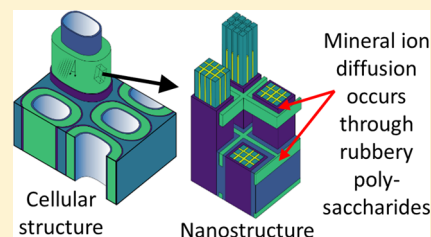


Mechanism for Diffusion through Secondary Cell Walls in Lignocellulosic Biomass

Joseph E. Jakes*

Forest Biopolymers Science and Engineering, Forest Products Laboratory, USDA Forest Service, One Gifford Pinchot Drive, Madison, Wisconsin 53726, United States

ABSTRACT: The future bioeconomy depends on the increased utilization of renewable lignocellulosic resources from trees and other bioenergy crops. However, considering that the diffusion of ions, chemicals, and enzymes into secondary cell walls is critical to effectively utilize lignocellulosic biomass, the unidentified mechanisms underpinning this diffusion have hindered progress. Here, nano-mechanical spectroscopy was used to measure changes in moisture-dependent relaxations of amorphous polysaccharides inside loblolly pine (*Pinus taeda*) cell wall layers. The comparison with recent ion mobility measurements made in similar cell wall layers revealed that the mineral ion diffusion occurs via interconnecting nanoscale pathways of rubbery amorphous polysaccharides. This result contradicts previous assertions of cell wall transport being an aqueous process occurring through simple interconnecting water pathways. Because polymer diffusion and aqueous transport via water channels are such different phenomena, the identification of the diffusion mechanism in this manuscript opens up a new paradigm in lignocellulosic research. The utilization of lignocellulosic resources is expected to be accelerated because the extensive polymer science literature can now be used to design the molecular architecture of lignocellulosic biomass to optimize diffusion properties for specific uses, including biorefinery feedstocks, advanced materials, and wood-based construction materials.



INTRODUCTION

Wood from trees and other sources of renewable lignocellulosic biomass is primed to a major role in meeting our future needs for materials, chemicals, fuel, and energy.^{1–3} In addition to continuing to provide wood-based building materials, wood is quickly becoming the basis and bioinspiration for new advanced materials including nanocellulose,⁴ green electronics,⁵ eco-friendly separation membranes,⁶ carbon,^{2,7} and smart materials.^{2,8} However, the development of technologies to fully utilize wood is hindered by the lack of fundamental material structure–property relationships, especially at the small length scales of individual cell wall layers. For example, despite the importance of material transport through cell walls to biorefinery pretreatments and enzymatic hydrolysis,⁹ the manufacture of engineered wood building materials,¹⁰ and decay and fastener corrosion degradation mechanisms in wood-based materials,^{11–13} the mechanisms for diffusion of different substances through wood cell walls are either poorly understood or unknown.

Material transport through lignocellulosic cell walls depends on the structure and composition of the cell wall, including the amount of absorbed water. Lignocellulosic biomass, such as softwood from coniferous trees, is, in general, an anisotropic cellular material composed of cells that can be thought of as hollow tubes with multilayer polymeric walls (Figure 1a). In wood, the secondary cell wall layers (S1, S2, and S3) are nanofiber-reinforced structures with helically wound cellulose fibrils embedded in a highly organized matrix of amorphous cellulose, hemicelluloses, and lignin (Figure 1b). Cells are held together by the compound middle lamella (CML), which is

proposed to be an open-cellular hemicelluloses nanostructure encrusted with lignin.¹⁴ Molecules of water are absorbed by all of the wood polymers, except between the highly ordered cellulose chains in the cellulose elementary fibrils.¹⁵ Wood moisture content (MC), defined as the mass of water divided by the oven-dry mass of wood, depends on ambient temperature and relative humidity (RH). At low MC, all water in wood are bound by the wood polymers inside cell walls. As MC increases, the maximum capacity for bound water is reached at the fiber saturation point (FSP), which depending on the definition is between 30 and 40% MC.¹⁶ Additional water added to the wood above the FSP forms free water in wood cavities, such as lumina.

