

## What was burning in the past? Charcoal identifications supplement an early-Holocene fire-history reconstruction in Yellowstone National Park, USA

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What was burning in the past? Charcoal identifications supplement an early-Holocene fire-1 2 history reconstruction in Yellowstone National Park, USA 3 Dominique MARGUERIE<sup>1\*</sup>, Teresa R. KRAUSE<sup>2,3</sup>, Cathy WHITLOCK<sup>2</sup> 4 5 <sup>1</sup>CNRS, UMR ECOBIO 6553, OSUR, University of Rennes 1, France 6 <sup>2</sup> Department of Earth Sciences, Montana State University, Bozeman, MT 59717, USA 7 <sup>3</sup> Department of Biology, Augsburg University, Minneapolis, MN 55454, USA 8 9 \* Corresponding author: dominique.marguerie@univ-rennes1.fr 10 11 Abstract: 12 Analysis of charcoal particles preserved in lake sediments has been frequently used to 13 reconstruct fire, vegetation and climate history. Larger macroscopic particles (>125 µm) are attributed to local fires, whereas microscopic charcoal particles (<125 µm), observed on 14 pollen slides, are a signal of regional fire. Taxonomic identification of the charcoal particles 15 16 adds to the fire-history reconstruction by providing information about fuel composition and past fire conditions. Identification of charcoal can unravel one of the longstanding questions 17 18 regarding past fire regimes, namely what was burning. This paper describes an analysis of charcoal particles preserved in the sediments of a small closed-basin lake in Yellowstone 19 20 National Park. Blacktail Pond (44.954°N, 110.604°W; 2012 m elev) is located in steppe 21 vegetation surrounded by conifer forest. Previous studies of the site have presented a multi-22 proxy environmental history of the last 14,650 years. The focus of this investigation was on the fire history of the early-Holocene (11,500-9900 cal yr BP). Thirty-nine sediment samples 23 were taken at 0.5 cm intervals to examine macroscopic charcoal particles, with attention on 24 25 two large charcoal peaks at 10,970 and 10,200 cal yr BP that registered large or near-site 26 fires. Twenty-eight samples contained 243 large-enough particles (180-250 µm) to be examined with scanning electron microscopy. We were able to identify charcoal of conifers 27 (Pinus, Picea, and Abies or Juniperus), Artemisia and unidentified deciduous shrubs, and 28 29 unidentified monocotyledons. The first fire burned a mixture of fuels, whereas the second episode burned Artemisia, shrubs and herbs but no conifers. The results are consistent with 30 previous pollen interpretations at Blacktail Pond that indicate an open mixed-conifer forest in 31 the early Holocene prior to 10,750 cal yr BP, replaced by Pinus contorta forest on the slopes 32 and steppe in the valley. The relative dominance of *Pinus contorta*-type charcoal points to 33 34 the importance of fire as a catalyst for the spread of this species. 35 36 Keywords: charcoal, wood anatomy, fire, early-Holocene, vegetation history, Yellowstone

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#### 41 **1. Introduction**

National Park, USA

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Charcoal data preserved in lake and wetland sediments are used around the world to reconstruct fire history, and the interest in fire as an Earth system process has led to the creation of a publicly available global charcoal database (Marlon et al, 2016). Most fire history reconstructions are based on changes in charcoal abundance through time, which are interpreted to reflect variations in fuel biomass, fire intensity, and area burned. Large charcoal peaks in individual records are attributed to fire episodes near the sites (Whitlock and Larsen, 2001; Marlon et al., 2006).

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51 We suggest that taxonomic identification of the charcoal particles preserved in lake 52 sediments provides additional information about past fire conditions that cannot be easily

53 inferred otherwise-namely, what burned? Knowing the fuel type, whether from conifers,

- hardwoods, shrubs, and/or herbs, and in some cases, assignment to genus or species help
- clarify the characteristics of the fire regime as well as the likely fire source area. This

56 information also provides better understanding of vegetation dynamics during times of

- 57 climatic transition.
- 58

59 Following Jones et al. (1997), the term "charcoal" is assigned here to all burned or

60 charcoalified (charred) plant remains produced during fires, not only woody fragments.

61 Charcoal particles are distinguished from other dark particles (especially the mineral ones)

by their black color, total opaqueness, high light reflectivity, and their angular and usually

planar shape with sharp edges. When poked, wood charcoal is easily broken into small

- 64 angular fragments that often show plant structures.
- 65

66 Charcoal can be transported in the atmosphere several meters to tens of kilometers before 67 final deposition and burial (Garstang et al., 1997; Clark et al., 1998; Pisaric, 2002). The travel 68 distance is a function of the fire characteristics, the conditions of transport, and the particle 69 size. Microscopic charcoal particles (<125  $\mu$ m) come from both local and distant fires 70 whereas larger macroscopic fragments (>125  $\mu$ m) are transported far less from the source 71 and thus provide a local fire history (Whitlock and Millspaugh, 1996; Carcaillet et al., 2001, 72 Whitlock and Larsen, 2001, Olsson et al., 2009).

73

74 The external morphology (size and shape) of macroscopic charcoal has been explored using 75 automated image analysis under a (stereo)microscope. Umbanhowar and McGrath (1998) 76 examined the area, length, and width of charcoal particles as an indicator of burned 77 vegetation type. They experimentally produced charcoal of wood, leaves and grasses by 78 burning samples in open fires and in a muffle furnace at 350°C. They distinguished these 79 three types of charcoal based on variations in length (along the major axis, µm), width (along the minor axis, µm) and area (µm<sup>2</sup>) of the particles. The elongation metric R (L/W) enabled 80 81 them to separate wood (R=2.13 in furnace and 2.23 in open fires) and leaf (R=1.91 and 2.15) particles from those of herbs (R=3.62 and 4.83). Grass charcoal was longer than either leaf 82 or wood charcoal. The anatomical structure of plant and the degree of elongation was 83 84 preserved even when the particle was broken, enabling separation of charcoal from deciduous versus herbaceous taxa. This morphological approach was a reasonable tool for 85 86 determining the nature of the burned vegetation.

