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Effects of particle size and pretreatment methods on the morphometry of grass charcoal particles: Implications for morphometric analysis of microcharcoal particles

Jun Inoue [1*] and Tatsuya Usuki [1]

[1] *Department of Geosciences, Graduate School of Science, Osaka Metropolitan University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan*

* Corresponding author: Jun Inoue, *Department of Geosciences, Graduate School of Science, Osaka Metropolitan University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan*

Tel +81-6-6605-2590, Fax +81-6-6605-2522

E-mail: inouej@omu.ac.jp

Abstract

The morphometry of sedimentary charcoal particles illuminates the source fuel types. This study explores the variation in morphometry (specifically, the length-to-width [L/W] ratio) of charcoal particles of different sizes, using charcoal collected from *Miscanthus sinensis* (Japanese pampas grass) fields following controlled burning. We also investigated the impact of pretreatment processes on sedimentary microcharcoals. The results indicate a general decrease in the L/W ratio with decreasing particle size, with the microcharcoal exhibiting a substantial decrease. The mean L/W ratios for size categories of 250 µm–1 mm, 125–250 µm, 63–125 µm, and <63 µm in size are 6.42, 5.54, 4.94, and 3.45, respectively. Furthermore, pretreatment processes for microcharcoal decrease the L/W ratio. Consequently, the L/W ratio of grass microcharcoal (<125 µm) likely falls within 50%–80% of the ratios observed in grass macrocharcoal (>125 µm), necessitating caution in interpreting microcharcoal data. Our findings emphasize the importance of considering particle size and pretreatment effects when interpreting paleofire records and highlight the need for further research to establish robust cutoff values for fuel type inference based on charcoal morphometry, particularly for microcharcoal.

Keywords

Sedimentary charcoal; Charcoal morphology; Length-to-width ratio; Fire history; Fuel type; Grass

1. Introduction

Wildfires play a crucial role in biome disturbance and development across ecosystems, posing threats to natural resources and human life. Paleofire records offer insights into the long-term interactions between fires and various factors, including climate change, vegetation type, and human activities. Charcoal particles in lake, bay, and peat sediments serve as primary proxies, enabling the reconstruction of paleofire records and clarifying not only fire occurrences, but also fire regimes, including type, frequency, intensity, size, distribution, and fuel types. Charcoal particles, classified as macrocharcoals (>125 μm) and microcharcoals (<125 μm), found in lacustrine sediments and other sediment types, facilitate historical fire regime reconstruction across local, regional, and global scales over extensive periods (e.g., Power et al., 2008; Daniau et al., 2012; Harrison et al., 2021). Here, charcoal particles are classified into microcharcoal and macrocharcoal, using a size threshold of 125 μm, following Vachula (2018). Although Vachula's original classification divides charcoals into macroscopic (>125 μm), mesoscopic (50–125 μm), and microscopic (10–50 μm) categories, we adopted a simplified classification for this study.

Given the considerable influence of fuel type on fire behavior, morphological analysis of charcoal particles is increasingly important for identifying both source fuel types and the fire types that generate them. Estimation of fuel type from charcoal involves morphological analysis, i.e., qualitatively assessment of particles based on shape, color, and microstructure, and morphometric analysis, involving quantitative measurements, such as circularity, length-to-width (L/W) ratio, and perimeter (Vachula et al., 2021). Advancements in image analysis have improved accessibility to morphometric methods, such as CharTool (Snitker et al., 2020); thus, they are widely applied to both micro- and macro charcoals in various sediment types (e.g., Thevenon et al., 2003; Daniau et al., 2007; Herrmann et al., 2010; Inoue et al., 2018; Miao et al., 2019; Haliuc et al., 2023). Conversely, morphological methods (i.e. qualitative classification by charcoal morphotypes) are used mainly to analyze macrocharcoal from lacustrine and peat sediments (e.g. Enache and Cumming, 2006; Jensen et al., 2007; Mustaphi and Pisaric, 2014; Frank-DePue et al., 2023).

