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Ilaria Fracasso[1,2#], Anna Dinella[1#], Francesco Giammarchi[1], Nadia Marinchel[3], Piotr Kołaczek[4], Mariusz Lamentowicz[4], Katarzyna Marcisz[4], Edyta Łokas[5], Magdalena Miecznik[5], Luca Bragazza[6], Matteo Girardi[7], Maurizio Ventura[1], Luigimaria Borruso[1], Giustino Tonon[1] and Cristiano Vernesi[2].

[1] Faculty of Science and Technology, Free University of Bolzano-Bozen, 39100 Bolzano, Italy

[2] Forest Ecology Unit, Research and Innovation Centre- Fondazione Edmund Mach, via E. Mach 1-S. Michele all'Adige (Trento), 38010 Italy

[3] Department of Pure and Applied Sciences, University of Urbino, Campus E. Mattei, 61029 Urbino, Italy

[4] Climate Change Ecology Research Unit, Faculty of Geographical and Geological Sciences, Adam Mickiewicz University, Poznań, Poland

[5] Institute of Nuclear Physics, Polish Academy of Sciences, Eliasza Radzikowskiego 152, 31-342 Kraków, Poland

[6] Agroscope, Field-Crop Systems and Plant Nutrition, Research Division Plant Production Systems, Route de Duillier 50, CH-1260 Nyon, Switzerland

[7]Conservation genetic unit, Research and Innovation Centre- Fondazione Edmund Mach, via E. Mach 1-S. Michele all'Adige (Trento), 38010 Italy

[#]**These authors contributed equally to the manuscript**

1. INTRODUCTION

Over the last decades, human activities are, directly or indirectly, driving severe environmental changes at both global and local scales (Allamano et al., 2009; Brunetti et al., 2004; Riberio et al., 2021; Simolo et al., 2010). Global warming is one of the major drivers, causing rapid biotic changes, especially in arctic and mountain regions where temperature increase is as much as twice that of the global rate (Brunetti et al., 2009). Upward migrations of alpine and nival species (Grabherr et al., 1994), changes in communities composition, and local extinctions are just a few examples of global warming impact (Lamprecht et al., 2018; Pauli et al., 2012, 2014; Rogora et al., 2018). Global warming effects are particularly deleterious for ecosystems like peatlands, that rely on a surplus of water to act as sinks of atmospheric carbon (Riberio et al., 2021). In addition, a change of species abundance and composition in peatlands, in response to climate warming, has been shown to affect the rate of peat accumulation and decomposition, leading to an increase in the wooded area of the peatland (Dinella et al., 2021; Heijmans et al., 2008).

Investigating the inter-play between climatic, environmental, and biotic changes is of fundamental importance for determining the future of an ecosystem and activating the most appropriate management policies and practices (Li et al., 2020). In this respect, the reconstruction of historical environmental changes could represent an important source of information (Andrews et al., 2022; Railsback et al., 2020). To this purpose, sediment depositions represent one of the most interesting matrices to study historical climatic and environmental alterations and their driving factors (Kaal et al., 2020; Stančikaitė et al., 2019; Zhang et al., 2020).

Ombrotrophic bogs, i.e. peatlands dominated by peat mosses (*Sphagnum* species), are valuable climatic and biological archives. These bogs are exclusively fed by external inputs (aerosol, rain, etc.), thus providing a signal primarily related to the atmosphere and, therefore, to climate

variability (Damman, 1986; Rydin and Jeglum, 2006). Moreover, the anoxic conditions are fundamental for preserving organic matter: the absence of oxygen in the deepest layers reduces degradation processes. Acid-resistant organic materials such as keratinized residues of vertebrates and invertebrates, pollen, plant residues and plant macro-fossils, and testate amoebae can be therefore analyzed to establish the presence of specific taxa (Gałka et al., 2015, 2018; Kajukało et al., 2016; Lamentowicz et al., 2015; Poto et al., 2013) and assess their function in the ecosystem (Birks, 2020; Marcisz et al., 2020). Moreover, the accumulation of organic matter allows measuring peat (= organic carbon) accumulation, a function of the rate of vegetation decay (Lindsay, 1995). Variations in peatland hydrology determine changes in the rate of peat accumulation/degradation (Cristea et al., 2014; Drollinger et al., 2020), affecting the survival of living organisms such as testate amoebae (Lamentowicz et al., 2008; Mitchell et al., 2008a) and peatland trees growing on the surface (Dinella et al., 2019).

Therefore, by combining information on hydrology and physicochemical characteristics of peatlands with the occurrence of specific taxa, it is possible to reconstruct a sufficiently long time series of climatic and biological information to assess changes in abiotic and biotic ecosystem components. Indeed, several studies confirmed the potential of this multi-proxy approach to get an overview of peatlands dynamics and past environmental changes (Lamentowicz et al., 2010; van der Knaap et al., 2011).

The main targets of taxonomic identification of peatland-derived organisms are plants and testate amoebae. The former, usually identified by pollen, represent key organisms in different ecosystems and can be used as bioindicators within the studied peatland and its surroundings habitat (Rolli et al., 2015; Smith, 1994; Zurayk et al., 2001). How testate amoebae communities are assembled is linked to peatland hydrology and this information can be accordingly used as

indicators of the degree of aridity/humidity of the ecosystem in response to local climate (Woodland et al., 1998; Mitchell et al., 2008b). In addition, both sub-fossil and living trees on peatlands can be used as proxies of past water table variations based on their tree-ring records (Edvardsson et al., 2016; Dinella et al. 2019).