87

88 Enache and Cumming (2006) analyzed charcoal particles from historic fires dating back to 89 1919 that occurred within a 20-km radius of Prosser Lake (British Columbia, Canada). The 90 authors observed the morphology of charcoal particles >150 µm diameter from a sediment core to infer the fuel type (bark, trunk, branch, leaf), temperature of formation, and 91 92 taphonomic modifications of the initial morphology. This work led to the establishment of seven charcoal morphotypes according to shape, ratio of major to minor axis, and 93 94 presence/absence of porosity and initial plant structure. The charcoal morphometric features suggested different fuels during each fire. Some morphotypes, rich in fibers, originated from 95 96 tree bark, whereas other types had parenchyma or vessels that originated from wood. Type F resulted from fibrous fragments or grass cuticles, and type B represented partially burned 97 fragments. Another type, M, was characterized by its irregular shape, its fragility and high 98 porosity, and was attributed to intense crown-fire burning of branches or leaves. Type M 99 100 charcoal in the core accurately matched the timing of known fires and was considered the 101 "most accurate record of fire events". Conversely, other types of charcoal (types B, C, M, P, 102 S) were thought to have been produced by older fires and redeposited or transported from 103 distant fires. Both Umbanhowar and McGrath (1998) and Enache and Cumming (2006) provide general insights about the vegetation burned, but they do not provide specific 104 taxonomic information. 105

106

Soil charcoal research or pedoanthracology focuses on large burned pieces >0.5 mm
 preserved in soils, often in association with archeological sites, to provide taxonomic
 information on local vegetation in the context of environmental change and human land use

110 (Carcaillet and Thinon, 1996; Nelle et al., 2013; Robin et al., 2013). Identification rarely

includes the charcoal preserved in lakes or wetlands, because these sedimentary particles 111 112 are usually small (<0.25 mm). Charcoal particles in lake and wetland deposits are often identified as conifer, hardwood, shrub, or herb and, in some cases, assigned to genera 113 (Quercus sp., Pinus sylvestris-type, Salix sp., Betula sp., Ericaceae) (Whitlock et al., 2006). 114 115 In a study of Holocene fire history, Olsson et al. (2009) identified charcoal particles from a 116 10.500 yr-long record from a peat and lake sediment deposit at Storasjö and Stavsåkra in southern Sweden. They suggested that both microscopic and macroscopic charcoal records 117 118 represented local fire history and showed three major phases of high fire activity controlled by climate during the early and middle Holocene and by humans during the late Holocene. 119 120 These charcoal identifications offered a partial list of fuels because a high quantity of the particles were unidentifiable. 121 122 123 The objective of this paper is to better understand past vegetation and fire conditions through the use of charcoal analysis on sediment cores taken from a small lake in northern 124 Yellowstone National Park, USA. Blacktail Pond (44.954°N, 110.604°W; 2012 m elevation) is 125 126 surrounded by steppe with conifer forest growing above the site on adjacent rocky buttes and at higher elevations. A ca. 14,650-yr-long record of vegetation and fire history was 127 128 reconstructed at this site and described in Krause and Whitlock (2013) and Huerta et al. (2009). Moreover, Krause and Whitlock (2013) present geochemical and isotopic data for the 129 late-glacial to early Holocene period, as part of a multi-proxy investigation of environmental 130 131 history. 132 The period of interest for the present study is the early Holocene from 11,500 to 9900 cal yr 133 BP at Blacktail Pond when summers were becoming progressively warmer and drier as a 134 result of increasing summer insolation (Krause and Whitlock, 2013). Early postglacial 135

warming shifted the vegetation from late-glacial parkland to early-Holocene mixed conifer
 forest and then steppe and closed pine forest (Krause and Whitlock, 2013) (Fig. 2). Fire

- activity, as evidenced by the charcoal record, was also increasing during this transition in
- response to warmer conditions and greater fuel biomass (Fig. 3). Two significant fire
- episodes occurred at ca. 10,970 and 10,200 cal yr BP, based on large charcoal peaks
- 141 (Krause and Whitlock, 2013). In this paper, we describe charcoal particle identifications that 142 reveal fuel and fire conditions of the early Holocene and supplement previous understanding
- of the vegetation and fire history at Blacktail Pond by answering the question: what was burning?
- 144 Dui 145

145

#### 147 2. Study area

# 148149 2.1. Current vegetation and fire regimes

150

151 The vegetation of northern Yellowstone is strongly influenced by elevation, aspect and geology (Despain, 1990). Today, grassland and steppe communities dominated by Artemisia 152 tridentata (big sagebrush). Festuca idahoensis. Ericameria nauseosa (rabbitbrush). 153 Sarcobatus vermiculatus (greasewood, on saline soils), and various herbs are present 154 155 generally below 1700 m elevation. Montane and subalpine forest occurs between 1700 and 2900 m elevation and is replaced by alpine tundra at elevations above 2900 m. Within the 156 157 forest zone, *Pinus flexilis* (limber pine) and *Juniperus scopulorum* (Rocky Mountain juniper) occur at low elevations (1700-1900 m elevation), Pseudotsuga menziesii (1900-2000 m 158 elevation) and Pinus contorta (2000-2400 m elevation) are present at middle to high 159 160 elevations. Picea engelmannii (Engelmann spruce), Abies lasiocarpa (subalpine fir), and Pinus albicaulis (whitebark pine) dominate high elevation forests and parkland (2400-2900 m 161 162 elevation). Juniperus communis is present in the understory at all elevations. 163 Blacktail Pond lies within calcareous glacial outwash and is surrounded by Artemisia 164

*tridentata* steppe, which grows at this elevation on fertile soils. *Pseudotsuga* forest is present

on adjacent rocky slopes of basalt and andesite, and *Pinus contorta* forest grows on local
rhyolite exposures and throughout the central part of Yellowstone National Park. Small
populations of *Abies* and *Picea* are found in valleys with cold-air drainage, and stands of *Populus tremuloides* (quaking aspen) grow on lower slopes in moist areas. Salix spp, Betula
occidentalis (water birch), Scirpus americanus, Carex and Typha latifolia are present along
the lake margin.

