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How To Efficiently Survey Floral Resource Availability and Diversity for Pollinating Insects in Semi-Natural Grasslands

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ABSTRACT

Floral resources are the foundation for insect pollinators, on which we in turn rely on for food and ecosystem functioning. Global and regional trends show that insect pollinators become increasingly endangered with modernization and intensification of land use, and thus the need to find ways of conserving insect pollinators and their habitats become more crucial. There has yet to be developed an agreed upon standard practice for surveying floral resources to assess habitat quality for pollinators. Here, I provide much needed information on the pros and cons of the two most common sampling approaches; transects and plots, and three observational metrics; inflorescence counts, occurrence in subplots (frequency) and percentage cover estimates. The comparisons were based on three basic diversity indices: species richness, abundance, and the Shannon-Weiner diversity index. The results mainly depended on the observation metrics, not the sampling approach. Both plot-based methods, i.e., occurrence in subplots and percentage cover estimates, were superior to transects with inflorescence counts, both in species detection and robustness to differences in vegetation and biomass. Occurrence in subplots seemed less prone to overestimation of clustered species and thus provided diversity measures that were more representative of the species composition at the different sites than the two other methods. Future surveys of floral resources in semi-natural grasslands should be based on vegetative cover, in order to increase efficiency of the surveys by reducing the need for repeated sampling throughout the flowering season. Vegetation surveys should also take into account the practical implications of surveyor ability of detecting species by adjusting sample unit size to allow the surveyor to get a good overview of the plot.

Keywords: Comparison, floral resources, inflorescence, plots, pollinators, semi-natural grasslands, transects.

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1. INTRODUCTION

Vegetation, the assemblage of plant species and the ground cover they provide, is a key component of most terrestrial ecosystems and the basis for many of the ecosystem services we rely upon. While we rely on plants to provide us with food such as grains, nuts, fruit and vegetables, 87.5% of all extant flowering (angiosperm) plant species depend on the interaction with animals and invertebrates to assist with reproduction through pollination (Kearns et al 1998; Ollerton et al. 2011). Plants with biotic pollination (PBP) make up about 35% of the global food production, whereas yields of about 75% of crop types worldwide are improved by biotic pollination (Midgley & Bond 1991; Allen-Wardell et al. 1998; Richards 2001; Klein et al. 2007; Gallai et al. 2009; Vanbergen 2013). Even though we are so dependent on these plants and their pollinators, they are in need of conservation measures (Van Swaay et al. 2013; Ollerton et al. 2014; Potts et al. 2016; Woodcock et al. 2016). Insects, the most important group of pollinators, are shown to have rapid declines in population and diversity with a review of 303 studies showing declines in species richness of 43% and 62% reduction in abundance with increasing urbanization and further 75% drop in pollinator abundance in pastures with increased intensification of land-use (Millard et al. 2021). A recent study from Germany showed that flying insect biomass had a seasonal decrease of 76% and mid-summer decline of 82% in the period 1989-2016 (Hallmann et al. 2017). These negative trends are also the case for plants, as shown by Wesche et al. (2012), the species richness and functional diversity of plants in managed grasslands in Central Europe have decreased by 30-50% over the last 50 years. The main cause of these declines is attributed to land-use intensification, particularly in agricultural landscapes (Wesche 2012; Van Swaay et al. 2013; Ollerton et al. 2014; Potts et al. 2016; Woodcock et al. 2016; Hallmann et al. 2017; Sánchez-Bayo & Wyckhuys 2019). As a response, Dicks et al. (2012) have identified key knowledge needs for evidence-based conservation of wild insect pollinators. One of which was the need to find out what floral resources are currently available to pollinators at landscape scale, and if these resources are changing. Another key knowledge need was the need for quantification of flower resources in the landscape collected alongside pollinator monitoring, including timing of flower blooming.

To meet the key knowledge needs proposed by Dicks et al., it is necessary with standardization of methods for acquiring the knowledge (2012). The methods used for assessing floral resources to date vary a lot among studies, as there is no agreed upon standard method. This causes a problem for nature managers and decision makers that are to apply and compare results from studies and monitoring. The methods vary in both spatial resolution and metrics of the explanatory variables, detectability of rare taxa, cost-efficiency, and study design (including the degree of bias). Szigeti et al. (2016b) did a review study of methods used in 158 studies that investigated floral resource availability for insect pollinators in the period 1981-2015. The study showed that regardless of the method used, the studies did not refer to existing protocols and had insufficient descriptions of their methodology. The most common sampling units were plots, or quadrats (used in 60.4% of the studies), followed by transects (used in 34% of the studies). The main difference between these two sampling units is the shape. Plots are most often smaller and squared and transects are longer (Küchler & Zonneveld 1988; Goslee 2006). These can be used separately or applied together, as seen in the Modified Whittaker non-nested plot and North Carolina Vegetation Survey nested plot (Stohlgren et al. 1995; Peet et al. 1998).

Sampling units may vary in characteristics such as size and shape. In the studies reviewed by Szigeti et al. (2016b) squared plots were the most widely used plots with 41.7% of the 60.4% and belt transects the most widely used type of transects with 87% of the 34% (Alarcón et al. 2008; Burkle et al. 2013).

The shape of the sample units is not necessarily too important, as long as they allow the surveyor to assess all parts of the sample unit area, which will become increasingly difficult with increasing sample unit size. With regard to size, studies show that an increase in size leads to a decrease in accuracy of abundance estimates, at the same time as it increases the number of species detected (McCune & Lesica 1992; Jalonen 1998). The area of sampling unit, site area and proportion of site covered differ greatly between the studies in the review, with area of sampling unit ranging from 0.3 m^2 to 310 000 m^2 and site area ranging from 8 m^2 to 125 km^2 (Wolf & Moritz 2008; Blaauw & Isaacs 2014; Wilkerson et al. 2014; Tadey 2015). With the wide range in spatial extent of study area in these studies, it is understandable that also resolution and methods will vary. However, some generalizations can and *should* be made to allow for better comparisons among studies and reuse of data.

Standardization of methods should be made at the level of sampling units and with respect to what metric to quantify, in addition to minimum requirements for sampling effort. The sampling units can mainly differ in size and shape, while observation metrics are more difficult as the large variation in plant morphology sometimes make it difficult to delimit applicable units, such as inflorescences and cover estimates. Some of the studies in the review from Szigeti et al. (2016b) used categorical values that could mediate this to some extent (Table 1). Another critical issue when it comes to observation metrics is the description of their delimitation and interpretation, which many of the studies reviewed by Szigeti et al. (2016b) failed to describe. This was particularly relevant for the metrics; "floral unit", "flower" and the cover estimates. Proper descriptions of how these metrics should be counted and estimated are necessary for repeatability and comparison among studies, as shown in the supplementary material of this study (Appendix I). An important concept for vegetation sampling is the species-area curve, coined by Gleason (1922), which relates to the saturation of species detected with increase in sample size, either by increase in sampling unit size or number of samples. Today the number of samples or sample area needed to meet the point of saturation for a given site or community, also known as the "minimum area", can be simulated with the use of statistical models, such as simulation-based sampling protocol (SSP) or rarefaction (Chao & Lost 2012; Chao et al. 2014; Andrade et al. 2019; Guerra-Castro et al. 2021).

Table 1: Observation metrics used and their frequency in the studies from the review of Szigeti et al. (2016b) with proportions that used categorical estimates and direct counts.

Metric	% of studies	% with categorical estimates	% with direct counts
Floral unit	28.8	15.21	84.78
Flower	24.4	36.11	63.89
Flowering shoot	13.5	9.52	90.48
Flower cover	12.8	95	5
Inflorescences	10.3	18.75	81.25
Green cover	7.7	100	0
Frequency of flowering shoots	1.9	0	100

Vegetation surveys and assessments of floral resources must also consider the phenology and seasonal changes in vegetation, particularly when it comes to the use of floral units. Several studies have stressed the importance of the timing and frequency of sampling (Alarcón et al. 2008; Hegland et al. 2010; Szigeti et al. 2016b). Hegland et al. (2010) showed that sampling with transects during the general peak of the flowering season would reduce the cost of sampling by 20%, while still detecting 70-85% of the most functionally important species for pollinators. However, a critique of this reduction of the temporal scale in studies and focus on common species is that it neglects the importance of rare species (Goulson & Darvill 2004; Szigeti et al. 2016b). The rare species often being a focal concern of conservation and nature management. Two of the relevant key knowledge needs stressed by Dicks et al. (2012), in addition to the ones previously mentioned, were the need for information about the timing of flower bloom and the effects of climate change. Both of which require information about floral unit estimates and repeated sampling throughout the vegetation season. This would increase the sensitivity to detection of changes in phenology as a response to climate change and potential mismatches in phenological interactions between species groups and migration patterns, causing imbalances in ecosystems (Hjort 1914; Cushing 1969, 1990; Kudo & Ida 2013). As we can see, there is a lot of research done to meet the key knowledge needs about plant-insect interactions. Yet, there is no agreed upon method for how to best assess floral resources.

The aim of this study was to provide knowledge about the pros and cons of two of the most common sampling units; plots and transects, in addition to test three different observation metrics. The research objective of this study is to compare the different survey methods' suitability for assessing floral resource availability using three common diversity indices within and among sites. The diversity metrics I have used here are species richness, abundance and diversity using the Shannon-Wiener diversity index (H) (Shannon 1948; Jalonen et al. 1998; Yorks & Dabydeen 1998; Little 2013; Andrade et al. 2019). Species richness was first coined by McIntosh and is one of the most fundamental measurements of ecological diversity and biodiversity and is simply a measure of species recorded in a unit of study (McIntosh 1967). Abundance, which can be divided into total abundance and relative abundance, is the quantity of the species present by a given metric (Peet 1974). When these indices are recorded within a plot, or square, the list with data is called a relevé (Küchler & Zonneveld 1988). The hypotheses of the study are 1) that plot-based methods are more efficient at describing the three diversity indices of semi-natural grassland than transects as they have lower resolution of the observation metrics, 2) that observational metrics based on vegetation cover detect more species due to more independence of phenology, since they do not depend on flowers blooming and 3) that the plot-based methods are more time effective than transects, as they cover less area and include smaller counts.

2. MATERIALS AND METHODS

2.1 Study area and field sites

The study area consisted of 16 sites dispersed in southeastern Norway in the counties of Viken and Innlandet (Figure 1). Each site was sampled once with each method, except for one site (site 18) where two transects were sampled, each with adjacent plots, leaving the total number of surveys per method at 17. The distance between sites varies from about 2 km to 137 km. The region is mostly comprised of intensified agricultural landscapes and production forests. The climatic conditions of the study area vary from oceanic with mild winters and high humidity in the southwest to transitional zones towards a more arid and continental climate with colder winters in the northeast (Ahti et al. 1968; Dahl et al. 1986; Moen 1998). The vegetation zones of the study area range from the boreonemoral to the mid-boreal zone. The sites have largely been chosen at random between semi-natural grasslands defined as mowed meadows by former mappings of nature types based on the nature classification system by Fremstad (1998). The sites have varying qualities and states of either bush encroachment from abandonment or effects of fertilization from intensified agriculture. A few sites are not even considered meadows but rather deteriorated or human-modified nature types in the new nature classification system – Nature in Norway (NiN) by Halvorsen et al. (2016).

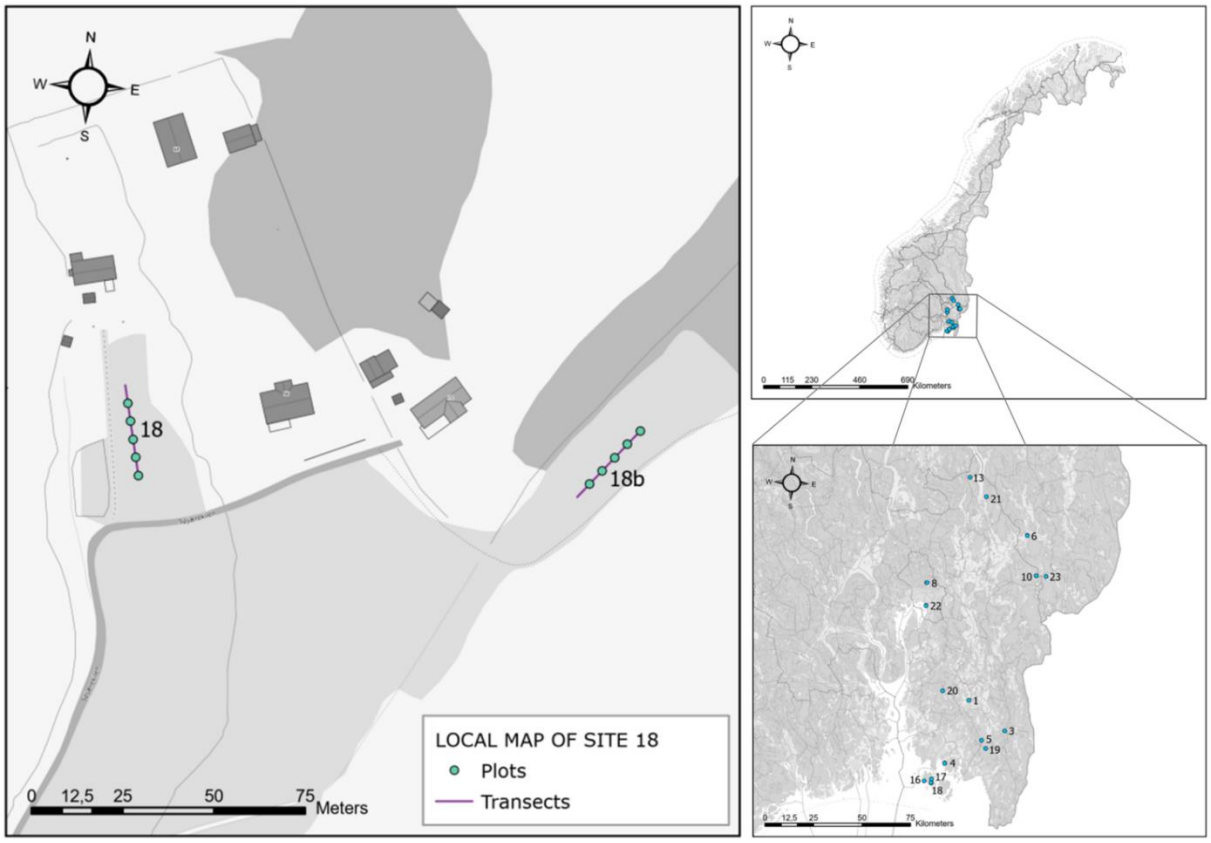


Figure 1: Map over site 18 with two transects and the positioning of plots along the transects (left), in addition overview maps of the 16 sampling sites shown with both regional (lower right corner) and national extent (upper right corner).