The L/W ratio, a widely used morphometric measurement, varies among macrocharcoals produced experimentally from grass plants and those from tree components (e.g., Umbanhowar and McGrath, 1998; Vachula et al., 2021). These findings extend to sedimentary charcoals, including microcharcoals (e.g. Daniau et al., 2023; Haliuc et al., 2023). However, applying these insights to microcharcoals may be challenging because of the potential morphometric alterations induced by fragmentation, combustion conditions, and post-depositional treatments (e.g. Umbanhowar and McGrath, 1998; Crawford and Belcher, 2014; Feurdean, 2021; Frank-DePue et al., 2023; Haliuc et al., 2023). To date, only one study has examined the L/W ratio in microcharcoal from experimental charcoals (Crawford and Belcher, 2014), albeit incompletely, as only residual microcharcoal on a 125 μm sieve was measured.

In the present study, we addressed this knowledge gap by focusing on charcoal particles from *Miscanthus sinensis* (Japanese pampas grass), which is characterized by a high L/W ratio (Ogura, 2007; Vachula et al., 2021), with the aim of determining the ratio variation across different particle sizes. The emphasis on a high L/W ratio arises from its potential sensitivity to fragmentation processes. Acknowledging the L/W ratio's susceptibility to fluctuations under varying temperature and combustion conditions (e.g. Feurdean, 2021; Feurdean et al, 2023), as well as our incomplete understanding of the optimal conditions required for such changes, we analyzed charcoals collected from *M. sinensis* fields following controlled burns. This approach allows for the exploration of the characteristics of charcoal produced under conditions similar to natural fires, an aspect overlooked in previous studies. Additionally, we investigated the impact of various treatments used for microcharcoal extraction from sediments, including chemical treatments, on the microcharcoal L/W ratio, aiming to discern potential alterations in this ratio due to the extraction processes. Methods for characterizing fuel types via microcharcoal analysis are limited; therefore, our examination of microcharcoal L/W ratios could markedly enhance our understanding of charcoal particle morphometry and refine the methodologies for paleofire reconstruction.

2. Materials and methods

2.1 Charcoal samples collected after a controlled burn of *M. sinensis* fields

To measure charcoals' L/W ratios, we used samples collected the day after a controlled burn of *M. sinensis* fields on the Soni plateau in central Japan, conducted on March 22, 2015. These grasslands have undergone annual spring burning. The plateau is primarily covered by *M. sinensis* and, to a lesser extent, *Sasa nipponica* (a dwarf bamboo species) along with other species (Figure 1a). For sampling, we specifically targeted an area predominantly covered by *M. sinensis* (Figure 1b). To obtain representative charcoal samples, we selected eight distinct collection sites, each spaced 50–200 m apart from the others. The geographical coordinates of these sites ranged from 34°31'07" to 34°31'20"N in latitude and from 136°09'45" to 136°09'58"E in longitude. Charcoal was collected from several hundred square centimeters at each site and stored in stainless-steel containers. Subsequently, the collected charcoal was cut with scissors into smaller pieces, each measuring approximately <1 cm in length. These samples were refrigerated at 4°C, and some samples were later used for Raman spectrum analysis following Inoue et al. (2017).

