Ecosystem responses to climate-related changes in a Mediterranean alpine environment over the last ~180 years

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ABSTRACT

The effect of recent climatic warming is significant in the Mediterranean region, especially in high mountain areas. This study uses multiple sedimentary proxies from Río Seco Lake, a remote alpine lake in the Sierra Nevada, southeastern Spain, to reconstruct recent environmental and ecological changes in the lake and catchment. Two main climatic periods can be distinguished during the past 180 years: Period One (1820- ~1920s) characterized by colder and wetter conditions than the more recent Period Two (~1920s to the present), characterized by warmer and drier conditions. Independent proxies such as subfossil chironomid assemblages, *n*-alkane indices, pollen data and/or spectrally-inferred chlorophyll-*a* concentrations indicate a longer ice-cover period, colder water temperature and more pronounced accumulation of snow in the catchment during Period One than in Period Two, likely producing water stress for catchment plant growth because of the low rate of ice melting in Period One. As temperatures increase and precipitation decreases from the 1920s onwards, a wider development of wetland plants observed, which is associated with the longer warm season that contributed to snow and ice melting in the catchment. This continuing temperature rise and precipitation decrease over the past 60-years by ~0.24 °C per decade and –0.92 mm/yr, respectively, lead to an important increase in chlorophyll-*a* and changes in lake biotic assemblages. Major chironomid community structure changes to warmer water taxa were recorded, resulting in a 2 °C increase in mean July air temperature inferred by chironomids from ~1950 onwards. An inferred increase in primary production for the past few decades is consistent with higher temperatures, whilst wider development of wetland plants is associated to longer warm season. The coherence between independent environmental proxies, each associated with distinct mechanistic linkages to climatic shifts, strengthens our interpretations of a recent warming trend and an intensification of summer drought in this high mountain area leading to distinct changes in the lake and its catchment.

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The impact of this climate change on the summits of Sierra Nevada and its influence transcends its geographical limits because these systems provide ecosystem services to a vast area.

INTRODUCTION

Over the last 150 years, the global average air temperature at the Earth's surface has increased by ~0.8 ºC, while in the Northern Hemisphere the last 30-year period was the warmest period on record (IPCC 2013). High mountain areas are among the most sensitive to anthropogenic climate change and are experiencing some of the highest rates of warming due to the elevation-dependent warming (EDW), i.e. the rate of warming is amplified with altitude (Pepin and others 2015). The Mediterranean region is considered to be the largest "climate change hot-spot" in the world (Giorgi 2006). In particular, Mediterranean high mountain ecosystems have been identified as especially susceptible to global warming (Lionello, 2012; Pauli and others, 2012). This susceptibility is partially associated to the increased risk of summer drought in this region, caused by the rise in average summer air temperature and the reduction in annual rainfall (Nogués-Bravo and others, 2012).

Remote areas, referred as unpopulated high altitude regions above the tree line as well as high latitude areas, are excellent sites to study climate change effects because the climate signal is not as strongly obscured by other human impacts as in more populated areas (Battarbee and others 2002) and because they are sensitive to both natural and anthropogenic factors (Pauli and others 1996; Adrian and others, 2009; Smol, 2008).High mountain ecosystems are strongly influenced by physical conditions and strongly limited by nutrients, exhibiting steep ecological gradients and narrow ecotonal boundaries.

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75   Therefore, changes in environmental conditions (e.g. temperature patterns, ice-free period duration, water

76   thermal regime, habitat and water availability) are expected to have great effects in them (Beniston 2003;

77   Rühland and others 2015).

78   One way to assess the pressures influencing these remote sites is through the study of lake

79   sediments, which are excellent archives of long-term environmental changes and allow environmental

80   and ecosystem conditions to be reconstructed from limnological, ecological and geochemical lake-

81   sediment proxies (Smol 2008). Ecosystem responses to warming are complex and show many direct and

82   indirect interactions, with numerous climate-related processes affecting to different biotic and abiotic

83   parameters. The analysis of independent proxies allows for the tracking of processes within lakes and

84   their catchment. Since individual proxies can have different mechanistic links that determine their

85   response to external stressors, studies based on multiple proxies offer a holistic approach to interpreting

86   past lake and catchment-related changes.

87   The Sierra Nevada of southernmost Spain is a protected high-mountain area situated where alpine

88   conditions and the influence of Mediterranean climate coexists. It is one of the most important

89   biodiversity hotspots in Europe and plant species loss in its summits attributed to climate change in the

90   last decades has been reported (Pauli and others 2012). This Mediterranean mountain range has shown a

91   rapid response to the recent warming with the disappearance of permanent ice from the highest north-

92   facing cirques (Oliva and others 2016). A trend in declining mean annual rainfall (Ruiz-Sinoga and others

93   2011) and a reduction of snow and ice cover since the 1960s (Pérez-Palazón and others 2015) has become

94   more pronounced since the onset of the 21st century (Bonet and others 2016). In addition, climate models

95   project an ongoing warming trend in this region for the end of the 21st century (Pérez-Luque and others

96   2016).

97   With this background, alpine ecosystems in the Sierra Nevada have become the focus of several

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98   paleoecological research projects over the Holocene in order to understand their post-glacial

99   environmental responses. Most of the wetlands and peatlands in the Sierra Nevada developed in

100   depressions carved during glacial times. Actually, the studied site, Río Seco Lake, bears the longest

101   sedimentary record in Sierra Nevada, registering the Pleistocene-Holocene transition (between ~11000-

102   12000 cal yr BP) (Anderson and others 2011). All the paleoenvironmental studies in Sierra Nevada alpine

103   wetlands agree with the Holocene climatic evolution of the western Mediterranean region, with an humid

104   early-middle Holocene that changes towards more arid conditions in the middle-late Holocene. Saharan

105   dust inputs in the Sierra Nevada after 6000 cal yr BP affected alpine aquatic primary production (Jiménez-

106   Espejo and others 2014; García-Alix and others 2017), as well as local vegetation and landscape

107   (Anderson and others 2011; Jiménez-Moreno and Anderson 2012; García-Alix and others 2017). Most

108   of the available long-term studies are not available at high resolution for the last hundred years, more

109   specifically for the period after the end of the Little Ice Age, when the current environmental conditions

110   of Sierra Nevada were established (García-Alix and others 2017; Oliva and others 2018). Some short-

111   term surveys (~150 years) based on biological proxies, such as cladocerans, diatoms and sedimentary

112   algal pigments (Jiménez and others 2015; Pérez-Martínez 2016; Jiménez and others 2018), have shown

113   significant response of biotic assemblages to direct and indirect effects of temperature increases at the

114   turn of 20th century, and especially over the past ~50 years. As yet, detailed comparisons of the communal

115   response of chironomids to climate variability have not been conducted in the Sierra Nevada.

116   Paleolimnological studies combining chironomids with organic geochemical indices may further our

117   understanding of climate and landscape processes in this alpine ecosystem.

118   This study aims to provide a high-resolution multi-proxy reconstruction of recent

119   palaeoenvironmental conditions from an alpine Mediterranean environment of the Sierra Nevada for the

120   first time, helping to understand past changes and constrain future environmental scenarios. The aim is to

121   put effects of recent climate changes in the context of a record extending back over the last 180 years to

122   assess the extent that lake and catchment-related processes are linked to direct and indirect climate-driven

123   changes. To accomplish this, we use a combination of stratigraphic records of sub-fossil chironomid

124   assemblages, leaf wax biomarkers (*n*-alkanes), spectrally-inferred chlorophyll-*a* concentrations, pollen,

125   cladocera, diatoms, organic matter content, organic matter C/N ratio and organic carbon isotopic

126   composition (Online Appendix 3).

127   **MATERIALS AND METHODS**

128   **Study site**

129   Sierra Nevada (SE Spain, maximum altitude 3482 masl) is the highest mountain range of the

130   Southern Europe. Sierra Nevada summits experience a high mountain Mediterranean semi-arid climate

131   characterized by a warm and dry season (from ~June to October). The meteorological station at the

132   summit (2507 masl) reports a mean annual temperature of 3.9 ºC and total precipitation of 693 mm, with

133   80% occurring as snow between October and April (Worldwide Bioclimatic Classification System, 1996-

134   2018). There are ~50 small lakes of glacial origin and many alpine meadows around lakes, streams and

135   depressions. These high-mountain meadow ecosystems represents a small area of the mountain range but

136   have a high rate of plant endemicity and host many threatened taxa (Pérez-Luque and others 2015).

137   Río Seco (RS) Lake (37º 03'N, 3º 20'W) is a small, low primary production and shallow lake of

138   glacial origin located above tree line at 3020 masl in the Sierra Nevada (southern Spain) (Fig. 1). The

139   catchment bedrock is siliceous and largely comprised of mica-schist with graphite and/or feldspar. The

140   soil is poorly developed and does not support agriculture or forestry. The catchment area is partially

141   covered (~15%) by alpine meadows, consisting primarily of sedges (Cyperaceae) and grasses (Poaceae).

142   The lake has no clearly differentiated littoral zone, but its shoreline is covered by bryophytes. With the

143   exception of the meadows, most of the catchment vegetation consists of scarce xerophytic shrubland (see

144   Anderson and others 2011).

145   The lake has diffuse inflows that provide water to the basin, and a small outlet. The inflow and
146   outflow of water can disappear as the ice-free season progresses. The lake is ice covered from around
147   October-November until June-July with a large interannual variability. Further details of physicochemical
148   and biological features are shown in Online Appendix 1. During the ice-free period, Secchi disk visibility
149   exceeds the water depth, the lake is not thermally stratified, and the maximum temperature is 16–18 ºC.
150   It is a fishless lake, with low plankton diversity. Among chironomids, the species *Psectrocladius*
151   *limbatellus* and *Micropsectra radialis* (as *M. coracina*) have been recorded in RS Lake (Laville and
152   Vílchez-Quero 1986).

