Does season matter for moss surface sample collection? A case study from Kungur forest-steppe, pre-Urals, Russia

Lyudmila S. Shumilovskikh[1,2], Irina Abdulmanova[3], Elena Efimik[3]

[1]Georg-August-University Göttingen, Wilhelm-Weber-Str. 2a, 37073 Göttingen, Germany

[2]Tomsk State University, prosp. Lenina 36, 634050 Tomsk, Russia

[3]Perm State University, Bukireva street 15, 614990 Perm, Russia

Corresponding author Lyudmila S. Shumilovskikh Shumilovskikh@yahoo.com

Abstract

Methodological papers advise to collect moss surface samples either at the beginning or at the end of the flowering season. In reality, such collections occur often within the flowering season for purposes of vegetation description or because of accessibility of remote study areas. Here we test whether the season of moss surface sample collection has an influence on pollen spectra. Ten moss/litter samples were collected in different habitats of the natural reserve "Spasskaya Gora" (Perm region, Russia) in July and September. The results show that pollen assemblages reflect well the present dominant vegetation of *Pinus*-*Betula*-forests and grasslands and demonstrate differences between open and forested areas as well as between abandoned fodder meadows and semi-natural hay meadows in use. Paired t-tests and Wilcoxon tests demonstrate that the dominant pollen taxa *Pinus diploxylon*-type, *Betula* and Poaceae as well as the rarefied number of pollen taxa do not differ significantly between seasons, while values of *Artemisia* are significantly higher in September. A significant decrease in pollen concentration in September indicates the importance of washing-out of pollen by precipitation. PCA results demonstrate the similarity of the pollen spectra between the seasons. Based on our results, we conclude that the season of surface sample collection does not significantly influence the pollen assemblages and appropriate field studies can be carried out also in summer during the flowering season. However, we strongly recommend to collect bulk samples of mosses with basal parts and/or litter with surface soils in order to ensure representation of the average pollen signal for the previous years and to dilute local extremes in the pollen deposition.

Keywords: surface samples; season; methodology; forest-steppe; ecotone; pollen-vegetation relationship

Introduction

Pollen surface samples are a useful tool for quantitative reconstruction of vegetation changes in the past. There are two principal approaches for using surface pollen spectra to interpret vegetation composition: (1) the matching of fossil pollen spectra with surface pollen spectra for identification of similar environments, and (2) the determination of correction factors to transform a percentage pollen diagram into a diagram showing the vegetation composition and changes accurately. The first approach provides a basis for transfer functions and the best modern analogue method (e.g. Overpeck et al. 1985). The second approach was developed from the concept of relative pollen productivity factors (Davis 1963; Andersen 1970) to model reconstructions based on relative pollen productivity estimates (Sugita 2007a, 2007b; Bunting et al. 2013). Semi-quantitative methods such as biomisation are used to display patterns in modern pollen spectra in comparison to current vegetation and thus interpret past vegetation changes (Prentice et al. 1996, 2000; Marinova et al. 2018). Furthermore, pollen spectra from surface soil samples are used in forensic palynology as evidence of the location of crimes (e.g. Horrocks et al. 1998, 1999; Mildenhall et al. 2006; Munuera-Giner and Carrión 2016).

Various methodological aspects of moss surface sample collection have been reviewed in previous studies, showing the influence of sieving effect by moss cushions (Crowder and Cuddy 1973), growth form (Boyd 1986), age of moss polster (Mulder and Janssen 1998, 1999), and season of moss collection (Cundill 1985; Farrell et al. 2016) on pollen representation. The studies provide different estimations of the time periods that pollen remain trapped in the moss (Pardoe et al. 2010), ranging from a few months (Cundill 1985, Farrell et al. 2016) to one-two (Caseldine 1981; Räsänen et al. 2004), five (Bradshaw 1981) and fifteen years (Crowder and Cuddy 1973). The moss sampling strategy varies in different studies (Pardoe et al. 2010), from the collection of a moss cushion at a single point for consistency in detailed pollen-vegetation analysis (Farrell et al. 2016) to bulk samples to minimise the influence of season and trapping capacities by different species, and to obtain an average for the previous years (Crowder and Cuddy 1973, Adam and Mehringer 1975). The method of collecting moss samples is therefore not yet standardised and opinions vary on the number of sub-samples, whether the dead basal moss parts should be included in the sample or not, and whether just one growth form of moss or a bulk sample of several growth forms should be used (Pardoe et al. 2010).

With respect to seasonality, the methodological papers advise to collect moss surface samples either at the beginning or at the end of the flowering season to ensure collection of the last entire season (Andersen 1970; Bradshaw 1981; Caseldine 1981; Bunting et al. 2013). In reality, such collections occur often within the flowering season for purposes of vegetation description or because of accessibility of remote study areas. In this study, we tested the influence of the season of surface sample collection on pollen assemblages. For this, we carried out a surface sample study in the natural reserve "Spasskaya Gora" (Perm region, Russia).

Materials and methods

The study was conducted in the natural reserve "Spasskaya Gora" (Perm region, Russia), a protected area in the northern Kungur forest-steppe situated within hemiboreal forest (Fig. 1). With a total area of 385 ha, it was established in 1965 for the protection of the Kungur forest-steppe. Forests cover about 44% of the area, mainly consisting of open *Pinus* forests, open *Betula* forests, *Betula-Ulmus* forests, *Tilia-Betula-Populus* forests and *Betula-Picea* forests. Open vegetation is mostly steppe-meadows (28%), alluvial meadows (21%) and petrophytic steppe (4%). The flora of "Spasskaya Gora" contains 438 species of vascular plants belonging to 274 genera and 73 families, and is characterized by a significant proportion (31%) of forest-steppe elements (Ovesnov and Efimik 2014).

Sample points were chosen based on Google Earth maps in forested and open habitats (Fig. 2). In 2018, 10 samples were collected in July with detailed vegetation descriptions following the protocol of Bunting et al. (2013). We present the simplified vegetation data in Table 1. The point of collection was marked and in September it was sampled again. Moss polsters, or litter in the absence of moss, were collected with the top few millimetres of surface soil. Because of a potential bias caused by pollen coming from a single plant, we collected five subsamples within 1 m^2 and mixed them in order to dilute any local extremes in pollen deposition (Adam and Mehringer 1975). The samples were stored in paper bags for drying and transported to the lab.

For laboratory analysis, 1 cm^3 of mixed material was taken from each sample. One tablet of *Lycopodium* spores (Batch number 1031) was added to the sample at the beginning of laboratory preparations to enable calculation of pollen concentration (Erdtman 1960). Samples were processed by treating with 10% HCl, 10% KOH for 5 minutes at 90°C, sieving through a metal sieve at 200 micron, cold 48% HF for one night, and acetolysis for 3 minutes. The remaining pellet was stored in glycerin and studied under 400× magnification. Pollen identification and taxonomy follows Beug (2004). Pollen was counted up to at least 500 grains (min. 501, max. 660). This pollen sum was used for calculation of pollen percentages, presented in Fig. 3. In order to check similarity of the entire

pollen assemblages between July and September for different samples, a principal component analysis (PCA) was applied using C2 (Juggins 2007).

