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Charcoal morphologies and morphometrics of a Eurasian grass-dominated system for robust interpretation of past fuel and fire type

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Abstract.

Reconstructing past fire regimes by quantifying charcoal fragments is a commonly used approach, and recent developments in morphological and morphometric analyses of charcoal particles have improved our ability to identify characteristics of burnt plant fuel and interpret fire-type changes. However, burning experiments linking known plants to these metrics are limited, particularly in open ecosystems. This study presents novel analyses of laboratory-produced charcoal of 22 plant species from the steppe regions of Eurasia (Romania and Russia), along with selected Holocene charcoal assemblages from the same areas. We characterize charcoal morphologies and morphometrics in these grass-dominated environments, thereby enabling more robust interpretations of fuel sources and fire types for palaeofire research. Our experiments demonstrate that fire temperature biases the amount of charcoal these plants produce. Grass charcoal production was significantly lower and decreased more strongly with fire temperature than forbs, suggesting an underrepresentation of graminoids in sedimentary charcoal assemblages. While charcoal morphologies enable finer distinctions between fuel types than morphometrics, both approaches are complementary for fuel identification. Morphometric analyses revealed that graminoid charcoal particles were more elongated (length-to-width ratio *L/W* = 4) and narrower (width-to-length ratio *W/L* = 0.38) than forbs (*L/W*=3.1 and *W/L*=0.42, respectively), in agreement with a global compilation for graminoids (*(L/W* = 4.3 for grass 5.4 grass and wetland graminoids) and forbs (*L/W* = 2.9). However, overlapping *L/W* values present a challenge for establishing absolute cut-off values for fuel type identification in charcoal assemblages with mixed fuel sources. Based on our analyses and compiled datasets from experimental burns, *L/W* values above 3.0 may indicate predominantly herbaceous morphologies in temperate grassland-dominated ecosystems, though values are likely to be higher for grass than forb-dominated vegetation. Notably, grasses exhibit shorter aspect ratios (4.3) than wetland graminoids (6.4), highlighting that the aspect ratio needs tailoring to the specific environment of its application i.e., dry vs wet open ecosystems. The long forms of graminoid charcoal particles also suggest their potential for longer-distance transport compared to more spherical particles produced from leaves, meaning they likely provide insights into regional fire history. An important finding is that charcoal morphology and morphometrics accurately reflected the dominant herb communities shown by the pollen record, highlighting a solid link between the dominant vegetation type and fuel burnt in grassland-dominated environments. However, the relationship between woody charcoal and pollen may be more complex for trees, as their pollen can travel longer distances compared to woody charcoal. Our results also highlight the complex interplay between local vegetation and charcoal composition with human fire use that needs to be considered when interpreting charcoal morphological records. Overall, these advancements in identifying fuel sources and changes in fire types make charcoal analysis highly relevant to studies of plant evolution and fire management. A critical takeaway from this study is the importance of not assuming the universality of previous research findings and instead employing experimental approaches to characterize charcoal particles in new ecosystems prior to the application of these techniques. For example, experimental charcoal research is needed in tropical grasslands and savannas.

1 Introduction

Fire plays a crucial role in shaping open ecosystems like grasslands and grass-tree mosaics (i.e., steppe, savanna, forest-steppe, woodlands) at evolutionary and ecological timescales (Bond et al., 2004). One of the largest extents of open systems is in Eurasia, which has been heavily impacted by human activities for millennia. Consequently, little is known about the natural occurrence and intensity of fires in relation to climate, biomass amount and composition in open ecosystems (Leys et al., 2018; Feurdean et al., 2013; 2021; Unkelbach et al., 2018; Feurdean and Vasiliev, 2019; Lukanina et al., 2023). Long-term records of wildfire activity are essential for understanding how fire regimes vary with changes in climate, vegetation, and human-vegetation interactions (Bowman et al., 2009).

Charcoal is the most widely used proxy for reconstructing past fire activity, and it is an inorganic carbon matrix resulting from the incomplete combustion of plant tissues (Whitlock and Larsen 2001). A few keys for identifying small charcoal particles in natural sedimentary archives have been made available. They are either based on quantitative characteristics of charcoal size and form (morphometrics such as aspect ratio, surface area, area-to-perimeter ratio), or qualitative morphological characteristics such as edge aspects, surface features, cleavage, lustre, tracheids with border pits, leaf veins, cuticles, etc. (Umbanhowar and Mcgrath, 1998; Enache and Cumming, 2006; Jensen et al., 2007; Courtney-Mustaphi and Pisaric, 2014; Pereboom et al., 2020; Feurdean, 2021). These keys can help link charcoal particles to characteristics of the burnt fuel from which they were produced, such as plant types (moss, graminoids, forbs, shrubs/trees) or plant parts (stems, branches, roots, leaves, wood). Most of the available keys for identifying charcoal morphologies and morphometries have been developed based on the description of sedimentary charcoal from boreal forests, with a few emerging studies focusing on experimentally produced charcoal of known plants or in other ecosystems (Umbanhowar and Mcgrath, 1998; Jensen et al., 2007; Crawford and Belcher, 2014; Li et al., 2019; Pereboom et al., 2020; Feurdean, 2021). As a result, the accuracy and applicability of charcoal morphometries in ecosystems with different dominant plant compositions still need to be determined. Moreover, the cut-off values of the aspect ratio i.e, length-to-width (*L/W*), or width-to-length (*W/L*) of sedimentary charcoal for fuel identification often contrast with those shown by laboratory experiments, highlighting the need for further research (see review by Vachula et al., 2021). Other metrics, such as surface area and area-to-perimeter ratio (*A/P*), have also been explored to describe regular versus irregular charcoal shapes (Lestienne et al., 2020; Pereboom et al., 2020; Feurdean, 2021), but their full potential requires further evaluation. Another uncertainty in charcoal-based fire reconstruction is how charcoal production is affected by the type of fuel biomass and fire temperature (Pereboom et al., 2020; Feurdean, 2021; Hudspith et al., 2018). Finally, charcoal shape and density can cause substantial differences in particle dispersal, and consequently, on the spatial representation of charcoal-based fire reconstructions (Clark and Hussey, 1988; Vachula and Richter, 2018; Vachula and Rehn, 2023). Thus, a better understanding of the factors influencing charcoal production, morphometries, and dispersal is essential for more accurate reconstructions of past fire activity.

This study presents the first analyses of laboratory-produced (muffle oven) charcoal from a variety of grass, forb, and shrub taxa from Eurasian steppe to better understand the diversity of charcoal morphologies and morphometrics in grassland-dominated ecosystems and facilitate more robust interpretations of fuel sources. By comparing the results to sedimentary charcoal morphologies, morphometrics, and pollen assemblages from the same regions, we aim to provide more accurate

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interpretations of past vegetation, fuel sources and fire types. The study also compares aspect ratio results to a compiled literature database to evaluate this metric's universality. Specifically, we determine i) morphometric and morphological distinction between taxa and plant parts, including photographic plates; ii) thresholds in charcoal morphometrics such as *L/W*, *W/L*, and *A/P* ratios indicative of open systems dominance; iii) a determination of the effect of combustion temperature on the charred mass of various taxa relative to the quantity of unburned biomass. Ultimately, our approach aims to provide an accessible tool for ecosystem managers to understand fire regimes in temperate grass-dominated ecosystems.

