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Stivrins, Normunds

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1 Towards understanding the abundance of non-pollen palynomorphs: A comparison of
2 fossil algae, algal pigments and *seda*DNA from temperate lake sediments

3
4 Normunds Stivrins^{a,b*}, Janne Soininen^b, Ilmar Tõnno^c, Rene Freiberg^c, Siim Veski^d, Veljo Kisand^e

5
6 ^aDepartment of Geography, Faculty of Geography and Earth Sciences, University of Latvia, Riga,
7 Jelgavas street 1, LV-1004, Latvia

8 ^bDepartment of Geosciences and Geography, University of Helsinki, P.O. Box 64, Helsinki, FI-
9 00014, Finland

10 ^cCentre for Limnology, Institute of Agricultural and Environmental Sciences, Estonian University
11 of Life Sciences, Rannu, 61117 Tartu County, Estonia

12 ^dInstitute of Geology, Tallinn University of Technology, Ehitajate tee 5, 19086 Tallinn, Estonia

13 ^eInstitute of Technology, University of Tartu, Estonia

14
15 *Corresponding author, E-mail: normunds.stivrins@gmail.com, Tel.: +371-2685-9295

16
17 Abstract

18 Given the increased interest in non-pollen palynomorphs (microscopic objects other than pollen
19 identified from the pollen slides) in palaeoecological studies, it is necessary to seek a deeper
20 understanding how reliable the obtained results are. By combining quantitative information of algal
21 pigments and phylotaxonomical resolution from sedimentary ancient DNA (*seda*DNA), we validate
22 the richness and abundance of aquatic non-pollen palynomorphs – fossil algae, in the sediment of a
23 small temperate lake. For the first time, fossil and *seda*DNA algae data were combined in a
24 composite data-set and algal turnover rates in time were reconstructed for the last 14,500 years.
25 This comparison will serve as an indication to what extent fossil algae can be used to answer

26 different research questions and to reveal if it is reliable to base palaeoecological interpretation
27 solely on fossil algae identified from the pollen slides.

28

29 Keywords: non-pollen palynomorphs; palaeopigments; *seda*DNA; community richness

30

31 1. Introduction

32 In addition to pollen identification, palynologists have recently started to consider the value of other
33 microscopic objects (e.g. fungi, algae, plant and animal remains) from pollen slides, referred to as
34 *sensu stricto* non-pollen palynomorphs (NPP). NPP are found in various environments such as in
35 sediments underlining their potential in palaeoecological studies (Shumilovskikh et al., 2016;
36 Lenarczyk et al., 2015; Aptroot and van Geel, 2006; Medeanic, 2006; Turner et al., 2014; Demske
37 et al., 2013). Whilst there are studies utilizing NPP in addressing significant research questions,
38 such as evaluating ecological impacts of the late Quaternary megaherbivore palaeodiet and
39 extinctions (Gill, 2014; van Geel et al., 2011), revealing fungi influence on forest dynamics
40 (Latałowa et al., 2013), and estimating biotic turnover rates during the Pleistocene-Holocene
41 transition (Stivrins et al., 2016), only a handful of studies deals with the comparison of NPP with
42 other proxies and the validation of NPP as a palaeobiological metric (Etienne and Jouffroy-Bapicot,
43 2014; Gill et al., 2013; Wood and Wilmshurst, 2013). Given the increased interest in NPP, it is
44 necessary to seek a deeper understanding how reliable metric it is in quantifying temporal changes
45 in ecosystems.

46

47 Based on Miola's (2012) review, there are more than 1300 NPP descriptions available up today and
48 the list of NPPs is still growing. Palaeoecologists are aware that there may be certain limitations in
49 NPP – for example, part of NPP do not preserve as fossils. Selective preservation and thus,
50 distribution, is an issue for most palaeoecological analyses. Therefore, the results must be

51 interpreted very carefully. Nevertheless, as all biological organisms prefer certain environmental
52 conditions, even scattered information about NPP in time and space can aid greatly the
53 palaeoecological reconstructions.

54

55 NPPs are formed both in terrestrial and aquatic environments and the bulk of them are characteristic
56 of the local environment, they thus do not travel long distances from their source of origin (van
57 Geel, 2001). Although, NPP identified alongside pollen analysis has been proven useful in
58 understanding of the changes in lakes in the past (van Geel, 2001; van Geel et al., 1994; Jankovská
59 and Komárek, 2000), majority of the studies have focused on paludified or terrestrial environments
60 (Dietre et al., 2017; Shumilovskikh et al., 2015; Chmura et al., 2006) leaving less attention to
61 aquatic environment. No matter whether NPP is discovered from terrestrial or aquatic sediments,
62 question remains – how reliable the results are?

63

64 Fortunately, regarding aquatic NPP (fossil algae) identified from gyttja, there are useful recent
65 methods to evaluate NPP performance, namely algal pigments and sedimentary ancient DNA
66 (*sedaDNA*). Algae contain pigments (lipid soluble chlorophylls and carotenoids), which are in most
67 cases well preserved in lake sediments (Leavitt and Hodgson, 2001). Hence, pigments can be used
68 to reconstruct the past quantitative phytoplankton community dynamics (Reuss et al., 2010; Leavitt
69 and Hodgson, 2001; Fietz et al., 2007; Tonno et al., 2013; Deshpande et al., 2014). Although algal
70 pigments provide quantitative estimations about the major phytoplankton groups, they are limited in
71 indicating the abundance of lower taxonomic groups such as genera or species (Leavitt and
72 Hodgson, 2001).

