1   Towards understanding the abundance of non-pollen palynomorphs: A comparison of

2   fossil algae, algal pigments and *seda*DNA from temperate lake sediments

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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

different research questions and to reveal if it is reliable to base palaeoecological interpretation

solely on fossil algae identified from the pollen slides.

1. Introduction

In addition to pollen identification, palynologists have recently started to consider the value of other

microscopic objects (e.g. fungi, algae, plant and animal remains) from pollen slides, referred to as

sensu stricto non-pollen palynomorphs (NPP). NPP are found in various environments such as in

sediments underlining their potential in palaeoecological studies (Shumilovskikh et al., 2016;

Lenarczyk et al., 2015; Aptroot and van Geel, 2006; Medeanic, 2006; Turner et al., 2014; Demske

et al., 2013). Whilst there are studies utilizing NPP in addressing significant research questions,

such as evaluating ecological impacts of the late Quaternary megaherbivore palaeodiet and

extinctions (Gill, 2014; van Geel et al., 2011), revealing fungi influence on forest dynamics

(Latałowa et al., 2013), and estimating biotic turnover rates during the Pleistocene-Holocene

transition (Stivrins et al., 2016), only a handful of studies deals with the comparison of NPP with

other proxies and the validation of NPP as a palaeobiological metric (Etienne and Jouffroy-Bapicot,

2014; Gill et al., 2013; Wood and Wilmshurst, 2013). Given the increased interest in NPP, it is

necessary to seek a deeper understanding how reliable metric it is in quantifying temporal changes

in ecosystems.

Based on Miola's (2012) review, there are more than 1300 NPP descriptions available up today and

the list of NPPs is still growing. Palaeoecologists are aware that there may be certain limitations in

NPP – for example, part of NPP do not preserve as fossils. Selective preservation and thus,

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 (*seda*DNA). 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 *seda*DNA, 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 *seda*DNA and NPP. *seda*DNA 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 *seda*DNA requires a complex targeted-

82   methodology/sequencing to represent more than one domain. In the current study, we compared

83   *seda*DNA 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   *seda*DNA, the comparison between NPP cyanobacteria and *seda*DNA bacteria should be done and

87   discussed elsewhere. Furthermore, by combining the semi-quantitative information from algal

88   pigments and higher taxonomical resolution from *seda*DNA, 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 *seda*DNA.

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 Svētiņu (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 km^2 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 Svētiņu.

110

111   Lake Lielais Svētiņu 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 *seda*DNA 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 *a*–Carotene was analysed to identify

149   the dynamics of Cryptophytes, while Peridinin indicated the abundance of Dinophytes (Leavitt and

150   Hodgson 2001).

151

152   *seda*DNA 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^{TM} DNA AWAY^{TM} 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 *seda*DNA 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 Svētiņu, indicate at least partly the spatiotemporal

171 variation in local in-lake productivity and population abundances. Algal data from *seda*DNA is

172 currently available as binary data that provide information on taxon presence in the lake. Note that

173 taxon absence from *seda*DNA 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 *seda*DNA 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 *seda*DNA (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

Relative proportions (percentages) of fossil algae vary significantly if they are estimated based on

sum of algae or pollen (Fig. 2 a,b). Pollen based proportions correlate with fossil algae

accumulation rates (Fig. 2 c) and can be an artefact due to used equation. However, proportions of

fossil algae based on algae sum, solely indicates changes within the algal population.