2 Material and methods

2.1 Laboratory analysis

We collected plant specimens from seven graminoid, fifteen forb, and one shrub species from the steppe area in the Dobrogea, Black Sea, Romania, and Konoplyanka, Trans-Urals, Russia (Table 1). Tree patches of *Quercus* and *Carpinus* occur in Dobrogea, and *Betula* and *Salix* are present near the Russian sites. However, at the Romania sites, the forest zone (thermophilous broadleaved) is closer (ca. 100 km) than for the sites (*Pinus-Betula*) in Russia (150 km). To determine the effect of increasing temperatures on the mass, morphometric characteristics, and morphologies of charred plant material, we dried these plants in a desiccator for 24 h, then burned them in a muffle furnace following the protocol of Feurdean (2021). Remains of individual plant species, combusted as either the entire plant or as divided plant parts (Table 1), were placed in ceramic crucibles, weighed, and covered with a lid to limit oxygen availability and avoid mixing of the charred particles. Samples were then combusted at 250, 300, 350, 400, and 450 °C. Samples were cooled in a desiccator, and the remaining charred sample was weighed to calculate the charred to pre-burning mass ratio. A small part of the charred mass was gently disaggregated with a mortar and pestle to mimic the natural breakage that charcoal particles experience over time through sedimentation processes (Umbanhowar and McGrath, 1998; Crawford and Belcher, 2014; Belcher et al., 2015). The charred and disaggregated sample was then washed through a 125 µm sieve to remove smaller fragments. This charcoal size fraction (>125 µm) is directly comparable to the size fraction analysed by the bulk of palaeofire studies (Vachula 2018). Morphometric measurements of individual charred particles were obtained from photographs taken at 4X magnification with a digital camera (KERN ODC 241 tablet camera). On average, more than 100 charcoal particles larger than 150 µm were automatically detected in most samples, except those burnt at higher temperatures, where particles were more prone to breaking or were ash. We measured the major (L) and minor (W) axes and surface area (A), and perimeter (P), of each particle following the algorithm of Feurdean (2021) and then calculated the aspect (*L/W; W/L*) and *A/P* ratios. Finer diagnostic features such as shape, and surface features (e.g., reticulates, leaf veins), the arrangement of epidermal cells, and cuticles with stomata were characterised by observing the charred particles under a stereomicroscope.

To demonstrate the applicability of experimental charcoal morphologies and morphometrics for the identification of fuel burnt in sedimentary records, we randomly selected five Holocene samples from a core taken from Mangalia Herghelie wetland, Romania, and six samples from wetland sites near the Karagaily-Ayat river (Tom and SH), where our plant material samples were collected. We first bleached the samples for 6 h and washed them using a 125 µm sieve.

2.2 Numerical analysis

The medians and standard deviations of the following charcoal morphometrics (*L/W*, *W/L*, *A/P*) were aggregated for each species, growth habit, and plant type for all burn temperatures and displayed as box plots. We used a two-tailed Mann–Whitney test to determine whether the medians of the charcoal morphometrics of the growth groups and plant types were equal (Table S1). This test does not assume a normal distribution, only similar distributions in both groups.

3 Results

3.1 Fuel-dependent influence of temperature on charred-mass production

All plant material burnt had a typical charcoal appearance after being subjected to a temperature of 250°C. Most materials turned to ash at 400°C, and all plant tissue became ash at 450°C. Plant material burnt at higher temperatures (400-450°C) tended to break more easily during manipulation. Grouped by growth habit, the percentage of charred mass retained at 300°C (intermediate temperature) was lower for graminoids (34%) than for shrubs (39%) and forbs (42%) (Fig. 1b; Table S2). Within the forb group, members of Asteraceae (*Anthemis, Xeranthemum, Achillea, Artemisia*) and *Chenopodium* had greater charred mass retained than other forb families (Lamiaceae, Fabaceae). Grouped by plant parts, the percentage of charred mass retained at 300°C was lower for grass stems and leaves (33%) than grass inflorescence (37%; Fig. 1b; Table S2). This trend in the loss of charred mass was consistent at all temperatures (Fig. 1af; Table S2).

3.2 Fuel-dependent variations in aspect (*L/W*; *W/L*) and area to perimeter (*A/P*) ratios

Grouped by growth habit, the aspect ratio (*L/W*) of grass charcoals at all temperatures combined were consistently more elongated (mean=4.0±2.5, median=3.1±1.4) than for forbs (mean=3.1±2.2, median=2.4±1.2), and twigs of shrubs (mean=3.5±2.3, median=3.1±2.3; Table S2). These trends in *L/W* are valid for all temperature increments (Fig. 2; Table S2). Within forbs, Asteraceae has more narrow leaves and shows high values in the *L/W* ratio relative to other forb families, i.e., Fabaceae and Lamiaceae (Fig. 2). When grouped by plant parts, the *L/W* ratio of grass stems and leaves (mean=4.2±2.8, median=3.4±2.8) at all temperatures combined was higher than that of grass inflorescence (mean=2.9±1.9, median=2.6±1.9; Fig. 2). Although individual measurements varied greatly, the mean values among species of grass and forbs are consistent (File S1; Table S2). The Mann–Whitney test confirmed that the *L/W* ratios of grass were significantly different from forbs and that grass stems and leaves were significantly different from those of grass inflorescence at all temperatures (Table S1). The width-to-length ratio (*W/L*), an inverse way to report the aspect ratio of charred particles in the literature, shows that grass charcoal is narrower (mean=0.38±0.2, median=0.3±0.2) than forbs (mean=0.42±0.2, median=0.4±0.2), and grass stems and leaves (mean=0.34±0.24, median=0.29±0.24) are narrower than graminoid inflorescence (mean=0.42±0.16, median=0.40±0.16) and twigs of shrub (mean=0.36±0.18, median=0.32±0.18; File S2; Table S2).

The area-perimeter-ratio (*A/P*), which describes more regular (smaller *A/P* values) versus irregular (larger *A/P* values) surfaces of charcoal, was only slightly smaller for grass (mean=50±30, median=45±30); stems and leaves (mean=47±29,

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median=40±29), and inflorescence (mean=52±25, median=40±25) than forbs (mean=53±25, median=43±34) and twigs of shrubs (mean=54±28, median=44±35; File S3; Table S2). There is also a clear tendency for *A/P* ratio to decrease with increased temperature.

3.3 Finer diagnostic features of the charcoal morphologies of various fuel types

Graminoid charred particles from stems and leaves were consistently flat, rectangular, and elongated (Fig. 3a). They mostly broke parallel to the long axis, resulting in elongated pieces with straight margins. They can also appear as featureless, long and thin filaments. The most common preserved surface features were rectangular epidermal cells or contained oval voids, reticulated or mesh patterns, and/or isolated veins.

Charcoal particles from the forb stems were cylindrical and rectangular, whereas fragments of leaves were mostly polygonal. Forbs with pinnate leaf shapes (Asteraceae, *Eryngium*), were generally more elongated than members of the Fabaceae and Lamiaceae families which had more rounded and polygonal leaf shapes (Fig. 3b). Edges were undulate, smooth, or denticulate. Surface textures were generally smooth (featureless), however, charcoal burnt at higher temperatures and those from sedimentary leaf charcoal had more frequently visible venation and ridges. When broken, these particles showed voids and reticulated mesh patterns. Twigs of *Ephedra* shrub showed both quadrilateral shape and layered or foliate structure. The edges and surface textures were smooth (Fig. 3b).

3.4 Morphometrics and finer diagnostic features of fossil charcoal

The aspect ratio of the sedimentary charcoal samples from Mangalia Herghelie, Romania varied between 3.0 and 5.5 (*L/W*) or 0.35-0.43 (*W/L*), whereas *A/P* ratio was between 28 to 40 (Table 2). Samples with elongated (*L/W*) and narrower particles (*W/L*) contained abundant morphologies of graminoid leaves (34-44%) and graminoid and forb stems (44-50%) but had no charred wood morphologies (Table 2). The Tom and SH sedimentary charcoal profiles from Russia showed aspect ratios ranging between 3.6 and 5.0 (*L/W*), 0.3-0.4 (*W/L*), and *A/P* ratios between 23 to 34 (Table 2). Samples with the highest *L/W* ratios showed the highest proportion of graminoid leaves (41-55%) and high proportions of graminoid and forb stem (37-50%) morphologies. There was a low proportion of wood charcoal morphologies (6-12%) but a highly variable tree pollen percentage (16-41%).