The most common technique for taxa identification is the morphological observation of biological residues. The success of these morphology-based techniques has been reported in many studies (Gałka et al., 2018; Lamentowicz et al., 2015; Parducci et al., 2015). However, it presents some limitations: i) it is highly dependent on the taxonomic expertise, ii) it is time-consuming; iii) especially for pollen, it is not so uncommon that the same morphological features are shared across different species.

The environmental DNA (eDNA) metabarcoding offers a solution to overcome these limitations (Thomsen et al., 2015). This approach has been widely applied for monitoring modern biological communities in water and soil (Edwards et al., 2018; Mayer et al., 2021; Rota et al., 2020; Sakata et al., 2020), and for the identification of plant taxa within palaeoecological archives such as lake sediments and glaciers (Alsos et al., 2018; Parducci et al., 2017). To the best of our knowledge, only one study (Parducci et al., 2015) has applied the eDNA metabarcoding approach to study plants in peatlands. Moreover, very few studies (only one as far as we know:Garcés-Pastor et al., 2019) applied eDNA metabarcoding to identify invertebrate communities, which are very abundant in peatlands and are not considered as proxies even though they have key roles and can be used as bioindicators of the ecosystem health (Gerlach et al., 2013; Paoletti et al., 1991). The present study pursues a two-fold goal. On one hand reconstructing the past climate variability of South Tyrol (Italy), while identifying possible human-induced perturbations based on the analyses of pollen and testate amoebae, and physico-chemical features of peat. On the other hand,

92 evaluating the effectiveness of the plastidial marker trnL (Taberlet et al., 1991) for the
93 identification of plant taxa in association with the use of a portion of the mitochondrial DNA
94 cytochrome oxidase 1 (CO1) for the classification of invertebrate taxa (Hebert et al., 2003).

95 **2. MATERIALS AND METHODS**

96 *2.1 Study site and peat coring*

97 The study site (Fig.1) is the "Biotop Wölflmoor", an ombrotrophic peatland (= bog) located in the
98 municipality of Nova Ponente (South Eastern Italian Alps, latitude 46,418586 – longitude
99 11,42801), Province of Bolzano (Italy). The study area is characterized by a temperate continental
100 climate with the highest precipitation during the summer season. The mean annual temperature is
101 6.0°C going from a minimum of 1.1°C to a maximum of 10.6 °C, while the total rainfall is about
102 780 mm per year (data from Nova Ponente meteorological station, period 1930-2017, Bolzano
103 province meteorological service).
104 The peatland is included in the Natura 2000 network and represents one of the highest settings of
105 peatlands in Europe with a minimum elevation of 1,291 m a.s.l. and a maximum elevation of 1,298
106 m a.s.l. Its surface area is 10 hectares, and the vegetation is typical of ombrotrophic bogs with a
107 dominance of *Sphagnum* mosses, surrounded by mountain pine (*Pinus mugo* Turra) (Alber et al.,
108 1996; Bragazza et al., 2005). The typical vegetation at this altitude is mainly characterized by the
109 presence of spruce (*Picea abies* (L.) H.Karst.), fir (*Abies alba* Mill.), scots pine (*Pinus sylvestris*
110 L.) and sporadically some beech (*Fagus sylvatica* L.) (Agriculture and forestry - administration of
111 Bolzano province).

112

The peat sampling, carried out during summer 2017, was performed by extracting a one-meter-long peat core using a Wardenaar peat sampler (Wardenaar, 1987). The core was then placed into a plastic tube and wrapped with a plastic foil. At the laboratory, the peat core was divided longitudinally into two halves. The first half was partitioned into slices of 1 cm so obtaining 100 peat subsamples used for radiocarbon and lead dating, and the measurement of bulk density, water content, ^{13}C and C:N ratio. Another subsample, taken every 4 cm, was then used for the palynological analysis and the testate amoeba analysis; the second half was further divided into 4 cm-long slices for environmental DNA extraction and amplification in order to obtain a total of 23 peat samples.

2.2 Dating and geochemical proxies

Peat dating was performed using ^{14}C (Beta Analytic Testing Laboratory, Miami, Florida, U.S.) and ^{210}Pb (Institute of Nuclear Physics Polish Academy of Science, Kraków, Poland). Eight ^{14}C (AMS-Accelerator Mass Spectroscopy) dates were carried out on carefully selected plant macro-fossils from 1-cm long peat slices of the profile (laboratory code: Beta; Tab. 1). Radiocarbon dates were then calibrated to receive calendar age using IntCal20 (Reimer et al., 2020) and post-bomb NH1 (Hua et al., 2021) atmospheric curve (Tab. 1). For the measurements of ^{210}Pb we selected 71 contiguous samples from a depth of 71–0 cm. The activity of ^{210}Pb was determined as the activity of its daughter radionuclide ^{210}Po (half-life 138 days), which is in radioactive equilibrium with ^{210}Pb. The ^{210}Po activities were measured using Alpha Duo spectrometer with Ortec detectors after sample preparation following Mroz et al. (2017). Blanks and the reference material (IAEA 447 moss soil) were analyzed to ensure the quality of measurements, while the age-depth relationship was estimated using the Constant Rate of Supply (CRS) model. Combining the two dating methods, we prepared the absolute chronology using i) Bayesian age-depth model based on three

