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How plot shape and dispersion affect plant species richness counts: implications for sampling design and rarefaction analyses

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Running head: Species richness vs. plot shape and dispersion

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Abstract

Questions: How does the spatial arrangement of sampling units influence recorded plant species richness values at small spatial scales? What are the consequences of these findings for sampling methodology and rarefaction analyses?

Location: Six semi-natural grasslands in Western Eurasia (France, Germany, Bulgaria, Hungary, Italy, Turkey).

Methods: In each site we established six blocks of 40 cm × 280 cm, subdivided into 5 cm × 5 cm micro-quadrats, on which we recorded vascular plant species presence with rooted (all sites) and shoot presence method (four sites). Data of these micro-quadrats were then combined to achieve larger sampling units of 0.01, 0.04 and 0.16 m² grain size with six different spatial arrangements (square, 4:1 rectangle, 16:1 rectangle, three variants of discontiguous randomly placed micro-quadrats). The effect of the spatial arrangements on species richness was then quantified as relative richness compared to the mean richness of the square of the same surface area.

Results: Square sampling units had significantly lower species richness than other spatial arrangements in all countries. For 4:1 and 16:1 rectangles, the increase of rooted richness was on average about 2% and 8%, respectively. By contrast, the average richness increase for discontiguous arrangements was 7%, 17% and 40%. In general, increases were higher with shoot presence than with rooted presence. Overall, the patterns of richness increase were highly consistent across six countries, three grain sizes and two recording methods.

Conclusions: Our findings suggest that the shape of sampling units has negligible effects on species richness values when the length-width ratio is up to 4:1 and the effects remain small even for more elongated contiguous arrangements. By contrast, results from discontiguous sampling units are not directly comparable with those of contiguous sampling units, and are strongly confounded by spatial extent. This finding is particularly problematic for rarefaction studies where spatial extent is often not controlled for. We suggest that the concept of effective area is a useful tool to report effects of spatial arrangement on richness values, and introduce species-extent relationships (SERs) to describe richness increases of different spatial arrangements of sampling units.

Keywords: Biodiversity; Discontiguous; Effective area; Grassland; Sampling unit; Scale dependence; Spatial autocorrelation; Spatial extent; Spatial grain; Species-area relationship (SAR); Species-extent relationship (SER); Vegetation plot

Abbreviations

A_e = effective area

Introduction

In ecology and conservation, species richness is probably the most frequently used metric of diversity because it is easily measurable in a multitude of different situations and comprehensible even for non-specialists. Accurate quantification of species richness requires appropriate sampling decisions regarding sample size, the selection and arrangement of sampling units (in vegetation science called quadrats, vegetation plots or just plots), as well as their size and shape (Kenkel et al. 1989; Bacaro et al. in press). Given that species richness on average increases with area (Arrhenius 1921; Connor & McCoy 1979; Dengler 2009), comparisons of species richness counts are usually only meaningful between sampling units of the same grain size. However, there are at least three other factors that can distort comparisons of species richness for a given area: (i) the shape of the sampling unit used to assess species richness (elongated vs. compact); (ii) the dispersion or contingency of subplots that constitute the overall area to be quantified (contiguous vs. discontiguous); and (iii) in the case of plants and other sessile organisms, the method by which an individual is considered present in the plot (shoot presence, rooted presence, grid-point presence) (Dengler 2008).

Essentially all geographic phenomena are subject to the distance decay of similarity ("the first law of geography": Tobler 1970; Nekola & Brown 2007), which is also true for ecological and biogeographical patterns, such as species composition (Harte et al. 1999; Nekola & White 1999). This means that two plant assemblages sampled geographically closer to each other, be it plant communities or be it regional floras, are on average more similar than those sampled at a larger distances. This is universally true for very local scales, such as a few meters (e.g. Dengler 2006), and for large distances such as several thousands of kilometres (e.g. Nekola & White 1999). The distance decay in plant species composition has two main drivers (Nekola & White 1999): First, the distance decay in climate, soil, topography, composition of species of other trophic levels as well as of human land-use patterns creates environmental filters that become, on average, more and more dissimilar with distance, thus selecting for increasingly different plant species composition. Second, biological processes of the plant species themselves are strongly distance-dependent, such as lateral spread, dispersal, gene flow and species-species interactions, including facilitation or parasitism. Such biological processes can even overrule – to some extent – environmental filtering, leading to the occurrence of species in ecologically suboptimal habitats which are spatially close to ecologically optimal source habitats. This phenomenon occurs both at local and at biogeographic distances and has been termed mass effect (Shmida & Wilson 1985) or vicinism (Zonneveld 1994). Generally, distance decay in compositional similarity of plant

assemblages should be relatively low when there are less pronounced environmental gradients and/or when species with good dispersal ability of diaspores and genes are considered, and *vice versa*. If we accept the universality of the distance decay, it is self-evident that sampling units, which cover a larger spatial distance ("extent" *sensu* Scheiner et al. 2000) yet have the same total area ("grain" *sensu* Scheiner et al. 2000), should on average comprise more species. This argument equally holds for elongated vs. compact shapes of releves and for discontiguous vs. contiguous arrangements.

While theoretically it is clear that less compact plots should lead to higher recorded species number, this factis rarely considered in sampling recommendations in vegetation science. For example, the methodological textbook of Kent (2012) does not mention plot shape at all, while Knapp (1984) discuss the pros and cons of squares vs. circles vs. rectangles mainly based on practical considerations, such as efforts needed to delimit the plot in the field and risk of overlooking species. In large homogenous stands, compact forms such as squares or circles are generally used for phytosociological sampling, whereas in vegetation mosaics rectangular and irregular plots are recommended to minimize the within-plot heterogeneity (Dierschke 1994). In the context of biodiversity monitoring, elongated shapes are sometimes recommended because they allow to capture of more species on the same surface area, which is considered more "efficient" (e.g. Stohlgren 2007; Bacaro et al. in press). However, the few studies examining impacts of different sampling unit shapes have generated contrasting results, and it is hard to assess the magnitude of "plot shape" effects. At small grain sizes (0.25–1 m²), for example, one study found increases of 1.4–1.6% in richness (Bossuyt & Hermy 2004), while another reported 40% higher richness (Stohlgren 2007) compared to squares of the same size. At a grain size of 16 m², Kunin (1997) found 5.5% more species in 16:1 rectangles than in either 4:1 rectangles or squares. By contrast, Keeley & Fotheringham (2005) found 4:1 rectangles of 1 m² and 100 m² to exhibit the same or even an insignificantly lower richness than squares of the same size. At intermediate grain sizes (habitat patches within 1-km² landscape segments), Heegaard et al. (2007) reported strong positive effects of the degree of elongation on species richness, with a more than doubled richness in the most elongated patches compared to circles on average. At much larger grain sizes of 32 km², 160 km² and 800 km² (distribution atlas data), Kunin (1997) found consistent and significant increases of about 6% for 4:1 rectangles and 16% for 16:1 rectangles in relation to squares.

