

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

Automated image analysis and hyperspectral imagery with enhanced dark field microscopy applied to biochars produced at different temperatures

This is the submitted version (pre peer-review, preprint) of the following publication:

Published Version:

Ilaria Piccoli, Armida Torreggiani, Chiara Pituello, Annamaria Pisi, Francesco Morari, Ornella Francioso (2020). Automated image analysis and hyperspectral imagery with enhanced dark field microscopy applied to biochars produced at different temperatures. WASTE MANAGEMENT, 105(15 March 2020), 457-466 [10.1016/j.wasman.2020.02.037].

Availability:

This version is available at: <https://hdl.handle.net/11585/762625> since: 2020-06-19

Published:

DOI: <http://doi.org/10.1016/j.wasman.2020.02.037>

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).
When citing, please refer to the published version.

(Article begins on next page)

Highlights

- Image analysis detected considerable modifications of PR particles after charring
- SEM micrograph showed nonporous surface on PL particles after 550°C pyrolysis
- EFDM image highlighted CD biochar formed by semi-crystalline aggregates
- Emerging imaging techniques are effective for characterizing biochar properties

1 **Automated image analysis and hyperspectral imagery with enhanced dark field**
2 **microscopy applied to biochars produced at different temperatures**

3

4 Iliaria Piccoli^{a*}, Armida Torreggiani^b, Chiara Pituello^a, Annamaria Pisi^c, Francesco
5 Morari^a, Ornella Francioso^c

6

7 ^aDepartment Agronomy, Food, Natural resources, Animals and Environment,
8 University of Padova, Viale Dell'Università 16, 35020 Legnaro, Italy

9

10 ^bInstitute of Organic Synthesis and Photoreactivity (ISOF), National Research
11 Council, Via P. Gobetti, 101 40129 Bologna, Italy

12

13 ^cDepartment of Agricultural and Food Sciences, University of Bologna, Viale Fanin 44,
14 40127 Bologna, Italy

15

16 *Corresponding author: ilaria.piccoli@unipd.it

17 **Abstract**

18 Biochar from agricultural biomasses and solid wastes represents a win-win solution for
19 a rationale waste management. Its sustainable usage requires identification and
20 standardization of biochar characteristics. The aim of this work was to identify the
21 physical-chemical and spatial characteristics of biochars from pruning residues (PR),
22 poultry litter (PL), and anaerobic cattle digestate (CD) at two pyrolysis temperatures
23 (350°C and 550°C). The biochar characterization was carried out by applying emerging
24 imaging techniques, the 2D automated optical image analysis and hyperspectral
25 enhanced dark-field microscopy (EDFM), and by SEM analysis. As predictable, the
26 feedstocks composition and the pyrolysis temperature strongly influence the physical
27 structures of the biochar samples. PR biochar was mainly characterized by broken and
28 fragmented structure with irregular and rough particle surface, completely different
29 from the original PR wood cell. The EDFM imaging analysis evidenced the thermal
30 degradation of PR vegetal products, composed primarily by hemicellulose, cellulose
31 and lignin. On the contrary, small and regular particles with smoot surface were
32 produced by the PL pyrolysis, especially at 550°C due to minor PL morphological
33 homogeneity in comparison with the other biomasses. Finally, CD charring was
34 characterized by changes in chemical composition, suggested by a lower pixel intensity.
35 In conclusion, the emerging imaging techniques used in this study showed to be very
36 effective in analyzing some properties of biochars then, they can be considered as
37 promising experimental strategies for detecting the feedstocks and pyrolysis
38 temperature of biochar.

39

40 KEYWORDS. Pyrolysis temperature, pruning residues, poultry litter, anaerobic cattle
41 digestate, discriminant analysis, hyperspectral imagery.
42

43 **1. Introduction**

44 Biochar is a carbon-rich material produced by burning agricultural biomasses (*e.g.*, crop
45 residues, wood biomass, animal litters) and solid wastes with little or no oxygen (*i.e.*,
46 pyrolysis or “charring”) (Sohi, 2012). The abundance of these biomasses and their
47 conversion into biochar are surely promising resources to improve waste management
48 and environment (Tan et al., 2015). In fact, a large number of investigations have
49 highlighted how biochar may have a positive impact on mitigating global warming, soil
50 amendment, enhancing crop yield and carbon storage (Khare and Goyal, 2013;
51 Lehmann, 2007; Lehmann and Joseph, 2009; Sohi, 2012; Verheijen et al., 2014;
52 Whitman et al., 2011; Woolf et al., 2010). The carbon sequestration potential of biochar
53 is attributed to its increase in soil stable C fraction (Kuzyakov et al., 2009; Major et al.,
54 2010) and hence long turnover time in soils. However, several studies have provided
55 examples where turnover times in soil are relatively short (<50 years) (Hilscher and
56 Knicker, 2011; Nguyen et al., 2009) and therefore, its longevity is still debated.

57 The biochar specific features, including porous structure and pore size distribution,
58 large specific surface area, active surface due to oxygen functional groups and presence
59 of minerals, make it as a possible adsorbent of nutrients or pollutant remover from
60 aqueous solutions, similar to activated carbon (Chun et al., 2004; Fu et al., 2012; Li et
61 al., 2008). There is lots of interest in understanding the behavior of biochar and precise
62 information on how the biochar is made from, and produced. All biochars do not have
63 the same properties since their chemical, structural and morphological characteristics
64 depend on the feedstock types, pyrolysis conditions, rate of heating-slow *versus* fast
65 pyrolysis and the duration of charring (Manyà, 2012; Pituello et al., 2015; Zimmerman

66 and Gao, 2013), of which, the temperature has been found to have a key role on
67 structural characteristics rather than the biomass feedstocks (Chen et al., 2012).

68 It is generally accepted that biochars are composed by more or less highly conjugated
69 aromatic ring. These structures become more polycondensed with increasing production
70 temperature (Preston and Schmidt, 2006). Depending on temperature, biochar produced
71 at low-temperatures have a greater reactivity in soils possibly due to their higher
72 available nutrients that may contribute to improve soil fertility (Chan et al., 2008; Day
73 et al., 2005) than biochar yield at high temperatures, enriched in material analogous to
74 activated carbon (Ogawa et al., 2006). The latter char is very brittle and prone to abrade
75 into fine fractions that may be incorporated into the soil mineral fraction or may be
76 easily transported in the environment.

77 Particle size distribution is critical for transport and distribution operations. A median
78 diameter of about 10 μm increases the dustiness of the very light fraction of biochar
79 (Blackwell et al., 2009) affecting the uniformity of spreading and increasing the health
80 risk for operators and particle drift in the environment. However, little is known about
81 the role of particle size in various soil processes such as the transport of adsorbed
82 contaminants (Oleszczuk et al., 2016), the nutrient release, the soil structure
83 aggregation, etc.. A greater contact area and thus a higher particle-to-particle
84 interactions are favoured by a small particle size and platy shape. However, Joseph et al.
85 (2009) postulated that the large inner porosity of a given biochar particle may make
86 particle size a redundant parameter especially for processes associated to water and
87 nutrient availability. Recently, Pituello et al. (2018) have reported contrasting effects
88 also on aggregate stability of different soils amended with biochar. Biochar application
89 increased the soil surface area in clay-poor soil providing additional interparticle

90 bonding while in clay-rich soil favored repulsive forces between particles with the same
91 charge and consequently, reduced soil particle aggregation.

92 Therefore, a particle size and shape characterization may improve the understanding
93 of the behaviour of a wide range of pyrolysis yields important for soil amendment
94 purposes.

95 The measurement of particle size alone is very complicate and may not be enough
96 sensitive to identify the differences between char samples because of heterogeneous
97 nature of feedstock and pyrolysis effect. Particles having very different shapes, but the
98 same area may be identified as identical. No instrument can really measure the particle
99 size distribution independently by the particle shape. Although it is possible to obtain
100 information about particle shape with laser diffraction (Ma et al., 2001), only imaging
101 analysis allows the real characterization of particle size and shape (Bittelli et al., 2019).
102 Imaging analysis technique is an effective technique both for particle size and shape and
103 can provide a real insight into the nature of particles under pyrolysis process. This
104 technology can quantify the size and shape of particles and it has been demonstrated to
105 be able to differentiate the particles (Polakowski et al., 2014). The system works by
106 using imaging every particle and can report particle size and shape data in terms of both
107 volume and number. The images are also screened using a range of morphological
108 filters to remove such things as partially imaged and/or overlapping particles.