^{14}C dates calculated for a depth of 103–50 cm using the OxCal v. 4.4 software (*P_Sequence* command: k=0.5, $log_{10}(k/k_0) = 1$; Bronk Ramsey, 1995, 2006, 2008) applying the IntCal20 (Reimer et al., 2020) atmospheric curve as the calibration set and ii) ^{210}Pb dates for a section of 50–0 cm. As water content, bulk density and C/N ratio revealed a strong difference between peat layers below and above a depth of ca. 66 cm, a boundary was applied in the ^{14}C-based Bayesian age-depth model. All ^{14}C dates retrieved from the section of 50–0 cm were used to validate the ^{210}Pb chronology. As the absolute chronology derived from the ^{14}C -based Bayesian age-depth model we used μ value (unit: cal. CE; CE-Common Era).

The analyses of water content, bulk density, ^{13}C isotopic signature ($\delta^{13}C$) and C:N ratio were conducted at the Free University of Bolzano. Water content and bulk density were calculated by measuring the wet weight and volume of each subsample and dry weight after oven drying at 65°C. The $\delta^{13}C$ and C:N ratio were measured with a continuous-flow isotopic ratio mass spectrometer (CF-IRMS; Delta V Advantage, Thermo Fisher Scientific, Bremen, Germany) coupled with a CHN elemental analyzer (Flash EA 2000 Thermo Fisher Scientific, Bremen, Germany), after drying subsamples at 105°C.

2.3 Pollen, testate amoebae and plant macro-fossils

Palynological analysis, testate amoeba and plant macro-fossils analysis were done at the Laboratory of Climate Change Ecology of the Adam Mickiewicz University in Poznań (Poland). A total of 27 samples were prepared for palynological analyses, using the standard laboratory procedures (Berglund and Ralska-Jasiewiczowa, 1986). To remove carbonates, samples were treated with 10% hydrochloric acid. This step was followed by digestion in hot 10% potassium hydroxide (to remove humic compounds). Next, acetolysis was performed. One *Lycopodium* tablet (produced by the Lund University) was added to each sample during the laboratory procedures to

159 calculate microfossil concentration (Stockmarr, 1971). Pollen, spores, and selected non-pollen
160 palynomorphs (NPPs) were counted under an upright microscope until the number of total pollen
161 sum (TPS) grains in each sample reached at least 500. Sporomorphs were identified with the
162 assistance of atlases and keys (Moore et al., 1991; Beug, 2004; van Geel and Aptroot, 2006). The
163 percentage diagram was drawn using the TILIA Graph program (Grimm, 1991).

164 For testate amoebae analysis, peat samples were washed under 300 μm sieves following the
165 method described by Booth et al. (2010). Testate amoebae were analyzed under a light microscope
166 with 200× and 400× magnifications, aiming at a minimum of 100 tests per sample (Payne and
167 Mitchell, 2009). Several keys and taxonomic monographs were used to achieve the highest
168 possible taxonomic resolution (e.g., Ogden and Hedley, 1980; Clarke, 2003; Mazei and Tsyganov,
169 2006; Siemensma, 2021). The results of testate amoeba analysis were used for the quantitative
170 depth-to-water table (DWT) reconstruction, which was performed in C2 software (Juggins, 2007)
171 using the European training set (Amesbury et al., 2016).

172 Plant macro-fossils analysis was based on the accessible literature (Grosse-Brauckmann, 1986;
173 Warner, 1990; Tobolski, 2000). The peat material was rinsed with water onto a sieve with mesh
174 size 0.25 mm. The residue was sorted under a stereoscopic microscope under 10–100 ×
175 magnification. Two randomly chosen samples from each section were examined at 200–400 ×
176 magnification to estimate the volume percentage of each plant taxon.

177 *2.4 Environmental DNA*

178 All laboratory procedures were carried out in the "Ancient DNA laboratory" (exclusively
179 dedicated to ancient DNA) at Fondazione Edmund Mach – Research and Innovation Center.
180 Sample preparation (cleaning and manual homogenization), DNA extraction and PCR

amplification were performed in three different rooms to avoid sample contamination. To further limit environmental contamination, the DNA extraction room was sterilized for 30 minutes using UV lamps, while all extraction steps were conducted under a biological hood and by sterilizing the equipment using bleach, ethanol, and UV for 20 minutes.

The DNA extraction was performed on 10 g of the sample using the DNeasy PowerMax Soil Kit (QIAGEN Inc.) and by following the manufacturer's instructions. All extraction batches included 9 samples and 1 blank extraction control.