4 Discussion

4.1 The influence of combustion temperature on charcoal production for graminoids and forbs

The relationship between charcoal production and the quantity of unburned biomass and fire temperature varies between fuel types due to their density, structure, and chemistry (Walsh and Li, 1994; Hudpith et al., 2018). While burning experiments only approximate some aspects of the heating conditions of vegetation, they provide valuable information on the amount of charcoal production with incremental rises in the fire temperature. Therefore, information on species-specific-charred mass production is critical for interpreting charcoal-based fire reconstruction. Our experimental charcoal production shows that grasses, particularly their leaves and stems, produced lower amounts of charcoal per unit biomass than grass inflorescences, forbs, and shrub wood (Fig. 1; Table S2). Grasses also lost their charred mass more rapidly with increasing burning temperature, from 45–55% at 250 °C to 1–3% at 450°C compared to forbs, 45–65% at 250°C to 5–15% at 450°C. Among forbs, members of the Asteraceae family and *Chenopodium* have higher charcoal production than members of the families Fabaceae and Lamiaceae. These results support laboratory burning experiments in a muffle oven and under open-flame conditions on several grass species from forest-steppe in North America showing that the charcoal mass retention of grass was significantly lower than that of wood (Umbanhowar and McGrath, 1998), a pattern also replicated in a calorimetric combustion study including graminoid, wood, needles, and deciduous leaves (Hudspith et al., 2018). Similarly, burning experiments in a muffle oven on graminoids from Alaska tundra (Pereboom et al., 2020) and Siberian taiga (Feurdean, 2021) demonstrate that these graminoids have significantly lower charred-mass retention per unit biomass than other growth habitats (forbs, shrubs/trees) or plant parts (leaves and wood).

The low bulk densities of graminoids may partly explain the observed pattern of low charcoal production (Hudspith et al., 2018). The chemical composition of plants, including their mineral constituents, lignin, and cellulose, also plays a crucial role in charcoal production. In laboratory experiments, forbs produced more charcoal than wood. Forbs are rich in cellulose and hemicellulose and pyrolyse at a lower and narrow temperature range (200 and 400°C), than wood, rich in lignin (160–900°C; Yang et al., 2007). However, in the oven, the plant tissue is more rapidly turned to ash (450-500°C) than under natural fire conditions (800°C) because of a reduced influence of flame dynamics and turbulent airflow (Belcher et al., 2015; Hudpith et al., 2018). One possible explanation for lower charcoal wood production may be an artifact of the faster combustion rates of oven experiments resulting in wood ashed at a lower side of its temperature ranges.

4.2 Fuel-dependent variability in charcoal morphometrics

We observed consistent *L/W* (elongation) or *W/L* (narrowness) ratios of charred fragments among our Eurasian species of the same plant group (genus or family), growth type, and plant parts at all temperatures (Fig. 2, Table S2). This consistency suggests that aspect ratio could be a useful morphometric for fuel type separation in palaeoenvironments dominated by grasslands. We found that the charred Eurasian grasses are more elongated (*L/W* 4.0±2.5) and narrower (*W/L* 0.38±0.2) than forbs (*L/W* 3.1±2.2, *W/L* 0.42±0.2), in agreement with a global compilation for graminoids (5.4±2.3; grass exclusively 4.3±1.7) and forbs (2.9±0.4; Fig. 4; Table 3). We also found differentiation of aspect ratio between parts of the same plant, with grass stems and leaves being more elongated (4.2±2.8) and narrower (0.34±0.24) than grass inflorescence (2.9±1.9; 0.42±0.1; Fig 2; Table S1, 2). Thinner-leaved forb species in the Asteraceae family also have a higher aspect ratio than members of the families Fabaceae and Lamiaceae, which have more polygonal or rounded leaves. Our *L/W* values for temperate grasses agree with individual experimental laboratory burning of *Poa trivialis* (3.7) from a UK botanical garden (Crawford and Belcher, 2014), and several temperate grass species from North America (3.6 from furnace versus 4.8 from open flame combustion; Umbanhowar and McGrath, 1998). Crucially, our global compilation shows that grasses, *Poa, Stipa, Agropyron,* etc., are shorter and bulkier (4.3±1.7) than wetland graminoids, Cyperaceae, *Eriophorum,* and *Phragmites* (7.6±0.6 for boreal and 6.4±3 global wetland graminoids; Fig. 4, Table 3). The consistency in *L/W* values for graminoids from temperate grasslands, on the one hand, and of wetland graminoids on the other hand, may suggest differences in the evolutionary developments in the physiology of dryland graminoids relative to that of wetlands graminoids (Hedges and Mann, 1979; Vachula et al., 2021). Both charcoal types have elongated vascular bundles connected to the occurrence of veins parallel to the long axis (Umbanhowar and McGrath, 1998). However, the stems of grasses are more robust, with developed mechanical tissue, than those of wetland graminoids. Wetland graminoids mass loss during senescence may explain their lesser robustness (Tanner, 2001; Vernescu et al., 2005).

In contrast to Lestienne et al. (2020), working in Mediterranean ecosystems, we found little differentiation in *A/P* between taxa and plant parts. The *A/P* for grass charcoal (50±25) shows that they only have a slightly more regulated form than forbs (52±30), suggesting that *A/P* may not be as helpful for fuel differentiation in ecosystems dominated by grasses and forbs (Table S2; File S3). However, there is a tendency for *A/P* ratio to decrease with increasing temperature, which is more evident for forbs (from 59 to 40) than for grass (from 53 to 44). This feature, combined with the fact that fossil samples have much lower *A/P* values (23-40) than do the experimental burns, suggests higher wildfire temperatures than experimental burns and some edge rounding (degree angles are eroded) during transportation and deposition. A decreased surface area and increased circularity with transportation have also been documented (Vannière et al., 2003; Crawford and Belcher, 2014; Courtney Mustaphi et al., 2015).

4.3 Finer diagnostic features of the charcoal morphologies for fuel-type identification comparison with keys from literature

Feurdean (2021) provided detailed examinations of the morphological characteristics of experimentally produced charcoal from various taxa and plant parts from the boreal zone, including wetland graminoids, leaves of mosses, ferns, forbs and trees, and the wood of trees and shrubs. The present study improves the link between the grass and forb species from Eurasian open ecosystems and their charcoal morphologies. Similar to charred wetland graminoids, charred temperate graminoids can be distinguished by their flat appearance and tendency to break into thin filaments (Fig. 3a). The grass stems and leaves were not burned separately in this study. However, comparison with previous publications and atlases (Schweingruber, 1987) indicates that grass leaves can often be distinguished by rectangular epidermal cells, reticulate meshes, and oval voids of former epidermal stomata, whereas grass stems display layered, foliated textures that share the appearance of wood (Fig. 3a). This feature contrasts with wetland graminoid charcoal which have a more elongated, fragile form (Feurdean, 2021). The grass inflorescence charcoal is irregular in shape with foliated polygons (Fig. 3a). The grass charcoals produced here are like type B (B2, B3, B4, B5), which is attributed to wood, type D (D1, D2, D3, F), which are attributed to monocotyledon leaves, and type E (E1, E2) mostly related to seeds (Fig. 4; Enache and Cumming, 2006; Courtney-Mustaphi and Pisaric, 2014).