Mass transport of mineral ions through secondary lignocellulosic cell walls is of particular interest in green electronics,⁵ manufacture of biomass-based activated carbon,⁷ bioinspiration for the design of new multifunctional smart materials,⁸ woody biomass pretreatments for biofuels and biochemical production,⁹ as well as wood degradation mechanisms, including fastener corrosion¹² and fungal decay.^{11,13} Previous models for ionic conduction in wood, which were developed over the past century to explain the strong dependence of bulk wood electrical properties with MC, assume that the mechanism for ion transport is an aqueous process occurring via interconnecting water pathways.^{20–24} These models are also applied for MC below FSP, which

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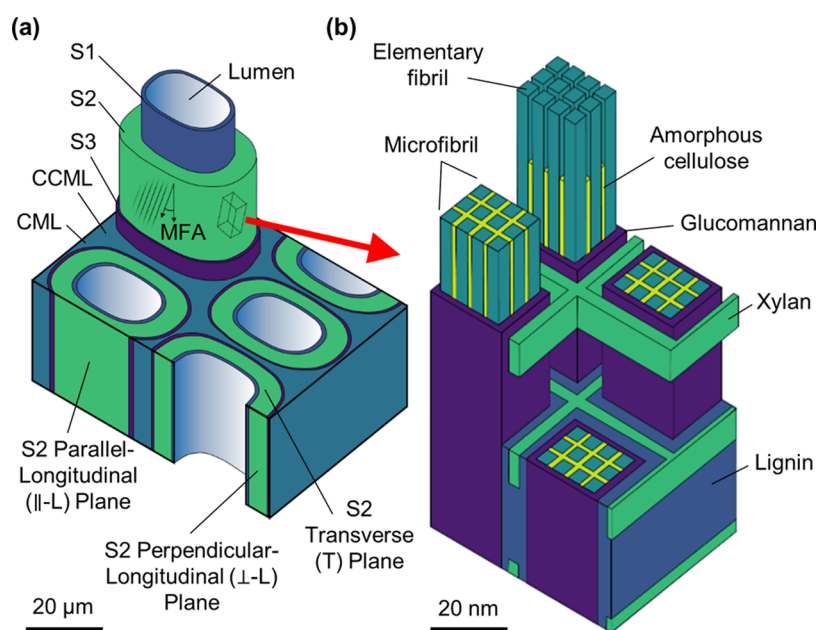


Figure 1. Schematics illustrating the breakdown of softwood, a lignocellulosic material. (a) Cellular structure showing secondary (S1, S2, and S3) and compound middle lamella (CML) cell wall layers. At the corner between multiple cells, the larger volume CML is termed the corner CML (CCML). The angle the helically wound cellulose fibrils make with the longitudinal cell axis is the microfibril angle (MFA). Of the secondary cell walls, the S2 is the thickest, with a relatively constant MFA typically between 5 and 30°. In this study, nanomechanical spectroscopy was performed in the CCML and in the S2 on the parallel–longitudinal (||–L), perpendicular–longitudinal (⊥–L), and transverse (T) planes. The parallel and perpendicular orientations refer to how the plane is oriented in the S2 with respect to the lumen surface. (b) A schematic illustrating a proposed S2 nanostructure. Cellulose is a linear polysaccharide and through intra- and interpolymer hydrogen bonding organizes into highly ordered elementary fibrils approximately 3 nm across.¹⁷ These elementary fibrils organize into microfibrils, such as in the three by four array proposed by Terashima and co-workers.¹⁸ There is an amorphous cellulose between elementary fibrils.¹⁹ Hemicelluloses are branched amorphous polysaccharides, of which xylan and glucomannan are two common types in wood cell walls. Their proposed nanostructure organization includes a glucomannan sheath around microfibrils and xylan perpendicular to the microfibrils.¹⁸ Lignin is a cross-linked aromatic polymer and encrusts the remaining cell wall volume.

implies that these water pathways somehow exist inside the wood cell walls. However, an experimental evidence verifying the existence of these water pathways is lacking.²⁵