73

74 Sequencing of environmental DNA, specifically *sedaDNA*, from lake sediments have recently
75 become more available that offers identification of specific palaeo communities and groups of

76 organisms (Willerslev et al., 2003; Graham et al., 2016; Coolen et al., 2013). There are two
77 significant differences between *sedaDNA* and NPP. *sedaDNA* can reconstruct species belonging to
78 all domains of Life: Eukaryota, Bacteria and Archaea, thus comprising in magnitudes higher
79 phylogenetic diversity than NPP, which due to taphonomical issues represents only selective
80 diversity of species. On the other hand, NPP usually covers representatives from two domains e.g.
81 Bacteria: cyanobacteria and Eukaryota: green algae, whilst *sedaDNA* requires a complex targeted-
82 methodology/sequencing to represent more than one domain. In the current study, we compared
83 *sedaDNA* and NPP algae from Eukaryota, which are one of the most abundant microscopic remains
84 from lacustrine palynological samples (Jankovská and Komárek, 2000; Stivrins et al., 2015;
85 Wacnik, 2009; Sarmaja-Korjonen et al., 2006). Since the domain of Bacteria was not targeted by
86 *sedaDNA*, the comparison between NPP cyanobacteria and *sedaDNA* bacteria should be done and
87 discussed elsewhere. Furthermore, by combining the semi-quantitative information from algal
88 pigments and higher taxonomical resolution from *sedaDNA*, it is now possible to validate the
89 abundance of NPP in lacustrine environments.

90

91 Here, we explore whether the amount of fossil algae (aquatic NPP) identified alongside pollen
92 analysis correlate with semi-quantitative and qualitative values of algal pigments and *sedaDNA*.
93 This evaluation will serve as an indication to what extent fossil algae can be used to answer
94 different palaeoecological research questions and is it sound to rely the interpretation solely on
95 fossil algae discovered from the pollen slides. In the current study, the term ‘fossil algae’ is used to
96 indicate NPP phytoplankton identified from the pollen slides.

97

98 2. Material and methods

99 2.1. Study area, sampling and chronology

100 Studied lake Lielais Svētīņu (mean water depth 2.9 m; maximum depth 4.9 m; area 18.8 ha) is
101 located in Latvia, eastern Baltic. The lake is a mesotrophic-dystrophic type drainage lake with a
102 relatively moderate and late human impact (Stivrins 2014; 2015). The present-day topography was
103 formed during the Weichselian glaciation and deglaciation (Zelčs and Markots, 2004). The bedrock
104 consists of Devonian dolomite covered by Quaternary deposits. The catchment area of 12 km² is
105 predominantly forested and partly covered by agricultural fields. The climate in the area is a
106 combination of continental and maritime influences, with mean annual temperature of +5.2 °C,
107 mean July temperature of +16.9 °C, and mean December temperature −4.1 °C (Stivrins et al., 2014).

108
109 **Fig.1.** Location of the studied Lake Lielais Svētīņu.

110
111 Lake Lielais Svētīņu was sampled in March 2009, and 2013 using a multiple parallel overlapping
112 sediment cores from ice using a Russian type corer with a diameter of 10 cm. The sediment
113 thickness reached to 1535 cm that comprise continual sediment record covering the last 14,500
114 years. The chronology of the core retrieved in 2009 is based on 20 radiocarbon dates (Stivrins et al.,
115 2015). Age-depth model was built using the OxCal 4.2.4 deposition model (Bronk Ramsey, 2009)
116 and the IntCal13 calibration set (Reimer et al., 2013). The sediment core used for algae pigments
117 and *sedaDNA* analyses was correlated with the year 2009 core according to the changes in lithology
118 and loss-on-ignition, enabling to adjust Stivrins et al. (2015) age-depth model using about 30

119 correlation levels (Kisand et al., under review). All calibrated ages in the text refer to years before
120 the present (cal. BP=AD 1950).

121

122 2.2. Fossil algae, algal pigments and *seda*DNA

123 Fossil algae were recovered from the core obtained 2009 and have been published in Stivrins et al.
124 (2015). Shortly, subsamples for microfossil analysis were prepared and analyzed along with pollen
125 analysis. Altogether 101 samples of known volume were treated using standard pollen preparation
126 method (10% HCl, 10% KOH and acetolyzed for 3 min). *Lycopodium* spores were added to
127 estimate phytoplankton accumulation rates. Commonly, the relative abundance of NPP is expressed
128 in percentages that are estimated against the sum of counted pollen. In the current study, relative
129 proportion (percentages) of phytoplankton is based on 1) the sums of phytoplankton and 2) the
130 sums of phytoplankton enabling comparison between these two approaches.

131 Palaeopigment subsamples were obtained from 2013 core with the resolution of one sample after
132 every 5 cm. Analysis of collected 93 palaeopigment subsamples followed the recommendations of
133 Leavitt and Hodgson (2001). Briefly, sediment samples were first freeze-dried and marked by
134 internal standard, thereafter sedimentary pigments were extracted with the mixture of acetone and
135 methanol (80:20 v:v) at -20 °C in the dark for 24 h. Finally, extracts were clarified before
136 chromatographic analysis by filtration through a 0.45 µm filter (Millex LCR, Millipore) to remove
137 any particles.

138 Reversed-phase high-performance liquid chromatography (RP-HPLC) was applied to separate the
139 pigments. Shimadzu Prominence (Japan) series binary gradient system with a photodiode array
140 (PDA) and fluorescence detectors was used (see Tamm et al. 2015 for more details). Peak
141 identification and quantification was made by commercially available external standards from DHI
142 (Denmark).

