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Image grey value analysis for estimating the effect of microorganism inoculants on straws decomposition

Yutao Liu [a,b,1,*], Sanqin Zhao [a,1], Qi Zhu [a,1], Weimin Ding [a,*]

[a] College of Engineering, Nanjing Agricultural University, Nanjing, Jiangsu 210032, PR China
[b] Biological and Agricultural Engineering Department, University of California at Davis, Davis, CA 95616, USA

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ABSTRACT

The colour of straw changing from lighter to darker after being incorporated into soil is one of the most important reflections of the degree of straw decomposition. To quickly and accurately identify the decomposition degree of wheat straw, a high-throughput quantitative image analysis method was developed based on the colour of the straw degraded in soil over a different number of days. The correlation between these values and the contents of the chemical components in the straw was also investigated. The results indicated that the grey value representing the colour of the straw had a significant negative correlation with the content of residual ash in the straw ($R^2 = 0.696$). The method was used to study the microorganism inoculants involved in straw decomposition. The results showed that there was a significant difference between the straw with and without microorganism inoculants in terms of the grey value after 30 days. The final colour of the straw with microorganism inoculants was much deeper than that of straw without inoculants. This suggests that the microorganism inoculants not only could accelerate the decomposition speed of the wheat straw incorporated into the soil but could also affect its final degree of mineralization.

1. Introduction

As an agricultural country, China has abundant biomass energy resources; straw accounts for 72.2% (Zeng et al., 2007). There is presently a surplus of crop residues in China, especially in developed regions such as JIANGSU province, due to the changing household energy structure (Wang et al., 2014) and the continuously increasing crop yields (National Bureau of Statistics of the People's Republic of China, 2004). Furthermore, because it is difficult to collect straw, many peasants have to discard it or burn it directly in fields (Zang et al., 2014; Cao et al., 2008), which leads to serious environmental pollution. According to statistics, the total amount of crop straw in China was approximately 1040 Mt in 2015 (Office of the Ministry of Agriculture of the People's Republic of China, 2015). Thus, it is urgent for researchers to devise a strategy for rationally dealing with excess straw. On the other hand, the constant decrease in organic matter content in Chinese soils is one of the most significant causes of degradation (Wang and Li, 2002), and only 24.3% of straw was returned to the fields in 2006 (Liu et al., 2010). Evidence has shown that the current content of organic carbon in the soil of arable land is only 51% of that in natural soil under an edaphic climax community (Wang and Li, 2002). However, to achieve a high crop yield, more chemical fertilizer must be used, which further deteriorates the soil structure and decreases the field quality. Field utilization in China has thus entered into a vicious cycle (Liu, 2014). Organic amendment is an effective way to improve the present situation (Dou et al., 2011; Zhang et al., 2012; Pascual et al., 1999), and there is evidence indicating that composted organic matter could be superseded by straw-returning treatments (Pascual et al., 1999; Zhichen et al., 2008).

Complex bio-chemical reactions occur during the decomposition process after straw is incorporated into soil. These lead to changes in various physical and chemical properties, such as straw colour, straw structure, absorption of water, the C:N ratio, the content of humus, etc., (McCalla, 1943; Piotrowska-Cyplik et al., 2013, 2009). It is important to identify the moment when the material reaches the stage of early maturity as soon as possible. At this stage, the decomposition processes become clearly inhibited (Kujawa et al., 2014). Straw colour is one of the most important reflections of the degree of decomposition and generally undergoes an appreciable change due to the decomposition process; it usually becomes a dark grey colour within 1–2 months under field condi-

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* Corresponding authors at: College of Engineering, Nanjing Agricultural University, Nanjing, Jiangsu 210032, PR China (Y. Liu).

E-mail addresses: lytnjau@163.com (Y. Liu), wmding@njau.edu.cn (W. Ding).

[1] These authors contributed to this work equally.

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tions (McCalla, 1943). Straw may also strongly affect the net radiative balance of soils, and therefore alter soil temperature and heat flux (Sharratt and Flerchinger, 1995). Several studies in recent years have used optical imaging techniques and computer technology to investigate these processes. Boniecki et al. proposed a method for estimating aerobic and anaerobic decomposition of organic matter based on neural image analysis of straw decomposition (Boniecki et al., 2012); Kujawa et al. also used neural image analysis to determine the maturity classification of sewage sludge composted with maize straw (Kujawa et al., 2014). Yet, these methods were established based solely on empirical data, and the linkage between the models and the contents of the chemical components in straw was not elaborated. The issue of using artificial neural networks to investigate the processes of biomaterial composting has not been fully acknowledged.

The aim of this study was to use an image-processing algorithm to rapidly obtain the characteristics of wheat straw and to establish the correlation between the grey values and the contents of the chemical components in straw. Furthermore, the method was used to determine the effect of microorganism inoculants on straw decomposition.

