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Standardised EDGG methodology for sampling grassland diversity: second amendment

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Abstract: The EDGG methodology of sampling multi-scale biodiversity in grasslands and other open habitats is widely applied and has proven to be a highly informative and effective way of recording high-quality data allowing for a multitude of different analyses. Based on our experiences with sampling, storing and analysing such data, here we propose three additions to the protocol: (1) We recommend to record also 1000-m² plots in addition to the hitherto seven standard grain sizes of 0.0001–100 m², as 1000 m² is a standard grain size in many international studies. (2) Recording species cover also for grain sizes larger than 10 m² (where hitherto only presence-absence was recorded) can be done efficiently by noting these values only for the additional species in the larger plot and for those that show a strong deviation from the average of the two 10-m² plots. (3) Finally, sampling biomass is valuable for analyses of the productivity/disturbance and of nutrient limitations. Both aspects can be covered by harvesting aboveground biomass in two random subplots of 20 cm x 20 cm (0.08 m² in total) and fractioning the material into necromass, living bryophytes and lichens, living herbs and living woody species. While Addition 2 hardly requires any additional time and thus should be implemented always, Additions 1 and 3 come with significant additional effort, which normally pays off, but suggests that in case of time limitations they might be restricted to a representative subset of plots in a study.

Keywords: biodiversity monitoring; biomass; cover estimate; EDGG Biodiversity Plot; grassland; multi-scale; nutrient limitation; open vegetation; productivity; scale dependence; standardised sampling; vegetation sampling.

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Introduction

Since 2009, the Eurasian Dry Grassland Group (EDGG) has been conducting annual Field Workshops (Dengler et al. 2009b; Dengler et al. 2016a) to collect standardised multi-scale and multi-taxon biodiversity data of grasslands and other non-forest habitats of the Palaearctic biogeographic realm. The basic concept of sampling was proposed by Dengler (2009), and a detailed protocol was later published by Dengler et al. (2016b). While initially these Field Workshops were focused on dry grasslands in the wider sense, other grassland types have been progressively included over the years, for example, alpine and mesic grasslands in the Field Workshop in Navarre (Biurrun et al. 2014) and a wide range of subalpine and alpine open habitats in the last Field Workshop in Switzerland (Dengler et al. 2020b). The Field Workshops have given rise to a series of regional studies of patterns and drivers of scale-dependent diversity of vascular plants, bryophytes and lichens (Turtureanu et al. 2014; Kuzemko et al. 2016; Polyakova et al. 2016; Dembicz et al. 2021a). Moreover, they together account for the core of EDGG vegetation-plot database GrassPlot (Dengler et al. 2018; Biurrun et al. 2019), which in turn provides the basis for comprehensive studies across the Palaearctic biogeographic realm (e.g. Dengler et al. 2020a; Dembicz et al. 2021b; Zhang et al. 2021). The standardised EDGG sampling approach has been also taken up by various other researchers in dry grasslands (Mardari & Tănase 2016; Talebi et al. 2021), dunes (Torca 2020), salt marshes (Campos et al. 2021), wet grasslands (Jensen et al. 2013) and for a multitude of vegetation types in the biodiversity monitoring program of South Tyrol, Italy (see Hilpold et al. 2020). Altogether this demonstrates how versatile and informative the approach is. In other words, it allows the creation of a set of high-quality data with moderate time effort, which, in turn, support a multitude of different analyses. Based on the experience gained during the Field Workshops and when analysing the data, we regularly refined the methodology (see Dengler et al. 2016b). More recently, a first formal amend-

ment was published, proposing a way to add orthopteroid insects as a fourth taxonomic group to the standard Field Workshop sampling (Hilpold et al. 2020). With this second amendment we want to propose three further additions, some of which were already (partly) implemented during recent Field Workshops: (1) 1,000-m² plots, (2) cover estimates also for larger plots, and (3) modified biomass sampling.