143

144 In the present study, it was assumed that markerpigments Fucoxanthin, Diadinoxanthin and
145 Diatoxanthin represent diatoms (Desphande et al 2014; McTigue et al 2015), while pigments
146 Zeaxanthin, Canthaxanthin and Echinenone were selected to represent cyanobacteria (Roy et al.
147 2011). Lutein and Chlorophyll *b* tracked green algae dynamics, although these pigments are also
148 present in higher plants (Waters et al. 2013). Alloxanthin and α -Carotene was analysed to identify
149 the dynamics of Cryptophytes, while Peridinin indicated the abundance of Dinophytes (Leavitt and
150 Hodgson 2001).

151

152 *sedaDNA* was extracted using PowerSoil® DNA Isolation Kit (MoBio) from sediment samples (0.3
153 g wet sample) in three biological replicates. DNA extraction was performed under positive-flow
154 hood (Kojair K-safety KR-125) exposed to UV light prior to extractions, surfaces were cleaned with
155 Thermo Scientific™ DNA AWAY™ Surface Decontaminant. Raw SE reads were quality trimmed
156 deleting all nucleotides below Q30 (Trimmomatic V0.32), paired and clustered (similarity threshold
157 of 97%) using CD-HIT-OUT to obtain molecular operational taxonomic units (mOTUs). Universal
158 18S rRNA primer pair covering V4 region (Tedersoo et al., 2015) was used to match the length of
159 fragment suitable for Illumina PE250 sequencing (<450-460 bp). Cluster sequences were aligned
160 and phylotaxonomy was determined using SINA aligner (Pruesse et al., 2012) and SILVA ssuRNA
161 database version 115 for 18S rDNA sequences (Quast et al., 2013; Pfeiffer et al., 2014). Clusters
162 with different domain affiliation (mostly Bacteria) and clusters with low number of reads (<4 reads)
163 were removed from further downstream analysis. More details on *sedaDNA* methodology see
164 Kisand et al. (in press). More details on *sedaDNA* methodology see Kisand et al. (in press).

165

166 2.3. Data analysis

167 As a whole, algal remains can be considered both as binary and quantitative data, but similarly to
168 terrestrial plant macrofossils and pollen, fossil algae records from the sediment samples do not

169 reflect the entire taxonomic diversity (Stivrins et al., 2016). Nevertheless, algae from small to
170 medium sized lakes, such as from Lielais Svētīņu, indicate at least partly the spatiotemporal
171 variation in local in-lake productivity and population abundances. Algal data from *sedaDNA* is
172 currently available as binary data that provide information on taxon presence in the lake. Note that
173 taxon absence from *sedaDNA* can be related to various other aspects, which are mostly caused by
174 low resolution of 18S rDNA sequences at genus and species level and is not necessarily reflecting
175 the true absence of species.

176

177 Considering their local source, we treated both fossil and *sedaDNA* algae as binary data and pooled
178 them into one composite algae dataset to obtain a more complete and reliable record of the algal
179 species richness. By doing so, for the first time, we demonstrate how fossil algae (Bacteria:
180 cyanobacteria and Eukaryota: green algae) counted from the pollen slides can be integrated with
181 *sedaDNA* (Eukaryota: green algae), thus introducing a new opportunity to solve future
182 palaeoecological questions, such as changes in biodiversity due to climate change. As an example,
183 we run Sørensen dissimilarity index that provides algal temporal turnover estimates (beta diversity
184 of species assemblages in time) for the last 14,500 years. Along with the Jaccard index, Sørensen
185 index is one of the most widely used dissimilarity indices and is regarded as one of the most
186 effective presence/absence measures (Magurran, 2004). Sørensen dissimilarity index is calculated
187 by $\beta_{SOR} = 1 - (2a / (2a + b + c))$, where ‘a’ indicates the total number of species present in both samples,
188 ‘b’ refers to the number of species present only in sample one and ‘c’ to the number of species
189 present only in sample two. The index ranges from zero to one, where zero indicates that the
190 communities have identical species composition while one indicates that two communities have no
191 shared species and thus full turnover.

192

193 3. Results

194 Relative proportions (percentages) of fossil algae vary significantly if they are estimated based on
195 sum of algae or pollen (Fig. 2 a,b). Pollen based proportions correlate with fossil algae
196 accumulation rates (Fig. 2 c) and can be an artefact due to used equation. However, proportions of
197 fossil algae based on algae sum, solely indicates changes within the algal population.

199 **Fig. 2.** Comparison of (a) fossil algae based on algal sum, %, (b) fossil algae based on pollen sum,
200 %, (c) fossil algae accumulation rate, $\text{cm}^{-2}\text{yr}^{-1}$, and (d) accumulation rates of algae pigments, $\text{cm}^{-2}\text{yr}^{-1}$.
201 ¹.

202
203 Phytoplankton accumulation rates (Fig. 2 c) were the lowest during the Lateglacial (14,500–11,700
204 cal. BP). Chlorophyta dominated in early Holocene (11,700–9000 cal. BP), after which their values
205 were low throughout the Holocene. Since 7500 cal. BP cyanobacteria accumulation rates increased
206 with a maximum from 4500 to 2500 cal. BP, whereas earlier their accumulation rates were
207 insignificant (no accumulation during the Late glacial).