2.2 Choice of model system

The model system chosen in this study was semi-natural grasslands. Floral resources for pollinators have been assessed and studied quite intensively over the years (Tepedino & Stanton 1981, 1982; Zimmerman & Pleasants 1982; Frankl et al. 2005; Hegland et al. 2010; Szigeti et al. 2016a). Most of the studies have focused on semi-natural grasslands, ecosystems that have a high diversity and abundance of flowering plants due to man-made disturbances through mowing and grazing by husbandry (Halvorsen et al. 2016; Szigeti et al. 2016a). Through small-scale disturbances with high frequency and nutrient depletion from the removal of biomass, the plant species that otherwise would have been outcompeted by tall-growth species with a high demand for nutrients are able to sustain and thrive, increasing species richness (McCook 1994; Young et al. 2001). The semi-natural grasslands have been an important part of civilization and a source of food for insect pollinators since the Neolithic Period and the domestication of the first animals created pastures (Poschlod et al. 2009). The hay-meadows, one of the most diverse terrestrial ecosystems, were introduced later by the mowing that started in the Roman Period. Since the industrial revolution and the beginning of the 20th century, pastures and hay-meadows have undergone drastic declines due to intensification through fertilization and more intensive soil management (Bignal & McCracken 1996). Seeing that semi-natural grasslands are considered biodiversity hotspots, in addition to having a cultural and aesthetical value, they are subject to intensive conservation measures (Öckinger & Smith 2007; Wilson et al. 2012; Dicks et al. 2012).

2.3 Transects and plot sampling

I surveyed floral resource diversity within 16 study sites, following three different methods: one transect-based method with direct flower counts and two plot-based methods with cover estimates based on frequency and percentage cover in subplots. Within each site, except the one with double sampling, I sampled one 25x1 m transect with additional five 1x1 m plots along the transect (Figure 1). The transects thus covered an area of 25 m^2, while the plot-based methods covered 5 m^2 each. All plots were placed with equal distances of 4 meters between each plot inside of the transect area. The positioning of the transects was limited by the shape and size of the meadows but was as far as possible established close to the core areas of the meadows. Due to sampling rather late in the season, August, eight of the originally planned 24 meadows had been mown and were therefore not suited for sampling. The remaining meadows had in some cases been mowed in parts of the meadows which further limited the possible positions. The equipment used for the sampling was a measuring band of 30 meters in length, square plots made of plywood and thread with the dimensions 1x1 m with subplots of 25x25 cm (Appendix I) and a digital tablet for notation of species data, time and positioning data. All the recordings were done in dry weather conditions between the hours of 07:00 and 19:00 in the period from 31st of July to the 14th of August 2020.

The flower counts along the 25 m transects included floral units of both blooming flowers, buds, and partially deteriorated flowers. This way, species in the end or beginning of their flowering season could be detected, thus allowing more species to be detected with less dependence on their phenology. The flower units varied depending on the flower type, although registered consistently within species. The floral units varied between species to make logical delimitations of floral units based on the morphology. The floral units used are described in detail in Appendix I, but varied from single flower, partial inflorescence and full inflorescence of the inflorescence types given by Jonsell (2004); scorpoid cyme, helicoid cyme, panicle, thyrsoid, dichasium, pleiochasium, thyrses, compound thyrses, corymb, umbel, compound umbel, spadix, head, capitulum, spike, catkin, raceme, and compound racemes. Due

to large numbers of floral units for some species, e.g. the lesser stitchwort *Stellaria graminea*, the maximum number of floral units per species at each transect was limited to 2000.

The two plot-based methods were based on five square plots of 1x1 m each consisting of 16 subplots of 25x25 cm per site, giving a total of 80 subplots per site. The plot size of 1x1 meter is recommended by (Andrade et al. 2019). For one site, site 20, there was one plot missing due to technical failure of uploading of the data from the tablet to the cloud for the percentage cover estimates leaving this site with only four plots. This site was excluded from the analysis when comparing methods among sites. The subplots were all sampled separately as individual sampling units, giving a total of 272 subplots for each site and a total of 1280 subplots across all sites. For occurrence in subplots (OS), the sampling method was simply to register presence of species within the subplots. OS could vary between 0, indicating an absence of the species in all subplots and 16 being presence in all subplots in a 1x1 m plot. The abundance measure thus being the frequency of subplots with species presence (Greig-Smith 1983).

Similarly, the percentage cover estimates (PCE) were registered per subplot. PCE were estimated per species on a scale of 0-100% per subplot, as applied for plots by Jalonen et al. (1998), thus ending up with the total per plot, given total dominance, the sum of 1600%. Due to species occurring in different heights and overlapping each other, the percentage cover per subplot for all species could exceed 100% (Peet et al. 1998). The cover estimates were based on canopy cover of live vegetation per species (Daubenmire 1959). For estimation of percentage cover, I applied a compromise between the Daubenmire method (1959) and the extended Daubenmire method suggested by Bonham et al. (2004), meaning I used percentage cover of estimates rounded to the nearest 5% rather than 1% as suggested in the extended Daubenmire method or the six scales in the Daubenmire method. Species present, yet with less of a cover than 3% were registered as presence with a cover of 1%. All methods were sampled separately, making it possible to record time usage per method. PCE and OS, however, was sampled in succession with OS before PCE for all plots to ensure that they were sampled in the exact same spot, but the time recordings were still taken separately. For all three methods the species identification was as detailed as reliability of identifications allowed, leaving a few taxa recorded at genus level (e.g. Scorzoneroides, Taraxacum etc.). The lowest taxonomic resolution applied was species level. The total number of taxa for all methods was 88, of which six were on genus level and the rest on species level. For further descriptions of the method used, see the supplementary material (Appendix I).

2.4 Statistical analysis

The plant species richness, abundance and diversity recorded using the different methods were compared in the R software environment (RStudio Team 2020). Species richness was compared by using the sum of species per plot and/or site, depending on what resolution the comparisons were for. Abundance is given by the different metrics as either 1) number of floral units, 2) frequency in subplots ranging from 0 (absent) to 16 (present in all subplots) or 3) percentage cover. The latter was estimated based on the mean cover of each subplot for the entire plot. Abundance was scaled before applied in models, by division of the maximum abundance estimate per method; 2000 for transects, 1600 for percentage cover and 16 for the occurrence survey. Diversity was based on the Shannon-Wiener diversity index (H), which is the relative abundance or evenness of species, requiring both a measure of species richness and their abundance, and is often referred to as “heterogeneity” measures (DeJong 1975; Good 1953; Magurran 2013). The diversity indices were compared on two spatial scales; within-site and among-site, the first being a comparison to see how the methods perform at describing

communities and the second being a comparison of how the methods perform when comparing communities. I also compared the effects of using different numbers of plots per site. In addition, I made a qualitative assessment of how the different species were recorded in terms of abundance and diversity, to infer how different traits affect the results. Prior to data analysis, the data was managed trough table preparations of the recorded data to ensure compatibility with the analyses in R. I used graphical verification of the data to check for residuals and see the distribution of the data, of which the data showed a Poisson distribution. I found no residuals of concern or data that were omitted from the dataset except for site 20 that was omitted due to one missing plot for PCE because of a fault in uploading from the digital tablet during sampling.

For within-site comparison I calculated the fraction for species richness and diversity (H) between the relevés from PCE and OS divided the relevés for transects. This was done to better see the relative difference between the methods, more specifically how the plot-based methods compared to transects when measuring α-diversity (Whittaker 1960). A fraction higher than 1 indicate that plot-based methods detect more species or describe a more diverse community. For this I used a linear mixed-model with the 'lme4' package in R, which is based on restricted (residual) maximum likelihood (Bates et al. 2007). In addition, I used the 'effects' package to get the lower and upper confidence intervals (CI) (Fox et al. 2016). In addition, I used functions from the 'Vegan' package to estimate diversity (The models were based on randomized sampling with 1000 permutations from the relevés of the different methods (seed = 1234).

Among-site comparison of sites, or comparison of β-diversity, was done by comparing species richness, abundance, and diversity (H) for all sites. This was compared using a simple linear regression model with log-transformation that in turn were applied with an "eff" object using the "effects" package in R. This "eff" object were used to plot the lines of the fitted model with upper and lower confidence limits for all three diversity indices. I did the analysis for 1-5 plots for both plot-based methods to see the effects of using different numbers of plots or increasing the sampling area, in total 30 models of five per method for the three diversity indices.

Among-site comparison for species was done using abundance and diversity (H) for each species based on the sum of the species across all sites. This was to see if some species differed in abundance and detectability in the plot-based methods compared to transects. The results were plotted with a simple linear regression model of the data, and log-transformation of the data.

3 RESULTS

3.1 Within-site comparison

The two plot-based methods, PCE and OS, detected the same number of species, as is expected when sampling at the same plots in succession (Figure 2). The species richness per subplots varied from none to 11 taxa. Compared to transects, they detected less species when using only one or two plots per site, shown by the fraction being less than 1. Increasing the number of plots to three or four, resulted in the two methods detecting about the same number of species as with the use of transects (Figure 2). However, when using five plots, the two methods detected significantly more species than transects, about 16.7% more species (coefficient of 1.167 and t-value of 22.56). The effect of increasing the number of plots was significantly higher when increasing from one plot (coefficient of 0.6026 and upper CI of 0.742) to three plots (coefficient of 0.975 and lower CI of 0.835). There was no significant effect when increasing the number of plots further, with a lower CI of 1.027 for five plots compared to

the upper CI of 1.114 for three plots. T-values range from 8.5 with one plot to 22.56 for five plots, with a steady increase for each plot added.

The comparison of diversity showed that there was no significant difference between the plot-based methods. The big difference of methods was between the two plot-based methods and transects, whereas the transects described less plant diversity at a community level than the other two methods (Figure 3). The difference between the two plot-based methods and transects was positively correlated with the increase in number of plots. The initial difference in diversity detected by the plot-based methods compared to transects was at about 19.4% with the use of one plot, however, with the increase to five plots the increase was about 52.8% compared to transects (Table 2). There was no significant difference in the diversity described by the plot-based methods with the increase in number of plots used, although, there was a clear trend with increasing diversity described.

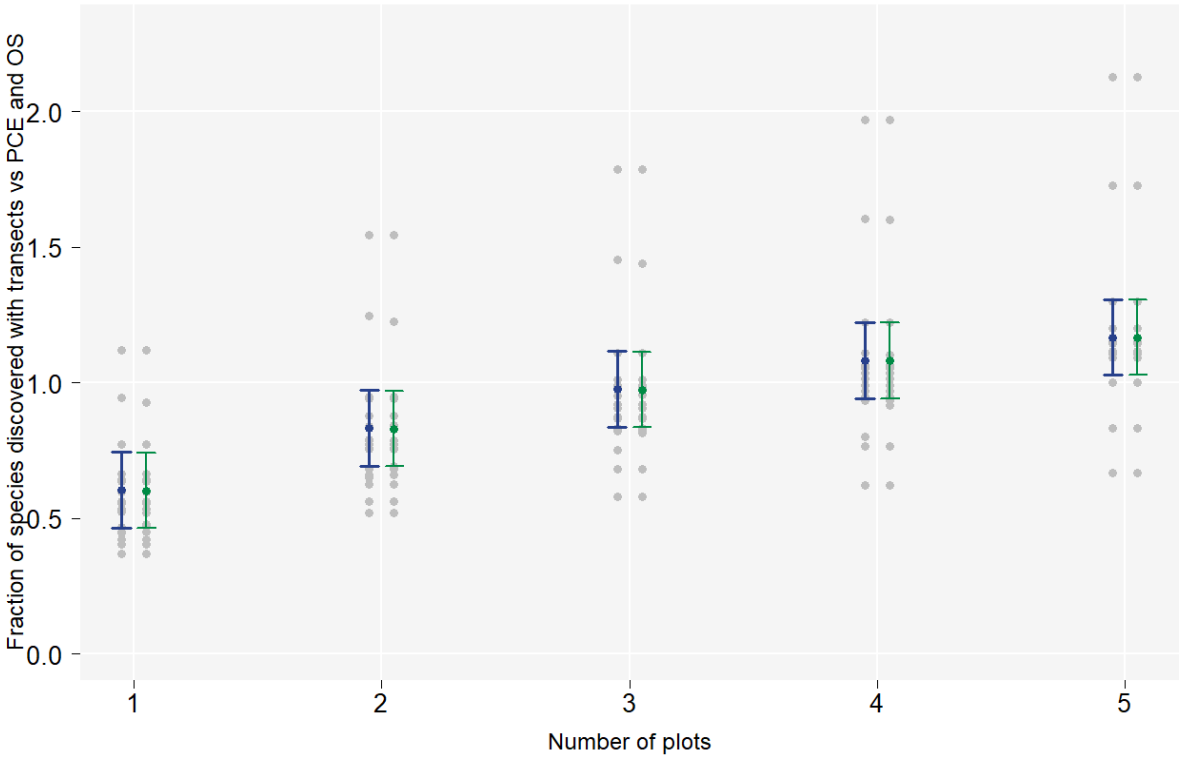


Figure 2: Plot based on a linear mixed-model for the fraction of species discovered by transects vs the two plot-based methods; occurrence survey (OS – blue bar) and percentage cover estimates (PCE – green bar). The bars represent the upper and lower confidence limit of the effect object based on the linear mixed-model, while the points represent the fraction of species discovered per site.

