

1 *Research Paper*

2 **Pollen productivity estimates from old-growth forest strongly differ from**  
3 **those obtained in cultural landscapes – evidence from the Białowieża National**  
4 **Park, Poland**

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26

27 **Abstract**

28 Pollen productivity estimates of individual plant taxa are necessary when determining changes of  
29 vegetation cover during the Holocene. To date, studies describing this parameter in lowland temperate  
30 Europe have been carried out in cultural landscapes showing low forest cover and dominated by human  
31 activities. However these may be of limited use when applied to reconstruct past land cover, for  
32 instance, from pre-agricultural landscapes. The aim of this paper is to ascertain whether pollen  
33 productivity from the closed canopy old-growth forest in the Białowieża National Park, Poland, where  
34 human impact has been minimal for nearly a century, is different from that calculated in much more  
35 open landscapes. We ask: how much does forest antiquity and structure influence the amount of pollen  
36 released from particular taxa? We implemented maximum likelihood estimation of relative pollen  
37 productivity for seven tree species and for *Poaceae* using 18 modern pollen assemblages and distance-  
38 weighted plant abundances. Our results demonstrate that the ratio of pollen productivity between high  
39 producers (*Pinus sylvestris* and *Quercus robur*) and low producers (*Poaceae*, *Corylus avellana*) is on an  
40 average six times greater in Białowieża than across other European cultural landscapes. Pollen from  
41 forest *Poaceae* and *C. avellana* is six times more underrepresented in old-growth forest than hitherto  
42 estimated from cultural landscapes. This finding reinforces the idea that pollen productivity can vary in  
43 response to changes in the prevailing environmental settings and we present for the first time a  
44 quantification of this variability, likely induced by differences in light availability.

45 **Key words**

46 canopy structure, ERV-models, landscape openness, moss polsters, old-growth forest, relative pollen  
47 productivity

48 **Introduction**

49 Advances in quantitative reconstruction of vegetation cover using fossil pollen over the last decade have  
50 made it possible to estimate land cover throughout the Holocene for most of Europe (Gaillard et al.,  
51 2008, 2010; Marquer et al., 2014; Mazier et al., 2012; Nielsen et al., 2012), an important parameter for  
52 climate modelling in relation to climate change (Gaillard et al., 2010; Strandberg et al., 2014; Trondman  
53 et al., 2015). Landscape openness is also critical to studies focusing on the impact of past civilisations on  
54 their environment, with respect to their cultural, economic and demographic dynamics (Nielsen et al.,  
55 2012).

56 These advances are due to the conceptual and technical progress in landscape reconstruction algorithms  
57 that translate pollen assemblages from small and large sedimentary basins into the estimation of  
58 percent vegetation cover or biomass (Sugita, 2007a, 2007b). Sugita's methodology used in these models  
59 solves a major difficulty in the analysis of fossil pollen, i.e. the confounding influence of spatial  
60 distribution of vegetation onto pollen proportion in sediments (Davis, 2000). One crucial parameter  
61 within Sugita's algorithms is the estimation of relative pollen productivity that is specific to different  
62 plant species (Broström et al., 2008; Hellman et al., 2008). Relative pollen productivity is the ratio  
63 between the pollen productivity of two species (Sugita et al., 1999).

64 To date, relative pollen productivity estimates (PPE) have been established for all the major tree species  
65 throughout cultural landscapes of temperate Europe where agriculture and urbanisation directly modify  
66 between 40 and 90% of the plant cover or from boreal and subalpine environments where climate is  
67 significantly colder and where lower pollen productivity is predictable (Table 1). Other studies estimate  
68 pollen productivity for various herbs but no trees (e.g. Broström et al., 2004; Bunting and Hjelle, 2010;  
69 Hjelle, 1998).

70 [Insert Table 1]

71 However, what is still unknown is whether results from cultural landscapes can be utilised to interpret  
72 fossil pollen assemblages from landscapes that have undergone different disturbance regimes such as  
73 old-growth forests or ancient landscapes with vegetation cover relatively untouched by agriculture. For  
74 instance, the composition and structure of vegetation before the advent of agriculture in Europe, as  
75 reconstructed using fossil pollen, has been at the heart of a polarized debate in recent decades following  
76 Vera's hypothesis in (2000). Are pre-agricultural landscapes best described as high-forest (i.e.  
77 continuous forest cover with a closed canopy wherever the relief and hydrology permits tree growth) or  
78 wood-pasture (i.e. patchy forest cover within a mosaic of regeneration stages driven by large herbivores,  
79 such as bison, aurochs, horses and other natives)? This question is of importance to guide nature  
80 conservation and forest management because ancient landscapes reconstructed with fossils are a  
81 source of inspiration and can serve as a baseline for restoration. Many palaeoecologists have argued for  
82 high forest on the basis of the fossil pollen record (eg Birks, 2005; Bradshaw et al., 2003; Mitchell, 2005)  
83 and the fossil beetle assemblages (Buckland, 2005; Smith et al., 2010). However, these studies and  
84 others (eg Svenning, 2002; Hall, 2008; Nielsen et al., 2012) also highlight the importance of natural  
85 forces such as wildfires, wind throws, hydrology and soil fertility, along with grazing, to create the  
86 dynamic necessary to open forest gaps and promote tree recruitment and regeneration of more light-  
87 demanding species. In this respect, validation of these competing hypotheses with more accurate  
88 quantitative reconstructions of past vegetation is still urgently needed.

89 The overarching aim of our study is to estimate new pollen productivity data from an old-growth forest  
90 that had minimum human impact over time and that can serve as an important contrast to the cultural  
91 landscapes previously used to calculate PPE. We chose the forest located in the Białowieża National Park  
92 (BNP) in Poland because this is often regarded as a recent analogue for primeval lowland woodland  
93 where large herbivores are among the most important disturbance factors (Bobiec, 2002; Faliński,  
94 1986). The BNP is covered by extensive old-growth forest not exploited by forestry or other human

95 activities since nearly 100 years and is nested at the heart of the largest fragment of temperate  
96 woodland in lowland Europe– the Białowieża Forest. Our key objectives are:

97 i. To establish relative pollen productivity estimates (PPE) for the main trees and *Poaceae* in the  
98 old-growth forest of Białowieża.

99 ii. To compare PPE from the Białowieża Forest to those obtained in cultural landscapes across  
100 Europe.

101 iii. To discuss any differences in the light of the disturbance regimes and environmental settings  
102 that prevail in old-growth forests and cultural landscapes.

### 103 **Study Area**

104 The Białowieża Forest is a flagship ecosystem in European nature conservation (Bobiec, 2002; Peterken,  
105 1996). It covers about 1450 km<sup>2</sup> (52°29′-52°57′N; 23°31′-24°21′E) straddling the border between Poland  
106 and Belarus (Figure 1). Białowieża Forest is the largest expanse of lowland temperate closed canopy  
107 forest in Europe and it is unique for the complete assemblage of native trees, large herbivores and  
108 carnivores, for its size and for its antiquity (Faliński, 1986).

109 [Insert Figure 1]

110 The whole area of Białowieża Forest has international protection as a UNESCO Biosphere Reserve and  
111 World Heritage site, a Ramsar site, and a Natura 2000 site as well as having protected status at the  
112 national and local level. Special protection applies to 105 km<sup>2</sup> within the Polish Białowieża National Park  
113 (BNP) and to 157 km<sup>2</sup> within the Belarus *Belovezhskaya Pushcha* National Park. Strict protection, where  
114 no human intervention is allowed and access is limited to research or restrictive tourism, started as early  
115 as 1921 for 47.5 km<sup>2</sup> of old growth forest within today's BNP. Outside this area and outside the  
116 numerous other smaller nature reserves present across Białowieża Forest, both hunting and forestry are

117 current practice, thereby directly influencing the populations of large herbivores and trees (Jędrzejewski  
118 et al., 2006; Kuijper et al., 2010a)

119 The Białowieża Forest has been under protection from agricultural development from the Middle Ages  
120 when it became a game reserve for Lithuanian and Polish rulers (Samojlik, 2005). As a result, it has  
121 remained remarkably untouched by human activities in contrast with the rest of Europe (Faliński, 1986;  
122 Latałowa et al., 2015). No large-scale agriculture or industry has ever been prominent. However, small-  
123 scale activities such as subsistence farming and various forest crafts are all reported from historical  
124 archives. Historical variation of these disturbance regimes explain much of the forest diversity today  
125 (Bobiec, 2012; Niklasson et al., 2010; Pigott, 1975). In spite of these activities, the area has experienced  
126 exceptional stability with regards to land cover for the last 200 years (Mikusińska et al., 2013). The main  
127 natural disturbance factors that have been shaping the forest composition and structure are: large  
128 herbivore selective browsing (Kuijper et al., 2010a; Smit et al., 2012), pathogen outbreaks such as the  
129 European spruce bark beetle (*Ips typographus*) (Bobiec et al., 2011; Miścicki, 2012) and wind throws  
130 (Faliński, 1978). Large herbivores and carnivores in this forest include bison (*Bison bonasus*), moose  
131 (*Alces alces*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), wild boar (*Sus scrofa*), wolves  
132 (*Canis lupus*), and lynx (*Lynx lynx*) (Kuijper et al., 2010a).