Typical features of forb leaves include their polygonal shapes (Fig. 3b). Netted venation with three branches diverging from a node and surfaces characterised by void spaces are visible in particles charred at higher temperatures as well as sedimentary particles. Forb stems are cylindrical (unbroken) and layered with foliated textures. Our forb charcoals are most like morphologies type A (A1, A2, A3, A4, A5), which are mostly attributed to leaves and wood, type B (B2, B3, B4), and type C (C5, C6), which are connected to leaves, stems, and veins (Courtney-Mustaphi and Pisaric, 2014), whereas some fragments resemble types M and P (Enache and Cumming, 2006).

In summary, our morphological analysis demonstrates that grass leaves are easily distinguished from forb leaves. However, there is an overlap between stems of grass and forb indicating that the two groups are difficult to discriminate based on charcoal morphologies. There is also some overlap between stems of grass and forb with wood morphologies, particularly in more mature plants, raising the possibility of misclassifying some stems of herbs and grass as wood morphologies (Fig. 3).

4.4 The morphometrics and morphologies of fossil charcoal particles: implications for fuel-type identification

All the sedimentary charcoal records we analysed indicate a significant presence of graminoid and forb morphologies, with aspect ratios ranging between 3.0 and 5.5, though the aspect ratio is slightly higher at the Russian sites (Table 2). These findings align well with the pollen records at these sites, which also demonstrate a high proportion of these plant types (Table 2). Nonetheless, notable discrepancies between the Romanian and Russian sites are also evident. At the Mangalia Herghelie site in Romania, samples with the highest aspect ratios (4.4-5.5) exhibit a considerable abundance of graminoid leaf morphologies (34-44%) and pollen from both dry (23-25%) and wetland (9-23%) graminoids. This suggests that fires likely occurred in dry grasslands and within the reed vegetation. The low percentages of wood morphologies (0-7%) and low to moderate tree pollen percentages (26-36%) in samples from this site indicate the scarcity of local woody vegetation, and the burning of woody fuel was limited. In contrast, the sites in Russia display a slightly higher percentage of woody morphologies (6-12%) that do not always correspond to the highly variable abundance of tree pollen percentages (19-41%), along with the occurrence of numerous large particles (ca.1 mm). Fortified and unfortified settlements and various kurgans have been documented near these sites from the Middle Bronze Age, and a nomadic lifestyle is postulated for the Iron Age, kurgans are present (Stobbe et al., 2016). Thus, building and household burning influenced the composition of local charcoal assemblages, likely leading to an increased representation of woody charcoal.

Our analysis indicates that the characteristics of charcoal morphologies and morphometrics largely reflect the dominant plant communities shown by the pollen record, particularly for herbaceous vegetation suggesting a robust link between the dominant fuel type and fuel burnt in grass-dominated environments. However, the proportion of tree pollen matches less than that of woody charcoal, which suggests that tree pollen is transported over longer distances than charcoal. These observations also highlight the complex interplay between local vegetation and charcoal composition with human activities and the need to consider site-specific factors when interpreting charcoal records.

4.5 Application of charcoal morphologies and morphometric for fuel- and fire-type reconstructions in grassland-dominated ecosystems

Our experimental production of charcoal has three primary applications for past fire reconstruction in grass-dominated systems. Firstly, it can improve the identification of fuel types based on charcoal morphologies and morphometrics. Secondly, it can

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determine the effect of biomass and fire temperatures on taxa-specific charcoal production. Thirdly, it can assess the influence of charcoal shape from different fuel types on the spatial scale of fire regime reconstructions.

Our analyses demonstrate that charcoal morphologies provide a finer distinction between fuel types than morphometrics. Grass leaf morphologies can be easily distinguished from forb leaves. However, morphologies of stems of grass and forb are more challenging to differentiate from each other. Charcoal morphometry also shows promising results in establishing threshold values for discriminating individual fuel types. Limitations, however, arise when analysing charcoal assemblages from mixed fuel sources due to overlapping *L/W* values. We identified a mean *L/W* value of 4.0 ±2.5 for grass and 3.1±2.2 for forbs charcoal (Fig. 4; Table 3). These values largely agree with the values generated from a literature compilation, 5.4±2.3 for graminoids (4.3±1.7 for grasses; 6.4±3 for wetland graminoids), 2.9±0.4 for forbs, and 2.2±0.1 for leaves (Fig. 4; Table 3). While our study used only a single woody shrub species (*Ephedra*; *L/W* 3.5±2.3), the literature compilation of woody particles revealed *L/W* 3.04±0.4, meaning an overlap with non-woody vegetation, especially forbs (Table 3; Fig.4). We propose that *L/W* values above 3 (*W/L* below 0.40) can indicate predominantly charcoal morphologies from grass and forbs in temperate grassy-dominated ecosystems. However, values can be higher in grass relative to forb-dominated vegetation within this ecosystem. Our proposed cut-off values to separate non-woody from woody fuels, based on experimental studies with known fuel types, are closest to those applied to a wide range of ecosystems in Europe (Marriner et al., 2019; Lebreton et al., 2019) and Canada (Umbanhowar and McGrath, 1998), and partially to those from the USA (Leys et al., 2017) and Asia (Miao et al., 2019, 2020), and differ the most from those commonly applied Africa (Aleman et al., 2013; Daniau et al., 2007, 2013, 2023; Thevenon et al., 2003), particularly those using *L/W* above 2 or *W/L* >0.5 for non-woody vegetation (see review by Vachula et al., 2021). Therefore, the morphometric cut-off values are not universally valid but vary with the dominant ecosystem type.

Validating sedimentary charcoal morphometrics and morphologies using pollen and plant macrofossils can aid in interpreting the fuel source, and our assessment in the Eurasian steppe sites shows great potential (Table 2). For example, a comparison of graminoid morphologies with pollen of grass (Poaceae, Cereals) and wetland graminoids (Cyperaceae, *Typha*) at Mangalia helped determine the approximate origin of the charred graminoids. Burning reed vegetation for water access was a common practice for past societies (Feurdean et al., 2013; Sim et al., 2023), and wetland graminoids may contribute significantly to the overall graminoid charcoal assemblage. Vice versa, variability in charred morphologies and morphometrics can indicate changes in the main vegetation composition. Morphologies of graminoids and forbs alongside high aspect ratios at our fossil sites appear to indicate the expansion of open grassy habitats. Woody expansion may be reflected by an increase in woody morphologies and a decrease in particle aspect ratio, however this relationship is less straightforward.

Secondly, the amount of charcoal produced varies depending on fuel type and fire temperature, resulting in likely bias in charcoal-based fire reconstructions. Grass fuels, for example, have a low post-fire charred-mass retention and rapidly decrease mass with temperature. This means grasses are likely to leave little charcoal or turn into ashes even in relatively low-intensity fires, resulting in an under-representation of grass charcoal in sedimentary records. In contrast, forbs tend to preserve a higher amount of their biomass even when burnt at high temperatures, suggesting that the forbs component of grasslands is more likely to be incorporated into sediments and likely also lower the *L/W* values. Thus, lower amounts of charred graminoids relative to forbs and wood may indicate high-intensity fires in a tree-grass matrix rather than an absence of grass fires. Thirdly, dispersal models accounting for shapes, sizes, and densities of charcoal, show that small differences in shape and density of charcoal can cause substantial differences in particle source area (Vachula and Richter, 2018; Vachula and Rehn, 2023). Elongated, lighter particles such as those from graminoids, have higher residence time in the atmosphere and can travel longer distances than spherical or heavier particles, such as those from leaves or wood (Clark and Hussey Vachula and Richter, 2018; Vachula and Rehn, 2023). Charcoal particles produced by grassland fires tend to be smaller than those produced by forest fires (Leys et al., 2017), and our results show that the size of charcoal particles decreased with the increased fire temperature. As a result, sedimentary charcoal in grass-dominated and mixed woody-grass ecosystems may represent a more regional fire history compared to forested ecosystems, thus affecting the reconstruction of local fire events using the decomposition method in peak and background charcoal (Vachula and Rehn, 2023). Nevertheless, knowledge of the influence of charcoal form, density, and transport can aid in interpreting the spatial scale of fire histories with changing dominant ecosystems.