Knowing that different plants flower at different times of the year, we expected to find differences in proportions of pollen taxa in the samples between July and September. We therefore tested the mean values for proportion of several taxa, total pollen concentration and number of pollen taxa. For this test, only taxa present in large quantities in the pollen spectra or those present in all samples were chosen, namely: *Betula* (flowering in May), *Pinus* (June), Poaceae (July) and *Artemisia* (August). We used rarefaction analysis with the vegan package (Oksanen et al. 2019) for standardisation of number of pollen taxa implemented for the lowest counts of 501 grains (Birks and Line 1992). All variables were tested for normality of distribution by visualisation methods such as density and q-q plots as well as Shapiro-Wilk test (Table 2). Depending on the results, a paired t-test was applied for normally distributed data or a paired Wilcoxon test to not normally distributed data. The paired tests were chosen because data arise from the same sample points, even though they were randomly collected within the plot. The null hypothesis "there is no difference between July and September" was tested with a statistical significance level $\alpha = 5\%$. In case $p < \alpha$, the null hypothesis is rejected and an alternative hypothesis "July differs from September" becomes true. We use boxplots for a visualisation of the results (Fig. 4). All tests were carried out using the car package (Fox and Weisberg 2019) in R version 3.6.3 (R Core Team 2020).

Results

Pollen assemblage characteristics

Overall, 85 pollen taxa including irregular forms such as tetraporate *Betula* or bicolporate *Tilia* were identified. The average number of pollen taxa per sample varies between 23 (rarefied 22) and 38 (rarefied 38) (Fig. 3). Grouped presentation of the pollen spectra of July and September in pollen diagram (Fig. 3) demonstrates comparable values between two studied months, although some deviations occur. The dominant taxa are *Betula* (13-53%), *Pinus* (8-42%), Poaceae (3-41%), Cerealia-type (0.3-24%) and Cichorioideae (1-26%). *Artemisia* occurs in all samples (0.3-6.5%). Pollen concentration varies considerably between the samples between 48,000 and 262,000 grains/cm^3.

Surface samples from forests are characterized by AP values exceeding 65% (Fig. 3). AP spectra are dominated by *Pinus diploxylon*-type and *Betula*. Sample 12 from *Tilia-Betula-Populus* forest shows up to 6% *Tilia*, while the sample from open *Betula*-forest has highest values of *Picea* (6%). NAP spectra have high amounts of Poaceae (2-11%) and *Artemisia* (1-6%), and *Mentha*-type occur only in these samples (Fig. 3).

Open vegetation types of xero-mesophilic and mesophilic meadows show AP values between 40 and 60%, dominated by *Betula* and *Pinus diploxylon*-type (Fig. 3). NAP is dominated by Poaceae (5-24%), Cerealia-type (2-24%), Cichorioideae (2-26%), *Medicago sativa*-type (up to 10%) and *Plantago major-media*-type (up to 7%).

Two samples from hay meadows within forests are characterized by lowest AP values of 23-39% and dominated by *Betula* and *Pinus diploxylon*-type (Fig. 3). Dominant taxa are Poaceae (23-42%), *Galium*-type (8-11%), *Plantago lanceolata*-type (7-12%), *Rumex acetosa*-type (4-9%).

Influence of sampling season

All tests of normality such as Shapiro-Wilk test (Table 2), density and q-q plots (not shown) reveal that with the exception of Poaceae in July, all other tested variables have a normal distribution. Therefore, in case of Poaceae we applied a paired Wilcoxon test.

In the tested taxa, paired t-tests and paired Wilcoxon tests (Fig. 4) demonstrate no significant differences between July and September values of *Betula*, *Pinus* and Poaceae, while *Artemisia* values are significantly higher in September. The rarefied number of pollen taxa does not significantly differ between July and September. Pollen concentration is significantly lower in September (105,000 pollen grains/cm^3) in comparison to July (155,000 pollen grains/cm^3).

For the pollen assemblages, the PCA demonstrates a close position of most sample pairs to each other (Fig. 5). This indicates a high similarity of pollen spectra in July and September. However, the sample pairs 5, 8, 10 and 12 have larger distances between each other on the first two axes.

Pollen-vegetation relationships

In terms of vegetation interpretation, the PCA groups the samples in three clusters (Fig. 5). The "hay meadow" group (samples 3 and 11) is characterized by Poaceae, *Plantago lanceolata*-type, *Galium*-type, *Rumex acetosa*-type and *Alchemilla pentaphyllea*-type. For the "fodder meadow" group (samples 2, 6, 8 and 10), *Medicago sativa*-type, Cichorioideae, Cerealia-type and *Pimpinella saxifraga* are important. The "forest" group (samples 5, 7, 9 and 12) is related to *Pinus diploxylon*-type, *Betula*, *Artemisia*, *Filipendula* and *Mentha*-type. Based on the distribution of the variables, the first axis is associated with open vs. forested vegetation types, while the second axis is connected to land-use form: abandoned cultivated fodder vs. semi-natural hay meadows in use.

Interpretation and discussion

Role of season in surface sample collection

The results of this study support the general assumption that moss polsters may preserve and integrate several years of pollen rain (Crowder and Cuddy 1973; Bradshaw 1981; Caseldine 1981; Räsänen et al. 2004; Pardoe et al. 2010). The statistical tests demonstrate that season of collection does not play a significant role for pollen spectra composition of moss surface samples. The dominant taxa in our samples – *Betula*, *Pinus diploxylon*-type and Poaceae – are similarly abundant in July and September samples. However, taxa with low percentages in the samples are more sensitive to the season of collection. In our case, *Artemisia* values are significantly higher in September after the flowering of *Artemisia* species in August and possibly before the signal is washed out from the moss polsters. Also, the PCA reveals the similarity of the July and September sample pairs. Larger distances in the sample pairs 5, 8, 10 and 12 can be explained by differences in Poaceae, *Pinus diploxylon*-type and *Betula* values within each pair (Fig. 5), since the Euclidian distances are sensitive to changes in the values of dominant taxa (Kindt and Coe 2005). Furthermore, the first two axes of the PCA plot reflect 46% of the variability in the dataset and representation of single samples vary considerably. Several factors are considered to influence the variability of pollen assemblages within the same vegetation plot, for example the combination of a few subsamples, and the heterogeneity of collected material and microhabitats. However, with this dataset the influence of these factors cannot be evaluated.