While for primary sampling vegetation ecologists normally use contiguous sample units (but see Dierschke 1994, who considers combining dispersed subplots into one virtual sampling unit admissible in phytosociology), the species data of several discontiguous

primary plots are in subsequent analytical steps often combined to form "virtual plots". This is particularly common for so-called species accumulation or rarefaction curves (hereafter referred to as rarefaction curves), which are a fashionable tool in biodiversity research (Gotelli & Colwell 2001, 2011), and are also widely used for comparison of different vegetation types (e.g. Stiles & Scheiner 2007) or floras (e.g. Koellner et al. 2004). However, the users of rarefaction curves often overlook the underlying assumptions of this technique. First, sampling units used for the construction of rarefaction curves need to be randomly distributed in the area of inference (Gotelli & Colwell 2011), and second, rarefaction curves of different types (vegetation, landuse,…) can only be statically compared when they are based on the same spatial extent (Chiarucci et al. 2009; Dengler & Oldeland 2010). The latter two studies showed with real and simulated data, respectively, that rarefaction curves of the same vegetation type have extremely different values depending on the spatial extent. This finding, an obvious consequence of the distance decay, questions results of many studies using rarefaction methods but not controlling for spatial extent. Due to the scarcity of methodological studies in this field, it is currently unclear how big the distorting effect of varying spatial extents is at the plot scale. However, in recent work with distribution data of different taxa (4–100 km²), Lazarina et al. (2014) nicely demonstrated that combining non-contiguous plots into richness counts leads to dramatically higher richness values than in contiguous areas.

Despite strong theoretical grounds for expecting significant impacts of sampling unit shape and contingency on species richness counts, the potential influence of differences in shape (degree of elongation) and contingency (degree of dispersion) are generally ignored in ecological studies. Here we aim to improve knowledge on effects of these two components of spatial arrangement on derived plant species richness values. In order to get results of high generality, we conducted a standardized study at six different grassland sites in six different Eurasian countries, examined three different spatial grain sizes and compared the two most frequently used recording principles in vegetation ecology (shoot vs. rooted). Specifically, we set out to (i) quantify the importance of sampling unit shape and dispersion for plant species richness counts and (ii) determine whether the effect sizes depend on grain size, recording principles and characteristics of the vegetation type being studied.

Methods

Study sites and plots

The sampling was conducted within the framework of the BiodivERsA project SIGNAL (http://www.bayceer.uni-bayreuth.de/signal/; see Jentsch et al. 2014). In each of six western Palaearctic countries along a steep climatic gradient (France, Germany, Bulgaria, Hungary, Italy, Turkey; see Appendix S1 for geo-locations and site characteristics) we established one experimental study site of approx. 30 m × 15 m in semi-natural grassland representative of their respective regions. The sites contained stand of vegetation managed agriculturally (mowing or extensive grazing) prior to the start of the SIGNAL project, selected to be as homogenous as possible. At each site we established six blocks of 280 cm × 40 cm (240 cm × 40 cm in Bulgaria), separated from each other by a minimum of 3 m and a maximum of 33 m.

Field sampling

Early in the growing season of 2013, we carefully placed and fixed iron frames subdivided into 10 cm × 10 cm grid cells into the vegetation. Vegetation recordings were carried out at peak biomass in 2013 (May in Italy, June in Bulgaria, France, Germany and Hungary, December in Turkey), and the 100-cm² grid cells were temporarily subdivided by inserting a thin wooden stick in the centre of each. This resulted in 448 5 cm × 5 cm micro-quadrats ("primary sampling unit") per block and 2,688 micro-quadrats per site. We recorded all vascular plants (including seedlings, juveniles and recently-senesced individuals) that occurred in each of the micro-quadrats. Two recording techniques were applied in parallel (Williamson 2003; Dengler 2008): (i) plant individuals with rooted presence only (i.e. rooting in the micro-quadrat) and (ii) plant individuals with shoot-presence (i.e. the plants' superficial parts fall inside the micro-quadrat when vertically projected; not recorded for Bulgaria and France).

Scales, cell arrangement and statistical analyses

Species composition and thus richness for secondary sampling units (short: sampling units) of 4, 16 and 64 cells size (0.01, 0.04 and 0.16 m²; "grain" *sensu* Scheiner et al. 2000) of different shape and spatial arrangements were derived by combining micro-quadrats in various ways. For the comparison of elongated vs. square plots, we first divided each block into 112 4-cell squares (arrangement A), 28 16-cell squares and seven 64-cell squares (96, 24

and 6 in Bulgaria) (Fig. 1). Next, we used full tessellation into 4:1 rectangles (arrangement B) with parallel orientation to the shape of the block. For 16:1 thin, elongated plots (arrangement C) no full tessellation was possible; instead the maximum possible number of non-overlapping plots were used, spread as widely as possible across each block.

For the comparison of contiguous vs. discontiguous sampling units, we used the micro-quadrats described above and randomly drew the same number of these (without replacement) to derive combined richness values for discontiguous sampling units (Fig. 1). Three cases of dispersion and thus spatial extent were considered: random draw from within a subblock of 8 × 8 cells (arrangement D; maximum distance: 0.50 m), from within a block (arrangement E; maximum distance: 2.80 m) and from within a site (arrangement F; maximum distance: 33 m). For arrangements D and E, we applied a nested random draw where first a random subblock or block were determined, and then the required random micro-plots were drawn within this unit.

Species richness analyses were carried out separately for rooted presence in each of the six countries, and for shoot presence in the four countries with available data (Germany, Hungary, Italy, Turkey). We tested effects of sampling unit shape separately for the three grain sizes (4, 16 and 64 cells), using linear mixed-effect models with block as random factor. To test the effect of the three discontiguous arrangements vs. squares of the same grain size, we calculated simple linear models. Mixed effect models were not possible in the latter case because arrangement F contains micro-quadrats of more than one block. To make absolute richness differences comparable across sites (countries), we calculated relative richness values as $S_{\text{shape } i} / S_{\text{square}}$, where S_{shape} is the mean species richness of a certain grain size and shape. Finally, we tested whether the values of relative richness obtained for each country differed between different sampling unit arrangements (i.e. different degrees of elongation or dispersion). These comparisons were carried out separately for each of the grain sizes using analyses-of-variance (ANOVAs).

All analyses were carried out in the R statistical environment (v.2.15.2). Residuals of the derived models were visually inspected for normality and homoscedasticity and they did not show problematic deviations.

Results

Effects of shape: elongated vs. square plots

Mean richness sampled as rooted presences in square plots ranged from 4.9 to 8.0 species for 0.01 m² (4 cells) and from 10.6 to 26.5 for 0.16 m² (64 cells) (Table 1). France had the lowest species richness at all grain sizes, whereas Germany had the highest species richness at 0.01 m² and Italy had the highest value of species richness at 0.04 and 0.16 m².