109 There is a need for characterization and analytical tools that can deal with
110 heterogeneous samples with minimum sample preparation. Several different techniques
111 such as Fourier transform infrared (FT-IR) spectroscopy have been applied for
112 evaluating various pyrolysis products (Cantrell et al., 2012; Pituello et al., 2015;

113 Srinivasan et al., 2015). However, the lack of spatial information makes FT-IR
114 spectroscopy not successful when chemical composition distribution is needed.

115 Hyperspectral imaging analysis systems combine conventional imaging and
116 spectroscopy for the identification and quantification of chemical constituents, as well
117 as their location or spatial distribution simultaneously. Hyperspectral enhanced dark-
118 field microscopy (EDFM) is a relatively new inspection technology that provides both
119 spectral and spatial information from the product with high spectral resolution through
120 the analysis of scattered light at pixel-by pixel level (Grahm and Geladi, 2007). Samples
121 are imaged by acquiring hundreds of continuous wavelengths or bands, producing
122 extensive spatial and spectral data for each pixel. Hyperspectral EDFM is specifically
123 designed to give quantitative mapping of surfaces and material identification for
124 heterogeneous samples (Badireddy et al., 2012; Torreggiani et al., 2014; Verebes et al.,
125 2013) as biochars. As most of products, biochar needs to be classified in order to
126 conform to a standard related to its usage. Some classification criteria for other
127 pyrolysis products are available, while none for biochar. For example, according to
128 Australian Environmental Protection Authority biosolids can be classified according to
129 contaminant and stabilization grade which, in turn, determine the permitted uses
130 (NSWEPA, 1997). A desired classification would relate biomass and pyrolysis type
131 with agronomic properties but unfortunately data to develop an appropriate biochar
132 classification framework are still lacking (Joseph et al., 2009). Some authors proposed
133 to cluster feedstock properties according to biochar characteristic, as percentage of
134 organic compounds (Demirbaş, 2001), inorganics composition (Nik-Azar et al., 1997),
135 particle size (Zanzi et al., 2002) or moisture content (Moghtaderi, 2006). Another
136 possible biochar classification has been proposed by Joseph et al. (2009) and includes

137 biochar properties (*e.g.*, ash content, labile C, pH) as a function of pyrolysis conditions.
138 The authors pointed out the need of more studies to fully characterize the range of
139 biochars that may be applied to soil.

140 The major objective of this work was to test the ability of new and emerging
141 imaging techniques such as 2D optical image and hyperspectral analysis, to provide
142 physical-chemical and spatial properties of biochars generated by the low temperature
143 pyrolysis from different feedstocks such as vineyard pruning residues, anaerobic
144 digestate and litter poultry. The obtained information will provide a wider picture on the
145 potential agronomic and environmental applications of biochar.

146

147 **2. Material and methods**

148 2.1 Feedstocks and Biochar Production

149 Feedstocks were collected from plants and experimental farms located in Veneto
150 Region, North-East Italy: (i) anaerobic digestate (CD) from a biogas plant that uses
151 cattle manure mixed with silage maize (30% c.a.), (ii) dry poultry litter (PL) from
152 Italtollina® Italtollina SpA, Verona and (iii) vineyard pruning residues (PR) from the
153 University farm. Feedstocks were dried overnight at 65 °C until the initial moisture
154 (ranging from 40 to 90 %) dropped to less than 7 % (except for dry poultry litter,
155 moisture content 12%) and then ground to a particle size of less than 2 mm. The
156 samples were pyrolyzed in lid-covered porcelain crucibles (Haldenwanger 79MF) in a
157 muffle furnace, preheated at 100 °C, to a highest heating temperature of, 350 and 550
158 °C with a heating rate between 16 and 19 °C/min and a residence time of 1 hour. The
159 crucibles were then moved with the lids on and left to cool down at room temperature to

160 prevent any loss in homogeneity due to accidental combustion. All experimental details
161 have been described in previous paper (Pituello et al., 2015).

162 The produced biochar was weighted and stored in air-tight Falcon vials prior to further
163 analysis.

164

165 2.2 Image analysis based on 2D technique

166 Size distribution and morphology descriptors of biochar particles were determined using
167 an automated particle system, Morphologi G3 (Malvern Instruments Ltd, Malvern, UK).

168 The instrument gave a detailed analysis by automatically capturing images of the
169 sample scanned with microscopic optics.

170 Biochar samples were dry dispersed on a glass plate by means of an automated
171 dispersion unit. Each sample was scanned using a 5x optics, on a circular scan area of
172 4.2 cm radius. Diascopic light was set at 80% intensity and focus was manually adjusted
173 before each measurement. To account for 3-dimensionality of particles, a z stacking
174 was used, resulting in an additional layer above focus of 48.9 μm . The size and shape
175 morphological descriptors were calculated by using the Malvern Morphologi G3
176 software analyzing at least 500.000 particles for each sample. Intensity mean (I) was
177 calculated as the average of the pixel greyscale levels in the particle, for greyscale
178 images I ranges between 0 (black) and 255 (white). Particle dimensions were quantified
179 in terms of volume (μm^3) and diameter (μm).

180 Particle shapes were quantified in terms of the following parameters:

181- Circularity (C), a measure of how well an object approximates a perfect circle, was
182 calculated as follows:

$$183 \quad C = \frac{2\pi A}{P^2} \quad (1)$$

184 where A is the particle area and P is the particle perimeter. Circularity ranged between 0
185 and -1, where 1 corresponds to a perfect circle while irregular objects approached 0.

186- Convexity (C_x) measures the edge roughness of a particle, and is the ratio between the
187 convex hull perimeter (P_c) and the actual perimeter of an object:

$$188 \quad C_x = \frac{P_c}{P} \quad (2)$$

189 Convexity ranges between 0 and 1. An object with a convexity of 1 indicates a smooth
190 shape because the convex hull perimeter equals actual perimeter in this instance.

191- Elongation index (E_i), a measurement of the overall symmetry/asymmetry of an object
192 is determined as noted below:

$$193 \quad E_i = 1 - \frac{width}{length} \quad (3)$$

194 where width and length are the shortest and longest object axes. Elongation indicates
195 the symmetry (close to 0) or asymmetry (close to 1) of an object in all directions.

196

197 2.3 Enhanced dark-field microscopy and hyperspectral imaging

198 Ground biochar samples (500 μm) were visualized, in air and at room temperature, *via*
199 their light scattering using an enhanced dark-field illumination system (CytoViva,
200 Auburn, AL) attached to an Olympus microscope. The system consisted of a CytoViva
201 150 dark field condenser in place of the microscope's original condenser, attached *via* a
202 fibre optic light guide to a Solarc 24 W metal halide light source (Welch Allyn,
203 Skaneateles Falls, NY). Improved optical performances are obtained by pre-aligned
204 Koehler and the main feature of Critical illumination. A 100X oil objective with an iris
205 (Olympus UPlanAPO fluorite, N.A. 1.35–0.55) was an integral part of the system.
206 Spectral data within each pixel of the scanned field of view were captured with a

207 CytoViva spectrophotometer and integrated CCD camera. The visible near-infrared
208 spectrophotometer operates in the range 400-1000 nm. Spectral data were analysed by
209 using the CytoViva Hyperspectral analysis software program (ENVI 4.4 and ITT Visual
210 Information Solutions). Image processing and analysis involved the building of spectral
211 libraries (spectral endmembers). The spectral endmembers were obtained by the
212 selection of a region of interest on the scanned sample. Finally, Spectral Angle Mapper
213 was used to measure the similarity between the image pixels and endmember pixels.

214

215 2.4 Scanning electron microscopy (SEM)

216 Dry samples of feedstocks (and their pyrolysis products at 550 °C) were mounted on
217 aluminium stubs with silver glue and coated with gold-palladium film using an ion
218 sputtering unit Balzer MED 010 (Balzers Union, Ltd, Balzers, Liechtenstein). The
219 samples were observed under a Philips SEM 515 scanning electron microscope (Philips,
220 Eindhoven, The Netherlands) at 7Kv and the pictures taken with a Nikon 5400 Coolpix
221 digital camera (Nikon, Chiyoda-ku, Tokyo, Japan).

