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Scale-dependent biases in species counts in a grassland

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Abstract. Numbers of plant species were recorded in species-rich meadows in the Bílé Karpaty Mts., SE Czech Republic, with the aim to evaluate the sampling error made by well-trained observers. Five observers recorded vascular plants in seven plots ranging from 9.8 cm^2 to 4 m^2 independently and were not time-limited. In larger plots a discrepancy of 10-20% was found between individual estimates, in smaller plots discrepancy increased to 33%, on average. The gain in observed species richness by combining records of individual observers (in comparison with the mean numbers estimated by single observers) decreased from the smallest plot (27-82% for two to five observers) to the largest one (13-25%). However, after misidentified and suspicious records were eliminated, the gain was much lower and became scale-independent; two observers added 12% species, on average, and the increase by combining species lists made by three or more observers was negligible (3% more on average). It is concluded that most discrepancies between individual observers were caused by misidentification of rare seedlings and young plants. We suggest that in species-rich meadows plants should be recorded by at least three observers together and that they should consult all problematic plant specimens together in the field, to minimize errors.

Keywords: Sampling error; Species richness estimate; Species-rich meadow.

Introduction

Plant species lists are increasingly needed for many purposes (Boulinier et al. 1998; Keating et al. 1998). In simple descriptive inventories, vegetation analyses and in biodiversity studies high quality plant censuses are required (Hawksworth 1996; Pickett et al. 1997). With notable exceptions species lists recorded in the field or compiled from literature are treated as exact; errors in species number estimates have rarely been considered or studied. However, it has been shown that even between two experienced persons collecting data in exactly the same area there can be a considerable discrepancy, called 'pseudoturnover' by Nilsson & Nilsson (1985), 'spurious turnover' by Rusch & van der Maarel (1992), sampling error by Lepš & Hadincová (1992) or bias in sampling by Wilson (1995). For example, Kirby et al. (1986) found that only 53-76% of species was recorded by different observers in the same forest and in the same season. Sampling errors reported by other authors are usually lower. From islands 0.3-2.19 ha in size Nilsson & Nilsson (1985) reported a mean sampling error of 11.4%. In fixed quadrats, 5 m × 5 m in size, situated in grasslands, Lepš & Hadincová (1992) found a mean difference in species-richness estimates between two observers of 13%.

Several factors have been reported to be responsible for the sampling errors. For example, the numbers recorded by the observers may be decreased because of problems distinguishing similar plants (Nilsson & Nilsson 1985; Kirby et al. 1986; Wilson 1995). Overlooking seedlings may also reduce the number of recorded species, whereas plant misidentification may result in an increase in species number if the seedling is identified as a plant not yet present in the list. Expectations of the species number may affect efficiency and sampling time of individual observers. Some authors have a tendency to increase their effort when the number of species so far recorded is unusually low (Lepš & Hadincová 1992) whereas others tend to search for species more thoroughly in quadrats with many species (Wilson 1995). Some other factors may affect the quality of census data, such as bad weather (either too hot or too cold, windy or rainy) and long working days, when the observer becomes tired. All these factors have a negative impact on the intentness to the work. Sampling efficiency is another factor which may affect species numbers (Nilsson & Nilsson 1985; Kirby et al. 1986). After all, collaborators may either disturb the observer or stimulate him/her, depending on the circumstances. Apparently, there are many factors which affect the quality of plant censuses, and in many cases their effect can be either negative or positive, depending on other factors.

In this paper we present data based on a simple

experiment in which we attempted to rule out most of the above factors. Five observers, intensively trained in plant searching and identification in the neighbourhood of the experimental plot before the experiment, made their censuses in small, exactly delimited plots within a few days, working independently. They were not allowed to disturb the vegetation in the studied plots and their work was not time-limited. Fortunately, the weather was not too bad to affect any of the observers. Therefore, most 'external disturbances' were ruled out and our comparison should reflect the differences in abilities of individual observers to find and identify plants, and not their background and training.