133 In terms of vegetation, this large forest complex is located at the transition between the boreal and the  
134 nemoral biogeographic zones. This is reflected by the co-occurrence of sub-oceanic, central-European  
135 and boreal forest communities, i.e. with deciduous trees and the evergreen spruce (*Picea abies*). The  
136 boreal elements of the flora and vegetation structure are promoted by a relatively short growing season  
137 (179 days) and long duration of snow cover (92 days) (Boczoń 2006; Faliński, 1986). For the period 1950-  
138 2003, the average annual precipitation was 627.5 mm and the temperatures were as follows: 6.8°C  
139 (annual), -4.3°C (January), 17.7°C (July) (Boczoń, 2006). However, in the last decade, precipitation has

140 systematically decreased, while temperatures have systematically increased, resulting in extension of  
141 growth seasons up to around 220 days long (Malzahn et al., 2009).

142 The Białowieża Forest is dominated by mesophile oak-linden-hornbeam forest community (*Tilio-*  
143 *Carpinetum*), having a multi-layered structure with *P. abies* in the top-most layer (up to 50m high), oak  
144 (*Quercus robur*), linden (*Tilia cordata*) and Norway maple (*Acer platanoides*) below it, and hornbeam  
145 (*Carpinus betulus*) in the lowest tree-layer closing space between other trees. Hazel (*Corylus avellana*)  
146 occurs in the shrub layer, together with juvenile forms of other trees. Ground water level and soil  
147 fertility determine the particular dominance and composition of the herb layer of this very biodiverse  
148 community (Faliński, 1986; Sokołowski, 1993). Other communities include mixed conifer forests with *P.*  
149 *abies* or pine (*Pinus sylvestris*) as dominant species, and lower abundances of *Q. robur* and birches  
150 (*Betula* spp.). These conifer forests appear to be in a process of transformation, manifested by a  
151 reduction of *Picea abies* and *Pinus sylvestris* and an increase in mesophile taxa, mainly *Carpinus betulus*  
152 and *T. cordata* (Bobic, 2012; Faliński, 1988). Also, small stands of pine forest (*Vaccinio vitis-idaeae-*  
153 *Pinetum*) are restricted to the nutrient-poor substrate found in areas of inland sand-dune systems.

154 Over 20% of the BNP is covered by forest growing on waterlogged soils, with a variety of fertility levels  
155 and hydrological regimes (Czerepko 2008). Ash-alder forest (*Circeo-Alnetum*) and ash-elm forest (*Ficario-*  
156 *Ulmetum*) occur along streams and in river valleys. These tree stands are dominated by alder (*Alnus*  
157 *glutinosa*), elm (*Ulmus glabra*), ash (*Fraxinus excelsior*) and *Picea abies*, while *Corylus avellana* grows in  
158 the shrub layer. In local depressions with stagnant water, alder carr develops with *A. glutinosa* as  
159 dominant species and lower abundances of *P. abies* and *B. pubescens*. Raised bogs and transitional  
160 peatlands support marshy forests dominated by either *Pinus sylvestris* or *Betula* spp. or a mixture of  
161 both, and have a lower abundance of *Picea abies*. Transitional bogs have the typical character of those  
162 found in the boreal zone, with spruce forests of the type *Sphagno girgensohnii-Piceetum* (Czerepko,

163 2008; Faliński, 1986). This is characterised by weak growth forms of *P. abies*, *B. pubescens* in lower  
164 abundances, a limited dwarf-shrub and herb layers, and a very rich moss layer. We refer the reader to  
165 the phytosociology literature for more information on the forest communities present in the Białowieża  
166 Forest and the BNP (eg Faliński, 1986; Sokołowski, 1993).

## 167 **Methods**

### 168 *Pollen*

169 All the field work was carried out within the BNP (52°45'07''N, 23°52'44''E) in August 2011. Moss  
170 polsters were collected from the forest floor, at 18 locations well-spread in space and showing diverse  
171 old forest stands (Figure 2, ESM Table S1). Twelve sampling sites were located in oak-linden-hornbeam  
172 forest and six in different types of coniferous forests. We sampled three or four moss sub-samples  
173 including both green and brown parts (Räsänen et al., 2004) within a 1 m<sup>2</sup> quadrat using a ring of 21.2  
174 cm<sup>2</sup>. The sub-samples were mixed in a plastic bag and kept in the dark at 4° C. The moss polsters were  
175 treated in the laboratory according to Hicks et al.'s (1999) protocol: rinsed in distilled water and sieved  
176 through 200 µm mesh, then boiled in 10% KOH for 5 minutes, followed by Erdman's acetolysis, staining  
177 and mounting in glycerine. Over 1000 pollen grains per sample were counted and identified with specific  
178 keys (Beug, 2004; Moore et al., 1991; Punt et al., 1976-2003) and the reference collection of the  
179 Laboratory of Palaeoecology and Archaeobotany, University of Gdańsk.

180 [insert Figure 2]

### 181 *Vegetation survey*

182 The vegetation survey was conducted at three spatial scales and followed standard practice for the  
183 estimation of pollen productivity based on moss polsters (Broström et al., 2008), in particular Bunting et  
184 al. (2013) for the first 10m and Mazier et al. (2008) from 10m to 100m. It was conducted around each 1  
185 m<sup>2</sup> quadrat sampled for pollen. The percentage cover of ground flora was estimated visually using four

186 transects of four 1 m<sup>2</sup> quadrats to 10 m away from the sampling point (distances from 0.5, 1.5, 3.5 and  
187 7.5 m). Damage from large herbivores was recorded as present or absent for each species in each  
188 quadrat. All plant identifications followed *Flora Europaea* (Tutin et al., 1964-1980) and *Flora Vegetativa*  
189 (Eggenberg and Möhl, 2008) for diagnostic vegetative characters. The canopy cover was also recorded  
190 using four transects along a distance of 100 m. For each transect the canopy composition and  
191 percentage cover was recorded within four 5m-radius relevés (distance 15, 30, 50 and 90 m), while  
192 canopy directly above the sampling quadrat was recorded in a 10-m radius relevé. Only mature trees  
193 producing pollen were recorded during the canopy survey. The vegetation data within 1000 m radius  
194 from the sampling point was prepared on the basis of existing floristic and phytosociological studies  
195 (Sokołowski, 1993, 2004). For each forest type, we compiled a list of tree and *Poaceae* species and their  
196 average percent cover within the community. The maps of forest habitat types and tree stands were  
197 drawn using the Numerical Map of BNP 2001 and a GIS (Quantum GIS ver. 2.0). In a GIS environment  
198 (ArcGIS 10.1), we compiled the survey and forest map data into a single vegetation map and split it into  
199 adjoining concentric rings at regular distances from the sampling point (0.5, 1.5, 3, 6, 10, 23.7, 41.2,  
200 72.8, 100 meters and then every 50 m from 100 m to 1000 m). Thus, we obtained the surface cover of  
201 all tree and herb species (in m<sup>2</sup> or %) within each concentric ring.

## 202 *Data Handling and Modelling*

203 To calculate PPE of main trees at our research sites, we undertook a whole modelling approach similar  
204 to that of Prentice and Sugita (Parsons and Prentice, 1981; Prentice and Parsons, 1983; Sugita, 1993;  
205 Sugita et al., 1999). This approach calculates PPE by maximum likelihood using pollen counts as a  
206 response variable and vegetation data as explanatory variable. We implemented three types of  
207 distance-weighting transformation on the vegetation data (Prentice and Parsons, 1983; Prentice, 1985;  
208 Sugita, 1993; Sugita et al., 1999) by down-weighting plant species surface cover in the concentric rings,  
209 proportionally with distances from the sampling points. This method simulates pollen dispersal such that

210 pollen accumulation is influenced by the distance of a sampling point from the pollen source. The three  
211 methods are inverse distance ( $1/d$ ), inverse square distance ( $1/d^2$ ) and pollen-type specific (pt-s)  
212 distance-weighting (Prentice, 1985). Pollen-type specific distance-weighting is based on the travelling of  
213 small particles in turbulent air above the canopy and has been successfully applied in similar situations  
214 (Broström et al., 2008). To implement this type of distance-weighting, we used the pollen fall speed as in  
215 Sugita (1999).

216 Three maximum likelihood algorithms were used to obtain relative PPE. These were implemented using  
217 vegetation data, distance-weighted in three ways as presented above, resulting in nine combinations of  
218 algorithm-distance-weighting. The algorithms are named ERV 1, ERV 2 and ERV 3 (Prentice and Parsons,  
219 1983; Sugita, 1993) and make different assumptions regarding the background pollen contribution  
220 across sites (summarised in Broström et al., 2008). All three algorithms make reasonable assumptions in  
221 many situations and there is no *a priori* reason to favour one over another, so all three algorithms are  
222 usually applied (Broström et al., 2008). The assumptions underlying each algorithm are reviewed in  
223 detail in Broström et al. (2008). The relevant source area of pollen (Sugita, 1994) was established by  
224 implementing each of the nine combinations of algorithm-distance-weighting at the same regular  
225 distances used for distance-weighting. We used the log-likelihood indicator to select the distance and  
226 combination of algorithm-distance-weighting that fitted best our pollen and vegetation data. The  
227 relevant source area of this best combination of parameters is reached when the log likelihood indicator  
228 stops improving with distance (ie it plateaus off when plotted against distance).