A short fragment of the chloroplast trnL was amplified to identify plant DNA using the primer pair c-A49325 and h-B49466 [5'-CGAAATCGGTAGACGCTACG-3' and 5'-CCATTGAGTCTCTGCACCTATC-3'] (Taberlet et al., 2007), while to identify arthropod DNA, a short fragment of the CO1 was amplified using the universal primers ZBJ-ArtF1c [5'-AGATATTGGAACWTTATATTTTATTTTTGG – 3'] and ZBJ-ArtR2c [5' – WACTAATCAATTWCCAAATCCTCC – 3'] (Zeale et al., 2011), which allowed the amplification of fragments of 157 bp (reaction mix and conditions in Table S1).

Reagents and environmental contaminations were monitored by adding blank controls to each amplification batch consisting of 9 samples, 1 extraction control and 1 PCR blank control. Two PCR replicates were performed for each sample.

Both samples and blank controls were checked on the QIAxcel capillary electrophoresis, with DNA High Sensitive cartridge (Qiagen, GmbH, Hilden, Germany), and sequenced using MiSeq Reagent Kit v3 in an Illumina MiSeq platform. Only blank controls presenting a positive signal on the capillary electrophoresis were sequenced together with biological samples.

2.5 Bioinformatics

Raw sequences derived from trnL and CO1 markers were analyzed separately. The data were pre-processed, quality filtered, trimmed, de-noised, merged, and analyzed using QIIME2 (Bolyen et al., 2019) and DADA2 (Callahan et al., 2016). Then, the obtained Amplicon Sequence Variants (ASVs) were clustered into OTU with a cutoff of 97% similarity and classified using the VSEARCH algorithm (Rognes et al., 2016). OTUs derived from CO1 libraries were assigned using the BOLD reference database (Ratnasingham and Hebert, 2007). Plant OTUs were taxonomically classified with a custom-made reference database.

3. RESULTS

3.1 Absolute chronology and peat accumulation rate

The overall trend of ^{210}Pb activity versus depth displayed a progressive decline from the surface until 17 cm depth. However, there were significant departures from this simple monotonic decrease, presumably reflecting episodic variations in the rate of peat growth or decomposition. Below 17 cm, ^{210}Pb unsupported activity decrease followed an approximate exponential downward trend with the increasing profile depth.

The age of the samples was calculated for the middle of the depth of the layers. ^{210}Pb dating revealed that the peat section 0-51 cm was accumulated in 147 ± 42 years. Based on the total ^{210}Pb inventory in the cores, atmospheric ^{210}Pb fluxes were calculated as 136 Bq m^{-2} yr^{-1}. This value agreed well with ^{210}Pb flux reported by Mróz et al. (2017) for Southern Poland (131–160 Bq m^{-2} yr^{-1}, 50°N) or by Vaasma et al. (2017) for North-East of Estonia (133 ± 24 Bq m^{-2} yr^{-1}, 59°N) and similar ^{210}Pb flux reported by Baskaran (2011), who estimated the value 155 ± 75 Bq m^{-2} yr^{-1} for the latitude belt 40–50°N.

The Bayesian age-depth model for a depth of 103–50 cm revealed agreement of model (A_{model}) equal to 88.3 % which is above the recommended by Bronk Ramsey (2008; A_{model} >60 %). This

226 section of the profile spans a period of 550±50 – 1185±145 cal. CE. The σ error of modelled dates
227 ranges between 40 and 114 calibrated years (Fig.2).
228 The distinct change of age between dates at a depth of 55.5 and 50.5 cm (covering ca. 800–930
229 calibrated years) suggests a depositional gap (hiatus) at least in this section. Moreover, at a depth
230 of 50.5 cm the difference between ^{210}Pb and ^{14}C is 44–136 years, but from a depth of 45.5 cm
231 toward the top calibrated post-bomb ^{14}C dates overlap with ^{210}Pb ones. Hence, the absolute
232 chronology of the section between 55.5 and 45.5 cm is rather uncertain.
233 The section of peat below the hiatus accumulated with a rate of 0.1–0.09 cm yr-1 (ca. 550–910 cal.
234 CE; 103–66.5 cm) and ca. 0.06 cm yr-1 (ca. 910–1090 cal. CE; 66.5–55.5 cm). The section above
235 the hiatus revealed a peat accumulation rate ranging between 0.45 (ca. 1951±10 cal. CE) and 2 cm
236 yr-1 (ca. 2015 cal. CE).

237 *3.2 Geochemical analyses*

238 The abrupt change recorded in the peat profile at c. 50 cm was also detected by measuring the
239 water content, bulk density, ^{13}C isotope, and C:N ratio (Fig.3) and it displayed a consistent trend.
240 A large variation occurred in about 10 cm of the peat profile (ca. 1090-1952 cal. CE; 55.5 – 45.5
241 cm), where water content increased (from 83 to 96 %) and conversely bulk density values
242 decreased (from 0.18 to 0.03 to g/cm3). This change was detectable also in the carbon isotope
243 measure and the C:N ratio, but to a lesser extent. The δ^{13}C variation of bulk peat along depth can
244 be divided into three phases. From the bottom of the core, it showed a decrease of 4.8‰ from the
245 maximum of -20.9‰ (ca. 611 cal. CE; 96.5 cm) to the first minimum -25.7‰ (ca. 1109 cal. CE;
246 54.5 cm), then it increased of 3.0‰ until the value of -22.7‰ (ca. 1991 cal. CE; 22.5 cm) and,
247 finally, it reached the second minimum of -26.04‰ (ca. 2013 cal. CE; 6.5 cm) decreasing by
248 3.34‰. The C:N ratio followed a similar pattern consisting of three stages with a slow decrease

from the bottom of the core, an abrupt increase in the middle part (ca. 1912-1952 cal. CE; 49.5–39.5 cm) and then a relatively stable phase until the uppermost peat layer.