172

Fires, both lightning and human caused, are common in western U.S. forests, and 173 Yellowstone National Park became internationally famous for large fires in 1988 that burned 174 175 3213 km<sup>2</sup>, or about 40% of the park (Schullery, 1989). Regional studies, based on lakesediment studies, indicate that fire activity increased during the late-glacial and early 176 177 Holocene periods and then decreased after ca. 9000 and again at 5000 cal yr BP (Iglesias et 178 al., 2018). The conifers of the region have different adaptations to fire that allow for their 179 survival. For example, the thick bark of *Pseudotsuga* enables these trees to survive lowintensity surface fires. Similarly, Pinus flexilis and P. albicaulis tolerate surface fires as the 180 181 trees mature and their bark thickens. P. contorta is adapted to high-severity fires, because, 182 even though the trees are killed, its serotinous cones open immediately and release 183 thousands of seeds. Abies and Picea have thin bark with branches to the ground and thus are highly vulnerable to fires. These conifers grow in wet regions where fires tend to be 184 infrequent crown fires, and regeneration occurs by seed (Knight et al., 2014). 185

186

#### 187 2.2. Lithology and chronology

188

This study used core BTP08B of Krause and Whitlock (2013), which was collected at the 189 western margin of the lake from the fen. The sediment from 5.25 m to 8.10 m depth was the 190 191 focus of this study. The chronology for Blacktail Pond record was developed from seven AMS radiocarbon dates on pollen residues, charcoal, and plant macrofossils, and two known 192 tephra ages (Krause and Whitlock, 2013). The environmental reconstruction was based on 193 194 pollen, geochemical properties (elemental geochemistry, oxygen isotopes from authigenic carbonates), magnetic susceptibility, and macroscopic charcoal. The pollen and charcoal 195 data and radiocarbon ages are available at Neotomadb.org. 196

197

198 The original charcoal analysis focused on particles >125 µm in diameter to capture high-199 severity fires occurring within a few kilometers of the lake (Krause and Whitlock, 2013; Huerta et al., 2009). Charcoal accumulation rates (CHAR; particles cm<sup>-2</sup> yr<sup>-2</sup>) were calculated 200 using CharAnalysis (Higuera et al., 2008). Charcoal concentrations and deposition times 201 were interpolated into contiguous 25-yr bins (the median resolution of the record), and CHAR 202 was determined by dividing the time-interpolated concentrations. Long-term trends in CHAR 203 204 (background CHAR or BCHAR) were separated from positive deviations (charcoal peaks). with a 500-yr loess smoother. BCHAR is considered an indicator of woody fuel biomass 205 206 (Marlon et al., 2006). Charcoal peaks were assigned to the positive residuals remaining after BCHAR is removed from the time series and identified above a 95<sup>th</sup> percentile of the noise 207 distribution (Higuera et al., 2010). These peaks are interpreted as fire episodes (one or more 208 209 fires during the time span of the peak).

210

#### 211 **2.3. Previous information on fire and vegetation dynamics**

212 213 Pollen data from Krause and Whitlock (2013) describe the late-glacial to early-Holocene vegetation history at Blacktail Pond (Fig. 2). The period from ca. 14,650 to 11,500 cal yr BP 214 215 was a period of cooler-than-present summer conditions, but temperatures were steadily increasing with rising summer insolation. The postglacial landscape was initially sparsely 216 vegetated with little soil development. After ca. 12,900 cal yr BP, conditions were warm 217 enough for Picea engelmannii to form a parkland at the site, and after ca. 12,500 cal yr BP. 218 Picea, Abies, and Pinus (both P. contorta and P. albicaulis/flexilis pollen types) were present 219 in open mixed-conifer forest communities. Beginning at ca. 11,500 cal yr BP, the carbonate 220

- 221 d<sup>18</sup>O record from Blacktail Pond indicates a sharp increase in summer temperatures, and the
- pollen record suggests a more closed forest at ca. 11,350 cal yr BP. By 9900 cal yr BP,
- 223 Pinus contorta replaced Picea engelmannii as the dominant conifer on hill slopes near
- Blacktail Pond. Given soil constraints, *Artemisia* steppe probably still covered the valley floor.
- 225

The charcoal-based fire history followed the vegetation history closely, showing few fires before 12,000 cal yr BP when vegetation cover was sparse. This was followed by a steady CHAR rise, suggesting a maximum in fire activity between ca. 12,000 and 10,500 cal yr BP,

- and a slight decline thereafter. Noteworthy are the two large fire episodes at ca. 10,970 cal yr
   BP and 10,200 cal yr BP (Fig. 3).
- 231

It is likely rising fire activity due to increasing summer insolation facilitated the transition from mixed-conifer forest to *Pinus contorta*, given the fact that *Picea* and *Abies* have little adaptation to fire. The two prominent fire events may have been important triggers in this transition. The first peak occurred early in the period of mixed-conifer forest, while the second peak occurred just before *P. contorta* became the dominant species near Blacktail Pond at 9900 cal yr BP, where it likely occupied upslope rocky areas and steppe was locally present (Krause and Whitlock, 2013).

239

# 240241 **3. Methods**

#### 242

For this study, a new macroscopic charcoal analysis was undertaken on the sediments above and below the two charcoal peaks. Twenty-six samples of 5 cm<sup>3</sup> were taken every 0.5 to 1 cm in 0.5-cm-thick sections from 671.5 (ca. 11,510 cal yr BP) to 653.5 cm (ca. 10,620 cal yr BP) depth around the first peak and 13 samples were also collected every 0.5 to 1 cm in 0.5-cm-thick sections from 643 (ca. 10,230 cal yr BP) to 631 cm (ca. 9910 cal yr BP) depth around the second peak (Table 3).

249

The sediment samples were disaggregated in 30 ml of 5% sodium metaphosphate for 20 minutes before 30 ml of 6% bleach (sodium hypochlorite, NaClO) was added. The samples were soaked in this solution for another 24 hours to further deflocculate clays and oxidize organic matter. This procedure made the samples easier to analyze.