229 The final selection of plant species was obtained by repeating the above analysis until all PPE values  
230 returned by the modelling were plausible (between 0.01 and 100) and had reasonable standard errors  
231 (<10% of the PPE). The distance-weighting and maximum likelihood calculations were implemented with  
232 the 'ERV.Analysis.v1.3.0' programme (S. Sugita, unpublished).

233 To compare the PPE obtained in the BNP with those of other studies, we calculated three sets of PPE  
234 using *Pinus sylvestris*, *Quercus robur* and *Poaceae* as reference taxa, respectively (Table 2). In addition,  
235 we compiled from the published literature all relevant estimates obtained with similar methods and the  
236 most recent consensus values given by Mazier et al. (2012) (Table 3).

## 237 **Results**

238 Pollen proved abundant enough for our analysis in all samples collected. All pollen spectra were  
239 characterised by a very high arboreal/non-arboreal proportion, between 93.8 and 98.3% (ESM Figure  
240 S1). In most of the 12 samples from oak-linden-hornbeam forest, the pollen of *Carpinus* (5) and *Quercus*  
241 (3) absolutely dominated. However, in two other samples *Picea* and *Pinus* pollen were co-dominant with  
242 a lower percentage of deciduous tree pollen. In one other sample *Betula* was the most common pollen  
243 type. The pollen spectra from oak-linden-hornbeam forest had several tree taxa well represented,  
244 reflecting the complex floristic composition of this vegetation type. In all moss polsters from coniferous  
245 forests (6) *Pinus* pollen dominated; it reached 80-90% in four samples just under 40% in the two  
246 remaining ones.

247 Plant taxa abundance at each site is presented in ESM table S2. Across all vegetation plots, damage from  
248 herbivory was systematically absent from herbaceous plants (including *Poaceae*) and was common on  
249 juvenile woody plants in the shrub layer of the forest's undergrowth.

250 The taxa which displayed a positive relationship between pollen percent and distance-weighted percent  
251 cover were *A. glutinosa*, *Betula* spp. (including *B. pendula* and *B. pubescens*), *C. betulus*, *Corylus*  
252 *avellana*, *Pinus sylvestris*, *Poaceae*, *Q. robur* and *T. cordata* (ESM Figure S2 A and B). Other pollen-types  
253 were not suitable for modelling with the methodology used. Notably the models including *Picea abies*,  
254 an important component in Białowieża Forest, returned unrealistic results for that taxon (PPEs =  $\sim 10^{-6}$ )  
255 with very large standard errors. In addition, the inclusion of *P. abies* in models was significantly

256 increasing the PPE standard errors for all other taxa. Sedges (*Cyperaceae*) did not show a good enough  
257 spread of data (pollen between 0% and 0.33% of pollen sum) and produced very large standard errors  
258 and unrealistic results when included in models.

259 Amongst distance-weighting methods, 1/d and pt-s (pollen-type specific) returned nearly equally good  
260 results with all three algorithms (Figure 3 A-C), while 1/d<sup>2</sup> systematically returned worse log-likelihoods.

261 In such situations it is appropriate to recommend 1/d, the simplest distance-weighting method.

262 [Insert Figure 3]

263 When using *Poaceae* as a reference taxon, the ERV 1 algorithm did not perform as consistently as ERV 2  
264 and ERV 3, as shown by the variability of the log-likelihood when plotted against the distance gradient  
265 (Figure 3 D in comparison to Figure 3 E-F). Because this behaviour is not observed when using *Pinus* or  
266 *Quercus* as reference taxa, it was concluded that in our study, *Poaceae* are less suitable for this purpose  
267 (Figure 3) but we provide PPEs relative to *Poaceae*, for comparison with similar published analyses.

268 The relevant source area is within a 400 m radius distance from sampling points with ERV 1 and ERV 3.  
269 Using these algorithms, PPE for all species stabilise before 400m along a distance gradient (ESM Figure  
270 S3). In contrast, the PPE obtained with the algorithm ERV 2 do not show any stable pattern along the  
271 distance gradient while log-likelihood values do not reach a horizontal asymptote as expected with this  
272 method (Sugita, 1994). We decided not to retain results of model ERV 2 on this basis, although the PPE  
273 were in the same range of values than those obtained with models ERV 1 and ERV 3.

274 The PPEs relative to *Pinus*, *Poaceae* and *Quercus* obtained with the best setting identified for our data, ie  
275 algorithms ERV 1 and ERV 3, 1/d distance-weighting and wind speed of 3 m s<sup>-1</sup>, are presented in Table 2.

276 Results indicate that *A. glutinosa*, *Betula*, *Q. robur* and *P. sylvestris* are high pollen producers, *Carpinus*  
277 *betulus* is an intermediate pollen producer and *Poaceae*, *Corylus avellana* and *T. cordata* are relatively

278 very low producers. There is between 23- (ERV 3) and 50-fold (ERV 1) difference of PPE between the  
279 highest producers (*Pinus and Quercus*) and the lowest producers (*Poaceae and Tilia*).

280 [Insert Table 2]

281 Discussion

282 *Relative PPE from Białowieża Forest*

283 Comparing our results to PPE values from similar studies across Europe indicates that the difference  
284 between low and high producers is much higher at our research sites than in any other place in the rest  
285 of lowland Europe (Table 3). The ratio of PPE between the highest producers (*Pinus and Quercus*) and  
286 the lowest producers (*Poaceae and Corylus*) is on average six times greater in the BNP than across  
287 European cultural landscapes. This means that either in closed-canopy forest *Poaceae* and *Corylus* are  
288 six times more under-represented in the pollen rain than hitherto estimated from cultural landscapes, or  
289 *Pinus* and *Quercus* are six times more over-represented, or any mixture between these two extreme  
290 explanations. However, because it is based on ratios, the modelling method used here does not allow us  
291 to ascertain whether high producers in the BNP are higher producers in absolute terms, or whether low  
292 producers produce an absolute lower quantity of pollen. This main finding, however, remained true  
293 whether using *Pinus*, *Quercus* or *Poaceae* as reference taxa. There may be several reasons why this ratio  
294 is so high in our study, including different methodologies between studies, extrinsic abiotic factors such  
295 as climate influencing pollen productivity rates and intrinsic biotic factors such as the composition of the  
296 vegetation and the disturbance regimes that the forest has been undergoing. Each of these will be  
297 discussed in turn in the next sections.

298 [Insert Table 3]

299 [Insert Figure 4]

300 *Reference taxa*

301 Our results highlight how the choice of reference taxa may influence the data interpretation. As  
302 underlined by Sugita et al. (1999) and Bunting et al. (2013), in theory any taxa can serve as the reference  
303 unit, if it represents a wide range of values in both pollen and vegetation data, and is expected as an  
304 intermediate pollen producer. For practical reasons, most studies in Europe included *Poaceae* or *P.*  
305 *sylvestris* and utilised them as reference taxa (summarised by Mazier et al., 2012). For this reason, in  
306 Figure 4, we display our data using these reference taxa and compare them with all other existing  
307 studies. However, *Pinus* and *Poaceae* probably represent two extremes in the range of absolute pollen  
308 productivity in the BNP, so, in our case, a better intermediate pollen producer would have been  
309 *Carpinus*. Unfortunately, only two other European studies have produced PPE values for this taxon  
310 (Soepboer et al., 2007; Sugita et al., 1999) drastically limiting potential comparison. Nevertheless, the  
311 very high ratio between high and low pollen producers is independent from the choice of a reference  
312 taxon.

313 *Media sampled and vegetation survey*

314 Methodology has been highlighted by some authors as a potential source of result discrepancy between  
315 studies (eg Broström et al., 2008; Bunting et al., 2013; Bunting and Hjelle, 2010; Hellman et al., 2008;  
316 Hjelle and Sugita, 2012; Theuerkauf et al., 2012), in particular, firstly, the media sampled, secondly, the  
317 models for pollen dispersal and number of years of pollen accumulation found in mosses or lake  
318 sediments and, thirdly, the way vegetation is surveyed.

319 Most studies show that different media are more or less effective in trapping certain pollen types,  
320 however, it is also emerging that variation between pollen assemblages collected from different media  
321 are not always significant (eg Broström et al., 2008; Giesecke and Fontana, 2008; Lisitsyna et al., 2012;  
322 Pardoe et al., 2010; Räsänen et al. 2004; Wilmshurst and McGlone, 2005). Some herb taxa show lower

323 PPE values with lake sediment sampling than with moss polster sampling and this has been attributed to  
324 the poor dispersal of certain pollen types (Broström et al., 2008). Results from northern Europe have  
325 also shown the same pattern (Lisytsyna et al., 2012) and this may impact the estimation of pollen  
326 productivity values. In addition, occasional differences in PPE may arise as a result of strong over-  
327 representation of some taxa in the local littoral vegetation (eg *Alnus* spp., *Filipendula*) which, if  
328 underestimated in the vegetation survey, may produce higher than expected PPE from lake sediments  
329 (Broström et al., 2008).