208
209 Although five major algal pigment groups were retrieved, only four of them (diatoms,
210 cyanobacteria, chlorophytes, cryptophytes) can be considered as dominant (dinophytes were
211 excluded due to minor values). Period from 14,500 to 11,700 cal. BP was characterized by low
212 pigment accumulation, and the highest accumulation occurred from 8200 to 5000 cal. BP (Fig. 2 d).

213
214 For the first time, the fossil phytoplankton (including also Bacteria: cyanobacteria) and *sedaDNA*
215 algae data were combined into one composite diagram (Fig. 3) that comprises as many as 87 taxa.
216 The highest numbers of phyla, order and species were observed for *sedaDNA* (Fig. 4). Only minor
217 share of these were overlapping with the fossil algae.

218
219 Sørensen dissimilarity index indicated overall lower values for the Pleistocene, i.e. Lateglacial
220 (14,500–11,700 cal. BP) and early/middle Holocene (8400–7900 and 6300–4700 cal. BP) (Fig. 5)
221 indicating lower biotic turnover in time. The highest sample dissimilarities and thus highest
222 turnover rates were observed for early (11,700–8500 cal. BP), middle (7800–6300 cal. BP) and late
223 Holocene (4700 cal. BP–present).

224

225 **Fig. 3.** Composite diagram based on fossil algae (NPP) and algal *seda*DNA data, comprising 87
 226 taxa from 14,500 cal. BP to present. Fossil algae – *, *seda*DNA – **, overlapping – ***.

228

229 **Fig. 4.** Comparison of the taxonomical level (a) phyla, (b) order and (c) species identified by NPP
230 (fossil algal remains) and *sedaDNA*, and their overlap. NPP cyanobacteria were excluded from this
231 comparison.

232

233 4. Discussion

234 4.1. *Fossil algae accumulation rates are not direct reflection of biomass*

235 For fossil algae representation, researchers typically use percentages instead of concentration, and
236 even more seldomly, accumulation rates. Percentages are commonly estimated against pollen sum
237 that by default is a biased way to proceed, if NPP are not pollen. There are methods how to estimate
238 microscopic object concentration per sample (volume) and even per year (Stockmarr, 1971).
239 Recently, Wood and Wilmshurst (2013) showed that the interpretation based on percentages might
240 lead to incorrect interpretations of expansion or extinction events in sedimentary records. Therefore,
241 the accumulation rates of NPP can be a reasonable way to overcome this issue. However, as we
242 demonstrate here (see Fig. 2), estimated accumulation rates of fossil algae show a different pattern
243 compared with algal pigments. The reason is most likely linked to at least two factors and study
244 design: 1) natural processes such as decomposition and preservation of algae and 2) pollen sample

chemical treatment. Next, we will discuss these factors in detail and the groups “seen” in pigments versus NPPs (fossils need to have recognizable morphology as is discussed below).

Haselwander and Oboh-Ikuenobe (2017) study on algal preservation in shallow freshwater lakes in Missouri, USA, demonstrated that *Staurastrum* sp., *Botryococcus* sp., *Pediastrum simplex* var. *pseudoglabrum*, *P. integrum* and *P. boryanum* var. *pseudoglabrum* preserve well in lake sediments. In contrast, species such as *Sphaerocystis* and *Ceratium hirundinella* do not preserve well. These findings are in line with other studies indicating that *Scenedesmus* sp., *Tetraëdron* sp., *Pediastrum* sp., *Coelastrum* sp., *Staurastrum* sp. and cyanobacteria (e.g. *Anabaena* sp., *Aphanizomenon* sp. and *Gloeotrichia* sp.) are the most common fossil algae remains in the samples because their cell wall material contains compounds, which confer resistance to bacterial decay (Bellinger and Sigee, 2010; Fey et al., 2010; Jankovská and Komárek, 2000; Weckström et al., 2010). Therefore, reconstructed algal population and accumulation dynamics are biased *per se*, leading to overrepresentation of some taxa and underrepresenting the other.

Pigments stored in sediment have different chemical stability and preservation (Leavitt and Hodgson 2001). However, in upper sediment layers, which are not consolidated, the degradation of pigments is highest to compare with historical sediment layers (Tönno et al. 2013). Microbial activity and thus degradation processes of settled material in upper sediment layers are much more intensive than in deeper (historical) sediments (Wetzel 2001).

Another important aspect is palynological preparation that includes several steps of chemical usage – hence all NPP usually undergo the same treatment (Chambers et al., 2011). Riddick et al. (2017) demonstrated that the application of acetolysis, an oxidizing technique common in palynological preparation, significantly destructs dinoflagellate cysts. Even more significantly, it decreases the

270 abundance of desmids (green algae) with a reported mean 87% decrease. While observations of
271 destructive effect of acetolysis has been explored also for other wide group of NPP – coprophilous
272 fungi spores (van Asperen et al., 2016), the application of acetolysis shown to be useful in
273 phytoliths extraction (da Costa et al., 2016). We agree with previous suggestions (Riddick et al.,
274 2017; van Asperen et al., 2016) that step of acetolysis should be of limited use, if not excluded at
275 all, in a future fossil algae identifications. If fossil algae are the main study subject rather than a
276 side-project within a pollen routine work, counting NPP from smear slides seem to be the best
277 option.