Our results contrast with those of Cundill (1985) and Farell et al. (2016), who found strong differences in pollen assemblages from mosses collected in different months. In addition to the fact that our study covers just two seasons within one year, we think that different sampling strategy in the studies plays a crucial role. Cundill (1985) and Farrell et al. (2016) collected only green parts of the mosses without basal sections. This can result in a very young age of the mosses varying from a few months to one year as well as the lack of pollen in the sample that has been washed into a deeper part of moss cushion or soil. In our study, we collected moss or litter together with surface soil to ensure the presence of several years of pollen deposition. Furthermore, the different presentation and analysis of the data might play a role. In case of study of Cundill (1985), the data are shown as concentration per weight, whilst we present our data as percentages, eliminating problems of

variability in pollen concentrations during the season. As noted by Boyd (1986), while pollen values of moss surface samples expressed in absolute terms (grains/gr or grains/cc) are difficult to evaluate, the relative proportions of pollen taxa trapped by mosses are more useful. Nevertheless, Farrell et al. (2016) found distinct seasonal differences using percentages rather than concentrations. This study is based on comparison of the Bray-Curtis index reflecting ecological distances between samples (Bray and Curtis 1957). Pairs of samples collected in spring, summer and autumn within the same plot were compared for three locations and show significant differences between seasons. However, Farrell et al. (2016) do not compare the ecological distances between locations using clustering or ordination techniques, which means we cannot fully compare their results with ours. Such comparison using PCA in our study demonstrates that the pollen assemblages reflect the dominant vegetation around the sample point rather than the season of the surface sample collection (Fig. 5).

The results of our study highlight the importance of pollen relocation by precipitation, shown by the comparison of pollen concentrations between July and September. In almost all samples, pollen concentrations in September are lower than in July (Fig. 3), which we associate with the washing-out of pollen grains into the soil by rain. Cundill (1985) also found minimum pollen concentrations on the green part of moss in winter, highlighting rapid redepositing of pollen from the moss surface to its base. Other studies demonstrated that washing-out can play an important role in the trapping capacities of different mosses (Boyd 1986) or within different parts of a moss polster (Crowder and Cuddy 1973) due to their different forms and surface textures. We used bulk samples, therefore we cannot estimate the influence of the moss species. However, all but one sample (sample 2; Fig. 3) show considerably lower pollen concentrations in September, independently of the collected material (moss or litter), indicating the importance of this factor for pollen representation in the sample.

Despite considerable variation in the rarefied number of taxa (Fig. 3), the differences between July and September are not significant (Fig. 4). A closer look at the pollen diagram (Fig. 3) shows that presence of the very rare pollen taxa in the same plots differs between the seasons. We interpret this variation in presence to be an artefact of counting rather than a true seasonal differences. In contrast, we do not expect that counting artefacts affect the total number of pollen taxa detected because of the relatively high pollen counts in the samples.

Reflection of vegetation patterns in pollen spectra

The current vegetation in “Spasskaya Gora” is dominated by *Pinus*- and *Betula*-forests and open grass meadows. The general pattern is reflected well by the dominant pollen taxa of *Pinus diploxylon*-type, *Betula* and Poaceae. Samples taken within *Tilia-Betula-Populus*-forests show very low percentages of *Tilia* pollen, highlighting its underrepresentation in the pollen spectra due to low pollen production and pollination by insects (Pigott and Huntley 1980). *Ulmus, Populus* and *Alnus* occur irregularly in the spectra despite their presence in vegetation (Table 1; Ovesnov and Efimik 2014). *Picea* reaches 6% only in one sample that does not have spruce in surrounding vegetation. Possibly the location of the sampling point on a slope led to a higher amount of wind-transported pollen. *Abies* does not exceed 1% due to its low abundance in the local vegetation and poor pollen transport because of its large and heavy pollen grains (Poska and Pidek 2010; Pidek et al. 2013). *Juniperus* is present only in the samples from *Pinus* forest, where it is widespread in shrub layer, and is strongly underrepresented.

The pollen spectra of surface samples provide important insights into pollen representation in different vegetation types in “Spasskaya Gora”. Samples collected in forests have AP between 65 and 90%, reflecting the open character of forests and a rather high proportion of herbs (Fig. 3). The reason for low AP values in some samples might be that following the method suggested by Bunting et al. (2013) the position of sampling point was chosen in an opening within the forest and not under a closed canopy. Meadow vegetation in the opening brings a strong local component in the pollen assemblages. Indeed, the highest AP values of 80-90% are documented in sample 5, which is a small

opening located within the *Betula-Pinus* forest, while the other three forest samples with lower AP values (65-80%) come either from a larger opening (sample 12), from the edge of the forest (sample 7) or from forest with a very open character (sample 9) (Fig. 2). In NAP taxa, *Mentha*-type occurs only in samples from forests, reflecting the presence of *Origanum vulgare* as dominant species in the local meadow communities of the forest openings. Despite of the presence of *Origanum vulgare* in other plots (samples 8 and 10), its abundance in the vegetation is very low so that this pollen type did not appear in the samples.

In the studied meadows, AP values can reach 40-60%, indicating a well-known overrepresentation of *Betula* and *Pinus* in the spectra due to high pollen production and easy wind transport (e.g. Crowder and Cuddy 1973; Bradshaw 1981; Pardoe et al. 2010; Farrell et al. 2016). Poaceae is strongly represented here, as are Cerealia-type and Cichorioideae in some samples, reflecting a strong role of local vegetation in pollen spectra formation. Cerealia-type pollen is possibly produced by the dominant species *Bromus inermis*, which together with *Medicago sativa* was sown for fodder production. Cichorioideae is mainly produced by the ruderal species *Taraxacum officinale* that grows abundantly in mown sites. *Pimpinella saxifraga* is noted as an important species in traditionally managed hay meadows (Hjelle 1999). In our records, *Pimpinella saxifraga* together with *Phyteuma*-type occur only in meadow communities, highlighting their indicative value for the interpretation of palaeorecords.

Hay meadows located within forests (Fig. 2) are dominated by NAP, and especially Poaceae. This is in contrast to our expectations of higher AP values in comparison to more open meadows. Since both samples were collected from traditionally managed meadows, possibly land-use plays a special role here. Sample 3 was collected after mowing, while the site of sample 11 was not mown in 2018. However, both hay meadows are in use and mowing occurs in July during flowering of most of the grass and forb species. Mowing and further drying of cut plant material leads to pollen being shed from anthers and its very local preservation in moss and litter. Further pollen taxa important in the mown hay meadows are *Plantago lanceolata*-type, *Plantago major-media*-type, *Rumex acetosa*-type and *Galium*-type. These plants might be favoured by mowing. This is in accordance with studies from western Norway (Hjelle 1999) showing dominance of grasses, *Plantago lanceolata* and *Rumex acetosa*-type in mown in comparison to grazed communities.