330 Another consideration regarding the media is that mosses may better retain some pollen types than  
331 others. Several studies indicate that baccate pollen, especially *Pinus*, is usually much better  
332 represented in moss polsters than in Tauber traps (Caramiello et al. 1991; Lisytsyna et al., 2012; Pardoe  
333 et al., 2010; Vermoere et al., 2000). This bias was also noted in the BNP where seven pairs combining  
334 moss samples and Tauber traps (averaged 2-years pollen data) showed that *Pinus* proportions in moss  
335 polsters were in average two times higher (Zimny, 2014). These differences are usually explained by the  
336 specific structures of the moss cushions which results in different effectiveness in trapping and then  
337 preserving pollen of various size and form (Joosten and De Klerk. 2007). Pollen deposition in mosses may  
338 also be biased by occasional extreme meteorological events (Sjögren et al., 2006), or pollen addition by  
339 insects (Bunting et al., 2013) which induce variation in the PPE of some taxa and not others.

340 Because of the annual variability in pollen deposition, PPE calculations should be made on samples  
341 containing several years of accumulation (Bunting et al., 2013). However, in most studies the number of  
342 years in both moss polsters and sediment samples cannot be determined with great confidence (Pardoe  
343 et al., 2010; Hjelle and Sugita, 2012). In our study we addressed the above issues by sampling mosses  
344 forming thick mats, including the lower brown parts, thus representing as many years as possible.  
345 Moreover, we sampled similar types of forest floor mosses (pleurocarps or *Sphagnum*) for all our

346 samples so as to avoid large discrepancies in pollen rain accumulation time between samples (ESM Table  
347 S1).

348 The deposition environment may also be a source of uncertainty. Although pt-s (pollen-type specific)  
349 distance-weighting better takes into account the different flight abilities of different pollen-types, the  
350 pt-s model currently in use (Prentice, 1985; Sugita et al., 1999) only reflects pollen dispersal from a  
351 ground level source and above the forest canopy (Jackson and Lyford, 1999; Theuerkauf et al., 2012). As  
352 a result we preferred 1/d (inverse distance) distance-weighting for our results, a robust methods that  
353 does not make any assumption regarding the deposition environment. The forest in the BNP is unique  
354 for its complex structure and it remains unknown how this may have affected pollen deposition. More  
355 research regarding the penetration of pollen rain under the canopy will be required in order to better  
356 understand the nature of background pollen rain in our samples.

357 Methods used to survey the plant species abundances around pollen sampling sites may influence  
358 estimation of pollen productivity (Broström et al., 2008; Bunting et al., 2013) and this has been  
359 demonstrated for various herbs and *Ericaceae* species (Bunting and Hjelle, 2010). However, to avoid any  
360 discrepancies in this respect, we followed the methodology used in other similar studies (Mazier et al.  
361 2008; Bunting et al., 2013) for, respectively, 10 to 100 m and 0 to 10 m away from the sampling point. In  
362 addition, the relevant source area of pollen in our research (400 m) was similar to other research based  
363 on moss-polsters (Broström et al., 2004; von Stedingk et al., 2008) giving less weight to the field survey  
364 data in the estimation of pollen productivity. Our estimates of tree cover slightly diverge from most  
365 studies of PPE in that we explicitly excluded immature trees. This follows the recommendation of  
366 Matthias et al. (2012) who found a significant difference in PPE when immature trees are included or  
367 excluded from calculations. However, this difference remains too small to explain the discrepancy  
368 between our results and those of other studies.

369 Finally, another potential source of uncertainty comes from the fact that some of our sites were  
370 relatively close to each other, ie there could be a problem of spatial autocorrelation. Because of  
371 extremely difficult access in many parts of the BNP, in our study, sampling location was a compromise  
372 between safety, needs for adequate moss cushions and spatial distribution. It is not clear how much this  
373 factor could have impact on PPE calculations and how it may have influenced our relative source area of  
374 pollen (Twiddle et al. 2012).

375 We acknowledge that some of the discrepancies in PPE results from different studies can be attributed  
376 to the variety of vegetation survey methodology used. However considering all the above  
377 methodological issues, it can be inferred that the much higher ratio between high and low pollen  
378 producers observed in the BNP cannot be explained on methodological grounds only.

#### 379 *The influence of extrinsic abiotic factors such as climate*

380 Plant species experience limited fitness towards the extreme edge of their distribution range because of  
381 sub-optimal climatic conditions, and, as a result, produce pollen less abundantly (Barnekow et al., 2007;  
382 Sjögren et al., 2006). All the eight tree species dealt with here are more or less at the centre of their  
383 distribution range (Jalas and Suominen, 1973, 1976) and it can be expected that they experience optimal  
384 climatic conditions for growth and flowering. Potentially, this might result in higher pollen productivity  
385 of deciduous trees in Białowieża Forest than in northern Europe.

386 Climate may not only affect annual pollen production but also its year-to-year variation, including  
387 biological cycles responsible for frequency of years with high and low pollen production (Nielsen et al.,  
388 2010). Three of the taxa studied here (eg *Betula*, *Alnus* and *Quercus*) have a more or less distinct  
389 biennial or triennial alternating pattern in many aerobiological stations in Europe, however, in some  
390 others such regularities are not observed (Spieksma et al., 2003). In Białowieża Forest, *Carpinus* and *Tilia*  
391 also show fluctuation in intensity of flowering (Pawlaczyk, 2009). Annual airborne pollen counts in high  
392 pollen seasons may be more than ten times higher than in low ones as evidenced for *Betula* (Latałowa et  
393 al., 2002) or *Quercus* (Grewling et al., 2014). Therefore, presence or absence of the alternating patterns  
394 and frequency of high and low pollen years might be of importance when considering PPE. This potential  
395 issue may have affected *Picea abies* in particular, a tree showing a large variation of pollen production

396 from year to year. This may explain why our data for this taxon could not be modelled and requires  
397 further investigation. Also, some pollen types may comprise different species in different climatic regions  
398 of Europe. For instance, the *Quercus* pollen recorded in our samples comes from the local native *Q.*  
399 *robur*. However, in other studies (Soepboer et al., 2007; Theuerkauf et al., 2012) both *Q. robur* and *Q.*  
400 *petraea* were included for the calculation of *Quercus* PPE. There is a similar situation with *A. incana*, a  
401 common tree in central Europe that produces pollen morphologically similar to that of *A. glutinosa* but  
402 that is scarce in the BNP. However, it is sometimes included in PPE of *Alnus* spp. (Poska et al., 2011). We  
403 are not aware of any direct evidence specifically demonstrating that these pairs of species (*Q. robur-Q.*  
404 *petraea*, *A. glutinosa-A. incana*, *B. pendula-B. pubescens*, *T. platyphyllos-T. cordata*) have similar or  
405 different pollen productivity but it cannot be excluded that taxonomic and genetic diversity within the  
406 geographic range of a taxa might be among the important factors deciding on its different response to  
407 climate variability (Hjelle and Sugita, 2012). However, because the existing data from different parts of  
408 Europe shows PPE values within a similar range, there is no reason to believe that the tree species  
409 assemblage present in the climatic condition of Białowieża Forest is sufficient to explain the unique  
410 characteristics of our results. *Age of trees*

411 Matthias et al. (2012) suggest that in cultural landscapes where forests are managed, PPE are likely to be  
412 lower than expected from a closed canopy old-growth forest. They argue that trees reach sexual  
413 maturity only after 10 to 50 years depending on the species and hardly produce any pollen until then. As  
414 a result, young tree plantations would not produce pollen, yet this fraction of forest cover tends to be  
415 included in datasets for the calculation of PPE. The result would be an over-estimation of the tree  
416 species' surface cover, resulting in an under-estimation of PPE values.

417 The old-growth forest of the BNP is also a dynamic ecosystem where young trees are an integral portion  
418 of the vegetation cover (Faliński, 1988; Sokołowski, 1993) and this can be extended to the general  
419 dynamics of the forest (Faliński, 1988). There is an ample body of evidence showing that in Białowieża  
420 Forest, tree recruitment is increasing (Miścicki, 2012), and being constantly shaped by natural  
421 disturbance factors like browsing (Bobiec et al., 2011; Kuijper et al., 2010a, 2010b), disease or wind  
422 throws (Bobiec, 2002). However, in our study the impact of immature trees on PPE calculation was  
423 minimized due to their exclusion during the field inventory. Moreover, even if our estimation of  
424 mature/immature trees in the plant cover was not in line with previous studies (except Matthias et al.,  
425 2012), we can assume that proportion of young trees in the BNP is much lower than in cultural

426 landscapes – tall *Pinus*, *Quercus* and *Alnus* over 100 years old (the highest pollen producers in this study)  
427 are very common in the forest. It is also important to stress that in the deciduous forest communities in  
428 the BNP *P. sylvestris*, a key species in our study, occurs in the form of veteran trees (more than 300-  
429 years old) reaching up to 45 m in height (Faliński 1977) but the younger generation of this tree is almost  
430 absent (Bobiec 2012). The very high *Pinus* PPE found at our study sites may be partly explained by the  
431 presence of such trees in the deciduous forest we sampled.