Addition 1: 1,000-m² plots

The GrassPlot database considers 1,000 m² as the eighth standard grain size to be collected in addition to 0.0001, 0.001, 0.01, 0.1, 1, 10 and 100 m² (Dengler et al. 2018; Biurrun et al. 2019). However, in the EDGG Biodiversity Plots (Dengler et al. 2016b) this grain size was not included so far, mainly because of the high sampling effort. Sampling all the grain sizes up to 100 m² in species rich grasslands can take already several hours (pers. observation from Field Workshops), while Dolnik (2003) reported between five and seven hours needed for multi-scale sampling of not particularly species rich grassland habitats of the Curonian Spit up to 900 m² (one well experienced botanist, smaller grain sizes not duplicated as in standard EDGG sampling). In consequence, GrassPlot currently comprises only 187 plots of 1,000 m² (including those of 900 m² or 1,024 m²), while there are 6,321 plots of 100 m² and 10,531 plots of 10 m² (GrassPlot v. 2.10; https://edgg.org/databases/GrasslandDiversityExplorer). Moreover, the currently included plots of that grain size are biased towards species-poor regions and vegetation types (see Fig. 1), preventing reliable richness estimates of that grain size across the Palaearctic. This is particularly unfortunate, as in other widely applied biodiversity sampling methodologies, like Whittaker plots (Stohlgren et al. 1995), the Carolina Vegetation Survey (Peet et al. 2012) and BIOTA Africa (Jürgens et al. 2012), 1,000 m² is one of the main grain sizes. We thus propose to add 1,000 m² as an optional new grain size to the EDGG standard methodology. Because careful collection of all terricolous species in 1,000 m² is time-consuming, and collecting the data in a less comprehensive manner than the smaller grain sizes would be futile, we recommend adding this grain size only for a subset of EDGG Biodiversity Plots. They should be selected in a way that (a) they are representative for the whole range of vegetation types in a study (i.e. not only for those stands that are relatively poorer in species) and (b) the surroundings of the 100-m² plots belong to the same main vegetation type. Both points are only relevant for maximising the utility of the plots within the GrassPlot database. For other purposes, it could make sense to disregard them. We acknowledge that for some vegetation types included in GrassPlot, such as spring vegetation, it might be hard or impossible to find patches that allow sampling of a 1,000 m² plot. To ensure that the smaller plots are on average as representative for the 1,000 m² as possible, one should arrange the largest plot in a way that the 100-m² is in its centre, not in a corner (see Dengler 2009). In Figure 2, we propose how this can be done practically.

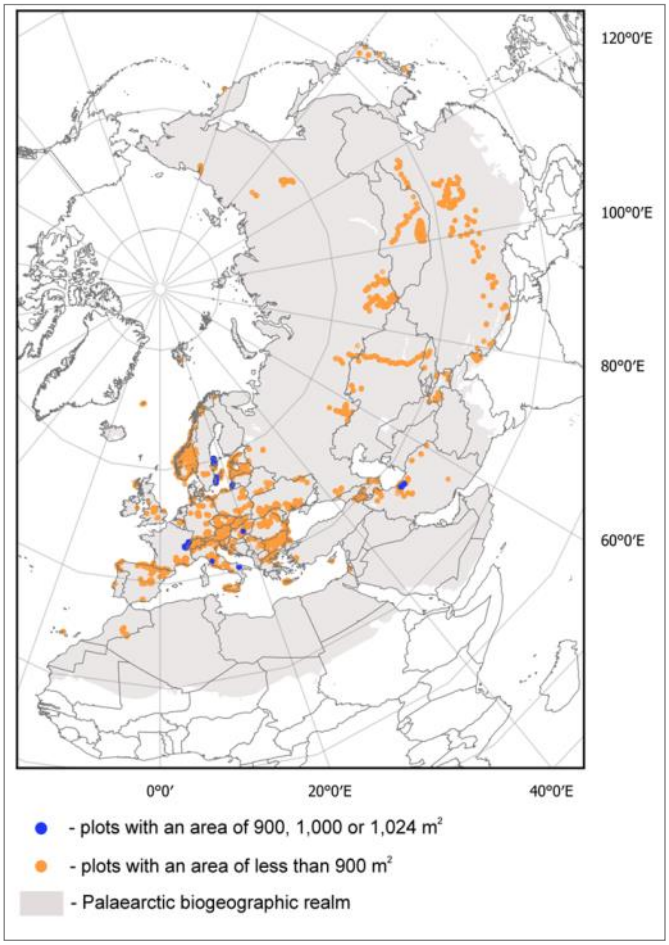

Fig. 1. Distribution of 1,000-m² plots (± 10%) in the GrassPlot database in relation to the distribution of plots of the grain sizes from 0.0001–100 m² (map based on GrassPlot version 2.10).