278

279 4.2. Taxonomic richness

280 As expected, our results demonstrate higher phylotaxonomic richness for the *sedaDNA* than for the
281 taxonomic richness of fossil algae. Small overlap at species level is not surprising due to low
282 resolution of 18S rDNA sequences in Eukaryotes in general. In addition, NPP sample preparation
283 includes sediment treatment using 10% KOH (potassium hydroxide) that dissolves all siliceous
284 material including diatoms, not to mention that diatom analysis have a different preparation method.
285 Hence, these are presented only in *sedaDNA*, and overall biomass showed by algae pigments (Fig.
286 2). Only bacteria and chemically resistant algae survive the whole cycle from their production in the
287 lake, through sedimentation to pollen sample preparation, identified under the microscope. In
288 contrast, *sedaDNA* and pigments identify even those algal species that have long lost their cells and
289 left their fingerprint at molecular level. Although it would seem that solely *sedaDNA* can be used to
290 reconstruct past algal diversity, unfortunately, it has its merits. For instance, to obtain information
291 of species, it is necessary to use higher resolution regions in genomes suitable for species detection,
292 for example ITS, also to have reference barcodes in genebanks against which to compare obtained
293 record. Methodology and process itself might be time consuming, and in most cases, also
294 expensive. In addition, taxon can be detected only if their molecular chains are not fragmented and

295 are long enough to detect by DNA sequencing. In current study, we did not analyse Bacteria
296 (including cyanobacteria) in *sedaDNA* and for *Pediastrum* resolution power in Archaeplastida was
297 relatively low. Probably mentioned circumstances of NPP and *sedaDNA* methods could be a reason
298 why only a few phyla, order and species were overlapping. Since the domain of Bacteria was not
299 targeted in *sedaDNA*, the comparison between NPP cyanobacteria and *sedaDNA* Bacteria should
300 be done and discussed elsewhere. Collectively, although each method has their pros and cons, they
301 both complement each other, as highlighted by the composite data-set we display (Fig. 3, 5) and
302 discuss further.

303

304 4.3. Implications and future prospect of non-pollen palynomorphs

305 There are several take-home messages from the current study that are implacable for future NPP
306 analyses. We underline that it is worth to keep tracking NPP alongside routine pollen analysis as it
307 gives additional insights for various ecological aspects (natural and anthropogenic). Combination of
308 fossil algae and *sedaDNA* record complete taxonomic richness and can be used to reconstruct for
309 instance functional groups or biodiversity through time. Our results show that fossil algae increased
310 overall algal diversity of phyla, orders and species. Sure enough, solely algal pigments of
311 cyanobacteria can be used to reconstruct their abundance, but that gives only quantitative biomass
312 estimates, while fossil algae provide partly from both – biomass and taxonomy. On the other hand,
313 algal pigments are the most quantitative, the *sedaDNA* data enables to estimate only proportions
314 and NPPs are the most selective with certain species as microfossils.

315

316

317 **Fig. 5.** Sørensen dissimilarity index estimated from composite data set of fossil algae (NPP) and
 318 *sedaDNA*. Background colors indicate main climatic and environmental changes such as
 319 Pleistocene-Holocene boundary, 8.2 ka cooling event and time of agriculture practice at Lake
 320 Lielais Svētīņū.

321

322 Based on composite information of algae taxonomy, we reconstructed algae turnover rates (beta
 323 diversity in time) for the last 14,500 years. Our estimates indicate higher turnover rates for warmer
 324 Holocene period (Fig. 5). During the Lateglacial, only significant shift in algal composition
 325 occurred at the beginning of the lake development stages. Otherwise, the end of Pleistocene was
 326 secluded from a distinct algal turnover, indicating stable aquatic and terrestrial environment that is
 327 in line with the study by Stivrins et al. (2016). The following swing at the boundary of Pleistocene-
 328 Holocene occurs when the rate of warming was 0.17 °C/decade that is comparable to the current
 329 warming in the Northern Hemisphere (Stivrins et al., 2016; Smith et al., 2015). Relatively low
 330 Sørensen dissimilarity indices from 8400 to 7900 cal. BP suggest stable algal composition. This
 331 time is known as the 8.2 ka cooling event that led to a drop of mean air temperature in winter by 2–
 332 3 °C (Seppä et al., 2007). It seems that algae did not react to this event, but still note high turnover
 333 rates right before and after 8.2 ka event. This suggests that algal turnover took place before and
 334 after, but during the cooling lake conditions and algal composition was stable in time .

335

336 Previous studies from Lake Lielais Svētīņu (Stivrins et al., 2015) and from Lake Højby Sø in
337 Denmark (Hede et al., 2010) indicate that disruption in thermophilous terrestrial vegetation and
338 increased erosional export of nutrients lasted for nearly 700 years. In addition, the 8.2 ka cooling
339 event marks the beginning of increased algae pigment accumulation of Cyanobacteria, Chlorophyta,
340 Cryptophytes and Diatoms that prosper until 5000 cal. BP. Increased abundance of these pigments
341 coincide with the warmest time in Holocene, namely Holocene Thermal Maximum (2.5–3.5 °C
342 above the present day mean temperature; Heikkilä and Seppä, 2010). In our data, algal turnover was
343 low from 6300 to 4700 cal. BP due to flourish of Cyanobacteria indicating prolonged period of
344 water column thermal stratification. Our finding suggests that possible future climate change could
345 shift Lake Lielais Svētīņu trophic state and algae composition back to the state similarly as
346 observed from 6300 to 4700 cal. BP. The last significant increase of algae turnover can be
347 associated with human activities such as agriculture practice (Stivrins et al., 2015) leading to trophic
348 change in lake. However, algae turnover has decreased towards present day that can be explained
349 by decreased population density and agricultural activities around the lake.

