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

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Abstract:

Analysis of charcoal particles preserved in lake sediments has been frequently used to reconstruct fire, vegetation and climate history. Larger macroscopic particles (>125 µm) are attributed to local fires, whereas microscopic charcoal particles (<125 µm), observed on pollen slides, are a signal of regional fire. Taxonomic identification of the charcoal particles 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 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 National Park. Blacktail Pond (44.954°N, 110.604°W; 2012 m elev) is located in steppe vegetation surrounded by conifer forest. Previous studies of the site have presented a multi-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 were taken at 0.5 cm intervals to examine macroscopic charcoal particles, with attention on two large charcoal peaks at 10,970 and 10,200 cal yr BP that registered large or near-site 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 (*Pinus*, *Picea*, and *Abies* or *Juniperus*), *Artemisia* and unidentified deciduous shrubs, and 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 previous pollen interpretations at Blacktail Pond that indicate an open mixed-conifer forest in the early Holocene prior to 10,750 cal yr BP, replaced by *Pinus contorta* forest on the slopes and steppe in the valley. The relative dominance of *Pinus contorta*-type charcoal points to the importance of fire as a catalyst for the spread of this species.

Keywords: charcoal, wood anatomy, fire, early-Holocene, vegetation history, Yellowstone National Park, USA

1. Introduction

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).

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

information also provides better understanding of vegetation dynamics during times of climatic transition.

Following Jones et al. (1997), the term “charcoal” is assigned here to all burned or charcoaled (charred) plant remains produced during fires, not only woody fragments. 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 angular fragments that often show plant structures.

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

The external morphology (size and shape) of macroscopic charcoal has been explored using automated image analysis under a (stereo)microscope. Umbanhowar and McGrath (1998) examined the area, length, and width of charcoal particles as an indicator of burned vegetation type. They experimentally produced charcoal of wood, leaves and grasses by burning samples in open fires and in a muffle furnace at 350°C . They distinguished these three types of charcoal based on variations in length (along the major axis, μm), width (along the minor axis, μm) and area (μm^2) of the particles. The elongation metric R (L/W) enabled 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 or wood charcoal. The anatomical structure of plant and the degree of elongation was preserved even when the particle was broken, enabling separation of charcoal from deciduous versus herbaceous taxa. This morphological approach was a reasonable tool for determining the nature of the burned vegetation.

Enache and Cumming (2006) analyzed charcoal particles from historic fires dating back to 1919 that occurred within a 20-km radius of Prosser Lake (British Columbia, Canada). The authors observed the morphology of charcoal particles $>150\ \mu\text{m}$ diameter from a sediment core to infer the fuel type (bark, trunk, branch, leaf), temperature of formation, and 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 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 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 fragments. Another type, M, was characterized by its irregular shape, its fragility and high porosity, and was attributed to intense crown-fire burning of branches or leaves. Type M charcoal in the core accurately matched the timing of known fires and was considered the “most accurate record of fire events”. Conversely, other types of charcoal (types B, C, M, P, S) were thought to have been produced by older fires and redeposited or transported from 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 taxonomic information.

Soil charcoal research or pedoanthracology focuses on large burned pieces $>0.5\ \text{mm}$ 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 (Carcaillet and Thion, 1996; Nelle et al., 2013; Robin et al., 2013). Identification rarely

includes the charcoal preserved in lakes or wetlands, because these sedimentary particles 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 (*Quercus* sp., *Pinus sylvestris*-type, *Salix* sp., *Betula* sp., Ericaceae) (Whitlock et al., 2006). In a study of Holocene fire history, Olsson et al. (2009) identified charcoal particles from a 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 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. These charcoal identifications offered a partial list of fuels because a high quantity of the particles were unidentifiable.

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 Yellowstone National Park, USA. Blacktail Pond (44.954°N, 110.604°W; 2012 m elevation) is 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 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 late-glacial to early Holocene period, as part of a multi-proxy investigation of environmental history.

The period of interest for the present study is the early Holocene from 11,500 to 9900 cal yr BP at Blacktail Pond when summers were becoming progressively warmer and drier as a result of increasing summer insolation (Krause and Whitlock, 2013). Early postglacial 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 (Krause and Whitlock, 2013). In this paper, we describe charcoal particle identifications that 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?

2. Study area

2.1. Current vegetation and fire regimes

The vegetation of northern Yellowstone is strongly influenced by elevation, aspect and geology (Despain, 1990). Today, grassland and steppe communities dominated by *Artemisia tridentata* (big sagebrush), *Festuca idahoensis*, *Ericameria nauseosa* (rabbitbrush), *Sarcobatus vermiculatus* (greasewood, on saline soils), and various herbs are present 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 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 elevation) and *Pinus contorta* (2000-2400 m elevation) are present at middle to high elevations. *Picea engelmannii* (Engelmann spruce), *Abies lasiocarpa* (subalpine fir), and *Pinus albicaulis* (whitebark pine) dominate high elevation forests and parkland (2400-2900 m elevation). *Juniperus communis* is present in the understory at all elevations.

Blacktail Pond lies within calcareous glacial outwash and is surrounded by *Artemisia 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.