Addition 2: Cover data also for larger grain sizes

According to standard EDGG sampling (Dengler et al. 2016b), species cover is only recorded for the 10-m² grain size, while for all other grain sizes only presence-absence information is collected. Based on recent paper projects using GrassPlot or the European Vegetation Archive (EVA; http://euroveg.org/eva-database; see Chytrý et al. 2016), we came to the conclusion that it would be highly valuable to have cover values also for other grain sizes. For example, the GrassPlot project #15 of W. Ulrich (pers. comm., see project description at https://edgg.org/databases/GrassPlot) studies species-abundance distributions across scales, but as datasets with cover data across multiple grains are hitherto very rare in GrassPlot, the authors created "virtual" plots of larger size by combining an increasing number of non-adjacent 10-m² plots of the same vegetation type in the same region. While this approach was considered acceptable for this specific question, it is certainly not optimal. EVA projects, on the other hand, often select only for certain grain sizes, and grain sizes of 10 m² and below are often excluded to limit the disturbing effects of varying plot sizes, for example, for classification (Dengler et al.

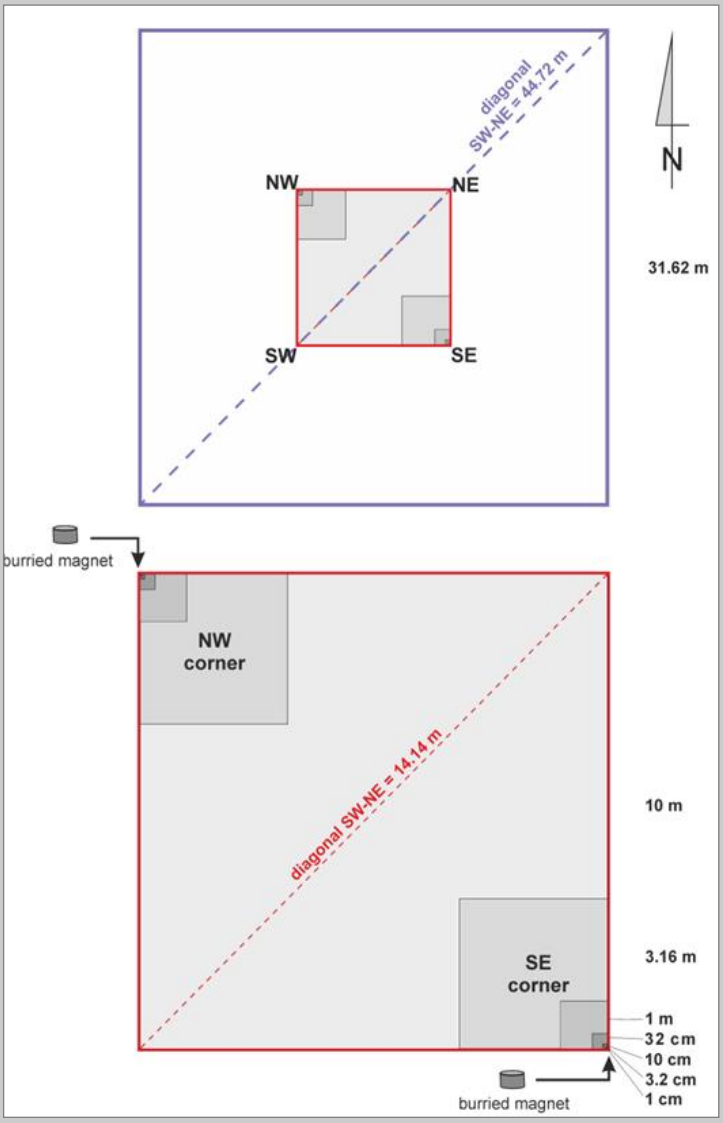

Fig. 2. Proposed arrangement of the 100-m² multiscale-sampling plots with additional sampling of 1,000 m². The delimitation needs one 50-m tape measure, two 10-m tape measures, two folding rules, a coloured string/tape of at least 124 m length, 10 metal pins and four larger pointed poles (from wood or metal). The procedure for setting up the plots is best conducted in the following sequence: Part A (100-m² plot). (1) Mark a suitable starting point with the metal pin, fix the 50-m tape there and direct a person with a compass into NE direction. (2) Mark the position 14.14 m on the tape with a second pin, but without fixing the tape in this corner. (3) Give way to slightly more than 20 m of tape. (4) One person with a third pin pulls the tape at the 10-m position to the NW. (5) Then a person at the NE corner pulls the tape carefully until it reaches exactly 20 m at the NE pin and fixes it there by turning it a few times around the pin. (6) The person at the NW position follows this inward movement, keeps both sides of the tape straight and carefully fixes the pin in the NW corner without disturbing the vertical structure of the vegetation. (7) Steps (3) to (6) are repeated analogously for the creation of the SE corner. Part B (nested subplot series of 0.0001–10 m²). (8) Mark the positions on the 50-m tape that are 3.16 m away from the NW corner with a pin each. (9) Fix the 10-m tape with the first pin at 0 m and the second pin at 6.32 m. (10) Pull the tape at the 3.16 m position inward until both sides are straight and fix it there with a pin. (11) Areas from 0.01 to 1 m² are temporarily laid out with folding rules bound in a 90° angle; areas of 0.0001 and 0.001 m² are normally not laid