool in each plot for all the 604 plots was constant, so that each species had the same probability to be recorded from squares and rectangles nested in each plot. Thus, the comparison between squares and rectangles was independent on the spatial dispersion of each subplot within plots, given the constraints of the plot perimeter. Therefore, this allowed to overcome issues due to the potential confounding effect of the spatial variation of plots on the shape of the plots, a criticism that Keeley and Fotheringham (2005) made to Stohlgren et al. (1995).

Finally, we addressed the ‘gradient problem’, i.e., the issue associated with the orientation of the sampling plots in relation to the main environmental gradients. Indeed, the orientation of plots may have a confounding effect and may generate contrasting results when comparing the sampling ‘power’ of multiple plot shapes (Dengler, 2008). More specifically, the tested hypothesis is that species variation in space increases with increasing distance within the main gradient: if the factor *F* causing the gradient has a partial derivative with respect to space that is not nil, then the longer the segment under consideration the wider the explored range of the *F* factor, i.e., if at one extreme of the sampling segment *X* we have *F=Fmin* and *F= Fmax* at the other extreme, with a mean gradient *(Fmax-Fmin)/X*, then the larger is the difference *Fmax-Fmin* the more species can be observed. In the case of regular shapes like rectangles or squares, the longest segment is not the longest side but the diagonal and this is the one that should be selected to be parallel to the gradient of the studied factor in order to maximize species variation (and collected species richness). However, it should acknowledged that this operation would be very impractical.

In our study we calculated mean species richness based on all possible combinations of rectangles and squares nested in each plot, thus avoiding any systematic effect of the position of the rectangles and squares in relation to one or more environmental gradients. However, it is possible that the higher species richness found in rectangles *versus* squares may partly have been due to a tendency of the former to encompass more environmental heterogeneity, as previously shown (Daubenmire, 1968; Stohlgren et al., 1995; Stohlgren, 2007; Dengler, 2009). This was also suggested by a larger number of species recorded by a random spatial arrangement of subplots nested in plots than when rectangles and squares were used. However, the confounding effect of environmental gradients is clearly remarkable in our analysis. We showed that the orientation of elongated plots does indeed affect the number of species recorded by the sampling design. Therefore, the structure of species gradients is a key to understanding the effect of plot shape. If there is only one relevant environmental gradient, and the long axes of rectangular plots follows that gradient, then species richness will exceed richness recorded from plots of a different orientation (or simply square plots). In their study, Keeley and Fotheringham (2005) observed that in the presence of a main environmental gradient, the number of species recorded could be higher for 1:4 rectangular plots compared to square plots when the long side of the rectangle was parallel to the environmental gradient, while an opposite pattern was observed when the orientation of the plot was perpendicular to such a gradient. However, it should be considered that if the environmental gradient influencing species turnover is perpendicular to the long axes of the rectangle, rectangular plots will result in less diversity (in this case rectangles double the length of the long axis while maintaining a constant area, thus losing 50% of the width of the perpendicular axis and producing a loss of sampled area along the main gradient in species turnover, i.e., less species diversity). Our results suggest that environmental gradients exist (and act) in more than a single dimension and while rectangles potentially capture larger environmental variability along one gradient, they lose variability along the gradient perpendicular to the long axis. These considerations imply that from a practical point of view, especially when the focus is on the comparison of plot shapes, the choice of the orientation of elongated plots should be carefully considered. Our results also suggest that, without the exact knowledge of primary gradients occurring in the field, we could easily operate wrong choice in the orientation of elongated plots resulting in a lost of recorded species. This study highlights that the number of species recorded in elongated plots may be affected simply by different orientations of plots along ecological gradients which may not be detected by the researcher, and this has important implications when comparing species richness among communities and sites. The choice of whether or not using elongated (rectangular) *versus* squared plots should therefore be dependent upon the objectives of survey, with squared plots being more suitable for assessing species composition of more homogeneous vegetation units and rectangular plots being more suited for recording more species in the pooled sample of a large area. If the aim of a survey is to evaluate the biodiversity and conservation value of a region/community, sampling protocols that maximise the number of recorded species given the same sampling effort should be used. Furthermore, considerations on the orientation of rectangular plots should be carefully made to enhance reproducibility of collected data (for a review on good practices in monitoring programs, see Bacaro et al., 2009) and correctly interpret the results of a survey. This information is central to develop cost-effective surveys and monitoring programmes aimed at maximising the number of species to be recorded. It is also important to improve our understanding of spatial patterns of biodiversity within small areas (plot/sampling unit), along elevation gradients and along north-south and east-west environmental gradients.