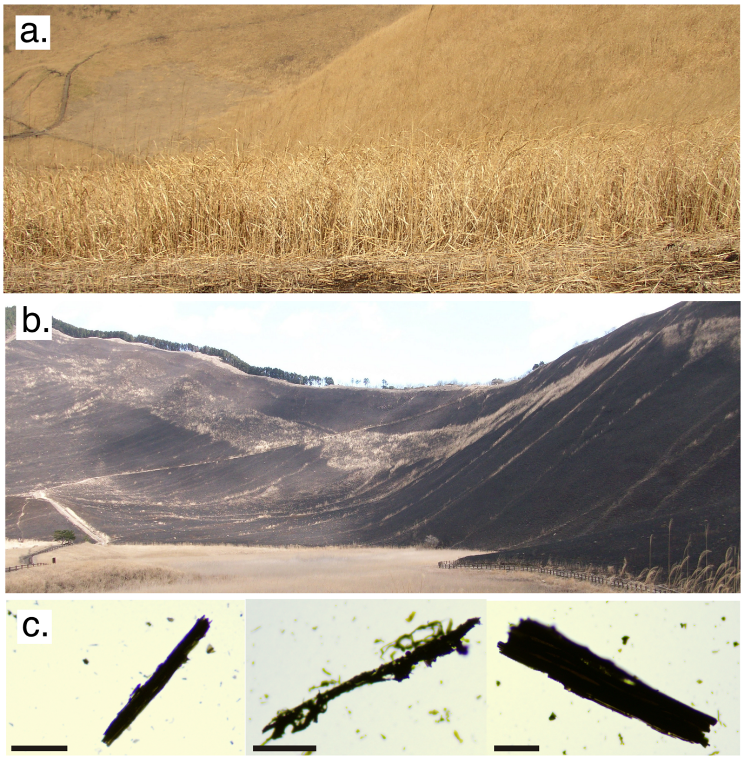

Figure 1. (a) *Miscanthus sinensis* fields on the Soni plateau in central Japan before controlled burning. (b) Panoramic view of the sampling area after controlled burning. The unburned area in the foreground is Okame-ike Pond. (c) Micrographs of charcoal particles collected after controlled burning. Scale bars: 100 µm.

2.2 Preparation of charcoal samples of various sizes

Untreated charcoal samples for microscopic observation, in contrast to the chemically and physically pretreated samples described later were produced. We combined 0.15 g of charcoal fragments from each sample and pulverized into finer particles using a medicine spoon. These finer charcoal particles were then sieved through mesh sizes of 1 mm, 250, 125, and 63 µm. Residues, including charcoal particles, from each sieve were collected. To measure the charcoals' L/W ratios, we used particles from the 250, 125, and 63 µm sieves as well as those that passed through a 63 µm sieve (i.e. 250 µm–1 mm, 125–250 µm, 63–125 µm, and <63 µm). For L/W ratio measurements, charcoal particle samples from each size category were dispersed in water, lightly stirred, and filtered using a mixed cellulose ester membrane filter (pore size: 0.2 μm; size: 25 mm).Filters containing charcoal particles were mounted on slides, which were subsequently examined under an optical microscope (Figure 1c).

2.3 Preparation of charcoal samples subjected to pretreatment for sedimentary charcoals

Approximately 0.1 g of <63 μm particles underwent a series of treatments for sedimentary microcharcoal, following the procedures outlined by Inoue et al. (2018; 2021). These treatments involved chemical processes using 10% potassium hydroxide and 70% hydrofluoric acid along with the addition of approximately 200,000 plastic microspheres (size: 15 μm) (Ogden, 1986). This was done because, to facilitate the calculation of microcharcoal concentration, exotic markers such as plastic microspheres are often added to samples during pretreatment processes (e.g., Inoue et al., 2018, 2021; Miao et al., 2019; Zhou et al., 2023). At each step, we applied centrifuge separation (2,000 rpm for 4 min) with skimming, and stirring was performed one to three times, for a total of seven times throughout the treatment. Given the challenge of settling charcoal particles, each centrifugation was preceded by a 1 h settling period to facilitate precentrifugation particle settling. Post-treatment, the charcoal particles were collected using a membrane filter to prepare a slide sample for measuring the L/W ratio.

To assess the physical effects of the treatments (i.e., centrifuge separation and stirring), a sample underwent only these processes, totaling seven centrifugal separations. By comparing the charcoal's L/W ratio after undergoing only physical treatments with that following the pretreatment, including chemical processes, we evaluated the isolated impact of these treatments on the ratio.

2.4 Measurement of charcoal particles' L/W ratios using image analysis

To measure charcoal particles' L/W ratios, samples were examined using an optical microscope (Nikon ECLIPSE LV100ND). Specifically, particles of size <63 μm and 63–125 μm were observed under a 40× objective lens with a 10× eyepiece, whereas 125–250 μm and 250 μm–1 mm particles were observed under a 10× objective lens with a 10× eyepiece. Charcoal particles were defined as black, fully opaque, angular particles within the samples. Images of charcoal particles were digitally captured on a personal computer using the WRAYCAM-NOA2000 video camera (WRAYMER INC., Japan). The L/W ratio of the ellipses approximating the charcoal particle shape was measured using ImageJ version 1.53k.

