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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

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(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
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17 Abstract

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

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

43 **1. Introduction**

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

The biochar specific features, including porous structure and pore size distribution, 57 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 aqueous solutions, similar to activated carbon (Chun et al., 2004; Fu et al., 2012; Li et 60 61 al., 2008). There is lots of interest in understanding the behavior of biochar and precise information on how the biochar is made from, and produced. All biochars do not have 62 the same properties since their chemical, structural and morphological characteristics 63 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

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

It is generally accepted that biochars are composed by more or less highly conjugated 68 aromatic ring. These structures become more polycondensed with increasing production 69 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.

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

bonding while in clay-rich soil favored repulsive forces between particles with the samecharge 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 sensitive to identify the differences between char samples because of heterogeneous 96 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 size distribution independently by the particle shape. Although it is possible to obtain 99 100 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 can provide a real insight into the nature of particles under pyrolysis process. This 103 technology can quantify the size and shape of particles and it has been demonstrated to 104 105 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;

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

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

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

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

146

147 **2. Material and methods**

148 2.1 Feedstocks and Biochar Production

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

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

162 The produced biochar was weighted and stored in air-tight Falcon vials prior to further163 analysis.

164

165 2.2 Image analysis based on 2D technique

Size distribution and morphology descriptors of biochar particles were determined using
an automated particle system, Morphologi G3 (Malvern Instruments Ltd, Malvern, UK).
The instrument gave a detailed analysis by automatically capturing images of the
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 4.2 cm radius. Diascopic light was set at 80% intensity and focus was manually adjusted 172 before each measurement. To account for 3-dimensionality of particles, a z stacking 173 was used, resulting in an additional layer above focus of 48.9 µm. The size and shape 174 175 morphological descriptors were calculated by using the Malvern Morphologi G3 software analyzing at least 500.000 particles for each sample. Intensity mean (I) was 176 177 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 in terms of volume (μm^3) and diameter (μm) . 179

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, was182 calculated as follows:

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

where A is the particle area and P is the particle perimeter. Circularity ranged between 0
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
$$C_x = \frac{P_c}{P}$$
(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
$$E_i = 1 - \frac{width}{length}$$
(3)

where width and length are the shortest and longest object axes. Elongation indicatesthe 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 their light scattering using an enhanced dark-field illumination system (CytoViva, 199 200 Auburn, AL) attached to an Olympus microscope. The system consisted of a CytoViva 150 dark field condenser in place of the microscope's original condenser, attached via a 201 202 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 Koehler and the main feature of Critical illumination. A 100X oil objective with an iris 204 (Olympus UPlanAPO fluorite, N.A. 1.35-0.55) was an integral part of the system. 205 206 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)

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

222

223 2.5 Statistical Analysis

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

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

matrix types \times three pyrolysis temperatures. The multiple discriminant functions, the 231 classification criteria, were determined by a measure of Squared Mahalanobis 232 233 Distances. Classification criteria were based on within-group pooled covariance matrix considering an equal prior probability of the groups. Multivariate F-tests was then 234 235 applied on pooled within-group variance and covariance matrices in order to determine whether or not there were any significant differences between groups. Canonical 236 correlation analysis was used to extract canonical roots and case scores. Only the roots 237 found statistically significant were used to plot group structure. 238

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 reaction, except CD that was alkaline (pH 8.3). Electrical Conductivity (EC) ranged 245 from 412 to 1642 µs cm⁻¹, respectively in PL and CD. In addition, ash content was 246 extremely variable; it ranged from 2.9 to 5.6 wt % in biomass-based feedstocks (CD and 247 248 PR). Total carbon was > 40 wt %, while total nitrogen content increased from 1.7 to 4.2 wt % going from CD to PL, and it was sensitively lower for PR. The temperature effect 249 on pH value was evident on all the samples but it was particularly relevant on PL 250 251 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 CD and PL samples, whereas in the case of PR gave rise to a decrease in EC values. 253 Ash content increased by increasing the temperature and was higher in the feedstocks 254 with low C content, reaching 32% in PL at 550°C (see Supplementary Material section 255 256 for further details).

257

258 *3.2 Image analysis based on 2D technique*

Size and shape captured by 2D images (Figure 1), detected considerable modifications for PR biochar particles at both pyrolysis temperatures. Some particles displayed heterogeneity in morphology and in grayscale intensity compared to native PR particles. Especially, agglomerates are prevalent at 550°C (see the top row of Figure 1). Polymerization/condensation reactions taking place during pyrolysis were responsible 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 particular, the color and shape of CD biochars appeared similar to unpyrolized material. 270 271 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 the least aromaticity compared to other matrices which could have caused an higher 273 274 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.