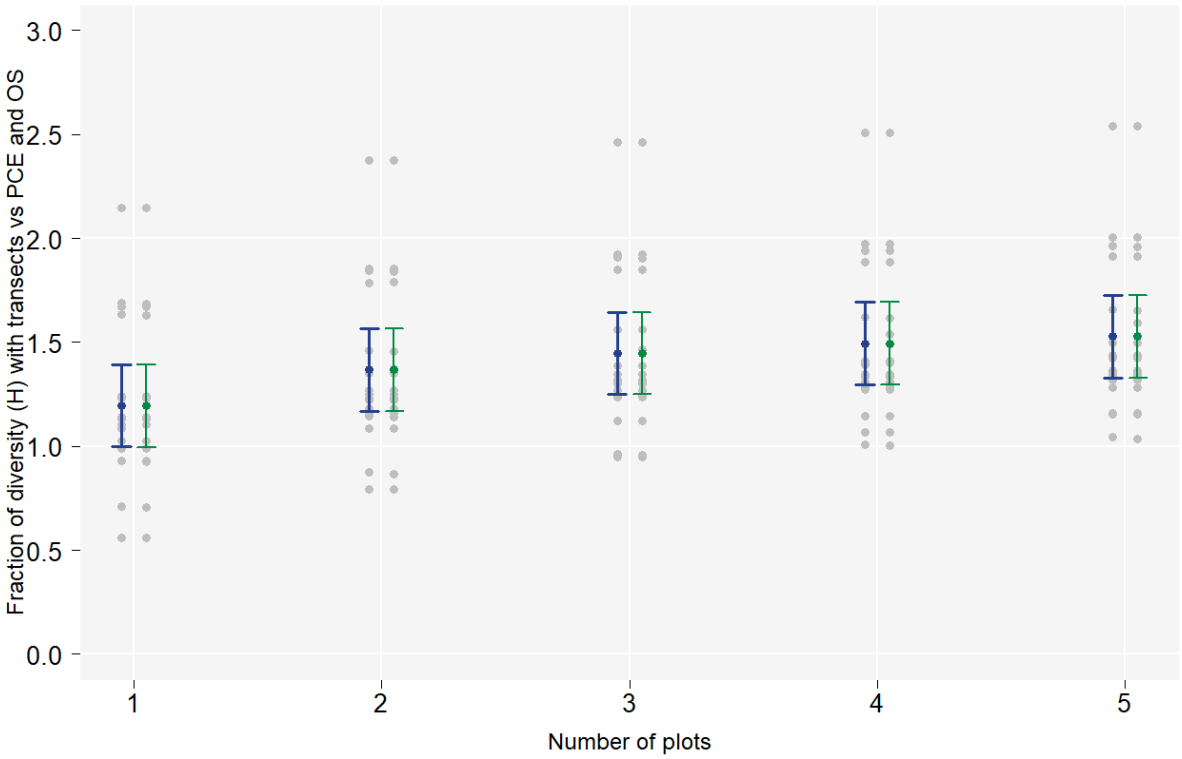


Figure 3: Plot based on a linear regression for diversity data provided by transects and the two methods; occurrence survey (OS – blue bar) and percentage cover estimates (PCE – green bar). The bars represent the upper and lower confidence limit of the effect object based on the linear mixed-model, while the points represent the fraction of species diversity (H) per site.

Table 2: Table of selected key results for comparison of diversity (H) from the summary of the "eff" object from the linear mixed-model (lmer) fit by restricted (residual) maximum likelihood (REML), showing coefficients and t-values from the lmer with lower and upper confidence interval (CI) from the "eff" object for the different number of plots per method compared to transects.

Method	# Plots	Coefficient	Lower CI (5%)	Upper CI (95%)	T value
Occurrence	1	1.194	0.995	1.392	11.881
	2	1.367	1.169	1.566	9.891
	3	1.447	1.249	1.646	14.437
	4	1.495	1.296	1.693	17.134
	5	1.528	1.329	1.726	19.027
Percentage	1	1.195	0.996	1.393	11.957
	2	1.366	1.368	1.565	9.864
	3	1.446	1.248	1.445	14.406
	4	1.494	1.295	1.692	17.114
	5	1.527	1.367	1.564	18.998

3.2 Among site comparison

The general results of among-site analysis when comparing the effects of different number of plots, are that 1) plot-based methods were close to identical for all three indices, 2) the plot-based methods detected more species at about three plots and more and 3) the plot-based methods described a more diverse species composition than transects.

I found that there was a significant positive correlation ($p < 0.05$) for all numbers of plots with both OS and PCE for abundance when comparing the abundance observed in transect surveys (Figure 4). The plots showed no major differences between methods or the number of plots. However, the abundance observed with the plot-based methods have a slight increase with the increase of abundance observed with transects per plot added. The coefficient intercept dropped steadily from about 3.3 to 1.6 when increasing the number of plots from one to five. The model slope was about 1.1 and t-value of 2.9-3.0 for all numbers of plots. This indicated that an increase in number of plots would make the abundance estimates more similar to transects, yet for more densely vegetated sites, i.e., higher abundance, the plot-based methods will have a higher relative abundance estimate compared to transects. The adjusted R^2-value was about 0.34 for all number of plots for both plot-based methods.

The plots for species richness showed a very strong positive correlation ($p < 0.001$) between the species detected by the transects and the plot-based methods (Figure 5). Transects detected more species than the plot-based methods with few numbers of plots, about three plots or less, but detect fewer species when increasing the plots to more than three plots. For some sites, the changes in species detection were more noticeable than others, whereas eight species were detected at site one using one plot, but then increased to 17 species detected when using five plots. The general trend for the plot-based methods was that the number of species are close to doubled using five plots compared to one, and the increase is more or less the same for each plot added. The decrease in slope for the model goes from 0.75 when using two plots and then steadily decreased to 0.74 when using five plots. The adjusted R^2-value varied between 0.54 for one plot and 0.66 for five plots.

The diversity comparison showed that both plot-based methods described a more diverse species composition than transects (Figure 6). There was only one site that stood out with a smaller diversity score when using plot-based methods compared to transects - site 3, albeit only when using three transects or less. The coefficient value increased from 0.29 to 0.65 for OS when increasing from one plot to five, more or less identical to PCE. The t-value was relatively low of about 1.7 for all number of plots. The amount of variation explained using the different plot-based methods compared to transects varied from 11,7% to 32,6%, as given by the adjusted R-squared. The adjusted R-squared value was positively correlated with the increase in number of plots.

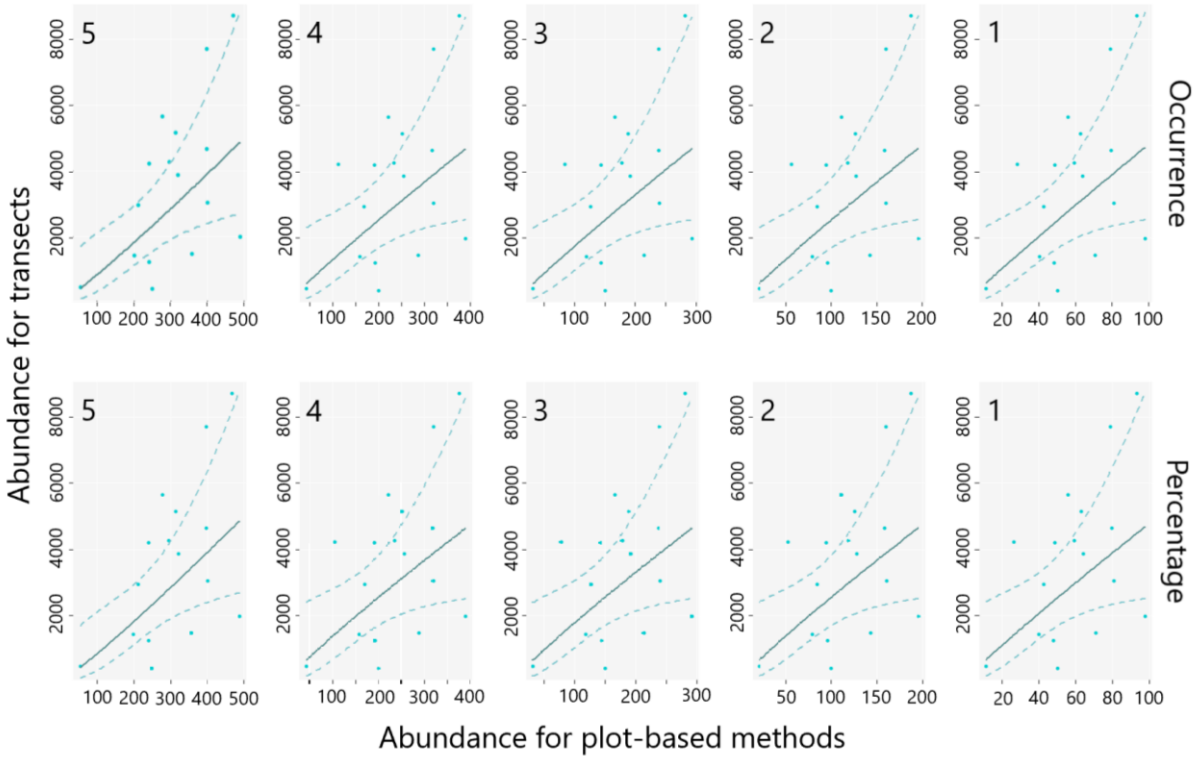


Figure 4: Plot based on a linear regression for abundance data provided by transects and the two methods; percentage cover estimates (PCE) and occurrence survey (OS), separated by the number of plots included per site to see effects on abundance at different plant densities. Each point is one site.

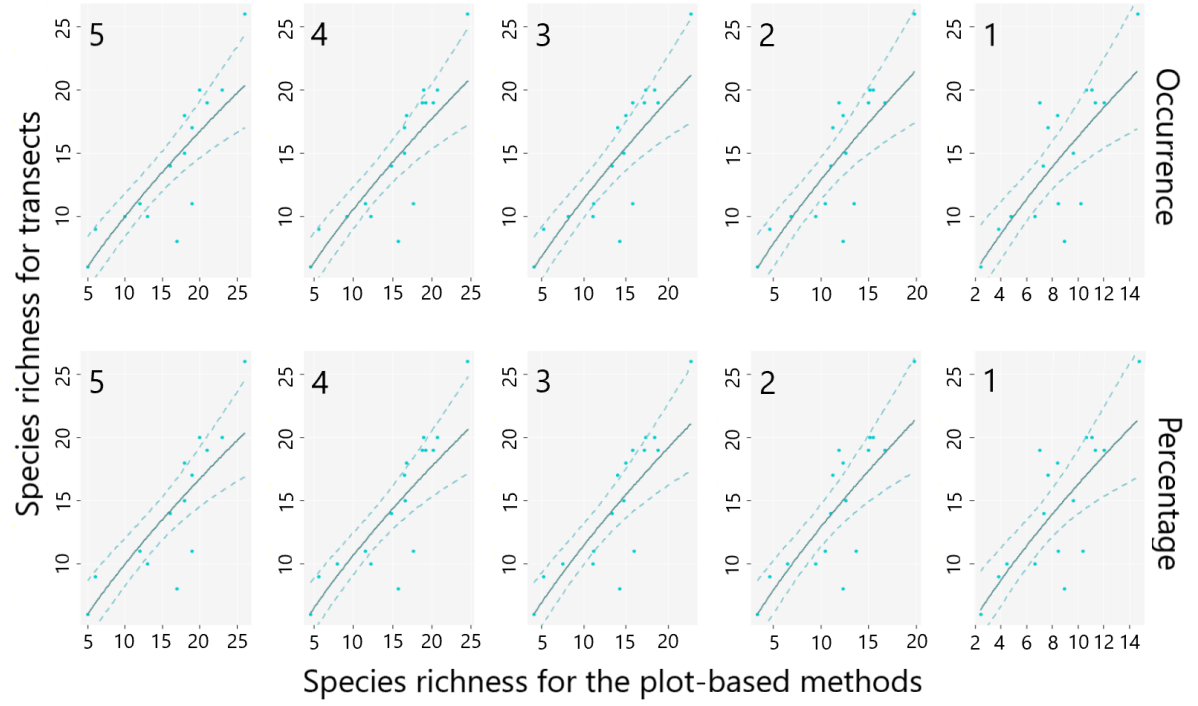


Figure 5: Plot based on a linear regression for species richness provided by transects and the two methods; percentage cover estimates (PCE) and occurrence survey (OS), separated by the number of plots included per site to see effects on species richness estimates between sites. Each point is one site.