Plots with more elongated shapes consistently contained more rooted species on average than more compact plots (16:1 > 4:1 > 1:1), irrespective of country and grain size (Table 1). Due to the high spatial variation in local richness, these differences were not always significant within a single country; in Bulgaria we even found in some cases slightly and insignificantly lower values. When subjecting the country-wise means of relative richness to ANOVAs, both 4:1 rectangles and 16:1 long thin plots were significantly richer than squares, except for 4:1 rectangles of 64 cells (Fig. 2). The relative increase was consistent among countries and largely scale-invariant between the three tested grain sizes, while the shape-dependent absolute differences varied (Table 1). In general, mean richness "gain" ranged from 2.1 to 2.3% and from 6.9 to 8.3% for comparisons between 4:1 vs. 1:1 and between 16:1 vs. 1:1 shapes respectively, with negligible and inconsistent effects of grain size. Site had some effect, with Turkey and Italy showing the strongest relative increase and for the two smaller grain sizes also France (Table 1). However, this did not change the overall consistent pattern, but just increased the variance towards more elongated shapes and larger grain sizes slightly (Fig. 2).

For shoot presence (Appendices S1 and S3), the richness values of the squares were consistently higher compared to rooted presence at all grain sizes. At 0.01 m², for example, the mean increase ranged between 0.6 in Hungary and 4.2 species in Italy (Appendix S2 vs. Table 1). While the overall pattern was very similar to that described for rooted presence, also the relative richness gain with decreasing compactness of the plots was higher for shoot presence than for rooted presence. For example, 16:1 plots had 10.5–12.0% more species compared to squares of the same size for shoot presence, whereas the gain was only 6.9–8.3% for rooted presence.

Effects of dispersion: discontiguous vs. contiguous micro-quadrats

The effect of discontiguous vs. contiguous arrangement of micro-quadrats to form a sampling unit was much stronger than that of different degrees of compactness in the case of contiguous plots. Differences between contiguous and discontiguous sampling approaches varied depending on the degree of dispersion (Table 2). Drawing from the whole site (arrangement F), yielded much higher species richness values than drawing from within a block (arrangement E) or a subblock (arrangement D) (Table 2, Fig. 2). These differences were highly significant both in the cross-country analysis (Fig. 2), and within countries (Table 2). As for the analyses of elongated vs. squared plot, the results for different degrees of dispersion were widely consistent among countries and across spatial scales. In general, drawing from a subblock (40 cm × 40 cm) produced 6.8–7.7% higher richness values, while drawing from a block (40 cm × 280 cm) yielded an increase of 13.0–21.5% and drawing from the whole site an increase of 28.3–46.3% on average (Table 2). As with sampling unit shape, the relative effects of dispersion were bigger in Turkey, Italy and France than in the other three countries.

Patterns of response for shoot presence data (Appendices S2 and S3) mirrored those presented for rooted presence, although the effect sizes were even higher than for rooted presence (Appendix S3 vs. Table 2). On average, a random draw from the site increased species richness values by 41.0–61.6% for shoot presence data compared to a 28.3–46.3% increase with root presence data.

Discussion

Effects of shape

In line with predictions, we found that plot shape, i.e. the degree of elongation of the plot, had a positive effect on species richness. In the case of rooted presence, the magnitude of elongation effects were quite small in relation to effect sizes researchers typically find when studying ecological rather than methodological drivers of biodiversity (about 2% increase for 4:1 and less than 10% for 16:1 rectangles compared to squares). For shoot presence the values were slightly higher (about 5% and 12%, respectively), but values for 4:1 shapes were still in a range that does not normally distort ecological inferences. Our findings are consistent with values reported in previous work with similar (Nosek 1976), slightly larger (Kunin 1997, Bossuyt & Hermy 2004) and much larger grain sizes (up to 800 km², Kunin 1997). By

contrast, Stohlgren (2007) found a much higher increase (40%) in 4:1 rectangles of 1 m² size, but this might be attributable to the heterogeneity of their site, which they emphasize.

For practical sampling of plots in vegetation science, it is always preferable to compare species composition and diversity in sampling units with standardized shapes (preferably compact like circle or square). However, our study indicates that deviations from this recommendation, up to a length-width ratio of 4:1, are also acceptable. Including elongated plots with length-width ratios larger than 4:1 in the same study is also possible if the expected effect size of the factor of interest is clearly larger. Since vegetation ecologists rarely use more elongated shapes than 4:1 this issue normally can be ignored when taking data for example from large vegetation-plot databases (Dengler et al. 2011). There are, however, well-established methods like the "Gentry plots", frequently applied in tropical (and sometimes other) forests, that use such "extreme" shapes as 25:1 rectangles for primary sampling (Phillips et al. 2003), where much stronger differences compared to squares are to be expected.

Effects of dispersion

Increasing dispersion, i.e. bigger distances between the micro-quadrats led to an increase in recorded richness for a given grain size. While this is a direct and inevitable consequence of the distance decay in practically any ecological or biogeographic phenomenon (Harte et al. 1999; Nekola & White 1999), it is rarely taken into account in studies operating with such discontiguous subplots (but see Chiarucci et al. 2009; Dengler & Oldeland 2010). Remarkably, the effect of dispersion was far more pronounced than that of elongated sampling units. Contiguous plots with a length-width ratio of 16:1 generally showed richness increases of around 10%. In contrast, discontiguous sampling generated up to 90% more species (Appendix S3), despite sampling in homogenous vegetation with a maximum distance between combined micro-quadrats of only 33 m.

Effects of dispersion have rarely been quantified in the literature. Bacaro et al. (in press) studied this effect at the plot scale (a few square metres). While they also report higher richness for plots composed of dispersed subplots, their paper does not allow direct comparison because they only analysed the effect when combining contiguous or dispersed sampling units across a large region. Lazarina et al. (2014) conducted an extensive study on the effect of different degrees of dispersion on richness values for different taxa (plants, birds, butterflies) and cell sizes (mostly distribution atlas data with grid cells of 4–100 km², but also one dataset with plot-scale data and cells of 4 m²). Their figures for British plant atlas data

indicate an increase of about 10% in richness between contiguous square and a random sampling where about 10% of the cells within the extent were sampled. This corresponds to a degree of dispersion between our arrangements D (25% cell filling) and E (3.6%; Fig. 1), where we found increases for rooted presence of 6–8% and 13–22% respectively at the different scales (Table 2). Finally, Dengler & Oldeland's (2010) simulation study demonstrated that the relative difference of recorded richness for contiguous plots ("true species-area relationships") and discontiguous/dispersed plots ("species-sampling relationships") is biggest for low to intermediate degrees of filling. For a filling of 16 cells out of 4096 (0.3%) their figure indicates a more than 2-fold increase.