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

282

Overall, the shape descriptors as circularity, convexity and elongation are shown inFigure 2.

In PR samples the circularity median values showed statistical difference (P ≤ 0.017) and in biochars they exhibited smaller values than in their original state (0.504, 0.586 and 0.669 for biochar 350, 550°C and feedstock particles, respectively) A decrease of circularity value suggests that the irregular particles formation was consistent in PR biochar. Similarly, the convexity exhibited a significant (P ≤ 0.017) decrease in both biochars, 0.807 for 350°C and 0.849°C for 550°C, with respect to PR particles in their original state (0.934). Thus, the PR particles shape of both biochars considerably became irregular and increased the surface roughness as an effect of the temperature. These changes are also supported by a significant (P=0.017) decrease of elongation median values in both biochars, 0.319 and 0.311 for 350° C and 550° C *vs* 0.366 in native particles. The outliers, appearing only after heating, denoted elongation values closer to 0 and therefore, they can be classified as "not elongate". This change may be due to the volatilisation process of the external components of the particles, which may lead to a decrease in stability of this outer part of the particles and a subsequent disintegration.



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

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



313

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

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

particles and biochar at 350°C. About elongation median values there were statistical differences (P=0.017) between samples. Especially, elongation values decreased from 0.326 in native particles to 0.261 for 350°C and 0.256 for 550°C biochar, the latter two might be classified as "not elongate". Overall, the pyrolysis temperatures produced smaller particles with regular and smooth surface compared to the poultry litter in 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.

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

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

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

- biochar. These parameters may be considered effective tool for identifying thetemperature experienced by feedstock particles at low pyrolysis temperature.
- 347

348 3.3 Hyperspectral analysis

A series of initial measurements were performed in order to evaluate the ability of the 349 Hyperspectral imagery analysis to characterize and differentiate the biochars. All the 350 351 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 feedstock gave rise to five spectra (Figure 4b). These spectral endmembers were 353 354 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 abundance (Figure 4d). Bright areas in Figure 4a, corresponding to the black areas in the 357 spectral mapping of Figure 4c, are spectrally unresolved because of their extremely high 358 brightness. Among five spectra, one (Endmember 1) represented the main contribution 359 to the total scattering of PR (~60%). Consequently, it can be considered the most 360 representative for PR feedstock. 361



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

362

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



379

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

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

397 As regards to CD, the further increase in the pyrolysis temperature induced progressive changes in the biochar. In particular, at 550°C bright spots, reflecting light more 398 399 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 bands at 1437 cm⁻¹ (it increases with the number of amorphous carbon structures) and 404 at 1582 cm^{-1} , and it is sharpened as the degree of graphitization increase (Kaufman et 405 406 al., 1989).



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

The progressive modifications occurring in the CD can be easily followed by 415 416 hyperspectral analysis: light scattering of the CD feedstock is due to only one Endmember 1, probably for the presence of big and lengthened particles. The latter is 417 418 still present at the end of the thermal treatment but in a less relevant amount (from \sim 419 75% to \sim 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 PL in comparison with other feedstocks. As in PR biochar, a significant contribution 426 427 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).





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

3.4 Relationships between image analysis-derived characteristics and different biocharmatrices

To determine if the biochar image analysis-derived characteristics can be valid criteria to classify biochar from one another and feedstocks, we carried out the discriminant analysis (DA). The discriminant function was described by circularity, convexity, elongation and intensity mean predictors. Mahalanobis distance among groups pointed 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 the first two accounted for 99.9 % (P < 0.001) of the total variance of variables. 449 450 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 particle shape resulting correlated with circularity, convexity and elongation (see 454 455 Supplementary Material section for further details).

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



464 Figure 8. Scatterplot of canonical Root 1 and 2 for the nine pre-defined groups
465 according to the interaction matrix × 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 poultry litter-derived biochar underwent the strongest morphological variations after 470 471 charring, allowing the easiest classification. On the contrary, the analysis of cattle manure digestate, which exhibited smaller changes in its properties after pyrolysis, 472 473 indicated that other parameters may be helpful for a correct classification of such 474 matrices.

475 The emerging imagining 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 other hand, hyperspectral analysis of scattered light, thanks to its property to detect 478 changes in light scattering properties, demonstrated that it is possible to build-up a 479 library of spectral data characteristics of the different morphologies correlated to the 480 481 chemical composition and heating treatment of the biomasses. On the other hand, image analysis, which is the less expensive technique, showed to be very useful in 482 detecting the original matrix and pyrolysis temperature of biochar, suggesting its 483 484 potential usage in bio-waste traceability.

486 Acknowledgments

- 487 The authors are extremely grateful to Dr. Carla Marzetti (Antigenia srl Unipersonale),
- 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.

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