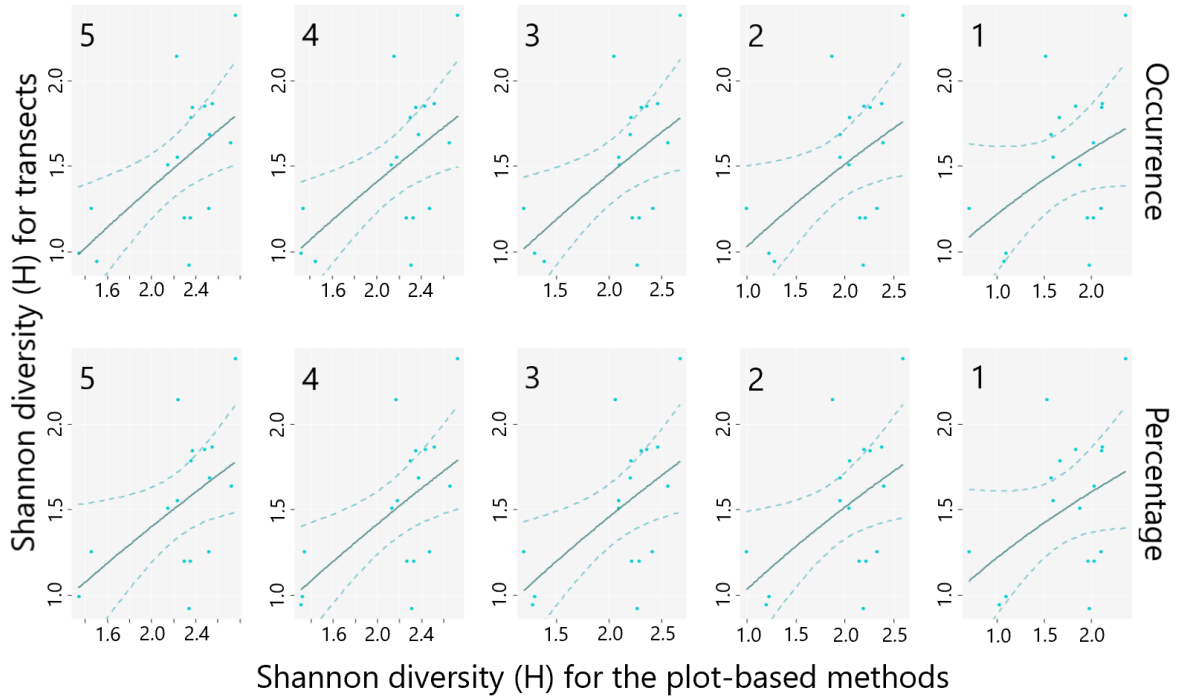


Figure 6: Plot based on a linear regression for Shannon diversity index (H) provided by transects and the two methods; percentage cover estimates (PCE) and occurrence survey (OS), separated by the number of plots included per site to see effects on diversity estimates between sites. Each point is one site.

The relationship between abundance estimates from transects and the two plot-based measures were highly significant ($p < 0.001$). The slope of the coefficients was 0.918 for OS and 0.653 for PCE. The adjusted R^2-values was 0.37 for OS and 0.32 for PCE, while the t-values were 6.2 and 5.6. PCE generally described a higher abundance than OS for most species, however, this could be partly due to the difference in scales (Figure 7). There was some variation in abundance described for the different species by the different methods, such as *Fragaria vesca* that had a high abundance using plot-based methods and a low abundance using transects. The same goes for *Ranunculus repens* and *Rumex acetosa*. There was no apparent correlation between the floral units applied and the abundance estimates. The exception were a few species with the floral unit "single flower" – *Potentilla argentea* and *Rubus idaeus,* which had a high transect abundance and low abundance with OS, although transect abundance was more similar to PCE for these species.

The relationship between the diversity for transects and plot-based methods were also highly significant ($p < 0.001$). The slope of the coefficient was 0.387 for OS and 0.440 for PCE. The adjusted R^2-values for both PCE and SO were 0.63, in addition to t-values of about 10.4 for both methods. Species with low diversity among sites with transects and higher diversity using plot-based methods were *Fragaria vesca*, *Veronica officinalis* and *Ranunculus repens*. Oppositely, *Chamaenerion angusifolium* and *Vicia sepium* had a low diversity among sites using plot-based methods and a higher diversity using transects. Most noticeable was *Ranunculus acris* that had a high diversity of about 2.0 for OS and PCE, and a low diversity of about 0.7 with transects.

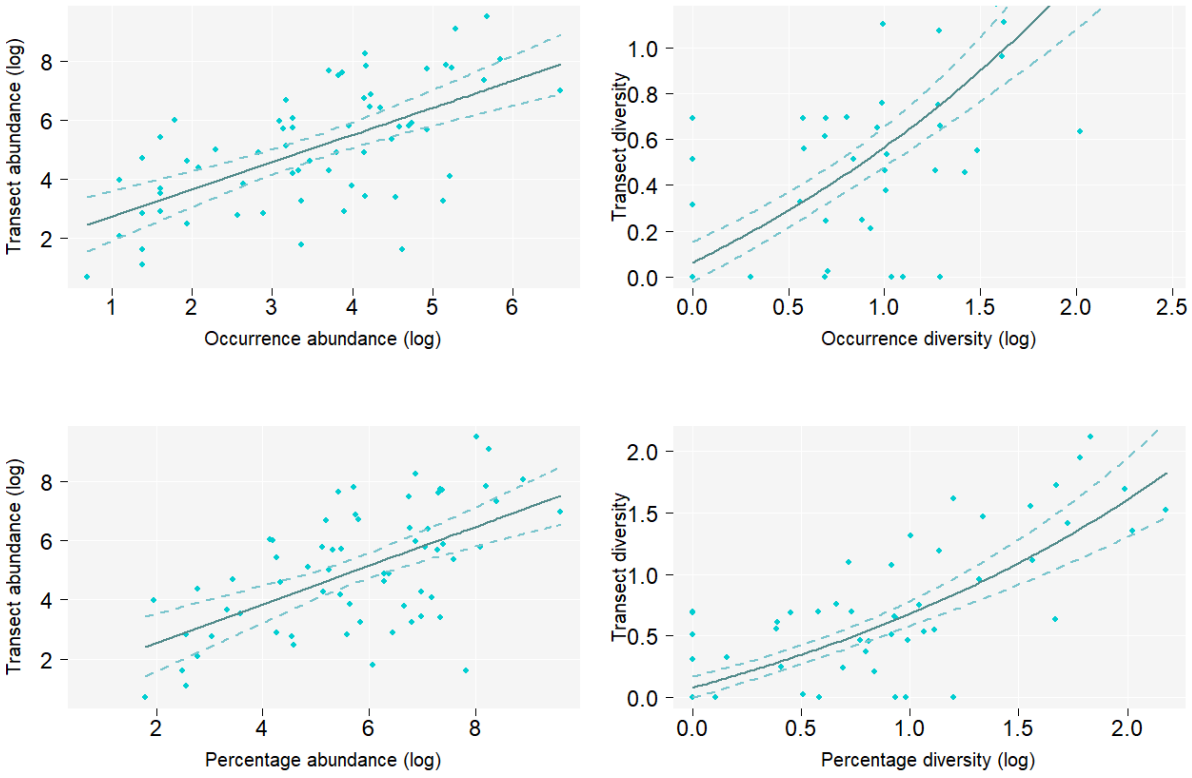


Figure 7: Plots for comparison of species data (points) across all sites based on "eff" objects of a simple linear regression for log-transformed abundance data and Shannon diversity index (H) provided by transects and the two methods; percentage cover estimates (PCE) and occurrence survey (OS).

3.3 Sampling duration

The duration of sampling for the methods was almost identical for the plot-based methods (Table 2). The transects were a lot more time consuming. While the two plot-based methods were similar in duration, PCE duration was biased by the sampling being done in succession of OS per plot. PCE also had a higher standard deviation. Transects took longer to sample, both per transect and when comparing the duration per square meter. Transects were on average 63% more time consuming per site, even though the duration per square meter was a lot shorter than the plot-based methods (< 3 times faster).

Table 2: Time duration per method for sites and square meters.

METHOD	MEAN DURATION PER SITE (IN SECONDS)	STANDARD DEVIANCE PER SAMPLING UNIT (IN SECONDS)	MEAN DURATION PER M^2 (IN SECONDS)
OCCURRENCE SURVEY	2306	217	461
PERCENTAGE COVER	2299	242	460
TRANSECTS	3748	2534	150

4. DISCUSSION

I found that the plot-based methods based on green cover detected more species and described a more diverse species composition than transects with direct flower counts for both within-site and among-site comparisons, confirming my first hypothesis. Considering the efficiency of the methods as well, the plot-based methods come off as superior to transects in assessment of floral resource availability, thus confirming the third hypothesis. However, it is important to stress that the differences were probably not due to the sampling unit of plots or transects, but rather the different observational metrics of direct floral counts, frequency based on green cover and percentage cover estimates of green cover, in line with the third hypothesis. The two latter of these observational metrics appeared to have a higher degree of phenological independence and thus detected plant species regardless of their flowering season, as initially believed.

Diversity indices

When looking more closely at the two plot-based methods, occurrence survey may seem a reasonable choice compared to percentage cover estimates as it appears less affected by surveyor bias when assessing the abundance, similar to the study by Ringvall et al. (2005). A surveyor bias that is of lesser importance when using a single surveyor per project, but that can be more profound when using several surveyors. The results from the among-site comparison also shows that OS have a closer linear relationship to transects with an estimated slope of the coefficient of 0.918 compared to PCE with 0.653. This can be partly explained by the difference in count variables. As seen with *Potentilla argentea*, *Rubus idaeus* and *Cirsium palustre*, which have "single flower" as floral unit, they have low abundance scores with OS compared to transects while they have higher abundance scores with PCE. These species had both many flowers per plant and rather large canopies due to clustered growth, although they were quite limited in extent which caused them to have a low frequency in subplots at the same time as they were dominating in the subplots they appeared. Had the floral unit or count variable been different for the transects, e.g. flowering shoot, then it is likely that the abundance estimates of OS and transects would be more similar compared to PCE and transects. For estimating floral resource availability, it is important to have accurate abundance estimates. Otherwise, the assessments will not provide the necessary information for floral resource availability for pollinators (Tepedino & Stanton 1982; Szigeti et al. 2016b).

The two plot-based methods had similar results for species richness, as expected. However, it is a bit surprising to see the same results when comparing the diversity. The diversity being an estimate based on both species richness and the relative abundance of species (Shannon 1948). This contradicts the idea that abundance estimates for PCE would be more like transects than OS, as seen with the less linear relationship between PCE and transects compared to OS and transects. More surprisingly, OS have no significant difference in abundance compared to PCE, which would indicate that OS could be used instead of PCE.

Sampling duration

The sampling duration in this study showed that the transect with flower counts were more time-consuming per site than the plot-based methods, although transects were faster per square meter. This compares to the study by Leis et al. (2003) that showed a higher time duration for contiguous plots, that had a larger sampling area, compared to modified Whittaker plot and point-intercept with smaller sampling area and lower resolution. The overall experience from the sampling shows that the

transects had more uncertainty in duration, as the increase in flower abundance and biomass made the counts increasingly difficult. While sites with low biomass and low flower abundance gave a good overview that made the flower counts quite efficient. The time consumption for the plot-based methods was more consistent as seen with the low standard deviation, which can ease the assessment of time needed for fieldwork – an important part of any project that includes fieldwork. The similar duration of the two plot-based methods is biased by the PCE happening in succession of OS, which caused me to have an idea of the percentage cover based on the occurrence observations just a few minutes in advance. Assessing percentage cover is more time consuming, in addition to being a larger source of bias due to risk over- or underestimation of cover based on surveyor judgement. The uncertainty in sampling duration for transects may indicate that this method may be less suited for habitats with dense vegetation, while the plot-based methods are more robust. However, the sampling protocol and especially plot size must be adapted to different plant communities (Jalonen et al. 1998).