254

The samples were then gently washed through two sieves with mesh sizes of 180 and 250 µm (Whitlock and Larsen, 2001). The residues after sieving were subsequently placed in a gridded petri dish and examined under a stereomicroscope. Of the 39 samples examined under a stereomicroscope, 28 contained relatively large charcoal particles (>180 µm in diameter). A total of 243 particles were mounted on stubs for SEM observation.

#### 261 **3.1. Identification**

262

263 The classical anatomical criteria used to identify modern wood may also be applied to

- 264 charcoal (Marguerie and Hunot, 2007). The identification is based on diagnostic characters
- of the wood microstructure and comparison of fossil material with modern reference
- 266 collections (Trouy, 2015). Identification of charcoal requires examination of the three-
- 267 dimensional arrangement of the cell elements that comprise hardwood or softwood. The
- three anatomical planes of the ligneous structure are transverse (Tr), radial (R) and
- 269 tangential (Tg). The transverse plane is most easily discovered because of the visibility of the
- 270 growth rings. It also is the most important section for observing microscopic features and ring
- distinctness. Within a longitudinal section (i.e., the vertical orientation of the cells in tree
- trunk) are the radial and tangential planes. The radial plane is a vertical surface running from

the pith outwards towards the bark; the tangential plane is perpendicular to the rays, andtangential to the growth rings.

274 275

Given the small size of the charcoal particles (about 180-250 µm in diameter) preserved in the Blacktail Pond core, usual identification criteria were not systematically possible. The first step was to determine if a specimen was ligneous (woody) or not. This identification was done from exposed planar sections produced by natural breakage. Generally, the charcoal particles offered only one or two anatomical sections visible along the radial or tangential planes, and it was impossible to split the particle along a new surface. We had to work with the existing fractures to observe all the aspects of the wood microstructure.

Identification keys were consulted for the details of charcoal particle characteristics (e.g.,
pits, perforation plates). The more fragmented the particle was, the more difficult it was to
identify or even find diagnostic features for identification. In contrast, large "mesoscopic"
charcoal particles (180-500 µm) often contain two anatomical sections allowing distinction of
monocotyledons, dicotyledons and gymnosperms (Marquer et al., 2012). These longitudinal
sections (either radial or tangential) are particularly informative for identifying of some conifer
genera.

291

292 In addition, processes related to transport and deposition of charcoal in the lake lead to 293 fractures, deformities, and impurities that often obscure anatomical features. At Blacktail 294 Pond, a few mesoscopic charcoal particles were recovered, including isolated wood fibers 295 and vascular elements that enabled conifer identification. Attention to the anatomical criteria preserved on the longitudinal planes was critical, even when only a few bundles of fibers 296 297 were preserved. In conifers and broad-leaved trees and shrubs, axial tracheids and vessels 298 form the main part of the wood tissue and these groups cannot be separated. However, 299 longitudinal sections reveal different combinations of pits that are diagnostic.

300

301 All wood anatomical features preserved in the charcoal particles at Blacktail Pond were recorded according to the IAWA list of microscopic features for hardwood and softwood 302 identification (Wheeler et al., 1989; Richter et al., 2004). Taxonomic identification relied on 303 identification keys and illustrated atlases of microscopic thin sections (e.g. Schweingruber, 304 305 1990). To observe cross-field pitting in the radial section required high magnification, and the 306 charcoal particles were examined through a SEM using magnifications of up to 5000x to view 307 these very fine anatomical characters. The SEM sessions took place at the Montana State University (USA) and the University of Rennes 1 (France). Wood anatomical features were 308 309 compared with genera in the charcoal reference collection (Archeosciences Laboratory, CReAAH, CNRS, University of Rennes 1) and several wood anatomical atlases. 310

The low number of identified charcoal particles at Blacktail Pond (243 particles) did not allow a quantitative reconstruction, but rather documented the presence of some taxa and the type of vegetation burned. Charcoal accumulation rates (CHAR) were calculated for particles >125 in diameter and ranged from 0.2 to 2.6 particles cm<sup>-2</sup> yr<sup>-1</sup> (Krause and Whitlock, 2013). There were few particles in the >180 µm size fraction.

#### **318 3.2. Collection of fresh wood samples around the site**

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The characteristics of the fossil wood anatomy were checked against modern, charred, and wood specimens. In addition, a reference collection was also made of wood from living trees and shrubs in the vicinity of Blacktail Pond. Wood samples from several plants of each species were charred in an open furnace after drying at 50°C for 30 hours. After carbonization (at 350°C with the time depending on the size of the wood), three anatomical sections were obtained by scalpel or simply by breaking the charcoal by hand. The fragments of charred tissue were mounted on metal stubs for SEM examination. 328

### 329 4. Results and Discussion

330 331

332

#### 4.1 Wood anatomy of living trees and shrubs around Blacktail Pond

We collected modern wood specimens from three pines and four broad-leaved shrubs from 333 the northern Yellowstone region: Pinus albicaulis, P. contorta, P. flexilis, Artemisia tridentata, 334 335 and Sarcobatus vermiculatus. For these taxa, wood identifications were based on burned living woody plants. Other conifers were not sampled: Juniperus scopulorum, Picea 336 337 engelmannii, and Abies lasiocarpa. Information about the wood anatomy of the living conifers at the species or genus level, especially the unsampled taxa, was obtained from several 338 339 atlases (Panshin and de Zeeuw, 1980; Barefoot and Hankins, 1982; Hoadley, 1990). In 340 addition, we made use of the atlases of Schweingruber (1990) and Vernet et al. (2001), 341 although neither deals with western U.S. trees. The European collection of wood at the 342 laboratory of Rennes, France was also examined for this project. Two papers on 343 identification of North American wood species (Kukachka, 1960; Minnis, 1987) and a special issue of IAWA journal (Richter et al., 2004) were also consulted. For each charcoal type, 344 345 descriptions were made in accordance with the on-line InsideWood database (IAWA Committee, 1989; InsideWood, 2012). 346

347 249

348 4.1.1. Wood anatomy of the conifers

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The anatomical distinction between conifer (softwood) and angiosperm (hardwood) is straightforward. Conifer wood is less complex because tracheids function for both conduction and support, and their shape is rather different from that of hardwood vessels. Identification of different conifer taxa, however, is generally more difficult than for hardwoods (Kukachka, 1960). Wood anatomy criteria allow conifer identification to genus, but species-level identification is difficult or even impossible.