out at all (because of the disturbance caused at these small scales) – instead the folding rule is just used to measure whether a certain plant individual is inside or not. Part C (1000-m² plot). (12) Now you can remove the 50-m tape from the outline of the 100-m² plot, but keep it fixed in its SW corner (If you have an additional tape available, you can also leave the original tape in its position to keep the visibility of the already sampled parts). (13) Extend the tape in a straight line over the NE corner until 29.43 m (= 14.14 m + (44.72 m – 14.14 m) / 2) are reached, where you fix the large pole (NE corner of 1000-m² plot). (14) Repeat the procedure analogously to create the three other corners. (15) Mark the outline of the 1000-m² plot with the coloured string/tape.

2009a). To allow the usage of EDGG Field Workshop data in such cases, we started to "impute" cover values for the 100-m² grain of EDGG Biodiversity Plots in GrassPlot post hoc (i.e. assign the mean cover of the two 10-m² plots when present in at least one of these and an arbitrarily low value when only present in the remaining 80 m²). While this is a reasonable approximation in most cases, it is hard to decide what the arbitrarily low cover value of the additional species should be (0.1%, 0.01% or 0.001%?) and this approach does not account for the uneven distribution of species in space, even when the plots are selected for relative homogeneity. For the future we therefore suggest a way in which much better cover data for 100 m² (and potentially 1,000 m²) could be achieved with minimal extra effort: one should just estimate the cover at these larger grain sizes for the additional species and for those species where the average of the two 10-m² subplots would be much too high or much too low. To facilitate the cover estimation, we provide here a reference how percentage cover values correspond to filled squares of a certain edge length in case of 10-m², 100-m² and 1,000-m² plots (Table 1). While recording cover for the larger grain sizes in that way comes with very small additional effort, recording cover also for the grain sizes below 10 m² would be more additional time effort (because there all species would have to be estimated) and also less often needed. Therefore, we do not include the latter into the standard EDGG methodology; nevertheless data with such additional information can be included and appropriately stored in GrassPlot database (where they are highly welcome!).

Table 1. Indication of the size of a square (length of one side given in m) to which a certain % cover corresponds for the three given standard plot sizes. Example: 0.001% cover in a 10-m² plot corresponds to a square of 0.01 m (1 cm) fully covered by one plant species.

Plot size	0.001%	0.01%	0.1%	0.2%	0.5%	1%	2%	5%	7%	10%	15%	20%
10 m²	0.01	0.03	0.10	0.14	0.22	0.32	0.45	0.71	0.84	1.00	1.22	1.41
100 m²	0.03	0.10	0.32	0.45	0.71	1.00	1.41	2.24	2.65	3.16	3.87	4.47
1,000 m²	0.10	0.32	1.00	1.41	2.24	3.16	4.47	7.07	8.37	10.00	12.25	14.14