5. Conclusions

This study provides novel insights into charcoal mass production, morphometrical aspects, and morphologies of grass and forb species from the Eurasian grasslands. It highlights the practical application of morphometry and morphology in enhancing the understanding of fuel composition and fire dynamics in grass-dominated ecosystems. The impact of fire temperature on charcoal production varies according to fuel type, with grasses exhibiting significantly lower charcoal production and decreasing more prominently with increasing fire temperature compared to forbs. This suggests an under-representation of grass morphologies in the sedimentary charcoal record relative to forbs.

Charcoal morphologies offer a finer distinction between fuel types than morphometrics. However, both morphologies and morphometrics can significantly complement each other in fuel identification. Leaf morphologies of grass can be easily distinguished from forb, but stem morphologies of grass and forb are challenging to differentiate. Morphometric analysis indicates that graminoid charcoal particles are more elongated (4.0±2.5) than forbs (3.1±2.2). Consequently, *L/W* values above 3 (*W/L* below 0.40) may indicate predominantly herbaceous morphologies in temperate grassy-dominated ecosystems. However, values can be higher in grass-dominated vegetation and this cut-off should not be applied to other ecosystems without a reasonable rationale. However, the growth habits of graminoids influence charcoal morphological aspects, with grasses exhibiting shorter aspect ratios (4.3±1.7) than wetland graminoids (6.4±3). For research performed in grass-dominated and mixed woody-grass systems, lower thresholds for aspect ratios may be applied compared to wetland graminoids from the tundra, boreal zones, or even drier regions where wetland assemblages dominate the local vegetation. This feature and the decrease in the size of herbaceous charcoal particles with the increased fire temperature indicate that reconstructing fire regimes in grass-dominated and mixed woody-grass ecosystems may provide insights into more regional fire history than forested systems.

Validations of morphologies and morphometrics from pollen and plant macrofossils can aid in interpreting the fuel sources. At the same time, the variability in charred morphology assemblages and aspect ratios also holds promise in reconstructing changes in vegetation composition, particularly in places with poor pollen preservation. Our results suggest that morphologies of graminoids and forbs are more closely linked to their abundance as indicated by pollen and likely the local vegetation composition. However, the relationship between woody charcoal and pollen may be more complex for trees, as their pollen can travel longer distances compared to woody charcoal. Furthermore, human modification of local vegetation can significantly distort the composition of charcoal assemblages.

A critical point arising from this study is the need to avoid assuming the universality of research findings and instead apply experimental approaches to characterizing charcoal particles in new ecosystem types. Future efforts to determine fuel sources based on analyses of charcoal fragment morphologies and morphometries should consider the investigation of taxa and plant parts from tropical grasslands, which have been largely overlooked in these studies. Determining the influence of particle shape on dispersal, transportation, and fragility, with the implication of spatial scale of charcoal reconstructions, is another area needing further research. The utilization of new image recognition or artificial intelligence software to collect data on charcoal could aid in the differentiation of fuel types at the particle scale. Whereas analyses of sedimentary charcoal require a degree of aggregation and/or human interpretation related to fuel type changes, automated approaches could allow for the fuel source of individual particles to be identified.

Data availability: All raw measurements of length, width, area, perimeter, and ratios of *L/W, W/L*, and *A/P* for all taxa and temperatures are presented in File S1

Sample availability: A limited amount of burnt plant material can be made available upon request.

Author contribution: AF designed the study and performed the burning experiments, the morphometrical, morphological and numerical analyses, and data presentation; DH, AS, MG, and AF collected the modern plants and performed pollen analysis. RSV compiled the morphometrics database from literature; AF wrote the manuscript with contributions from RSV; all the authors reviewed and edited the manuscript and agreed with its content.

Competing interests: The author declare that they have no conflict of interest.

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Figure legends and embedded figures

Figure 1. The percent of charred mass retained after burning individual plant taxa in a muffle oven at 250°C, 300°C, 350°C, 400°C, and 450°C (a-e). The abbreviations used in the figure are S & L – stem and leaf, I-inflorescence; where this is not noted it implies mixed plant parts. Mean charred mass values grouped by grasses and forbs, the main growth habits, at increasing temperature from 250°C to 450°C are also presented (f). Grass taxa are represented in yellow (light yellow for S & L, dark yellow for I), forbs in orange, and shrubs in grey. The full names of plant species burnt, and their origins are presented in Table 1.

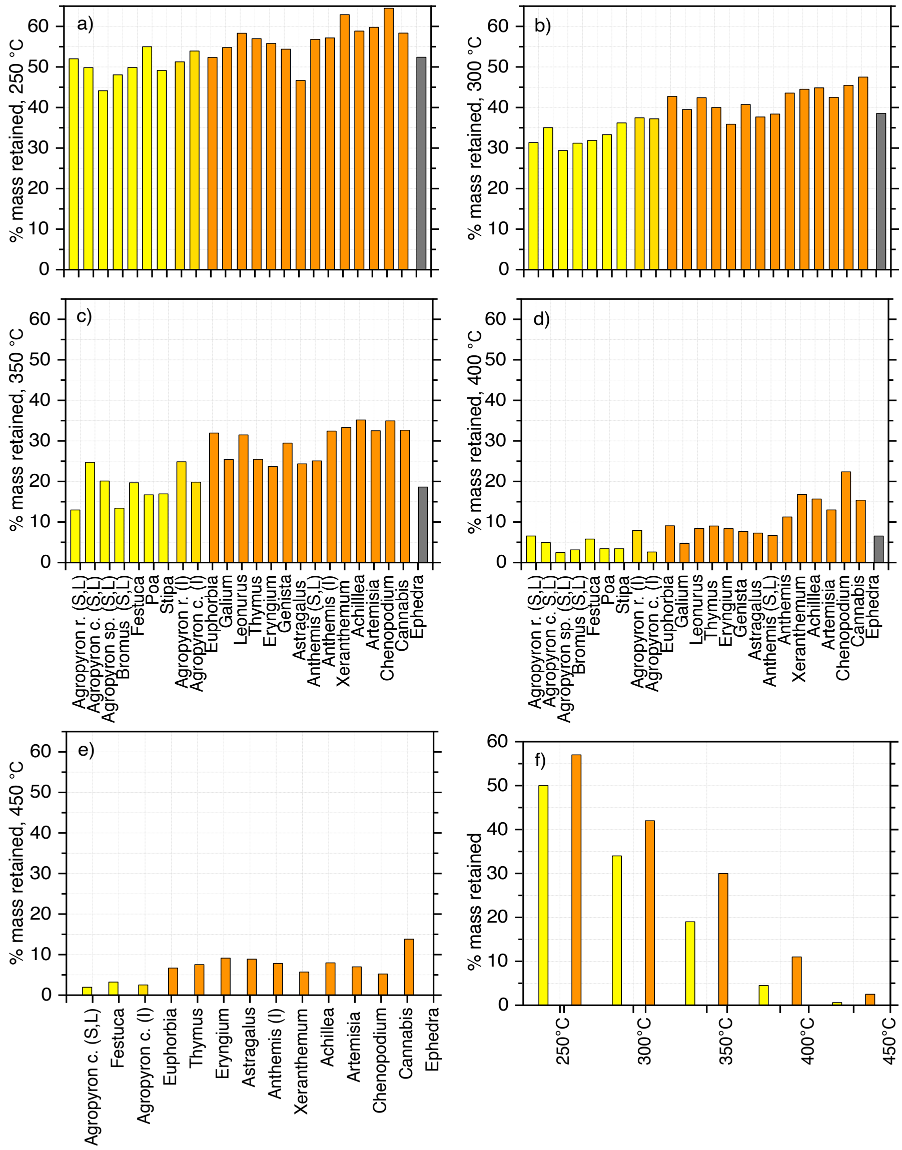

Figure 2. The median aspect ratios (*L/W*) of charred particles from the individual taxa combusted at 250°C, 300°C, 350°C, and 400 °C, respectively. Insets in each panel represent the median values of grass and forb taxa, with the same colour scheme as in individual measurements. Box plots represent the distribution of data as follows: the horizontal line in each box denotes the median, the upper quartile is the median value of the upper half of the data points, the lower quartile is the median value of the lower half of the data points, and whiskers represent the respective minimum and the maximum values. Individual and mean *L/W* values for each temperature are presented in Table S2, whereas mean *L/W* values for all temperatures combined are shown in Fig. 4. Abbreviations and colour coding as in Fig. 1.

