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# In and out: Effects of shoot- vs. rooted-presence sampling methods on plant diversity measures in mountain grasslands

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ABSTRACT

Plant diversity measures (e.g., alpha- and beta-diversity) provide the basis for a number of ecological indication and monitoring methods. These measures are based on species counts in sampling units (plots or quadrats). However, there are two alternative conventions for defining a vascular plant species as "present" in a plot, i.e. "shoot presence" (a species is recorded if the vertical projection of any above-ground part falls within the plot) and "rooted presence" (a species is recorded only when an individual is rooted inside the plot). Very few studies addressed the effects of the two sampling conventions on species richness and diversity indices. We sampled mountain dry grasslands in Italy across different plot sizes and vegetation types to assess how large is the difference in alpha- and beta-diversity values and in sample-based rarefaction curves between the two methods. We found that the difference is greatly dependent on plot size, being more relevant, both in absolute and percentage values, at smaller grain; it is also dependent on habitat type, being larger in shallow-soil communities, as they have a sparser vegetation structure and host life-form types with a larger lateral spread. At fine spatial scales (<1 $m^2$) the difference is large enough to bias statistical inference, and we conclude that at such scales one should not attempt to compare plant diversity indices if they were not obtained with the same sampling convention.

## 1. Introduction

Plant diversity measures, based on species counts in plots (called also quadrats or relevès) delimited within the vegetation, are widely used in ecological monitoring, management and assessment (Elzinga et al., 1998; Yoccoz et al., 2001; Laurila-Pant et al., 2015). The size of such plots usually ranges between 0.01 and 500 $m^2$, depending on vegetation physiognomy and research aims. The simplest diversity measures include the species richness at the plot scale or alpha-diversity, and the variation in the identities of species between plots or beta-diversity (Jost, 2007; Anderson et al., 2011). For instance, alpha- and/or beta-diversity measures of vascular plants have been used for monitoring the effects of grazing animals on ecosystems (Hanke et al., 2014; Schrautzer et al., 2016), for assessing the impact of land-use changes (Gillet et al., 2016), for evaluating the effectiveness of protected areas (Chiarucci et al., 2012; Beauvais et al., 2016), and for monitoring the effects of alien species (Selvi et al., 2016).

Although counting plant species in a plot may seem a conceptually simple and operationally clear measurement, there actually are many potential sources of bias and a number of alternative methodological options: it is often overlooked that biodiversity metrics and indices are comparable only if data are collected with the same methods and conventions through the whole sampling process (Chiarucci et al., 2011; Brunialti et al., 2012). In particular, for environmental monitoring through time, or for comparing the effects of different management regimes, it is necessary that the observed species richness data are not systematically biased by a difference in sampling protocol or quality across the considered time span or treatments (Archaux et al., 2009; Bacaro et al., 2009; Kercher et al., 2003; Morrison, 2016). Many studies dealt with the influence on plant biodiversity data of methodological issues such as plot size and shape (e.g. Keeley and Fotheringham, 2005; Bacaro et al., 2015; Güler et al., 2016), field workers number (e.g. Vittoz and Guisan, 2007; Archaux et al., 2009) and skill level (Bacaro et al., 2009), species misidentification (Scott and Hallam, 2003) and differences between individual botanists (e.g. Kercher et al., 2003; Milberg et al., 2008; for an up-to-date review, see Morrison, 2016).

However, only very few studies addressed the effects of the two alternative conventions for defining a species as "present" in a plot (Dengler, 2008). One is termed "shoot presence" (Greig-Smith, 1983) or "any-part system" (Williamson, 2003): this will record a

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species as present if the vertical projection of any above-ground part of an individual falls within the plot. The other method is known as "rooted presence" (Greig-Smith, 1983), and will record a species only when an individual or part of an individual is rooted in the sample area.

To our knowledge, the only published study performing a thorough comparison of biodiversity values between shoot- and rooted-presence data obtained from the same plots was a paper by Kilburn (1966), who compared the results across many different plot sizes in both forest and prairie communities from North America. Sykes et al. (1994) reported very briefly, and without providing the complete results, on an unpublished comparison between the two methods across different plot sizes in Swedish grasslands. Williamson (2003) argued that the two methods will differently and significantly affect the properties of species-area curves, especially the shape of the left end (see also Dengler, 2009b), but his comparison was based on datasets surveyed in two different regions, each with one of the two alternative conventions. A very recent paper by Güler et al. (2016), comparing the effects of different plot shapes on species richness metrics in low-elevation grasslands from various European countries, took into account also the additional effect of using shoot- vs. rooted presence.

Both Kilburn (1966) and Sykes et al. (1994) reported that the differences were important only at small plot sizes ($\leq 1\,m^2$) and were relatively small in absolute values. However, such differences may become relevant when they are compared in percentage values (Dengler, 2008). For instance, in the study by Kilburn (1966), at $0.01\,m^2$ scale, shoot presence resulted in an average richness increase of 14.3% at the grassland site, and of 200% at the oak forest site. However, the difference obtained for the forest site is a trivial result: within a wooded ecosystem, small plots will obviously have a small probability of hosting rooted trees or shrubs. The differences between the two methods are more interesting when referring to grassland vegetation, or when only the herb layer of a forest is considered. In semi-natural grasslands, Güler et al. (2016) found, for squared plots of $0.01\,m^2$, that shoot presence resulted in an average richness increase between 11.3% (in Hungary) and 64.6% (in Italy), corresponding to 0.6 and 4.2 species, respectively.