5. Conclusion

The question whether or not the shape of sampling plots affects the number of species recorded in vegetation surveys at different spatial scales has received increasing attention due to its implications for the development of efficient biodiversity assessments for conservation and management purposes, including the design of biodiversity reserves.

Compact sample units have been described as a good solution since they have short edges relatively to their areas, thus minimising the number of decisions to be made on the inclusion or exclusion of individuals that overlap the edge. In fact, the ‘edge effect’, a sampling artifact, has often been advocated to be one of the main reasons for observed differences between squared and rectangular sampling units (Barbour et al., 1999). On the other hand, strips may be easier to sample, for example in aerial surveys, on steep ground or on cliffs, and they can be useful for collecting a higher number of species when the environmental gradient is clearly directional (Greenwood and Robinson, 2006).

The achieved results showed that, overall, the shape and orientation of the sampling units affect the number of species collected in a vegetation survey at the regional scale. Each plot shape has advantages and drawbacks. Rectangles, on average, tend to capture more species compared to squares, independently of the orientation of the plots, likely due to a longer perimeter, which then results in a higher probability to sample environmental variability and, in turn, a higher species richness. However Dengler (2008) argued that squares and not rectangular plots should be used in vegetation surveys, as they tend to mitigate the potential confounding effect of environmental heterogeneity on species richness.

As a conclusive remark, however, the proposed evaluations on the efficacy of different sampling shapes mirrored those obtained by Keeley and Fotheringham (2005) for the Mediterranean area as well: given the occurrence of complex and interacting environmental gradients, the random placement of sampling units differently shaped -squares or rectangles- do not produce appreciable differences in the number of collected species. Considering that an a-priori analysis of the main gradients in the field is practically impossible, we strongly believe that, whenever complex environmental variables interact (as in the Mediterranean area), the use of squares in vegetation sampling is advisable for sampling plant diversity. However, it should be considered that the square is not the most compact figure: from a geometrical point of view, the one plane shape that takes into account every direction with the same weight is, in fact, the circle even if circles cannot cover completely any surface. The best approximation of a circle that can totally cover a surface is the hexagon (more sophisticated forms are represented by the Penrose's tiles, 1974, which are inapplicable in real field conditions). Future efforts on this topic could address, for example, how hexagonal shapes compare with the traditional square/rectangle ones in collecting species richness.

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Tables

Table 1. Descriptive data for the investigated network of protected area (both Sites of Community Importance and nature reserves) within the Siena Province.

	Whole Dataset	North-South oriented plots	West-East oriented plots
Number of plots	604	93	98
Number of subplots	9664	1488	1568
Total species richness	1041	561	583
Mean number of species per plot	31.5 (0-122)	30.2(4-87)	29.7(0-90)
Mean number of species per subplot	12.3(0-60)	12.1(0-55)	11.8(0-44)

Table 2. Effect of the shape of the sampling unit on total species richness.

Source	df	SS	MS	*F*	*P*
Shape	2	1054340	527170	10201	<0.001
Residuals	17997	930036	52		
Total	17999	1984376			

Table 3. Effect of orientation of rectangular plot (parallel *versus* perpendicular) with respect to the main environmental gradient on sampled species richness. The ANOVA analysis was carried out separately for plots with North-South and East-West aspect

Aspect	Factors	df	SS	MS	*F*	*P*
North-South	Orientation	1	4.5	4.5	0.076	0.793
	Residuals	6	357.5	59.58		
	Total	7	362.0			
East-West	Orientation	1	128.0	128.0	1.197	0.316
	Residuals	6	641.5	106.9		
	Total	7	769.5			