222

223 2.5 Statistical Analysis

224 Temperature effect on biochar morphological parameters was compared by applying the
225 non-parametric paired sign test. Bonferroni correction was then adopted to account for
226 multiple comparisons considering significant at $P \leq 0.05/3 = 0.017$.

227 To assess the ability of morphological parameters to best predict biochar origin,
228 backward stepwise discriminant analysis (DA) was employed. The descriptors for the
229 initial DA were circularity (C), elongation (E_i), convexity (C_x) and intensity (I). Nine
230 pre-defined groups were taken into account as result of the linear combination of three

231 matrix types × three pyrolysis temperatures. The multiple discriminant functions, the
232 classification criteria, were determined by a measure of Squared Mahalanobis
233 Distances. Classification criteria were based on within-group pooled covariance matrix
234 considering an equal prior probability of the groups. Multivariate F-tests was then
235 applied on pooled within-group variance and covariance matrices in order to determine
236 whether or not there were any significant differences between groups. Canonical
237 correlation analysis was used to extract canonical roots and case scores. Only the roots
238 found statistically significant were used to plot group structure.
239 Statistical analyses were carried out using Statistica version 10 (Stat Soft. Inc., Tulsa,
240 OK, USA).

241 **3. Results and discussion**

242 *3.1 Property of feedstocks and Biochar*

243 Chemical, physical and structural compositions of biochars at different pyrolysis
244 temperatures were described by Pituello et al. (2015). All the feedstocks had a neutral
245 reaction, except CD that was alkaline (pH 8.3). Electrical Conductivity (EC) ranged
246 from 412 to 1642 $\mu\text{s cm}^{-1}$, respectively in PL and CD. In addition, ash content was
247 extremely variable; it ranged from 2.9 to 5.6 wt % in biomass-based feedstocks (CD and
248 PR). Total carbon was > 40 wt %, while total nitrogen content increased from 1.7 to 4.2
249 wt % going from CD to PL, and it was sensitively lower for PR. The temperature effect
250 on pH value was evident on all the samples but it was particularly relevant on PL
251 samples, as it varied from 6.9 up to 10.2 with an increase in the pyrolysis temperature.
252 The temperature increase caused a U-shaped response trend in the EC values of both
253 CD and PL samples, whereas in the case of PR gave rise to a decrease in EC values.
254 Ash content increased by increasing the temperature and was higher in the feedstocks
255 with low C content, reaching 32% in PL at 550°C (see Supplementary Material section
256 for further details).

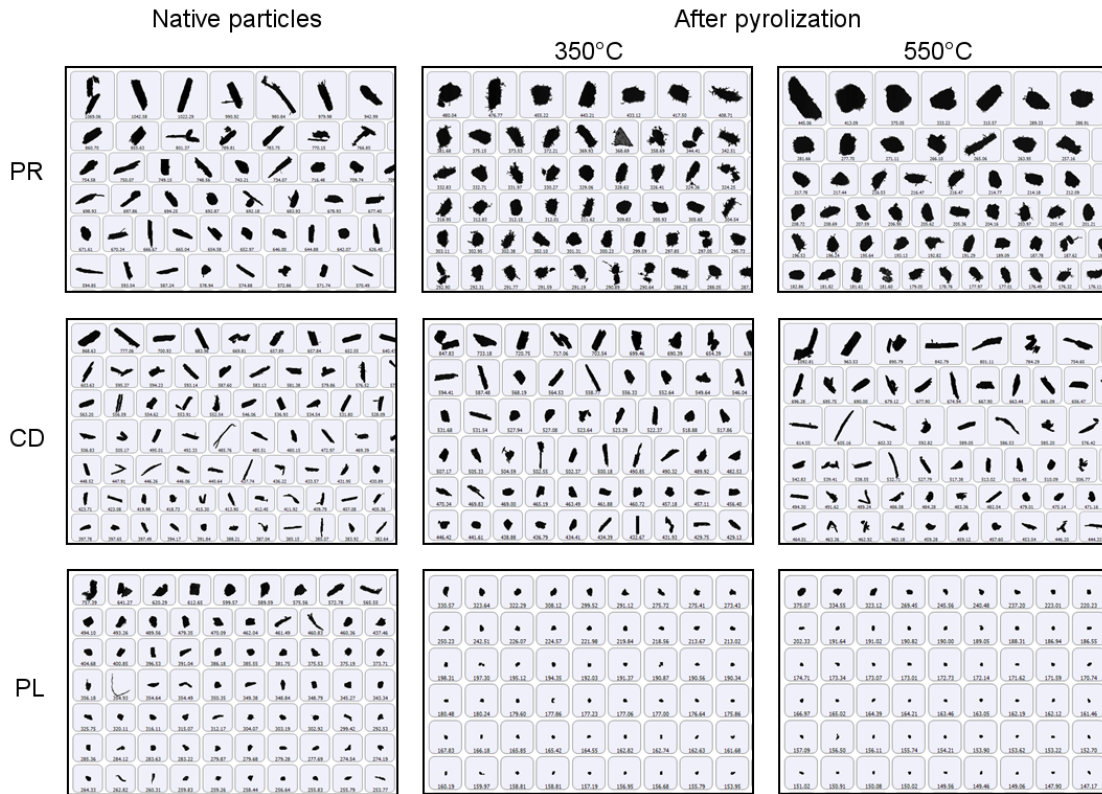
257

258 *3.2 Image analysis based on 2D technique*

259 Size and shape captured by 2D images (Figure 1), detected considerable modifications
260 for PR biochar particles at both pyrolysis temperatures. Some particles displayed
261 heterogeneity in morphology and in grayscale intensity compared to native PR particles.
262 Especially, agglomerates are prevalent at 550°C (see the top row of Figure 1).
263 Polymerization/condensation reactions taking place during pyrolysis were responsible
264 for different heterogeneous aggregates formation in biochar. Furthermore the increased

265 alkalinity observed at high temperatures was probably related to
266 polymerization/condensation reactions (Gascó et al., 2005; Liang et al., 2016), release
267 of low molecular weight compounds (*i.e.*, water and acids), high concentration in base
268 cations and carbonates (Fidel et al., 2017).

269 Conversely, these changes were not so strong for the CD and PL biochar particles. In
270 particular, the color and shape of CD biochars appeared similar to unpyrolyzed material.
271 Instead PL biochar particles displayed a consistent and regular reduction of particles
272 size at both temperatures. Cantrell et al. (2012) observed PL-derived biochar to exhibit
273 the least aromaticity compared to other matrices which could have caused an higher
274 fragmentation with the pyrolysis temperature. These observations suggest that the
275 variability of biochar response to the temperature depends on the feedstock
276 composition, which can be converted into a wide range of shapes from irregular to
277 spherical.



278

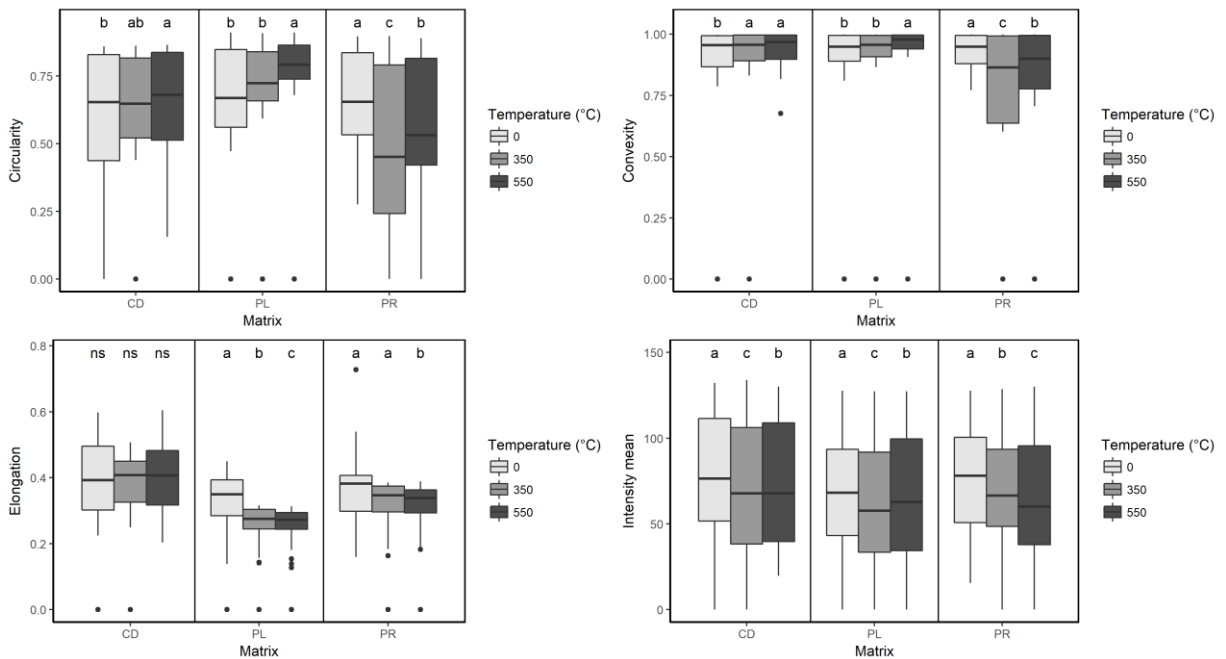
279 **Figure 1.** Set of images of different feedstocks, vineyard pruning residues (PR), cattle
 280 anaerobic digestate (CD) and poultry litter (PL) before and after pyrolysis at 350°C and
 281 550°C. The biochar particles displayed morphological heterogeneity in PR and CD.