356

Among the conifer genera likely to be identified in the Blacktail Pond core, the primary diagnostic features was the presence or not of resin ducts, the height of rays, and the nature of cross-field pitting (i.e., pits intersecting walls of an axial tracheid and a ray parenchyma cell) (Table 1).

361

Five types of pits occur in the earlywood or springwood and vary in terms of frequency, arrangement, form and size (Ritcher et al., 2004; Schweingruber, 1990, 2011). The types range from a single large pit that occupies most of the area of the cross-field, as in the case of some pines, to very small pits that occupy only a small portion of the cross-field areas, as in *Picea*.

367

368 In the case of small charcoal particles where it is impossible to observe the three anatomical sections, the radial plane is often the most obvious one. It is also the crucial section for 369 discerning decisive features for the identification of conifer wood. For example, the 370 characteristics used to identify small fragments of Abies are the absence of resin canals, 371 372 transversal tracheids, nodular ray parenchyma, and the presence of two to four taxodioid pits 373 per cross-field. Crystals appearing in the form of regular prisms, cubes, or octahedra are 374 commonly found in Abies species (Schweingruber, 1990). In Juniperus wood, resin canals 375 and transversal tracheids are also absent, and key features are ray parenchyma that have abundantly nodular ends walls and cupressoid cross-field pitting. In Picea, resin canals are 376 377 surrounded by rather thick-walled epithelial cells, the ray tracheids are present with a smooth and wavy wall, ray parenchyma cell walls are thick, and cross-field pitting is piceoid-type 378 (Marguerie et al., 2000; Schweingruber, 1990). Crystals in ray cells occur occasionally in 379 Picea wood. Finally, small fragments of Pinus wood in radial section show abundant and 380 381 large resin canals with thin-walled epithelium cells, ray tracheids that are dentate to reticulate, and fenestriform or pinoid pits in the cross-fields (Table 1). 382

383 384 Species identification of *Pinus* is difficult or impossible. However, the genus can be subdivided into two groups on the basis of anatomical traits like ray cells, ray tracheids and 385 386 the type of cross-field pitting, all of which can be discerned under high magnification (Table 387 2). *Pinus contorta* wood has rays of medium height in tangential section (1 to 12 cells, mainly 388 5) (Fig. 4b), ray tracheids that are distinctly dentate to reticulate walls, and one to four, but generally two, pinoid ray pits per cross-field. Each pit has an irregular shape and size (Fig. 4c 389 390 and 4d). *Pinus albicaulis* wood has short rays with only one to ten cells (although sometimes more), with an average height of three to four cells (Fig. 4f). It also generally has one large, 391 simple, fenestriform (window-like) ray pit per cross-field, rarely two (Fig. 4g and 4h). Pinus 392 flexilis wood has short rays in tangential section with one to eight cells (three to four on 393 394 average) (Fig. 4i). Cross-field pitting is also fenestriform, with on average one or two pits per 395 earlywood cross-field (Fig. 4k and 4l). 396 397 Using these criteria, we were able to identify Pinus, Picea, and Juniperus or Abies genera in 398 the charcoal particles at Blacktail Pond. It was also possible to separate Pinus contorta from P. albicaulis and/or P. flexilis on the basis of the cross-field pitting and the walls of ray 399 400 tracheids. 401 402 4.1.2. Wood anatomy of the broad-leaved shrubs 403 404 In contrast to gymnosperms, the xylem of angiosperm woods contains vessels and is thus easy to distinguish from conifers by its more complex wood anatomy. Four common shrubs 405 found in the steppe vegetation of northern Yellowstone National Park were examined. 406 407 The wood of Artemisia tridentata, an Asteraceae shrub, is semi-ring porous (Fig. 5a and 5b) 408 and contains vessels with spiral thickenings and simple perforations. Rays are large (6-8 409 cells) and heterogenous (Fig. 5c and 5d). In the case of small charcoal particles in the 410 411 sedimentary record, the key feature was vessel walls: numerous, big, lentoid, with lined-up pits (Fig. 5e). 412 413 *Ericameria nauseosa*, another Asteraceae, has ring porous wood with large (5 to 11 cells) 414 (Fig. 5f, 5g and 5h), heterogenous (square cells) rays (Fig. 5j), and simple perforations in the 415 416 vessels (Fig. 5i). Vessel walls are adorned by rounded pits (Fig. 5i). 417 *Purshia tridentata*, a Rosaceae shrub, has a semi-ring porous wood (Fig. 5k and 5l) with thin 418 (1-3 cells) homogenous rays (Fig. 5m and 5n). Perforations inside the vessels are simple and 419 inter-vessel walls bear medium sized and few pits (Fig. 5m and 5n). 420 421 Sarcobatus vermiculatus (greasewood), an Amaranthaceae shrub, has semi-ring porous 422 wood (Fig. 50), thin rays (1 cell) (Fig. 5p), simple perforations in vessels (Fig. 5r and 5s), and 423 rounded pits frequently organized into lenticular openings (Fig. 5q, 5r and 5s). 424 425 So, in the case of the wood of broad-leaved shrubs near Blacktail Pond, the density, 426 427 arrangement, shape, and size of vessel pits were diagnostic features for identification. 428 4.2. Charcoal particle analysis in the Blacktail Pond sediment core 429 430 Charcoal >180 um in diameter was present in low amounts and variable condition and 431 432 comprised 28 of the 243 samples. Despite the small size of most particles, wood anatomy was often well preserved. Most of the charcoal particles were pieces of burned wood (67.1% 433 of wood + herb), and some could be identified to genus (11.4%). Samples from the two large 434 CHAR fire peaks had the most identifiable particles and also were the most taxonomically 435 436 diverse. These two fire episodes suggest large fires or fires near the lake over a short period

of time. Samples from the period before the first peak that dated from ca. 11,350 to 11,170
cal yr BP also contained abundant and diverse wood (Table 3).