Fires, both lightning and human caused, are common in western U.S. forests, and Yellowstone National Park became internationally famous for large fires in 1988 that burned 3213 km², or about 40% of the park (Schullery, 1989). Regional studies, based on lake-sediment studies, indicate that fire activity increased during the late-glacial and early Holocene periods and then decreased after ca. 9000 and again at 5000 cal yr BP (Iglesias et al., 2018). The conifers of the region have different adaptations to fire that allow for their survival. For example, the thick bark of *Pseudotsuga* enables these trees to survive low-intensity surface fires. Similarly, *Pinus flexilis* and *P. albicaulis* tolerate surface fires as the trees mature and their bark thickens. *P. contorta* is adapted to high-severity fires, because, even though the trees are killed, its serotinous cones open immediately and release 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 infrequent crown fires, and regeneration occurs by seed (Knight et al., 2014).

2.2. Lithology and chronology

This study used core BTP08B of Krause and Whitlock (2013), which was collected at the western margin of the lake from the fen. The sediment from 5.25 m to 8.10 m depth was the 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 tephra ages (Krause and Whitlock, 2013). The environmental reconstruction was based on pollen, geochemical properties (elemental geochemistry, oxygen isotopes from authigenic carbonates), magnetic susceptibility, and macroscopic charcoal. The pollen and charcoal data and radiocarbon ages are available at Neotomadb.org.

The original charcoal analysis focused on particles >125 µm in diameter to capture high-severity fires occurring within a few kilometers of the lake (Krause and Whitlock, 2013; Huerta et al., 2009). Charcoal accumulation rates (CHAR; particles cm⁻² yr⁻²) were calculated using CharAnalysis (Higuera et al., 2008). Charcoal concentrations and deposition times were interpolated into contiguous 25-yr bins (the median resolution of the record), and CHAR was determined by dividing the time-interpolated concentrations. Long-term trends in CHAR (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 (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 95th percentile of the noise distribution (Higuera et al., 2010). These peaks are interpreted as fire episodes (one or more fires during the time span of the peak).

2.3. Previous information on fire and vegetation dynamics

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 was a period of cooler-than-present summer conditions, but temperatures were steadily increasing with rising summer insolation. The postglacial landscape was initially sparsely vegetated with little soil development. After ca. 12,900 cal yr BP, conditions were warm enough for *Picea engelmannii* to form a parkland at the site, and after ca. 12,500 cal yr BP, *Picea*, *Abies*, and *Pinus* (both *P. contorta* and *P. albicaulis/flexilis* pollen types) were present in open mixed-conifer forest communities. Beginning at ca. 11,500 cal yr BP, the carbonate

$d^{18}O$ 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, *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.

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).

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).

3. Methods

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 cm³ 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).

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.

The samples were then gently washed through two sieves with mesh sizes of 180 and 250 μ m (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 μ m in diameter). A total of 243 particles were mounted on stubs for SEM observation.

3.1. Identification

The classical anatomical criteria used to identify modern wood may also be applied to charcoal (Marguerie and Hunot, 2007). The identification is based on diagnostic characters of the wood microstructure and comparison of fossil material with modern reference collections (Trouy, 2015). Identification of charcoal requires examination of the three-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 tangential (Tg). The transverse plane is most easily discovered because of the visibility of the 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, and tangential to the growth rings.

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.

In addition, processes related to transport and deposition of charcoal in the lake lead to fractures, deformities, and impurities that often obscure anatomical features. At Blacktail Pond, a few mesoscopic charcoal particles were recovered, including isolated wood fibers 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 were preserved. In conifers and broad-leaved trees and shrubs, axial tracheids and vessels form the main part of the wood tissue and these groups cannot be separated. However, longitudinal sections reveal different combinations of pits that are diagnostic.

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 identification (Wheeler et al., 1989; Richter et al., 2004). Taxonomic identification relied on identification keys and illustrated atlases of microscopic thin sections (e.g. Schweingruber, 1990). To observe cross-field pitting in the radial section required high magnification, and the charcoal particles were examined through a SEM using magnifications of up to 5000x to view 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 compared with genera in the charcoal reference collection (Archeosciences Laboratory, CReAAH, CNRS, University of Rennes 1) and several wood anatomical atlases.

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 μm in diameter and ranged from 0.2 to 2.6 particles $\text{cm}^{-2} \text{yr}^{-1}$ (Krause and Whitlock, 2013). There were few particles in the >180 μm size fraction.

3.2. Collection of fresh wood samples around the site

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.

4. Results and Discussion

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 the northern Yellowstone region: *Pinus albicaulis*, *P. contorta*, *P. flexilis*, *Artemisia tridentata*, and *Sarcobatus vermiculatus*. For these taxa, wood identifications were based on burned living woody plants. Other conifers were not sampled: *Juniperus scopulorum*, *Picea 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 atlases (Panshin and de Zeeuw, 1980; Barefoot and Hankins, 1982; Hoadley, 1990). In addition, we made use of the atlases of Schweingruber (1990) and Vernet et al. (2001), although neither deals with western U.S. trees. The European collection of wood at the laboratory of Rennes, France was also examined for this project. Two papers on 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, descriptions were made in accordance with the on-line InsideWood database (IAWA Committee, 1989; InsideWood, 2012).