Recently, it was proposed that instead of mineral ion transport occurring through water pathways, transport occurs via pathways of interconnecting regions of amorphous polysaccharides (hemicelluloses and amorphous cellulose) that have passed through their moisture-induced glass transition and are in their rubbery state.²⁶ In amorphous polymers, the diffusion of chemicals like mineral ions is mostly nonexistent when the polymer is in its glassy state.^{27,28} However, above the glass transition in the rubbery state, chemical diffusion occurs and is strongly correlated with the segmental motion of polymers. It is often interpreted that diffusion and polymer relaxations are correlated because they are being controlled by the same free volume increases in the polymer structure above the glass transition.^{27,29} The involvement of amorphous polysaccharides was proposed because of the wood polymers at room temperature, only they pass through a moisture-induced glass transition. Experiments on intact wood³⁰ and amorphous polysaccharides extracted from wood^{31,32} find that amorphous polysaccharides pass through a glass transition when conditioned at room temperature in the range of 60–80% RH (11–15% bulk wood MC). In contrast, even water-saturated lignin remains glassy at room temperature with an approximate 70 °C glass transition temperature observed in lignin both in intact wood³⁰ and extracted.³³ The similarities between the glass transitions measured in intact wood and in extracted materials support

that the lignin and amorphous polysaccharides are not a miscible blend in the wood cell wall and exhibit distinct thermal transitions.³⁰ Consequently, mineral ion diffusion would be mostly nonexistent through lignin at room temperature. Examples of potential interconnecting diffusion pathways composed of amorphous polysaccharides in the S2 (Figure 1b) include the glucomannan sheath around microfibrils,¹⁸ xylan perpendicular to the microfibrils,¹⁸ and amorphous cellulose between elementary fibrils.¹⁹ In the CML, the proposed pathway is through the “ribs” of the open-cellular hemicelluloses nanostructure.¹⁴

The proposed mechanism of mineral ion transport through rubbery polysaccharide pathways in wood cell walls is supported but not confirmed by recent experiments examining the effects of moisture on the diffusion of implanted ions in wood cell wall layers.³⁴ In these experiments, the implanted mineral ions potassium, copper, zinc, and chloride diffused through S2 and CML only when conditioned above the 60–80% RH range over which the amorphous polysaccharides pass through their moisture-induced glass transition. However, cell wall diffusion has never been causally linked to the segmental motion of amorphous polysaccharides, which would confirm the diffusion occurring through rubbery amorphous polysaccharides.

A direct comparison of molecular relaxations to ion mobility would determine if the diffusion is causally linked to polymer segmental motion.^{28,29,35,36} In this study, the moisture-dependent amorphous polysaccharides’ relaxations were assessed in the S2 and CCML of loblolly pine (*Pinus taeda*)

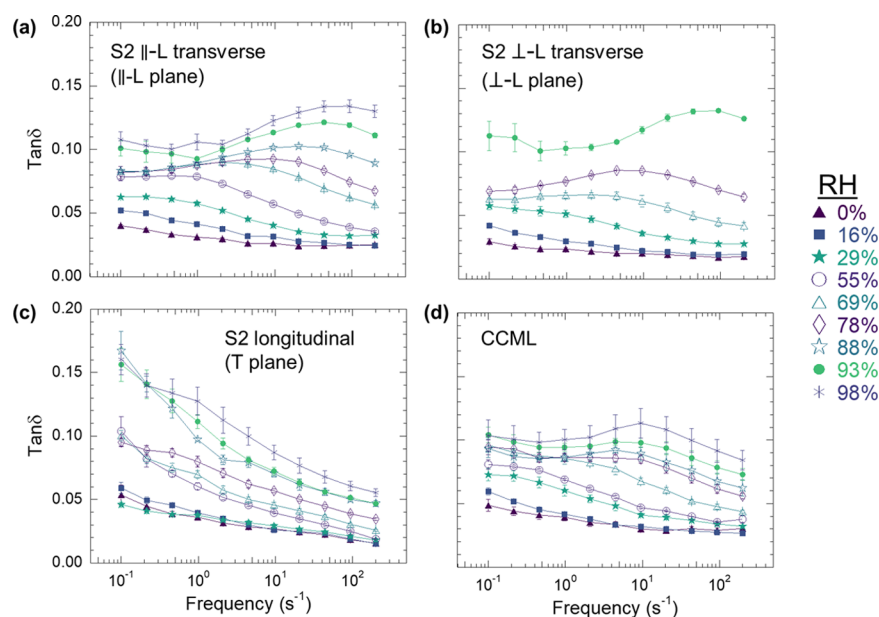


Figure 2. Mechanical damping ($\tan \delta$) spectra assessed in loblolly pine S2 and CCML as a function of relative humidity (RH) using nanoindentation-based mechanical spectroscopy. In the S2, $\tan \delta$ spectra in the transverse direction were assessed by placing nanoindentations in both the (a) parallel–longitudinal (||–L) plane and (b) perpendicular–longitudinal (⊥–L) plane. The S2 longitudinal $\tan \delta$ spectra (c) were assessed placing nanoindentations on the transverse (T) plane. The CCML $\tan \delta$ spectra (d) were assessed by nanoindentations placed in the CCML of a surface prepared in the transverse orientation. Error bars are the standard error.