Sampling unit

The sampling unit used in this study, 1x25 m transects and 1x1 m square plots, appear to have had no major effect on the diversity indices and results of this study as the plots were dispersed with equal distance within the transect covering one fifth of the area in the transects. Even though the plot-based methods covered a smaller portion of the site, they did provide more information about the floral resources. However, had the plots been randomly dispersed, they would probably have given a more representative description of the overall species composition per site (Küchler & Zonneveld 1988). This would be more difficult with transects, unless reducing the length quite drastically and then resembling more of a rectangular plot rather than a transect. A simulation-based study of spatial autocorrelation by Goslee (2006) showed that random sampling using plots was more efficient at detecting rare species than transects, although both were likely to miss rare species unless applying a sufficient number of sampling units per site. The transects in this study were also positioned by subjective placement due to the width of the meadows being insufficient for the 25 m transects and thus the plots were also subjectively placed, whereas they preferably should be placed randomly to detect more species richness and diversity within the site by detecting more of the variation in ecological conditions within the site (Swacha et al. 2017). The size of the sampling units, however, is considered good as it allows the surveyor to get a decent overview of the sampling area without having to move too much around and thereby trampling the vegetation. Albeit there is a trade-off with using this rather moderate size or width of one meter – the risk of bias towards rare and/or clumped species (Elzinga et al. 1998; Szigeti et al. 2016b).

Observation metrics

The observation metrics were one of the most crucial part of this study and I dare say for most studies concerning floral resource availability. While this study used direct flower counts, frequency based on presence in subplots and percentage cover estimates, it has given rise to ideas concerning other count variables as well. The transects with direct flower counts were generally more difficult due to the large quanta of certain species making it difficult not to miscount, in addition to the difficulty of delimiting the floral units in the field as the flowers are often entangled or less distinctly separated than the schematic illustrations shown in the supplementary material. The floral units used for the flower counts have a large variation in the actual number of flowers present within each floral unit, making the flower counts less informative for the assessment of floral resources. It would therefore be more rational to count flowering shoots and then extrapolate the flower abundance per flowering shoot of

each species based on trait databases and information in floras if available or based on measures of a few flowering shoots from each species during sampling (Liu et al. 2021). This would both ease the sampling and give more accurate estimates of the floral resources. In this study the flower counts also included buds and flowers during blooming as well as deflowered flowers, to mitigate the fact the sampling happened late in the season and thus increased the 'phenological detection window'. This phenological, or temporal, dependence comes of as a weakness with the flower counts.

The plot-based methods had in common that they were based on presence regardless of flowering, which enables the detection of the species present throughout the vegetative stage of their life history. This is the reason for more species being detected by the plot-based methods. The phenological independence could reduce the need for sampling effort throughout the season (Alarcón et al. 2008; Hegland et al. 2010; Szigeti et al. 2016b). However, the cover estimates do not necessarily give an accurate estimate of floral resources as it provides information more related to the leaf area index (LAI) (Chen et al. 1992). However, LAI is closely related to the floral resources and can still be considered relevant, as seen in the modeling of yield based on LAI estimated from remote sensing (Dente et al. 2008; Duchemin et al. 2008). An assumption supported by the strong correlation between the flower counts in the transects and the plot-based methods in this study. OS with frequency in subplots intuitively provides less detailed information about the floral resources as it has a lower resolution by simply detecting presence within 25x25 cm subplots and abundance estimates based on 1x1 meter plots as sampling unit. Although, this might not be the case as it could also reduce the risk overestimation of rare and clustered species (Elzinga et al. 1998; Szigeti et al. 2016b).

This can be seen in the among-site comparison of species, which shows that differences between methods are largely a result of the methods themselves. OS have higher diversity scores for the species than transects and PCE, and that some of the species with lower diversity scores are species with high abundance at single or few sites. This indicates that some species are more sensitive to the floral units used in this study in relation to estimates based on vegetative cover of the species. In comparison percentage cover estimates could result in high abundance estimates for the clustered species that would not be representative of the overall species composition at the site. This bias could be reduced by lowering the resolution through increasing the size of the sampling unit. The downside with this, would then be the issues of practical sampling being increasingly difficult as the surveyor must step within the sampling unit to get accurate estimates or to reduce the risk of missing species present. In addition, the increase in sampling unit size would decrease the reliability of the cover estimates (McCune & Lesica 1992; Jalonen 1998). Lowering the resolution to simple abundance estimates based on frequency in subplots could also increase the consistency between surveyors, making the data more suited for comparison and reuse (Ringvall et al. 2005).

5. CONCLUSION

In this study I found that plot-based methods using vegetation cover of flowering species detect more species and describe a more diverse species composition than transects with direct flower counts, despite the plot-based methods covering a fifth of the area covered by transects. Study designs should account for surveyor bias with respect to observational metrics and floral units, as well as the size of sampling units. There is a need for future research that looks into sampling effort relative to temporal scales, such as phenology of flowering plants and interannual variation within sites. For achieving results relevant for decision makers and nature managers integrative methods should be used, including a variation in spatial scales from remote sensing with landscape scales to local scales with species compositions at sites and nectar production.

This general assessment of the methods has led to the understanding that future floral resource assessments should be based on frequency of vegetation cover for flowering plant species regardless of flowering as this can be extrapolated from trait databases based on information about the LAI and flower/inflorescence types per species or based on a few such measures as a part of the study. This should provide sufficient information to assess species composition on a local scale within sites and information necessary to compare different sites. The sampling unit should not exceed a size that allows the surveyor assess species abundance and presence without having to move within the sampling unit. The sampling units should be randomly dispersed within sites to ensure a representative sampling of the ecosystems' species composition. To increase the resolution of the floral resource estimates it is advisable to include extrapolations of nectar production for the different species, when and if this becomes available in accessible trait databases (Tepedino & Stanton 1981, 1982; Zimmerman & Pleasants 1982; Szigeti et al. 2016b). For larger spatial scales other methods can complement the vegetation surveys, such as remote sensing in combination with modeling of both yields and ecosystems (Dente et al. 2008; Feilhauer et al. 2016; EcoMap wp3 – led by Rune Halvorsen (ongoing)). This would enable an approach relevant for the importance of landscape scale for insect pollinators as these forage on a landscape scale (Frankl et al. 2005). The sampling should have a temporal scale representative of the total flowering season of the focal ecosystem and be done over several years in response to the interannual variation in species composition (Alarcón et al. 2008; Szigeti et al. 2016b).

There is a need for future studies that provides more information about the necessary sampling effort throughout the total flowering season and the relative sampling effort in terms of proportion of total site area that needs to be sampled to get sufficient information about species composition and floral resources. 'Sufficient information' referring to the point at which further sampling stops providing significant changes in results, i.e. reaches the minimum area (Gleason 1922). It is important to detect as many species as possible, and if not, account for rare taxa to get as accurate measures of species compositions and floral resources as possible. This is especially important for conservation of rare taxa and for decision makers and nature managers to assess habitat stability as species-rich communities are more stable than species-poor communities (Goulson & Darvill 2004; Nicholls & Altieri 2013; Dorado & Vázques 2014). There is also a need for more comprehensive trait databases with information about nectar production and floral resources.

6. REFERENCES

Alarcón, R., Waser, N. M., & Ollerton, J. (2008). Year-to-year variation in the topology of a plant–pollinator interaction network. Oikos, 117(12), 1796-1807.

Allen-Wardell G, Bernhardt P, Bitner R, Burquez A, Buchmann S (1998). The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. Conservation Biology, 12:8–17.

Andrade, B. O., Boldrini, I. I., Cadenazzi, M., Pillar, V. D., & Overbeck, G. E. (2019). Grassland vegetation sampling-a practical guide for sampling and data analysis. Acta Botanica Brasilica, 33(4), 786-795.

Bates, D., Sarkar, D., Bates, M. D., & Matrix, L. (2007). The lme4 package. R package version, 2(1), 74.

Bignal, E. M., & McCracken, D. I. (1996). Low-intensity farming systems in the conservation of the countryside. Journal of Applied Ecology, 413-424.

Blaauw, B. R., & Isaacs, R. (2014). Flower plantings increase wild bee abundance and the pollination services provided to a pollination-dependent crop. Journal of Applied Ecology, 51(4), 890-898.

Burkle, L. A., Marlin, J. C., & Knight, T. M. (2013). Plant-pollinator interactions over 120 years: loss of species, co-occurrence, and function. Science, 339(6127), 1611-1615.

Chao, A., & Jost, L. (2012). Diversity measures. In Encyclopedia of theoretical ecology (pp. 203-207). University of California Press.

Chao, A., Gotelli, N. J., Hsieh, T. C., Sander, E. L., Ma, K. H., Colwell, R. K., & Ellison, A. M. (2014). Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. Ecological monographs, 84(1), 45-67.

Chen, J. M., & Black, T. A. (1992). Defining leaf area index for non-flat leaves. Plant, Cell & Environment, 15(4), 421-429.

Cushing, D. H. (1969). The regularity of the spawning season of some fishes. ICES Journal of Marine Science, 33(1), 81-92.

Cushing, D. H. (1990). Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. In Advances in marine biology (Vol. 26, pp. 249-293). Academic Press.

DeJong, T. M. (1975). A comparison of three diversity indices based on their components of richness and evenness. Oikos, 222-227.

Dente, L., Satalino, G., Mattia, F., & Rinaldi, M. (2008). Assimilation of leaf area index derived from ASAR and MERIS data into CERES-Wheat model to map wheat yield. Remote sensing of Environment, 112(4), 1395-1407.

Dicks, L. V., Abrahams, A., Atkinson, J., Biesmeijer, J., Bourn, N., Brown, C., ... & Sutherland, W. J. (2013). Identifying key knowledge needs for evidence-based conservation of wild insect pollinators: a collaborative cross-sectoral exercise. Insect Conservation and Diversity, 6(3), 435-446.

Dorado, J., & Vázquez, D. P. (2014). The diversity–stability relationship in floral production. Oikos, 123(9), 1137-1143.

Duchemin, B., Maisongrande, P., Boulet, G., & Benhadj, I. (2008). A simple algorithm for yield estimates: Evaluation for semi-arid irrigated winter wheat monitored with green leaf area index. Environmental Modelling & Software, 23(7), 876-892.

Ebeling, A., Klein, A. M., Schumacher, J., Weisser, W. W., & Tscharntke, T. (2008). How does plant richness affect pollinator richness and temporal stability of flower visits? Oikos, 117(12), 1808-1815.

Elzinga, C. L., & Salzer, D. W. (1998). Measuring & monitoring plant populations. US Department of the Interior, Bureau of Land Management.

Feilhauer, H., Doktor, D., Schmidtlein, S., & Skidmore, A. K. (2016). Mapping pollination types with remote sensing. Journal of vegetation science, 27(5), 999-1011.

Fox, J., Weisberg, S., Friendly, M., Hong, J., Andersen, R., Firth, D., ... & Fox, M. J. (2016). Package 'effects'. Url: http://www.r-project.org, http://socserv.socsci.mcmaster.ca/jfox.

Frankl, R., Wanning, S., & Braun, R. (2005). Quantitative floral phenology at the landscape scale: Is a comparative spatio-temporal description of ''flowering landscapes''possible? Journal for Nature Conservation, 13(4), 219-229.

Fremstad, E. 1998. *Vegetasjonstyper i Norge*. NINA, Norsk Institutt for Naturforskning.

Gallai N, Settele JM, Vaissiere BE (2009). Economic valuation of the vulnerability of world agriculture confronted with pollination decline. Ecological Econonomics, 68:810–821.

Goulson, D., & Darvill, B. (2004). Niche overlap and diet breadth in bumblebees; are rare species more specialized in their choice of flowers? Apidologie, 35(1), 55-63.

Greig-Smith, P. (1983). Quantitative plant ecology (Vol. 9). University of California Press.

Guerra-Castro, E. J., Cajas, J. C., Simões, N., Cruz-Motta, J. J., & Mascaró, M. (2021). SSP: an R package to estimate sampling effort in studies of ecological communities. Ecography, 44(4), 561-573.

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., ... & de Kroon, H. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PloS one, 12(10), e0185809.

Halvorsen, R., co-workers and collaborators, (2016). NiN – typeinndeling og beskrivelsessystem for natursystemnivået. – Natur i Norge, Artikkel 3 (versjon 2.1.0): 1–528 (Artsdatabanken, Trondheim; http://www.artsdatabanken.no.)

Hegland, S. J., Dunne, J., Nielsen, A., & Memmott, J. (2010). How to monitor ecological communities cost-efficiently: The example of plant–pollinator networks. Biological Conservation, 143(9), 2092-2101.

Hjort, J. (1914). Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. ICES.

Jalonen, J., Vanha-Majamaa, I., & Tonteri, T. (1998, January). Optimal sample and plot size for inventory of field and ground layer vegetation in a mature Myrtillustype boreal spruce forest. In Annales Botanici Fennici (pp. 191-196). Finnish Zoological and Botanical Publishing Board.

Kearns, C. A., Inouye, D. W., & Waser, N. M. (1998). Endangered mutualisms: the conservation of plant-pollinator interactions. Annual review of ecology and systematics, 29(1), 83-112.

Klein AM, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA (2007). Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B: Biological Sciences, 274:303–313.

Küchler, A. W., & Zonneveld, I. S. (1988). Floristic analysis of vegetation. In Vegetation mapping (pp. 51-66). Springer, Dordrecht.

Kudo, G., & Ida, T. Y. (2013). Early onset of spring increases the phenological mismatch between plants and pollinators. Ecology, 94(10), 2311-2320.