#### 432 *Canopy structure and light limitation*

433 Our results may be explained by the multi-layered forest and highly diversified structure occurring in the  
434 BNP (Bobiec, 2012; Faliński, 1986). The highest pollen producers in our study (*Pinus sylvestris*, *Q. robur*  
435 and *A. glutinosa*) reach the upper forest layer (Faliński, 1977) where their large crowns are fully exposed  
436 to sunlight enabling good flowering conditions and pollen dispersal. In addition, these taxa have  
437 relatively low pollen fall speed (Sugita et al., 1999) meaning that they may be over-represented in the  
438 pollen rain in relation to the surveyed vegetation (Theuerkauf et al., 2012). *Betula*, a light demanding  
439 tree, is also among the highest pollen producers in our data. In the BNP, *Betula* is frequent in the forest  
440 gaps (*B. pendula*) and is common on peat bogs (*B. pubescens*) where it grows in full light and is exposed  
441 to wind - good conditions for high pollen production and dispersal. Again, we cannot exclude that its  
442 pollen is over-represented because pollen dispersal in those conditions is higher than expected.

443 In Białowieża Forest, *Corylus avellana* is restricted to very shaded forest undergrowth and never reaches  
444 the canopy (Sokołowski, 1993), therefore light limitation is likely to reduce its reproductive fitness. This  
445 is in direct contrast with open or semi-open habitats promoting flowering such as hedgerows and forest  
446 edges where it is generally found in cultural landscapes (eg Ellenberg, 1988). We suggest that this might  
447 well account for the much lower PPE of *Corylus* than hitherto assumed. The same may concern *T.*  
448 *cordata*. Although some of the *T. cordata* trees in Białowieża Forest reach the canopy level, many are

449 located under the canopy, where they do not reach their full potential for pollen production (Bobiec  
450 2012; Keczyński 2005). *Carpinus* is among the low pollen producers but its PPE is higher than those of  
451 *Poaceae*, *Corylus* and *Tilia*. When calculated in relation to *Poaceae* (ERV 3), the value (4.48) is very  
452 similar to that calculated for the Swiss Plateau (4.56) (Soepboer et al., 2007).

453 In the BNP, *Poaceae* flowering is also possibly reduced by the low light levels in the forest understorey in  
454 comparison to situations where agriculture prevails. It has been highlighted that *Poaceae* comprises  
455 numerous species and as a result, their PPEs from different areas may be different (Broström et al.,  
456 2008). In fact, many of the *Poaceae* taxa found in Białowieża Forest during our survey are specialists of  
457 forest environments (*Poa nemoralis*, *Festuca altissima*, *Deschampsia cespitosa* subsp. *parviflora*, *Dactylis*  
458 *aschersoniana*, *Brachypodium sylvaticum* and *Melica nutans*) that are expected to be lower pollen  
459 producers than grass species growing in full light conditions outside forest. In addition, other more  
460 ubiquitous *Poaceae* recorded during our survey (*Phragmites australis*, *Molinia caerulea*, *Festuca ovina*,  
461 *Deschampsia flexuosa*, *Danthonia decumbens*, *Calamagrostis arundinacea*) are expected to show  
462 reduced fitness as a result of the shading from the canopy.

463 Large grazers directly impact on herbaceous plant flowering, either by physically suppressing flowering  
464 in a continuous way (Groenman-van Waateringe, 1993; Vera, 2000) or by enhancing the production of  
465 inflorescences and lengthening the flowering season, a phenomenon known as over-compensation (e.g.  
466 Massad, 2013). However, in Białowieża Forest grazing is unlikely to explain the low *Poaceae* PPE for  
467 three reasons. Firstly, we did not find any signs of grazing during our survey and *Poaceae* cover is low in  
468 the quadrats near our sampling points (mean= 3.1 %, n=306), i.e. it never consists in a grazing lawn likely  
469 to sustain regular grazing. Secondly, ungulates in Białowieża Forest are primarily browsers and tend to  
470 graze and browse preferentially in forest gaps (Kuijper et al., 2009) not covered in our study. Thirdly,  
471 European bison, the only large herbivore truly adapted to grazing within the local faunal assemblage,  
472 occurs in a low density of 0.45 individuals km<sup>-2</sup> (Kuijper et al., 2010a), therefore its impact on grass  
473 flowering within the BNP may be neglected. Browsing has a pervasive effect on the forest composition  
474 in Białowieża, where herbivores literally select the canopy trees of tomorrow (Kuijper et al., 2010a).  
475 However they can only reach young seedlings and saplings and in this respect, browsing herbivores do  
476 not impact pollen productivity in a direct way in the short term.

477 *Nutrient cycling*

478 Nitrogen availability is an important factor stimulating higher pollen productivity in some species. Long-  
479 term manipulation field experiments in the UK show that flowering can increase up to three times in  
480 woody plants with nitrogen addition (Phoenix et al., 2012). Similar results have been obtained for North  
481 American *Quercus* spp. (Callahan et al., 2007). The current nitrogen levels in the BNP area are increasing  
482 with a large amount of dead wood decomposition (Paluch, 2001) and the deposition of atmospheric  
483 nitrogen at the current rate of 11 kg ha<sup>-1</sup> year<sup>-1</sup> (Malzahn, 2009). In addition, the long-term  
484 transformation of some conifer forests into oak-linden-hornbeam (Bobiec, 2012) is likely to change  
485 nitrogen cycling at those locations. On the whole it is not known whether the nitrogen availability levels  
486 currently experienced by trees in Białowieża Forest may differ from those in cultural landscapes but this  
487 is likely. However this is a subject that deserves further consideration.

488 Also, relatively high density of ungulates in the BNP may be of importance, because large herbivores  
489 redistribute nitrogen in a more heterogeneous way (Augustine and Frank, 2001; Bump et al., 2009;  
490 Hobbs, 2006; Murray et al., 2013) and modify the cycling of nutrients (reviewed in Pastor et al., 2006). It  
491 is difficult to assess the impact of nutrient manipulation by large vertebrates on our results, however,  
492 this is another factor that probably needs further consideration.

493 *Potential implications of the PPEs from Białowieża Forest for palaeoenvironmental reconstructions and*  
494 *conservation science*

495 Pollen productivity estimates are the core element in quantitative reconstructions of past vegetation  
496 cover based on mechanistic models developed by Sugita (2007a,b; Sugita et al., 2010); one underlying  
497 assumption of these models is that PPE are constant in space and time. However, our results reinforce  
498 the idea that pollen productivity can vary in response to changes in the prevailing environmental  
499 settings and we present for the first time a quantification of this variability, likely induced by differences

500 in tree cover and canopy structure. Our results indicate that it would be constructive to use, in parallel  
501 consensus PPEs calculated mostly from cultural landscapes (Mazier et al., 2012) and PPEs from an old-  
502 growth forest, when running the landscape reconstruction models. We suspect that our PPEs will result  
503 in interpreting higher proportions of open-land than hitherto estimated eg for the mid-Holocene  
504 landscapes in the temperate zone of lowland Europe (Nielsen et al., 2012; Trondman et al., 2015).

505 Tree cover during the Holocene is an important parameter in a number of studies on climate change  
506 modelling (Gaillard et al., 2010; Strandberg et al., 2014; Trondman et al., 2015). In fact, vegetation is an  
507 inherent part of climate systems influencing circulation of energy, water and greenhouse gases between  
508 land and the atmosphere. Therefore, climate change studies will benefit from a more informed choice of  
509 PPEs to quantify past changes in vegetation cover.

510 As there is a growing consensus about the importance of vegetation history for conservation science and  
511 ecosystem management (Froyd and Willis, 2008; Jeffers et al., 2015), palynological tools need continuing  
512 improvement. Applying our new set of PPE to pollen-based quantitative reconstructions of past  
513 vegetation will help to better understanding vegetation cover in temperate Europe prior to  
514 development of agriculture. Landscape openness and the drivers shaping vegetation structure for this  
515 period is the focus of a long-standing debate that has a direct impact on different nature conservation  
516 concepts (eg Bradshaw et al., 2003; Mitchell, 2005; Vera, 2000).

## 517 **Conclusions**

518 To date, pollen productivity has only been estimated in temperate Europe from cultural landscapes  
519 where human activities such as agriculture, industrialisation and urbanisation are a preponderant source  
520 of disturbance. We found, that pollen productivity in the closed canopy old-growth forest of Białowieża  
521 National Park (where disturbance by human activities is minimal) was different from that measured in  
522 cultural landscapes. In fact, the ratio of pollen productivity estimates between high producers (*Pinus*

523 *sylvestris* and *Quercus robur*) and low producers (*Poaceae* and *Corylus avellana*) is on an average six  
524 times greater in the BNP than across European cultural landscapes. We discuss several potential factors  
525 likely to explain our distinctive results, including the methodology used and the environmental settings  
526 in the BNP.