153   There are a few signs of significant human activity in the area. RS Lake is relatively remote, with
154   local human activity currently limited to infrequent sheep herding around surrounding meadows during
155   summer months. A dirt road, constructed between 1964 and 1965 and lying upgradient from RS Lake,
156   experiences only foot traffic since the establishment of the Sierra Nevada National Park in 1999. A
157   mountain hut was situated close to the shoreline and operated for three decades (1967-1997). The
158   demolition of the hut at 1997 produced a large amount of inorganic material, which clouded the lake and
159   had a major effect on biota and geochemical variables (Jiménez and others 2015).

160   **Sediment sampling, analyses and dating**

161   A sediment core was collected from the deepest part of the lake in September 2008 using a slide-
162   hammer gravity corer (Aquatic Research Instruments, Hope, Idaho, USA) with an inner core-tube
163   diameter of 6.8 cm. The core (16 cm long), was extracted in a methacrylate cylinder and immediately
164   wrapped in a dark bag to keep it protected from the light, sectioned into 0.5 cm slices using an extruder,
165   and sealed in sterile Whirlpak® bags, which were stored and transported in a cold box to the laboratory.
166   Subsamples were collected at each interval in the laboratory and kept in a cold (4 ºC) and dark room until

167   analysis. The sediment was dated by gamma (^{210}Pb, ^{137}Cs, and ^{226}Ra) and alpha spectroscopy (^{210}Pb in

168   deepest part of core) (Eakins and Morrison 1978) establishing a chronology for the past ~180 years. The

169   dating and sedimentation rate were calculated by using the constant flux: constant sedimentation (cf:cs)

170   model (Appleby and Oldfield 1983). The core was analyzed at the Center for Research, Innovation and

171   Technology (CITIUS) of the University of Sevilla, Spain.

Instrumental climate data

173   We use MAAT Madrid (mean annual air temperature series from Madrid station) and AP San

174   Fernando (annual precipitation series from San Fernando station) as representative of air temperature and

175   precipitation tendencies of the larger region around the Sierra Nevada during the last 170 years throughout

176   the analyses. Geographical distance between Madrid and San Fernando climate stations from RS Lake

177   are 376 and 262 km, respectively, while altitude differences are 2356 and 2992 m, respectively (Online

178   Appendix 2).

Sedimentary proxy record

180   Sedimentary chlorophyll-*a* was inferred by visible reflectance spectroscopy using a FOSS

181   NIRSystems Model 6500 series Rapid Content Analyzer (Tidestone Technologies, Inc.) to measure

182   spectral reflectance of sediments that had been freeze-dried and sieved through a 125 μm-mesh, following

183   the methods described by Michelutti and others (2005). The chlorophyll-*a* concentration includes native

184   chlorophyll-*a*, as well as all chlorophyll isomers and its major derivatives (pheophytin *a* and

185   pheophorbide *a*), and therefore accounts for the major diagenetic products (Michelutti and Smol 2016).

186   Loss on ignition (LOI) was measured to calculate the organic matter and carbonate content in the

187   sediments (Heiri and others 2001). LOI was assessed sequentially on all core intervals (every 0.5 cm)

188   using a muffle furnace. Samples were dried in an oven at 105 ºC for 24 h and weighed. The content of

8

189 the organic and carbonate matter was analysed by incinerating the samples at 550 ºC for 4 h and at 900

190 ºC for 2 h, respectively (see detailed methods in Dean 1974 and Heiri and others 2001).

191   Total and inorganic carbon, and nitrogen content were analyzed with a CARLO ERBA EA 1108

192 CHNSO Elemental Analyzer system. The organic fraction was determined as the difference between the

193 total and the inorganic carbon fraction (Meyers and Teranes 2001). The carbon/nitrogen (C/N) ratio was

194 calculated from the mass data and expressed as atomic ratio.

195   Prior to the carbon isotope analysis from the bulk sediment organic matter ($\delta^{13}C_{org}$), 1 g of freeze-

196 dried sediment was extracted by drying the samples (50 ºC) for 24h. The carbonate fraction was then

197 removed by addition of 10% HCl to the solution. The C isotopic composition ($^{13}C/^{12}C$) of acid-treated

198 samples was analyzed using a mass multicollector spectrometer (Isoprime; GV Instruments) equipped

199 with a EuroVector elemental analyzer (mod. Euro EA 3000) and continuous flow inlet. The results are

200 expressed as $\delta^{13}C_{org}$ in the conventional delta (δ) notation versus Vienna PeeDee Belemnite (V-PDB).

201 Reproducibility measured for working standards during each run was better than ± 0.15 ‰.

202   The total lipid extract (TLE) from 32 freeze-dried sediment samples was extracted with a Thermo

203 Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor system using 9:1 DCM:methanol. The

204 obtained TLE was separated in neutral and acid fractions by aminopropyl-silica gel chromatography using

205 1:1 DCM:isopropanol and ether with 4% acetic acid, respectively. Afterwards, the *n*-alkanes were

206 obtained by eluting the neutral fraction with hexane through a 230-400 mesh/35-70 micron silica-gel

207 chromatographic column and analyzed using a GC-FID (Shimadzu 2010) and a GC-MS (Shimadzu

208 QP2010-Plus Mass Spectrometer interfaced with a Shimadzu 2010 GC). To check the reproducibility of

209 the measurements and to quantify the *n*-alkane content, a mixture of *n*-alkanes (C_{16}, C_{18}, C_{19}, C_{20}, C_{23},

210 C_{25}, C_{26}, C_{28}, C_{30}, C_{32}, and C_{37}) was measured every five samples. The measurement error was lower than

211 1.5%.

212   For fossil pollen, a modified Faegri and Iversen (1989) procedure was followed using 1 cm^3 of

213   sediment. Pre-treatment included (NaPO$_3$)$_6$ to deflocculate clays and the addition of *Lycopodium* spores

214   for calculation of pollen concentration. Sediments were suspended in Na$_4$P$_2$O$_7$ and sieved, then treated

215   with HCl, HF and acetolysis solution. Samples were stained and suspended in silicone oil and identified

216   at 400-1,000x to their lowest taxonomic level – mostly genus, sometimes family or other grouping – using

217   a light microscope. For more details on the methodology see Anderson and others (2011).

218   For chironomid analysis, samples (~0.3 g dry weight) for each analysed interval of sediment were

219   immersed in 10% KOH for 2-3 hours and subsequently sieved through a 100 µm fraction. The head

220   capsules were sorted from other sieve residue under a dissection microscope. Chironomids were prepared

221   in Eurapal mounting medium and identified at 100-400x magnification with a compound microscope.

222   The minimum count threshold was 40 (range 43-83.5), except for the uppermost sample, which only

223   consisted of 32 head capsules. Taxonomy mainly followed Oliver and Roussel (1983) and Brooks and

224   others (2007). Comparatively, fewer intervals were counted for chironomids (10 samples) than for the

225   other proxies (32 samples). Chironomid samples were analyzed every 2 cm from 0 to 16 cm.

226   Cladoceran and diatom assemblages from the sediment core of RS Lake were previously analyzed

227   and published in Pérez-Martínez (2016) and Jiménez and others (2018). In this study we use the PCA

228   axis 1 sample scores of both assemblages to compare with the rest of the proxies.

229   Interpretation of selected sedimentary proxies in RS Lake can be found in Online Appendix 3.

230   **Data analyses**

231   Principal component analysis (PCA) was used to summarize the dominant pattern of assemblage

232   variability in chironomid, cladocera and diatom assemblages, as detrended correspondence analysis

233   (DCA) indicated relatively short lengths of the first two compositional gradients (1.43 and 0.73 standard

234   deviation units on the DCA axis 1 and 2, respectively). Chironomid relative abundances were square root

235   transformed prior to analyses to equalize variance among taxa. Ordinations were conducted using the

236   vegan package (Oksanen and others 2015) for the R software environment (R Development Core Team,

237   2015). The annually resolved climate series (MAAT Madrid and AP San Fernando) were averaged over

238   the period of accumulation for each dated interval, thereby integrating the instrumental data with the

239   paleolimnological data (Sorvari and others 2002). The relationships between sedimentary proxies

240   (downcore PCA axis 1 sample scores, organic geochemical proxies and sedimentary chlorophyll-*a*

241   record) and changes in climatic series (MAAT Madrid and AP San Fernando) were then examined.

242   STATISTICA v.7 (Statsoft) software was used to test the data normality and calculate Pearson

243   correlations. The Kolmogorov-Smirnov test with Lilliefor's correction was performed to determine the

244   normality of the data distribution. Pearson correlation coefficients were used to provide an indication of

245   the strength of the relationships between parameters. For the sedimentary chlorophyll-*a* record, the

246   uppermost sedimentary interval (0-0.5 cm) was excluded because it could not be reliably identified as

247   exclusively representing sedimentary chlorophyll-*a* due to the presence of algal mat material. Lake

248   sediment records can be affected by bioturbation and other factors which can to some extent smooth out

249   short-term variability (e.g. between year variability). We therefore do not report *p* values for correlations

250   between lake sediment records as well as between lake sediment records and instrumental data series,

251   since statistical testing of correlation coefficients assumes statistical independence of the data points.

252   However, all of the discussed relationships would have been statistically significant if tested using

253   standard tests for statistical significance of *p* values.