Taking together the comprehensive findings of Lazarina et al. (2014) for biogeographic grain sizes and ours for vegetation ecological grain sizes with the study of Dengler & Oldeland (2010) on a fictive scale, it is clear that richness counts for dispersed subplots are nearly always higher than for a contiguous sampling unit of the same surface area. The richness increases range from about 6% for very little dispersion (filling of the extent by 25%) to more than 100% in the so far studied examples. These values for richness increase in the case of dispersed subplots can be considered to represent the lower margin of what typically is to be expected in rarefaction analyses, where vegetation is not homogenous and where the dispersion is greater. Strong differences can also occur among different dispersed arrangements (see Table 2 and Appendix S3 as well as Fig. 2 of Dengler & Oldeland 2010: contrast between their SSR full and SSR centre). This indicates that comparison between different categories (vegetation types, treatments,…) in rarefaction analyses are only sensible when not only the sampled area but also the sampled extent and the spatial arrangement are kept identical. In many situations it is hard to keep extent and dispersion patterns constant, which questions the appropriateness of rarefaction methods in such cases. Chiarucci et al. (2009) and Bacaro et al. (2012) have proposed "spatially constrained rarefaction" as a method to overcome these limitations, which corrects for different spatial extent provided the coordinates of the individual sampling plots are known, but this method has yet to become commonplace in vegetation studies.

Beyond rarefaction, our findings have also implications for reporting species richness. In the literature, authors often speak of species richness even when they refer to the richness derived from the combination of several discontiguous quadrats. Since we have demonstrated that "conventional" richness (for contiguous areas) is sometimes extremely different from such values, we recommend to use the term "cumulative species richness" for richness values

from discontiguous areas, with a clear indication not only of the cumulative surface area (grain) but also the spatial extent from which they have been drawn.

What this means in practice shall be shortly discussed with a typical example from the literature: Öster et al. (2007) reported a "mean species density on 10 m²" of 57.1 vascular plants for Swedish grasslands, what seems to be close to the "world record grasslands" at the 10 m²-grain size in Romania (Wilson et al. 2012), which have a mean richness of 70.2 vascular plants (Dengler et al. 2012). At closer look, however, both values are incomparable because the areas of Öster et al. (2007) are composed of 10 subplots randomly drawn from areas of 0.2–18.9 ha (mean: 5.6 ha). This corresponds to a "cell filling" of on average less than 0.02%, which is far sparser than in the examples discussed before so that we can assume that reported value of 57.1 species is higher than the average richness in a contiguous 10-m² plot in their area. While the authors correctly reported these details of their methods in the text, the shortened terminology of the diversity variables in their table could prompt misunderstandings. A clear and explicit terminology would help to avoid this. Likewise, the term "vegetation plot" or short "plot" should be restricted to contiguous sampling units. Accordingly, the "Gentry plots", one of the most widespread sampling approaches for tropical forests (Baraloto et al. 2013) should not be termed "plots" any longer (and be stored as single 0.1-ha plots in vegetation databases) as it is widespread practice, but named as what they are: complex sampling schemes where 10 discontiguous 100-m² subplots are combined to form a secondary sampling unit (Phillips et al. 2003). Based on the points discussed here, such 0.1-ha Gentry "plots" are not comparable to conventional (contiguous) 0.1-ha plots as regards species composition and diversity metrics.

Generalities and idiosyncrasies

Overall, the observed increases in richness with decreasing compactness of the arrangements of micro-quadrats were highly consistent across sites, grain sizes and recording methods (rooted vs. shoot presence). The fact that we included grasslands from two zonobiomes (Nemoral and Mediterranean, as well as a transition Nemoral-Steppic) with quite different climates and land use history underlines the generality of the results. Since we selected areas within the grassland sites that were relatively homogenous in terms of topography and vegetation physiognomy, our values for richness increase can be considered to be at the lower margin of what can be found in randomly located plots. Higher gains should be expected in more heterogeneous vegetation (Bartha & Horváth 1987).

The slight differences between countries regarding the richness gain with decreasing compactness could thus be attributable to the different levels of homogeneity that could be achieved locally. Consistently high richness gains with decreasing compactness across all 13 comparisons were found (in this sequence) for Turkey, France and Italy in the case of rooted presence (Tables 1 and 2) and for Italy and Turkey in the case of shoot presence (Appendices S2 and S3), while the sites in Germany, Bulgaria and Hungary usually showed the lowest increase. While we did not attempt to measure abiotic site heterogeneity, this ranking coincides with the particularly high visible site heterogeneity of the Turkish site (many stone of different size at or near the surface, variable microtopograhy) and the known small-scale heterogeneity in historic land use in the Italian site. Taking a simple β-diversity measure (cumulative richness of all blocks of a country / mean rooted richness of 10 cm × 10 cm; Appendix S1), Italy had also by far the most heterogeneous vegetation, but France and Italy were only in the middle range. On the other side, Germany with the on average lowest richness gains, was also the country with the lowest β-diversity value and a visually particularly homogenous stand.

Regarding the recording methodology, the relative increase of richness (in %) for the same spatial arrangement was nearly always higher for shoot presence than for rooted presence, typically with a factor of approx. 1.5 (see Tables 1 and 2, Appendices S2, S3 and S5). This could be explained by the increasing length of the margin in less compact sampling units, which influences the richness in case of rooted presence directly (e.g. Dengler 2003), but only indirectly via vicinism (i.e. atypical species that occur inside the plot only due to high diaspore pressure from neighbouring communities) in the case of rooted presence.

Grain size had limited effects on relative richness gains compared with site-specific factors or recording methodology. Indeed, mean richness gains for 4:1 vs. 1:1 plots were nearly indistinguishable between grain sizes of 0.01, 0.04 and 0.16 m² for rooted presence (see Table 1), and varied only moderately in response to dispersion (see Table 2). While for logistical reasons (work effort) we could study only very small grain sizes, this relative scale invariance indicates that the patterns will likely remain similar for grain sizes that are one to three orders of magnitude larger, thus in the normal range of vegetation plots in herbaceous vegetation (Chytrý & Otypková 2003). Other studies have demonstrated that the slope of the species-area relationship (which is closely related to the distance decay) often remains relatively constant over many orders of magnitude (Dengler & Boch 2008; Wilson et al. 2012).

Consequences for future studies

Clear guidelines on vegetation recording are critical for accurate assessments and monitoring of species richness and biodiversity responses to global change. Our key findings are that richness values of sampling units with very different compactness and dispersion are not directly comparable. The concept of "effective area" may however help overcome this problem and allow robust cross-site comparisons (Lazarina et al. 2014)). Effective area A_e is here defined as the equivalent square-shaped area that contains the same number of species as an elongated, dispersed or otherwise irregular sampling unit. While Lazarina et al. (2014) required A_e only to be contiguous, we more precisely specify it to be square-shaped to allow also comparison between contiguous sampling plots of different compactness. While circles are even more compact than squares, their richness values in reality differ only negligibly from those of squares (Stohlgren 2007 and see extrapolation below); moreover, circular sampling units are rare for vegetation data and inexistent for atlas data, so that using squares as baseline is sensible.