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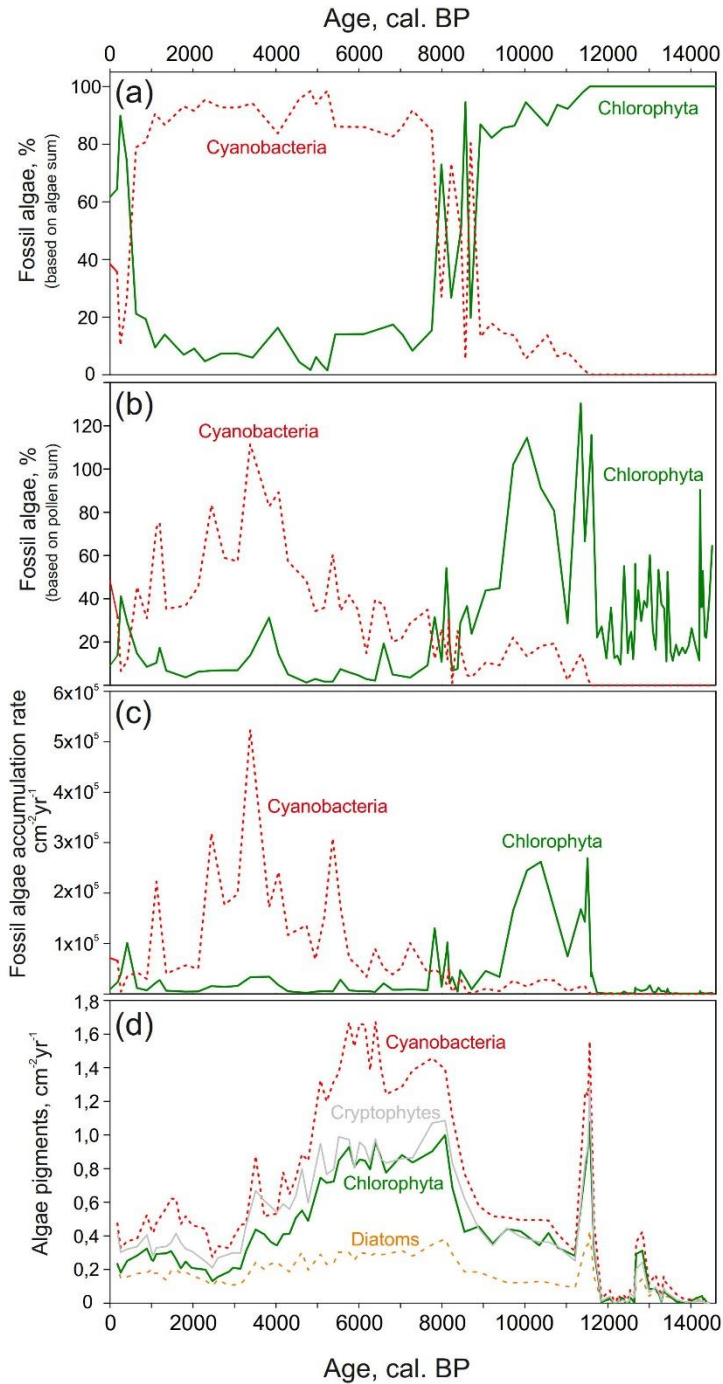
199　**Fig. 2.** Comparison of (a) fossil algae based on algal sum, %, (b) fossil algae based on pollen sum,

200　%, (c) fossil algae accumulation rate, $cm^{-2}yr^{-1}$, and (d) accumulation rates of algae pigments, $cm^{-2}yr^{-1}$

201　.

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 *seda*DNA

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 *seda*DNA (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).

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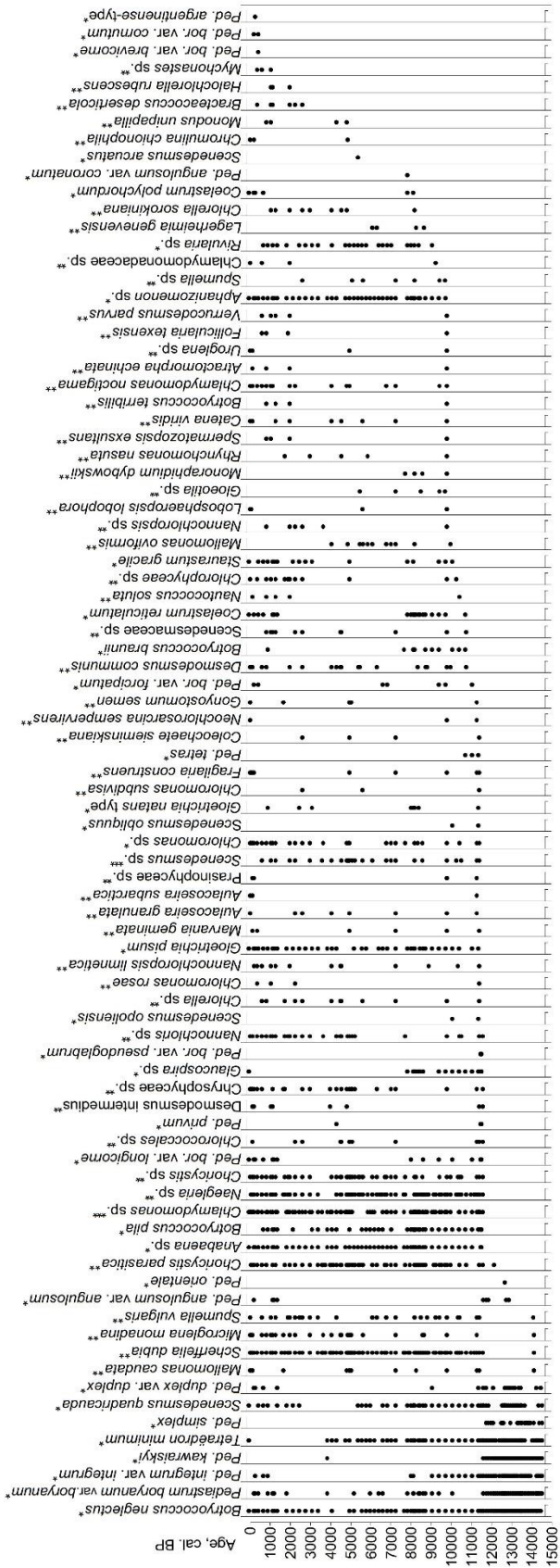
Fig. 3. Composite diagram based on fossil algae (NPP) and algal *seda*DNA data, comprising 87

taxa from 14,500 cal. BP to present. Fossil algae – *, *seda*DNA – **, overlapping – ***.