4.1.1. Wood anatomy of the conifers

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.

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).

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*.

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 discerning decisive features for the identification of conifer wood. For example, the characteristics used to identify small fragments of *Abies* are the absence of resin canals, transversal tracheids, nodular ray parenchyma, and the presence of two to four taxodioid pits per cross-field. Crystals appearing in the form of regular prisms, cubes, or octahedra are commonly found in *Abies* species (Schweingruber, 1990). In *Juniperus* wood, resin canals 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 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 (Marguerie et al., 2000; Schweingruber, 1990). Crystals in ray cells occur occasionally in *Picea* wood. Finally, small fragments of *Pinus* wood in radial section show abundant and 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).

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 the type of cross-field pitting, all of which can be discerned under high magnification (Table 2). *Pinus contorta* wood has rays of medium height in tangential section (1 to 12 cells, mainly 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 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, simple, fenestriform (window-like) ray pit per cross-field, rarely two (Fig. 4g and 4h). *Pinus flexilis* wood has short rays in tangential section with one to eight cells (three to four on average) (Fig. 4j). Cross-field pitting is also fenestriform, with on average one or two pits per earlywood cross-field (Fig. 4k and 4l).

Using these criteria, we were able to identify *Pinus*, *Picea*, and *Juniperus* or *Abies* genera in 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 tracheids.

4.1.2. Wood anatomy of the broad-leaved shrubs

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 found in the steppe vegetation of northern Yellowstone National Park were examined.

The wood of *Artemisia tridentata*, an Asteraceae shrub, is semi-ring porous (Fig. 5a and 5b) and contains vessels with spiral thickenings and simple perforations. Rays are large (6-8 cells) and heterogenous (Fig. 5c and 5d). In the case of small charcoal particles in the sedimentary record, the key feature was vessel walls: numerous, big, lentoid, with lined-up pits (Fig. 5e).

Ericameria nauseosa, another Asteraceae, has ring porous wood with large (5 to 11 cells) (Fig. 5f, 5g and 5h), heterogenous (square cells) rays (Fig. 5j), and simple perforations in the vessels (Fig. 5i). Vessel walls are adorned by rounded pits (Fig. 5i).

Purshia tridentata, a Rosaceae shrub, has a semi-ring porous wood (Fig. 5k and 5l) with thin (1-3 cells) homogenous rays (Fig. 5m and 5n). Perforations inside the vessels are simple and inter-vessel walls bear medium sized and few pits (Fig. 5m and 5n).

Sarcobatus vermiculatus (greasewood), an Amaranthaceae shrub, has semi-ring porous wood (Fig. 5o), thin rays (1 cell) (Fig. 5p), simple perforations in vessels (Fig. 5r and 5s), and rounded pits frequently organized into lenticular openings (Fig. 5q, 5r and 5s).

So, in the case of the wood of broad-leaved shrubs near Blacktail Pond, the density, arrangement, shape, and size of vessel pits were diagnostic features for identification.

4.2. Charcoal particle analysis in the Blacktail Pond sediment core

Charcoal >180 µm in diameter was present in low amounts and variable condition and 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% of wood + herb), and some could be identified to genus (11.4%). Samples from the two large CHAR fire peaks had the most identifiable particles and also were the most taxonomically 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).

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 cross-section (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.

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).

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

4.2.3. Remains of conifers

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.

The radial section was best for distinguishing among conifers. This plane is achieved through natural breakage along the rays and was found in several charcoal fragments from Blacktail 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 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 *Pinus albicaulis* and/or *P. flexilis* share this feature (Fig. 4g, 4h, 4k, 4l). The second type, pinoid pitting, is made by ray pits that are simple and more or less large. Usually two to four pinoid ray pits can be observed per cross-field. This type of pitting is characteristic of *Pinus contorta* (Fig. 7k, 7l, 7m).

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

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. 7o to 7r).

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.

4.2.4. Unidentifiable charcoal

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.

4.3. Charcoal analysis results

The charcoal particles that could be identified include *Pinus*; *Picea*; *Abies* or *Juniperus*; a deciduous shrub, most likely *Artemisia*; and unidentifiable monocotyledons. *Pinus albicaulis* 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. 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 *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. 10,780 cal yr BP. *Artemisia* charcoal was identified at ca. 10,990, 10,180, and tentatively at 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 fires burned both forest and steppe. However, remains of hardwood and monocotyledons comprised most of the charcoal from two samples dating to ca. 10,650 cal yr BP, suggesting that steppe was the main fuel at that time.

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).

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

5. Conclusion and perspectives

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 during the late-glacial and early-Holocene periods. The pollen and charcoal records show that increased fire activity played a significant role in the transition from late-glacial *Picea* parkland to early-Holocene vegetation of *Pinus contorta* forest and steppe. The charcoal identifications enrich this reconstruction by showing that large fire episodes may have facilitated the transition. In the case of the fire at ca. 10,970 BP, conifers and shrubs were both fuel types near the lake. The second fire episode at ca. 10,200 cal yr BP, the presence of hardwood charcoal and no softwoods implies the development of steppe vegetation near 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 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 *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 early Holocene.