The sampling errors can possibly be reduced if records by individual observers are combined. We used this approach to estimate the gain in species number by a combination of records by two to five observers. Some literature data suggest that sampling errors may depend on the spatial scale (Nilsson & Nilsson 1985). Therefore, we performed our experiment in plots ranging from 9.8 cm^2 to 4 m^2, to see the effect of various plot sizes.

For the experiment we selected a species-rich grassland, where plant identification is relatively difficult because most plant individuals are perennial and usually sterile, multiplying vegetatively. Moreover, most individuals belonged to grasses and *Carex* species which are often difficult to identify. Due to these circumstances we believe that in other plant communities of the temperate zone the sampling errors made by well-trained observers under optimal conditions should be lower than those reported here. On the other hand, the discrepancies in the census data, shown in this paper, are likely close to the minimum which can be achieved in similar plant stands because the presented results reflect an ideal situation in which the observers minimize all possible factors causing discrepancies between their census data.

The study area

The experiment was carried out in the core of the National Nature Reserve of Čertoryje, Bílé Karpaty Mts., Czech Republic (48° 54' N, 17° 25' E). Mean monthly temperatures were 9.4 °C and the mean annual precipitation was 464.1 mm during the last 10 years (meteorological station at Strážnice, 8 km from the plot). The experimental area was situated in a grassland with scattered *Quercus* spp. trees, at an altitude of 440 m a.s.l., on a SW-faced slope with an inclination of 5°. Soil conditions were as follows: pH [H_2O]: 5.99; total nitrogen: 0.47%; total carbon: 5.45%. Ca: 8.42 mg/g; K: 0.05 mg/g; Mg: 0.72 mg/g; Na: 0.052 mg/g; P: 0.234 mg/g (estimated in a 1M $BaCl_2$ extract, with the ICP OES method). The grassland has not been fertilised in the past decades. The following species attained a mean cover of 2% or more in June 1998: *Bromus erectus* (21%), *Carex montana* (16%), *Molinia arundinacea* (6%), *Cirsium pannonicum* (4%), *Prunella grandiflora* (3%), *Viola hirta* (2%), *Potentilla alba* (2%), and *Brachypodium pinnatum* (2%) (estimated in 25 plots, 0.5 m^2 each, situated 20 m from the studied area; Klimeš unpubl.). The maximum above-ground biomass was about 250 g/m^2 in 1998 and the total cover of vascular plants was about 70%. A more detailed description of the species composition of the grassland and its abiotic environment is given in Jongepierová et al. (1994), Klimeš et al. (1995) and Klimeš (1999).

Methods

Seven non-overlapping plots were randomly placed and fixed in an apparently homogeneous and species-rich grassland area, 8 m × 8 m in size. The largest plot was 4 m^2 in size. The next plots were always 4 × smaller than the previous one, so that the smallest plot was about 9.8 cm^2 in size. The three smallest plots were circular and delimited by a wire; their diameters were ca. 3.5, 7.1 and 14.1 cm, respectively. The medium plots were quadrats, 25 cm × 25 cm and 50 cm × 50 cm in size, delimited by a rope. The two largest plots were rectangles, 0.5 m × 2 m and 0.5 m × 8 m in size, also delimited by a rope. The observers were not allowed to enter the plots to prevent trampling and other disturbance. Therefore, species censuses were made while lying or kneeling on the ground.

In June 1998, before the experiment started, the observers spent about a week identifying plants in a large number of small plots in the surroundings of the experimental plots. Therefore, they saw nearly all plants occurring in the studied area many times, including different ontogenetic stages. They also repeatedly discussed uncertain identifications and corrected their records, if necessary. The five observers were either professional botanists or PhD students with at least three-years of experience with the flora of the Bílé Karpaty Mts. Therefore, their backgrounds were similar.