439440 4.2.1. Remains of monocotyledons

Charred remains of herbs, including parenchymatous organs that resembled stems, were
commonly observed in three-dimensional views under high-magnification SEM (Fig. 6a to 6f)
(Hather, 1993). Some elements were identified as stems when viewed in trilobal crosssection (Fig. 6b) and laterally (Fig. 6a and 6d).

The vascular tissue or xylem of herbs was preserved, almost intact and without serious distortion in some specimens. Phloem was also recognized by its position relative to the xylem. In Figure 6b, the phloem is internal and forms a cylinder surrounded by a crown of xylem. The xylem elements have a polygonal transversal section (Fig. 6c). Sometimes, deformation of the xylem revealed its staged elements laterally (Fig. 6e). Figure 6d shows stomata in the lower epidermis of an aerial stem.

453454 4.2.2. Remains of broad-leaved taxa

Among the burned wood of broad-leaved shrubs in the Blacktail Pond record, two specimens
(at ca. 10,970 and 10,185 cal yr BP) had well preserved vessels and wall ornamentation
similar to the pitting structure observed in *Artemisia tridentata* wood (Fig. 5e). Numerous
lined-up, lentoid pits were easily distinguished from other plant tissue (Fig. 6g, 6h, 6i).

- 460 461 Several vessel wall pits were very simple and could not be identified (Fig. 6j, 6k, 6l).
- 462

463 4.2.3. Remains of conifers

464

The presence of vertical or longitudinal tracheids with uniseriate (very common) or biseriate opposite big bordered pits was characteristic of conifer wood (Fig. 7a to 7e). Small charcoal particles with tangential sections showed resin canals with epithelial cells and high rays (Fig. 7f, 7g). Some tracheids had helical cracks in their walls, which is a common feature of compression wood in conifers.

470

The radial section was best for distinguishing among conifers. This plane is achieved through 471 natural breakage along the rays and was found in several charcoal fragments from Blacktail 472 473 Pond. Pinus species were identified by the cross-field ray pits. Two types of pits were observed in the Pinus wood. The first type was fenestriform pitting, which is window-like, with 474 475 a large, simple ray pit occupying almost the entire cross-field. Rarely do wood fragments have two fenestriform pits per cross-field (Fig. 7h, 7i, 7j). However, in the Yellowstone region 476 *Pinus albicaulis* and/or *P. flexilis* share this feature (Fig. 4g, 4h, 4k, 4l). The second type, 477 pinoid pitting, is made by ray pits that are simple and more or less large. Usually two to four 478 479 pinoid ray pits can be observed per cross-field. This type of pitting is characteristic of *Pinus* 480 contorta (Fig. 7k, 7l, 7m).

- 481
- Wood fragments with ray tracheids and without fenestriform or pinoid pits in the cross-fields were assigned to *Picea* (Fig. 7d, 7n).
- 484

A third type of cross-field ray pits was taxodioid: large pit aperture with narrow border. When charcoal particles had such pits and no tracheids, we assigned them to either *Abies* or *Juniperus* (Fig. 70 to 7r).

- 487
- In conifer tracheids, two types of cell contents were present, one of which forms pyramidal crystals or druses (Fig. 7s, 7t). This common feature was not diagnostic.
- 491

#### 492 4.2.4. Unidentifiable charcoal

493

16.5% of charcoal particles in the large size range (180 - 250 µm) lacked any discernible
cellular structure. Remains of leaves were often found as part of charred plant tissues
(11.5%), and hairs (trichomes) and stomata were also observed (Fig. 6m, 6n, 6o). Similarly,
small fragments of vesicular charcoal were noted and could have come from any
parenchymous tissue.

499 500

#### 501 **4.3. Charcoal analysis results**

502 503 The charcoal particles that could be identified include *Pinus*; *Picea*; *Abies* or *Juniperus*; a 504 deciduous shrub, most likely Artemisia; and unidentifiable monocotyledons. Pinus albicaulis 505 and/or *P. flexilis* (these species cannot be distinguished by their pollen) was present in the vegetation at ca. 11,350 cal yr BP, during a time of mixed-conifer forest (Table 3). P. 506 507 albicaulis and/or P. flexilis charcoal was also noted at ca. 9980 cal yr BP, when these taxa were less abundant locally and may have come from more distant fires. The presence of 508 509 Pinus contorta charcoal at ca. 11,180 cal yr BP aligns well its rising abundance in the pollen record. P. contorta and/or Picea were also identified in three charcoal samples dating to ca. 510 10,780 cal yr BP. Artemisia charcoal was identified at ca. 10,990, 10,180, and tentatively at 511 512 9920 cal yr BP, but shrub charcoal was present throughout the record, as was charcoal from herbaceous taxa. The presence of both hardwood and softwood charcoal suggests that most 513 fires burned both forest and steppe. However, remains of hardwood and monocotyledons 514 comprised most of the charcoal from two samples dating to ca. 10,650 cal yr BP, suggesting 515 516 that steppe was the main fuel at that time.

517

It is interesting that the first large fire episode at 10,970 cal yr BP consisted of burned conifers (*Picea, Abies* and/or *Juniperus*), shrubs and herbs, but no *Pinus* charcoal was identified. This assemblage is consistent with an open mixed-conifer forest, in which pine was not dominant. The second fire episode at 10,200 cal yr BP contained shrub and herb charcoal and no conifer charcoal, implying a local fire that primarily burned steppe vegetation around the lake (Table 3).

524

525 Two centuries before the first fire peak (from ca. 11,150 to 10,970 cal yr BP) and one century 526 after the second fire peak (from ca. 10,200 to 10,085 cal yr BP), the cores contained no 527 charred macroscopic plant remains.