527 We conclude that our results cannot be explained on methodological grounds only. We followed  
528 standard methods for the vegetation survey and moss polsters, as recommended by previous studies of  
529 cultural landscapes. In addition, we excluded from our vegetation dataset trees not mature enough to  
530 produce pollen, a potential bias highlighted by Matthias et al. (2012).

531 From the environmental factors considered we proposed that light availability is the most important.  
532 This is the direct result of the forest structure and age, a unique characteristic of the BNP forest. All low  
533 pollen producers in our data (*Corylus*, *Poaceae* and *Tilia*) grow in relatively shaded situations and do not  
534 receive sufficient light for their pollen production to be as abundant as in cultural landscapes. In  
535 addition, dense, multi-layered forest may limit the dispersal of their pollen, while high producers (*Pinus*  
536 *sylvestris* and *Quercus robur*) are tall trees that reach the canopy where they receive sufficient light for  
537 abundant pollen production and where pollen gets freely dispersed.

538 Besides light, we highlight that enhanced nitrogen availability induces a significant increase in pollen  
539 production in some species and that nitrogen cycling depends on atmospheric pollution levels, dead  
540 wood decomposition and spatial redistribution by large herbivores amongst other factors. We believe  
541 this is an aspect of pollen productivity that deserves more attention in the future.

542 The PPE for high pollen producers relative to *Pinus* and PPE for low pollen producers relative to *Poaceae*  
543 are comparable with estimates previously reported in other studies, but none of these reference taxa  
544 give consistent results for the whole range of taxa used in this study. This is an interesting finding which

545 not only underlines specific characters of the data from an old-growth forest, but also indicates that  
546 more attention should be drawn to the role of a reference taxa used for relative PPE calculation.

547 Our results reinforce the idea that pollen productivity can vary depending on the prevailing  
548 environmental settings, ecological conditions and disturbance regimes. We present for the first time a  
549 quantification of this variability and suggest our PPE results to be used in parallel to consensus PPE from  
550 cultural landscapes (Mazier et al., 2012) when interpreting pollen assemblages potentially coming from  
551 closed canopy forest. Applying the two PPE sets should help to test hypothesis for instance regarding  
552 the interpretation of pollen spectra from the mid-Holocene forest maximum at the heart of the "Vera  
553 debate" (Mitchell 2005) or different scenarios of human induced changes in land-cover used for climate  
554 modelling (Marquer et al. 2014; Trondman et al. 2015). These improved insights into vegetation  
555 reconstruction are necessary to better understand the drivers of change that occurred in the past and  
556 the consequences they had on land-cover and climate.

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## 569 **References**

- 570 Abraham V and Kozáková R (2012) Relative pollen productivity estimates in the modern  
571 agricultural landscape of Central Bohemia (Czech Republic). *Review of Palaeobotany and*  
572 *Palynology* 179: 1–12.
- 573 Augustine D and Frank D (2001) Effects of migratory grazers on spatial heterogeneity of soil  
574 nitrogen properties in a grassland ecosystem. *Ecology* 82(11): 3149–3162.
- 575 Barnekow L, Loader NJ, Hicks S, Froyd CA and Goslar T (2007) Strong correlation between  
576 summer temperature and pollen accumulation rates for *Pinus sylvestris*, *Picea abies* and *Betula*  
577 spp. in a high-resolution record from northern Sweden. *Journal of Quaternary Science* 22(7):  
578 653–658.
- 579 Beug H (2004) *Leitfaden der Pollenbestimmung für Mitteleuropa und angrenzende Gebiete*.  
580 München: Publisher Verlag Friedrich Pfeil.
- 581 Birks HJB (2005) Mind the gap: how open were European primeval forests? *TRENDS in*  
582 *Ecology and Evolution* 20(4): 154-156.
- 583 Bobiec A (2002) Białowieża Primeval Forest. *International Journal of Wilderness* 8(3): 33.
- 584 Bobiec A (2012) Białowieża Primeval Forest as a remnant of culturally modified ancient forest.  
585 *European Journal of Forest Research* 131(5): 1269–1285.
- 586 Bobiec A, Jaszcz E and Wojtunik K (2011) Oak (*Quercus robur* L.) regeneration as a response to  
587 natural dynamics of stands in European hemiboreal zone. *European Journal of Forest Research*  
588 130(5): 785–797.
- 589 Boczoń A (2006) Characteristics of thermal and pluvial conditions in the Białowieża Primeval  
590 Forest between 1950 and 2003. *Leśne Prace Badawcze* 1: 57-72.
- 591 Bradshaw RHW, Hannon GE and Lister AM (2003) A long-term perspective on ungulate –  
592 vegetation interactions. *Forest Ecology and Management* 181: 267-280.
- 593 Broström A, Sugita S and Gaillard M-J (2004) Pollen productivity estimates for the  
594 reconstruction of past vegetation cover in the cultural landscape of southern Sweden. *The*  
595 *Holocene* 14(3): 368–381.

- 596 Broström A, Nielsen AB, Gaillard M-J et al. (2008) Pollen productivity estimates of key  
597 European plant taxa for quantitative reconstruction of past vegetation: a review. *Vegetation*  
598 *History and Archaeobotany* 17(5): 461–478.
- 599 Buckland PC (2005) Palaeoecological evidence for the Vera hypothesis. In: *Large herbivores in*  
600 *the wildwood and modern naturalistic grazing systems*. English Nature Research Report 648,  
601 pp.62-116, Peterborough.
- 602 Bump JK, Webster CR, Vucetich JA et al. (2009) Ungulate carcasses perforate ecological filters  
603 and create hotspots in forest biogeochemical herbaceous layers allowing trees a competitive  
604 advantage. *Ecosystems* 12(6): 996–1007.
- 605 Bunting MJ and Hjelle KL (2010) Effect of vegetation data collection strategies on estimates of  
606 relevant source area of pollen (RSAP) and relative pollen productivity estimates (relative PPE)  
607 for non-arboreal taxa. *Vegetation History and Archaeobotany* 19(4): 365–374.
- 608 Bunting MJ, Armitage R, Binney HA and Waller MP (2005) Estimates of “relative pollen  
609 productivity” and “relevant source area of pollen” for major tree taxa in two Norfolk (UK)  
610 woodlands. *The Holocene* 15(3): 459–465.
- 611 Bunting MJ, Farrell M, Broström A et al. (2013) Palynological perspectives on vegetation survey:  
612 a critical step for model-based reconstruction of Quaternary land cover. *Quaternary Science*  
613 *Reviews* 82: 41-55.
- 614 Callahan HS, Del Fierro K, Patterson AE and Zafar H (2007) Impacts of elevated nitrogen inputs  
615 on oak reproductive and seed ecology. *Global Change Biology* 14(2): 285–293.
- 616 Caramiello R, Siniscalco C and Piervittori R (1991) The relationship between vegetation and  
617 pollen deposition in soil and in biological traps. *Grana* 30(2): 291-300.
- 618 Czerepko J (2008) A long-term study of successional dynamics in the forest wetlands. *Forest*  
619 *Ecology and Management* 255: 630-642.
- 620 Davis MB (2000) Palynology after Y2K - Understanding the source area of pollen in sediments.  
621 *Annual Review of Earth and Planetary Sciences* 28: 1–18.
- 622 Eggenberg S and Möhl A (2008) *Flora Vegetativa*. Lausanne: Rossoli.
- 623 Ellenberg H (1988) *Vegetation ecology of Central Europe*. Cambridge: Cambridge University  
624 Press.
- 625 Faliński JB (1977) Research on vegetation and plant population dynamics conducted by  
626 Białowieża Geobotanical Station of the Warsaw University in the Białowieża Primeval Forest  
627 and in the environ (1952-1977). *Phytocoenosis* 6(1/2): 1-132.