During the experiment each observer worked completely independently in each of the seven plots, without discussing the numbers of recorded species or difficulties with plant identification. The order of the plots was not fixed. Some observers did their work within one day, others during two days. However, sampling time per plot and for all seven plots was not limited to remove the effect of differences in efficiency of individual observers. The sampling time, i.e. the time required to census plants in a plot, was recorded. After the five observers finished their censuses, the first author combined the species lists and tried to confirm in the field species occurrences in particular plots recorded by a single observer (further on called

singletons) or by two observers (doubletons) only.

Data analysis was performed using an original data set and a data set from which suspicious records – singletons and doubletons – not found by the first author at the end of the experiment were removed (further on called corrected data). Note that the corrected data are not necessary free of errors because some species could have been overlooked at the end of the experiment, especially in larger plots. Therefore, the real species numbers probably are slightly higher than the corrected estimates.

The gain in species number by a combination of records by two to five observers was estimated from all possible combinations of the given number of observers and compared with the mean numbers estimated by single observers.

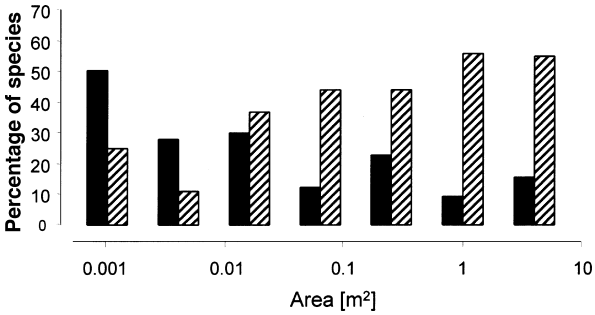

Fig. 1. Percentage of species recorded by one observer (singletons; full bars) and by all five observers (hatched bars) in plots of various sizes. 100% corresponds to the total number of species recorded in a particular plot.

Results

Species richness

A total of 112 vascular plants were recorded by the five observers in the seven plots (based on uncorrected data). None of the species were found in all plots and by all observers. The most frequent species, *Viola hirta*, was found in 4.9 plots on average (out of 7), followed by *Leontodon hispidus, Linum catharticum, Brachypodium pinnatum, Bromus erectus* and *Carex caryophyllea.* 12 species were found once and by a single observer. Altogether 48 singletons were recorded in the seven plots. Two species were recorded as singletons in three plots, nine species in two plots, and 37 species in one plot. The percentage of singletons decreased with increasing plot size whereas the percentage of plants recorded by all five observers increased (Fig. 1). In the smallest plot about 50% of species belonged to singletons, whereas in the 4 m^2 plot 16% were singletons.

The percentage of singletons was approximately the same with four observers, one observer (E) recorded about two times more singletons in smaller plots than the others. The percentage of singletons recorded in the smallest plot increased with sampling time ($r = 0.836$, $P < 0.02$; regression analysis).

The number of species recorded in small plots was relatively low. However, in the largest plots a high species richness was found. In 1 m^2 58 to 67 species were recorded by individual observers, in the 4-m^2 plot, 71 to 88 species were found (Table 1).

Combining uncorrected species lists by individual observers increased the species numbers considerably, especially in larger plots. The difference between the corrected and uncorrected data increased if data from more observers were combined. Mean species gain by a combination of the uncorrected data from two observers was nearly 40% in small plots and 12% in the largest plot (Fig. 2A). The gain by combining the data of three, four or five observers was also considerable – nearly 20% per observer more in the smallest plots. In larger plots the gain decreased if data from more observers were combined, being about 2% when the last observer was added (Fig. 2A). However, after the data were corrected for suspicious records, the scale-dependency became poor and the gain decreased dramatically (Fig. 2B). The gain in species number by combining records of two observers decreased to 9-20% and adding data of one more observer further increased the species number by 2.6% only. The fourth observer added about 1% and the fifth observer only 0.3% of species, on average (Fig. 2B).

Table 1. Number of species found by the five observers (A to E) in plots of different sizes. Based on the uncorrected data. Sampling time (minutes) in brackets.