528 529

#### 530 **5. Conclusion and perspectives**

531

532 The Blacktail Pond study contributes new information to our understanding of the fire history of northern Yellowstone, and the development of vegetation in the northern Rocky Mountains 533 during the late-glacial and early-Holocene periods. The pollen and charcoal records show 534 that increased fire activity played a significant role in the transition from late-glacial Picea 535 536 parkland to early-Holocene vegetation of *Pinus contorta* forest and steppe. The charcoal 537 identifications enrich this reconstruction by showing that large fire episodes may have 538 facilitated the transition. In the case of the fire at ca. 10,970 BP, conifers and shrubs were 539 both fuel types near the lake. The second fire episode at ca. 10,200 cal yr BP, the presence 540 of hardwood charcoal and no softwoods implies the development of steppe vegetation near 541 the site with conifers restricted to more distant settings on rocky buttes and at higher elevations. The second fire must have been fairly local but probably intense to produce so 542 543 much charcoal. Another interesting insight is the presence of *Pinus contorta* charcoal as early as ca. 11,180 cal yr BP, even before it became the dominant vegetation type. Fire and 544 545 P. contorta are closely linked at present, given its ability to rapidly regenerate after fire. The

record at Blacktail Pond suggests that fire facilitated its increasing dominance in the earlyHolocene.

548

549 This study shows that it is possible to identify a part of the very small charcoal remains 550 preserved in the lake sediments with SEM identification. The results provide unique insights 551 as to the fuel composition. The next phase of research will focus on examining charcoal from 552 other sites in the Yellowstone region to see if comparable shifts in fuel were occurring at 553 times of changing climate, vegetation, and fire activity.

554

#### 555 **5.1. Perspectives on the identification of very small fragments of burnt wood**

- Identification of charcoal fragments is a valuable tool in reconstructing past environments
  and determining disturbances. This paper illustrates the potential for the identification of
  charcoal particles to be part of paleoenvironmental reconstructions. We believe that this
  approach deserves consideration in other settings.
- 561

The analysis of charcoal in lake or wetland sediment cores is challenged by the smallness and relative scarcity of suitable material as well as preservation issues related to charcoal transport and deposition into such sites. These data complement other quantitative proxies in building an environmental reconstruction (e.g., pollen, macroscopic charcoal, lithologic and geochemical data).

567

568 Other techniques can improve the results in this study. Reflected Light Microscopy (RLM) is 569 used to identify sizable and numerous charcoal fragments by studying fresh break surfaces 570 (Marguerie and Hunot, 2007). In case of very small fragments, Scanning Electron 571 Microscopy (SEM) is best suited for detailed observation of small irregular surfaces with high 572 relief. The increasing number of tabletop SEMs in laboratories now enables this type of 573 analysis at high magnifications and great depth of field.

574

575 Because it is difficult to make transversal or longitudinal sections by breaking, splitting or 576 slicing to observe wood anatomical features on small charcoal particles, High-Throughput Xray Computed Tomography (HT-µCT) also opens up new opportunities to reveal fine 577 578 anatomical characters. It has been shown to be effective in imaging anatomical features that 579 cannot be observed on exposed surfaces (Haneca et al., 2012). The ability to yield images of 580 internal wood structure without having to break or cut the sample is a powerful tool to identify diagnostic features (3D observation) (Haneca et al., 2012). The technique allows the 581 582 production of unlimited virtual sections and clean digital cuts in any direction on very small samples (Van den Bulcke et al., 2009; Hubau et al., 2013). The resolution obtained is about 583 584 2 µm, which is sufficient to reveal many features of interest for wood anatomy (Mayo et al., 2010). However, the resolution of HT-µCT is not as high as with SEM, and fine details in the 585 586 vessels and fibres, and pitting may not be visible (Hubau et al., 2013). Another related technique, nanotomography or X-ray nano Computed Tomography (nanoCT), offers higher 587 resolution and is also excellent for seeing features that are not clearly visible under SEM 588 because they are concealed by coatings or mineral deposits (Van den Bulcke et al., 2009; 589 590 Hubau et al., 2013).

- 591
- 592 593
- 594

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596

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- 607 -----
- 608

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- examination of modern charcoal accumulation in Yellowstone National Park, USA. The 730 731 Holocene. 6, 7-15.
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- 733 -----
- 734

#### Figure captions 735

- 736 Figure 1. Location of Blacktail Pond.
- Figure 2. Pollen diagram from Blacktail Pond (ex. Krause and Whitlock, 2013) 737
- Figure 3. Fire proxies at Blacktail Pond (ex. Krause and Whitlock, 2013) 738

- Figure 4. Scanning electron microphotographs of burnt wood of three pine species in the
- northern Rocky Mountains: a. Pinus contorta, transverse section; b. P. contorta, tangential
- section; c, d. P. contorta, radial section; e. P. albicaulis, transverse section; f. P. albicaulis,
- tangential section; g, h. P. albicaulis, radial section; i. P. flexilis, transverse section; j. P.
- 743 flexilis, tangential section; k, l. P. flexilis, radial section
- Figure 5. Scanning electron microphotographs of burnt wood of four shrubs in the northern
- Rocky Mountains: a, b. Artemisia tridentata, transverse section; c. A. tridentata, tangential
   section; d, e, A. tridentata, radial section; f, g. Ericameria nauseosa, transverse section; h. E.
- 747 nauseosa, tangential section; i, j. E. nauseosa, radial section; k, l. Purshia tridentata,
- transverse section; m. P. tridentata, tangential section; n. P. tridentata, radial section; o.
- 749 Sarcobatus vermiculatus, transverse section; p, q, r. S. vermiculatus, tangential section; s. S.
- 750 vermiculatus, radial section
- 751 Figure 6. Charred remains of monocotyledons, broadleaf woods and leafs from Blacktail
- Pond by a scanning electron microscope: a. Stem of monocotyledon; b, c. Trilobal cross-
- section stem of monocotyledon with visible phloem and xylem; d, e. Details of xylem
- elements of stem; f. Xylem and phloem in longitudinal section; g, h, i. Particular pits of the
- vessel walls Artemisia sp.; j, k, i. Simple pits of the vessel walls in unidentifiable broadleaf; m,
- n, o. Fragments of leaves with hairs (trichomes) and stomata
- 757 Figure 7. Charred remains of conifers from Blacktail Pond
- 758 a, b, c, d, e. Woody fragments of conifers; d, n. wood fragment of conifer with ray tracheids; f.
- 759 Tangential section showing low rays of conifer; g. Possible resin canal and rays in tangential
- section; h, i, j. Pinus with fenestriform cross-field pitting; k, l, m. Pinus with pinoid cross-field
- *pitting; o, p, q, r. Conifer with taxodioid cross-field pits; s. Crystals in tracheids; t. Other type*
- 762 of crystals in tracheids
- Table 1. Main wood anatomical features of the common genera of conifers of the northernRocky Mountains around Blacktail Pond
- Table 2. Main key characteristics of microscopic anatomical features in the wood of threenorthern Rocky Mountains pines around Blacktail Pond
- 767 Table 3. Results from charcoal analyses of the sediment of Blacktail Pond