282

283 Overall, the shape descriptors as circularity, convexity and elongation are shown in
 284 Figure 2.

285 In PR samples the circularity median values showed statistical difference ($P \leq 0.017$)
 286 and in biochars they exhibited smaller values than in their original state (0.504, 0.586
 287 and 0.669 for biochar 350, 550°C and feedstock particles, respectively) A decrease of
 288 circularity value suggests that the irregular particles formation was consistent in PR
 289 biochar. Similarly, the convexity exhibited a significant ($P \leq 0.017$) decrease in both
 290 biochars, 0.807 for 350°C and 0.849°C for 550°C, with respect to PR particles in their
 291 original state (0.934). Thus, the PR particles shape of both biochars considerably

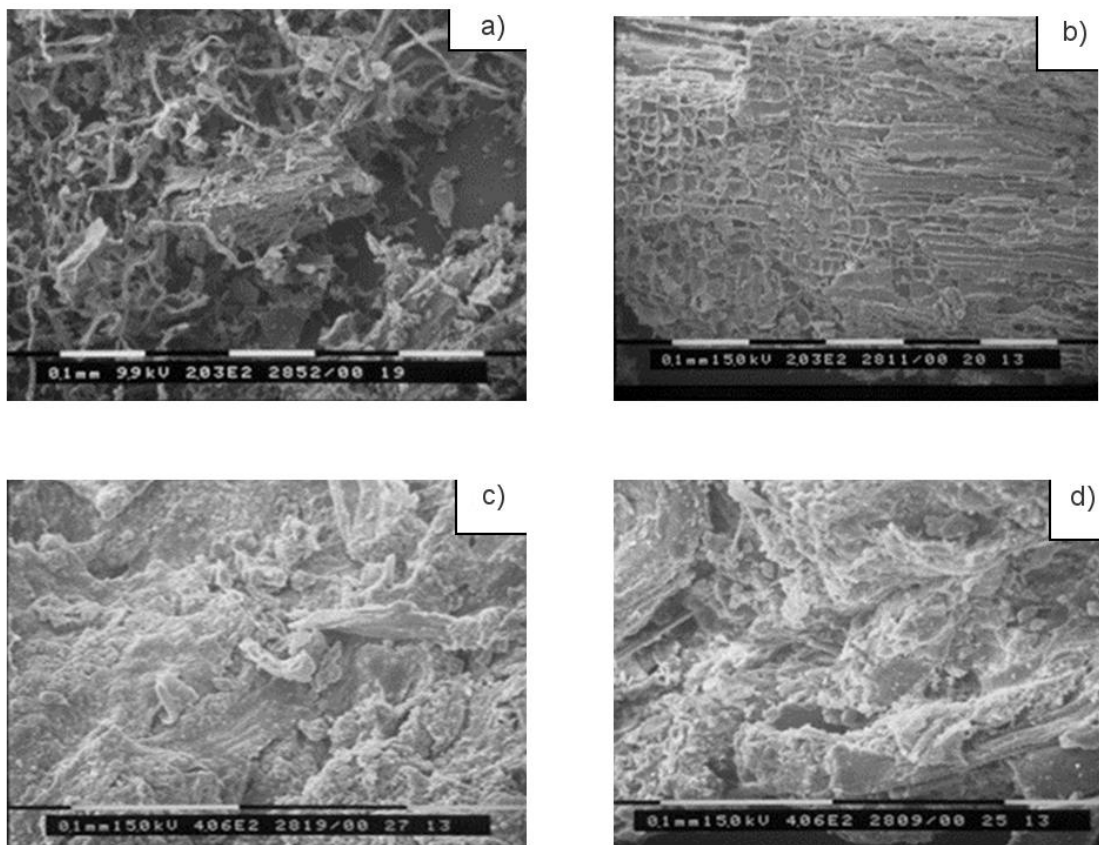
292 became irregular and increased the surface roughness as an effect of the temperature.
 293 These changes are also supported by a significant ($P=0.017$) decrease of elongation
 294 median values in both biochars, 0.319 and 0.311 for 350°C and 550°C vs 0.366 in native
 295 particles. The outliers, appearing only after heating, denoted elongation values closer to
 296 0 and therefore, they can be classified as “not elongate”. This change may be due to the
 297 volatilisation process of the external components of the particles, which may lead to a
 298 decrease in stability of this outer part of the particles and a subsequent disintegration.



299 **Figure 2.** Box plot of vineyard pruning residues (PR), cattle anaerobic digestate (CD)
 300 and poultry litter (PL) and biochars at 350° and 550°C descriptors. Different letters
 301 indicate statistical differences, according to Bonferroni correction for multiple
 302 comparisons consider ($P \leq 0.017$). The box represents the upper (75%) and lower
 303 quartile (25%) of the data termed the interquartile range, the horizontal line inside the
 304 box is the median of the data, and the ends of the whiskers show the highest and lowest
 305 data points.

307

308 The observed modification was also detectable by SEM micrograph of biochar at 550°C
309 where is clearly visible how the pyrolysis treatment totally modified the wood cell
310 morphological structure originally present, substituting it with a structure completely
311 broken and fragmented (Figure 3a and b). This furthermore supported the reduction in
312 elongation and circularity descriptors.



313
314 **Figure 3.** Scanning electron micrographs (SEM) of A) biochar from vineyard pruning
315 residues (PR) yielded at 550 °C; B) unpyrolyzed PR; C) biochar from poultry litter (PL)
316 yielded at 550°C and D) unpyrolyzed PL.

317

318 In PL samples only circularity and convexity median values statistically differed ($P =$
319 0.017) in biochar at 550°C (Figure 2). Moreover, both descriptor values were bigger
320 than PL particles in their original state. No statistical difference was found between PL

321 particles and biochar at 350°C. About elongation median values there were statistical
322 differences ($P=0.017$) between samples. Especially, elongation values decreased from
323 0.326 in native particles to 0.261 for 350°C and 0.256 for 550°C biochar, the latter two
324 might be classified as “not elongate”. Overall, the pyrolysis temperatures produced
325 smaller particles with regular and smooth surface compared to the poultry litter in
326 original state. This phenomenon was more evident at 550°C.

327 The SEM micrograph of PL biochar at 550°C (Figure 3c) gave information about the
328 formation of agglomerates with an irregular and nonporous surface.

329 As regards to CD, no statistical differences were found among the circularity, convexity
330 median and elongation values between biochar obtained at 350°C and 550°C.
331 Conversely, circularity and convexity descriptors increased and statistically ($P=0.017$)
332 differed from unpyrolyzed CD particles. Biochar particles at 550°C were mostly regular
333 in size and shape. We can infer that the regularization of biochar particles at 550°C
334 might be as a consequence of enhanced aromaticity and/or occurred minerals
335 calcination (e.g., calcite) as described by Hung et al. 2017).

336 Pixel intensity showed median values exhibited statistical difference ($P = 0.017$) in all
337 samples. Their values progressively decreased from 79 in feedstock particles to 72 in
338 both biochars because of the pyrolysis temperature. The variations in pixel intensity
339 could be an indication of differences in composition. Particles containing high-density
340 components may produce higher intensity images. However, because little is known
341 about the application of this technique on biochar, no attempts were made to quantify
342 these differences from the images.