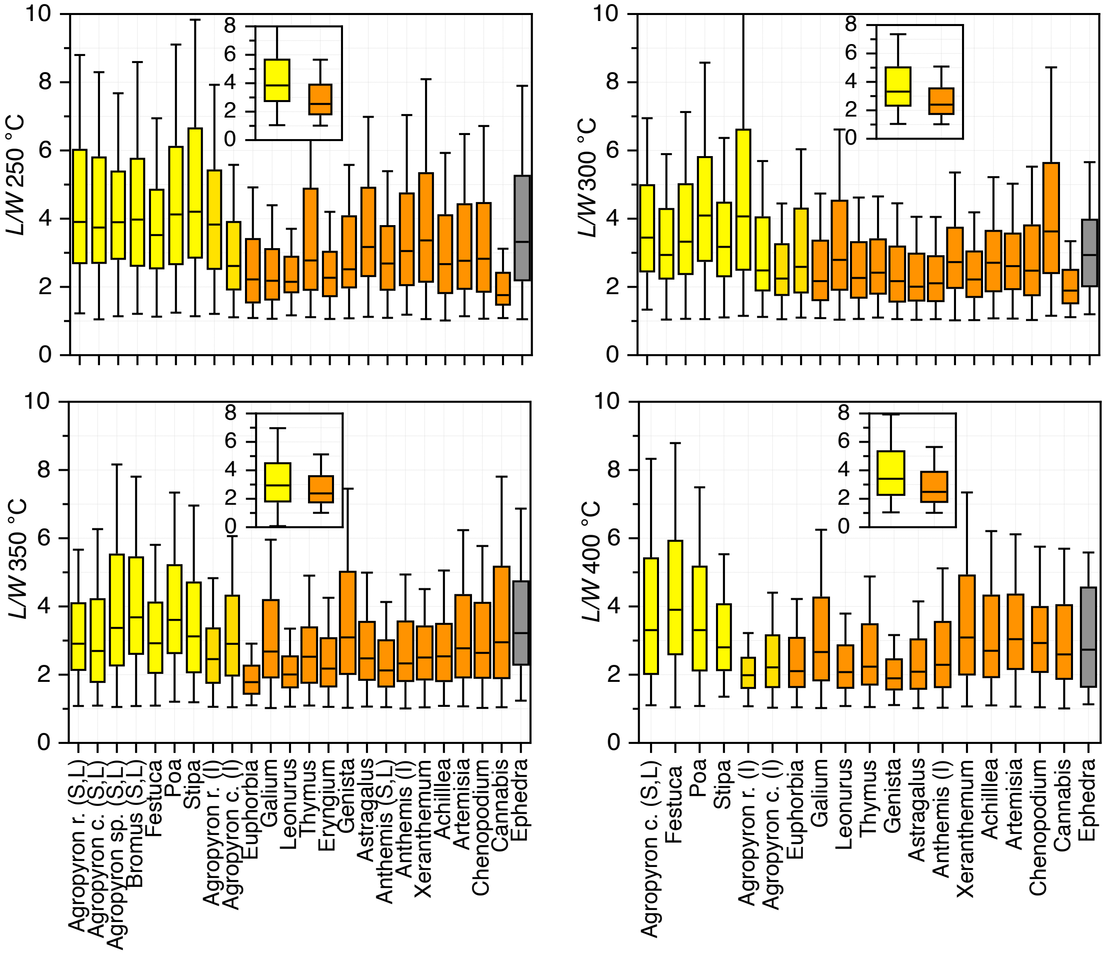

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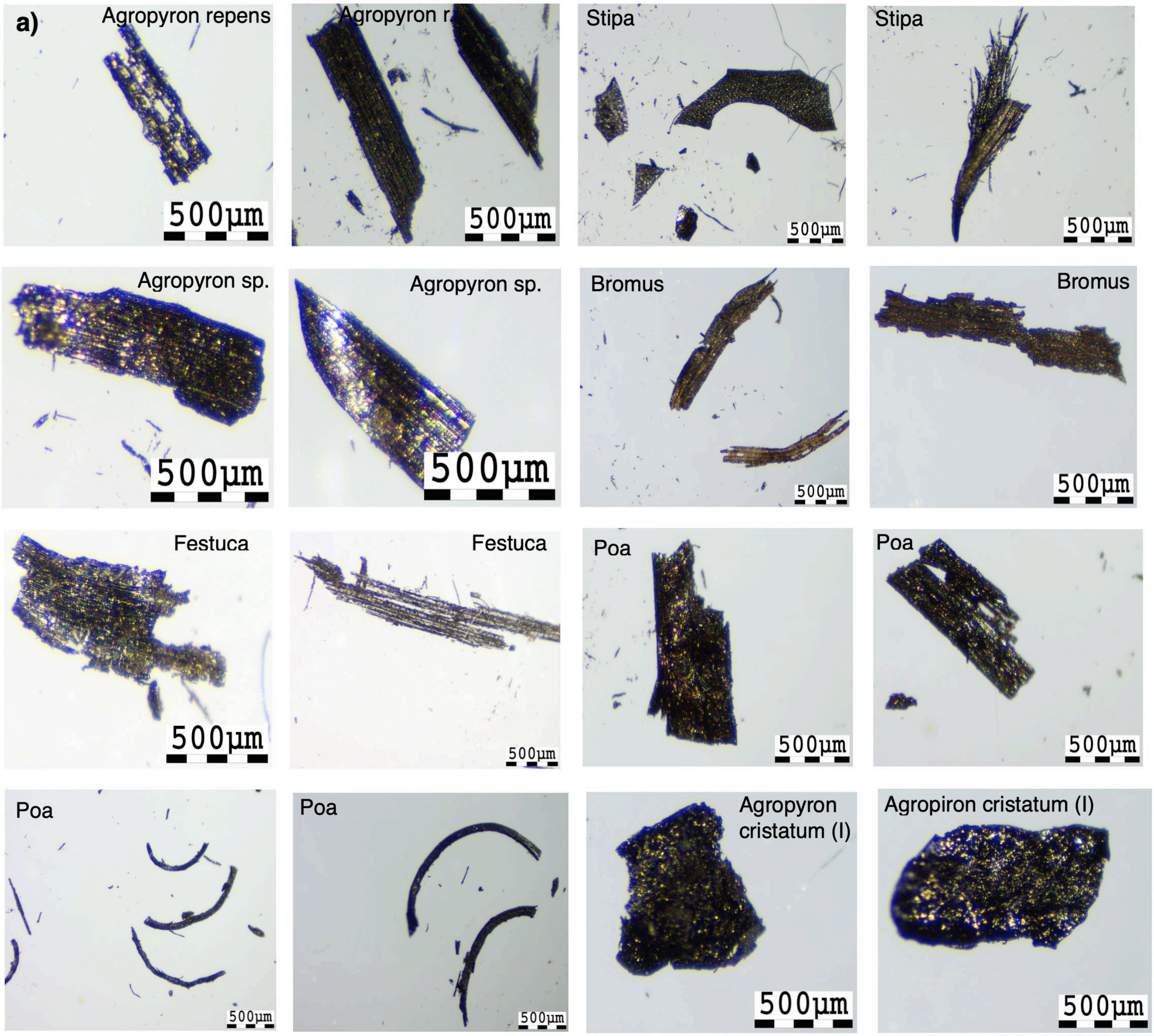
Figure 3. Photomicrographs of characteristic charcoal morphotypes under stereomicroscope (4 ×). (a) Grass and (b) Forbs. The last raw on panel b) presents sedimentary charcoal. L-Leaf, S-Stem, I=Inflorescence.