Upon observing charcoal particles in the samples of each size, we found that some particles did not fall within their size categories. Consequently, we restricted the L/W ratio measurement to particles with lengths within the specified size range for each size category. Additionally, the L/W ratio measurements of <63 μm charcoal particles were limited to those with lengths exceeding 20 μm. Regarding the pretreatment effect on charcoal particles' L/W ratios, measurements were confined to particles with lengths of 20–50 μm, following Inoue et al. (2018).

3. Results

Figure 2a and Table 1 show the L/W ratios of the charcoal particles within each size range (N = 400 in each range; N = 1600 in total). Both the mean and median ratios suggested that the ratio decreases as particle size decreases. The *t*-test and *U*-test results for the L/W ratio pairs in each range (Supplementary Table S1) indicate that most *p*-values are <0.01, implying a generally significant trend of increasing L/W ratios with particle size. This trend was particularly pronounced in the microcharcoal size range (<125 μm). Figure 2b displays circular charts showing the percentages of each L/W ratio category in each size range. Although the percentages of L/W ratios between 3.5 and 10 varied slightly across the size ranges, those exceeding 10 and those below 3.5 varied substantially. Substantial changes in the percentages of high L/W ratios (>10) likely contributed to the more pronounced changes in mean L/W ratios with particle size compared to changes in median ratios.

Figure 3a and Table 1 show the L/W ratios of charcoal particles without treatment (i.e., “untreated” charcoal particles; N = 1200), those subjected to chemical and physical treatments (N = 1200), and those that underwent only physical treatment (N = 400). Charcoal particles’ L/W ratios after both pretreatments and only physical treatments were smaller than those of untreated particles. Statistical analysis revealed that p-values from t-tests and U-tests for all L/W ratio pairs in each sample were <0.01 and (Supplementary Table S1). As shown in Figure 3b, the percentages of L/W ratios between 3.5 and 10 and those exceeding 10 were lower in particles following pretreatment and physical treatment (>10: 1%–2% and 10%–20%, respectively) compared with untreated particles (>10: ~5% and ~25%, respectively). Conversely, the percentages of ratios <3.5 after these treatments (80%–90%) were higher than those in untreated particles (~70%).

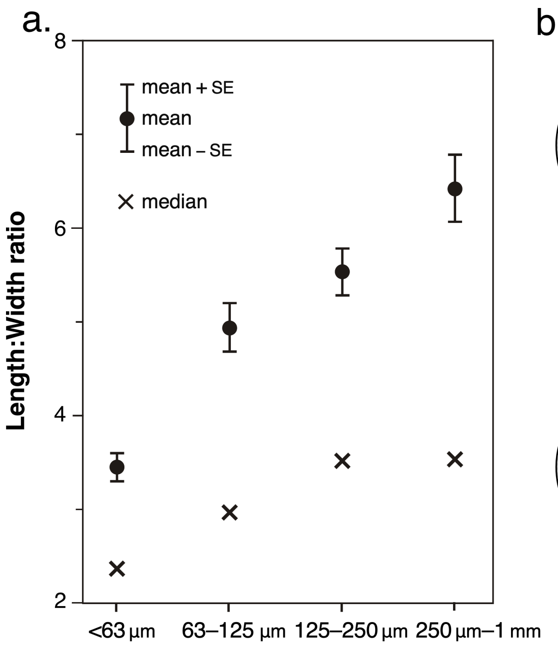

Figure 2. (a) Mean ± standard error (SE) and median length-to-width (L/W) ratios of charcoal particles across size classes (<63 µm, 63–125 µm, 125–250 µm, and 250 µm–1 mm). (b) Percentage distribution of charcoal particles by L/W ratio category (<3.5, 3.5–10, and >10) across particle size classes.

Table 1. Mean, standard deviation, standard error, and median values of charcoal particle length-to-width (L/W ratios across particle size classes, and charcoal particle (20–50 µm) L/W ratios of untreated particles and those subjected to chemical treatment with physical treatment or physical treatment alone.