Figures

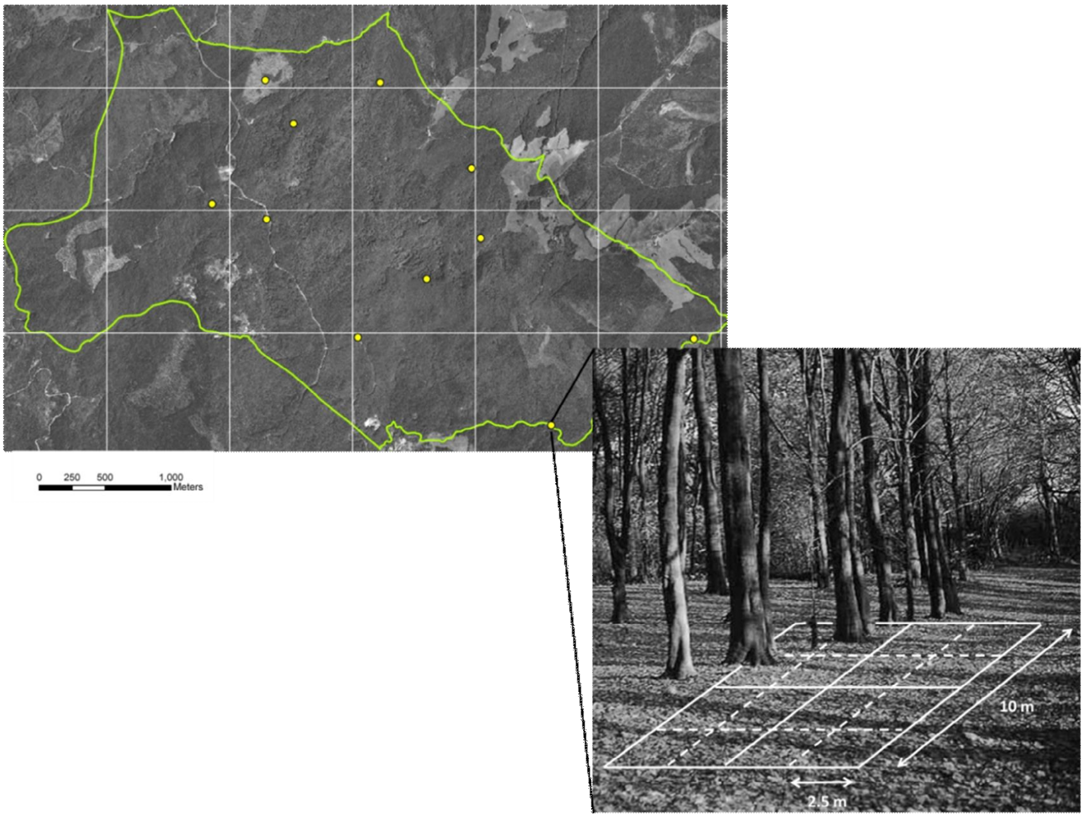

Fig. 1. Description of the adopted sampling design; the whole territory was divided into a grid with cells of 1 m × 1 km and one random point was selected for each cell. Points falling within SCIs surface were used as sample. At each sampling site, one 10 m^2 square plot was used to measure vegetation structure and sample plant species composition. The plot was further divided into 16 contiguous subplots (2.5 m × 2.5 m) in which the presence/absence of species was recorded.