Conclusions

Our study carried out in the natural reserve “Spasskaya Gora” (Perm, Russia) demonstrates that pollen assemblages of surface samples reflect well the general pattern of the vegetation of the Kungur forest-steppe and are not significantly influenced by the season of collection. Furthermore, we show that pollen deposition process is strongly influenced by the washing-out of pollen grains from mosses or litter surface in the soil by precipitation. Based on these findings, we conclude that the season of surface sample collection does not play a significant role in pollen assemblages and field studies can be carried out also in summer during the flowering season. However, mosses or litter should be collected with basal parts or rather surface soils in order to ensure representation of the average pollen signal for the previous years. We also recommend collecting bulk samples to minimise the influence of differences in trapping capacities between species and to dilute local extremes in the pollen deposition.

Acknowledgements

The authors thank Laura Sutcliffe for polishing the English. This work was supported by the German Research Foundation (DFG) under Grant SH 836/3-1.

References

Adam DP, Mehringer PJ. 1975. Modern pollen surface samples – an analysis of subsamples. Jour. Research U.S. Geol. Survey 3(6): 733-736.

Andersen S. 1970. The relative pollen productivity and pollen representation of north European trees, and correlation factors for tree pollen spectra determined by surface pollen analysis from forests [dissertation]. Copenhagen: Reitzels Forlag.

Beug HJ. 2004. Leitfaden der Pollenbestimmung [Guideline for pollen identification]. München: Verlag Dr. Friedrich Pfeil. German.

Birks HJB, Line JM. 1992. The use of rarefaction analysis for estimating palynological richness from Quaternary pollen-analytical data. The Holocene 2: 1-10.

Bohn U, Neuhäusl R, Gollub G, Hettwer C, Neuhäuslová Z, Schlüter H, Weber H. 2003. Map of the Natural Vegetation of Europe. Münster: Landwirtschaftsverlag.

Boyd WE. 1986. The role of mosses in modern pollen analysis: the influence of moss morphology on pollen entrapment. Pollen et Spores 28: 243-256.

Bradshaw RHW. 1981. Modern pollen representation factors for woods in southwest England. J. Ecol. 69: 45-70.

Bray JR, Curtis JT. 1957. An ordination of the upland forest communities of southern Wisconsin. Ecological Monographs 27: 325-349.

Bunting MJ, Farrell M, Broström A, Hjelle KL, Mazier F, Middleton R, Nielsen AB, Rushton E, Shaw H, Twiddle CL. 2013. Palynological perspectives on vegetation survey: a critical step for model-based reconstruction of Quaternary land cover. Quaternary Science Reviews 82: 41-55.

Caseldine CJ. 1981. Surface pollen studies across Bankhead Moss, Fife, Scotland. J. Biogeogr. 8: 7-25.

Crowder AA, Cuddy DG. 1973. Pollen in a small river basin. In: Birks HJB, West RG, editors. Quaternary Plant Ecology. Oxford: Blackwell Scientific Publications; p. 61-77.

Cundill PR. 1985. The use of mosses in modern studies at Morton Lochs, Fife. Transactions of the Botanical Society of Edinburgh 44: 375-383.

Davis M. 1963. On the theory of pollen analysis. Am. J. Sci. 261: 897-912.

Erdtman G. 1960. The acetolysis method. Svensk Botanisk Tidskrift 54: 561–564.

Farrell M, Bunting MJ, Middleton R. 2016. Replicability of data collected for estimation of relative pollen productivity. Review of Palaeobotany and Palynology 232: 1-13.

Fox J, Weisberg S. 2019. An R Companion to Applied Regression, Third edition. Thousand Oaks CA: Sage.

Hjelle KL. 1999. Modern pollen assemblages from mown and grazed vegetation types in western Norway. Review of Palaeobotany and Palynology 107: 55-81.

Horrocks M, Coulson S, Walsh K. 1998. Forensic Palynology: Variation in the Pollen Content of Soil Surface Samples. Journal of Forensic Sciences 43: 320-323.

Horrocks M, Coulson S, Walsh K. 1999. Forensic Palynology: Variation in the Pollen Content of Soil on Shoes and in Shoeprints in Soil. Journal of Forensic Sciences 44: 119-122.

Juggins S. 2007. C2 Version 1.5 User guide. Software for ecological and palaeoecological data analysis and visualisation. Newcastle upon Tyne: Newcastle University.

Kindt R, Coe R. 2005. Tree diversity analysis. A manual and software for common statistical methods for ecological and biodiversity studies. Nairobi: World Agroforestry Centre (ICRAF).

Marinova E, Harrison SP, Bragg F, Connor S, de Laet V, Leroy SAG, Mudie P, Atanassova J, Bozilova E, Caner H, Cordova C, Djamali M, Filipova-Marinova M, Gerasimenko N, Jahns S, Kouli K, Kotthoff U, Kvavadze E, Lazarova M, Novenko E, Ramezani E, Röpke A, Shumilovskikh L, Tantau I, Tonkov S. 2018. Pollen-derived biomes in the Eastern-Mediterranean-Black Sea-Caspian Corridor. J. Biogeogr. 45(2): 484-499.

Mildenhall DC, Wiltshire PEJ, Bryant VM Jr. 2006. Forensic palynology: why do it and how it works. Forensic Sci Int 163:163-172.

Mulder C, Janssen CR. 1998. Application of Chernobyl caesium-137 fallout and naturally occurring lead-210 for standartization of time in moss samples: recent pollen-flora relationships in the Allgäuer Alpen, Germany. Review of Palaeobotany and Palynology 103: 23-40.

Mulder C, Janssen CR. 1999. Occurrence of pollen and spores in relation to present-day vegetation in a Dutch heathland area. Journal of Vegetation Science 10: 87-100.

Munuera-Giner M, Carrión JS. 2016. Chapter 1. Forensic Palynology: Checking Value of Pollen Analysis as a Tool to Identify Crime Scene in Semiarid Environments. In: Kars H, van den Eijkel L, editors. Soil in Criminal and Environmental Forensics. Switzerland: Springer International Publishing; p. 3-16, DOI: 10.1007/978-3-319-33115-7_1.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H. 2019. Community Ecology Package. Version 2.5-6. URL: https://cran.r-project.org, https://github.com/vegandevs/vegan.

Overpeck JT, Webb T, Prentice IC. 1985. Quantitative interpretation of fossil pollen spectra - dissimilarity coefficients and the method of modern analogues. Quat. Res. 23: 87-108.

Ovesnov SA, Efimik EG. 2014. Flora istoriko-prirodnogo kompleksa “Spasskaya Gora” (Permskiy kray) [Flora of the historico-natural complex “Spasskaya Gora” (Perm region)]. Vestnik Udmurtskogo universiteta 4: 18-26. Russian.

Pardoe HS, Giesecke T., van der Knaap WO, Svitavská-Svobodová H, Kvavadze EV, Panajiotidis S, Gerasimidis A, Pidek IA, Zimny M, Święta-Musznicka J., Latałowa M., Noryśkiewicz AM, Bozilova E, Tonkov S, Filipova-Marinova MV, van Leewen JFN, Kalniņa L. 2010. Comparing pollen spectra from modified Tauber traps and moss samples: examples from a selection of woodlands across Europe. Vegetation History and Archaeobotany 19: 271-283.