254   A stratigraphically constrained cluster analysis was carried out in the R software environment (R

255   Development Core Team 2015), using the Rioja package (Juggins, 2012) to identify the periods with

256   homogeneous response of the different proxies. Stratigraphic zonation was done by a cluster analysis with

257   a constrained incremental sum of squares (coniss method), using the chclust() function (method='coniss')

258   of the Rioja package. The cluster analysis was applied using the combination of all biogeochemical

259   proxies. To characterize the timing of largest change in MAAT Madrid and AP San Fernando series data,

260   breakpoint analyses using a two-segment piecewise linear regression were applied to each series to

261   identify where the slope changes (Toms and Lesperance 2003).

262   Zonation of the stratigraphic profiles of chironomid data was performed by a cluster analysis with

263   a constrained incremental sum of squares (CONISS), square root transformation of data and chord

264   distance as the dissimilarity coefficient, using Tiliagraph View (TGView) version 2.02 (Grimm 2004)

265   and determining the number of significant zones by means of the broken stick model (Bennett 1996).

266   Chironomid-inferred mean July air temperature (MJAT) reconstruction was performed using the program

267   C2 (Juggins 2007) based on a 274-lake chironomid-temperature calibration dataset from Switzerland and

268   Norway (Heiri and others 2011) and a temperature inference model (transfer function) developed from

269   these data. The calibration dataset covers a mean July air temperature gradient from 4 to 18.4°C and a

270   wide range of arctic, alpine, subalpine and temperate lowland lakes. The applied transfer function was

271   based on weighted averaging-partial least squares regression (WA-PLS; ter Braak and Juggins 1993; ter

272   Braak and others 1993). The model featured a cross-validated r^2 of 0.84 and a root mean square error of

273   prediction (RMSEP) of 1.55°C. RMSEP, r^2, and sample-specific errors of prediction (eSEPs) were

274   calculated based on 9999 bootstrapping cycles in C2. Chironomid assemblage percentage data were

275   square root transformed before calculation of WA-PLS and distance metrics. To assess the trajectory of

276   RS chironomid assemblages relative to summer temperature, we compare the RS data to mountain lakes

277   in the Swiss Alps, see details in Online Appendix 4 (Fig S2).

278   **RESULTS**

279   **Chronology**

280   The ^{210}Pb dating of the sediment core from RS Lake shows sedimentation rates of 0.9-1.1 mm

281   year^{-1} from 0 to 6 cm depth (from ca. 2008 to 1948) and 0.7-0.8 mm year^{-1} from 6 cm to 15.5 cm depth

282   (from ca. 1948 to 1821) (Jiménez and others 2018). For further details see Online Appendix 5.

283   **Instrumental climate data**

284   Over the 143-year record, mean annual air temperature (MAAT) from the Madrid climate station

285   indicates a warming trend beginning at the turn of the 20th century (Fig. 2 and Fig. S1 in Online Appendix

286   2). Total annual precipitation (AP) from the San Fernando climate station indicates that over the 172-year

287   record, the second half of the 19th century was wet, reaching a maximum around 1860-70 and then

288   decreasing from the late 19th century to the present, interrupted only by positive anomalies in the 1960s

289   (Fig. 2 and Fig. S1 in Online Appendix 2). The last 40 years of the AP San Fernando record exhibit

290   persistent low precipitation values that were particularly low from 1985-1995.

291   According to main shifts in climatic data, consistent with noticeable changes in direction,

292   magnitude and timing in the major paleoenvironmental proxies in the present study, two distinct climatic

293   periods are indicated for the climate data: Period One from 1820 to ~1920s, a period of relatively high

294   precipitation and low and decreasing temperature; and Period Two from ~1920s to the present, a warmer

295   and drier period, particularly since the mid-70s (Fig. 2).

296   **Sedimentary proxy record**

297   A similar trend is observed in % organic matter content (estimated by Loss on ignition; LOI$_{550}$)

298   and sedimentary chlorophyll-*a* through the entire profile (Pearson r=0.52, n=32). The two variables show

299   a progressive decrease from 1820 to ~1920s followed by an increase to the present, except for the abrupt

300   decrease of LOI$_{550}$ in the 1990s (Fig. 2). The trend of sedimentary chlorophyll-*a* is parallel to MAAT

301   Madrid for the entire record (Pearson r=0.76, n=24), and also similar between LOI$_{550}$ and MAAT Madrid

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302 until the late 1980s (Pearson r=0.46, n=20). The abrupt decrease of LOI_{550} values from ~1990s is

303 responsible for the decoupling of trends between LOI_{550} and sedimentary chlorophyll-*a* record and MAAT

304 Madrid, and is associated with the demolition of the mountain hut in 1998, altering sediment composition

305 as is also apparent in the ^{210}Pb activity profile (Jiménez and others 2015) (Fig. S3; Online Appendix 5).

306   From 1860 to the ~1920s, the C/N ratio shows the lowest values of the entire period (11.7±2.1,

307 mean±SD), coincident with high values of AP San Fernando and low values of MAAT Madrid, while

308 higher values are recorded from ~1920s to the present (15.6±2.6) coincident with a warmer and drier

309 period. C/N ratio and $\delta^{13}C_{org}$ show opposite tendencies for almost all the record. C/N peaks in the mid-

310 19th century (1860-1870s) and ~1920s, associated with $\delta^{13}C_{org}$ decreases, except for the last four decades

311 (from mid-1970s to the present), a period in which the C/N ratio shows the highest persistent values and

312 $\delta^{13}C_{org}$ exhibits a decreasing trend after maximum values (Fig. 2).

313   The RS Lake record shows a predominance of *n*-alkanes with odd carbon chains. The CPI ranges

314 from 3.6 to 4.4 and P_{aq} values are higher than 0.28 for the whole period (Fig. 2). From 1820 to ~1920, P_{aq}

315 shows the lowest and very homogeneous (~0.3) values, while ACL shows the highest values of the record.

316 ACL values show an opposite trend than P_{aq} values (Pearson r=-0.97, n=32) for the entire record. The

317 main difference between ACL and P_{aq} values is observed during the first period, between ~1850 and

318 ~1880, with a small increase (0.32) of P_{aq} in the 1860s, agreeing with decreasing ACL values during this

319 period (~1850-1880) and coincident with the highest persistent precipitation. CPI and P_{aq} do not show

320 any correspondence before ~1915, but from ~1920s to the present CPI and P_{aq} have an opposite trend.

321 The most important change in the *n*-alkane record is indicated by the maximum P_{aq} value (~0.48) and

322 minimum ACL values (~28.30) recorded at ~1963, agreeing with increase in the AP San Fernando record,

323 followed by a P_{aq} decrease and ACL increase after ~1970s (Fig. 2). This P_{aq} decrease is coeval with the

324 drop of $\delta^{13}C_{org}$ and increase of the C/N ratio of bulk sediment around 1978. Just after ~1988, the increase

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325 in CPI and ACL values, as well as the decrease in P_{aq} agrees with the MAAT Madrid increase and AP

326 San Fernando decrease which reflects a severe and long drought period from the late 1980s to mid-1990

327 (Fig. 2).

328   In the pollen assemblages, the Cyperaceae/Poaceae (C/P) ratio shows low values (0.17±0.04)

329 from 1820 to 1920s coincident with low values of P_{aq} and MAAT Madrid, together with high values of

330 AP San Fernando. Higher values (0.27±0.1) are recorded from ~1920s to the present, agreeing with

331 MAAT Madrid increase and AP San Fernando decrease. A similar trend is observed between C/P ratio

332 and P_{aq} (Pearson r=0.41, n=31). In addition, the appearance of the green alga *Pediastrum* from ca. 1950

333 onwards is noticeable (Fig 2).

334   Major changes in aquatic organism groups are observed for the last 60 years preceded by a period

335 of minor changes. A total number of 7 morphotypes of chironomids were identified. The taxa

336 *Micropsectra radialis*-type and *Psectrocladius sordidellus*-type are abundant throughout the sedimentary

337 intervals (Fig. 3). Based on cluster analysis, the most significant change is observed around 1950s and

338 consists in the new arrival of *Chironomus plumosus*-type, *Heterotrissocladius marcidus*-type and

339 *Micropsectra insignilobus*-type (Fig. 3). One major significant zone boundary in the chironomid record

340 ~1940-1950 was identified based on comparison with the broken-stick model, coincident with a major

341 change of PCA axis 1 sample scores for chironomids (which explain 51% of the variance). The

342 chironomid-based MJAT reconstruction suggests a trend of increasing MJAT from ~1940-1950s

343 onwards, presumably driven by increasing summer water temperature in RS from this period onwards

344 (Fig. 3 and 4, and Fig. S2 in Online Appendix 4).

345   Cladoceran PCA axis 1 sample scores identified the greatest change at ~1990, while a

346 significantly shift in diatom PCA axis 1 sample scores is observed from ~1960 onwards (Fig. 4). These

347 changes consisted in changes in species relative abundance but also in appearance and disappearance of

15

certain species. The taxon-specific cladoceran and diatom changes (not shown) started at the turn of the 20th century (~1920s), but became especially striking in the last five decades (Pérez-Martínez and others 2012; Jiménez and others 2018). The first PCA axis explained 38% (cladocera) and 26% (diatoms) of the variance of the biological assemblage data. Cladoceran and diatom PCA axis 1 sample scores are consistent with major changes observed for MAAT Madrid (increasing trend) and AP San Fernando (decreasing trend).

Overall, major changes for geochemical proxies started after the ~1920s consistent with the rise in temperature and preceded by a period of minor changes; however the main changes in biological proxies seem to be delayed, and their response intensified after the ~1940-1950s onwards, consistent with recent intensification of warming.