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

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.

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).

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

Because it is difficult to make transversal or longitudinal sections by breaking, splitting or slicing to observe wood anatomical features on small charcoal particles, High-Throughput X-ray Computed Tomography (HT- μ CT) also opens up new opportunities to reveal fine anatomical characters. It has been shown to be effective in imaging anatomical features that cannot be observed on exposed surfaces (Haneca et al., 2012). The ability to yield images of 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 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 2 μ m, which is sufficient to reveal many features of interest for wood anatomy (Mayo et al., 2010). However, the resolution of HT- μ CT is not as high as with SEM, and fine details in the 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 resolution and is also excellent for seeing features that are not clearly visible under SEM because they are concealed by coatings or mineral deposits (Van den Bulcke et al., 2009; Hubau et al., 2013).

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734
735 **Figure captions**

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

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. 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. 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. *Sarcobatus vermiculatus*, transverse section; p, q, r. *S. vermiculatus*, tangential section; s. *S. vermiculatus*, radial section

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, l. Simple pits of the vessel walls in unidentifiable broadleaf; m, n, o. Fragments of leaves with hairs (trichomes) and stomata

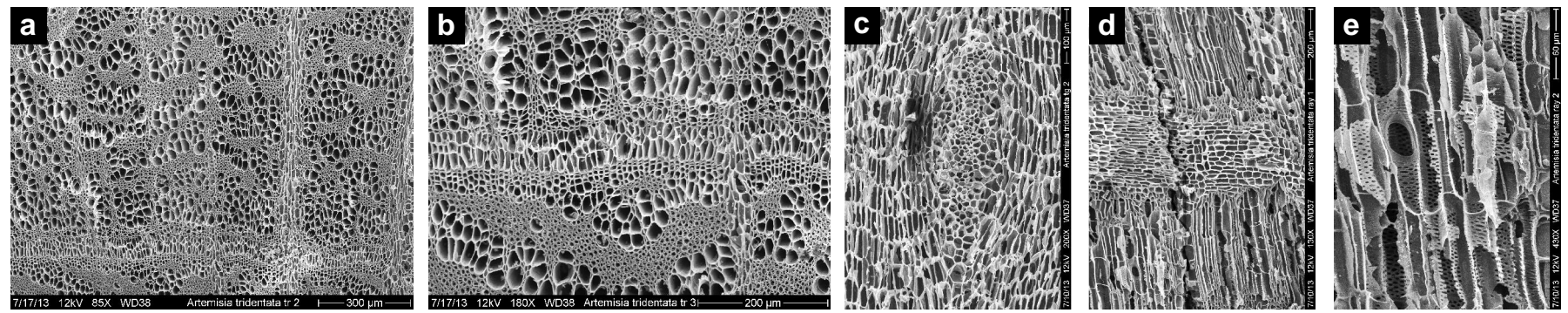
Figure 7. Charred remains of conifers from Blacktail Pond
a, b, c, d, e. Woody fragments of conifers; d, n. wood fragment of conifer with ray tracheids; f. Tangential section showing low rays of conifer; g. Possible resin canal and rays in tangential section; h, i, j. *Pinus* with fenestriform cross-field 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 of crystals in tracheids

Table 1. Main wood anatomical features of the common genera of conifers of the northern Rocky Mountains around Blacktail Pond

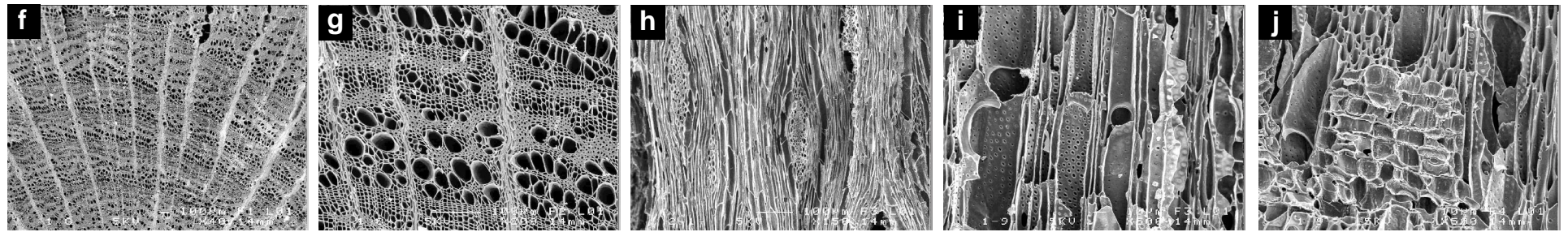
Table 2. Main key characteristics of microscopic anatomical features in the wood of three northern Rocky Mountains pines around Blacktail Pond