570

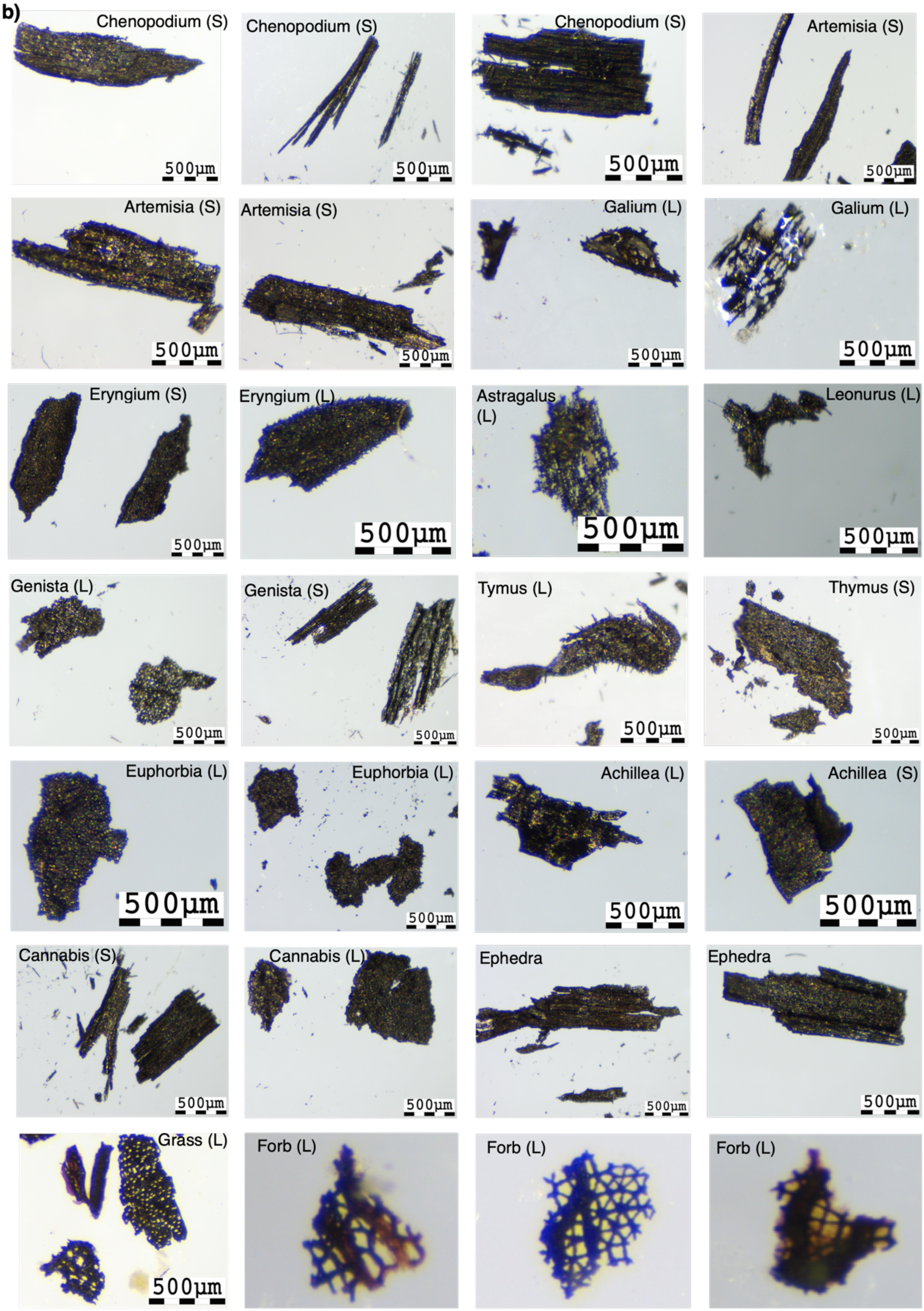
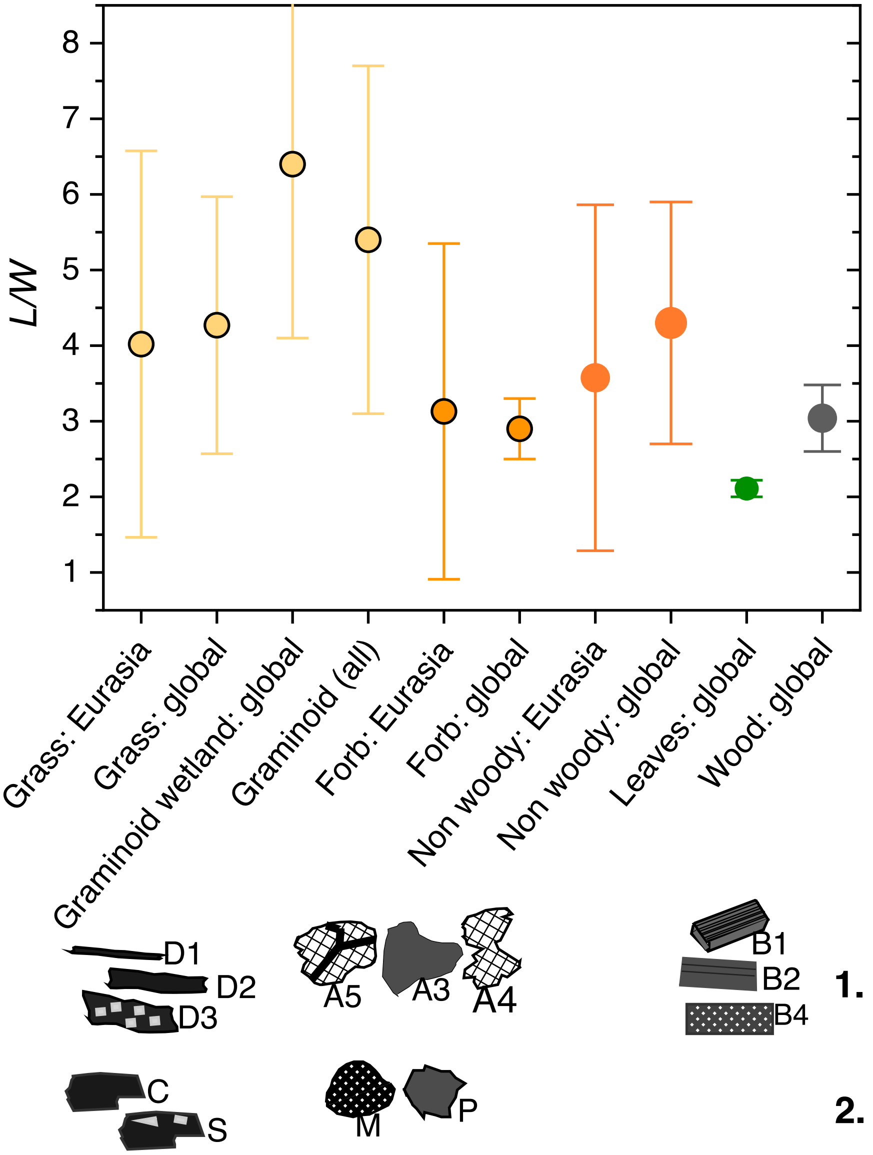