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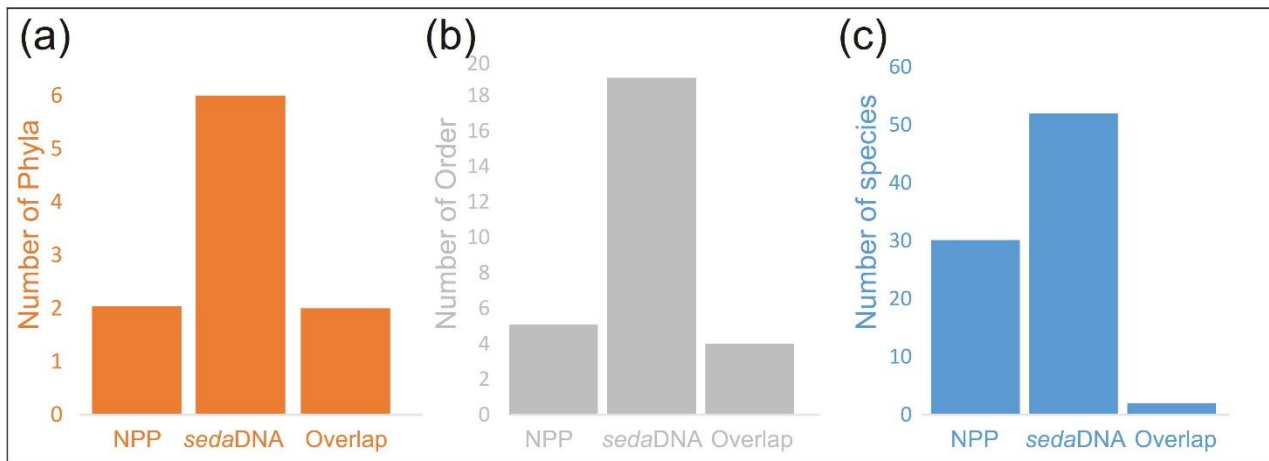
Fig. 4. Comparison of the taxonomical level (a) phyla, (b) order and (c) species identified by NPP

(fossil algal remains) and *seda*DNA, and their overlap. NPP cyanobacteria were excluded from this

comparison.

4. Discussion

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

For fossil algae representation, researchers typically use percentages instead of concentration, and

even more seldomly, accumulation rates. Percentages are commonly estimated against pollen sum

that by default is a biased way to proceed, if NPP are not pollen. There are methods how to estimate

microscopic object concentration per sample (volume) and even per year (Stockmarr, 1971).

Recently, Wood and Wilmshurst (2013) showed that the interpretation based on percentages might

lead to incorrect interpretations of expansion or extinction events in sedimentary records. Therefore,

the accumulation rates of NPP can be a reasonable way to overcome this issue. However, as we

demonstrate here (see Fig. 2), estimated accumulation rates of fossil algae show a different pattern

compared with algal pigments. The reason is most likely linked to at least two factorsand study

design: 1) natural processes such as decomposition and preservation of algae and 2) pollen sample

245 chemical treatment. Next, we will discuss these factors in detail and the groups "seen" in pigments

246 versus NPPs (fossils need to have recognizable morphology as is discussed below).

247

248 Haselwander and Oboh-Ikuenobe (2017) study on algal preservation in shallow freshwater lakes in

249 Missouri, USA, demonstrated that *Staurastrum* sp., *Botryococcus* sp., *Pediastrum simplex* var.

250 *pseudoglabrum*, *P. integrum* and *P. boryanum* var. *pseudoglabrum* preserve well in lake sediments.

251 In contrast, species such as *Sphaeorocystis* and *Ceratium hirundinella* do not preserve well. These

252 findings are in line with other studies indicating that *Scenedesmus* sp., *Tetraëdron* sp., *Pediastrum*

253 sp., *Coelastrum* sp., *Staurastrum* sp. and cyanobacteria (e.g. *Anabaena* sp., *Aphanizomenon* sp. and

254 *Gloeotrichia* sp.) are the most common fossil algae remains in the samples because their cell wall

255 material contains compounds, which confer resistance to bacterial decay (Bellinger and Sigee,

256 2010; Fey et al., 2010; Jankovská and Komárek, 2000; Weckström et al., 2010). Therefore,

257 reconstructed algal population and accumulation dynamics are biased *per se,* leading to

258 overrepresentation of some taxa and underrepresenting the other.