Observer/area [m^2]	0.000977	0.003906	0.015625	0.0625	0.25	1	4
A	4 (5)	11 (19)	19 (27)	30 (40)	39 (49)	58 (54)	74 (61)
B	4 (2)	9 (9)	17 (9)	29 (16)	43 (26)	65 (59)	88 (111)
C	5 (5)	12 (13)	19 (23)	30 (36)	39 (50)	67 (79)	84 (180)
D	4 (2)	10 (5)	18 (22)	29 (15)	38 (30)	61 (43)	76 (78)
E	5 (8)	8 (14)	21 (13)	27 (23)	40 (38)	60 (50)	71 (80)
Observers A-E combined	8	18	29	41	54	75	99

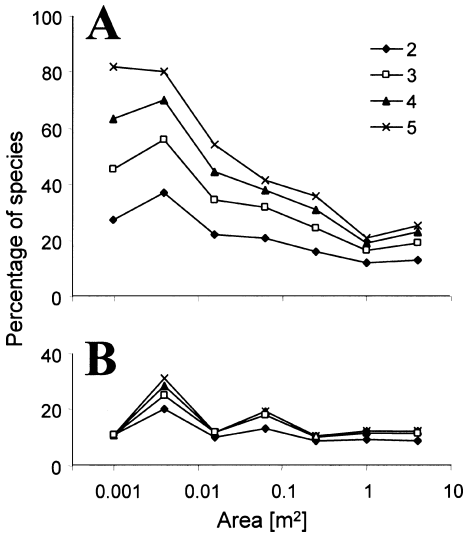

Fig. 2. Mean gain in species richness (%) by combining records of two to five observers. 100% corresponds to the mean number estimated by single observers. **A.** Original data. **B.** Corrected data.

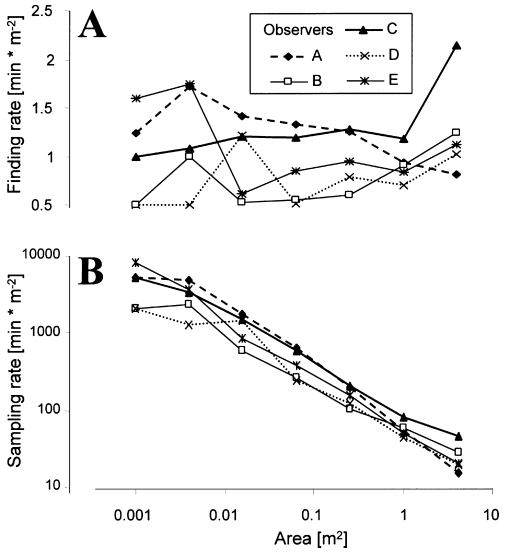

Fig. 3. Scale-dependent finding rate (mean number of species recorded per minute) (**A**) and sampling rate (mean time needed to census plants in 1 m^2) (**B**) by the five observers.

Sampling efficiency

The time needed for a plant census in individual plots markedly differed between individual observers (Table 1). For example, plant census in the smallest plot was made in 2 to 8 minutes and in the 1-m^2 plot in 43 to 79 minutes. The variation of the sampling time decreased with increasing plot size, from the smallest plot to the 1-m^2 plot. However, it then increased in the largest plot where 61 to 180 minutes were needed. Consequently, the scale-dependent finding rate (mean time needed to find a species) also differed between individual observers (Fig. 3A). In smaller plots the pattern was unclear but in the largest plots the finding rate usually increased. Observer A showed an opposite trend; except for the smallest plot, his finding rate decreased monotonously with plot size (Fig. 3A).

The sampling rate (mean time needed to census plants on 1 m^2) decreased with increasing area according to the power function in all observers (Fig. 3B). However, in some plots the observers behaved unpredictably, spending either too little time (all observers except for C, in the smallest plot) or unexpectedly much time there (observer D in the 0.016-m^2 plot, observer C in the 4-m^2 plot).