DISCUSSION

The combination of analysis of C/N ratio, $\delta^{13}C_{org}$ values and n-alkanes indices from the sediments from the RS Lake core provides an opportunity to identify the main sources of organic matter in the study area. The values of C/N ratio (9.5 to 19.7), $\delta^{13}C_{org}$ (-23.63 to -20.57 ‰) and CPI (~3.6 to 4.5) indicate the deposition of a mixed source of algal-derived and terrestrial organic matter for the entire record. This is expected for this system due to the small lake size (0.42 ha) and catchment area (9.9 ha), together with its partial coverage (~15%) by alpine meadows. It is worth noting that the mixed sources that led to the observed $\delta^{13}C_{org}$ values in RS Lake are also in accordance with a water-column study of this system (Pulido-Villena and others 2005), and with long-term and modern surveys of plants and lacustrine algae in other alpine lakes of the Sierra Nevada (García-Alix and others 2012; Jiménez-Moreno and others 2013).

Three factors suggest a predominance of wet environments in the catchment basin, in agreement with the previously discussed C/N ratio values. These include the low CPI values (<4.5), which show a

371 dominance of *n*-alkanes with odd carbon chains indicating vascular plant input (Bush and McInerney

372 2013) and algal contributions (Han and Calvin 1969) and the P_{aq} values, which exceed 0.28 pointing to

373 an emergent aquatic plant predominance (Ficken and others 2000) for the entire record. In RS Lake there

374 are no emergent aquatic plants, but this P_{aq} value likely indicates bryophyte and others semi-aquatic

375 vegetation, that formed the alpine meadows and surrounded the catchment (García-Alix and others 2017).

376 However, variations in the different proxies indicate differences in the degree of wet conditions in the RS

377 catchment basin over the study period (discussed below).

378 Related to biological proxies, the observed low taxonomic richness in chironomids is consistent

379 with an oligotrophic alpine lake, and is similar to conditions found in other alpine lakes (Heiri and others

380 2011). The chironomid assemblages were heavily dominated by the two taxa: *Psectrocladius sordidellus*-

381 type (~77%), which often dominates in alpine lakes (Heiri and Lotter 2010) and *Micropsectra radialis*

382 (~15%), which in small lakes is restricted to cold arctic and alpine habitats (Heiri and others 2011).

383 However, the arrival of chironomid groups and the most marked changes in chironomid community

384 composition are observed for the last 60 years, coincident with the main observed changes in cladoceran

385 and diatom assemblages. The similar timing in changes indicates a parallel response of the lacustrine

386 biota to the effects related to climate change. Most of the chironomid taxa are typical for cold, nutrient

387 poor and oxygen rich environments (Lotter and others 1998; Heiri and others 2011). However,

388 *Chironomus plumosus*-type is typically found in more nutrient rich, warmer and oxygen poor

389 environments (Lotter and others 1998; Heiri and others 2011). The appearance of this taxon agrees with

390 higher nutrient availability and higher oxygen depletion (e.g. in the sediments) in RS Lake from ~1960

391 onwards.

392 The general environmental trends deduced from RS Lake organic proxies follow the long-term

393 late Holocene changes described in the same site and in neighbor alpine wetlands (Anderson and others

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394   2011; Jiménez-Moreno and Anderson 2012; Jiménez-Moreno and others 2013; García-Alix and others

395   2017). The Period One, from 1820 to the 1920s, with colder and wetter conditions, registers the

396   environmental response to the warming after the LIA conditions, and Period Two, from 1920s to the

397   present, with drier and warmer conditions, records the response of RS Lake to the recent and ongoing

398   climate change. The identification of these two different environmental periods agrees with an abrupt

399   environmental change registered in other alpine peatlands in the region (Borreguil de la Virgen and

400   Borreguil de la Caldera) that occurred at ~1920s (García-Alix and others, 2017). Furthermore, high-

401   resolution cladoceran records from 6 lakes of the Sierra Nevada region, including RS Lake (Jiménez and

402   others 2018), indicate that the onset cladoceran changes also occurred at the turn of the 20th century and

403   intensified in the past 50 years. All these results indicate a regional-scale response to climate change.

404   Period One: *period between 1820 and ~1920s*

405   The paleolimnological changes observed in Period One may have been promoted by the seasonal

406   character of the Mediterranean precipitation, mainly concentrated during winter, as well as a longer cold

407   season in southern Iberia coeval with lower temperature than experienced during Period Two. Hence,

408   because precipitation occurs mainly during the cold winter season, the combined effects of relatively high

409   precipitation and decreasing air temperatures (Fig. S1; Online Appendix 2) during the Period One

410   probably led to later seasonal lake ice-off period, colder water temperatures and larger accumulation of

411   snow in the catchment basin, and as a consequence, the reduction of aquatic and/or terrestrial primary

412   production, as can be observed by the gradual decrease of LOI_{550} and sedimentary chlorophyll-*a* in

413   parallel with MAAT Madrid. However, despite the overall decrease of aquatic primary production during

414   Period One, the C/N ratio indicates a higher contribution of algae to the bulk organic matter than in the

415   most recent climatic period, which agrees with the homogeneous P_{aq} values (~0.3), which indicate there

416   is a predominance of emergent aquatic plants (Ficken and others 2000). This is probably the consequence

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417 of a reduced catchment surface and growing season for wetland plants (lower C/N values) and a reduced

418 water availability (lower P_{aq} values in Period One than in Period Two). This may have been caused by

419 delayed ice and snow melting in the catchment basin and higher snow accumulation by the precipitation

420 increase and the presumably low temperatures. Besides, any shortening of the ice-free period in the

421 catchment may also hinder input of terrestrial organic matter into the lake.

422 The highest ACL and lowest C/P ratio values of the entire record also indicate less water

423 availability (Fig. 2). The main difference in Period One between P_{aq} and ACL values is characterized by

424 a small increase (0.32) of P_{aq} in the decade of 1860, concomitant with the ACL decrease (average of

425 28.93) from 1850 to 1880, indicating a preponderance of *n*-alkanes with lower chain length. This response

426 of P_{aq} and ACL can be read as indicating relatively wetter environment compared to the rest of Period

427 One, likely induced by the combination of persistent high precipitation coeval with milder temperature.

428 This scenario (cold and wet conditions) presumably fostered the maintenance of glacial and other

429 perennial ice banks in the highest north-facing cirque of Sierra Nevada during the final periods of the

430 Little Ice Age (LIA) (Oliva and Gómez-Ortiz 2012), which began to disappear around the ~1920s

431 (Grunewald and Scheithauer 2010). Colder water conditions would explain the chironomid community

432 composition in Period One (Fig. 3), with a high abundance of cold-tolerant taxa such as *M. radialis*-type

433 and *P. sordidellus*-type, a very low diversity and the absence of warm-water chironomids. This is also

434 supported by the dominance of the cladoceran species *Chydorus sphaericus*, which has been mainly

435 associated with long ice cover period in the Sierra Nevada (Jiménez and others 2018). PCA axis 1 sample

436 scores of cladocerans, diatoms and chironomids show minor changes during this period (Fig. 4).

437 *Period Two: period from ~1920s to the present*

438 Warmer and drier climate conditions during Period Two produced substantial changes in

439 biological and organic geochemical proxies. The increasing values of the sedimentary chlorophyll-*a* and

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440   LOI$_{550}$ may indicate a progressive increase of aquatic and terrestrial primary production probably

441   associated with longer growing seasons and higher water temperatures with the onset of the 20th century

442   rise in air temperature (Fig. 2). An increase in aquatic primary production in remote lakes by warming

443   has been reported (Adrian and others 2009). Warming may cause a longer ice-free season which increases

444   light availability and mean water temperature, while also increasing water lake residence time through

445   reduced inflows and increasing evaporation but enhanced melting of snow and weathering (increasing

446   lake solute inputs; Preston and others 2016). These processes may enhance biological production in lakes,

447   and a longer growing season could also increase annual biomass accumulation (Fee and others 1992) in

448   lakes and catchment. This effect may have been enhanced by atmospheric deposition of Saharan dust at

449   these low-productive lakes. For example, the delivery of atmospheric P-rich Saharan dust during the last

450   50 years may partially explain the trends in the sedimentary chlorophyll-*a* record in RS Lake, a

451   phenomenon that has been demonstrated in Sierra Nevada lakes (Morales-Baquero and others 2006;

452   Jiménez and others 2018). Hence, it is likely that the combination of warmer temperatures, longer growing

453   seasons and increased delivery of P-laden dust has resulted in notable increases in chlorophyll-*a* in RS

454   Lake. These observations are consistent with the appearance of the green alga *Pediastrum* from ~1950

455   onwards and the chironomid *Chironomus plumosus*-type from ~1960 onwards, also agreeing with an

456   increase of primary production (Lotter and others 1998; Weckström and others 2010).

457   The previous findings are consistent with the higher C/N values from the entire period indicating

458   a higher contribution of vascular land plants to bulk organic matter. A longer warm season with increased

459   temperatures probably enhanced snow and ice melting in the catchment basin and, as a consequence, the

460   catchment surface and growing season for wetland plants. This is supported by the increasing values of

461   P$_{aq}$ after ~1921, reaching 0.38 at ~1928 simultaneous to the decreasing values of ACL. The maximum P$_{aq}$

462   value (~0.48) and minimum ACL values (~28.30) are recorded by ~1963, agreeing with recorded periods

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463 of elevated precipitation (Fig. 2). Unlike conditions with higher precipitation and colder temperatures

464 around ~1850 and the 1880s, climate during the 1960s shows high precipitation with higher temperatures,

465 thereby enhancing the melting season and providing more net water availability. This combination of a

466 longer growing season and greater water availability triggered the development of larger wetland areas,

467 as shown by higher C/P ratio values. This is in concordance with Pérez-Palazón and others (2015)

468 indicating a decreasing extent and persistence of the ice and snow covered area over the Sierra Nevada

469 from the 1960s onwards.