Little, A. (2013). Sampling and Analyzing Wetland Vegetation. In Wetland Techniques (pp. 273-324). Springer, Dordrecht.

Liu, Y., Li, G., Wu, X., Niklas, K. J., Yang, Z., & Sun, S. (2021). Linkage between species traits and plant phenology in an alpine meadow. Oecologia, 195(2), 409-419.

McCook, L. J. (1994). Understanding ecological community succession: causal models and theories, a review. Vegetatio, 110(2), 115-147.

McCune, B., & Lesica, P. (1992). The trade-off between species capture and quantitative accuracy in ecological inventory of lichens and bryophytes in forests in Montana. Bryologist, 296-304.

McIntosh, R. P. (1967). An index of diversity and the relation of certain concepts to diversity. Ecology, 48(3), 392-404.

Midgley, J. J., & Bond, W. J. (1991). How important is biotic pollination and dispersal to the success of the angiosperms? Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 333(1267), 209-215.

Millard, J., Outhwaite, C. L., Kinnersley, R., Freeman, R., Gregory, R. D., Adedoja, O., ... & Newbold, T. (2021). Global effects of land-use intensity on local pollinator biodiversity. Nature Communications, 12(1), 1-11.

Moen, A. 1998. Nasjonalatlas for Norge: Vegetasjon (Norwegian national atlas: vegetation). *Norwegian Mapping Authority, Hønefoss*.

Nicholls, C. I., & Altieri, M. A. (2013). Plant biodiversity enhances bees and other insect pollinators in agroecosystems. A review. Agronomy for Sustainable development, 33(2), 257-274.

Öckinger, E., & Smith, H. G. (2007). Semi-natural grasslands as population sources for pollinating insects in agricultural landscapes. Journal of applied ecology, 44(1), 50-59.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R. B., ... & Oksanen, M. J. (2013). Package 'vegan'. Community ecology package, version, 2(9), 1-295.

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? Oikos, 120(3), 321-326.

Ollerton, J., Erenler, H., Edwards, M., & Crockett, R. (2014). Extinctions of aculeate pollinators in Britain and the role of large-scale agricultural changes. Science, 346(6215), 1360-1362.

Peet, R. K. (1974). The measurement of species diversity. Annual review of ecology and systematics, 285-307.

Peet, R. K., Wentworth, T. R., & White, P. S. (1998). A flexible, multipurpose method for recording vegetation composition and structure. Castanea, 262-274.

Poschlod, P., Baumann, A., & Karlik, P. (2009). Origin and development of grasslands in Central Europe. In Grasslands in Europe (pp. 15-25). KNNV Publishing.

Potts, S. G., Imperatriz-Fonseca, V., Ngo, H., Biesmeijer, J. C., Breeze, T., Dicks, L., ... & Viana, B. F. (2016). Summary for policymakers of the assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) on pollinators, pollination and food production (No. hal-01946814).

Richards A. J. (2001). Does low biodiversity resulting from modern agriculture practice affect crop pollination and yield? Annals of Botany, 88:165–172

Ringvall, A., Petersson, H., Ståhl, G., & Lämås, T. (2005). Surveyor consistency in presence/absence sampling for monitoring vegetation in a boreal forest. Forest Ecology and Management, 212(1-3), 109-117.

RStudio Team (2020). RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.

Sánchez-Bayo, F., & Wyckhuys, K. A. (2019). Worldwide decline of the entomofauna: A review of its drivers. Biological conservation, 232, 8-27.

Shannon, C. E. (1948). A mathematical theory of communication. The Bell system technical journal, 27(3), 379-423.

Stohlgren, T. & Falkner, M. & Schell, L.D. (1995). A Modified-Whittaker nested vegetation sampling method. Plant Ecology. 117. 113-121. 10.1007/BF00045503.

Swacha, G., Botta-Dukát, Z., Kącki, Z., Pruchniewicz, D., & Żołnierz, L. (2017). A performance comparison of sampling methods in the assessment of species composition patterns and environment–vegetation relationships in species-rich grasslands. Acta Societatis Botanicorum Poloniae, 86(4), 3561.

Szigeti, V., Kőrösi, Á., Harnos, A., Nagy, J., & Kis, J. (2016a). Comparing two methods for estimating floral resource availability for insect pollinators in semi-natural habitats. In Annales de la Société entomologique de France (NS) (Vol. 52, No. 5, pp. 289-299). Taylor & Francis.

Szigeti, V., Kőrösi, Á., Harnos, A., Nagy, J., & Kis, J. (2016b). Measuring floral resource availability for insect pollinators in temperate grasslands–a review. Ecological Entomology, 41(3), 231-240.

Tadey, M. (2015). Indirect effects of grazing intensity on pollinators and floral visitation. Ecological Entomology, 40(4), 451-460.

Tepedino, V. J., & Stanton, N. L. (1982). Estimating floral resources and flower visitors in studies of pollinator-plant communities. Oikos, 384-386.

Yorks, T. E., & Dabydeen, S. (1998). Modification of the Whittaker sampling technique to assess plant diversity in forested natural areas. Natural Areas Journal, 185-189.

van Swaay, C., van Strien, A., Harpke, A., Fontaine, B., Stefanescu, C., Roy, D., ... & Warren, M. (2013). The European grassland butterfly indicator: 1990–2011. EEA Technical Reports, 11.

Vanbergen, A. J., & Initiative, T. I. P. (2013). Threats to an ecosystem service: pressures on pollinators. Frontiers in Ecology and the Environment, 11(5), 251-259.

Whittaker, R. H. (1960). Vegetation of the Siskiyou mountains, Oregon and California. Ecological monographs, 30(3), 279-338.

Wilkerson, M. L., Ward, K. L., Williams, N. M., Ullmann, K. S., & Young, T. P. (2014). Diminishing returns from higher density restoration seedings suggest trade-offs in pollinator seed mixes. Restoration Ecology, 22(6), 782-789.

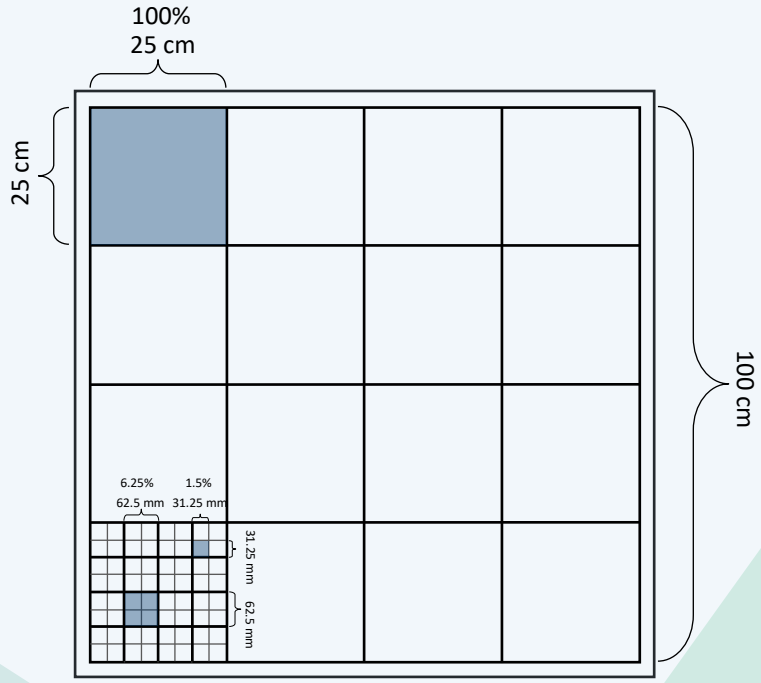
Wilson, J. B., Peet, R. K., Dengler, J., & Pärtel, M. (2012). Plant species richness: the world records. Journal of vegetation Science, 23(4), 796-802.

Wolf, S., & Moritz, R. F. (2008). Foraging distance in Bombus terrestris L. (Hymenoptera: Apidae). Apidologie, 39(4), 419-427.

Woodcock, B. A., Isaac, N. J., Bullock, J. M., Roy, D. B., Garthwaite, D. G., Crowe, A., & Pywell, R. F. (2016). Impacts of neonicotinoid use on long-term population changes in wild bees in England. Nature Communications, 7(1), 1-8.

Young, T. P., Chase, J. M., & Huddleston, R. T. (2001). Community succession and assembly comparing, contrasting and combining paradigms in the context of ecological restoration. Ecological restoration, 19(1), 5-18.

Zimmerman, M., & Pleasants, J. M. (1982). Competition among pollinators: quantification of available resources. Oikos, 381-383.



APPENDIX I

SAMPLING PROTOCOL

TRANSECT DESIGN AND PLOT POSITIONING

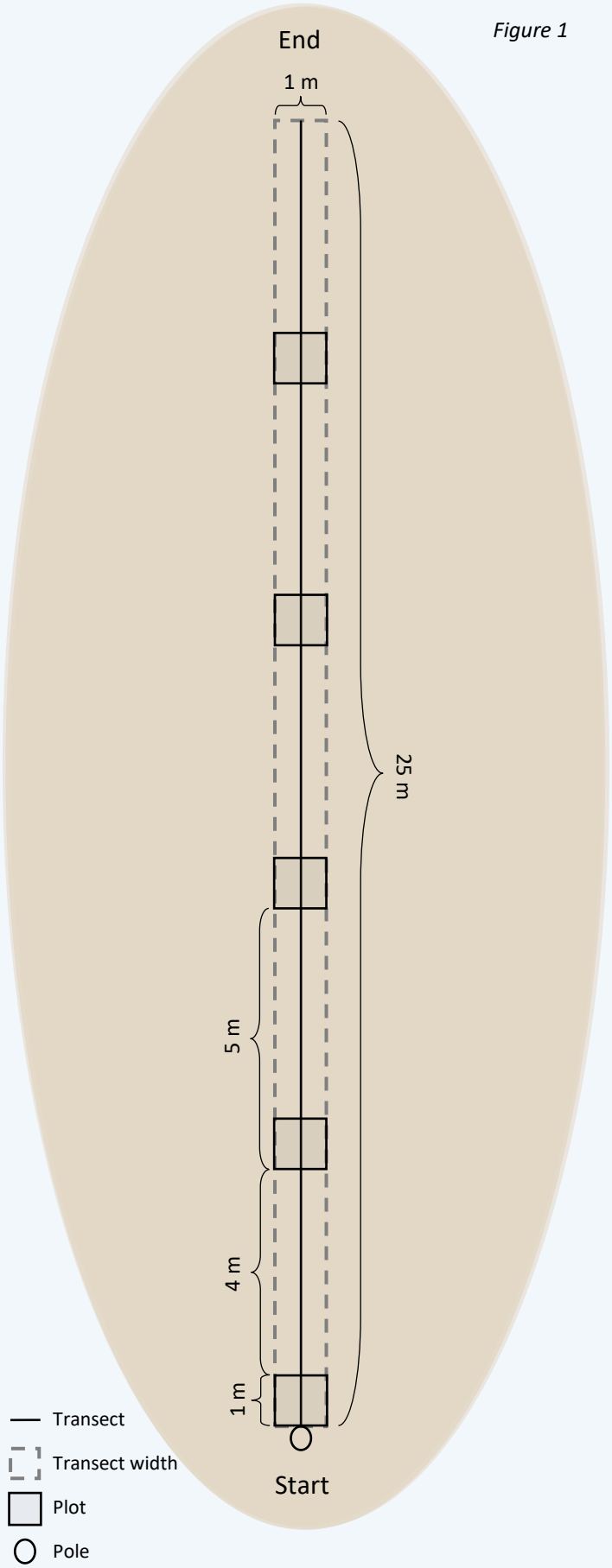


Figure 1

Transect design

Transect length: 25 m

Transect width: 1 m

Transect positioning

The transects were placed next to poles that was previously placed in the core parts of the selected meadows for the purpose of sampling pollinating insects. The poles were used as starting points for the transects. Transects were then extended 25 meters in a direction that covered as much of the core parts as possible. The length was ensured by measure bands of lengths > 25 meters that was attached to the upper part of the poles. When deploying the transects it is important to not step within the transect area, so the plants in the transect area is unharmed. Therefore it is important walk around the planned transect in a curve and then tighten the measure band for accurate measures. The transects were always straight lines, although topography caused some elevational variation that could give minor alteration of total length.

Plot positioning

The plots were positioned along the transect in order to best compare the different methods. The plots were always positioned with a distance of 4 meters between each other, giving the total distance of 5 meters for the plot and gap between them (figure 1). The plots were usually positioned with the first plot starting by the poles. However, in some cases the transects were extended with the end being in a more central part of the meadow than the poles. Then the plots were positioned in the end of the transect, so the plots would cover as much of the core of the meadow as possible. The plots could be opened and closed, which allowed the plots to be placed under trees and bushes if necessary.

