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Short communication

Density mapping of decaying wood using X-ray computed tomography

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ABSTRACT

Wood biodegradation is a central process at the crossroads of several disciplines. It is not only important for carbon storage in forests, but it is also important for wood conservation, wood protection and wood transformation products. Many methods already exist for studying wood biodegradation; however, they present several drawbacks, being time-consuming or destructive. Moreover, they provide little information regarding the complexity of the degradation process and the heterogeneity of the wood substrate. Based on a kinetic study of the biodegradation of *Fagus sylvatica* by the white-rot fungus *Phanerochaete chrysosporium*, we developed an X-ray computed tomography method coupled with an in-house plugin for fast, non-destructive and accurate measurement of the density variations of decaying wood. This method allowed us to examine the spatial heterogeneity of woody decayed material at the millimeter scale, providing information about the fungal pattern of degradation. Thus, X-ray computed tomography is an efficient tool that can be used for measuring the degradation of a variety of wood substrates ranging from small normalized wood blocks to fallen logs in the forest.

1. Introduction

Understanding the wood biodegradation process is important at the theoretical level to better understand wood biogeochemistry (Boddy and Watkinson, 1995), as well as at the applied level (Råberg et al., 2013). Numerous fungal species live in association with wood. However, saprotrophic basidiomycetes have been described as the most important decomposers in environments ranging from forest ecosystems (Kubartová et al., 2012) to indoor buildings (Schmidt, 2007). These fungi use a large set of extracellular enzymes to mineralize the wood lignin and carbohydrates such as cellulose and hemicelluloses (Baldrian, 2008). Decomposition of wood by these fungi causes economic losses (Schwarze et al., 2004) and problems for the conservation of historic woods (Blanchette, 2000). However, ligninolytic fungi also have biotechnological applications in the food and textile industries, as well as for soil bioremediation (Rodríguez Couto and Toca Herrera, 2006). In particular, these fungi are important in the field of wood science, where they are used for biopulping and biobleaching (Blanchette et al., 1988; Singh et al., 2010).

Various methods have been developed to evaluate wood degradation at different scales. Visual estimation and mass loss have been traditionally used to estimate the stage of alteration on a global scale. For example, the European standard EN113 (EN113, 1996), which is the reference method for evaluating the efficacy of wood preservatives, is based on the mass loss of standardized wood blocks incubated with wood-decaying fungi. This method is fast but not very informative with regard to the biodegradation process. The evaluation of wood alteration can also rely on the physical, mechanical and chemical properties of the wood. Based on mechanical tests, the strength loss of the wood can be used to measure degradation (Winandy and Morrell, 1993). Meanwhile, chemical analyses (Freschet et al., 2012) can reveal variations in macromolecule concentration (lignin and cellulose) in the wood or can provide elemental compositions (carbon and nitrogen) following biodegradation. The delignification process has also been followed by near- and mid-infrared spectroscopy (Schwanninger et al., 2004). Unfortunately, while offering a wealth of information, these methods destroy the sample. At the cellular scale, wood

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degradation can also be monitored and quantified using microscopy (Schwarze, 2007). While extremely accurate, microscopic approaches require time for sample preparation and are not well adapted for quantitative studies. Thus, there is a need to develop new methods for monitoring wood biodegradation that can quickly detect and quantify the early signs of degradation so that preventative measures can be taken.

Both X-ray microdensitometry and electric resistivity tomography have been reported to be good techniques for studying wood decay, and these techniques are based on density loss analysis (Bucur et al., 1997; Macchioni et al., 2007) and variations in electric resistivity (Bieker et al., 2010) of the decaying wood, respectively. X-ray computed tomography (CT) is an imaging procedure that computes 3D maps of objects based on X-ray attenuation for estimation of their density (Freyburger et al., 2009). Interestingly, wood density has been described as an important wood functional trait and as an integrator of wood properties (Chave et al., 2009). X-ray CT is faster than X-ray microdensitometry and provides higher resolution than electric resistivity tomography. It is non-destructive and accurate to the millimeter scale or higher. X-ray CT data acquisition is fast, taking only a few seconds for a slice without the need for sample preparation. Finally, it provides fast 3D analysis of relatively important volumes (0.1 m^3 or more) with good enough precision for quantitative studies. Initially, the X-ray CT technique was developed for medical imaging, but it has recently been used for the analysis of various materials. According to recent literature reviews (Wei et al., 2011; Longuetaud et al., 2012), CT scanning has mainly been used in wood science for detecting internal log features such as pith, growth rings, heartwood/sapwood, knots and decay.