259

260 Pigments stored in sediment have different chemical stability and preservation (Leavitt and

261 Hodgson 2001). However, in upper sediment layers, which are not consolidated, the degradation of

262 pigments is highest to compare with historical sediment layers (Tõnno et al. 2013). Microbial

263 activity and thus degradation processes of settled material in upper sediment layers are much more

264 intensive that in deeper (historical) sediments (Wetzel 2001).

265

266 Another important aspect is palynological preparation that includes several steps of chemical usage

267 – hence all NPP usually undergo the same treatment (Chambers et al., 2011). Riddick et al. (2017)

268 demonstrated that the application of acetolysis, an oxidizing technique common in palynological

269 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 *seda*DNA 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 *seda*DNA, 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, *seda*DNA 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 *seda*DNA 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

are long enough to detect by DNA sequencing. In current study, we did not analyse Bacteria

(including cyanobacteria) in *seda*DNA and for *Pediastrum* resolution power in Archaeplastida was

relatively low. Probably mentioned circumstances of NPP and *seda*DNA methods could be a reason

why only a few phyla, order and species were overlapping. Since the domain of Bacteria was not

targeted in *seda*DNA, the comparison between NPP cyanobacteria and *seda*DNA Bacteria should

be done and discussed elsewhere. Collectively, although each method has their pros and cons, they

both complement each other, as highlighted by the composite data-set we display (Fig. 3, 5) and

discuss further.

4.3. Implications and future prospect of non-pollen palynomorphs

There are several take-home messages from the current study that are implacable for future NPP

analyses. We underline that it is worth to keep tracking NPP alongside routine pollen analysis as it

gives additional insights for various ecological aspects (natural and anthropogenic). Combination of

fossil algae and *seda*DNA record complete taxonomic richness and can be used to reconstruct for

instance functional groups or biodiversity through time. Our results show that fossil algae increased

overall algal diversity of phyla, orders and species. Sure enough, solely algal pigments of

cyanobacteria can be used to reconstruct their abundance, but that gives only quantitative biomass

estimates, while fossil algae provide partly from both – biomass and taxonomy. On the other hand,

algal pigments are the most quantitative, the *seda*DNA data enables to estimate only proportions

and NPPs are the most selective with certain species as microfossils.
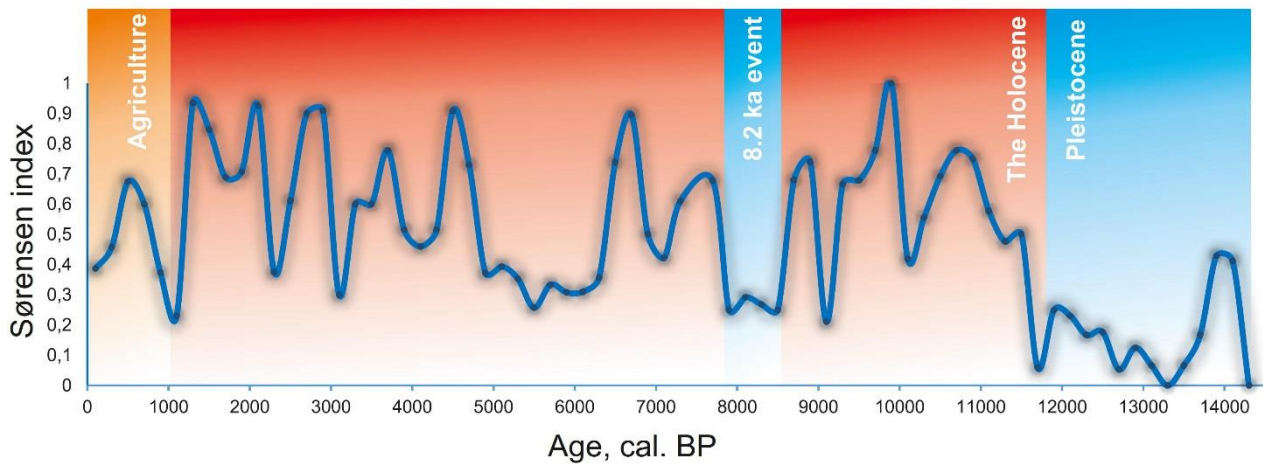
Fig. 5. Sørensen dissimilarity index estimated from composite data set of fossil algae (NPP) and *seda*DNA. Background colors indicate main climatic and environmental changes such as Pleistocene-Holocene boundary, 8.2 ka cooling event and time of agriculture practice at Lake Lielais Svētiņu.

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

335

336   Previous studies from Lake Lielais Svētiņu (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 Svētiņu 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

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

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