350

351

352 While palaeoecological proxies generally reflect changes in landscape, the dynamics in abundance
353 of planktonic NPPs must be viewed with respect to lake's ontogeny (Kisand et al., submitted).
354 Indeed, long-term NPP dynamics probably were dependent from several influencing aspects, such
355 as water level changes of the lake that can be driven also by infilling process (Belle et al., in press)
356 leading to transition from deep to unstratified lacustrine ecosystem.

357

358 Conclusions

359 In the current study, we explored a long-lasting question – how reliable are non-pollen
360 palynomorphs (fossil algae) data, recovered alongside routine pollen analysis. We used *sedaDNA*
361 and algal pigment data to validate the richness and abundance of fossil algae. In addition, for the
362 first time, we compiled a composite data-set from fossil and *sedaDNA* algae to show how fossil
363 algae can be integrated with other palaeo proxies and estimate algae turnover rates for the last
364 14,500 years from a small lake Lielais Svētīņu sediments. Our results revealed a mismatch between
365 reconstructed fossil algae accumulation rates between those obtained from algae pigments. As
366 predicted, taxonomically, fossil algae underrepresents species, but still aid those missing from
367 *sedaDNA*. Small amount of species were overlapping between fossil and *sedaDNA* algae and
368 possible reasons are discussed. Algae turnover rates estimated from a composite data-set indicate
369 lower biotic turnover rates for the Lateglacial (14,500–11,700 cal. BP) and higher for the Holocene
370 (11,700 cal. BP–present). By conducting this study, we encourage a growing number of
371 palynologists keep tracking NPP in their routine work and seek integration possibilities with other
372 ecological and palaeoecological disciplines/proxies in order to tackle important research questions.

373

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377

378 **References**

379

380 Aptroot, A., van Geel, B., 2006. Fungi of the colon of the Yukagir Mammoth and from
381 stratigraphically related permafrost samples. Review of Palaeobotany and Palynology
382 141, 225-230.

383 Belle, S., Tönno, I., Stivrins, N., Veski, S., in press. Lake deposits contribute to enhanced CH₄
384 availability for the benthic food web: evidence from chironomid paleo-diet
385 reconstruction. *Journal of Quaternary Science*, DOI: XXXXXXXXXXXXXXXX.

386 Bellinger, E.G., Sigee, D.C., 2010. *Freshwater algae, identification and use as bioindicators*. Wiley,
387 Chippengam, p. 271.

388 Bronk Ramsey, C., 2008. Deposition models for chronological records. *Quaternary Science Reviews*
389 27, 42-60.

390 Chambers, F.M., van Geel, B., van der Linden, M., 2011. Considerations for the preparation of peat
391 samples for palynology, and for the counting of pollen and non-pollen palynomorphs.
392 *Mires and Peat* 7, 1-14.

393 Chmura, G.L., Stone, P.A., Ross, M.S., 2006. Non-pollen microfossils in Everglades sediments.
394 *Review of Palaeobotany and Palynology* 141, 103-119.

395 da Costa, F.G.C.M., Souza, P.C.T., Klein, D.E., Bove, C.P., 2016. Application of acetolysis in
396 phytoliths extraction. *Review of Palaeobotany and Palynology* 228, 93-97.

397 Demske, D., Tarasov, P.E., Nakagawa, T., Suigetsu 2006 Project Members, 2013. Atlas of pollen,
398 spores and further non-pollen palynomorphs recorded in the glacial-interglacial late
399 Quaternary sediments of Lake Suigetsu, central Japan. *Quaternary International* 290-
400 291, 164-238.

401 Deshpande BN, Tremblay R, Pienitz R, Vincent WF (2014) Sedimentary pigments as indicators of
402 cyanobacterial dynamics in a hypereutrophic lake. *J Paleolimnol* 52: 171-184, DOI
403 10.1007/s10933-014-9785-3

404 Deshpande BN, Tremblay R, Pienitz R, Vincent WF (2014) Sedimentary pigments as indicators of
405 cyanobacterial dynamics in a hypereutrophic lake. *J Paleolimnol* 52: 171-184, DOI
406 10.1007/s10933-014-9785-3

407 Dietre, B., Walser, C., Kofler, W., Kothieringer, K., Hajdas, I., Lambers, K., Reitmaier, T., Haas,
408 J.N., 2017. eolithic to Bronze Age (4850-3450 cal. BP) fire management of the Alpine
409 Lower Engadine landscape (Switzerland) to establish pastures and cereal fields. *The*
410 *Holocene* 27, 181-196.

411 Etienne, B., Jouffroy-Bapicot, I., 2014. Optimal counting limit for fungal spore abundance estimation
412 using *Sporormiella* as a case study. *Vegetation History and Archaeobotany* 23, 743-
413 749.

414 Fey, S.B., Mayer, Z.A., Davis, S.C., Cottingham, K.L., 2010. Zooplankton grazing of *Gloeotrichia*
415 echinulate and associated life history consequences. *Journal of Plankton Research* 32,
416 1337-1347.

417 Fietz, S., Nicklisch, A. & Oberhansli, H. (2007) Phytoplankton response to climate changes in Lake
418 Baikal during the Holocene and Kazantsevo Interglacials assessed from sedimentary
419 pigments. *Journal of Paleolimnology*, 37, 177–203.

420 Gill, J.L., 2014. Ecological impacts of the late Quaternary megaherbivore extinctions 201, 1163-1169.

421 Gill, J.L., McLauchlan, K.K., Skibbe, A.M., Goring, S., Zirbel, C.R., Williams, J.W., 2013. Linking
422 abundances of the dung fungus *Sporormiella* to the density of bison: implications for
423 assessing grazing by megaherbivores in palaeorecords. *Journal of Ecology* 101, 1125-
424 1136.