Table 3. Results from charcoal analyses of the sediment of Blacktail Pond

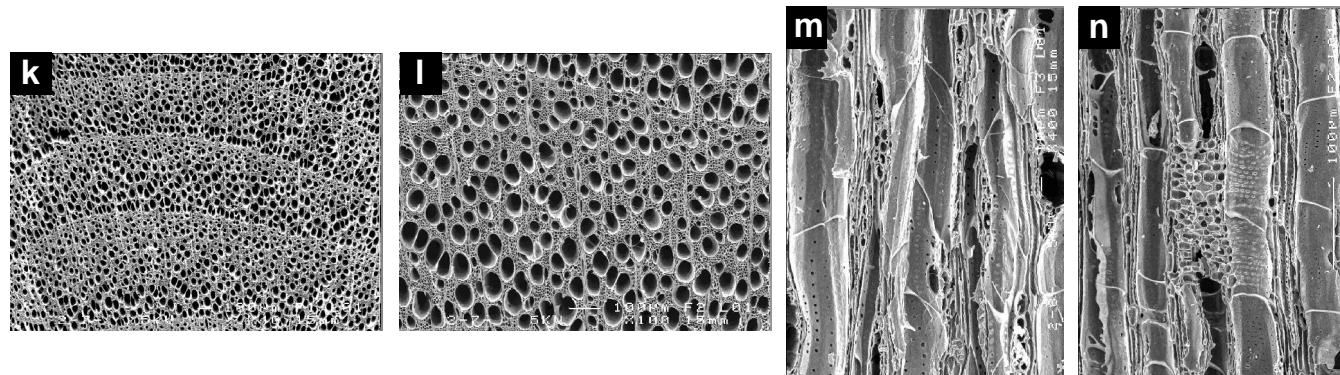
Figure



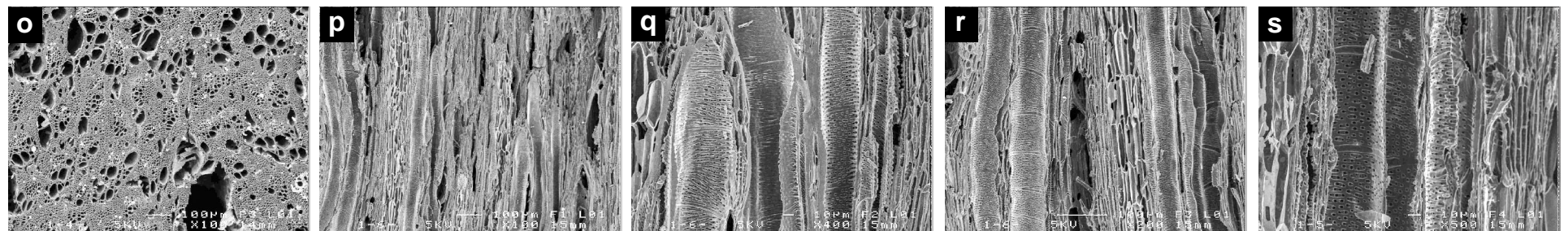
Artemisia tridentata



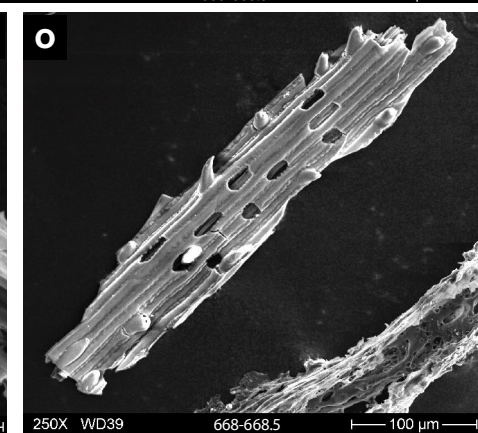
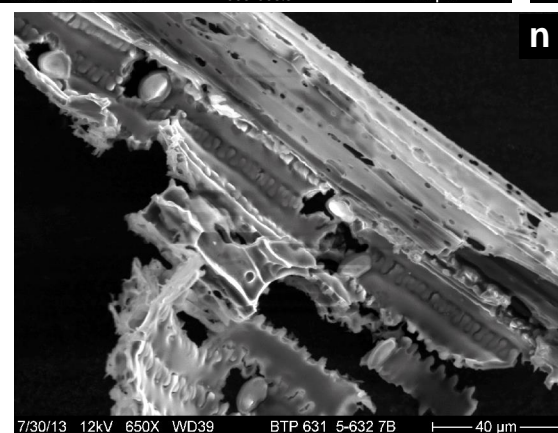