3.3 Pollen analysis

Phase I (102.5–74.5 cm; ca. 550–720 cal. CE)

The period was characterized by the highest forest cover (AP: 93–97.6 %). Norway spruce (*Picea abies*; 15.9–33.9%), silver fir (*Abies alba*; 3.4–14.1%), common beech (*Fagus sylvatica*; 6.5–14.3 %) and oak (I; 4.8–10.7 %) were the main components of local forests (Fig.4). Hop-hornbeam (*Ostrya)* and/or Oriental hornbeam (*Carpinus orientalis)*, European larch (*Larix*) and hazel (*Corylus*) occurred as additional forest components. Betula trees (*Betula alba* type) and alder trees (*Alnus glutinosa* type) probably occupied the peatland outskirt and/or damp habitats in lower altitudes. High values of *Pinus sylvestris* type (23.1–30.5%) result from the proximity of *Pinus mugo* shrublands. Cyperaceae (1.3–3.6%), *Vaccinium* groups (0.5–3.1%), and *Sphagnum* (0.2–3.1%) sporomorphs were the most common taxa related to peatland habitat.

Phase II (74.5–62.5 cm; ca. 720–970 cal. CE)

The forest cover declined (AP: 91.4–92.8%). Among dominant taxa in the previous zone, *P. abies* (to 11.4%) and *A. alba* (to 3.9%) retreated distinctly, while a gradual increase in values of Poaceae (2.7–3%), *Plantago lanceolata* (0.6–0.8%), Cerealia type, Secale cereale as well as *Betula alba* type and *Corylus* took place. Continuous, but not frequent presence of *Olea* and *Vitis vinifera* pollen grains were also detected.

Phase III (62.5–42.5 cm; ca. 970–1958 cal. CE, with the section with uncertain chronology)

Deforestation affected *F. sylvatica* (decline to 2%) and *Quercus* (decline to 2%) stands the most and to a lesser degree *P. abies* and *A. alba*. *Pinus mugo* probably spread (*P. sylvestris* type: 34.3–49%). *Pinus cembra* may have appeared in the peatland vicinity. Pollen percentages of *Olea*, cf. Phillyrea, *Vitis vinifera* and *Humulus/Cannabis* revealed maxima in the profile (1.8, 1.3, 0.8, and 1.5%, respectively), while values of Poaceae, *Plantago lanceolata*, *Artemisia*, Cerealia type and S. cereale (max. 0.8%) increased.

Phase IV (42.5–16.5 cm; ca. 1958–1987 cal. CE)

Decrease in AP values (77.1–84.2%) indicates continuous deforestation, which probably affected the most *F. sylvatica* and *Quercus*. Simultaneously *Ostrya* and/or *Carpinus orientalis* gradually spread (pollen type: 1.7–3.9%). At the end of Phase IV, *Betula alba* type reached its maximum (13.3%) while *Pinus mugo* (*P. sylvestris* type) retreated.

Phase V (16.5–0 cm; ca. 1987–2017 cal. CE)

Arboreal vegetation increased its cover, mainly by the expansion of *Ostrya* and/or *Carpinus orientalis* and partly *Pinus mugo* (*P. sylvestris* type). The decline of AP values at the top of the profile is a result of the bias related to the increase in Apiaceae values, which was probably a local event. Since ca. 1994 cal. CE a stable presence of pollen of *Ambrosia artemisiifolia* type was noted which is probably a result of the spread of invasive *Ambrosia sp*. in the region.

3.4 Testate amoebae and plant macro-fossils

Testate amoeba communities recorded a shift between the first and the second half of the peat core (Fig.5). Up to about 1005 cal. CE (~ 60.5 cm) the testate amoeba communities were dominated by several mixotrophic taxa: *Archerella flavum, Heleopera petricola, Heleopera sphagni* and *Hyalosphenia papilio*. During that time water table depth (DWT) at the peatland was high and