768



Artemisia tridentata



Ericameria nauseosa



Purshia tridentata



Sarcobatus vermiculatus





7/1/13 12kV 550X WD39 661-661.5

X WD39 BTP 666-666\_5 4A ⊢

⊢−−− 50 µm −−−−

- No conflict of interest regarding this work on "What was burning in the past? Charcoal identification supplements early-Holocene fire-history reconstruction in Yellowstone National Park, USA".
- Dr. Dominique Marguerie, Dr. Teresa Krause, Dr. Cathy Whitlock

6

- 1 All the authors signing this paper "Charcoal identifications supplement an early-Holocene
- 2 fire-history reconstruction in Yellowstone National Park, USA" declare that they approve the
- 3 submission of their work to *Quaternary International*.
- 4
- 5 Dr. Dominique Marguerie, dominique.marguerie@univ-rennes1;fr
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8

Wood anatomical features		Abies sp.	Juniperus sp.	Picea sp.	Pinus sp.			
Resin canals	Presence	No	No	Yes surrounded by rather thick-walled epithelial cells	Yes with thin-walled epithelium cells			
Rays	Tracheids			with smooth, wavy walls	with dentate to reticulate walls			
	Height (nb of cells)	15 to 25	2 to 5 (less than 15)	10 -15 cells, upper limit 25	8 to 15 cells			
Cross-field pitting	Fenestriform or "window like"				Х			
	Pinoid				Х			
	Piceoid			Х				
	Cupressoid		Х					
	Taxodioid	2 to 4	Χ?					

Wood anato	omical features	Pinus albicaulis	P. contorta	P. flexilis
Day calla	Height	1 to 10	1 to 12	1 to 8
Ray cells	(nb of cells)	(average 3-4)	(average 5)	(average 3-4)
Ray t	racheids	Yes	Yes with dentate to reticulate walls	Yes
One of field	Fenestriform	1 manaly 0		1 or 2 frequently
Cross-field	or "window like"	Trarely Z		2
pitting	Pinoid		1 to 4 frequently 2	

Pollen analysis data	Depth (cm)	Age BP	Pinus	Picea	Abies/Juniperus	Indistinct Conifer	Artemisia-type	Indistinct Broadleaf	Broadleaf hardwood	Undifferentiated wood	Herb / Monocotyledon	Leaf	Non-identifiable charred rest	Unburned rest
	631 - 631.5	9914									4	1		1
st.	631.5 - 632	9920					1?		1	2	6	3	3	
	632 - 632.5	9938				1 (Pinus/Picea no Abies/Juniperus)				3	2	1	1	
ore	632.5 - 633	9952				,						2		
efe	633.5 - 634	9977	1 (P. albicaulis-type)			1				1	1	1		
Pin	635 - 635.5	10017	, , , , , , , , , , , , , , , , , , , ,					1	1	1	3	2		
<del>D</del>	637.5 - 638	10085												Х
OSe	638 - 638.5	10099												Х
ō	639 - 639.5	10128												Х
	640 - 640.5	10157												Х
	641 – 641.5	10186					1	1	2	1	1	1	1	
Second fire peak	641.5 - 642	10200									4	5		
	642.5 - 643	10233									2	1	2	
	653.5 - 654	10625						1	1	1	2		<u> </u>	
st	654 - 654.5	10646				1		1	1	1	3	4	1	
ore	654.5 - 655	10667						8	2	2		2	3	
e T	655 - 655.5	10688				2 (Pinus/Picea no Abies/Juniperus)		1	1	1				
Pin	655.5 - 656	10711									5		2	
р р	656.5 - 657	10755	2 (P. contorta-type)			4				2			10	
OSe	657 - 657.5	10778	1 (P. contorta-type?)	1		1 (Pinus/Picea no Abies/Juniperus)				3			2	
ŏ	657.5 - 658	10801	1 (P. contorta-type)			1				1				
	658 - 658.5	10824								1	4		1	
	658.5 – 659	10848												Х
	659.5 - 660	10896								1	3			
	660 - 660.5	10920			1	1				1			<b> </b> '	
First fire peak	661 – 661.5	10970			1	2	1		1	1		$\perp$	<b> </b> '	<u> </u>
	661.5 - 662	10995												X
Swing Picea/Pinus	662 - 662.5	11020						1	1	1		1	<b> </b> '	
Pine forest	663.5 - 664	11098										<u> </u>	<b>_</b> '	<u> </u>
	664 - 664.5	11124											<b> </b> '	X
	664.5 - 665	11150										$\perp$	<b> </b> '	X
	665 - 665.5	11177	1 (P. contorta-type)			7		1	1	1	8	1	8	
	665.5 - 666	11204							7			1	<b> </b> '	<u> </u>
	666 - 666.5	11231				1				2	2			
	667 - 667.5	11285												X
	668 - 668.5	11340	1 (P.albicaulis-type)			2		5	2	1	4	2	5	
	669 - 669.5	11397						3	1	2	2		1	
Increased fire activity	670 - 670.5	11454				3				1				
Spruce forest peak	671 - 671.5	11511												X

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