Pidek IA, Svitavská-Svobodová H, van der Knaap WO, Magyari E. 2013. Pollen percentage thresholds of *Abies alba* based on 13-year annual records of pollen deposition in modified Tauber

traps: perspectives of application to fossil situations. Review of Palaeobotany and Palynology 195: 26-36.

Pigott CD, Huntley JP. 1980. Factors controlling the distribution of *Tilia cordata* at the northern limits of its geographical range. II. History in north-west England. New Phytologist 84: 145-164.

Poska A, Pidek IA. 2010. Pollen dispersal and deposition characteristics of *Abies alba, Fagus sylvatica* and *Pinus sylvestris*, Roztocze region (SE Poland). Vegetation History and Archaeobotany 19: 91-101.

Prentice IC, Guiot J, Huntley B, Jolly D, Cheddadi R. 1996. Reconstructing biomes from palaeoecological data: a general method and its application to European pollen data at 0 and 6 ka. Clim. Dyn. 12: 185-194.

Prentice IC, Jolly D, BIOME 6000 participants. 2000. Mid-Holocene and glacial maximum vegetation geography of the northern continents and Africa. J. Biogeogr. 27: 507-519.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Räsänen S, Hicks S, Odgaard BV. 2004. Pollen deposition in mosses and in a modified 'Tauber trap' from Hailuoto, Finland: what exactly do the mosses record? Rev. Palaeobot. Palynol. 129: 103-116.

Sugita S. 2007a. Theory of quantitative reconstruction of vegetation. I. Pollen from large sites REVEALS regional vegetation composition. Holocene 17: 229-241.

Sugita S. 2007b. Theory of quantitative reconstruction of vegetation. II. All you need is LOVE. Holocene 17, 243-257.

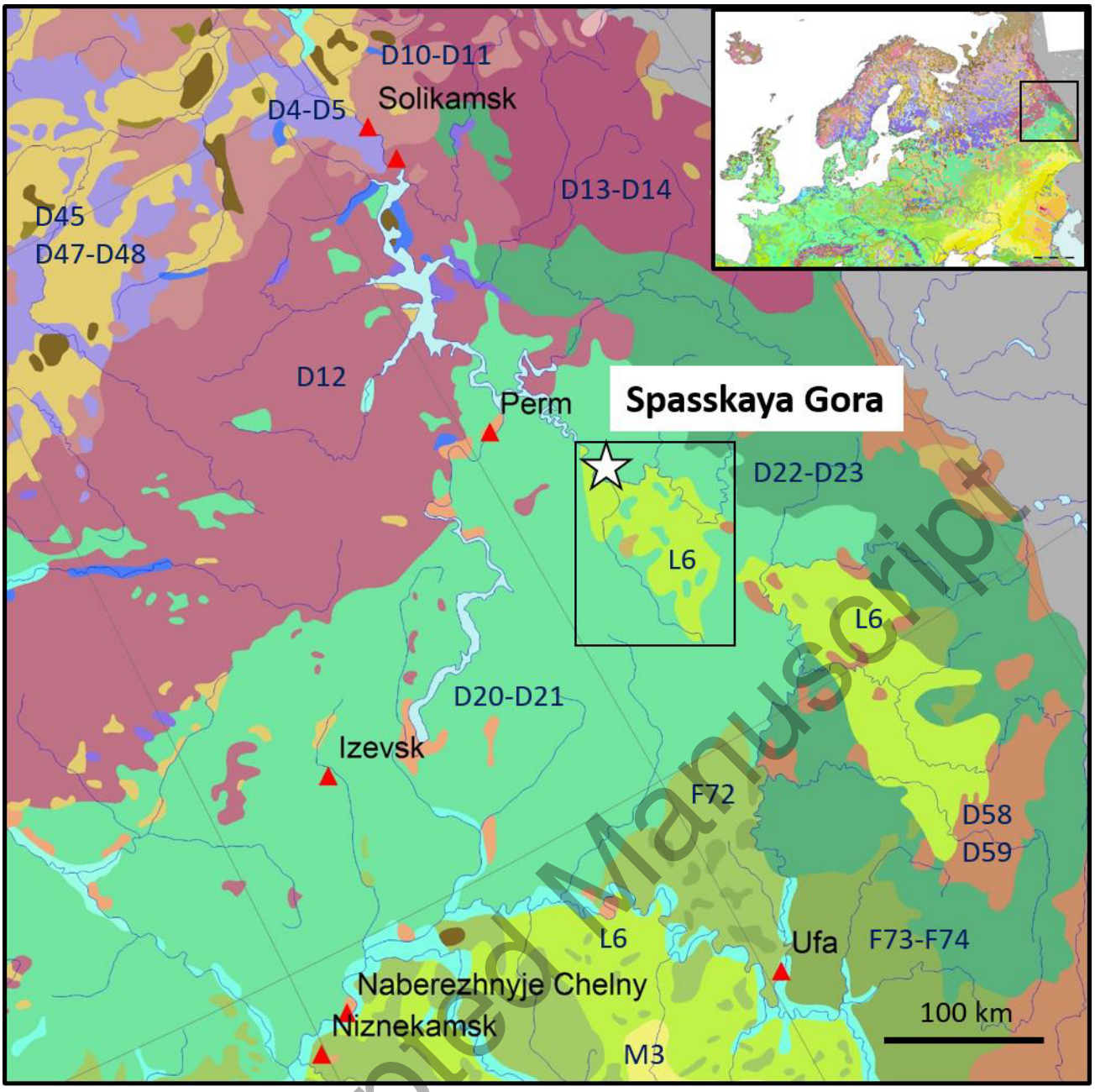

Fig. 1. Vegetation map of Europe with the location of the Kungur forest-steppe and natural reserve "Spasskaya Gora" (based on Bohn et al. 2003). Vegetation units: D4 - North European moss-rich spruce forests, D5 - Northeast European hygrophilous spruce forests, D10 - Pre-Ural fir-spruce forests, D11 - Pre-Ural hygrophilous pine-spruce forests, D12 - Pre-Ural fir-spruce forests, D13 - North Ural pine-spruce forests, D14 - Middle Ural spruce-fir forests, D20 - Pre-Ural herb-rich fir-spruce forests, D21 - Pre-Ural herb-rich fir-spruce forests, D22 - Middle and south Ural herb-rich spruce-fir forests, D23 - South Ural tall-herb-rich mixed spruce-fir forests, D45 - North European pine forests, D47 - North and east European hygrophilous pine forests, D48 - North and east European pine forests, D58 - Middle and south Ural herb-rich pine forests, D59 - South Ural herb-rich pine forests, F72 - Southeast Sarmatian lime-pedunculate oak forests, F73 - Pre-Ural lime forests, F74 - Ural maple-lime-pedunculate oak forests, L6 - Transvolgian-pre-Ural meadow steppes, M3 – Transvolgian herb-rich grass steppes.