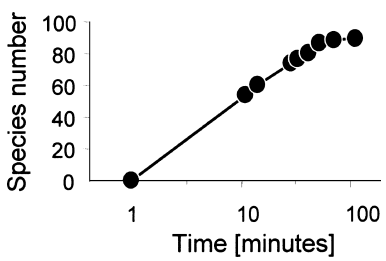

Fig. 4. Time-dependent accumulation of species numbers recorded by observer B in the 4-m^2 plot; accumulated number of species = 49.79 * log(time [minutes]) + 0.985, $R^2 = 0.998$, $P < 0.0001$; regression analysis, the two last values were removed from the analysis.

Discussion

Sampling efficiency and time-limitation

Sampling rate (mean time needed to census plants at 1 m^2) has been suggested to be one of the key factors responsible for the discrepancy between censuses of individual observers (Kirby et al. 1986; Wilson 1995). In our experiment the sampling rate was much lower than that by Lepš & Hadincová (1992), who spent maximally 40 minutes per plot of 25 m^2. However, in their plots only 18.6 species per plot occurred on average (combined records), whereas in our area more than 128 to 138 species can be expected in plots of the same size, as calculated from the species-area relationship. Still, sampling rate cannot explain this difference because the relationship between the time spent and the accumulated number of species recorded is curvilinear. During the first few minutes of a census the number of recorded species is limited by the observer's ability to write down plant names, whereas later on much of the time is used for searching for small and sterile specimens. After time was log-transformed and the two last values were removed, accumulation of species numbers in time becomes linear (Fig. 4). The fitted line indicates that 83% of species were ob-

served in 25% of the time and 97% in 46% of the time. During the next 40 minutes only one species was found. Therefore, even if the time was much reduced, the number of recorded species would remain high. These results indicate that time limitation need not be the main factor causing incompleteness of a species census (but see Kirby et al. 1986). A comparison of observers A and B supports this view. In the 4-m^2 plot observer A spent ca. 50% of the time observer B needed. The first observer found 14 species less than B. However, if B had stopped the search at the same time as A, he would have found, according to Fig. 4, only two or three species less than when recording species for 50 minutes more. Therefore, the great difference in sampling time between the two observers does not explain the discrepancy in their species numbers.

Fortunately, sampling time can be relatively easily controlled using the relationship between the number of species so far recorded and time passed (Fig. 4). According to the curve in Fig. 4 and our experience from other species-rich meadows a plant census should not be finished unless no new plant species is found in an interval of about 5 minutes in plots 0.25 to 4 m^2 in size. For larger plots this limit should be increased because more time is spent by walking.

In our experiment variation of the sampling time decreased with increasing plot size, as one may expect. However, in the largest plot the minimum and maximum sampling time differed with a factor three. Moreover, except for one observer the finding rate increased in the largest plot. These data indicate that in the largest plot some observers needed a long time before they considered their work finished. As expected, the observers markedly differed in the time they spent in searching for the last few species. The data by a single observer (A) indicate that a low finding rate in the largest plot could limit the number of recorded species. His species numbers in smaller plots were above the mean whereas in larger plots they were below it. All other observers spent sufficient time at the plots recording all species which they were capable of recognizing.

Plant misidentification and data correction

The meadows of the studied area are often called 'flower-rich'. However, this does not mean that most plant individuals are regularly flowering. For example, in a plot 2.25 m^2 in size only 5.9% shoots of vascular plants were fertile in June 1998 (Klimeš unpubl.). Therefore, extensive knowledge of all plants at sterile stages is necessary before a plant census can be started in meadows similar to those we have studied. Identification of recently established plants may cause serious problems, especially in smaller plots where adult conspecifics can rarely be found in the plot. In June, the best time to do a plant census, in the plot reported above, 4.0% of the shoots were seedlings. Three of them were not present as adult conspecifics (Klimeš unpubl.). Therefore, we attempted to identify all young plants, including the seedlings.

