



Original research article

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 × 10 m), each divided in a grid of 16 contiguous subplot units (2.5 m × 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 depend 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.

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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,

Acronyms: AC, accumulation curve; RC, rarefaction curve.

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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; Stohlgren, 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 (1685 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 9661 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 61,945 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 sampling plots were located in the study area following the procedure used by the Italian National Forest Inventory (Gasparini et al., 2010) (Fig. 1). 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 km², were used to locate sampling plots. Each plot, 10 m × 10 m in size, was located using a high precision GPS and was then divided into four quadrants and sixteen 2.5 m × 2.5 m subplots (four per quadrant). In total, we recorded plant occurrence data within 604 plots and 9664 subplots (see Table 1). Field sampling was performed during years 2005–2009.

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

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