291 stable (DWT of ca. 6 cm). Between 1056 and 1954 cal. CE (ca. 57.5-44.5 cm) we recorded a shift
292 in the relative abundance of testate amoeba species and much lower concentration of individuals
293 in the sediment (see testate amoeba sum on Fig.5). Mixotrophic taxa decrease rapidly (except for
294 *Heleopera petricola*), whereas small taxa (with shells smaller than 60 μm, *Cryptodifflugia*
295 *oviformis* and *Trinema enchelys*) and the dry indicator species *Bullinularia indica* appeared. This
296 shift is related to rapid water table lowering (drop from ca. 6 cm to 20 cm). From 1960 cal. CE
297 (ca. 41.5 cm) up to the top of the profile some of the mixotrophic species reappeared in the
298 communities (*H. sphagni* and *H. papilio*) accompanied by dry indicator taxa, such as *Alabasta*
299 *militaris, Nebela collaris* and *Nebela tincta*. The water table remained low in this period (mean
300 value: 14 cm).
301 The analysis of plant macro-fossils provides insightful clues on local vegetation changes. The
302 diagram (Fig.6) can be divided into two sections. The bottom part of the core till 72.5 cm (ca. 841
303 cal. CE) is represented by the domination of *Sphagnum fuscum/rubellum*, Ericaeae root and
304 herbaceous plant remain with the addition of *Sphagnum papilosum*, *Sphagnum cuspidatum* and
305 *Andromeda polifolia* that occurs in form of leaves and seed on depth 90-87 cm. Also, Pine and
306 *Rhynchospora* seeds were recorded in this section. The horizon 65.5-50.5 cm (ca. 919-1185 cal.
307 CE) consisted of a critical threshold in the peatland development, the strongest change at 61.5 cm
308 (ca. 988 cal. CE) represented by the peak of the unidentified organic matter being simultaneously
309 the most decomposed part. Above 61 cm the plant communities were dominated by *Sphagnum*
310 *magellanicum* and *Sphagnum papilosum,* while herbaceous plants disappeared at a depth of 52.5
311 cm (ca. 1148 cal. CE). *Rhynchospora* seeds were recorded until the depth 41 cm (ca. 1960 cal.
312 CE). This section consisted of a low percentage of Ericaceae roots.

313 *3.5 Environmental DNA taxonomic classification*

The analysis of plant eDNA provides a similar trend as that from all the other proxies. The highest abundance is distributed between three main families: Sphagnaceae, Cyperaceae and Ericaceae (Fig.7). Sphagnaceae and Ericaceae are dominant between 45.5 and 36.5 cm depth (ca. 1952-1968 cal. CE) but present a strong decrease of the abundances in superficial and deep samples, particularly for Ericaceae as already presented by the macro-fossil analysis. Cyperaceae followed an opposite trend, missing completely between 45.5–36.5 cm and dominating the superficial and deep samples. All other taxa are much less represented; nevertheless, they appear to present slightly more variability in the most superficial samples than in the intermediate and deepest ones. Finally, Betulaceae were found only at a depth of 63.4 cm (ca. 954 cal. CE), whereas they were found in almost all samples with the pollen analysis.

The analysis of the mt DNA CO1 revealed the high abundance of the Adinetidae family (Fig.8a), which belongs to the Rotifera phylum. Among all the other taxa, belonging to the Arthropoda phylum (Fig.8b), Carabidae and Blastobasidae represented the most abundant families, with the first one occurring only in deep samples, and the second one in some superficial and deep samples, showing a similar pattern to the other proxies before and after the hiatus. The other families were recorded in traces, but they seem to have been influenced by the environmental conditions since the Gnaphosideae family, together with Tabanidae and Cecidomyiidae, appeared only in samples corresponding to the drier period.

4. DISCUSSION

Peat bogs from mountain areas are of particular interest for reconstructing climatic and associated ecological changes through time. Pet bogs can be precisely dated, and a series of well-established proxies offers the opportunity to evaluate changes in abiotic and biotic components (Lamentowicz et al., 2010) by integrating physicochemical, hydrological, and taxonomical information. The latter

is, however, sometimes difficult, and time-consuming to obtain, especially when targeting both fauna and flora. In this study of the Wölflmoor peat bog, situated in the Southern Alps (South Tyrol, Italy), we aimed at, on one hand, reconstructing climatic and associated ecological changes with established proxies and methodologies, and, on the other hand, assessing the effectiveness of eDNA metabarcoding for taxonomic identification of plant and invertebrate taxa.

4.1 Climatic and ecological changes

From our analysis, a substantial change in peatland conditions before and after the hiatus occurred. Before the hiatus, between ca. 920-1186 cal. CE (65.5-50.5 cm) water content and bulk density decrease and increase, respectively, highlighting a period of important changes in peatland conditions (Fig.3). The clear trend in the water content and bulk density may be interpreted as a shift from moist to dry environment, which persisted for almost a century (ca. 1090-1186 cal. CE; 55.5-50.5 cm). As already recorded in other peatlands across Europe (Lamentowicz et al., 2008, 2009) and other natural archives (Esper et al., 2002; Tiljander et al., 2003), the dry phase may be explained by the Medieval Warm Period (WMP), which was characterized by temperatures comparable to the current ones (Bradley et al. 2003; Crowley and Lowery, 2000; Goosse et al. 2006).

The other proxies also highlighted drier conditions and a more mineral soil substrate shift in the same time window. Plant macro-fossils (Fig.6) revealed a dry phase at ca 60.5 cm (ca. 1005 cal. CE; critical transition) described by the decomposed peat and indicate a disturbance that started at ca. 65.5 cm (920 cal. CE) and intensified at ca. 60.5 cm (1005 cal. CE). The site recovered from the disturbance until 55.5 cm, when peat initiated again a very rapid accumulation. The testate amoeba communities (Fig.5), especially in terms of individual concentration (lower testate amoeba sums analyzed in this period) and testate amoeba functional trait composition, showed a significant

drop in the relative abundance of mixotrophic species during the critical period. A high abundance of mixotrophs is linked to high water tables and an open landscape that allows high light intensity on the surface of the bog (Heal, 1964; Payne et al., 2016; Marcisz et al., 2016; Creevy et al., 2018). Water table lowering and shading due to the overgrowth of the peatland with trees, as confirmed by the presence of wood in this phase, could harm phototrophic metabolism, leading to the disappearance of mixotrophs from testate amoeba communities (Jassey et al., 2015; Payne et al., 2016; Marcisz et al., 2020) and promoting smaller taxa, usually indicator of dry conditions (Marcisz et al., 2016).