470   Warmer conditions are likely responsible for the transition in cladocera, diatoms and chironomid

471 assemblages from the 1940-1950s onwards, following the shifts in P_{aq} and ACL values after the 1920s.

472 Assemblage shifts as a consequence of the rise of temperature in the first part of the 20th century have

473 been observed in many others remote areas (Sorvari and others 2002; Rühland and others 2015). Over

474 the last ~60 years the most notable changes in lacustrine biota are shown by the trend of PCA axis 1

475 scores coincident with major shift in MAAT Madrid and AP San Fernando (Fig. 4). Lacustrine biota

476 apparently exhibited a delayed response to changes in air temperatures and precipitation, and significant

477 responses to climate change are observed when the climatic shift intensified for the last ~60 years.

478   Temperature is particularly important in determining shifts in chironomid assemblage

479 composition (Heiri and others 2003; Bigler and others 2006). Cluster and PCA analyses indicate that the

480 most relevant changes were characterized by the reduction of cold-tolerant taxa *P. sordidellus*-type and

481 *M. radialis*-type and the increase of taxa better adapted to warmer condition such as *C. plumosus*-type,

482 *H. marcidus*-type, *M. insignilobus*-type in the uppermost section of the sediment core. The taxon *C.*

483 *plumosus*-type includes a number of species, and is generally considered to be thermophilic and indicative

484 of relatively warm lakes (Brooks and Heiri 2013), although it can also occur in lakes in the subalpine

485 vegetation belt at low abundances (Heiri and Lotter 2010). Hence, the new arrival and increase of *C.*

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486 *plumosus*-type in RS Lake is probably, at least partially, related to water temperature rise and possibly

487 promoted by the increase in in-lake nutrient availability discussed above. This represents the first

488 occurrence of the genus *Chironomus* in alpine lakes of Sierra Nevada (Laville and Vílchez-Quero 1986;

489 Real and others 2000), yet it does occur in subalpine lakes of Central Europe (Heiri and Lotter 2010).

490 Similarly, *M. insignilobus*-type has its maximum abundances at lower altitudes in the Alps (Bigler and

491 others 2006). Overall, the timing of appearance and major contribution to change of these two taxa in RS

492 Lake suggest warmer summer water temperature, which is also reflected in a warming of chironomid-

493 inferred mean July air temperatures by about 2 ºC from ~1950s onwards. This warming in climatic

494 conditions is consistent with changes in cladoceran and diatom community composition at RS Lake

495 (Pérez-Martínez and others 2012; Jiménez and others 2018). The similar timing and direction of changes

496 in chironomid community composition as observed for cladoceran and diatom assemblages, coincident

497 with changes in other Sierra Nevada lakes (Jiménez and others 2018), corroborate the hypothesis of

498 climate-driven shifts in the ecological status of distinct trophic levels in these alpine lakes. These changes

499 are also in good agreement with changes in aquatic community structure in others remote ecosystems

500 (Rühland and others 2014), coinciding with recent warming.

501 Maximum values of C/N ratio and decreasing $\delta^{13}C_{org}$ values from mid-1970s to the end of the 80s

502 are interpreted as a major vascular land plant contribution to bulk organic matter. There was a decrease

503 in this time in the P_{aq} values (simultaneous with ACL values increase), and therefore apparently a decrease

504 in the water availability in the catchment, agreeing with unprecedented high temperatures and a

505 precipitation decrease. This suggests lesser water availability induced by greater evaporation rates,

506 enhanced by higher frequency of intense summer droughts as a consequence of intensified warming in

507 the Mediterranean area (Giorgi 2006). Even though this warming promoted a longer growing season and

508 increased lake primary production (more *Pediastrum*, higher sedimentary chlorophyll-*a* and LOI_{550}), the

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509   C/N ratio suggests a higher vascular land plant contribution to the bulk organic matter.

510   After ~1988, CPI and ACL values sharply increase and P_{aq} decreases, which agrees with the

511   extreme droughts of the early 1990s in the Mediterranean region, and is in concordance with the

512   temperature rise and precipitation decrease. It suggest less water availability in the catchment likely

513   affecting those meadow plant species with highest water requirements. These observations, together with

514   the decrease of the C/N ratio values, point towards a relative decrease in terrestrial vegetation production

515   in the catchment, probably related to the regional drought. By contrast, these conditions of increasing

516   temperatures and less water availability in the catchment likely enhanced biological production in the

517   lake by different processes such as longer ice-free season, higher mean water temperature and higher

518   solute concentrations. Cladocera results strongly support this findings considering that the main shift

519   within the sedimentary cladoceran assemblages occurred at the 90s in RS Lake (Jiménez and others 2018)

520   and is coincident with the severe periods of drought during the late-1980s and 1990s in Southern Spain

521   (Udelhoven and others 2009).

522   This short-core multiproxy study provides a valuable high-resolution record of

523   paleoenvironmental and paleolimnological change for the last ~180 years. The climate-driven changes

524   from Period One to Period Two – shorter duration of ice-cover period, higher summer water temperature

525   and greater water availability for catchment plant growth – are responsible for the primary changes in

526   lake and catchment history between both distinguished periods. From ~1970 forward the steep rising in

527   temperature and decrease in precipitation likely lead to a drier ambient in the catchment, with less water

528   availability for plant growth and increasing drought as the summer advances. Pauli and others (2012)

529   evidence that flora species richness has declined on the southern European summits (including Sierra

530   Nevada) within the 2000s but increased in European boreal-temperate mountain regions. Differences are

531   attributed to the decrease of the availability of water in the European south. In this study, we show a

532 tendency to increased aridity in the Sierra Nevada, starting at the turn of the 20th century and intensified

533 from the 70s onward. Consequences for the vegetation are serious in terms of species loss considering

534 that the flora of Sierra Nevada summits comprises a sizable percentage of endemic species in Europe,

535 implying therefore an important loss of endemic European species.

536   Consequences of the changes observed in climate for lakes are also pronounced. We show an

537 increase in chlorophyll-*a* and changes in biota assemblages from the mid-20th century onward, mainly

538 governed by different processes as longer growing season, increasing water temperature and reduced

539 water level because of higher evaporation rates and reduced water inflow. All these processes, and the

540 additional P enrichment due to Saharan dust affecting this region, may lead to further trophic state changes

541 of the Sierra Nevada lakes. An increase in algal biomass and the appearance and disappearance of lake

542 species signify deep changes in the ecosystem functioning as both primary producer biomass and lake

543 trophic web are major components of the ecosystem structure. The similar timing and direction of changes

544 in chironomid community composition as observed for cladoceran and diatom assemblages, coincident

545 with changes in other Sierra Nevada lakes (Jiménez and others 2018), corroborate the hypothesis of

546 climate-driven shifts in the ecological status of distinct trophic levels in these alpine lakes. Hence, it is

547 likely the ecological thresholds for biotic communities were crossed after the intensification of

548 temperature and precipitation changes since the last decades.

549   If, as predicted by climate models, the rising of temperature and decrease in rainfall continue in

550 the Sierra Nevada region and drought processes observed in this study intensify, physical and biological

551 transformations can be expected in the catchment ecosystem of RS Lake and in other glacial valleys of

552 Sierra Nevada, even modifying the Sierra Nevada summits' image of glacier valleys with clear water

553 lakes and green alpine meadows. The impact of this climate change on the summits of Sierra Nevada and

554 its influence transcends its geographical limits because these systems provide ecosystem services as

555 important as being the largest source of water for the population living in the lowlands, for agricultural

556 uses, for the generation of hydroelectric power, habitat for the species (many of them endemic),

557 ecotourism, and the aesthetic value and source of scientific knowledge (Palomo and others 2013). The

558 beneficiaries of water resources are primarily the inhabitants of the large cities near Sierra Nevada

559 (Granada and Almería) and many other smaller towns since this is a very populated area. Moreover, South

560 East of Spain is a preeminently agricultural and tourist area and the numerous rivers whose sources are

561 in Sierra Nevada supply water for these activities. For millennia, humans have inhabited the Sierra

562 Nevada environment and have benefited from these services, however the magnitude of human pressures,

563 including climate change, could exceed the resilience of these ecosystems.

564   This is the first study at short-time scale (180 years) to use multiples proxies to provide an

565 integrated view of how this and similar alpine ecosystems are responding to climate change. Because so

566 little is known concerning the effects of recent warming on these alpine ecosystems, further investigations

567 on similar lakes in the region are needed to provide a more comprehensive understanding of the effect of

568 climate change on these vulnerable ecosystems and their surroundings.

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766   **FIGURES**

767   **Figure 1**. Geographical location of the study site. A) Inset map: Contour map of Iberian Peninsula

768   showing the location of the study area; Contour of Sierra Nevada National Park indicating the study area;

769   B) map of the Sierra Nevada mountain range showing locations of Río Seco (RS) Lake (circle) and highest

770   mountain peaks (white triangles); C) RS Lake bathymetry (digitized map of bathymetry report from

771   Egmasa S.A.); D) photo of RS Lake (August 2012).

772   **Figure 2**. Comparison of the downcore sedimentary proxies. Profiles of organic matter content (LOI_{550}),

773   sedimentary chlorophyll *a* (Chl a) (mg g^{-1} DW); $\delta^{13}C_{org}$ (V-PDB), atomic C/N ratio, biomarkers (CPI, P_{aq}

774   and ACL) and pollen data (C/P ratio and *Pediastrum* %) from 1820 to 2008 A.D. Two distinct climatic

775   period are defined based on the climate data: Period One (from 1820 to ~1920s) and Period Two (from

776   ~1920s to the present). Stratigraphically constrained cluster analyses using biological and geochemical

777   proxies is also shown. The mean annual air temperature anomaly from Madrid climate station (MAAT

778   Madrid) and annual precipitation anomaly from San Fernando climate station (AP San Fernando) is

779   shown since 1860 and 1840, respectively. Temperature anomalies are related to the period 1961-1990

780   and precipitation anomalies are related to the whole period. A LOESS smoother (span = 0.2) was applied

781   to all the variables (bold line). Applying a two-segment, piecewise linear regression to the MAAT Madrid

782   series identified a threshold change to higher mean temperatures in the early 1970s (breakpoint= 1972 ±

783   4.7, $p < 0.0001$), while a potential additional breakpoint, not considered statistically significant, is also

784   identified in the time interval of 1912-1915. For precipitation data, no significant breakpoint was

785   identified.