425 Haselwander, R.D., Oboh-Ikuenobe, F.E., 2017. Preliminary observations on the preservation of
426 organic-walled algae in shallow, freshwater lakes from south–central Missouri, USA.
427 *Palynology* 41, 72-88.

428 Hede, M.U., Rasmussen, P., Noe-Nygaard, N., Clarke, A.L., Vinebrooke, R.D., Olsen, J., 2010.
429 Multiproxy evidence for terrestrial and aquatic ecosystem response during the 8.2 ka
430 event as recorded at Højby Sø, Denmark. *Quaternary Research* 73, 485-496.

431 Jankovská, V., Komárek, J., 2000. Indicative value of *Pediastrum* and other coccal green algae in
432 Palaeoecology. *Folia Geobotanica* 35, 59-82.

433 Kisand, V., Talas, L., Kisand, A., Stivrins, N., Reitalu, T., Alliksaar, T., Vassiljev, J., Liiv, M.,
434 Heinsalu, A., Seppä, H., Veski, S., in press. From microbial eukaryotes to metazoan
435 vertebrates: complete inventory in sedimentary ancient DNA over the last ~14,500
436 years. *Molecular Ecology Resources*, DOI: XXXXXXXXXXXX.

437 Latałowa, M., Pędziszewska, A., Maciejewska, E., Święta-Musznicka, J., 2013. *Tilia* forest
438 dynamics, *Kretzschmaria deusta* attack, and mire hydrology as palaeoecological
439 proxies for mid-Holocene climate reconstruction in the Kashubian Lake District (N
440 Poland). *The Holocene* 23, 667-677.

441 Leavitt, P.R. & Hodgson, D.A. (2001) Sedimentary pigments. In: Tracking environmental change
442 using lake sediments. Volume 3: Terrestrial, algal, and siliceous indicators. (Smol, J.
443 P., Birks, H. J. B. & Last, W. M., eds.), Kluwer Academic Publishers, Dordrecht, The
444 Netherlands, pp 295–325.

- 445 Lenarczyk, J., Kołaczek, P., Jankovská, V., Turner, F., Karpińska-Kołaczek, M., Pini, R.,
446 Pedziszewska, A., Zimny, M., Stivrins, N., Szymczyk, A., 2015. Palaeoecological
447 implications of the subfossil *Pediastrum argentinense*-type in Europe. Review of
448 Palaeobotany and Palynology 222, 129-138.
- 449 Magurran, A.E., 2004. Measuring Biological Diversity. Blackwell Science Ltd., Oxford, p. 215.
- 450 McTigue ND, Bucolo P, Liu Z, Dunton KH 2015 Pelagic-benthic coupling, food webs, and organic
451 matter degradation in the Chukchi Sea: Insights from sedimentary pigments and stable
452 carbon isotopes. Limnology and Oceanography 60: 429-445
- 453 Medeanic, S., 2006. Freshwater algal palynomorph records from Holocene deposits in the coastal
454 plain of Rio Grande do Sul, Brazil. Review of Palaeobotany and Palynology 141, 83-
455 101.
- 456 Miola, A., 2012. Tools for Non-Pollen Palynomorphs (NPPs) analysis: A list of Quaternary NPP
457 types and reference literature in English language (1972–2011). Review of
458 Palaeobotany and Palynology 186, 142-161.
- 459 Reimer, P.J., Bard, E., Bayliss, A., Beck, J.W., Blackwell, P.G., Bronk Ramsey, C., Buck, C.E.,
460 Cheng, H., Edwards, R.L., Friedrich, M., Grootes, P.M., Guilderson, T.P., Haflidason,
461 H., Hajdas, I., Hatte, C., Heaton, T.J., Hoffmann, D.L., Hogg, A.G., Hughen, K.A.,
462 Kaiser, K.F., Kromer, B., Manning, S.W., Niu, M., Reimer, R.W., Richards, D.A.,
463 Scott, E.M., Southon, J.R., Staff, R.A., Turney, C.S.M., van der Plicht, J., 2013. Intcal13
464 and marine13 radiocarbon age calibration curves 0–50,000 years cal BP. Radiocarbon
465 55,1869-1887.
- 466 Reuss, N., Leavitt, P.R., Hall, R.I., Bigler, C. & Hammarlund, D. (2010) Development and application
467 of sedimentary pigments for assessing effects of climatic and environmental changes
468 on subarctic lakes in northern Sweden. Journal of Paleolimnology, 43, 149–169.
- 469 Riddick, N.L., Volik, O., McCarthy, F.M.G., Danesh, D.C., 2017. The effect of acetolysis on desmids.
470 Palynology 41, 171-179.
- 471 Roy S, Llewellyn CA, Egeland ES, Johnsen G (2011) Phytoplankton pigments. Characterization,
472 Chemotaxonomy and Applications in Oceanography. Cambridge University Press
- 473 Sarmaja-Korjonen, K., Seppänen, A., Bennike, O., 2006. *Pediastrum* algae from the classic late
474 glacial Bølling Sø site, Denmark: Response of aquatic biota to climate change. Review
475 of Palaeobotany and Palynology 138, 95-107.

476 Seppä, H., Birks, H.J.B., Giesecke, T., Hammarlund, D., Alenius, T., Antonsson, K., Bjune, A.E.,
477 Heikkilä, M., MacDonald, G.M., Ojala, A.E.K., Telford, R.J., Veski, S., 2007. Spatial
478 structure of the 8200 cal yr BP event in Northern Europe. *Climate of the Past*
479 *Discussions* 3, 165-195.