Addition 3: Biomass sampling

Several years ago, Dengler et al. (2016b: C.4) recommended sampling aboveground biomass of representative subplots of defined surface within the 10-m². At that time, we left it open whether it should be a pooled biomass and necromass sample or whether and how it should be fractioned. We also did not give clear reasons why to sample biomass at all. One motivation for biomass data is to use it as a proxy of productivity and (absence) of disturbance. The more living and dead aboveground biomass is there, the higher is the productivity and/or the lower is the disturbance. According to Grime (2001), one should expect a unimodal relationship of species richness to this combined value of biomass. In this respect, it would make sense to pool all aboveground fractions, which is the easiest way of sampling. Another option would be to separate into three fractions already in the field: (a) living vascular plants, (b) living non-vascular plants and (c) necromass. It is a bit more time-consuming, but would also allow us to distinguish between the two dimensions productivity (positively related to peak living biomass in communities dominated by life forms other than phanerophytes) and disturbance (negatively related to necromass). However, recently Wassen (2021) brought another aspect of biomass to our attention: dried biomass is a very effective means to determine the type of nutrient (co-) limitation under which a plant community grows by determining the ratios of different elements (Wassen et al. 2005, 2021). This is much easier than soil analyses (which are impeded by the fact that it is hard to determine which fraction of an element is really available for plants). Contents and ratios of P, N and K can be determined from ground samples of air-dried biomass with standard elemental analysers, not requiring any further treatment in the field (Wassen 2021). The only limitation for that type of analysis is that it requires subsetting to only the living biomass of the non-woody vascular plants, i.e. excluding dwarf shrubs, young phanerophytes, bryophytes, lichens and necromass. Taking both possible analyses of sampled biomass together we now recommend the following procedure:

Aboveground biomass should be sampled in two randomly placed subplots of 20 cm x 20 cm (0.04 m²) within the 10-m² plots applying the rooted presence method and using a scissor or a knife. We are not aware of an established standard of cutting height. Evidently, there is a trade-off between collecting the above-ground biomass as completely as possible and having a big by-catch of non-desired materials, such as soil particles which are heavy and thus could bias the results more than small pieces of missing stems. We recommend that researchers apply a pragmatic solution, which might be approximately 1 cm above soil surface. In the field, the biomass should be split into the four fractions (i) necromass (litter + dead wood), (ii) living bryophytes and lichens, (iii) living herbs and (iv) living woody species (dwarf shrubs and young phanerophytes). During the expedition the biomass should be air-dried and prior to analysis dried at 65 °C to constant weight. The four fractions should then be weighed and recorded as dry mass per m². Fraction (iii) should be ground and then used for determination of elemental contents and ratios. If the sampling of biomass should be too time consuming for all plots of a Field Workshop (or another project), we recommend to do it only in one of the corners of the EDGG Biodiversity Plots and in a random subset of the normal plots. Biomass sampling should preferentially be done around the annual peak biomass, and not shortly after a severe disturbance event (mowing, intensive grazing, fire).

If sampled during 2021, M. Wassen (pers. comm., see Wassen 2021) would generally be interested in receiving the (iii) samples for a pan-Palaearctic project and then conduct the lab analyses. If interested in this offer, please contact him beforehand (m.j.wassen@uu.nl).

Conclusions and outlook

In this article we propose three potential additions to the EDGG standard sampling methodology. They are inspired by the general philosophy of this approach (a) to base every methodological step on careful considerations and not on blindly following traditions, and (b) to counterbalance additional time effort vs. gained additional information. While we consider all three elements as optional, we are convinced that in most cases the additional efforts (meaning the reduced number of plots that could be sampled) is more than compensated by additional analytical options gained. In Appendix S1 in Supporting Information we provide an updated form to incorporate the three additional elements in field recording.

While Addition 2 comes with almost negligible additional effort, Additions 1 and 3 have significant additional effort. Therefore, in case of Addition 1 and possibly also Addition 3, we recommend implementing them only for a subset of plots. If you opt for this solution, however, it will be crucial that you do this for a representative subset. We hope that this second Amendment to the EDGG sampling methodology (Dengler et al. 2016b) after Hilpold et al (2020) will be followed widely and prove to yield important additional insights. Being involved in many broad-scale analyses using plot data, we will continue to observe needs for analyses,

and, if we find a certain modification or addition to have a good cost-return ratio propose them in future amendments.

Author contributions

All three authors are jointly involved in the organisation of EDGG Field Workshops and in the governance of the GrassPlot database. J.D. had the idea of the paper and drafted the manuscript, I.D. prepared the figures, while I.B. and I.D. revised and augmented the text.

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Supporting Information

Appendix S1. Updated recording form for EDGG Biodiversity Plots.