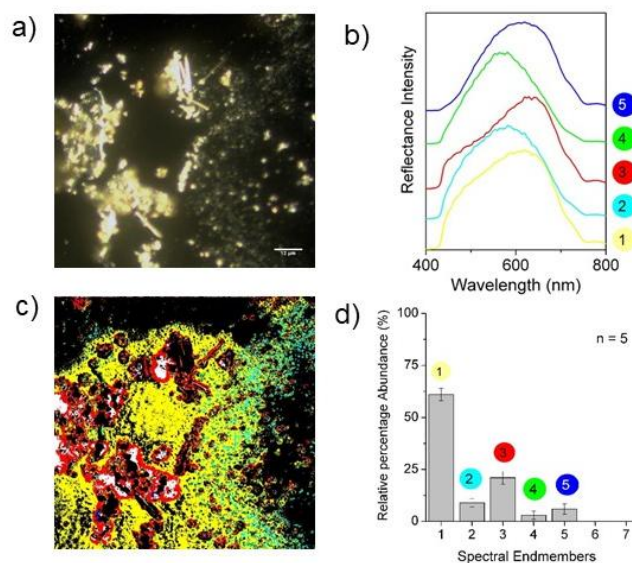
343 These results suggested that automated particle imaging provided a rapid evaluation
344 between physical descriptors of different feedstocks, pyrolysis temperature and resulting

345 biochar. These parameters may be considered effective tool for identifying the
346 temperature experienced by feedstock particles at low pyrolysis temperature.

347

348 *3.3 Hyperspectral analysis*

349 A series of initial measurements were performed in order to evaluate the ability of the
350 Hyperspectral imagery analysis to characterize and differentiate the biochars. All the
351 samples were first imaged *via* light scattering using the EDFM system (as example,
352 Figure 4a) and then the Hyperspectral analysis was performed. Light scattering from PR
353 feedstock gave rise to five spectra (Figure 4b). These spectral endmembers were
354 successively used in the image scenes of all samples to perform the spectral mapping.
355 The Spectral Angle Mapper (SAM) classification image showed the distribution of all
356 the five endmembers in the PR image (Figure 4c) and the quantification of their relative
357 abundance (Figure 4d). Bright areas in Figure 4a, corresponding to the black areas in the
358 spectral mapping of Figure 4c, are spectrally unresolved because of their extremely high
359 brightness. Among five spectra, one (Endmember 1) represented the main contribution
360 to the total scattering of PR (~60%). Consequently, it can be considered the most
361 representative for PR feedstock.

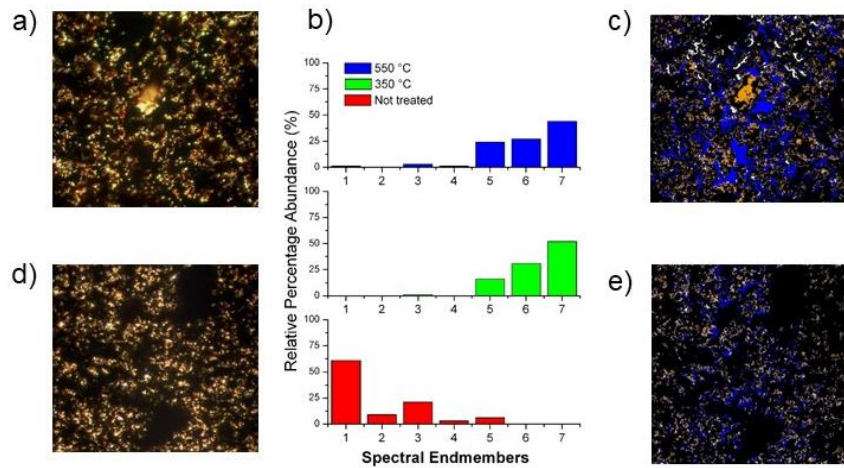


362

363 **Figure 4.** (a) Hyperspectral image (EDFM), (b) Spectral signatures in the 400-800 nm,
 364 and (c) Map of the spectral Endmembers in the hyperspectral image of the vineyard
 365 pruning residues (PR) obtained by SAM analysis (coloured areas indicate the matching
 366 with the spectral profiles); (d) Histogram reporting the relative percentage abundance of
 367 the spectral patterns in the maps of hyperspectral images of four PR samples (n standing
 368 for the number of the analysed samples). Images were acquired by using 40x objective.
 369 All images are $60 \mu\text{m} \times 60 \mu\text{m}$.

370

371 As expected, the pyrolysis at $350 \text{ }^\circ\text{C}$ induced strong morphological changes in PR
 372 biochar, well visible in the EDFM image (Figure 5a). This was also confirmed by the
 373 disappearance of the four endmembers contributing to the total scattering of PR
 374 feedstock and the presence of two new spectral profiles (Figure 5b). Conversely, little
 375 morphological modifications appeared at 550°C (Figure 5d). In fact, the three
 376 endmembers mainly contributing to the scattering of the biochar at 350 and 550°C are
 377 the same (5, 6, and 7), and only some slight changes in their relative percentage
 378 abundance were found (Figure 5b).



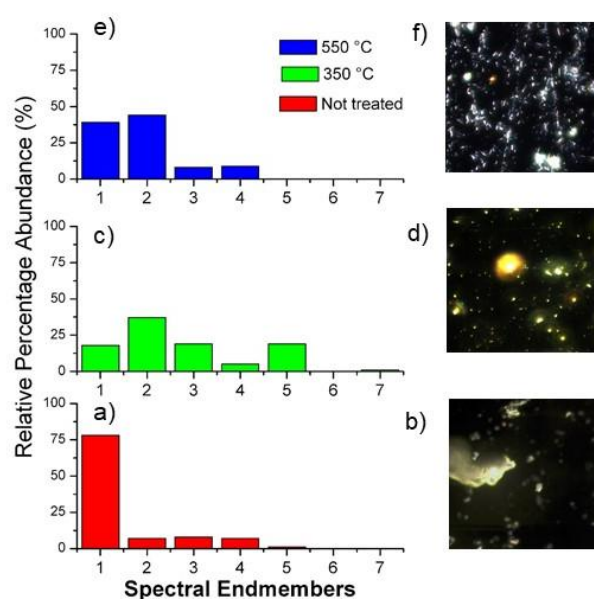
379

380 **Figure 5.** Spectral mapping of vineyard pruning residues (PR) after pyrolysis at 350°
 381 and 550°C. (a), (d): EDFM images; (b) Relative percentages abundance of the spectral
 382 profiles, revealed by the SAM analysis of the hyperspectral images. For comparison,
 383 also the relative percentages obtained before the pyrolysis treatment are reported; (c)
 384 and (e): spectral mapping. (a), (c): treated at 350°C; (d), (e): treated at 550°C.

385

386 This spectral pattern is characteristic of biochar and can be ascribed to the thermal
 387 degradation products of vegetal biomass, composed primarily of hemicellulose,
 388 cellulose and lignin. Our results are consistent with thermal analyses under pyrolysis
 389 conditions of raw biomass (Rutherford et al., 2012). The major decomposition processes
 390 take place from 200 to 500 °C and they are characterized by different steps: i) partial
 391 hemicellulose decomposition, (ii) complete hemicellulose decomposition and partial
 392 cellulose decomposition, (iii) full cellulose and partial lignin decomposition, and (iv)
 393 successive decomposition and increasing degree of carbonization (Rutherford et al.,
 394 2012). The disappearance of the Endmember 1, on the basis of descriptor shapes, can be
 395 associated to the absence of big particles and the formation of particles classified as “not
 396 elongate”.

397 As regards to CD, the further increase in the pyrolysis temperature induced progressive
 398 changes in the biochar. In particular, at 550°C bright spots, reflecting light more
 399 efficiently, were visible in the EFDM image, indicating the formation of semi-
 400 crystalline aggregates, compared with the amorphous structure prevailing in PR biochar
 401 (Figure 6). This result is also supported by previous FT-IR spectral profile of biochar
 402 from CD which showed a typical band resembling graphite-like carbon or with a low
 403 degree of disorder (Pituello et al., 2015). This is based on the relative intensity of the
 404 bands at 1437 cm^{-1} (it increases with the number of amorphous carbon structures) and
 405 at 1582 cm^{-1} , and it is sharpened as the degree of graphitization increase (Kaufman et
 406 al., 1989).