- 628 Faliński JB (1986) *Vegetation dynamics in temperate lowland primeval forests: ecological*  
629 *studies in Białowieża forest*. Dordrecht: Junk.
- 630 Faliński JB (1988) Succession, regeneration and fluctuation in the Białowieża Forest (NE  
631 Poland). *Vegetatio* 77: 115-128.
- 632 Froyd C and Willis KJ (2008) Emerging issues in biodiversity and conservation management :  
633 the need for palaeoecological perspective. *Quaternary Science Reviews* 27: 1723-1732.
- 634 Gaillard M-J, Sugita S, Bunting MJ et al. (2008) The use of modelling and simulation approach  
635 in reconstructing past landscapes from fossil pollen data: a review and results from the  
636 POLLANDCAL network. *Vegetation History and Archaeobotany* 17(5): 419–443.
- 637 Gaillard M-J, Sugita S, Mazier F et al. (2010) Holocene land-cover reconstructions for studies on  
638 land cover-climate feedbacks. *Climate of the Past* 6(4): 483–499.
- 639 Giesecke T and Fontana SL (2008) Revisiting pollen accumulation rates from Swedish lake  
640 sediments. *The Holocene* 18(2): 293-305.
- 641 Grewling Ł, Jackowiak B and Smith M (2014) Variations in *Quercus* sp. pollen seasons (1996–  
642 2011) in Poznań, Poland, in relation to meteorological parameters. *Aerobiologia* 30: 149-159.
- 643 Groenman-van Waateringe W (1993) The effects of grazing on the pollen production of grasses.  
644 *Vegetation History and Archaeobotany* 2(3): 157–162.
- 645 Hall S (2008) A comparative analysis of the habitat of the extinct aurochs and other prehistoric  
646 mammals in Britain. *Ecography* 31: 187–190.
- 647 Hellman SE, Gaillard M-J, Broström A and Sugita S (2008) The REVEALS model, a new tool to  
648 estimate past regional plant abundance from pollen data in large lakes: validation in southern  
649 Sweden. *Journal of Quaternary Science* 23(1): 21–42.
- 650 Hicks S, Tinsley H, Pardoe H and Cundill P (1999) *European Pollen Monitoring Programme.*  
651 *Supplement to the Guidelines*. Oulu: University of Oulu.
- 652 Hjelle KL (1998) Herb pollen representation in surface moss samples from mown meadows and  
653 pastures in western Norway. *Vegetation History and Archaeobotany* 7: 79–96.
- 654 Hjelle KL and Sugita S (2012) Estimating pollen productivity and relevant source area of pollen  
655 using lake sediments in Norway: How does lake size variation affect the estimates? *The*  
656 *Holocene* 22(3): 313-324.
- 657 Hobbs NT (2006) Large herbivores as sources of disturbance in ecosystems. In: Danell K,  
658 Bergström R, Duncan P and Pastor J (eds) *Large Herbivore Ecology, Ecosystem Dynamics and*  
659 *Conservation*. Cambridge: Cambridge University Press.

- 660 Jackson ST and Lyford ME (1999) Pollen dispersal models in Quaternary plant ecology:  
661 Assumptions, parameters, and prescriptions. *The Botanical Review* 65(1): 39–75.
- 662 Jalas J and Suominen J (1973) *Atlas Florae Europaeae. Distribution of Vascular Plants in*  
663 *Europe. 2. Gymnospermae (Pinaceae to Ephedraceae)*. Helsinki: The Committee for Mapping  
664 the Flora of Europe & Societas Biologica Fennica Vanamo.
- 665 Jalas J and Suominen J (1976) *Atlas Florae Europaeae. Distribution of Vascular Plants in*  
666 *Europe. 3. Salicaceae to Balanophoraceae*. Helsinki: The Committee for Mapping the Flora of  
667 Europe & Societas Biologica Fennica Vanamo.
- 668 Jeffers ES, Nogué S and Willis KJ (2015) The role of palaeoecological records in assessing  
669 ecosystem services. *Quaternary Science Reviews* 112: 17-32.
- 670 Jędrzejewski W, Spaedtke H, Kamler JF, Jędrzejewska B and Stenkewitz U (2006) Group Size  
671 Dynamics of Red Deer in Białowieża Primeval Forest, Poland. *Journal of Wildlife Management*  
672 70(4): 1054–1059.
- 673 Joosten H and de Klerk P (2007) In search of finiteness: the limits of fine-resolution palynology  
674 of *Sphagnum* peat. *The Holocene* 17(1): 1023-1031.
- 675 Keczyński A (2005) Zmiany struktury drzewostanów wykształconych na glebach  
676 hydrogenicznych – na przykładzie wybranych powierzchni badawczych w Białowieskim Parku  
677 Narodowym. *Leśne Prace Badawcze* 4: 87-102.
- 678 Kuijper DPJ, Cromsigt JPGM, Churski M, Adam B, Jędrzejewska B and Jędrzejewski W (2009)  
679 Do ungulates preferentially feed in forest gaps in European temperate forest? *Forest Ecology and*  
680 *Management* 258(7): 1528–1535.
- 681 Kuijper DPJ, Cromsigt JPGM, Jędrzejewska B et al. (2010a) Bottom-up versus top-down control  
682 of tree regeneration in the Białowieża Primeval Forest, Poland. *Journal of Ecology* 98(4): 888–  
683 899.
- 684 Kuijper DPJ, Jędrzejewska B, Brzeziecki B, Churski M, Jędrzejewski W and Żybura H (2010b)  
685 Fluctuating ungulate density shapes tree recruitment in natural stands of the Białowieża Primeval  
686 Forest, Poland. *Journal of Vegetation Science* 21(6): 1082–1098.
- 687 Latałowa M, Miętus M and Uruska A (2002) Seasonal variations in the atmospheric *Betula*  
688 pollen count in Gdańsk (Southern Baltic coast) in relation to meteorological parameters.  
689 *Aerobiologia* 18: 33-43.
- 690 Latałowa M, Zimny M, Jędrzejewska B and Samojlik T (2015) Białowieża Primeval Forest – 2000-  
691 year interplay of environmental and cultural forces in Europe’s best preserved temperate woodland.  
692 In: Kirby K and Watkins C (eds) *Europe’s Changing Woods and Forests: From Wildwood to*  
693 *Cultural Landscapes*. CABI, pp. 243-264.

- 694 Lisitsyna OV, Hicks S and Huusko A (2012) Do moss samples, pollen traps and modern lake  
695 sediments all collect pollen in the same way? A comparison from the forest limit area of  
696 northernmost Europe. *Vegetation History and Archaeobotany* 21(3): 187–199.
- 697 Malzahn E (2009) Bio-monitoring of the forest environment of the Białowieża Primeval Forest.  
698 *Ochrona Środowiska i Zasobów Naturalnych* 40: 439-447.
- 699 Malzahn E, Kwiatkowski W and Pierzgalski E 2009. II. Przyroda nieożywiona. In: Okołów C,  
700 Karaś M and Bołbot A. (eds) *Białowiecki Park Narodowy. Poznać – Zrozumieć – Zachować*.  
701 Białowieża: Białowiecki Park Narodowy, pp.18-36.
- 702 Marquer L, Gaillard M, Sugita S et al. (2014) Holocene changes in vegetation composition in  
703 northern Europe: why quantitative pollen-based vegetation reconstructions matter. *Quaternary*  
704 *Science Reviews* 90(15): 199-216.
- 705 Massad TJ (2013) Ontogenetic differences of herbivory on woody and herbaceous plants: a  
706 meta-analysis demonstrating unique effects of herbivory on the young and the old, the slow and  
707 the fast. *Oecologia* 172(1): 1–10.
- 708 Matthias I, Nielsen AB and Giesecke T (2012) Evaluating the effect of flowering age and forest  
709 structure on pollen productivity estimates. *Vegetation History and Archaeobotany* 21(6): 471–  
710 484.
- 711 Mazier F, Broström A, Gaillard M-J, Sugita S, Vittoz P and Buttler A (2008) Pollen productivity  
712 estimates and relevant source area of pollen for selected plant taxa in a pasture woodland  
713 landscape of the Jura Mountains (Switzerland). *Vegetation History and Archaeobotany* 17(5):  
714 479–495.
- 715 Mazier F, Gaillard M-J, Kuneš P, Sugita S, Trondman A-K and Broström A (2012) Testing the  
716 effect of site selection and parameter setting on REVEALS-model estimates of plant abundance  
717 using the Czech Quaternary Palynological Database. *Review of Palaeobotany and*  
718 *Palynology* 187: 38–49.
- 719 Mikusińska A, Zawadzka B, Samojlik T, Jędrzejewska B and Mikusiński G (2013) Quantifying  
720 landscape change during the last two centuries in Białowieża Primeval Forest. *Applied*  
721 *Vegetation Science* 16(2): 217–226.
- 722 Miścicki S (2012) Structure and dynamics of temperate lowland natural forest in the Białowieża  
723 National Park, Poland. *Forestry* 85(4): 473–483.
- 724 Mitchell FJG (2005) How open were European primeval forests? Hypothesis testing using  
725 palaeoecological data. *Journal of Ecology* 93: 168-177.
- 726 Mitchell FJG and Cole E (1998) Reconstruction of long-term successional dynamics of  
727 temperate woodland in Białowieża Forest, Poland. *Journal of Ecology* 86(6): 1042–1059.