Charcoal size class/ treatment	Mean L/W ratio	Standard deviation	Standard error	Median L/W ratio	N
<63 µm	3.45	3.08	0.15	2.36	400
63–125 µm	4.94	5.21	0.26	2.96	400
125–250 µm	5.54	4.93	0.25	3.51	400
250 µm–1 mm	6.42	7.23	0.36	3.54	400
Untreated charcoals	3.57	3.62	0.10	2.32	1200
Chemical treatment with physical treatment	2.76	2.05	0.06	2.09	1200
Physical treatment alone	2.37	1.68	0.08	1.90	400

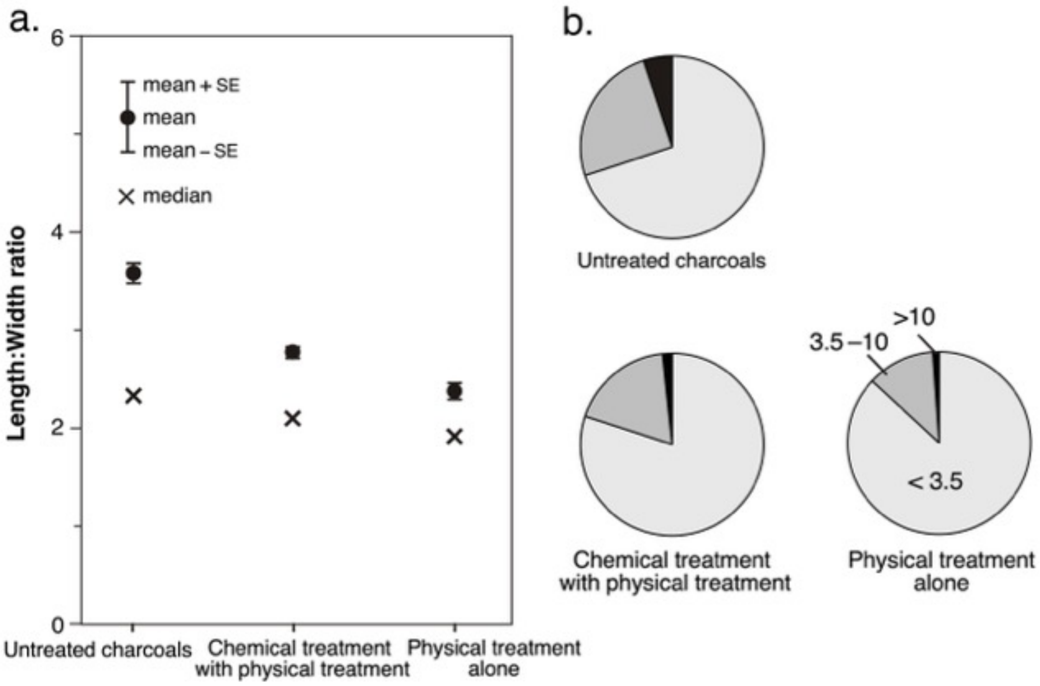

Figure 3. (a) Mean ± standard error (SE) and median length-to-width (L/W) ratios of charcoal particles in different treatment groups: nontreatment, chemical treatment with physical treatment, and physical treatment alone. (b) Percentage distribution of charcoal particles by L/W ratio categories (<3.5, 3.5–10, and >10) following nontreatment, chemical treatment with physical treatment, or physical treatment alone.