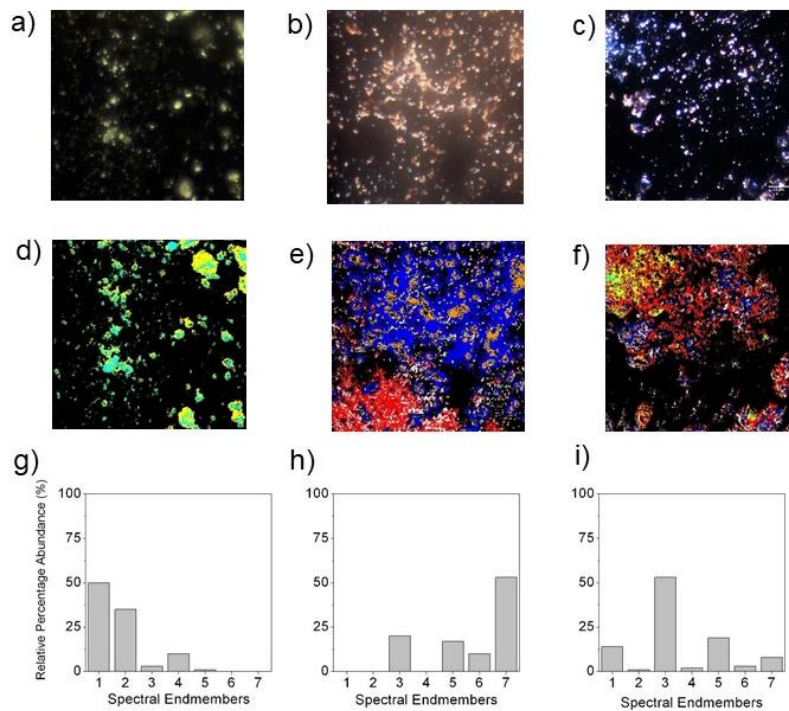


407
 408 **Figure 6.** Spectral mapping of cattle anaerobic digestate (CD) samples before and after
 409 pyrolysis. (a, c, e) Relative percentages abundance of the spectral profiles, revealed by
 410 the SAM analysis of the hyperspectral images, respectively for poultry litter (PL)
 411 samples before the heat treatment and after pyrolysis at 350° and 550°C. (b, d, f) EDFM
 412 images, respectively for PL samples before and after pyrolysis at the two different
 413 temperatures.

414

415 The progressive modifications occurring in the CD can be easily followed by
416 hyperspectral analysis: light scattering of the CD feedstock is due to only one
417 Endmember 1, probably for the presence of big and lengthened particles. The latter is
418 still present at the end of the thermal treatment but in a less relevant amount (from ~
419 75% to ~ 40%). In addition, the Endmember 2 sensitively increases its contribution,
420 probably because of the formation of anaerobic digestion products in the biochar. Thus,
421 these two spectral profiles (1 and 2) can be considered the most representative for this
422 biochar.

423 The pyrolysis of PL feedstock induced the strongest changes, clearly visible in the
424 EDFM images of the different spectral patterns at 350 and 550°C (Figure 7). The great
425 susceptibility to the temperature might depend on minor morphological homogeneity of
426 PL in comparison with other feedstocks. As in PR biochar, a significant contribution
427 from the Endmembers 5, 6, 7 was found at 350°C. We can infer that vegetal residues of
428 PL can contribute to this effect. Moreover, PL at 550°C exhibited different spectral
429 patterns in comparison with PR biochar. A spectroscopic characteristic of PL biochar is
430 the progressive increase in the contribution to the total light scattering of the sample
431 from the Endmember 3 (from 3% to 20%, and finally 53%): this behavior can be
432 attributed to the increase of the mineral component (CO_3^{2-}) (Pituello et al., 2015).



433

434 **Figure 7.** Spectral mapping of poultry litter (PL) samples before and after pyrolysis. (a,
 435 b , c): EDFM images of PL samples before the heat treatment and after pyrolysis
 436 respectively at 350° and 550°C; (d, e, f) Maps of the spectral Endmembers in the
 437 hyperspectral images; (g, h, i) Relative percentages abundance of the spectral profiles,
 438 revealed by the SAM analysis of the EDFM images, respectively for PL samples before
 439 the heat treatment and after pyrolysis at 350° and 550°C.

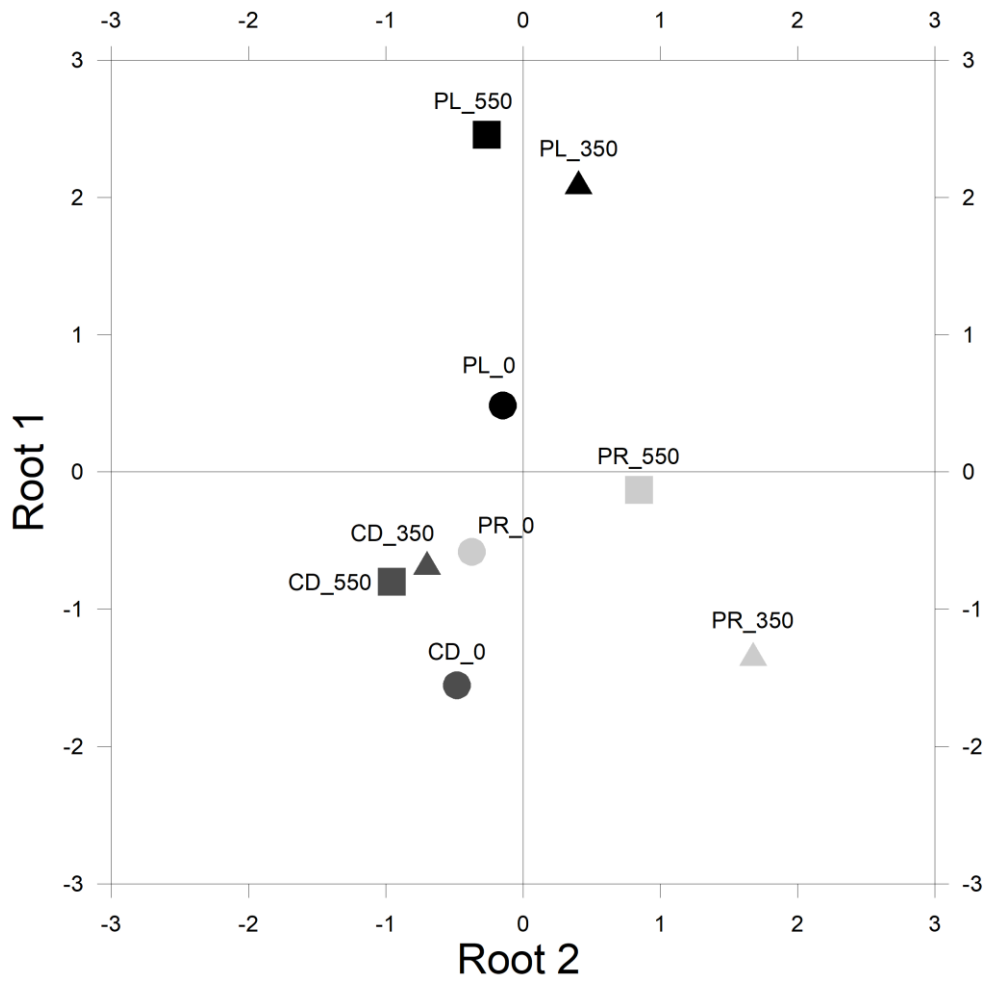
440

441 3.4 Relationships between image analysis-derived characteristics and different biochar
 442 matrices

443 To determine if the biochar image analysis-derived characteristics can be valid criteria
 444 to classify biochar from one another and feedstocks, we carried out the discriminant
 445 analysis (DA). The discriminant function was described by circularity, convexity,
 446 elongation and intensity mean predictors. Mahalanobis distance among groups pointed
 447 out no difference between CD biochars while pyrolysis temperatures significantly

448 discriminates PR and PL biochars. Four roots were extracted from the matrix and only
449 the first two accounted for 99.9 % ($P < 0.001$) of the total variance of variables.
450 Canonical analysis involved the construction of discriminant functions called “canonical
451 Roots” allowing n-dimensional space objects to be represented on 2D space preserving
452 objects distance order. Root 1 was associated with particle sphericity being positively
453 correlated with circularity and negatively with elongation. Root 2 was representative of
454 particle shape resulting correlated with circularity, convexity and elongation (see
455 Supplementary Material section for further details).