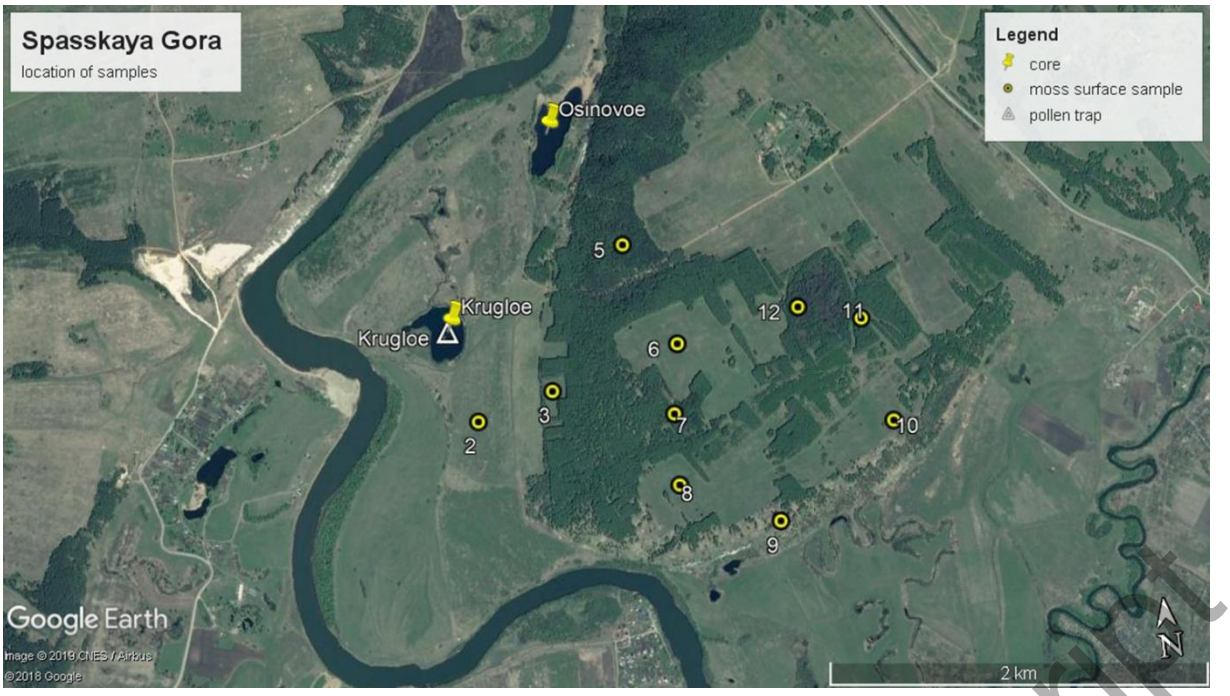

Fig. 2. Location of surface samples collected in the natural reserve “Spasskaya Gora”.

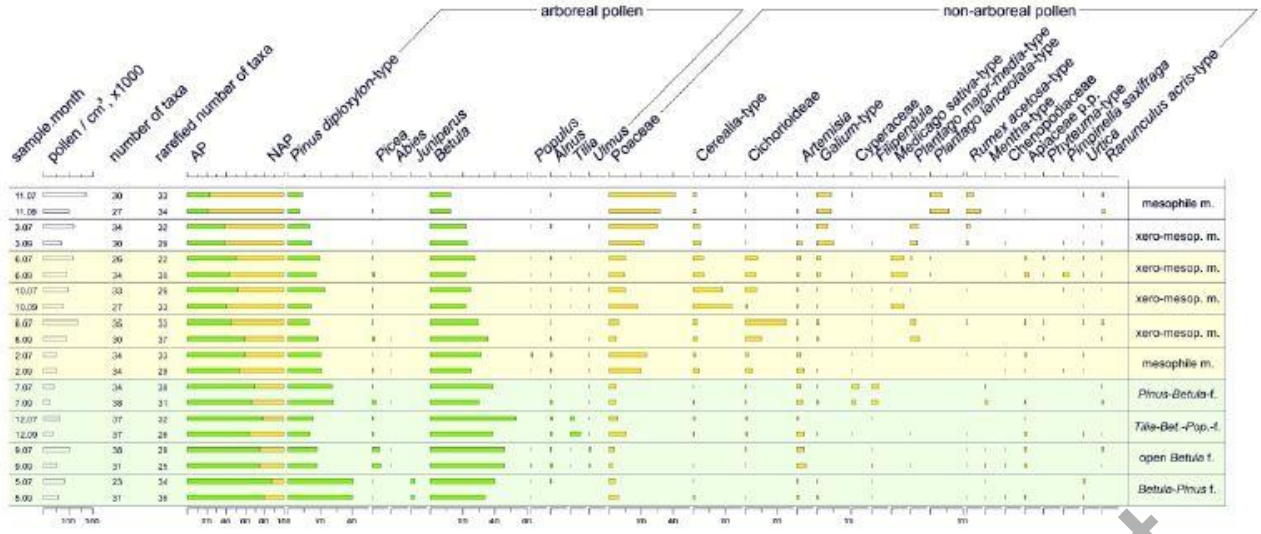

Fig. 3. Selected curves of pollen spectra from forested and open habitats of the natural reserve "Spasskaya Gora".