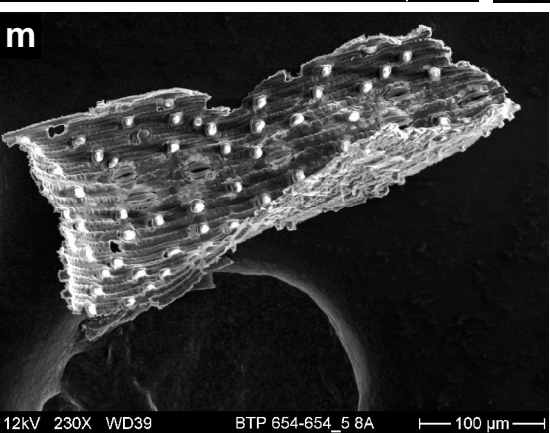
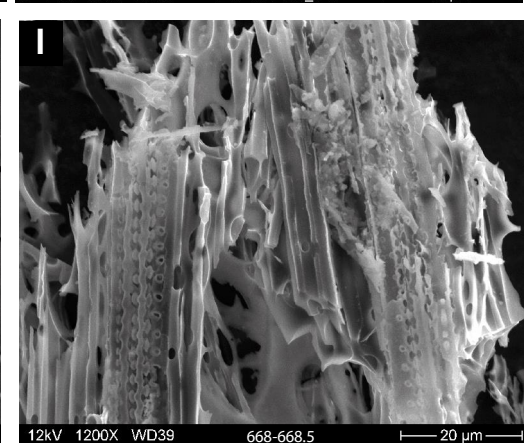
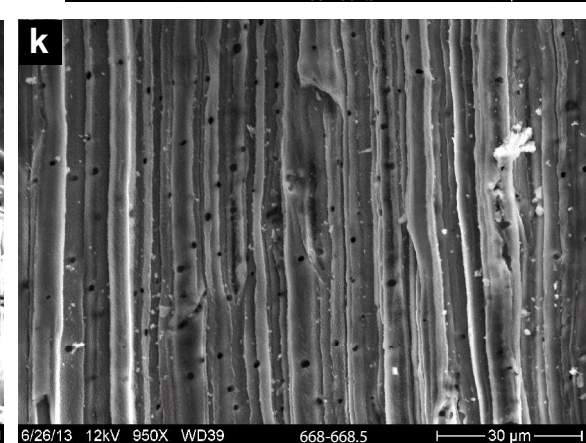
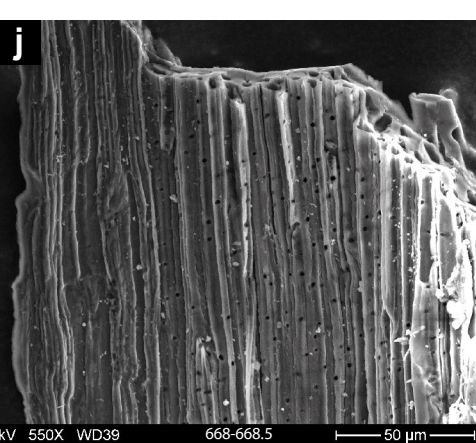
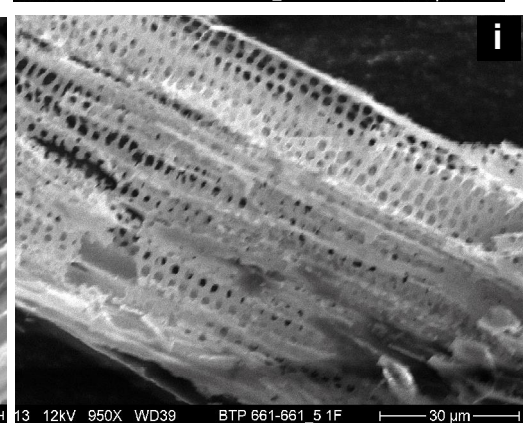
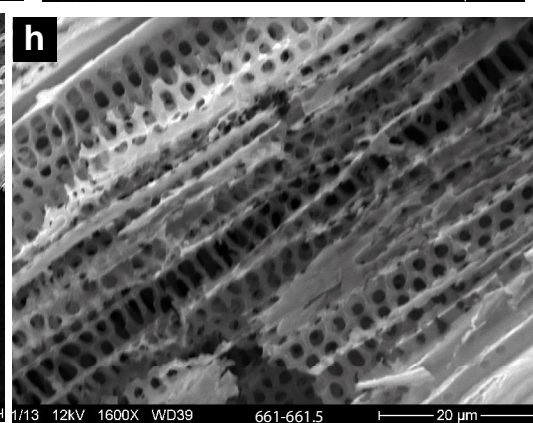
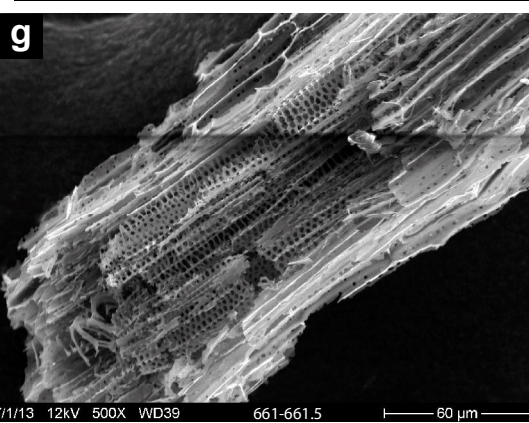