456 Biochar groups are shown in Figure 8 in the coordinate system of Root 1 (ordinate axis)
457 and 2 (abscissa axis). Root 1 discriminated PL compounds from PR and CD ones being
458 mostly particles characterized by regular circular shape. Root 2 separated PR biochar
459 from its native matrix confirming that pyrolysis strongly affected morphological
460 properties of PR particles. On the contrary CD-derived compounds laid in a clustered
461 area highlighting no differentiation between the CD compounds. Indeed as previously
462 reported, CD particle has been less modified by charring procedure.



463

464 **Figure 8.** Scatterplot of canonical Root 1 and 2 for the nine pre-defined groups

465 according to the interaction matrix \times temperature.

466 **4. Conclusions**

467 The findings show the potentialities of spectral libraries and image analysis to classify
468 biochar according to their feedstocks and the pyrolysis temperature, which strongly
469 influence the physical structures of the biochar samples. In fact, pruning residues and
470 poultry litter-derived biochar underwent the strongest morphological variations after
471 charring, allowing the easiest classification. On the contrary, the analysis of cattle
472 manure digestate, which exhibited smaller changes in its properties after pyrolysis,
473 indicated that other parameters may be helpful for a correct classification of such
474 matrices.

475 The emerging imaging techniques used in this study showed to be very effective in
476 analyzing some properties of biochars, and they can represent promising experimental
477 strategies for detecting the feedstocks and pyrolysis temperature of biochars. On the
478 other hand, hyperspectral analysis of scattered light, thanks to its property to detect
479 changes in light scattering properties, demonstrated that it is possible to build-up a
480 library of spectral data characteristics of the different morphologies correlated to the
481 chemical composition and heating treatment of the biomasses. On the other hand,
482 image analysis, which is the less expensive technique, showed to be very useful in
483 detecting the original matrix and pyrolysis temperature of biochar, suggesting its
484 potential usage in bio-waste traceability.

485

486 **Acknowledgments**

487 The authors are extremely grateful to Dr. Carla Marzetti (Antigenia srl Unipersonale),
488 the Italian contact person of Cytoviva company (University of Auburn, AL, USA) for
489 Hyperspectral System Imaging, and to Dr. Carla Ferreri (ISOF-CNR) for providing the
490 CytoViva® hyperspectral microscope. This research did not receive any specific grant
491 from funding agencies in the public, commercial, or not-for-profit sectors.

492 **References**

- 493 Badireddy, A.R., Wiesner, M.R., Liu, J., 2012. Detection, characterization, and
494 abundance of engineered nanoparticles in complex waters by hyperspectral
495 imagery with enhanced darkfield microscopy. *Environ. Sci. Technol.* 46, 10081.
496 <https://doi.org/10.1021/es204140s>
- 497 Bittelli, M., Andrenelli, M.C., Simonetti, G., Pellegrini, S., Artioli, G., Piccoli, I.,
498 Morari, F., 2019. Shall we abandon sedimentation methods for particle size
499 analysis in soils? *Soil Tillage Res.* 185, 36–46.
500 <https://doi.org/10.1016/J.STILL.2018.08.018>
- 501 Blackwell, P., Riethmuller, G., Collins, M., 2009. Biochar Application to Soil, in:
502 Lehmann, J., Joseph, S. (Eds.), *Biochar for Environmental Management*.
503 Earthscan, Gateshead, UK, pp. 207–222.
- 504 Cantrell, K.B., Hunt, P.G., Uchimiya, M., Novak, J.M., Ro, K.S., 2012. Impact of
505 pyrolysis temperature and manure source on physicochemical characteristics of
506 biochar. *Bioresour. Technol.* 107, 419–428.
507 <https://doi.org/10.1016/j.biortech.2011.11.084>
- 508 Chan, K.Y., Van Zwieten, L., Meszaros, I., Downie, A., Joseph, S., 2008. Using poultry
509 litter biochars as soil amendments. *Aust. J. Soil Res.* 46, 437–444.
510 <https://doi.org/10.1071/SR08036>
- 511 Chen, Z., Chen, B., Zhou, D., Chen, W., 2012. Bbsolute sorption and thermodynamic
512 behavior of organic pollutants to biomass-derived biochars at two pyrolytic
513 temperatures. *Environ. Sci. Technol.* 46, 12476–12483.

514 <https://doi.org/10.1021/es303351e>

515 Chun, Y., Sheng, G., Chiou, G.T., Xing, B., 2004. Compositions and sorptive properties
516 of crop residue-derived chars. *Environ. Sci. Technol.* 38, 4649–4655.
517 <https://doi.org/10.1021/es035034w>

518 Day, D., Evans, R., Lee, J., Reicosky, D., 2005. Economical CO, SO, and NO capture
519 from fossil-fuel utilization with combined renewable hydrogen production and
520 large-scale carbon sequestration. *Energy* 30, 2558–2579.
521 <https://doi.org/10.1016/j.energy.2004.07.016>

522 Demirbaş, A., 2001. Carbonization ranking of selected biomass for charcoal, liquid and
523 gaseous products. *Energy Convers. Manag.* 42, 1229–1238.
524 [https://doi.org/10.1016/S0196-8904\(00\)00110-2](https://doi.org/10.1016/S0196-8904(00)00110-2)

525 Fidel, R.B., Laird, D.A., Thompson, M.L., Lawrinenko, M., 2017. Characterization and
526 quantification of biochar alkalinity. *Chemosphere.*
527 <https://doi.org/10.1016/j.chemosphere.2016.09.151>

528 Fu, P., Hu, S., Xiang, J., Sun, L., Su, S., Wang, J., 2012. Evaluation of the porous
529 structure development of chars from pyrolysis of rice straw: effects of pyrolysis
530 temperature and heating rate. *J. Anal. Appl. Pyrolysis* 98, 177–183.
531 <https://doi.org/10.1016/j.jaap.2012.08.005>

532 Gascó, G., Blanco, C.G., Guerrero, F., Lázaro, A.M.M., 2005. The influence of organic
533 matter on sewage sludge pyrolysis. *J. Anal. Appl. Pyrolysis* 74, 413–420.
534 <https://doi.org/10.1016/j.jaap.2004.08.007>

- 535 Grahn, H.F., Geladi, P., 2007. Techniques and Applications of Hyperspectral Image
536 Analysis, Techniques and Applications of Hyperspectral Image Analysis. John
537 Wiley & Sons, Ltd, Chichester, West Sussex.
538 <https://doi.org/10.1002/9780470010884>
- 539 Hilscher, A., Knicker, H., 2011. Carbon and nitrogen degradation on molecular scale of
540 grass-derived pyrogenic organic material during 28 months of incubation in soil.
541 *Soil Biol. Biochem.* 43, 261–270. <https://doi.org/10.1016/j.soilbio.2010.10.007>
- 542 Hung, C.Y., Tsai, W.T., Chen, J.W., Lin, Y.Q., Chang, Y.M., 2017. Characterization of
543 biochar prepared from biogas digestate. *Waste Manag.* 66, 53–60.
544 <https://doi.org/10.1016/j.wasman.2017.04.034>
- 545 Joseph, S., Peacocke, C., Lehmann, J., Munroe, P., 2009. Developing a biochar
546 classification and test methods, in: Lehmann, J., Joseph, S. (Eds.), *Biochar for*
547 *Environmental Management*. Earthscan Ltd, London.
- 548 Kaufman, J.H., Metin, S., Saperstein, D.D., 1989. Symmetry breaking in nitrogen-
549 doped amorphous carbon: Infrared observation of the Raman-active G and D
550 bands. *Phys. Rev. B.* <https://doi.org/10.1103/PhysRevB.39.13053>
- 551 Khare, P., Goyal, D.K., 2013. Effect of high and low rank char on soil quality and
552 carbon sequestration. *Ecol. Eng.* 52, 161–166.
553 <https://doi.org/10.1016/j.ecoleng.2012.12.101>
- 554 Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., Xu, X., 2009. Black carbon
555 decomposition and incorporation into soil microbial biomass estimated by ¹⁴C
556 labeling. *Soil Biol. Biochem.* 41, 210–219.