786   **Figure 3**. Chironomid remains in the sediment core from RS Lake, together with a cluster analysis of

787   assemblage data using Constrained Incremental Sum of Squares (CONISS). Light grey silhouettes show

788   ×10 exaggeration. The horizontal grey-shaded area represent the period post-1820 A.D.

789   **Figure 4.** Comparison of Cladocera, diatom and chironomid PCA axis 1 sample scores for RS Lake

790   sediment core, together with chironomid-inferred mean July air temperatures based on the chironomid

791   records (see text for details). The error bar lines indicate the sample-specific estimated standard error of

792   prediction**.** Note the inverted scales in the axis scores for Cladocera and chironomids.
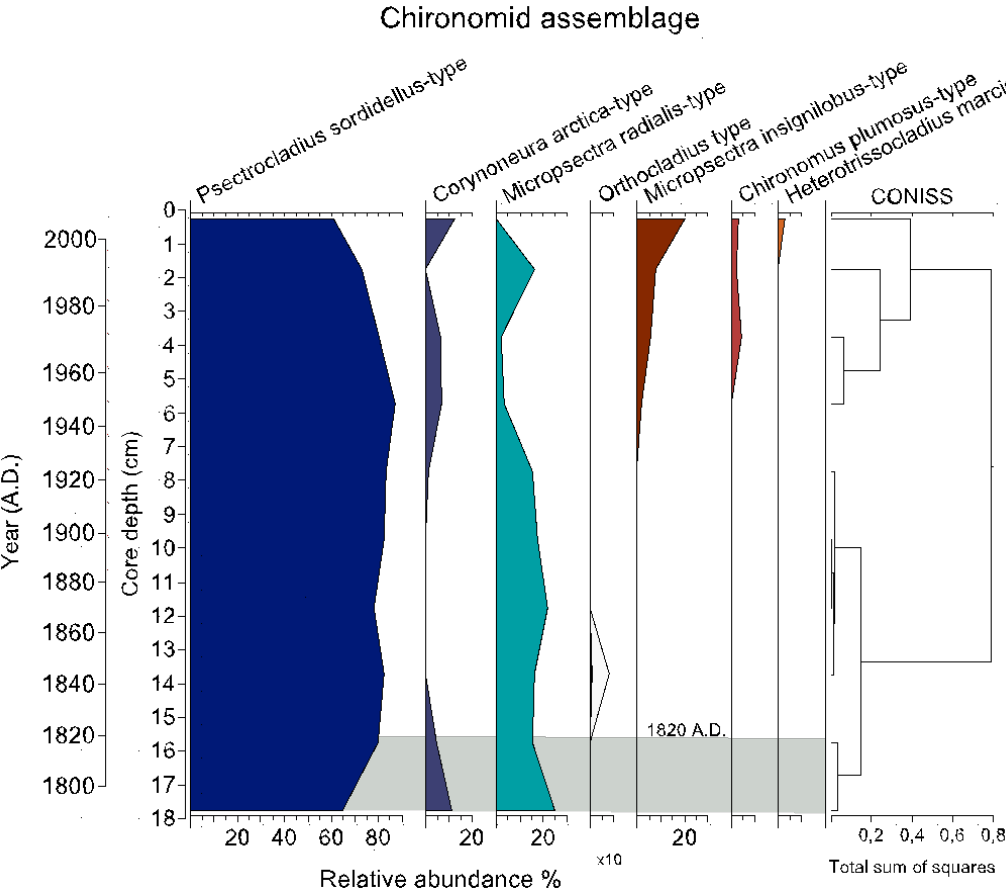
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796   **Fig 1.**

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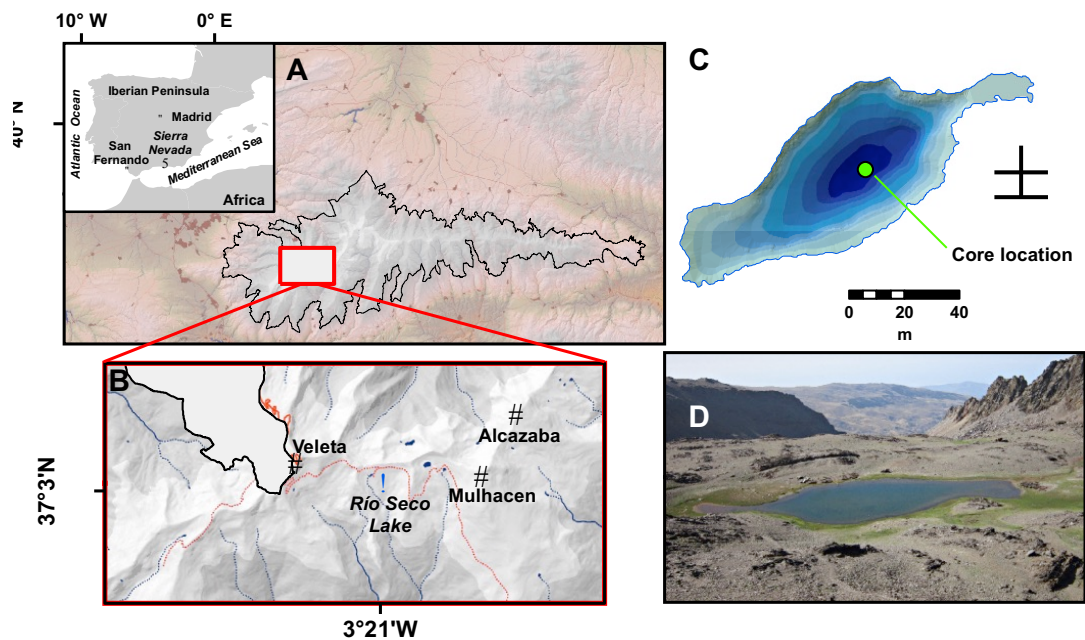
800   **Fig 2.**

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802   **Fig 3**.

Chironomid assemblage
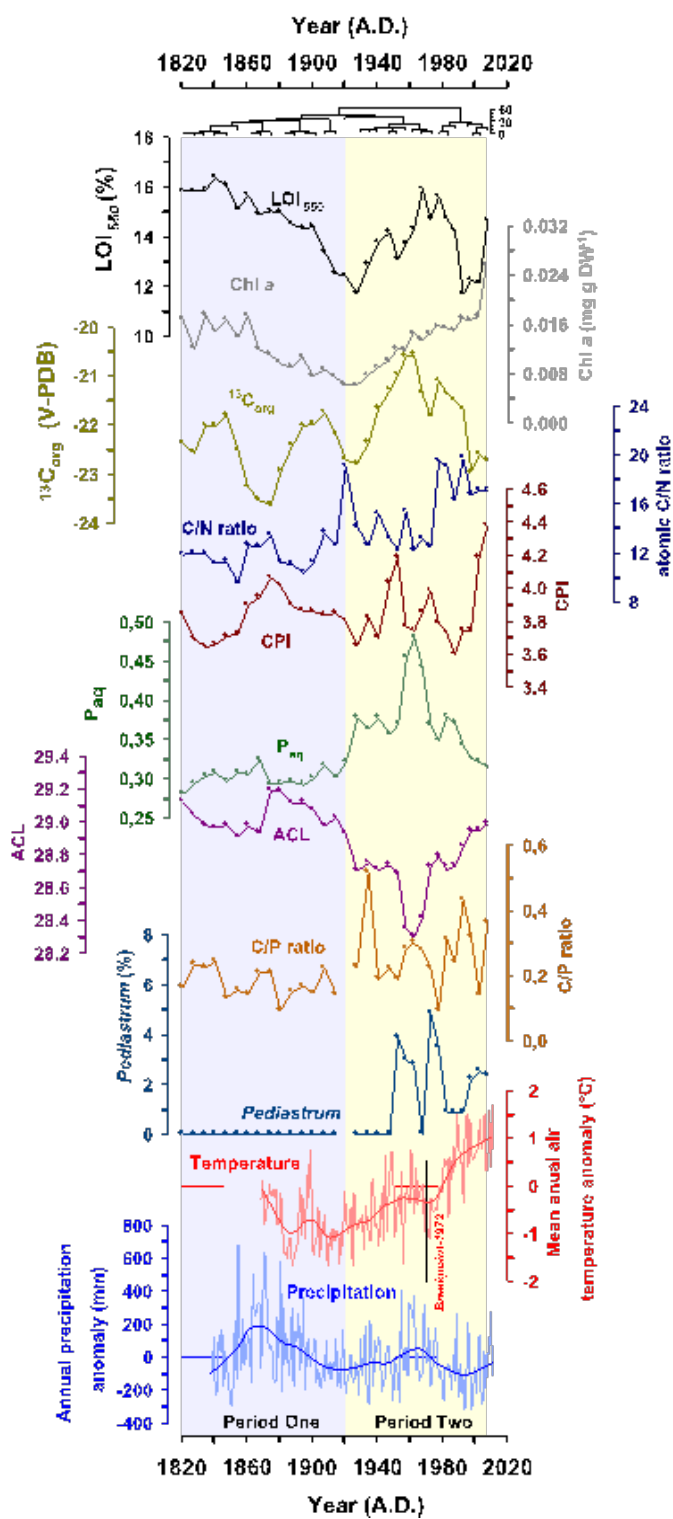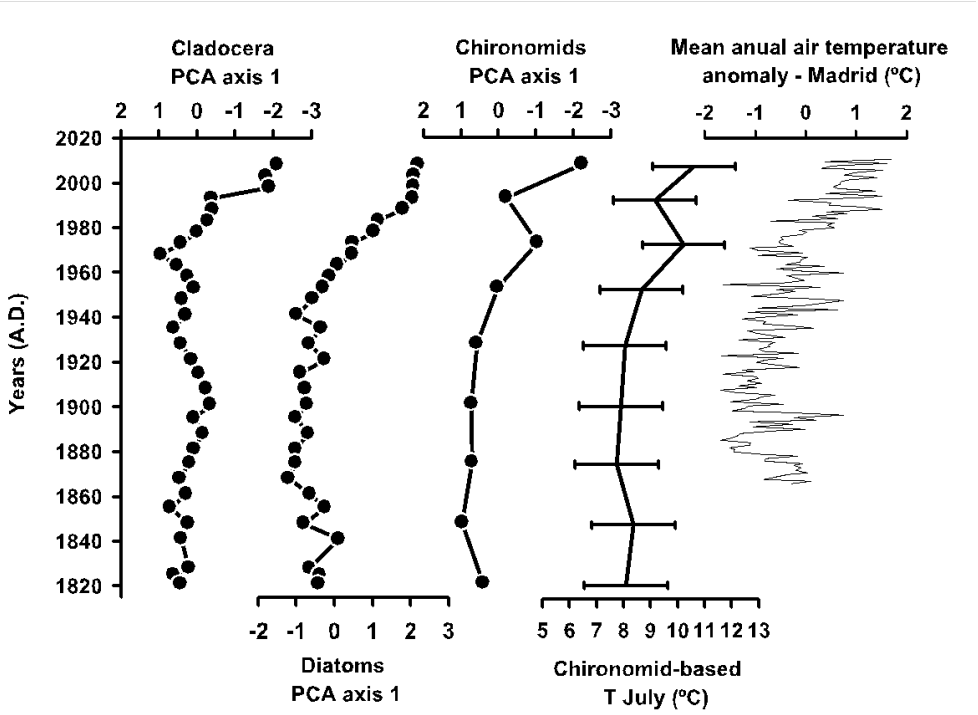
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810   **Fig 4.**