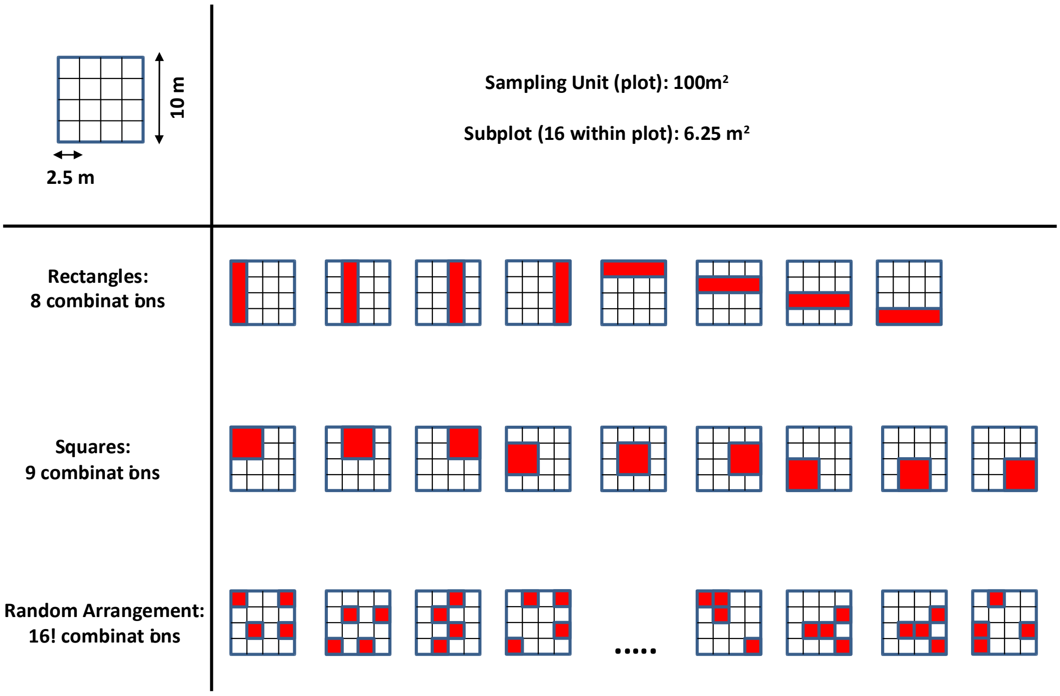

Fig. 2. Description of three possible spatial arrangements of subplots (here constraining the total plot area to 25 m^2 to visualise one of the possible combinations of subplots within each plot) used to test the effect of subplot shape on total species richness. Total species richness for each design (rectangular, squared and random subplots) was evaluated as the mean of 1000 random selections multiplied by each possible combination of rectangles (8), squares (9) and (16!) random subplots for the 604 plots.