480 Shumilovskikh, L.S., Schlütz, F., Achterberg, I., Bauerochse, A., Leuschner, H.H., 2015. Non-pollen
481 palynomorphs from mid-Holocene peat of the raised bog Borsteler Moor (Lower
482 Saxony, Germany). *Studia Quaternaria* 32, 5-18.

483 Shumilovskikh, L.S., Seeliger, M., Feuser, S., Novenko, E., Schlütz, F., Pint, A., Pirson, F., Brückner,
484 H., 2016. The harbor of Elaia: A palynological archive for human environmental
485 interactions during the last 7500 years. *Quaternary Science Reviews* 149, 167-187.

486 Stivrins, N., Kalnina, L., Veski, S., Zeimule, S., 2014. Local and regional Holocene vegetation
487 dynamics at two sites in eastern Latvia. *Boreal Environment Research* 19, 310-323.

488 Stivrins, N., Kołaczek, P., Reitalu, T., Seppä, H., Veski, S., 2015. Phytoplankton response to the
489 environmental and climatic variability in a temperate lake over the last 14,500 years in
490 eastern Latvia. *Journal of Paleolimnology* 54, 103-119.

491 Stivrins, N., Soininen, J., Amon, L., Fontana, S.L., Gryguc, G., Heikkilä, M., Heiri, O., Kisielienė,
492 D., Reitalu, T., Stančikaitė, M., Veski, S., Seppä, H., 2016. Biotic turnover rates during
493 the Pleistocene-Holocene transition. *Quaternary Science Reviews* 151, 100-110.

494 Stockmarr, J., 1971. Tablets with spores used in absolute pollen analysis. *Pollen et Spores* 13, 615-
495 621.

496 Tamm, M.; Freiberg, R.; Tönno, I.; Nõges, P.; Nõges, T. (2015). Pigment-based chemotaxonomy - a
497 quick alternative to determine algal assemblages in large shallow eutrophic lake? *PLoS*
498 *ONE* 10(3): e0122526. doi: 10.1371/journal.pone.0122526

499 Tönno I, Kirsi A-L, Freiberg R, Alliksaar T, Lepane V, Kõiv T, Kisand A, Heinsalu A (2013)
500 Ecosystem changes in a large shallow Lake Võrtsjärv, Estonia - evidence from sediment
501 organic matter and phosphorus fractions. *Boreal Environ Res* 18: 195-208

502 Turner, F., Pott, R., Schwarz, A., Schwalb, A., 2014. Responses of *Pediastrum* in German floodplain
503 lakes to Late Glacial climate changes. *Journal of Paleolimnology* 52, 293-310.

504 van Asperen, E.N., Kirby, J.R., Hunt, C.O., 2016. The effect of preparation methods on dung fungal
505 spores: Implications for recognition of megafaunal populations. *Review of*
506 *Palaeobotany and Palynology* 229, 1-8.

- 507 van Geel, B., 2001. Non-pollen palynomorphs. In: Smol, J.P., Birks, H.J.B., Last, W.M. (Eds.),
508 Terrestrial, algal and siliceous indicators: Tracking Environmental Changes using Lake
509 Sediments, Vol. 3. Kluwer Academic Press, Dordrecht, pp. 99-119.
- 510 van Geel, B., Fisher, D.C., Rountrey, A.N., van Arkel, J., Duivenvoorden, J.F., Nieman, A.M., van
511 Reenen, G.B.A., Tikhonov, A.N., Buigues, B., 2011. Palaeo-environmental and dietary
512 analysis of intestinal contents of a mammoth calf (Yamal Peninsula, northwest Siberia).
513 Quaternary Science Reviews 30, 3935-3946.
- 514 van Geel, B., Mur, L.R., Ralska-Jasiewiczowa, M., Goslar, T., 1994. Fossil akinetes of
515 *Aphanizomenon* and *Anabaena* as indicators for medieval phosphate-eutrophication of
516 Lake Gościąg (Central Poland). Review of Palaeobotany and Palynology 83, 97-105.
- 517 Wacnik, A., 2009. Vegetation development in the Lake Milkowskie area, north-eastern Poland, from
518 the Plenivistulian to the late Holocene. Acta Palaeobotanica 49, 287-335.
- 519 Waters, M.N., Smoak, J.M., Saunders, C.J., 2013. Historic primary producer communities linked to
520 water quality and hydrologic changes in the northern Everglades. Journal of
521 Paleolimnology 49, 67-81. DOI 10.1007/s10933-011-9569-y
- 522 Weckström, K., Weckström, J., Yliniemi, L.-M., Korhola, A., 2010. The ecology of *Pediastrum*
523 (Chlorophyceae) in subarctic lakes and their potential as paleobioindicators. Journal of
524 Paleolimnology 43, 61-73.
- 525 Wetzel R.G. (2001) Limnology. Lake and river ecosystems. Academic Press, USA
- 526 Wood, J.R., Wilmschurst, J.M., 2013. Accumulation rates or percentages? How to quantify
527 *Sporormiella* and other coprophilous fungal spores to detect late Quaternary
528 megafaunal extinction events. Quaternary Science Reviews 77: 1-3.
- 529 Zelčs, V., Markots, A., 2004. Deglaciation history of Latvia. In: Ehlers, J., Gibbard, P.L. (Eds.),
530 Quaternary Glaciations—extent and chronology of glaciations. Elsevier, Amsterdam, pp.
531 225-243.