4. Discussion

To evaluate charcoal particles' L/W ratios, we analyzed charcoals collected from *M. sinensis* fields following controlled burns, reflecting semi-natural conditions. The mean L/W ratios of microcharcoals are smaller, at 3.45 (<63 µm) and 4.94 (63–125 µm), whereas those of macrocharcoals are larger, at 5.54 (125–250 µm) and 6.42 (250 µm–1 mm) (Figure 3a and Table 1). Previous studies have indicated that the mean L/W ratio of 125–250 µm macrocharcoal particles produced from *M. sinensis* through open-burn processes is 7.4 (Ogura, 2007; Vachula et al., 2021). However, the mean L/W ratio for similarly sized charcoal particles in the present study was 5.54. Despite disparities in L/W ratios between our study and previous studies assessing experimentally produced charcoal, we observed higher L/W ratios (>5) compared to those found in most other plant charcoals. For instance, Vachula et al. (2021) noted that L/W ratios greater than approximately 3.5 signify grass and other non-woody fuels, while ratios less than approximately 2.5 indicate charcoal derived from woody sources. Additionally, Feurdean et al. (2023) indicated that L/W ratios above 3.0 may represent predominantly herbaceous morphologies in temperate grassland-dominated ecosystems. Therefore, our observed ratios of *M. sinensis* charcoals align broadly with those of experimentally produced charcoal, suggesting that the morphologies of experimentally produced and naturally formed charcoals are comparable. Variations in L/W ratios between the charcoals examined in the current study and those in previous experimental studies may be attributed to the inclusion of samples from mixed plants, despite the predominant presence of *M. sinensis* at our study sites. Achieving a vegetation distribution composed exclusively of one plant species in natural settings is impossible, inevitably leading to differences in comparison with experimental charcoal derived from a single plant species.

We observed that charcoal particles' L/W ratios decreased as the particle size decreased, a trend that was also reported by Umbanhowar and McGrath (1998) and Crawford and Belcher (2014). In the present study, the percentages of L/W ratios exceeding 10 decreased substantially, suggesting that elongated charcoal particles tended to become shorter during subdivision. This finding supports the notion that charcoal particles predominantly break in a widthwise direction, likely because the force applied to an elongated charcoal piece fractures the charcoal at its weakest point, typically near the long axis center, perpendicular to the axis (Umbanhowar and McGrath, 1998).

The difference in mean L/W ratios between untreated (3.57) and pretreated (2.76) charcoal particles suggests that sediment extraction processes could alter these ratios. Clark (1984) noted that various processes in microcharcoal extraction (i.e. the pollen slide method) can cause breakages. The reduction in particles' L/W ratios post-treatment, compared with untreated particles, likely results from breakage during the process, as suggested by Clark (1984). Charcoal particles of *M. sinensis*, being elongate and thin, tend to break widthwise. Notably, the similar L/W ratios observed between post-treated and solely physical-treated charcoal particles imply that physical processes predominantly affect the ratios. However, Clark (1984) found that centrifugation alone minimally affected charcoal particles. This disparity may be attributed to differences in the assessed charcoal materials: Clark (1984) used mixed wood and grass charcoal particles, whereas we used highly elongated charcoal from *M. sinensis*, which is likely more prone to breakage. This assumption is consistent with the findings of Zhang and Lü (2006), which show that L/W ratios decrease due to breakage caused by physical treatments, such as shaking and centrifuging, with grass charcoals exhibiting a substantial reduction.

Our findings suggest that L/W ratio cutoff values, commonly used as thresholds for distinguishing between vegetation types (e.g. grass and woody plants) based on macrocharcoal, cannot be directly applied to microcharcoal. Based on compiled L/W ratios from various experimentally produced macrocharcoals, Vachula et al. (2021) proposed that ratios exceeding 3.5 indicate grass and nonwoody fuels, whereas ratios below 2.5 indicate tree and shrub fuels. Although they argue that the L/W ratio cutoff values used in existing literature to distinguish fuel types from sedimentary charcoal particles are not supported by their experimental measurement dataset, more than half of the studies examined microcharcoal or micro- and macrocharcoal (e.g. Herrmann et al., 2010; Daniau et al., 2013; Inoue et al., 2018; Miao et al., 2019). Although L/W ratios are used as a reference for evaluating fuel types based on microcharcoal (e.g. Daniau et al., 2023; Haliuc et al., 2023), directly applying L/W ratios from experimentally produced macrocharcoal to sedimentary microcharcoal could result in inaccurate fuel type estimations.