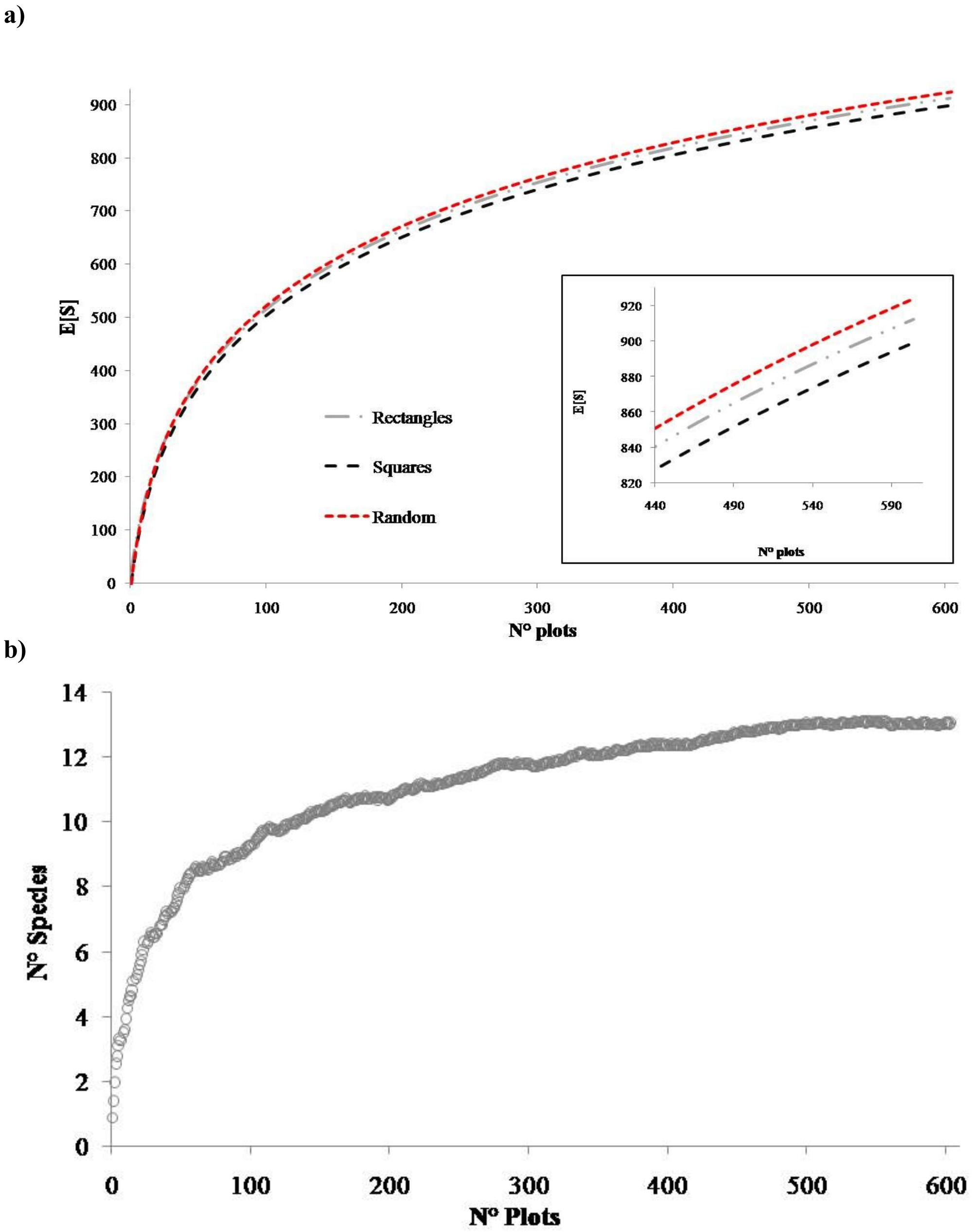

Fig. 3. Effect of the shape of the sampling unit on the number of species using rarefaction curves (a). Each curve was calculated as the mean of 1000 accumulation curves obtained by combinations of subplots for each shape. b) Mean species differences between squares and rectangles expressed as a function of the sampling effort (number of accumulated plots).