THE PLOT DESIGN AND COUNT UNITS

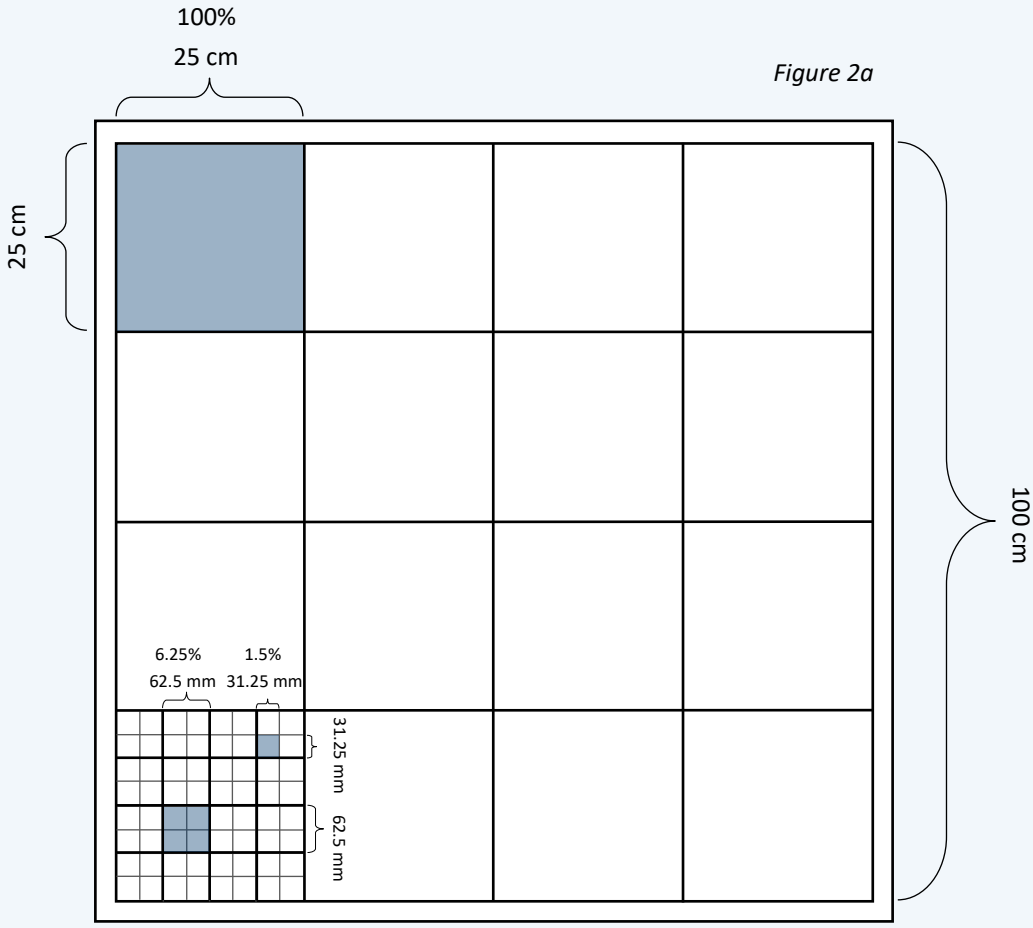


Figure 2a

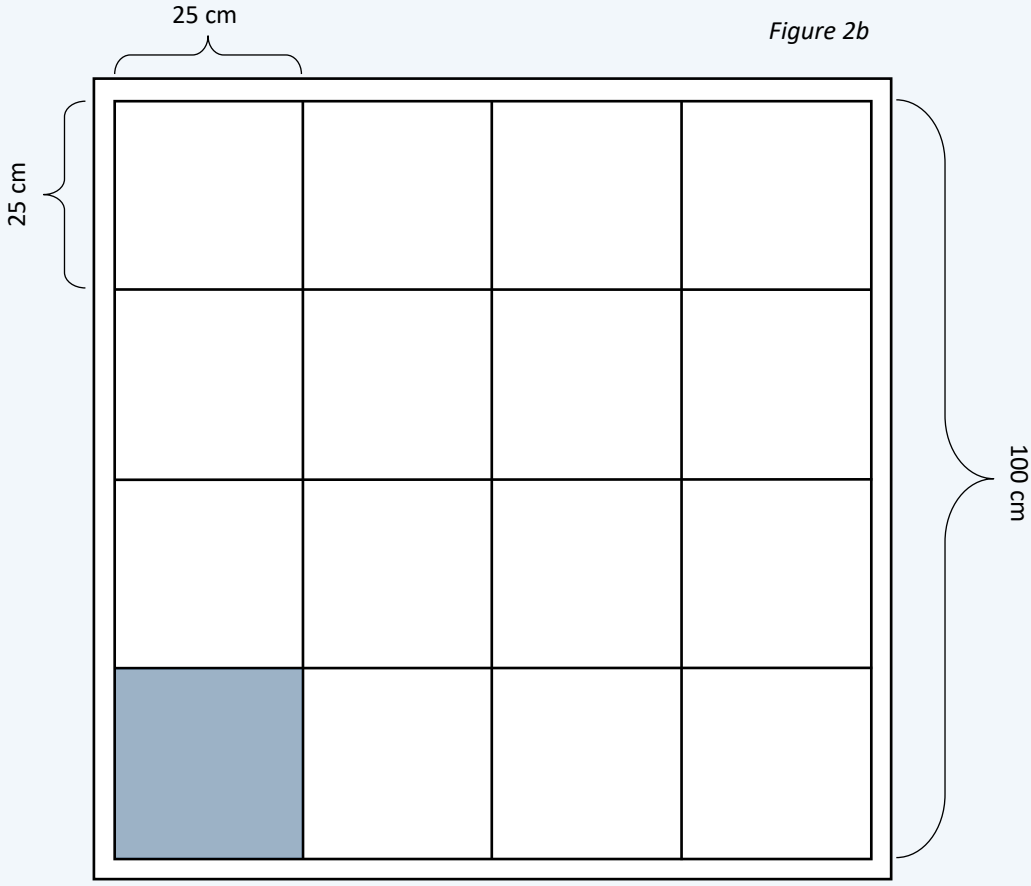


Figure 2b

Percentage cover estimates (PCE)

Plot frame: 100x100 cm

Subplot frame: 25x 25 cm

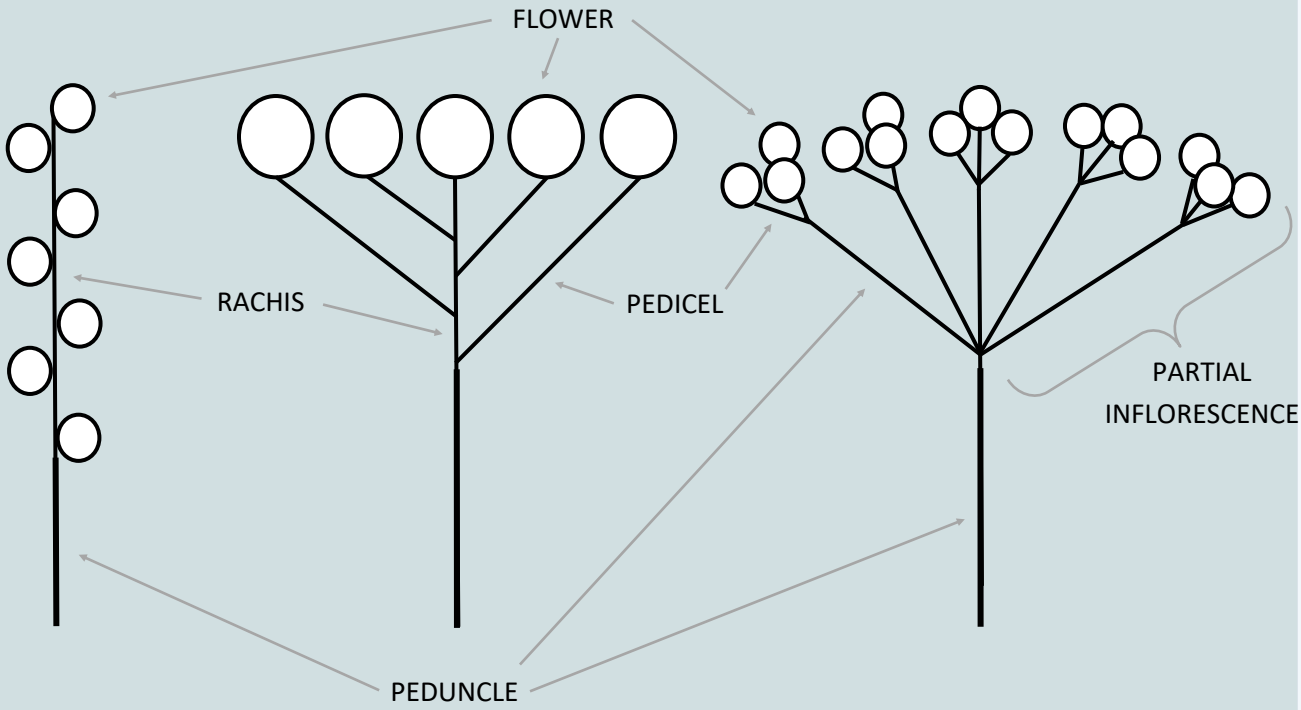
Each subplot was assessed individually. This means that each subplot can have a cover of < 100% per species. Due to differences in length of species canopies, the total cover can be > 100%.

To estimate the percentage cover in subplots, a visualized non-physical grid similar to the main plot grid was used by the surveyor (figure 2a). This made the estimations easier. Species with presence or cover < 1.5% was counted as 1%. The percentage was otherwise always rounded off to nearest 5% in the counts. Percentage cover was estimated on the basis of aerial view of the plot.

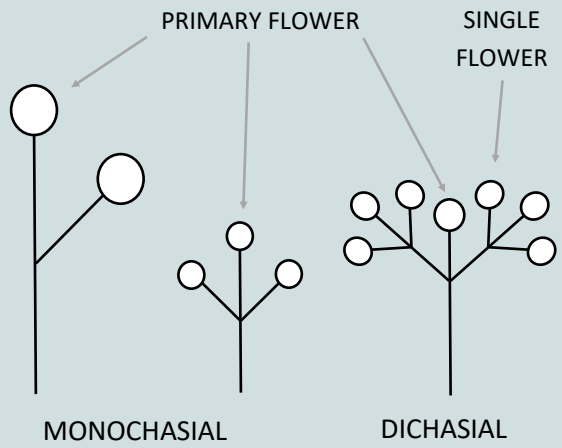
Occurrence in subplots (OS)

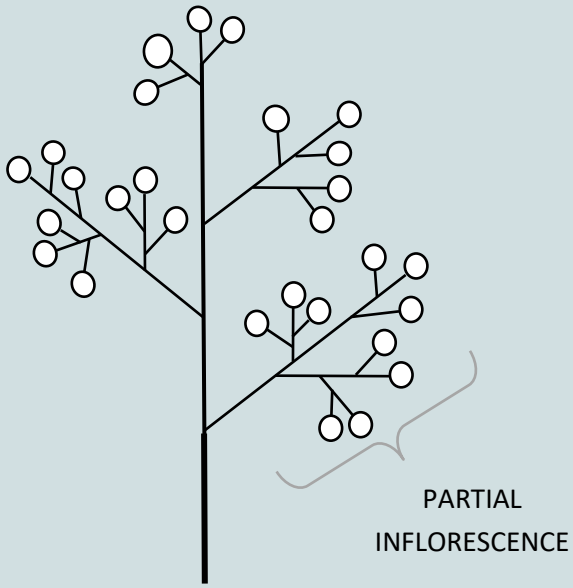
Occurrence in subplots was based on presence in the subplots without any relation to species densities per subplot. Total count per plot was < 16 (figure 2b).

THE INFLORESCENCE & FLORAL UNITS FOR TRANSECTS

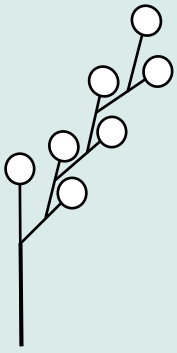


CYMES

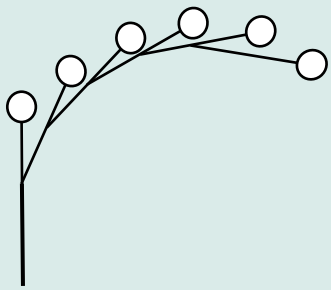




BOTANICAL TERMS USED FOR DESCRIBING FLORAL UNITS



SCORPIOID CYME



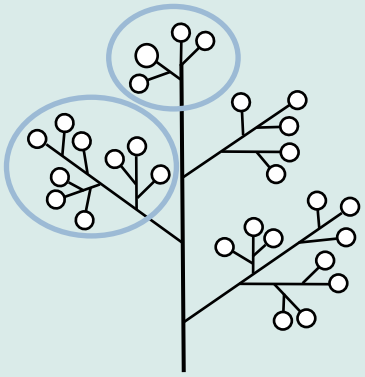
HELICOID CYME

DETERMINATE INFLORESCENCES

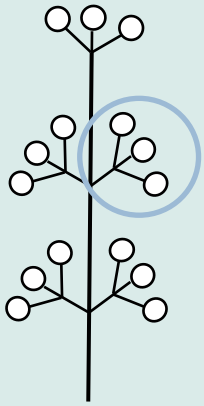
Based on the illustrations in:
B. jonsell (ed.) 2004: Flora Nordica, General Volume, Stockholm.