Applying the concept of effective area to our results (Appendix S5) provides an easily understandable interpretation of the effects of different arrangements of sampling units. For rooted presence and 0.01 m², for example, a 4:1 rectangle was on average as rich as a square of the 1.06-fold area, while randomly dispersed micro-quadrats within the whole site correspond to a square of the 1.93-fold area. The largest relative A_e for means across countries of 4.51 was found for the latter arrangement in the case of 0.04 m² grain size and shoot presence (Appendix S5). The maximum value for an individual site was even 6.05 for this arrangement and 0.01 m² grain size in Turkey (not shown). Among others, Appendix S5 demonstrates that 16:1 rectangles and a sampling unit consisting of 16 micro-quadrats randomly distributed within an 8 × 8 square had a similar effective area of 1.23 times that of a contiguous square (rooted presence; 1.45 times for shoot presence).

Another way to compare different spatial arrangements of sampling plots is to quantify and test the effects of their spatial extents A_{extent}. One of the easiest ways of making A_{extent} of any spatial arrangement comparable is to use the size of the smallest circle that encompasses the complete sampling unit. When at the same time the grain size is kept constant, this allows to calculate species-extent relationships (SERs) similar to species-area relationships (SARs), which we introduce here as a new concept. Doing so for the mean values of rooted presence at 0.01 m² grain size across all six countries, yields an unexpectedly tight relationship with R^2 = 0.994 (Fig. 3), despite the very different spatial arrangements involved. With a *z*-value (slope in double-logarithmic space) of only 0.039 the species increase with increasing spatial extent

is much lower than with increasing grain size (there we had a z-value of 0.378), but still appreciable. Since this relationship is so tight, one can use it for predicting richness differences of any spatial arrangement of sampling units totalling 0.04 m² relative to a square of that size. Using the regression function, for example, a circle of 0.04 m² in our grasslands would only have 1.7% fewer species than a square – no wonder that Stohlgren (2007) with his relatively few replicates could not find any difference in such a comparison. Taking species-area and species-extent relationships together and assuming power functions (as they were well supported here and in many other studies), one gets:

$$\log S = \log c + z \log A + z_{\text{extent}} \log A_{\text{extent, relative}},$$

with S = species richness, A = surface area of the sampling unit, $A_{\text{extent, relative}}$ = area of the circle that comprises the whole sampling unit, standardised by the area of a circle that comprises a square of the same surface area, z = slope of the species-area relationship, z_{extent} = slope of species-extent relationship.

Finally, considering the typical richness gains of various spatial arrangements of sampling units, how should species richness data then be sampled best? Some researchers have suggested that a sampling approach is preferable over another if it finds more species on the same area A of the combined sampling units (e.g. Stohlgren 2007; Bacaro et al. in press). They argue that spatial arrangements with maximum ratio of A_e / A (i.e. with high length-width ratio or high dispersion) would be preferable because one would find more species on the same area. This line of reasoning is however questionable for two reasons. Firstly the additional effort for delimitating more complicated sampling units with increased border length will often increase the overall time needed to record one species on average. Secondly, obtaining high richness values is generally less important than the ability to compare values with those from similar studies. We believe that a square sampling unit, despite having a very low effective area, is the most advantageous shape. This, together with the fact that the large majority of legacy data has been sampled on squared plots, makes compact squares in most cases the best choice for sampling units.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Characterisation of the study sites.

Appendix S2. Species richness (shoot presence) for square (1:1) plots of 4, 16 and 64 cells size (0.01 m², 0.04 m² and 0.16 m²) and the relative richness increase of rectangles (4:1 and 16:1) compared to squares of the same size.

Appendix S3. Species richness (shoot presence) for square (1:1) plots of 4, 16 and 64 cells size (0.01 m², 0.04 m² and 0.16 m²) and the relative richness increase for discontiguous "plots" of the same size drawn randomly from within different spatial extents.

Appendix S4. Relative increase in species richness (shoot presence) of various contiguous (B–C) and discontiguous (D–F) arrangements of micro-quadrats of total areas of 4, 16 and 64 cells (0.01, 0.04 and 0.16 m²).

Appendix S5. Effective areas that correspond to the five different spatial arrangements of sampling units used for richness counts in this study.

628 **Table 1.** Species richness (rooted presence) for square plots (1:1) of 4, 16 and 64 cells in size (0.01
629 m^2, 0.04 m^2 and 0.16 m^2) and the relative richness increase of rectangles (4:1 and 16:1) compared to
630 squares of the same size. Values are means for the six study sites (FR: France; DE: Germany; BG:
631 Bulgaria; HU: Hungary; IT: Italy; TR: Turkey) and an overall mean. Significance of differences is
632 given according to a mixed linear model per site (n.s.: $p \geq 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p <$
633 0.001).

Cells	Parameter	FR	DE	BG	HU	IT	TR	Mean
4	Richness (1:1)	4.9	8.0	5.3	5.3	6.5	7.0	6.2
	4:1 vs. 1:1	2.9% *	2.6% *	-0.3% n.s.	1.8% n.s.	2.5% n.s.	4.1% **	2.3%
16	Richness (1:1)	7.3	11.9	10.5	10.0	14.5	11.2	10.9
	4:1 vs. 1:1	2.4% n.s.	2.3% n.s.	0.4% n.s.	1.2% n.s.	2.7% n.s.	3.8% n.s.	2.1%
	16:1 vs. 1:1	9.6% ***	7.7% ***	5.0% n.s.	7.6% *	9.0% ***	11.1% ***	8.3%
64	Richness (1:1)	10.6	16.0	19.4	16.5	26.5	17.1	17.7
	4:1 vs. 1:1	3.3% n.s.	1.2% n.s.	-1.4% n.s.	5.0% n.s.	2.1% n.s.	2.7% n.s.	2.1%
	16:1 vs. 1:1	8.1% n.s.	5.6% n.s.	-1.2% n.s.	7.6% *	10.1% **	11.3% **	6.9%

634

635 **Table 2.** Species richness (rooted presence) for square (1:1) plots of 4, 16 and 64 cells size (0.01 m², 0.04 m² and
636 0.16 m²) and relative richness increase for discontiguous sampling units of the same size drawn randomly from
637 within subblocks of 8 × 8 cells (Sub), within blocks (Block) or within sites (All). Values are means for the six
638 study sites (country acronyms according to Table 1) and an overall mean. Significance of differences is given
639 according to a mixed linear model per site (n.s.: $p \geq 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