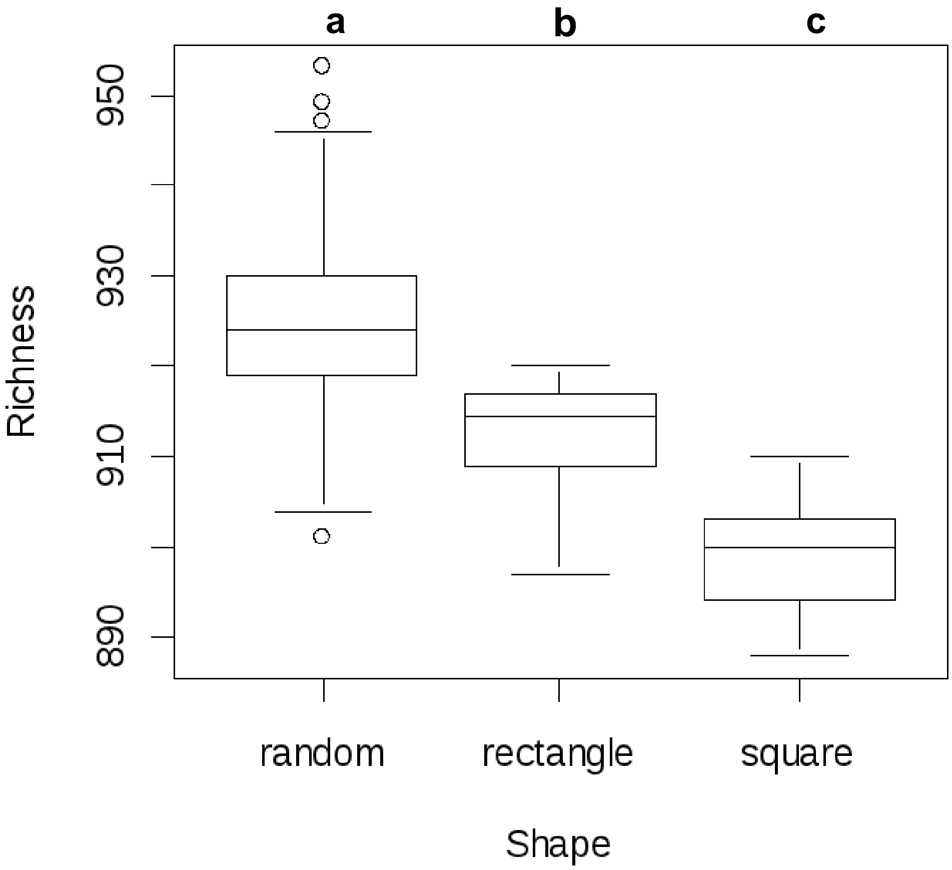

Fig.4. Box-and-whisker plot showing the effect of the different shapes of the sampling units on species richness (box-plots are based on species richness values obtained by accumulation curves). The box shows the interquartile range that contains values between 25th and 75th percentile. The line inside the box show the median. The two "whiskers" show adjacent values. The upper adjacent value (upper mark) is the value of the largest observation that is less than or equal to the upper quartile plus 1.5 the length of the interquartile range. The lower adjacent value (lower mark) is the value of the smallest observation that is greater than or equal to the lower quartile less 1.5 times the length of interquartile range.Dots represent outliers (values outside lower-upper mark range).

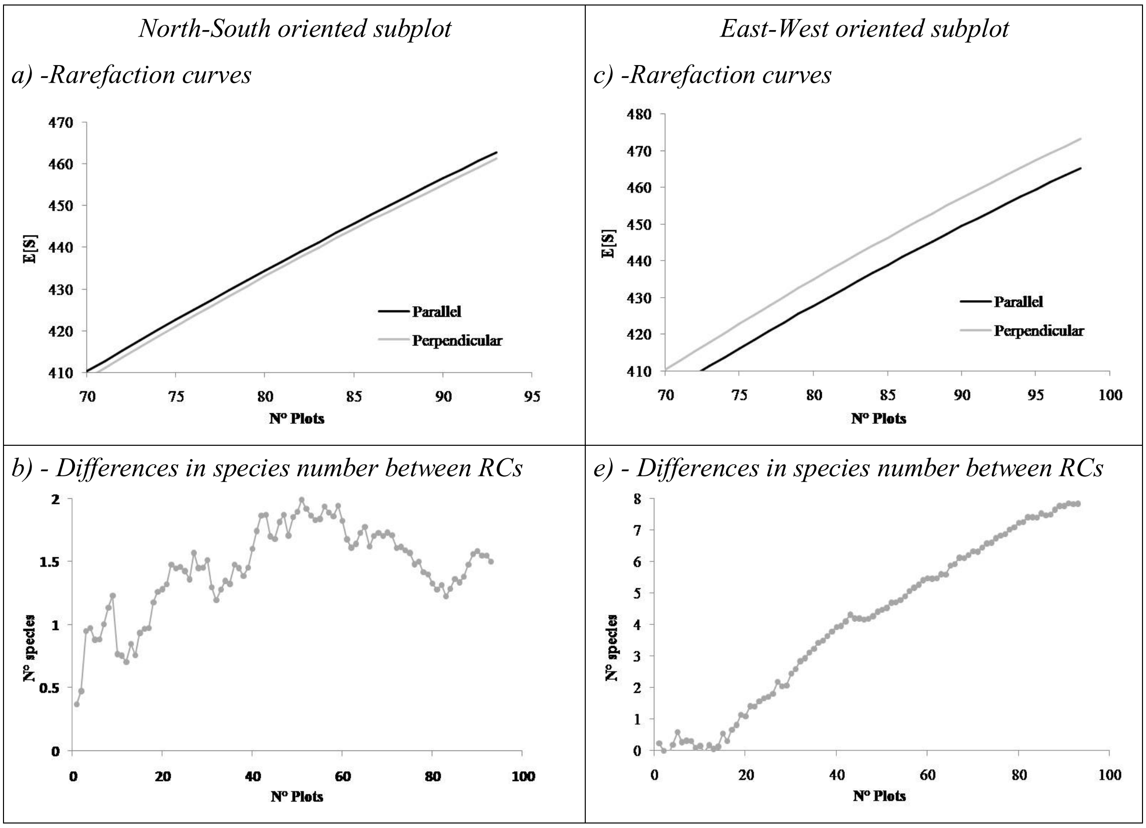

Fig. 5. Rarefaction analyses performed on the number of estimated species in a) North-South and c) East-West oriented subplots. Observed differences (in term of the absolute number of species) between the two rarefaction curves are represented in b) and e) for North-South and East-West oriented plots respectively.