According to the inspection done at the end of the experiment most singletons belonged to seedlings and young, poorly differentiated plants. Out of them some were misidentified or overlooked by several observers. Many problems can be solved if a lens is used (e.g., *Leontodon hispidus* vs. *Silene nutans* vs. *Taraxacum* sect. *Ruderalia*; *Centaurea jacea* vs. *C. scabiosa*; *Filipendula vulgaris* vs. *Potentilla heptaphylla*), which however becomes more difficult if plants cannot be removed from a plot and the number of seedlings is high.

The experience of individual observers may vary substantially. Even observers with a lot of experience but from different regions or trained in different vegetation types may have serious problems with identifying sterile plants. This was shown by Tüxen (1972), who presented species lists recorded by 11 phytosociologists from different European countries in a 12-m^2 grassland plot in Germany. Some of them had serious problems identifying even the dominant grass of the stand. They recorded between 17 and 33 species, and in one case only 7 species were shared by two observers.

In our experiment, those who took part in the experiment were trained in plant identification in the studied area at least one week before the experiment started. With a few exceptions the observers did not have any problems with the identification of adult plants, even if sterile. However, some species pairs are well known to be difficult to identify if they are represented by sterile individuals and untypical or young specimens only. In our plots plants belonging to several species pairs were repeatedly confused. For example, *Poa angustifolia* can be misidentified with young plants of *Festuca rupicola*, unless the folded leaves are opened, which itself is not easy. If the number of shoots belonging to the two grasses is a few hundred, checking them all is impossible. Similarly, just emerging sterile shoots of *Carex caryophyllea* produced by hypogeogeneous rhizomes can be confused with shoots of *C. michelii* or *C. tomentosa*, and atypical shoots of *C. flacca* can be misidentified with *C. panicea* shoots. The fact that in some plots some observers recorded one of the three *Carex* species and other observers a different one, indicates that there can be confusion.

It is very likely that most dubious records in our data were among singletons. Some of the plants recorded in particular plots by two observers also appeared to be misidentifications, whereas all plants recorded in a plot by three or more observers were found to be correct. In small plots some observers had the tendency to identify even poorly developed seedlings. Therefore, the proportion of singletons was relatively high there and increased with

sampling time. Most of these singletons were later proved to be misidentifications. The effort to identify each single plant at any ontogenetic stage therefore seems to be contra-productive, because it results in a high number of errors. Similarly, the difference between the number of species in combined records of several observers based on uncorrected and corrected data increased if records of more observers were combined. This also indicates that by a combination of several uncorrected records the species numbers increased mainly because of an accumulation of errors. This problem can partly be solved if individual observers work together and consult all problems in the field. Our data suggest that in this case three observers may find up to 98% of species which seems to be satisfying for most purposes.

In some cases the difficulties with plant identification are solved by merging similar species (e. g. Rusch & van der Maarel 1992). Species aggregates represent a special problem because proper identification of microspecies represented by sterile plants is usually impossible. The situation is even worse with some apomictic taxa which do not differ much in their environmental demands and may occur therefore together. In this case even fertile plants are usually not identified to species (e.g., *Taraxacum* sect. *Ruderalia* in our plots; *Hieracium* subg. *Pilosella* and *Rubus fruticosus* agg. in other stands).

Conclusions

1. The discrepancy in species number estimates of vascular plants between individual observers ranged between 10 and 20%.

2. The gain in species richness caused by combining species lists recorded by five observers decreased from the smallest plot (82%) to the largest plot (25%). However, after suspicious and misidentified records were corrected, the gain became scale-independent, being 12% for two observers combined, on average. The increase due to the combining of species lists recorded by three or more observers was negligible.

3. Most discrepancies between individual observers were caused by misidentification of rare seedlings and young plants.

4. It is suggested that in species-rich meadows at least three observers should record plants together and problematic records should be checked in the field, to minimize errors.

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