THE INFLORESCENCE & FLORAL UNITS FOR TRANSECTS

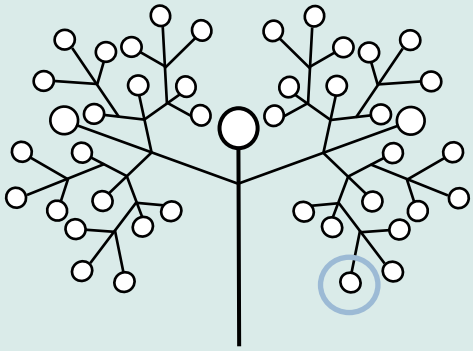
DETERMINATE INFLORESCENCES



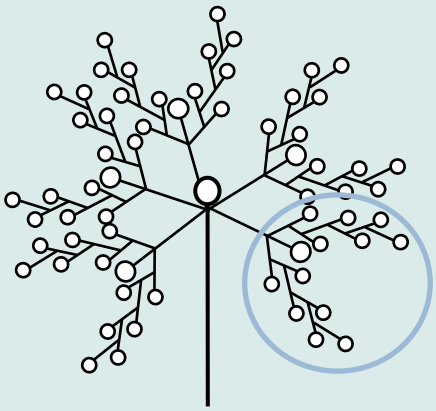
PANICLE



THYRSOID

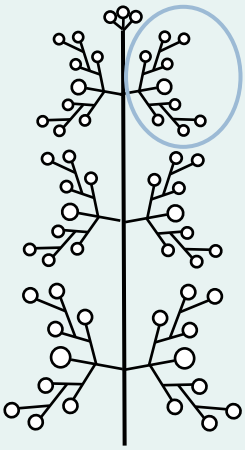


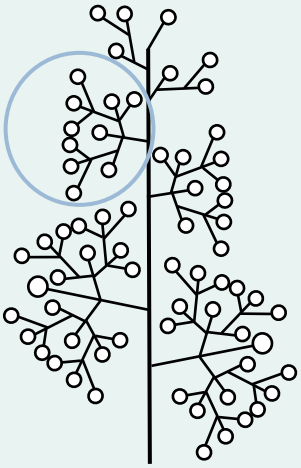
DICHASIUM



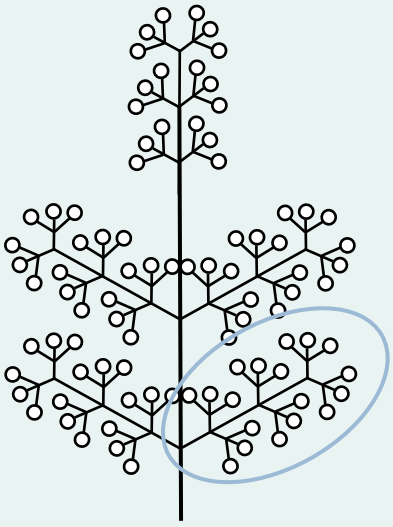
PLEIOCHASIUM

INDETERMINATE INFLORESCENCES





THYRSES



COMPOUND THYRSES

= Floral unit

Based on the illustrations in:
B. jonsell (ed.) 2004: Flora Nordica, General Volume, Stockholm.

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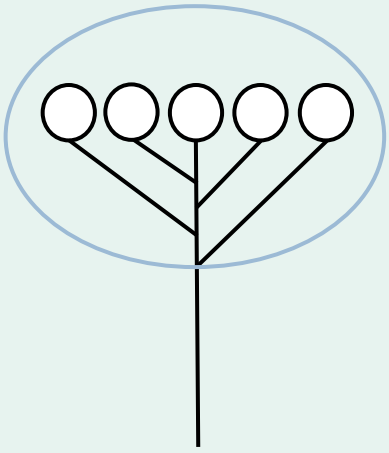
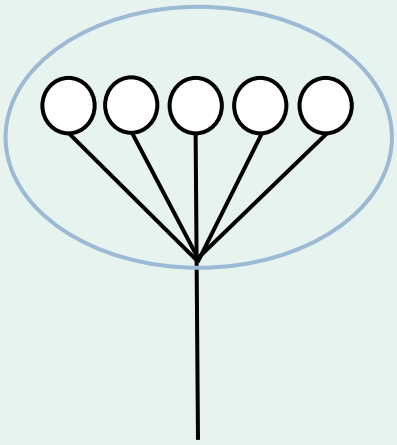
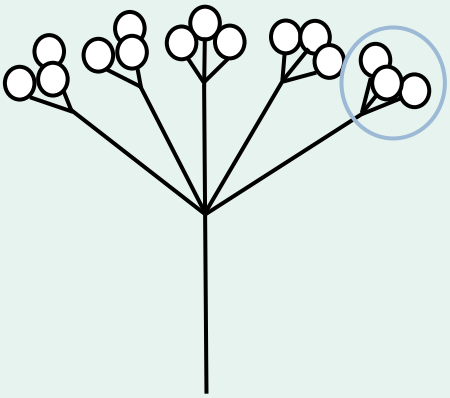


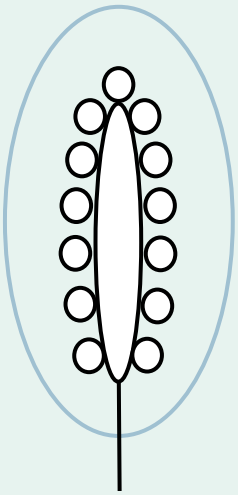
CORYMB



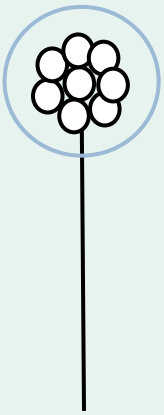
UMBEL



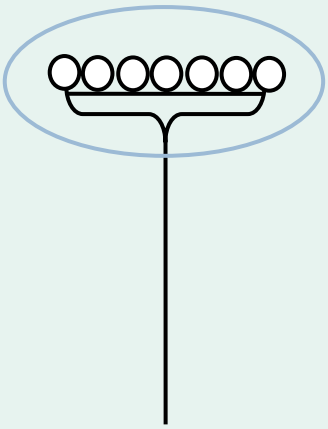
COMPOUND UMBEL



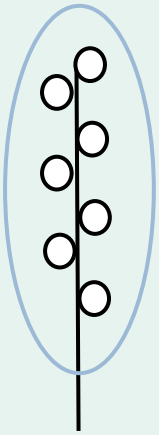
SPADIX



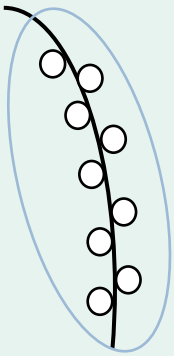
HEAD



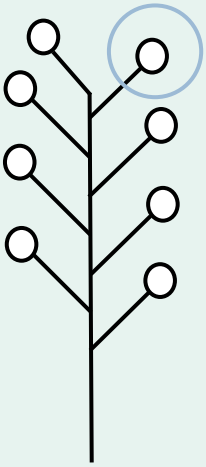
CAPITULUM



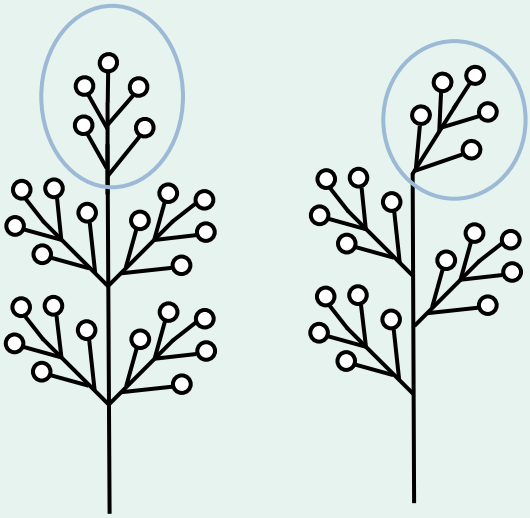
SPIKE



CATKIN



RACEME



COMPOUND RACEMES

= Floral unit

Based on the illustrations in:
B. jonsell (ed.) 2004: Flora Nordica, General Volume, Stockholm.

INDETERMINATE INFLORESCENCES

Table 1: Overview of the species occurring in the study sorted by alphabetical order within the category of inflorescence they are placed in. There are also included some relevant notes for some species based on the experiences made during the fieldwork.

SPECIES	INFLORESCENCE TYPE	FLORA UNIT & NOTES
Achillea millefolium	**CORYMB**	Full inflorescence
Achillea ptarmica		Full inflorescence
Allium oleraceum	**UMBEL**	Full inflorescence
Anthriscus sylvestris	**COMPOUND UMBEL**	Full inflorescence
Carum carvi		Full inflorescence
Peucedanum palustre		Full inflorescence
Pimpinella saxifraga		Full inflorescence
Selinum carviflora		Full inflorescence
Aegopodium podagraria		Full inflorescence
Campanula persicifolia	**RACEME**	Flower
Campanula rotundifolia		Flower
Capsella bursa-pastoris		Flower
Chamaenerion augustifolium		Full inflorescence
Convolvulus arvensis*		Flower. * Uncertain designation of inflorescence type.
Linaria vulgaris		Flower
Platanthera bifolia		Full inflorescence
Rhinanthus minor		Flower
Veronica officinalis		Full inflorescence
Calluna vulgaris*	**COMPOUND RACEME**	Partial inflorescence. *Uncertain designation of inflorescence type.
Euphrasia sp*		Only identified to genus-level
Hypericum maculatum		Flower
Hypericum perforatum		Flower
Lathyrus linifolius		Partial inflorescence
Lathyrus pratensis		Partial inflorescence
Myosotis sp.*		Flower. *Only identified to genus-level
Rumex acetosa		Full inflorescence
Rumex acetosella		Full inflorescence
Solidago vigaurea		Full inflorescence
Veronica chamaedrys		Full inflorescence
Vicia cracca		Partial inflorescence
Vicia sepium		Partial inflorescence

SPECIES	INFLORESCENCE TYPE	NOTES
Anthyllis vulneraria	HEAD	Full inflorescence
Trifolium medium		Full inflorescence
Trifolium pratense		Full inflorescence
Trifolium repens		Full inflorescence
Medicago lupulina		Full inflorescence
Plantago lanceolata	SPIKE	Full inflorescence
Plantago major		Full inflorescence
Plantago media		Full inflorescence
Prunella vulgaris		Full inflorescence
Galium album	THYRSES	Partial inflorescence
Galium verum		Partial inflorescence
Glechoma hederacea*		Partial inflorescence. *Uncertain designation of inflorescence type
Lamium album*		Partial inflorescence. *Uncertain designation of inflorescence type
Lotus corniculatus*		Partial inflorescence. *Uncertain designation of inflorescence type
Urtica dioica*		Partial inflorescence. *Uncertain designation of inflorescence type
Valeriana sambucifolia		Partial inflorescence
Hylotelephium maximum*		Partial inflorescence. *Uncertain designation of inflorescence type
Knautia arvensis	THYRSOID	Partial inflorescence
Melampyrum sylvaticum		Partial inflorescence
Centaurea jacea	CAPITULUM	Counted each capitulum
Cirsium arvense		Counted each capitulum
Cirsium palustre		Counted each capitulum
Hieracium pilosella*		Counted each capitulum. *Uncertain identification and only to the subgenus H. pilosella
Hieracium sp.*		Counted each capitulum. *Only identified to genus-level
Hieracium umbellatum*		Counted each capitulum. *Uncertain identification
Hieracium vulgatum*		Counted each capitulum. *Uncertain identification
Leucanthemum vulgare		Counted each capitulum
Scorzoneroides sp.*		Counted each capitulum. *Only identified to genus-level
Taraxacum sp.*		Counted each capitulum.*Only identified to genus-level
Tragopogon pratensis		Counted each capitulum

SPECIES	INFLORESCENCE TYPE	NOTES
Alchemilla sp.*	**DICHASIUM**	Only identified to genus-level
Cerastium fontantum		Flower
Lychnis flos-cuculi*		Flower. *Uncertain designation of inflorescence type
Stellaria graminea		Flower
Filipendula ulmaria	**PLEIOCHASIUM**	Partial inflorescence
Geranium sylvaticum*		Flower
Fragaria vesca	**PANICLE**	Flower
Geum rivale		Flower
Potentilla argentea		Flower
Potentilla crantzii		Flower
Potentilla erecta		Flower
Ranunculus acris		Flower
Ranunculus auricomus		Flower
Ranunculus repens		Flower
Rosa canina*		Flower. *Uncertain designation of inflorescence type.
Rosa villosa mollis*		Flower. *Uncertain designation of inflorescence type.
Rubus idaeus		Flower
Vaccinium uliginosum		Flower
Vaccinium vitis-idaea		Flower
Viola canina		Flower
Viola raviniana		Flower
Viola tricolor		Flower
Salix caprea	**CATKIN**	Full inflorescence
Dianthus deltoides	**HELICOID CYME**	Full inflorescence

Inflorescence types were illustrated on the basis of the illustrations shown in Flora Nordica General Volume (Jonsell 2004). Species were dessignated to the different types of inflorescence based on personal judgement and the use of available information in floras (Elven et al. 2005; Mossberg 2018).

References

Jonsell B. (2004). Flora Nordica General Volume. Swedish Royal Academy of Sciences. 274 p.

Elven, R., Alm, T., Berg, T., Båtvik, J. I. I., Fremstad, E., & Pedersen, O. (2005). Johannes Lid & Dagny Tande Lid: Norsk flora. Det norske samlaget.

Mossberg, B. (2018). Gyldendals store nordiske flora. Gyldendal.

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