Decreases in the L/W ratio with smaller particle sizes have been observed in sedimentary charcoal particles (Miao et al., 2020; Zhou et al., 2023). For example, Miao et al. (2020) categorized sedimentary charcoal particles across size classes into two L/W ratio categories: >2.5 and <2.5. The distribution of charcoal concentrations in each category across size classes indicated that a relatively high number of particles exceeding 100 μm exhibited L/W ratios greater than 2.5, whereas a significantly higher number of particles smaller than 30 μm exhibited L/W ratios below 2.5. Variations in L/W ratios by particle size were observed during the course work of Inoue et al. (2018), promoting the restriction of L/W ratio measurements to charcoal particles in the size range of 20–50 μm. These observations suggest that for the same fuel type, the L/W ratio may vary with changes in sedimentary charcoal size. Therefore, limiting the particle sizes or classifying the particles into different size categories for measurement may be necessary.

The present findings, along with those of Crawford and Belcher (2014), indicate that the L/W ratios of microcharcoal to macrocharcoal in grass-derived charcoal are markedly reduced, amounting to only 50%–80% of macrocharcoal ratios. Although the universality and general applicability of these results remain uncertain, they may serve as a preliminary guide for future assessment. Crawford and Belcher (2014) suggested that the L/W ratios of grass charcoal are likely larger than those of wood charcoal, even for microcharcoal. Therefore, relative changes in sedimentary microcharcoal L/W ratios could be used to infer changes in fuel types. Notably, accurate estimation of fuel types via microcharcoal L/W ratios requires access to ratios from experimentally produced microcharcoal across diverse fuel sources.

4. Conclusion

We investigated the L/W ratio variation across size ranges of charcoal particles and evaluated the impact of pretreatment on particles collected after controlled burns in *M. sinensis* fields. The observed macrocharcoal L/W ratios were comparable to previous experimental findings. As the particle size decreased, the L/W ratio generally decreased, with a particularly pronounced trend noted for microcharcoal. The mean L/W ratios of macrocharcoals are greater than 5, whereas those of microcharcoals are less than this value. Additionally, the pretreatment processes reduced the microcharcoal L/W ratios from greater than 3.5 to less than 3. Consequently, the grass microcharcoal L/W ratios likely fall within 50%–80% of the grass macrocharcoal ratios. Overall, these findings imply that cutoff values for L/W ratios, established through experimental studies on macrocharcoal, may not directly apply to microcharcoals. Therefore, our study emphasizes the need for further investigation to enhance the understanding of charcoal morphometry, particularly regarding microcharcoal.

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Supplementary Table S1.
(a) p-values from Welch's t-test (upper) and Mann–Whitney U test (lower) for charcoal particle length-to-wi (L/W) ratios across particle size classes.
(b) p-values from Welch's t-test (upper) and Mann–Whitney U test (lower) comparing L/W ratios among charc particles (20–50 µm) remaining untreated, subjected to chemical treatment with physical treatment, or undergo physical treatment alone.

a	<63 µm	63–125 µm	125–250 µm	250 µm–1 mn
<63 µm		1.28×10^{-6}* 2.65×10^{-4}*	2.06×10^{-12}* 4.38×10^{-14}*	2.04×10^{-13}* 1.01×10^{-16}*
63–125 µm	1.28×10^{-6}* 2.65×10^{-4}*		0.09 7.35×10^{-4}*	9.16×10^{-4}* 4.30×10^{-5}*
125–250 µm	2.06×10^{-12}* 4.38×10^{-14}*	0.09 7.35×10^{-4}*		0.04 0.45
250 µm–1 mm	2.04×10^{-13}* 1.01×10^{-16}*	9.16×10^{-4}* 4.30×10^{-5}*	0.04 0.45	

*p-value <0.01

b	Untreated charcoa	Chemical treatment with physical treatment	Physical treatment alone
Untreated charcoals		2.43×10^{-11}* 1.14×10^{-7}*	1.09×10^{-18}* 6.43×10-14*
Chemical treatment with physical treatmen	2.43×10^{-11}* 1.14×10^{-7}*		1.42×10^{-4}* 7.69×10-5*
Physical treatment solely	1.09×10^{-18}* 6.43×10^{-14}*	1.42×10^{-4}* 7.69×10^{-5}*	

*p-value <0.01