At this stage, also the pollen stratigraphy confirmed important vegetational changes (Fig.4). During Phase I (102.5–74.5 cm; ca. 550–720 cal. CE), the high forest cover dominated by *Picea abies*, *Abies alba* and *Fagus sylvatica* is related to a wet environment (Lamentowicz et al., 2008, 2015; Gałka et al., 2015), presumably with low human pressure. Phase II, on the other hand, seems to have been a scenario of a forest decline, characterized by the retreat of *Picea abies* and *Abies alba* and an increase of Poaceae, Cerealia type, Secale cereale as well as *Betula* and *Corylus* (Phase II: 74.5–62.5 cm; ca. 720–970 cal. CE), which may suggest adaptation of deforested areas to cultivated land and pastures as well as colonization of some deforested abandoned lands by birch and hazel (within forest gaps). Subsequent phase III (62.5–42.5 cm; ca. 970–1958 cal. CE) revealed a progression in the deforestation process with a further increase of Poaceae, *Plantago lanceolata*, Cerealia and *Secale cereale* values, indicating a high human pressure. At the same time, an increase in Cyperaceae percentages compared to *Sphagnum* suggests a shift to drier environmental conditions.

After the mentioned section, the age-depth model showed the presence of a hiatus which is a consequence of a disturbance that completely transformed the bog vegetation. Similar transitions

383 in time were connected with a human impact and described from the Polish Carpathians (peat
384 cutting) (Kołaczek et al., 2018) as well as Pomerania (unknown origin) (Lamentowicz et al., 2008).
385 Judging from the C:N, bulk density, and testate amoeba data this depositional gap is highly related
386 to strong and prolonged desiccation of peat, which caused faster decomposition of the topmost
387 layer. However, due to the high human pressure, mechanical removal of the upper peat layer should
388 be also taken into consideration as a cause of such distinct hiatus at a depth of ca. 50 cm. Results
389 from layers after the hiatus were compared with data produced by the monitoring station, which
390 identified dry periods in 1945, in the early 1970s, around 2005, and in the last five years (Fig.9),
391 while short episodes of moist conditions occurred in 1979 and before the beginning of 2000. These
392 observations are confirmed by the testate amoeba communities, that generally followed the
393 precipitation pattern. From 1960 cal. CE mixotrophic species reappeared inside the communities,
394 which combined with the presence of dry indicator taxa corroborate the alternation of dry and wet
395 periods. The pollen data (Phases IV and V) highlight continuous deforestation, with an increase of
396 *Betula alba* type ca. 1987 cal. CE, which may indicate birch colonization ca. 1984 cal. CE on
397 abandoned open-lands which were mainly occupied by grasslands.

398 *4.2 eDNA metabarcoding of plant and invertebrate taxa*

399 As the other proxies, eDNA metabarcoding revealed an abrupt change in the peatland conditions
400 dominated by the disappearance of Cyperaceae around the depth of 45.5 cm (ca. 1925 cal. CE)
401 together with the appearance of Ericaceae. As shown by Fig.7, the identified wet periods are
402 dominated by Cyperaceae, a family of vascular plants related to wet environments (Gałka et al.,
403 2018), while the abundance of Sphagnaceae and Ericaceae, more adapted to drier conditions
404 (Gałka et al., 2018), decrease drastically. In contrast, the dry period is characterized by a high
405 abundance of the last two families, while Cyperaceae completely disappeared.

406 Because of their strong adaptation to peatland conditions, Sphagnaceae, Ericaceae and Cyperaceae
407 were certainly present in higher proportions (Farrick and Price, 2009; Svensson, 1986; Vitt, 2006).
408 Therefore, DNA from these families could have been preferentially amplified, causing an under-
409 representation of all the other species (Fulton, 2012).
410 Among the metazoans, the most abundant taxa were assigned to the Rotifera phylum (Fig.8a). This
411 phylum is known to include organisms able of coping with the most diverse environmental
412 conditions (Arora, 1966; Bērziņš and Pejler, 1989). This ability allows them to inhabit the deepest
413 layers of peatlands characterized by low oxygen concentrations, which usually precludes the
414 survival of many other organisms. For this reason the under-representation of many taxa can be
415 related to the preferential amplification of DNA from modern organisms belonging to Rotifera
416 (Garcés-Pastor et al., 2019). Nevertheless, excluding rotifers (Fig.8b), two families are the most
417 common among all samples: Carabidae and Blastobasidae. The presence of the first family was
418 established only for the deepest samples. This finding supports the identification of a shift from
419 wet to dry conditions, as this family was found to be related to deciduous forests (Calatayud et al.,
420 2016). Therefore, the presence of forest species around the peatland favored the establishment of
421 Carabidae, which then was not detected with the increase of temperatures at the beginning of the
422 dry period (Fig.8b). The second family belongs to the order of Lepidoptera. This order represents
423 a group of organisms spread worldwide and in all terrestrial ecosystems (Lopez-Vaamonde et al.,
424 2010). The occurrence of the Blastobasidae family is not indicative of specific environmental
425 conditions, but according to Spitzer et al. (1993) Lepidoptera living in non-disturbed environments
426 are specialized taxa that may be damaged by any source of the disturbance. According to Fig.8b,
427 this family was detected in samples related to periods long before and long after the event that
428 caused the hiatus. Therefore, it could be hypothesized that this event, in addition to causing a loss

of material from the peatland, resulted in a disturbance of these taxa, which were able to recover after a few years.