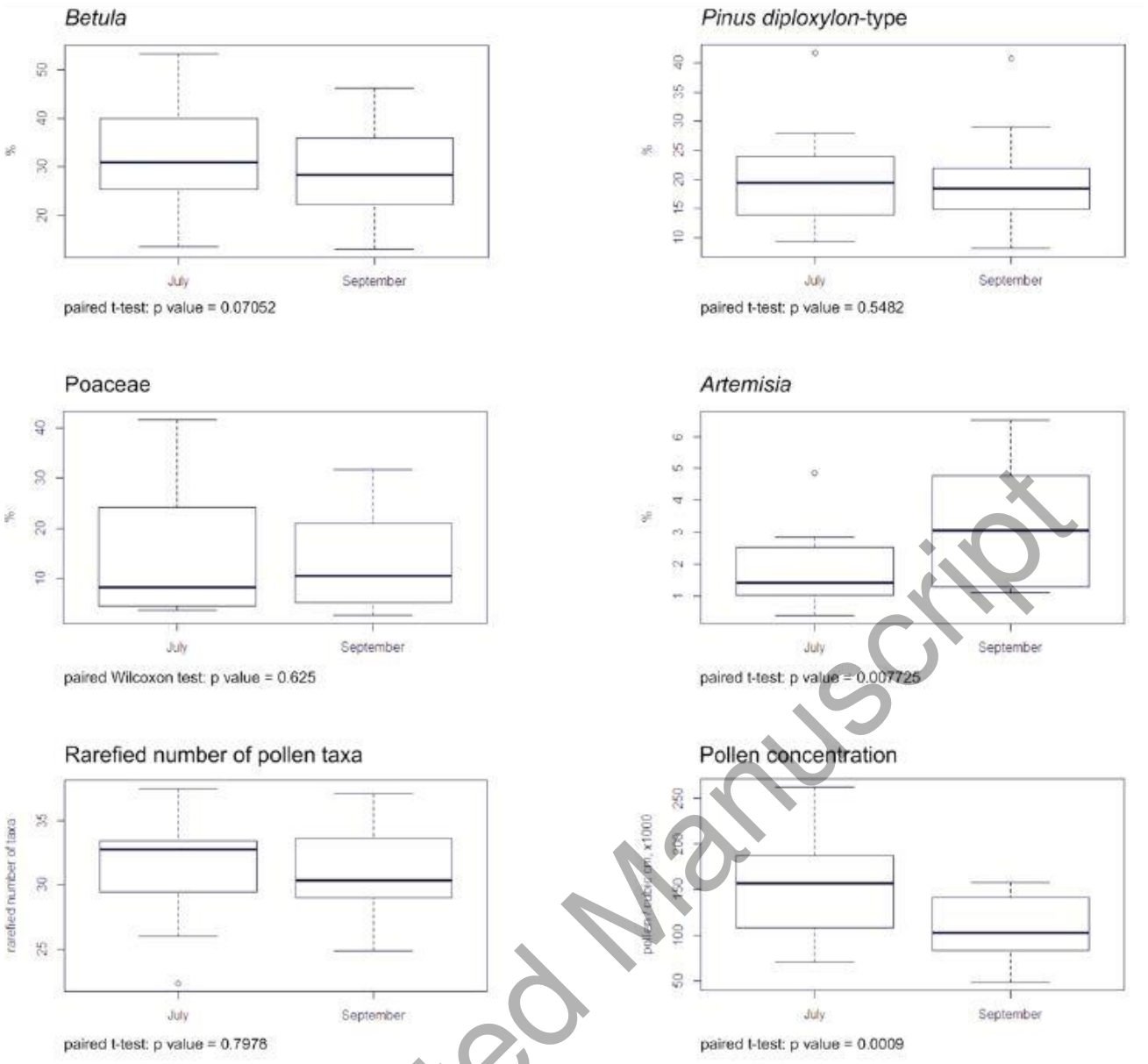

Fig. 4. Boxplots of t-tests and Wilcoxon test for selected taxa (*Betula*, *Pinus diploxylon*-type, Poaceae, *Artemisia*), number of species and concentration.

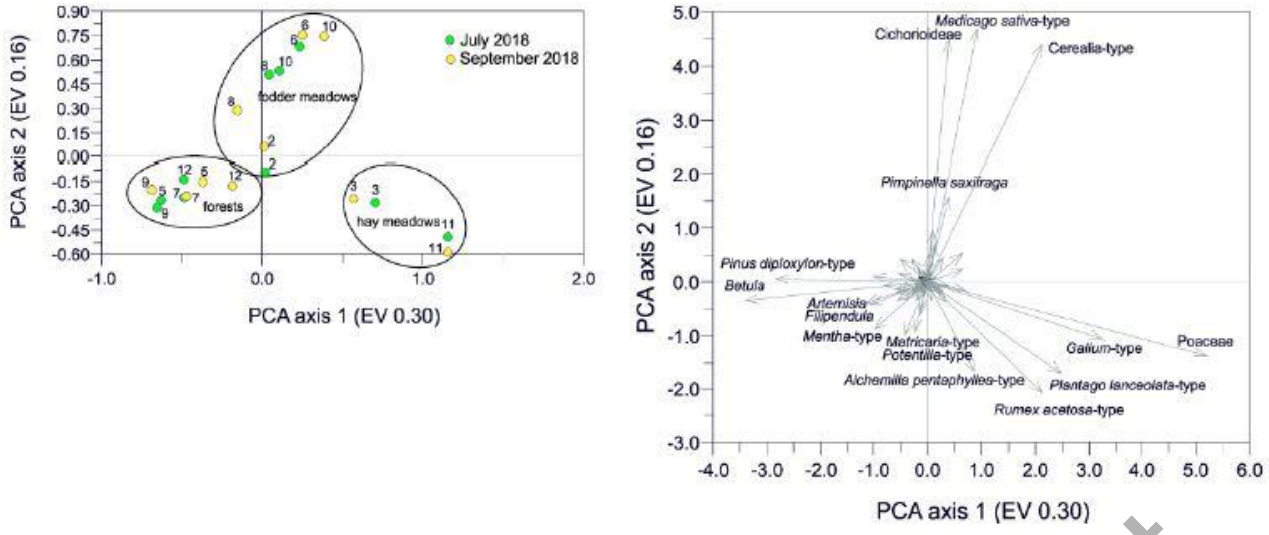

Fig. 5. Principal component analysis (PCA) of pollen spectra of surface samples from "Spasskaya Gora".

Table 1. Description of vegetation at sampling points.