b)
Chenopodium (S)
500µm
Chenopodium (S)
500µm
Chenopodium (S)
500µm
Artemisia (S)
500µm
Artemisia (S)
500µm
Artemisia (S)
500µm
Galium (L)
500µm
Galium (L)
500µm
Eryngium (S)
500µm
Eryngium (L)
500µm
Astragalus (L)
500µm
Leonurus (L)
500µm
Genista (L)
500µm
Genista (S)
500µm
Tymus (L)
500µm
Thymus (S)
500µm
Euphorbia (L)
500µm
Euphorbia (L)
500µm
Achillea (L)
500µm
Achillea (S)
500µm
Cannabis (S)
500µm
Cannabis (L)
500µm
Ephedra
500µm
Ephedra
500µm
Grass (L)
500µm
Forb (L)
Forb (L)
Forb (L)

Figure 4. Mean *L/W* values from experimental charcoal produced from known plants for main growth habits (grass, wetland graminoids, forbs) and plant types (leaves, wood) from this study (Eurasia) and the compilation from literature (see details Table 3). Comparison with selected charcoal morphotypes of 1. Courtney-Mustaphi and Pisaric (2014) and 2. Enache and Cumming (2006) and is also shown.



TABLES

Table 1. List of plants burned from Dobrogea, the Black Sea, Romania (Ro) and Konoplyanka, Trans-Urals, Russia (Ru).

Plant type	Scientific name	Family	Common name	Plant burned
Grass				
Grass_RO	*Agropyron repens/ Elymus repens*	Poaceae	Couch grass	Stem/leaves
Grass_RO	*Agropyron repens*	Poaceae	Couch grass	Inflorescence
Grass_RO	*Agropyron cristatum*	Poaceae	Crested wheatgrass	Stem/Leaves
Grass_RO	*Agropyron cristatum*	Poaceae	Crested wheatgrass	Inflorescence
Grass_RO	*Agropyron sp.*	Poaceae	Wheatgrass	Stem/Leaves
Grass_RO	*Festuca sp.*	Poaceae	Creeping bent grass	Stem/Leaves
Grass_RO	*Poa sp.*	Poaceae	Meadow-grass	Stem/Leaves
Grass_RO	*Bromus sterilis / tectorum*	Poaceae	Bromegrass	Stem/Leaves
Grass_RU	*Stipa capilata*	Poaceae	Feder grass	Whole
Forbs				
Forb_RO	*Anthemis/Cota tinctoria*	Asteraceae	Golden marguerite	Whole
Forb_RO	*Artemisia campestris*	Asteraceae	Field wormwood	Whole
Forb_RO	*Xeranthemum annuum*	Asteraceae	Annual everlasting	Whole
Forb_RO	*Achillea sp.*	Asteraceae	Yarrows	Whole
Forb_RO	*Leonurus sp.*	Lamiaceae		Whole
Forb_RO	*Eryngium campestre*	Apiaceae	Field eryngo	Whole
Forb_RO	*Chenopodium*	Chenopodiaceae	Goosefoot	Whole
Forb_RO	*Euphorbia glareosa*	Euphorbiaceae	Spurge	Whole
Forb_RU	*Genista tinctoria*	Fabaceae	Dyer's greenweed	Whole
Forb_RU	*Astragalus sp.*	Fabaceae	Milkvetch	Whole
Forb_RU	*Galium sp.*	Rubiaceae	Bedstraw	Whole
Forb_RU	*Cannabis ruderalis*	Cannabaceae	Hemp	Whole
Forb_RU	*Thymus cf. hirsutus*	Lamiaceae	Thyme	Whole
Shrubs				
Shrub_RU	*Ephedra distachya*	Ephedraceae	Joint pine	Twigs

Table 2. Charcoal morphometrics: the mean aspect ratio (*L/W, W/L*) and area to the perimeter (*A/P*) ratio, and percentage of main charcoal morphotypes in the Holocene samples from Mangalia Herghelie, Black Sea, SE Romania, and Konoplyanka, Trans-Urals, Russia. The pollen percentages of main vegetation groups (graminoids, forbs, shrubs, and trees) are similar to those used for charcoal morphologies.

Age (cal yr BP)	**2350**	**2950**	**3150**	**3950**	**5850**	
Mangalia Hergehlie, Romania						
Charcoal morphometrics						
L/W	5.5	4.4	3.2	3.3	3.0	
W/L	0.37	0.34	0.43	0.41	0.35	
A/P	40	34	37	37	28	
Charcoal morphologies (%)						
Wood	0	0	7	6	4	
Leaf (forb & tree)	7	22	12	15	7	
Leaf graminoid	43	34	26	34	48	
Stem graminoid & forb	50	44	55	45	41	
Pollen (%)						
Trees	26	26	29	34	36	
Shrub	0.0	0.5	1	1	0.6	
Grass	23	25	38	7	23	
Graminoid wetlands	23	9	11	2	25	
Forb	52	48	32	58	40	
Konoplyanka, Russia	**1700**	**1800**	**1750**	**2050**	**2830**	**4140**
Charcoal morphometrics						
L/W	5.0	3.7	3.6	4.0	4.8	4.1
W/L	0.3	0.4	0.36	0.3	0.3	0.34
A/P	24	34	31	27	23	29
Charcoal morphologies (%)						
Wood	6	10	10	12	7	8
Leaf (forb & tree)	2	0	0	1	1	2
Leaf graminoid	55	17	17	23	41	29
Stem graminoid & forb	37	73	73	64	50	61
Pollen (%)						
Trees	20	41	16	31	36	19
Shrub	0	0	0	0	0	0
Grass	31	24	50	16	19	23
Graminoid wetlands	7	3	2	9	1	2
Forb	49	35	34	53	45	58

Table 3. Comparative *L/W* values of experimental charcoal produced from known plants in this study with those compiled from literature by Vachula et al. (2021) and new Feurdean (2021) results. Graminoid all global includes grass and graminoid wetland type. *L/W* value for non-woody type combines charcoal of grass and forb, whereas the *L/W* value in parenthesis combines grass, wetland graminoid and forbs. The wood sums the *L/W* values of trunks and twigs.

Fuel type	**Zone**	***L/W***	**References**
Graminoid (grass)	Eurasia	4.0±2.5	This study
Graminoid (grass)	Global	4.27±1.7	This study; Vachula et al., 2021
Graminoid (wetland)	Boreal	7.64±0.6	Pereboom et al. 2020, Feurdean, 2021
Graminoid (wetland)	Global	6.4±3	
Graminoid (all)	**Global**	**5.4±2.3**	See above
Forb	Eurasia	3.1±2.2	This study
Forb	**Global**	**2.9±0.4**	This study; Vachula et al., 2021; Feurdean, 2021
Non-woody (graminoid & forb)	Eurasia	3.6±2.4	This study
Non woody (graminoid & forb)	**Global**	**3.6 ±1.1** **(4.3±1.6)**	This study; Vachula et al., 2021 Feurdean, 2021
Leaf (forbs, shrub & trees)	**Global**	**2.1±0.1**	Vachula et al., 2021; Feurdean 2021
Wood (shrub & tree)	**Global**	**3.4±0.4**	This study; Vachula et al., 2021; Feurdean 2021