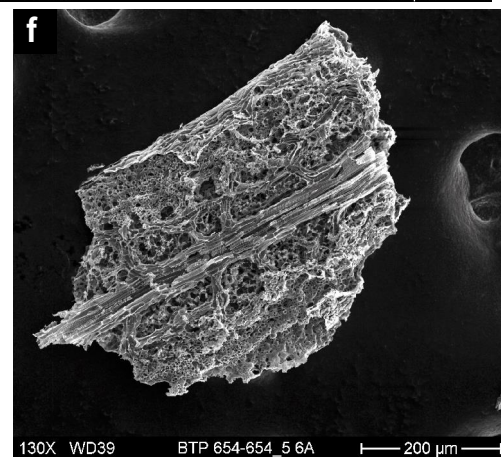
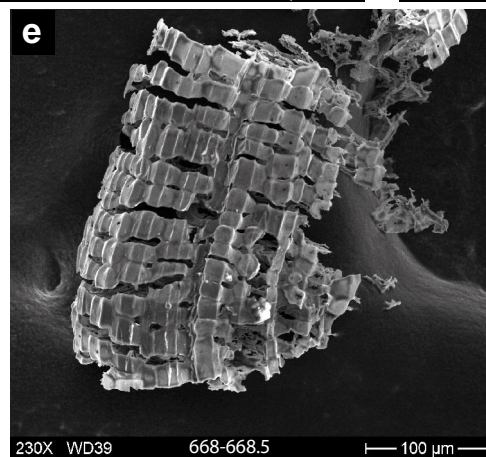
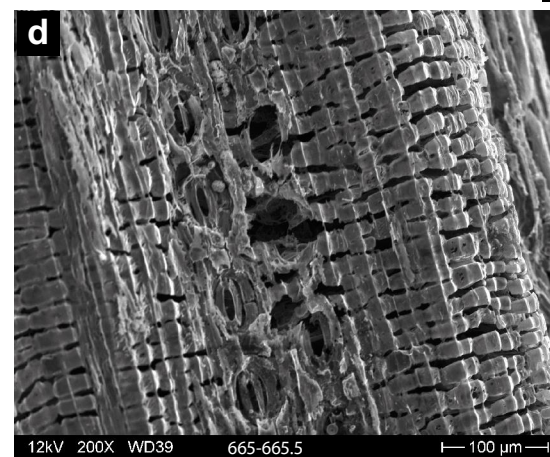
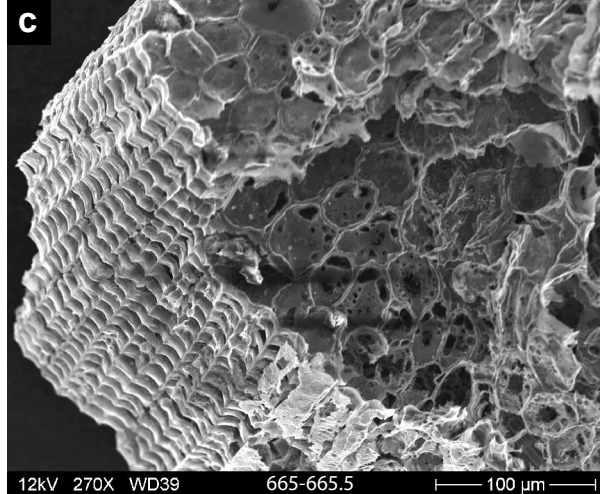
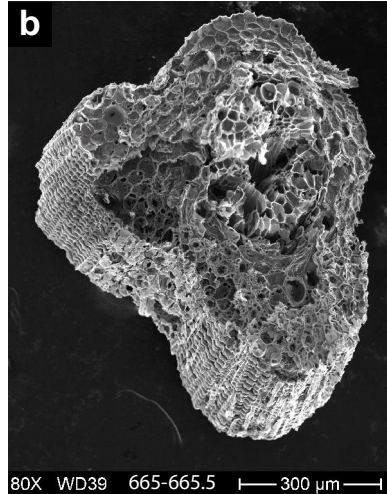
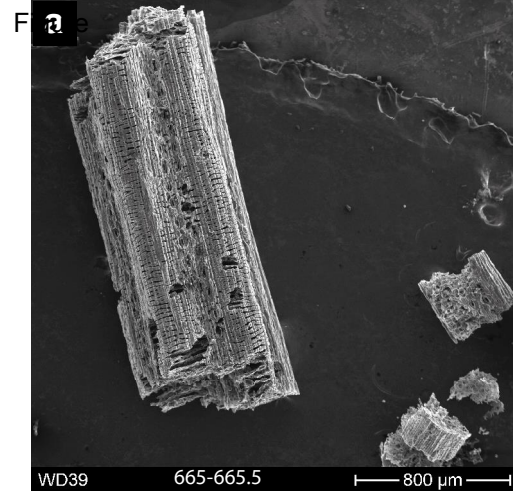
Ericameria nauseosa