using nanoindentation-based mechanical spectroscopy. The time-scales of the molecular relaxations were assessed directly from glass transition peaks in the mechanical damping ($\tan \delta$) spectra. These relaxations were directly compared to moisture-dependent ion mobility provided by recent electrical conductivity measurements in similar S2 and CCML.³⁷ The comparison revealed a causal link between the transport of mineral ions through cell walls and the segmental motion of rubbery amorphous polysaccharides.

METHODS

Surfaces of unembedded loblolly pine (*P. taeda*) were prepared for the nanoindentation following previously developed methods.^{38,39} In brief, first a hand razor was used to carefully trim a pyramid with an apex in the region of interest. Then, the oriented blocks were fitted into a Leica EM UC7 Ultramicrotome (Wetzlar, Germany) equipped with a diamond knife. Surfaces were prepared by removing 200 nm-thick sections from the apex until an appropriately sized surface was prepared, typically hundreds of microns on a side. Surfaces in the transverse and tangential–longitudinal orientations were prepared. In the transverse surface, nanoindents were placed in the compound corner middle lamellae and in the tangential side of the S2 along a single row of daughter cells. In the tangential–longitudinal surface, all nanoindents were placed along a single S2 secondary cell wall layer in either the perpendicular–longitudinal plane or parallel–longitudinal plane of the S2. See Figure 1a for S2 plane labeling.

The Bruker–Hysitron (Minneapolis, Minnesota) TriboIndenter upgraded with the nanoDMA III package and equipped with a Berkovich probe was used. The relative humidity (RH) inside the nanoindentation enclosure was controlled between 0 and 98% RH with an InstruQuest (Coconut Creek, FL) HumiSys HF RH generator. Specimens were conditioned inside the enclosure for at least 36 h at each condition. During the experiments, the RH was maintained to

within $\pm 0.5\%$ RH using the feedback control and a temperature and humidity sensor held approximately 5 cm from the specimens. The ambient temperature inside the enclosure was also measured and in the range of 27–29 °C for all experiments. An open-loop reference frequency load function was employed to measure mechanical dampening ($\tan \delta$).^{40,41} It consisted of an initial lift-off and approach to accurately detect the specimen surface, initial preload to 4% of maximum load, hold at preload for 2 s to begin 100 Hz dynamic loading, followed by a 0.1 s^{-1} constant strain rate loading segment at 100 Hz to the maximum load. The hold at maximum load began with a 17 s 220 Hz dynamic segment, followed by a set of reference frequency segments and a final unloading. The reference frequency segments consisted of alternating 7 s 220 Hz reference frequency segments and 10 test frequencies that were spaced on a logarithmic scale from 200 to 0.01 Hz. The $\tan \delta$ results reported in this paper are from the first 220 Hz reference frequency segment and each test frequency segment. For a given RH and cell wall layer, maximum loads were chosen so the resulting maximum depths were 150–200 nm, and load amplitudes were adjusted so the displacement amplitudes were 3–4 nm. Nanoindents (3–8) were performed at each RH condition. The $\tan \delta$ peak frequencies were visually measured from all $\tan \delta$ spectra with well-defined peaks.

RESULTS AND DISCUSSION

Nanoindentation was performed on surfaces prepared in the three S2 planes defined in Figure 1a and the CCML of loblolly pine. During nanoindentation, a carefully shaped probe is pressed into a material following a prescribed loading function. An advantage of nanoindentation is the ability to probe the mechanical properties of very small volumes of materials, such as the S2 and CCML,⁴² under various RH conditions.^{43,44} Viscoelastic properties, including mechanical damping ($\tan \delta$), can be measured over a range of frequencies by superimposing

a small sinusoidal signal on the static load.^{45–47} Peaks in nanoindentation $\tan \delta$ spectra arising from thermal transitions, including glass transitions in polymers, have been shown to appear similarly as in conventional dynamic mechanical analysis $\tan \delta$ spectra.^{48,49}