- 728 Moore PD, Webb JA and Collinson ME(1991)*Pollen Analysis*. Second Edition. Oxford:  
729 Blackwell Scientific Publications.
- 730 Murray B, Webster C and Bump J (2013) Broadening the ecological context of ungulate-  
731 ecosystem interactions: the importance of space, seasonality, and nitrogen. *Ecology* 94(6): 1317-  
732 1326.
- 733 Nielsen AB (2004) Modelling pollen sedimentation in Danish lakes at c.ad 1800: an attempt to  
734 validate the POLLSCAPE model. *Journal of Biogeography* 31(10): 1693–1709.
- 735 Nielsen AB, Giesecke T, Theuerkauf M et al. (2012) Quantitative reconstructions of changes in  
736 regional openness in north-central Europe reveal new insights into old questions. *Quaternary*  
737 *Science Reviews* 47: 131-149.
- 738 Nielsen AB, Møller PF, Giesecke T, Stavngaard B, Fontana SL and Bradshaw RHW (2010) The  
739 effect of climate conditions on inter-annual flowering variability monitored by pollen traps  
740 below the canopy in Draved Forest, Denmark. *Vegetation History and Archaeobotany* 19: 309-  
741 323.
- 742 Niklasson M, Zin E, Zielonka T et al. (2010) A 350-year tree-ring fire record from Białowieża  
743 Primeval Forest, Poland: implications for Central European lowland fire history. *Journal of*  
744 *Ecology* 98(6): 1319–1329.
- 745 Paluch R (2001) Wpływ drewna martwego na zasobność gleby określonej za pomocą roślinności  
746 runa w Białowieskim Parku Narodowym. *Parki Narodowe i Rezerваты Przyrody* 20(4): 15-25.
- 747 Pardoe HS, Giesecke T, van der Knaap WO et al. (2010) Comparing pollen spectra from  
748 modified Tauber traps and moss samples: examples from a selection of woodlands across  
749 Europe. *Vegetation History and Archaeobotany* 19(4): 271-283.
- 750 Parsons RW and Prentice IC (1981) Statistical approaches to R-values and the pollen-vegetation  
751 relationship. *Review of Palaeobotany and Palynology* 32: 127–152.
- 752 Pastor J, Cohen Y and Hobbs NT (2006) The roles of large herbivores in ecosystem nutrient  
753 cycling. In: Danell K, Bergström R, Duncan P and Pastor J (eds) *Large Herbivore Ecology,*  
754 *Ecosystem Dynamics and Conservation*. Cambridge: Cambridge University Press, pp.289–325.
- 755 Pawlaczyk P (2009) III. Zbiorowiska Leśne. In: Okołów C, Karaś M, Bołbot A (eds) *Białowieski*  
756 *Park Narodowy. Poznać – Zrozumieć – Zachować*. Białowieża: Białowieski Park Narodowy,  
757 pp.38-58.
- 758 Peterken G (1996) *Natural Woodland: Ecology and Conservation in Northern Temperate*  
759 *Regions*. Cambridge: Cambridge University Press.

- 760 Phoenix GK, Emmett BA, Britton AJ et al. (2012) Impacts of atmospheric nitrogen deposition:  
761 responses of multiple plant and soil parameters across contrasting ecosystems in long-term field  
762 experiments. *Global Change Biology* 18(4): 1197–1215.
- 763 Pigott CD (1975) Natural Regeneration of *Tilia cordata* in Relation to Forest-Structure in the  
764 Forest of Białowieża, Poland. *Philosophical Transactions of the Royal Society B: Biological*  
765 *Sciences* 270(904): 151–179.
- 766 Poska A, Meltsov V, Sugita S and Vassiljev J (2011) Relative pollen productivity estimates of  
767 major anemophilous taxa and relevant source area of pollen in a cultural landscape of the hemi-  
768 boreal forest zone (Estonia). *Review of Palaeobotany and Palynology* 167(1-2): 30–39.
- 769 Prentice IC (1985) Pollen representation, source area, and basin size: toward a unified theory of  
770 pollen analysis. *Quaternary Research* 23(1): 76–86.
- 771 Prentice IC and Parsons RW (1983) Maximum Likelihood Linear Calibration of Pollen Spectra  
772 in Terms of Forest Composition. *Biometrics* 39(4): 1051.
- 773 Punt W, et al. (1976–2003) *The Northwest European Pollen Flora, vols I–*  
774 *VIII*. Amsterdam:Elsevier.
- 775 Räsänen S, Hicks S and Odgaard B (2004) Pollen deposition in mosses and in a modified  
776 “Tauber trap” from Hailuoto, Finland: what exactly do the mosses record? *Review of*  
777 *Palaeobotany and Palynology* 129(1-2): 103–116.
- 778 Räsänen S, Suutari H and Nielsen AB (2007) A step further towards quantitative reconstruction  
779 of past vegetation in Fennoscandian boreal forests: Pollen productivity estimates for six  
780 dominant taxa. *Review of Palaeobotany and Palynology* 146(1-4): 208–220.
- 781 Samojlik T (2005) *Conservation and Hunting. Białowieża Forest in the Time of Kings*.  
782 Białowieża: Zakład Badania Ssaków PAN.
- 783 Sjögren P, van Leeuwen JFN, van der Knaap WO and van der Borg K (2006) The effect of  
784 climate variability on pollen productivity, AD 1975–2000, recorded in a *Sphagnum* peat  
785 hummock. *The Holocene* 16(2): 277–286.
- 786 Smit C, Kuijper DPJ, Prentice D, Wassen MJ and Cromsigt JPGM (2012) Coarse woody debris  
787 facilitates oak recruitment in Białowieża Primeval Forest, Poland. *Forest Ecology and*  
788 *Management* 284: 133–141.
- 789 Smith D, Whitehouse N, Bunting MJ and Chapman H (2010) Can we characterise “openness” in  
790 the Holocene palaeoenvironmental record? Modern analogue studies of insect faunas and pollen  
791 spectra from Dunham Massey deer park and Epping Forest, England. *The Holocene* 20(2): 215-  
792 229.

- 793 Soepboer W, Sugita S, Lotter AF, van Leeuwen JFN and van der Knaap WO (2007) Pollen  
794 productivity estimates for quantitative reconstruction of vegetation cover on the Swiss Plateau.  
795 *The Holocene* 17(1): 65–77.
- 796 Sokołowski AW (1993) *Phytosociological characteristics of forest communities in the*  
797 *Białowieża National Park*. Białowieża: Białowieża National Park, p.190.
- 798 Sokołowski AW (2004) *Lasy Puszczy Białowieskiej*. Warszawa: Centrum Informacyjne Lasów  
799 Państwowych.
- 800 Spieksma FTM, Corden JM, Detandt M et al. (2003) Quantitative trends in annual totals of five  
801 common airborne pollentypes (*Betula*, *Quercus*, Poaceae, *Urtica*, and *Artemisia*), at five pollen-  
802 monitoring stations in Western Europe. *Aerobiologia* 19: 171–184.
- 803 Strandberg G, Kjellström E, Poska A et al. (2014) Regional climate model simulations for  
804 Europe at 6 k and 0.2 k yr BP: sensitivity to changes in anthropogenic deforestation. *Climate of*  
805 *the Past* 10: 661–680.
- 806 Sugita S (1993) A Model of Pollen Source Area for an Entire Lake Surface. *Quaternary*  
807 *Research* 39(2): 239–244.
- 808 Sugita S (1994) Pollen Representation of Vegetation in Quaternary Sediments: Theory and  
809 Method in Patchy Vegetation. *The Journal of Ecology* 82(4): 881.
- 810 Sugita S (2007a) Theory of quantitative reconstruction of vegetation I: pollen from large sites  
811 REVEALS regional vegetation composition. *The Holocene* 17(2): 229–241.
- 812 Sugita S (2007b) Theory of quantitative reconstruction of vegetation II: all you need is LOVE.  
813 *The Holocene* 17(2): 243–257.
- 814 Sugita S, Gaillard M-J and Broström A (1999) Landscape openness and pollen records: a  
815 simulation approach. *The Holocene* 9(4): 409–421.
- 816 Svenning JC (2002) A review of natural vegetation openness in north-western Europe.  
817 *Biological Conservation* 104: 133–148.
- 818 Theuerkauf M, Kuparinen A and Joosten H (2012) Pollen productivity estimates strongly depend  
819 on assumed pollen dispersal. *The Holocene* 23(1): 14–24.
- 820 Trondman A-K, Gaillard M-J, Mazier F et al. (2015) First pollen-based quantitative  
821 reconstructions of Holocene regional vegetation cover (plant functional types and land-cover  
822 types) in Europe suitable for climate modelling. *Global Change Biology* 21: 676–697.
- 823 Tutin TG, Heywood VH, Burges NA et al. (1964–1980) *Flora Europaea*. Cambridge: Cambridge  
824 University Press.

- 825 Twiddle CL, Jones RT, Caseldine CJ and Sugita S (2012) Pollen productivity estimates for a  
826 pine woodland in eastern Scotland: The influence of sampling design and vegetation patterning.  
827 *Review of Palaeobotany and Palynology* 174: 67–78.
- 828 Vera FWM (2000) *Grazing ecology and forest history*. Wallingford: CABI.
- 829 Vermoere M, Vanhecke L, Walelkens M, Smets E (2000) A comparison between modern pollen  
830 spectra of moss cushions and Cundill pollen traps. *Grana* 39 :146-158.
- 831 Von Stedingk H, Fyfe RM and Allard A (2008) Pollen productivity estimates from the forest--  
832 tundra ecotone in west-central Sweden: implications for vegetation reconstruction at the limits of  
833 the boreal forest. *The Holocene* 18(2): 323–332.
- 834 Wilmshurst JM and McGlone M (2005) Origin of pollen and spores in surface lake sediments:  
835 Comparison of modern palynomorph assemblages in moss cushions, surface soils and surface  
836 lake sediments. *Review of Palaeobotany and Palynology* 136(1-2): 1–15.
- 837 Zimny M (2014) *Późnholoceńska historia roślinności Puszczy Białowieskiej*. PhD Thesis,  
838 University of Gdańsk, Poland.
- 839