Here, we applied the X-ray CT technique for imaging density of wood during fungal biodegradation. We performed a kinetic study of beechwood (*Fagus sylvatica* L.) degradation by the model white-rot fungus *Phanerochaete chrysosporium* RP78 (Martinez et al., 2004) under controlled conditions. The biodegradation process was monitored both by traditional mass loss analysis and by density loss analysis using the X-ray CT technique.

2. Materials and methods

2.1. Fungal strain

P. chrysosporium (strain RP78) (Martinez et al., 2004), a well-known white-rot fungus (Burdsall and Eslyn, 1974), was obtained from the Center for Forest Mycology Research, Forest Products Laboratory (Madison, Wisconsin, USA). This strain was maintained at 25 °C on 3% malt agar slants.

2.2. Experimental design

The kinetics of wood decay was evaluated using beech (*Fagus* sylvatica) wood blocks (50 × 24 × 6 mm) incubated in the presence or absence of *P. chrysosporium* in 90-mm Petri dishes containing malt (30 $g \cdot l^{-1}$) agar (20 $g \cdot l^{-1}$) medium. Wood blocks were all sampled from the same heartwood plank (24 mm thick). The blocks were free of knots and other visual defects (Fig. 1). Samples ($n = 108$) were cut with a circular saw in the radial (50 mm) and axial directions (6 mm). In the axial direction, two samples in a row were considered as biological replicates because of their proximity.

The wood blocks were autoclaved (20 min, 120 °C, dry cycle) twice with 2 days in between each autoclave cycle. Half of the wood samples were inoculated with a 5-mm-diameter fungal plug of *P. chrysosporium* RP78, which was placed 3 cm from the periphery of the block. For each of these wood samples, the corresponding biological replicate received a wet sterile piece of cotton only as a control. The sterile wet cotton was used to maintain constant moisture inside the Petri dish and to detect any microbial contamination growing in the dishes. No microbial growth was detected during the 150 days of incubation. Plates were sealed with adhesive tape and incubated at 25 °C in the dark for 0, 15, 30, 60 or 150 days.

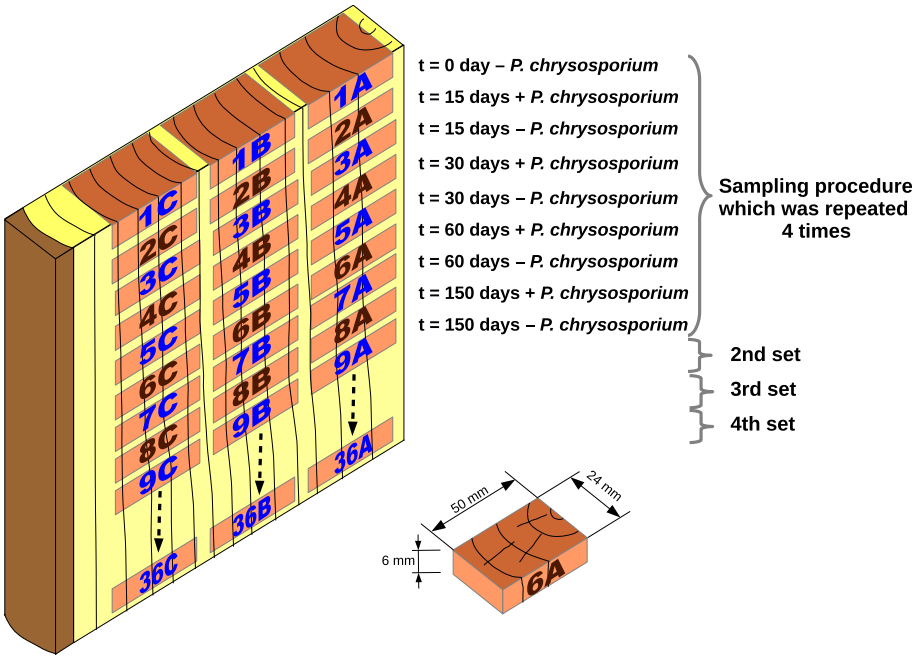

Fig. 1. Diagram of the experimental design. In total, 108 samples of 50 mm × 24 mm × 6 mm in size (radial, tangential and longitudinal directions, respectively) were cut within a plank and divided into 4 sets of 36 samples (numbered 1A to 9C, 10A to 18C, 19A to 27C and 28A to 36C). There were 9 conditions for time (0, 15, 30, 60 and 150 days) and type of inoculation (with or without the white-rot fungus *P. chrysosporium*), with $n = 12$ replicates for each condition (from the 3 rows (called A, B and C) and the 4 sets). In the axial direction, two consecutive samples from a row were considered as biological replicates because of their proximity. These replicates were incubated with or without *P. chrysosporium* (e.g., samples 4A and 5A).