Despite their presence in traces, also the other families seems to corroborate the shift in the environmental conditions; in fact, Gnaphosidae is a family of organisms with a predilection for drier conditions (Gajdoš et al., 2016), while the Culicidae family (more adapted to wet habitat (Costa et al., 2010)) was replaced by Tabanidae, a family inside the same order of Culicidae, but with a preference versus drier environmental conditions (Herczeg et al., 2015).

4.3 Taxonomic identification: comparison between eDNA Metabarcoding and morphology

Pollen and eDNA confirmed trends shown by the physicochemical analyses. However, it seems that the results of the eDNA metabarcoding are somehow distorted due to the signal saturation by modern and/or highly abundant taxa. This determined this method to be less effective than the morphological taxonomic identification of pollen and not fully explanatory for arthropods.

The reasons for this performance could be explained by the different amounts of material needed by the approaches to establish the presence of a taxon inside samples. For the morphological identification, the presence of a single pollen grain is sufficient, while for the eDNA the amount of starting material must be sufficient to get several reads able to pass all the filtering steps. However, this goal can be hampered by several factors: the under-representation of taxa due to the preferential amplification of abundant taxa (Shirazi et al., 2021), the accuracy of the reference databases (i.e. ideally, they should include reference sequences for each OTU in our dataset) (Schenekar et al., 2020), the degeneration of primers (i.e. having degenerate primers able to amplify DNA sequences from species also quite distant from each other) (Elbrecht et al., 2019), and the variability of the selected markers within target species (Meusnier et al., 2008; Taberlet et al., 1991).

452 It is important to recall that, insofar, very few eDNA studies (Garcés-Pastor et al., 2019; Parducci
453 et al., 2015) have been performed on peat bogs. For this reason, we argue that eDNA
454 metabarcoding is expected to be significantly improved. For both plants and arthropods, reference
455 databases are becoming more comprehensive year by year, and other sets of primers (Chen et al.,
456 2010; CBOL Plant Working Group: Hollingsworth et al. 2009; Elbrecht et al., 2019; Hadziavdic
457 et al., 2014) can be used in place of or in support of those used in this study in order to increase
458 the range of taxa identified at high taxonomic levels.
459 For plants, eDNA and pollen can provide complementary information able to improve the
460 classification of taxa identified with the morphological approach alone.
461 About arthropods, they have never been investigated for historical reconstructions, probably due
462 to the difficulties in the taxonomic assignment with the morphological identification (Thomsen et
463 al., 2015). These difficulties can be caused by the similar appearances of some species or because
464 morphological keys can be useful only for specific genders or life stages.
465 The eDNA can overcome these limitations, allowing the inclusion of arthropods and other
466 invertebrates in studies based on the historical reconstruction of biodiversity since they play a
467 fundamental role inside lots of ecosystems and can be used as bioindicators of soil quality
468 (Hodkinson and Jackson, 2005; Maleque et al., 2009; Nakamura et al., 2007; Paoletti, 1999;
469 Seastedt and Crossley, 1984).

470 **5. CONCLUSIONS**

471 The first 1-m long peat core extracted from the Wölflmoor bog in South Tyrol (Italy) is more than
472 a millennium old, suggesting a long history of this peatland during the Holocene. The stratigraphy
473 showed a consistent difference in the composition and structure of the material along the peat core
474 and, consequently, the peat dating revealed a lack of material halfway through the sample, which

475 determined an interruption of about 600 years. This may suggest that the Wölflmoor bog was
476 exploited between the end of the 19th century and the beginning of the 20th. Proxies from
477 Wölflmoor bog showed converging trends over time with visible differences in recorded values
478 between the two portions of the peat core. Pollen, water content, bulk density, and testate amoebae
479 indicate a shift from moisture to warmer, drier conditions between 920 and 1186 cal. CE, possibly
480 attributed to the initial stage of the Medieval Warm Period, while the increase in Poaceae, Secale
481 cereale and other Cerealia type highlight a high human pressure started ca. 720 cal. CE. The eDNA
482 metabarcoding analyses confirmed this trend, showing the coherence with climatic proxies already
483 widely used in climate reconstruction studies.
484 In recent decades, the comparison of peatland proxies allowed detecting an alternation of dry to
485 wet period, particularly warmer and drier conditions during the last years.
486 Overall, eDNA metabarcoding was less effective than pollen morphological identification, while
487 for the Arthropoda the signal was likely saturated by living organisms, showing that this technique
488 needs further methodological developments. Further studies are foreseen to explore the full
489 potential of this technique in peatland archives studies to produce data that can effectively
490 complement those derived by morphological analyses.
491

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493 This study is dedicated to the memory of Giustino Tonon.
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