2. Materials and methods

2.1. Soil and wheat straw preparation

Dry topsoil (yellow-brown soil) was collected on April 4, 2014, from the 0–15 cm depth range at the Agronomy Test Station of Nanjing Agricultural University (32.034505°N, 118.630553°E) in Nanjing, Jiangsu, P.R. China. It was then sieved through a 2 mm screen and stored at room temperature. The constituents of the soil were as follows: 16.89 g/kg organic carbon, 0.689 g/kg total nitrogen, 13.87 mg/kg NO_3^--N, and 12.92 mg/kg NH_4^+-N.

Wheat straw (Huaimai 19) was collected from Huanghai Ranch (34.329418°N, 119.922956°E), Xiangshui County, Yancheng City, Jiangsu Province. It was dried in an oven at 45 °C to decrease the moisture content from approximately 30% to 16% and was then cut into pieces approximately 5 cm long. The constituents of the straw were as follows: total carbon 526.2 g/kg, total nitrogen 7.23 g/kg, total potassium 10.7 g/kg, and total phosphorus 2.9 g/kg.

2.2. Microorganism inoculant preparation

The microorganism inoculants contained 2 cellulose degradation bacterium strains (*Bacillus subtilis* and *Bacillus licheniformis*), 2 nitrogen fixation yeast strains (*Hansenula sp.* and *Schizosaccharomyces sp.*) and 1 lignin degradation actinomyces strain (*Thermoactinomyces sp.*); they were isolated from fermented dairy manure, straw, or leaves, and the function of each strain was tested (Cao, 2003). The bacterium strains, yeast strains and the actinomyces strain were streaked on LB, PDA and GM1 media plates, respectively, and then a single colony was chosen to be cultivated in the corresponding liquid media until it was in the stable phase. The individual fermented liquid media of *Bacillus subtilis*, *Bacillus licheniformis*, *Hansenula* sp., *Schizosaccharomyces* sp. and *Thermoactinomyces* sp. were mixed according to the volume ratio 4:3:1:1:1 to obtain the microorganism inoculants used in this study.

2.3. Experimental design and sampling

The straw was decomposed in PVC columns (Internal Diameter: 7.5 cm; Length: 30 cm), as shown in Fig. 1. The columns were divided into three parts: soil on the top 20 cm, 6 g of straw mixed with soil in the middle 5 cm, and soil at the bottom 5 cm. There were two treatments for the middle parts: (1) straw mixed with soil, 0.1 g $Co(NH_2)$ and 10 mL microorganism inoculant liquid (MI); and (2) straw mixed with soil, 0.1 g $Co(NH_2)$ and 10 mL tap water as a control check (CK). The columns full of soil and straw were incubated at room temperature after the wheat harvest (from June 2, 2014, to September 9, 2014), and the relative humidity level was maintained at roughly 65%. Every ten days, 3 columns of each treatment were sampled and stored at 4 °C in a refrigerator for further use. The straw in columns were removed, cleaned, dried at 105 °C for 2 h, scanned for images and analysed for chemical components after the incubation time ended.

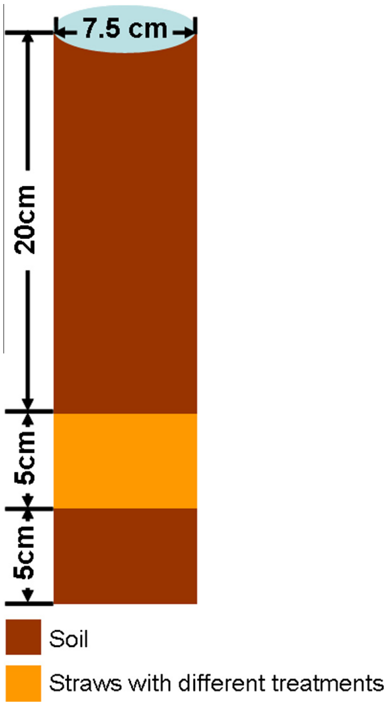

Fig. 1. PVC pipe used for straw that decomposed in it.

2.4. Image acquisition

The image acquisition steps are shown in Fig. 2. Straw was spread out and scanned to acquire the image (300 dpi, RGB colour; Fig. 2a) with a scanner (MICROTEK, ScanMaker E900). An image analysis program was designed using MATLAB 2013a (The MathWorks, Inc., Natick, MA). The image analysis steps were as follows: the raw RGB image (Fig. 2a) was converted to greyscale (Fig. 2b) using the (R + G + B)/3 components; a binary image (Fig. 2c) was obtained using the Otsu automatic threshold method; all connected components with fewer than 100 pixels were removed (Fig. 2d); and the grey images of the straw regions were extracted using the (b × d) operation. The extraction results are shown in Fig. 2e. In each step of the extraction, the functions used included 'rgb2gray', 'im2bw', and 'bwareaopen' (as shown in the Supplementary material). The grey value was calculated from the region (except black parts) in Fig. 2e. The final value was the average of grey values for the images of straw from 3 different columns sampled on the same day.

2.5. Analysis of chemical components content

The content of hemicellulose, cellulose, lignin and residual ash was determined by the neutral detergent fibre (NDF) method (Van Soest et al., 1991). The chemical properties of straw from col-