Cells	Parameter	FR	DE	BG	HU	IT	TR	Mean
4	Richness square	4.9	8.0	5.3	5.3	6.5	7.0	6.2
	Sub vs. 1:1	8.8% ***	6.8% ***	-0.6% n.s.	5.5% **	12.3% ***	7.7% ***	6.8%
	Block vs. 1:1	18.6% ***	10.1% ***	9.6% ***	9.7% ***	15.3% ***	14.7% ***	13.0%
	All vs. 1:1	33.2% ***	23.5% ***	18.3% ***	18.4% ***	31.1% ***	45.2% ***	28.3%
16	Richness square	7.3	12.0	10.5	10.1	14.5	11.2	10.9
	Sub vs. 1:1	11.9% ***	4.3% *	6.6% *	7.3% ***	9.7% ***	6.2% *	7.7%
	Block vs. 1:1	29.2% ***	15.5% ***	16.4% ***	16.0% ***	26.6% ***	25.4% ***	21.5%
	All vs. 1:1	51.0% ***	30.0% ***	36.7% ***	31.9% ***	54.9% ***	63.6% ***	44.7%
64	Richness square	10.6	16.0	19.4	16.5	26.5	17.1	17.7
	Block vs. 1:1	23.4% ***	14.5% ***	10.1% **	14.3% ***	19.7% ***	23.9% ***	17.6%
	All vs. 1:1	47.2% ***	31.0% ***	39.7% ***	35.7% ***	57.2% ***	66.7% ***	46.3%

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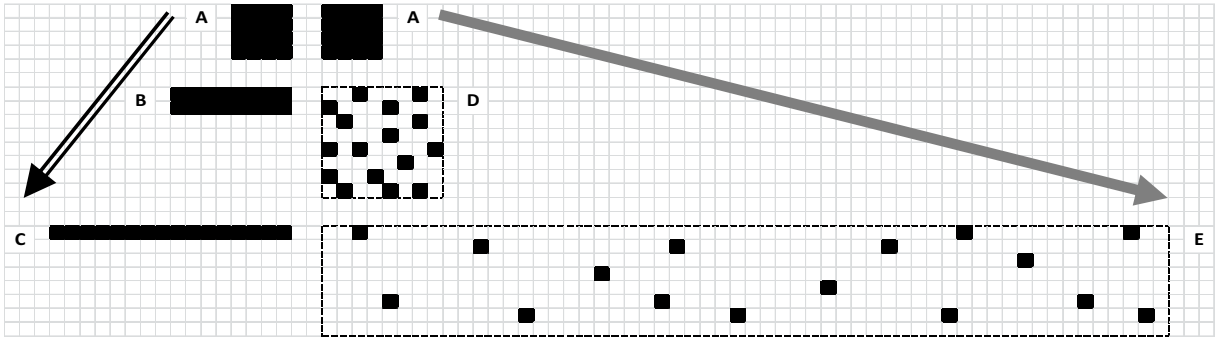
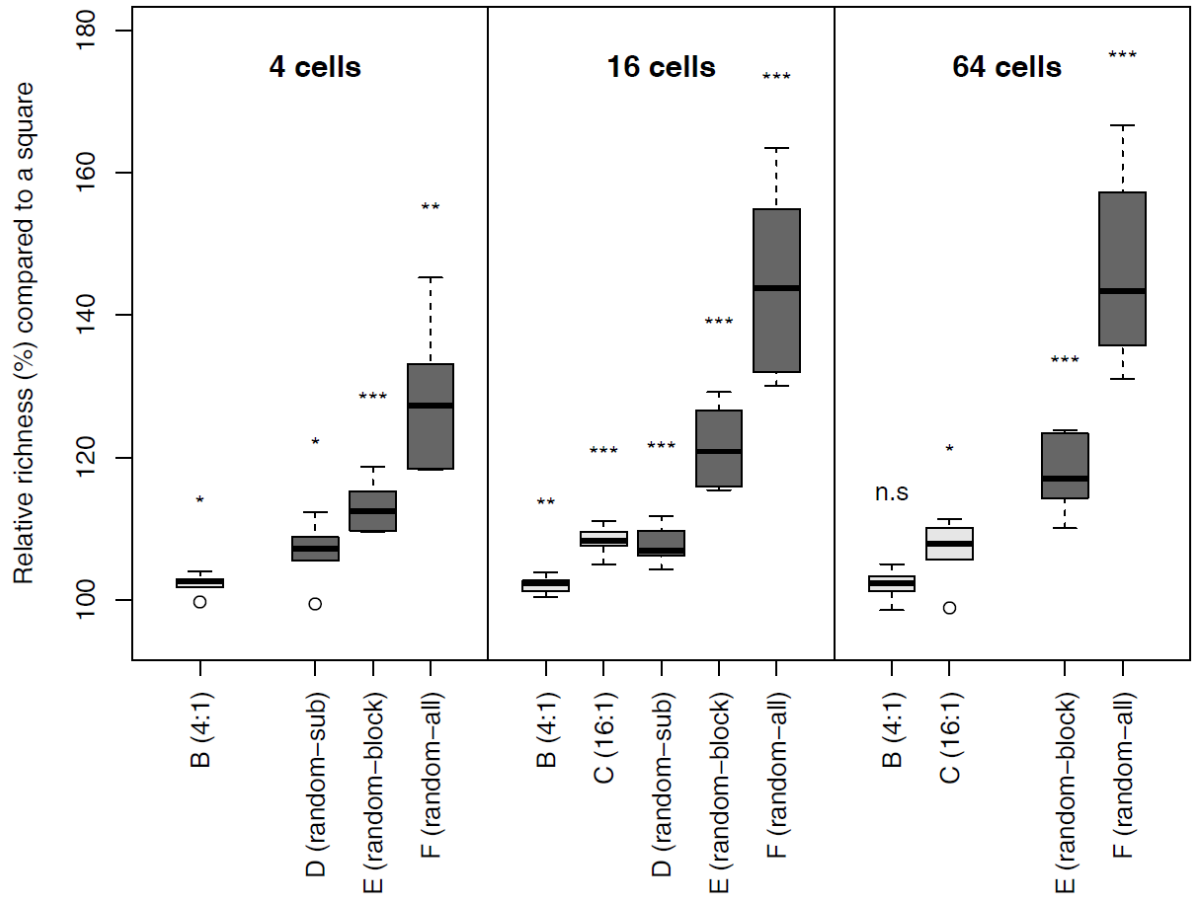
641



642
643

644 **Fig. 1.** Schematic visualisation of the arrangement of micro-quadrats that form a 16-cell sampling unit in the
645 case of different shapes (A: 1:1; B: 4:1; C: 16:1) and dispersions (A: contiguous; D: discontiguous from
646 subblock; E: discontiguous from block; not shown F: discontiguous from all six blocks of a site). The black
647 arrow symbolises the transition from a compact shape to more and more elongated shapes and the grey arrow the
648 transition from a contiguous arrangement to more and more discontiguous (dispersed) arrangements.