557 <https://doi.org/10.1016/j.soilbio.2008.10.016>

558 Lehmann, J., 2007. Bio-energy in the black. *Front. Ecol. Environ.*

559 [https://doi.org/10.1890/1540-9295\(2007\)5\[381:BITB\]2.0.CO;2](https://doi.org/10.1890/1540-9295(2007)5[381:BITB]2.0.CO;2)

560 Lehmann, J., Joseph, S., 2009. Biochar for environmental management: science and

561 technology, *Science And Technology*.

562 Li, W., Yang, K., Peng, J., Zhang, L., Guo, S., Xia, H., 2008. Effects of carbonization

563 temperatures on characteristics of porosity in coconut shell chars and activated

564 carbons derived from carbonized coconut shell chars. *Ind. Crops Prod.* 28, 190–

565 198. <https://doi.org/10.1016/j.indcrop.2008.02.012>

566 Liang, C., Gascó, G., Fu, S., Méndez, A., Paz-Ferreiro, J., 2016. Biochar from pruning

567 residues as a soil amendment: Effects of pyrolysis temperature and particle size.

568 *Soil Tillage Res.* 164, 3–10. <https://doi.org/10.1016/j.still.2015.10.002>

569 Ma, Z., Merkus, H.G., Scarlett, B., 2001. Extending laser diffraction for particle shape

570 characterization: Technical aspects and application. *Powder Technol.* 118, 180–

571 187. [https://doi.org/10.1016/S0032-5910\(01\)00309-6](https://doi.org/10.1016/S0032-5910(01)00309-6)

572 Major, J., Lehmann, J., Rondon, M., Goodale, C., 2010. Fate of soil-applied black

573 carbon: Downward migration, leaching and soil respiration. *Glob. Chang. Biol.* 16,

574 1366–1379. <https://doi.org/10.1111/j.1365-2486.2009.02044.x>

575 Manyà, J.J., 2012. Pyrolysis for biochar purposes: A review to establish current

576 knowledge gaps and research needs. *Environ. Sci. Technol.*

577 <https://doi.org/10.1021/es301029g>

578 Moghtaderi, B., 2006. The state-of-the-art in pyrolysis modelling of lignocellulosic
579 solid fuels. *Fire Mater.* 30, 1–34. <https://doi.org/10.1002/fam.891>

580 Nguyen, B.T., Lehmann, J., Kinyangi, J., Smernik, R., Riha, S.J., Engelhard, M.H.,
581 2009. Long-term black carbon dynamics in cultivated soil, in: *Biogeochemistry*.
582 pp. 163–176. <https://doi.org/10.1007/s10533-008-9248-x>

583 Nik-Azar, M., Hajaligol, M.R., Sohrabi, M., Dabir, B., 1997. Mineral matter effects in
584 rapid pyrolysis of beech wood. *Fuel Process. Technol.* 51, 7–17.
585 [https://doi.org/10.1016/S0378-3820\(96\)01074-0](https://doi.org/10.1016/S0378-3820(96)01074-0)

586 NSW EPA, 1997. Environmental guidelines: use and disposal of biosolids products.
587 Environment Protection Authority, Chatswood, NSW, Australia.

588 Ogawa, M., Okimori, Y., Takahashi, F., 2006. Carbon sequestration by carbonization of
589 biomass and forestation: Three case studies, in: *Mitigation and Adaptation*
590 *Strategies for Global Change*. pp. 429–444. [https://doi.org/10.1007/s11027-005-](https://doi.org/10.1007/s11027-005-9007-4)
591 [9007-4](https://doi.org/10.1007/s11027-005-9007-4)

592 Oleszczuk, P., Ćwikła-Bundyra, W., Bogusz, A., Skwarek, E., Ok, Y.S., 2016.
593 Characterization of nanoparticles of biochars from different biomass. *J. Anal.*
594 *Appl. Pyrolysis* 121, 165–172. <https://doi.org/10.1016/J.JAAP.2016.07.017>

595 Pituello, C., Dal Ferro, N., Francioso, O., Simonetti, G., Berti, A., Piccoli, I., Pisi, A.,
596 Morari, F., 2018. Effects of biochar on the dynamics of aggregate stability in clay
597 and sandy loam soils. *Eur. J. Soil Sci.* 69, 827–842.
598 <https://doi.org/10.1111/ejss.12676>

599 Pituello, C., Francioso, O., Simonetti, G., Pisi, A., Torreggiani, A., Berti, A., Morari, F.,
600 2015. Characterization of chemical–physical, structural and morphological
601 properties of biochars from biowastes produced at different temperatures. *J. Soils*
602 *Sediments* 15, 792–804. <https://doi.org/10.1007/s11368-014-0964-7>

603 Polakowski, C., Sochan, A., Bieganski, A., Ryzak, M., Földényi, R., Tóth, J., 2014.
604 Influence of the sand particle shape on particle size distribution measured by laser
605 diffraction method. *Int. Agrophysics* 28, 195–200. [https://doi.org/10.2478/intag-](https://doi.org/10.2478/intag-20014-0008)
606 [20014-0008](https://doi.org/10.2478/intag-20014-0008)

607 Preston, C.M., Schmidt, M.W.I., 2006. Black (pyrogenic) carbon: A synthesis of current
608 knowledge and uncertainties with special consideration of boreal regions.
609 *Biogeosciences* 3, 397–420. <https://doi.org/10.5194/bg-3-397-2006>

610 Rutherford, D.W., Wershaw, R.L., Rostad, C.E., Kelly, C.N., 2012. Effect of formation
611 conditions on biochars: Compositional and structural properties of cellulose, lignin,
612 and pine biochars. *Biomass and Bioenergy* 46, 693–701.
613 <https://doi.org/10.1016/j.biombioe.2012.06.026>

614 Sohi, S.P., 2012. Carbon storage with benefits. *Science* (80-.).
615 <https://doi.org/10.1126/science.1225987>

616 Srinivasan, P., Sarmah, A.K., Smernik, R., Das, O., Farid, M., Gao, W., 2015. A
617 feasibility study of agricultural and sewage biomass as biochar, bioenergy and
618 biocomposite feedstock: Production, characterization and potential applications.
619 *Sci. Total Environ.* 512–513, 495–505.
620 <https://doi.org/10.1016/j.scitotenv.2015.01.068>

621 Tan, X., Liu, Y., Zeng, G., Wang, X., Hu, X., Gu, Y., Yang, Z., 2015. Application of
622 biochar for the removal of pollutants from aqueous solutions. *Chemosphere*.
623 <https://doi.org/10.1016/j.chemosphere.2014.12.058>

624 Torreggiani, A., Tinti, F., Savoini, A., Melchiorre, M., Po, R., Camaioni, N., 2014.
625 Hyperspectral imaging of polymer/fullerene blends. *Org. Photonics Photovoltaics*
626 2. <https://doi.org/10.2478/oph-2014-0003>

627 Verebes, G.S., Melchiorre, M., Garcia-Leis, A., Ferreri, C., Marzetti, C., Torreggiani,
628 A., 2013. Hyperspectral enhanced dark field microscopy for imaging blood cells. *J.*
629 *Biophotonics*. <https://doi.org/10.1002/jbio.201300067>

630 Verheijen, F.G.A., Graber, E.R., Ameloot, N., Bastos, A.C., Sohi, S., Knicker, H., 2014.
631 Biochars in soils: New insights and emerging research needs. *Eur. J. Soil Sci.* 65,
632 22–27. <https://doi.org/10.1111/ejss.12127>

633 Whitman, T., Nicholson, C.F., Torres, D., Lehmann, J., 2011. Climate change impact of
634 biochar cook stoves in western kenyan farm households: System dynamics model
635 analysis. *Environ. Sci. Technol.* 45, 3687–3694. <https://doi.org/10.1021/es103301k>

636 Woolf, D., Amonette, J.E., Street-Perrott, F.A., Lehmann, J., Joseph, S., 2010.
637 Sustainable biochar to mitigate global climate change. *Nat. Commun.* 1.
638 <https://doi.org/10.1038/ncomms1053>

639 Zanzi, R., Sjöström, K., Björnbom, E., 2002. Rapid pyrolysis of agricultural residues at
640 high temperature. *Biomass and Bioenergy* 23, 357–366.
641 [https://doi.org/10.1016/S0961-9534\(02\)00061-2](https://doi.org/10.1016/S0961-9534(02)00061-2)

642 Zimmerman, A.R., Gao, B., 2013. The stability of biochar in the environment, biochar
643 and soil biota. CRC Press, Boca Raton, FL.
644