Nr.	Lat, N	Long, E	Alt, m	Vegetation type	Land use	Dominant taxa
2	57.4811	56.8981	116	Mesophile herb-grass meadow	Fallow arable land, hay	*Dactylis glomerata* L., *Poa pratensis* L., *Taraxacum officinale* Wigg., *Tanacetum vulgare* L., *Cirsium arvense* (L.) Scop., *Artemisia vulgaris* L., *Picris hieracioides* L., *Sonchus arvensis* L., *Potentilla anserina* L., *Aegopodium podagraria* L., *Pimpinella saxifraga* L., *Lathyrus pisiformis* L., *L. pratensis* L., *Veronica longifolia* L., *V. teucrium* L., *Trifolium medium* L., *Galium mollugo* L.
3	57.4820	56.9051	127	Xero-mesophile herb-grass meadow within *Populus-Betula* forest	Traditional hay	*Achillea millefolium* L., *Hieracium caespitosum* Dumort., *Cirsium arvense* (L.) Scop., *Taraxacum officinale* Wigg., *Alchemilla vulgaris* L., *Fragaria vesca* L., *Galium mollugo* L., *Leucanthemum vulgare* Lam., *Plantago major* L., *P. media* L., *Agrostis tenuis* Sibth., *Bromus inermis* Leyss., *Dactylis glomerata* L., *Festuca pratensis* Huds., *Phleum pratense* L., *Poa pratensis* L., *Veronica chamaedris* L., *V. teucrium* L., *Ranunculus acris* L.
5	57.4884	56.9130	181	Opening in *Betula-Pinus* forest	-	*Pinus sylvestris* L., *Betula pendula* Roth, *Juniperus communis* L., *Lonicera xylosteum* L., *Aegopodium podagraria* L., *Agrimonia eupatoria* L., *Dactylis glomerata* L., *Calamagrostis canescens* (Weber) Roth, *Phleum pratense* L., *Cirsium arvense* (L.) Scop., *C. heterophyllum* (L.) Hill, *Filipendula vulgaris* Moench, *Fragaria vesca* L., *F. viridis* (Duchesne) Weston, *Galium mollugo* L., *Hieracium umbellatum* L., *Lathyrus sylvestris* L., *Origanum vulgare* L., *Prunella vulgaris* L., *Pulmonaria mollis* Wulfen ex Hornem., *Rubus saxatilis* L.
6	57.4835	56.9166	181	Xero-mesophile herb-grass meadow with *Betula* patches	Cultivated fodder	*Arrhenatherum elatius* (L.) J.Presl et C. Presl, *Bromus inermis* Leyss., *Dactylis glomerata* L., *Festuca pratensis* Huds., *Phleum pratense* L., *Poa pratensis* L., *Fragaria viridis* (Duchesne) Weston, *Galium mollugo* L., *Picris hieracioides* L., *Medicago sativa* L., *Pimpinella saxifraga* L., *Prunella vulgaris* L., *Ranunculus acris* L., *R. polyanthemos* L., *Taraxacum officinale* Wigg., *Vicia cracca* L., *Vicia sepium* (L.) Moench
7	57.4802	56.9155	173	Opening in *Pinus-Betula* forest	-	*Betula pendula* Roth, *Pinus sylvestris* L., *Populus tremula* L., *Alchemilla vulgaris* L., *Anthoxanthum odoratum* L., *Arrhenatherum elatius* (L.) J.Presl et C. Presl, *Calamagrostis arundinacea* (L.) Roth, *Dactylis glomerata* L., *Deschampsia cespitosa* (L.) P. Beauv., *Heracleum sibiricum* L., *Phleum pratense* L., *Poa pratensis* L., *Carex contigua* Hoppe, *Centaurea scabiosa* L., *Cirsium arvense* (L.) Scop., *Filipendula vulgaris* Moench, *Fragaria viridis* (Duchesne) Weston, *Galium boreale* L., *Geranium pratense* L., *G. sylvaticum* L., *Picris*

						hieracioides L., *Lathyrus pisiformis* L., *L. pratensis* L., *L. sylvestris* L., *Origanum vulgare* L., *Pyrethrum corymbosum* (L.) Scop., *Trifolium montanum* L., *Verbascum nigrum* L., *Veronica teucrium* L.
8	57.4768	56.9151	166	Xero-mesophile grass-herb meadow with *Betula* patches	Cultivated fodder	*Picris hieracioides* L., *Leucanthemum vulgare* Lam., *Arrhenatherum elatius* (L.) J.Presl et C. Presl, *Bromus inermis* Leyss., *Dactylis glomerata* L., *Festuca pratensis* Huds., *Phleum phleoides* (L.) H. Karst., *Achillea millefolium* L., *Campanula patula* L., *Fragaria vesca* L., *Galium mollugo* L., *Medicago sativa* L., *Plantago media* L., *Prunella vulgaris* L., *Pyrethrum corymbosum* (L.) Scop., *Taraxacum officinale* Wigg., *Trifolium montanum* L., *T. pratense* L., *Veronica teucrium* L.
9	57.4745	56.9235	170	Open *Betula* forest with xero-mesophile herb-rich meadows	-	*Brachypodium pinnatum* (L.) Beauv., *Calamagrostis epigeios* (L.) Roth, *Dactylis glomerata* L., *Phleum phleoides* (L.) H. Karst., *Poa angustifolia* L., *Astragalus danicus* Retz., *Filipendula vulgaris* Moench, *Fragaria viridis* (Duchesne) Weston., *Galium boreale* L., *Inula hirta* L., *Artemisia sericea* Weber ex Stechm., *Hieracium umbellatum* L., *Origanum vulgare* L., *Libanotis krylovii* V.N. Tikhom., *L. montana* Crantz , *Trommsdorfia maculata* (L.) Bernh., *Trifolium medium* L., *T. pratense* L., *Veronica teucrium* L.
10	57.4785	56.9346	170	Xero-mesophile herb-grass meadow close to *Betula* forest	Cultivated fodder	*Anthoxanthum odoratum* L., *Arrhenatherum elatius* (L.) J.Presl et C. Presl, *Bromus inermis* Leyss., *Festuca pratensis* Huds., *Phleum pratense* L., *Poa pratensis* L., *Alchemilla vulgaris* L., *Centaurea scabiosa* L., *Cirsium arvense* (L.) Scop., *Fragaria viridis* (Duchesne) Weston, *Galium boreale* L., *Hieracium umbellatum* L., *Lathyrus pisiformis* L., *L. pratensis* L., *Medicago sativa* L., *Melilotus albus* Medik., *Pyrethrum corymbosum* (L.) Scop., *Taraxacum officinale* Wigg., *Trommsdorfia maculata* (L.) Bernh., *Trifolium pratense* L., *T. medium* L., *Veronica spicata, V. teucrium* L., *Vicia cracca* L.
11	57.4835	56.9330	186	Mesophile herb-grass meadow close to *Tilia-Betula-Populus*-forest	Traditional hay	*Agropyron reflexiaristatum* Nevski, *Agrostis tenuis* Sibth., *Arrhenatherum elatius* (L.) J.Presl et C. Presl, *Dactylis glomerata* L., *Phleum pratense* L., *Alchemilla vulgaris* L., *Cirsium arvense* (L.) Scop., *Galium mollugo* L., *Hieracium caespitosum* Dumort., *Lathyrus pratensis* L., *Leucanthemum vulgare* Lam., *Plantago lanceolata* L., *Ranunculus acris* L., *Rumex acetosa* L., *Rumex crispus* L.
12	57.4844	56.9275	191	Opening in *Tilia-Betula-Populus*-forest	-	*Tilia cordata* Mill., *Betula pendula* Roth, *Populus tremula* L., *Padus avium* Mill., *Aconitum septentrionale* Koelle, *Aegopodium podagraria* L., *Alchemilla vulgaris* L., *Cirsium heterophyllum* (L.) Hill, *Geranium sylvaticum* L., *Dactylus glomerata* L., *Deschampsia cespitosa* (L.) P. Beauv., *Millium*

						effusum L., *Polygonum bistorta* L., *Sanguisorba officinalis* L.

Table 2. p-values of Shapiro-Wilk test (normal distribution of $p > 0.05$).

Variable	July	September
Betula	0.9924	0.9446
Pinus diploxylon-type	0.2387	0.1267
Poaceae	0.01116	0.2834
Artemisia	0.1731	0.2294
Rarefied number of taxa	0.1805	0.8788
Pollen concentration	0.9334	0.784