The measured RH-dependent $\tan \delta$ spectra are shown in Figure 2. Overall, $\tan \delta$ increased with RH, which demonstrated that the absorbed water acted to increase mechanical energy dissipative processes. Only the $\tan \delta$ spectra in the S2 transverse directions (Figure 2a,b) and CCML (Figure 2d) exhibited peaks. A peak in $\tan \delta$ is indicative of molecular-scale dissipation processes with an intrinsic periodicity matching the experimental frequency at the observed peak.²⁷ Based on previous reports of glass transitions in amorphous polysaccharides^{30–32} and lignin,³³ the inescapable explanation is that these $\tan \delta$ peaks arose from the moisture-induced glass transition of the amorphous polysaccharides inside the wood cell wall layers. The S2 transverse and CCML $\tan \delta$ spectra had similar magnitudes and shapes below 50% RH. Above about 30% RH, the peaks began to form, first as shoulders in the spectra at a lower frequency, and then as well-formed peaks that moved to higher magnitudes and frequencies with increasing RH.

In contrast to the S2 transverse and CCML $\tan \delta$ spectra, no peaks were observed in the S2 longitudinal $\tan \delta$ (Figure 2c). Measured mechanical damping in a composite material like the S2 depends on the composition and orientation of its components.⁵⁰ Evidently, in the longitudinal direction, in which the probe loading direction is parallel to the stiff cellulose fibrils, another dissipative mechanism was activated and dominated over the mechanical damping arising from configurational rearrangements of the amorphous polysaccharides at their glass transition.

In Figure 3, $\tan \delta$ peak frequencies are plotted as a function of RH. In the S2 transverse orientations, the $\tan \delta$ peaks have

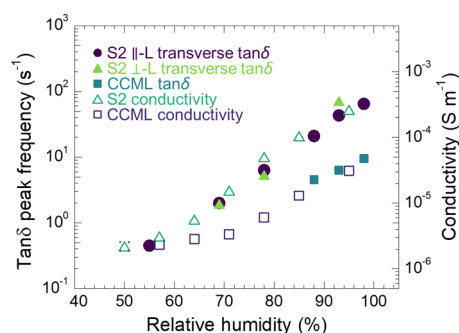


Figure 3. Comparison of mechanical damping ($\tan \delta$) peak frequencies assessed in this study to conductivity measurements.³⁷ Both sets of experimental data were made in the S2 and CCML of loblolly pine as a function of relative humidity (RH). The left and right axes both span 4 orders of magnitude and are offset to show how the data overlap.

similar trends with RH. However, in the CCML, well-formed $\tan \delta$ peaks form only at higher RH, and peak frequencies are lower than in the S2. This difference in peak frequencies between the S2 and CCML indicates different molecular relaxation time-scales between amorphous polysaccharides in the two cell wall layers. Although it is uncertain whether differences in time-scales arise from variances in amorphous polysaccharide structure or MC, these differences provide the

opportunity to directly compare molecular relaxations to ion mobility in different cell wall layers of the same specimen.

The RH-dependent ion mobilities in S2 and CCML of loblolly pine are provided by recent electrical conductivity measurements,³⁷ which are also plotted in Figure 3. Experimental evidence shows that electrical conductivity in wood is ionic.^{23,51} Therefore, the ion conductivity (σ) through wood cell walls is expected to obey the usual relation⁵²

$$\sigma = \sum \mu_i n_i z_i \quad (1)$$

where μ_i is the mobility of ion i , n_i the concentration of ion i , and z_i the charge of ion i . Furthermore, at higher levels of moisture, such as at and above the RH range over which amorphous polysaccharides pass through their moisture-induced glass transition, the electrical charge in wood is carried by the movement of endogenous mineral ions.^{51,53,54} Of the endogenous minerals in wood, potassium, calcium, and magnesium are by far the most abundant,⁵⁵ and potassium has the highest mobility.^{53,54} Therefore, potassium ions are most likely to control measured electrical conductivity. In X-ray fluorescence microscopy maps of potassium in S2, CML, and CCML, similar potassium concentrations were observed in all cell wall layers in loblolly pine,¹³ suggesting that n_i should be similar between the S2 and CCML. Thus, to a close approximation, any differences in measured σ between the S2 and CCML, such as those plotted in Figure 3, can be ascribed to changes in ion mobility.