649

650 **Fig. 2.** Relative increase in species richness compared to square plots (1:1) of the same size (= 100%) (rooted
651 presence) of various contiguous (B–C) and discontiguous (D–F) arrangements of micro-quadrats of total areas of
652 4, 16 and 64 cells (0.01, 0.04 and 0.16 m²). The boxplots are based on the mean values of the six study sites;
653 asterisks indicate the significance of differences compared to squares (100%), based on *t*-tests. The sampling
654 designs are: B = rectangle with 4:1 ratio; C = thin elongated with 16:1 ratio; D = discontiguous with random
655 draw from within a subblock of 8 × 8 cells; E = discontiguous with random draw from within a block; F =
656 discontiguous with random draw across all blocks of a site.

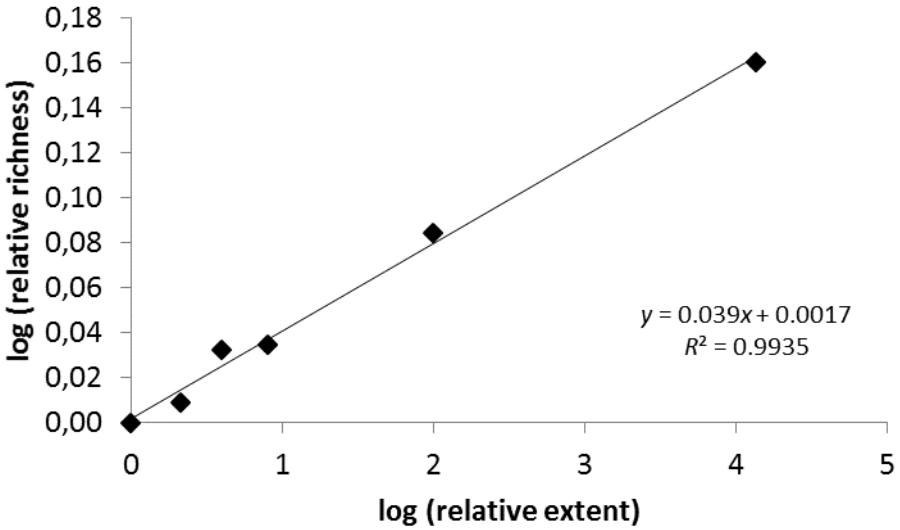


Fig. 3. Example of a species-extent relationship for a comparison of our six different spatial arrangements (square, two types of rectangles, three types of dispersed plots) for 0.04 m², shoot presence and means across all six countries. Both axes are standardised by the values of a square, i.e. the square appears in the origin of the graph. Note that the relative extent for the least compact arrangement (micro-quadrats dispersed across all blocks of a site) varies somehow across countries and is here given as the maximum. If the exact block arrangement had been identical in all countries, the point would lie further to the left and thus the relationship would be even tighter.

Appendix S1. Characterisation of the study sites, arranged according to increasing mean annual temperature. Mean annual temperature and mean annual precipitation are based on Worldclim 5' data (Hijmans et al. 2005).

Site	Latitude (°N)	Longitude (°E)	Elevation (m a.s.l.)	Mean annual temperature (°C)	Mean annual precipitation (mm)	Dominant graminoids (frequency order)	Total species richness (rooted) of a 100-cm² square (α)	Total species richness of all six blocks (γ)	β diversity (γ / α)
France (FR): Laqueuille	45.6	2.7	1040	7.0	1200	*Poa pratensis* agg., *Poa trivialis, Lolium perenne*	4.9	28	5.7
Germany (DE): Bayreuth	49.9	11.6	365	8.2	724	*Festuca rubra, Luzula campestris* agg, *Antoxanthum odoratum.*	8.0	33	4.1
Bulgaria (BG): Sofia	42.7	23.3	650	10.2	559	*Poa pratensis* agg., *Cynodon dactylon, Dactylis glomerata,*	5.3	61	11.5
Hungary (HU): Tiszaalpar	46.8	20.0	100	10.5	550	*Cynodon dactylon, Festuca pseudovina, Poa pratensis* agg.	5.3	41	7.7
Italy (IT): Camerino	43.2	13.1	546	12.1	880	*Dactylis glomerata, Lolium perenne, Elymus repens*	6.5	114	17.5
Turkey (TR): Manisa	38.7	27.3	70	17.0	695	*Bromus chrysopogon, Taeniatherum caput-medusae* agg., *Poa timoleontis*	7.0	45	6.4

669 **Appendix S2.** Species richness (shoot presence) for square (1:1) plots of 4, 16 and 64 cells size (0.01 m², 0.04
670 m² and 0.16 m²) and the relative richness increase of rectangles (4:1 and 16:1) compared to squares of the same
671 size. Values are means for the four study sites (country acronyms according to Table 1) and an overall mean.
672 Significance of differences is given according to a mixed linear model per site (n.s.: $p \geq 0.05$, *: $p < 0.05$, **: $p <$
673 0.01, ***: $p < 0.001$).