In general, plant diversity patterns at very fine spatial scale ($\leq 1\,m^2$) have to be interpreted with caution because of many potential pitfalls, the most prominent being the artifacts originated by the rarefaction effect, i.e. by the relationship between the number of individuals and the number of species (Palmer et al., 2008 and references therein). Nevertheless, in grassland ecosystems, biodiversity patterns at fine spatial grain are important, because it is at such scales that plants interact with each other (Dengler, 2009a; Wellstein et al., 2014); moreover, temperate secondary grasslands are probably the most diverse plant communities globally for plot sizes $<100\,m^2$ (Wilson et al., 2012).

Surprisingly, as already noticed by Dengler (2008), in many (perhaps most) biodiversity studies it is rarely mentioned which one of the two recording conventions was followed, even when dealing with fine-scale richness measures. Although it could be often assumed that, when the method is not specified, it was rooted presence that was followed, this might not be always true. For instance, in the interesting study by Klimeš et al. (2001) on inter-observer misidentification rates in fine-scale plots in grasslands, the recording method is not specified despite the very small size of the smallest plots ($0.001\,m^2$): however, judging from the richness figures reported in their Table 1, it might be that shoot presence was used. Thus, it may be possible that some monitoring works or comparative studies unintentionally mixed data collected with the two methods.

For these reasons, there still is a need for exploring the effects of the two sampling conventions on biodiversity metrics, across different ecosystem types, biogeographic areas and spatial scales. The work by Kilburn (1966) took place in very low-diversity prairies, while the study by Güler et al. (2016) was carried in a pre-existing network of permanent plots that had been preferentially placed in homogenous grassland patches. In the present work, we test the different performances of the two sampling methods with randomly located nested plots in high-diversity grasslands, across different plot sizes and vegetation types. Our research questions are: *i)* how large is the difference in alpha- and (intra-plot)beta-diversity values between shoot- and rooted-presence, and is this difference large enough to bias statistical inference? *ii)* how does the importance of this difference vary with plot size? *iii)* is the difference more relevant in some grassland types than others and/or correlated with structural properties of the vegetation?

## 2. Methods

Data were gathered in the "Abruzzo Lazio e Molise" National Park (Central Apennine mountains, Italy) (Fig. 1), during a wider study on biodiversity patterns (Primi et al., 2016). Five patches of semi-natural montane grassland and one patch of subalpine grassland, surrounded by beech (*Fagus sylvatica*) forest, were chosen as study sites. Elevation of the study areas ranges between 1300 and 1900 m; bedrock is limestone at all sites, but geomorphology includes both mountain slopes and karst plateaux (these latter are composed in turn by different landforms such as dolines, hummocks, rocky outcrops, etc.). Climate is sub-Mediterranean, with a marked precipitation minimum in summer (Filibeck et al., 2015). Dominant grasses include *Festuca circummediterranea*, *Koeleria lobata, Bromus erectus, Avenula praetutiana;* most frequent forbs include *Cerastium tomentosum, Viola eugeniae, Hieracium pilosella* (Primi et al., 2016). The communities growing on karst hummocks and mountain slopes belong to various associations within the phytosociological class *Festuco-Brometea* (Lucchese et al., 1995; Primi et al., 2016), while the (sub)acidophilous swards in the doline bottoms are classified in *Nardetea strictae* (Di Pietro et al., 2005).

Sampling was performed in early summer in 2013 and 2014. Sampling design was based on a grid of cells of 300 × 300 m, overlaid on the National Park area, with one random point selected within each cell. All the random points falling within the six chosen grasslands were selected, for a total of 70 points (Fig. 1). At each GPS-located point, vascular plants were surveyed using a nested plot based on the design proposed by Dengler (2009a): one $1\,m^2$ squared plot was centred on the random point; two $0.1\,m^2$ subplots were placed at two opposite corners of the main plot, and two $0.01\,m^2$ subplots were placed within each of the $0.1\,m^2$ plots (Fig. 2).

For each plot and subplot, we listed all vascular plant species, taking note whether each species had at least one individual, or a part of it, rooting within the plot ("rooted presence") or was present only with projecting aerial parts ("shoot presence"). Plant names were standardized following Conti et al. (2005). For each plot and subplot we recorded vegetation height (defined as the measure in cm of the highest plant individual), total vegetation cover (visually estimated in percentage values) and topographical position. This latter was defined as a categorical variable with three values: doline hollows (i.e. flat or concave areas at the bottom of karst landforms), slopes (i.e. both the proper mountain slopes and the sloping sides of the karst landforms), and humps (i.e. the hummocks and rocky outcrops between dolines in the karst plateaux).

At each sampling point, we calculated beta-diversity between the subplots of the same plot using Whittaker's multiplicative formula (Anderson et al., 2011), i.e. dividing gamma-diversity by alpha-diversity: this approach, when applied to presence/absence data, ensures that alpha and beta are free to vary independently (Jost, 2007). For beta-diversity at $0.1\,m^2$ scale, gamma-diversity