As shown in Figure 3, the $\tan \delta$ peak frequencies in S2 increased by about 2.5 orders of magnitude from 55 to 98% RH, which is similar to the increase in conductivity over the same RH range. In the CCML, the $\tan \delta$ peak frequencies from 88 to 98% RH are about an order of magnitude lower than the S2 over the same RH range, which are again similar to the CCML conductivity. Following previous work identifying ion conduction mechanisms in polymers,^{35,36} these similarities in RH-dependence of the $\tan \delta$ peak frequencies and conductivity in Figure 3 reveal that molecular relaxations of amorphous polysaccharides are intimately linked to σ and therefore to the diffusion of mineral ions, such as potassium ions. Therefore, mineral ion transport in cell walls is not occurring through water pathways, as previously asserted,^{20–24} but rather through interconnected pathways of rubbery amorphous polysaccharides.

The identification of diffusion via rubbery amorphous polysaccharides as a material transport mechanism through lignocellulosic cell wall layers provides a new paradigm for modeling and designing lignocellulose materials for specific uses. The extensive literature of diffusion through amorphous materials and polymers, much of which is based in fundamental frameworks like free volume and fragility, can now be effectively employed to accelerate progress. Moreover, these frameworks provide fundamental models to inform the design of molecular architectures. For instance, although efforts to overcome recalcitrance in biorefineries have often focused on lignin, modifying hemicelluloses to promote the diffusion of pretreatment chemicals and enzymes into and depolymerization products out of cell walls may provide new avenues for improvements. Furthermore, modelers could identify molecular structures that promote diffusion using in silico studies. This information could then be used by molecular biologists to design plants to grow the lignocellulosic biomass with the desired molecular structure.⁵⁶ Conversely, for

preventing wood degradation in building materials, inhibiting cell wall diffusion would decrease susceptibility to fastener corrosion and fungal decay. Again, molecular biology approaches could be used to design trees to grow wood with inhibited diffusion and improved durability, or post-treatments could be used to modify the amorphous polysaccharide structures in wood. One such treatment is acetylation, which is a chemical modification known to impart protection to wood from fungal decay without using biocidal chemicals.¹⁰ During acetylation, hydroxyl groups on wood polymers are replaced with larger, less hydrophilic acetyl groups. Indeed, it has already been shown that onset RH for potassium ion diffusion through wood cell walls increases with the level of acetylation,⁵⁷ which supports the concept that preventing diffusion through wood cell walls is an avenue to protect the wood from degradation. The improved understanding of diffusion presented here and deployment of future predictive models based on polymer science will accelerate efforts to increase utilization of lignocellulosic biomass for our future bioeconomy.

CONCLUSIONS

The transport of mineral ions through lignocellulosic cell walls was revealed to occur by diffusion through interconnecting pathways of rubbery amorphous polysaccharides. This transport mechanism supplants the previous assertion that cell wall transport was an aqueous process occurring through interconnecting water pathways. From a polymer science perspective, aqueous transport via water channels and diffusion through rubbery amorphous polymers are very distinct phenomena. In a water pathway, the transport depends on the chemical species' diffusion coefficient in water and is largely independent of the properties of the material creating the water pathway. In contrast, in diffusion through amorphous polymers, the rate of diffusion is directly correlated with the segmental motions of polymers. Therefore, to control transport, the polymers themselves need to be modified. Researchers now know that the segmental motions of the amorphous polysaccharides need to be targeted to modify and control diffusion through lignocellulosic secondary cell walls. Although the work in the paper was specifically related to mineral ions, it is expected to also be relevant to the cell wall transport of other chemicals with similar sizes, such as wood adhesive components, because the interconnecting pathways of rubbery amorphous polysaccharides are the only current plausible transport mechanism. This paper also only utilized unmodified wood cell walls. It remains likely that after cell wall modification, such as the removal of wood polymers during biorefinery pretreatments, that water pathways form in the modified cell walls. These water pathways are likely important for the cell wall transport of larger substances, such as the enzymes used in enzymatic hydrolysis of lignocellulosic biomass.

AUTHOR INFORMATION

Corresponding Author

*E-mail: jjakes@fs.fed.us.

ORCID

Joseph E. Jakes: 0000-0001-9030-5753

Notes

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