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Supplementary information

Appendix 1. Basic geographical and limnological characteristics of the study lake

	RS Lake
Latitude	37º03'07.63''N
Longitude	3º20'43.92''W
Altitude (m asl)	3020
Lake area (ha)[a]	0.42
Catchment area (ha)[a]	9.9
Maximum depth (m)	2.9
Maximum volume (m^3)[b]	4772
Catchment area/surface area[a]	21.5
pH	6.0-7.6 (6.9)
Conductivity (μS cm^{-1})	10-77 (24)
Alkalinity (meq L^{-1})	0.05-0.16 (0.11)
TP (μg L^{-1})	7-27 (16)
TN (μg L^{-1})	99-732 (403)
Chl a (μg L^{-1})	0.3-1.1 (0.6)
Phytoplankton biomass (μgC L^{-1})	20
DOC (mg L^{-1})	0.7-2.7 (1.8)
Calcium (mg L^{-1})	0.5-2.1 (1.2)

[a] Data from Morales-Baquero and others (1999).
[b] Data from Egmasa S.A.

Supplementary Table S1. Data are derived from a monitoring study over 10 years. Range and mean values (in brackets). Abbreviations: TP, Total phosphorus; TN, Total nitrogen; Chl *a*, Chlorophyll- *a;* DOC, Dissolved Organic Carbon.

Appendix 2. Instrumental climate data for Sierra Nevada summits

Long-term temperature and precipitation records do not exist for Sierra Nevada summits. One of the longest temperature series in the Sierra Nevada is provided by Cerecillo Station (Láujar, Almería), which is located at 1800 masl and provides data since 1960, although there are numerous gaps. A strong correlation was found between the Cerecillo temperature series and short series of homogenized mean annual temperature records dating back to 1960 and available from meteorological stations (Armilla and

Lanjarón, http://www.aemet.es) located at lower altitudes and less than 20 km from the Sierra Nevada summits (Armilla r= 0.67, $p < 0.001$, n= 35; Lanjarón r= 0.78, $p < 0.001$, n= 35). A record of precipitation (dating back to 1940) from Armilla is also available. A comparison between these nearby short series of temperature data with several longer homogenized air temperature records from Central and South Spain (Staudt and others 2007), and precipitation records of five long series from southern Spain (Esteban-Parra and others, 1998), indicated that the strongest correlations were with the Madrid climate station (AEMET 3195 since 1864, 664 masl) (Armilla r = 0.72, $p < 0.001$, n = 51; and Lanjarón r = 0.78, $p < 0.001$, n = 54) for temperature, and with San Fernando series (Naval Base of the Spanish Army, Cádiz, since 1839, 28 masl; r = 0.62, $p < 0.001$, n= 65) for precipitation.
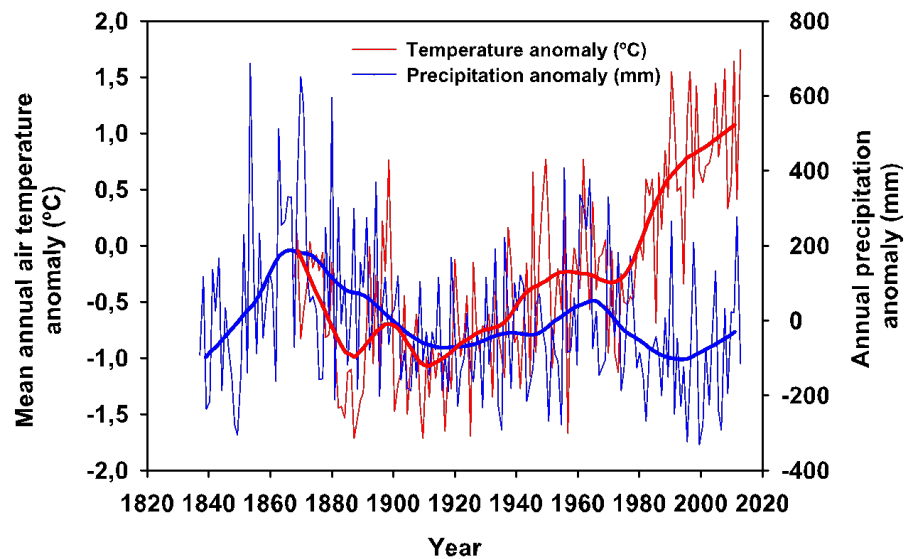
Supplementary Figure S1. The mean annual air temperature anomaly from Madrid climate station (MAAT Madrid) and annual precipitation anomaly from San Fernando climate station (AP San Fernando) since 1860 and 1840, respectively. Temperature anomalies are related to the period 1961-1990 and precipitation anomalies are related to the whole period. A LOESS smoother (span = 0.2) was applied to all the variables to improve the clarity of the figure and highlight trends.

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Appendix 3. Interpretation of selected sedimentary proxies in RS Lake

Among the proxies used in this study, subfossil assemblages of biota communities (chironomids, cladocera and diatoms) are frequently used as indicators of climate-related changes in lakes since aquatic organisms inhabiting lakes respond to climate changes by altering their community structures (Smol 2008). Chironomid assemblage changes are reliable temperature indicators because many chironomid taxa are very sensitive to water temperature changes (Brooks 2006). Chironomids are also used as indicators of lake nutrient concentrations and oxygen availability in lakes (Lotter and others 1998; Heiri and others 2011). Cladocera inhabit a wide range of habitats in mountain lakes and its taxa are sensitive indicators of lake water temperatures (Korhola 1999; Fischer and others 2011) or growth period duration (Catalan and others 2009; Jiménez and others 2018), among others. Diatom is a usually dominant algal group in lakes, occurring across a broad range of limnological conditions. For example, diatom assemblages respond to changes in water column stability (Sorvari and others 2002), to changes associated with decreases in ice cover duration (Douglas and Smol 1999; Lotter and others 1999) or to the habitat availability as many diatom taxa show habitat specificity (Rühland and others 2015). The biota assemblages analyzed may reinforce each other if provide evidences of climate-related changes in the same direction and timing.

Sedimentary chlorophyll-*a* concentration is used to track past trends in aquatic and terrestrial primary production (Leavitt and Hodgson 2001; Hundey and others 2014; Michelutti and Smol 2016). In mountain lakes, warming may cause a longer ice-free season which increases light availability and mean water temperature, while also increasing water lake residence time through reduced inflows and increasing evaporation but enhanced melting of snow and weathering (increasing lake solute inputs; Preston and others 2016; Sommaruga-Wögrath and others 1997). These processes may enhance biological

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production in lakes, and a longer growing season could also increase annual biomass accumulation (Fee and others 1992) in lakes and catchment. LOI_{550} values were used as an estimate of organic matter content for each sediment interval (Heiri and others 2001). LOI_{550} has been widely used as indicative of primary production (Dean 1974; Battarbee and others 2001; Smol 2008), but can also be influenced by changes in catchment erosion (Battarbee and others 2002). Hence, sedimentary chlorophyll-*a* and LOI_{550} values have been used to infer changes in both aquatic and terrestrial primary production in the present study.

The atomic C/N ratio is used as an indicator of organic matter source in lacustrine sediments (Meyers and Ishiwatari 1993; Kaushal and Binford 1999). Organic matter from terrestrial vascular land plants (cellulose-rich and protein-poor) is usually characterized by C/N values higher than 20, while algal-derived OM (cellulose-poor and protein-rich) typically features values between 4-10. Intermediate values (C/N rates between 10 and 20) are typical for sediments influenced by both sources (Meyers and Teranes 2001).