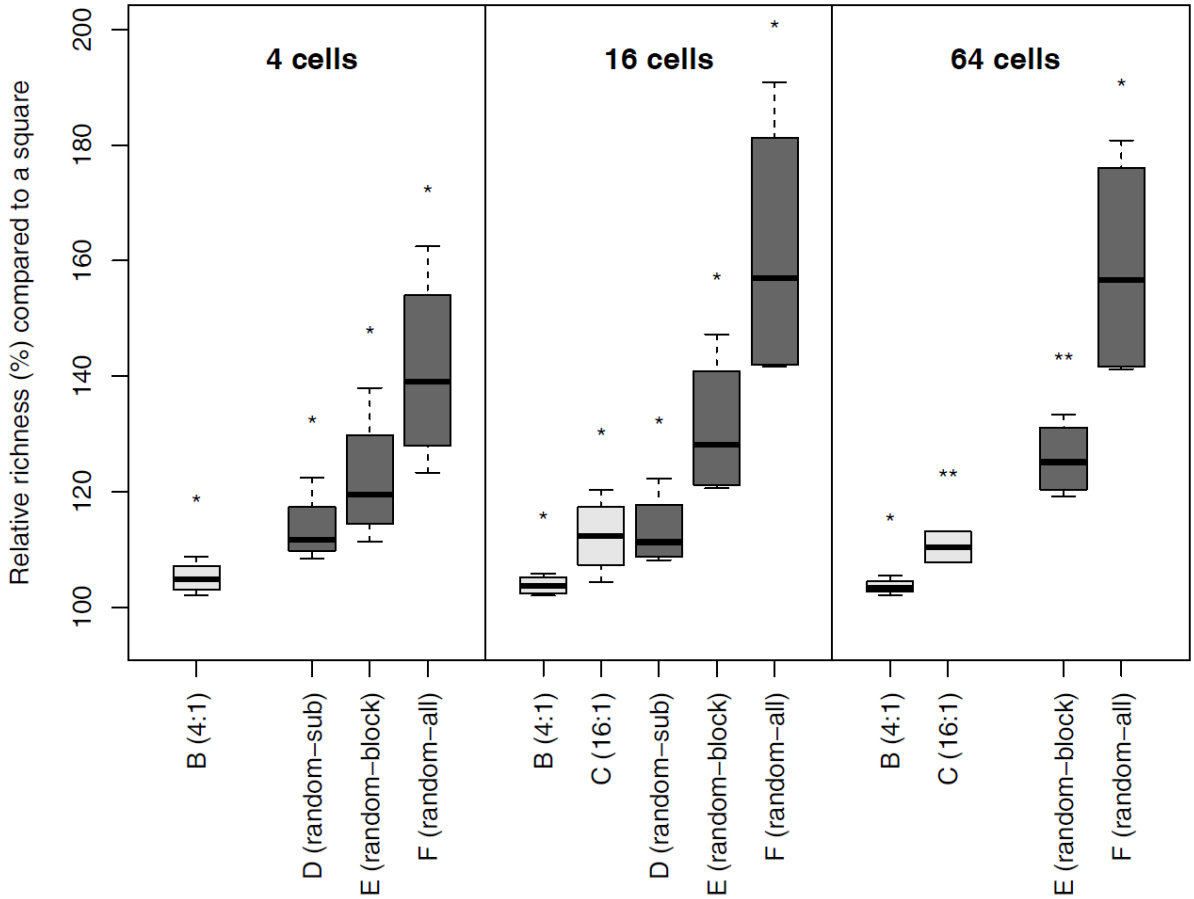
Cells	Size	DE	HU	IT	TR	Mean
4	Richness square	9.6	5.9	10.7	8.3	8.6
	4:1 vs. 1:1	4.1% ***	2.0% n.s.	8.8% ***	5.5% ***	5.1%
16	Richness square	13.0	10.7	18.6	12.9	13.8
	4:1 vs. 1:1	2.7% n.s.	2.0% n.s.	5.9% *	4.7% *	3.8%
	16:1 vs. 1:1	4.4% ***	10.3% ***	20.4% ***	14.3% ***	12.0%
64	Richness square	16.6	17.0	30.4	19.1	20.8
	4:1 vs. 1:1	3.4% n.s.	5.5% n.s.	2.2% n.s.	3.3% n.s.	3.6%
	16:1 vs. 1:1	7.9% *	7.7% *	13.2% ***	13.0% ***	10.5%

674

675 **Appendix S3.** Species richness (shoot presence) for square (1:1) plots of 4, 16 and 64 cells size (0.01 m², 0.04
676 m² and 0.16 m²) and the relative richness increase for discontiguous sampling units of the same size drawn
677 randomly from within subblocks of 8 × 8 cells (Sub), within blocks (Block) or within sites (All). Values are
678 means for the four study sites (country acronyms according to Table 1) and an overall mean. Significance of
679 differences is given according to a mixed linear model per site (n.s.: $p \geq 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p <$
680 0.001).

Cells	Parameter	DE	HU	IT	TR	Mean
4	Richness square	9.6	5.9	10.7	8.3	8.6
	Sub vs. 1:1	12.3% ***	8.5% ***	22.5% ***	11.1% ***	13.6%
	Block vs. 1:1	17.6% ***	11.4% ***	38.0% ***	21.5% ***	22.1%
	All vs. 1:1	32.7% ***	23.3% ***	62.5% ***	45.5% ***	41.0%
16	Richness square	13.0	10.7	18.6	12.9	13.8
	Sub vs. 1:1	9.3% ***	8.1% ***	22.3% ***	13.3% ***	13.3%
	Block vs. 1:1	21.8% ***	20.6% ***	47.3% ***	34.5% ***	31.1%
	All vs. 1:1	41.6% ***	42.3% ***	90.9% ***	71.8% ***	61.6%
64	Richness square	16.6	17.0	30.4	19.1	20.8
	Block vs. 1:1	19.3% ***	21.4% ***	33.4% ***	28.8% ***	25.7%
	All vs. 1:1	42.2% ***	41.2% ***	80.8% ***	71.2% ***	58.9%

681



682

683 **Appendix S4.** Relative increase in species richness (shoot presence) of various contiguous (B–C) and
684 discontiguous (D–F) arrangements of micro-quadrats of total areas of 4, 16 and 64 cells (0.01, 0.04 and 0.16 m²)
685 compared to squared plots (1:1) of the same size (A). The boxplots are based on the mean values of the four
686 study sites; asterisks indicate the significance of differences compared to squares (100%), based on *t*-tests. The
687 sampling designs are: B = rectangle with 4:1 ratio; C = thin elongated with 16:1 ratio; D = discontiguous with
688 random draw from within a subblock of 8 × 8 cells; E = discontiguous with random draw from within a block; F
689 = discontiguous with random draw across all blocks of a site.

Appendix S5. Effective areas that correspond to the five different spatial arrangements of sampling units used for richness counts as compared in this study. For each of the two recording schemes (rooted presence, shoot presence) and for each of the three grain sizes, this table reports the area of a square that would contain the same species richness on average. Both richness and effective area are given relative to the square as the most compact arrangement included in the study. The values are means of six countries (rooted presence) and four countries (shoot presence), respectively. The calculations are based on power-law regressions through the mean richness values of squares of 0.01, 0.04 and 0.16 m² (in the double-log representation). The regression functions were (with logarithms to the base of 10; S = species richness; A = area in m²): $\log S = 1.5548 + 0.3847 \log A$; $R^2 = 0.9981$ (rooted presence) and $\log S = 1.5761 + 0.3185 \log A$; $R^2 = 0.9983$ (shoot presence).

	Rooted presence						**Shoot presence**					
	0.01 m²		**0.04 m²**		**0.16 m²**		**0.01 m²**		**0.04 m²**		**0.16 m²**	
Arrangement	**Relative richness**	**Relative effective area**	**Relative richness**	**Relative effective area**	**Relative richness**	**Relative effective area**	**Relative richness**	**Relative effective area**	**Relative richness**	**Relative effective area**	**Relative richness**	**Relative effective area**
B: 4:1 Rectangle	1.023	1.06	1.021	1.06	1.021	1.06	1.051	1.17	1.038	1.12	1.036	1.12
C: 16:1 Rectangle	NA	NA	1.083	1.23	1.069	1.19	NA	NA	1.120	1.43	1.105	1.37
D: Dispersed within subblock	1.068	1.19	1.077	1.22	NA	NA	1.136	1.49	1.133	1.48	NA	NA
E: Dispersed within block	1.130	1.38	1.215	1.67	1.176	1.53	1.221	1.87	1.311	2.34	1.257	2.05
F: Dispersed within site	1.283	1.93	1.447	2.66	1.463	2.73	1.410	2.94	1.616	4.51	1.589	4.28