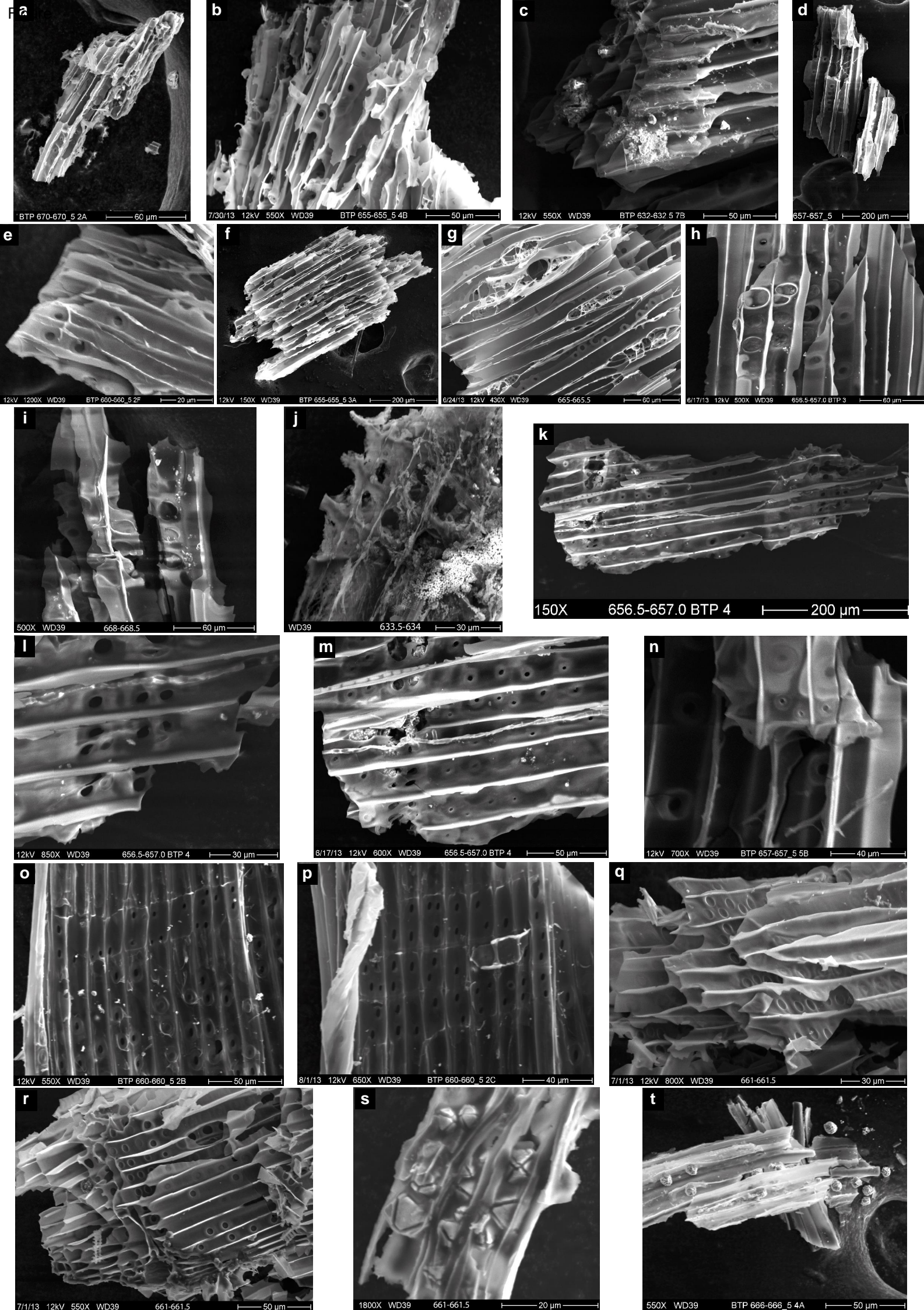


Purshia tridentata



Sarcobatus vermiculatus





1 No conflict of interest regarding this work on “What was burning in the past? Charcoal
2 identification supplements early-Holocene fire-history reconstruction in Yellowstone National
3 Park, USA”.

4

5 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

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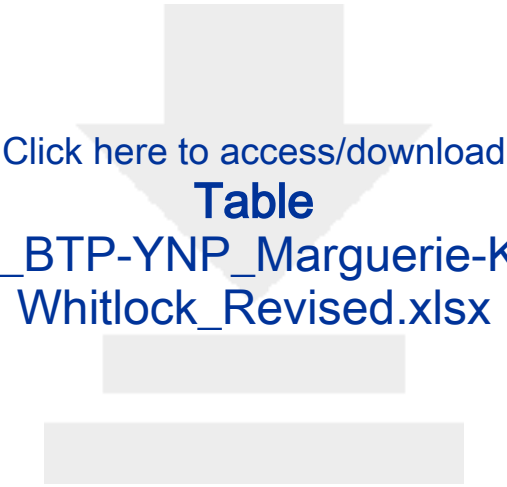
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Wood anatomical features		<i>Abies sp.</i>	<i>Juniperus sp.</i>	<i>Picea sp.</i>	<i>Pinus sp.</i>
Resin canals	Presence	No	No	Yes surrounded by rather thick-walled epithelial cells	Yes with thin-walled epithelium cells
Rays	Tracheids			with smooth, 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"				X
	Pinoid				X
	Piceoid			X	
	Cupressoid		X		
	Taxodioid	2 to 4	X ?		

Wood anatomical features		<i>Pinus albicaulis</i>	<i>P. contorta</i>	<i>P. flexilis</i>
Ray cells	Height (nb of cells)	1 to 10 (average 3-4)	1 to 12 (average 5)	1 to 8 (average 3-4)
Ray tracheids		Yes	Yes with dentate to reticulate walls	Yes
Cross-field pitting	Fenestriform or "window like"	1 rarely 2		1 or 2 frequently 2
	Pinoid		1 to 4 frequently 2	

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Table

Table3_BTP-YNP_Marguerie-Krause-Whitlock_Revised.xlsx



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Table

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