The $\delta^{13}C_{org}$ values of sediment cores indicate past changes in productivity levels in lacustrine environments (Schelske and Hodell 1991, 1995), with higher $\delta^{13}C$ of organic matter resulting from increased aquatic productivity (Hodell and Schelske 1998). Lacustrine algae preferentially take up the light carbon isotope (^{12}C) from the water's dissolved inorganic carbon (DIC) pool during photosynthesis. However, with enhanced primary production, this discrimination leads to an increase in the heavier isotope in the water DIC pools. Consequently, the algae that use ^{13}C are enriched in the heavier isotope (O'Leary 1988; Wolfe and others 2001) and show, therefore, higher values of $\delta^{13}C$. Nevertheless, the carbon isotopic composition of algal-derived organic matter is similar from that of organic matter from C_3 vascular plants (Meyers and Teranes 2001), where heavier isotopic compositions are usually related to a decrease in the water-use efficiency under dry conditions (Farquhar and others 1982). Hence, the additional use of the atomic C/N ratio to support the interpretation of $\delta^{13}C$ values allows better

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discrimination of organic matter sources in systems where both algal and vascular plants organic matter are present (Meyers 1994). Thus, C/N ratio adds new information to the previous proxies, chlorophyll-*a* and LOI_{550}, and also helps to interpret the organic carbon isotopic data.

The *n*-alkanes indices permit to investigate the water availability for the catchment vegetation and, combined with the above proxies, to comprehend the causes of the observed changes in primary production. The main source of the *n*-alkanes in the sediment are the epicuticular leaf waxes of plants that protect the water balance of leaves, reduce the mechanical damage to leaf cells, and prevent fungal and insect attack (Eglinton and Hamilton 1967; Post-Beittenmiller 1996). *n*-alkane carbon chains from leaf waxes usually range from 21 to 37 C atoms, with a predominance of odd carbon numbers. Long chain *n*-alkanes (higher than C_{27}) are usually found in vascular terrestrial plants (Eglinton and Hamilton 1967). Aquatic and semiaquatic plants, such as macrophytes, usually maximise the C_{21}, C_{23}, and C_{25} *n*-alkanes (Cranwell 1984; Ficken and others 2000). Shorter *n*-alkanes, such as C_{15}, C_{17}, and C_{19} as well as low odd/even carbon ratios are usually related to autochthonous aquatic organic matter, originating from organisms such as algae and bacteria (Cranwell 1982; 1984). Several *n*-alkane indices have been calculated to summarize their distributions. The Average Chain Length (ACL) is a measurement of the weighted average of the carbon chain lengths. The portion aquatic (P_{aq}) is the ratio between typical aquatic plant *n*-alkanes (C_{23} and C_{25}) and terrestrial plant waxes (C_{29} and C_{31}) (Ficken and others 2000). For modern plants, terrestrial plants < P_{aq} 0.23 > emergent aquatic plants < P_{aq} 0.48 > floating/submerged plants (Ficken and others 2000). Although other *n*-alkane indices can also provide information on the aquatic or terrestrial source of organic matter (e.g. the ratio of short odd *n*-alkanes vs. long odd *n*-alkanes; C_{17-25}/C_{29-35}), the present study focuses on P_{aq} to simplify the discussion. However, P_{aq} shows a very similar trend as the C_{17-25}/C_{29-35} ratio in the Río Seco *n*-alkane record (Pearson r= -0.96; n=32).

The Carbon Preference Index (CPI) represents the relative abundance of odd vs. even carbon

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chain lengths (see Bush and McInerney 2013 for a review). Values lower than 2 suggest an even *n*-alkane preference (indicating diagenetic alteration or algal/bacterial influence), and values higher than 2 point towards an odd preference (indicating plant sources, and thermal immaturity). *n*-alkane distributions usually vary depending on the environmental conditions, such as water availability, precipitation or temperature (Ficken and others 2000; Schefuß and others 2003). However, since *n*-alkanes can be also affected by regional conditions, we will follow a regional interpretation for *n*-alkanes based on a *n*-alkane plant survey in high elevation wetlands in the Sierra Nevada (García-Alix and others 2017). This pointed out that there is a predominance of the short carbon chains distribution in and near water pools, and a predominance of longer *n*-alkane chains furthest away from such pools. Therefore, lower ACL values are usually recorded in areas closer to the water pools, where the P_{aq} values are usually high, pointing towards higher water availability. The same data showed that plants in environments with high water availability usually recorded low CPI values (6.5±2.5), while plants from fully terrestrial environments in Sierra Nevada, with lower water availability, usually recorded higher CPI values (higher than 13.6±8.6).

The Cyperaceae/Poaceae (C/P) pollen ratio is used in this study as a sedimentary proxy of changes in climatic conditions (Turney and others 2004) and so reinforce the information obtained from the above proxies. High C/P ratio usually indicates wetter conditions, whilst low C/P ratios represent drier environments (Jiménez-Moreno and others 2008; Ramos-Román and others 2016).

Appendix 4. **Comparison of RS chironomid assemblages to mountain lakes in the Swiss Alps relative to summer temperature**

As a supporting analysis, the modern distribution of chironomid assemblages in lakes in the Swiss Alps (Heiri and Lotter 2010) was used to assess the trajectory RS chironomid assemblages relative to

6

summer temperature. For this purpose fossil samples were plotted passively in a Detrended Canonical Correspondence Analysis (DCCA) with mean July air temperature as the sole constraining variable. Variations of site scores of RS assemblages towards high or low DCCA axis values 1 represent changes in assemblage composition towards assemblages typical for cold or warm mountain lakes in the Swiss Alps, respectively. All fossil RS chironomid samples had a close analogue in the chironomid assemblage dataset from the Swiss Alps if assessed following Birks and others (2010), with both chi-square and squared chord distance metrics to the closest analogue lower than the 1st percentile of all distances calculated between chironomid samples in the Alpine dataset. Chironomid assemblage percentage data were square root transformed before calculation of DCCA. Results indicated that before ~1940-1950 DCCA axis 1 values represent assemblages presently found in small Swiss lakes in the alpine zone with temperatures mostly below 10°C. Between ~1940-1970 DCCA axis 1 scores change to values more typical for Swiss lakes in the upper subalpine zone with July air temperatures in the 10-12°C range.
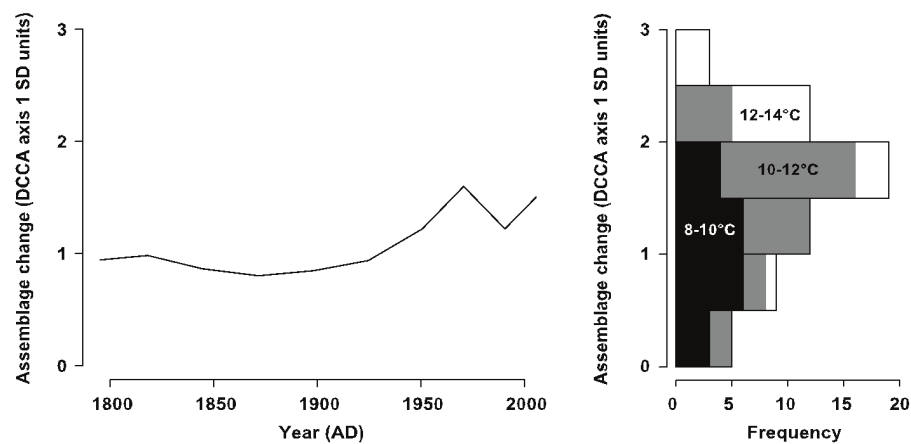

Supplementary Figure S2. Axis 1 scores of the RS chironomid samples added passively to a Detrended Canonical Correspondence Analysis (DCCA) of chironomid surface sediment samples from 117 sites in the Alps with July air temperature as only constraining variable. For comparison the distribution of DCCA

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axis 1 scores of the surface sediment samples for three groups of lakes with mean July air temperatures in the range of 8-10, 10-12 and 12-14°C are shown, which represents the transition of chironomid assemblages in lakes across the treeline elevation.

Appendix 5. Chronology of RS Lake

The ^{210}Pb activity profile shows a typical exponential decline towards the deepest part of the core. Based on the sedimentation rate, the ^{210}Pb dated sediment core indicates 187 years of accumulation and each interval of the sediment core represents approximately 5 and 7 years of accumulation from 0 to 6 cm and from 6 to 15.5 cm depth, respectively. A significant peak in ^{137}Cs activity profile is observed between 4 and 4.5 cm of sediment samples, recording the 1963 fallout maximum from atmospheric nuclear weapons testing.
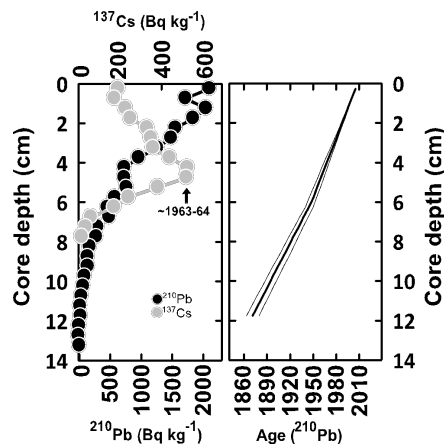
Supplementary Figure S3. Radiometric chronology showing ^{210}Pb (black circle) and ^{137}Cs (grey circle) activity (Bq Kg^{-1} dried sediment). On the right, A. D. year for RS Lake sediment core; continuous lines represent the dating errors (1 SD in sediment age) associated with each dated interval (Jiménez and others, 2018).

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