Estimating carbon loss due to internal decay in living trees using tomography: implications for forest carbon budgets

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Keywords: carbon cycle, internal decay, forest biomass, forest carbon, forest dynamics, tomography

Abstract
The world’s forests sequester and store vast amounts of atmospheric carbon, playing a crucial role in climate change mitigation. Internal stem decay in living trees results in the release of stored carbon back into the atmosphere, constituting an important, but poorly understood, countervailing force to carbon sequestration. The contribution of internal decay to estimates of forest carbon stocks, though likely significant, has yet to be quantified, given that an accurate method for the non-destructive quantification of internal decay has been lacking. To that end, we present here a novel and potentially transformative methodology, using sonic and electrical resistance tomography, for non-destructively quantifying the mass of stored carbon lost to internal decay in the boles of living trees. The methodology was developed using 72 northern hardwood trees (Fagus grandifolia, Acer saccharum and Betula alleghaniensis) from a late-successional forest in northwestern Connecticut, USA. Using 105 stem disks corresponding to tomographic scans and excised from 39 of the study’s trees, we demonstrate the accuracy with which tomography predicts the incidence and severity of internal decay and distinguishes active decay from cavities. Carbon mass fractions and densities, measured and calculated from 508 stem disk wood samples corresponding to density categories, as predicted by sonic tomography, were used with stem disk volumes to generate indirect estimates of stem disk carbon mass accounting for decay, $C_{SD}$, or assuming no decay, $C_{ND}$; these indirect estimates were compared with direct estimates calculated using stem disk mass, $C_{mass}$, and carbon mass fraction data. A comparison of three linear regression models with $C_{mass}$ as the response variable and $C_{SD}$ or $C_{ND}$ as the predictor variable ($C_{mass} \sim C_{SD}$, $R^2 = 0.9733$, Model 1; $C_{mass} \sim C_{ND}$, $R^2 = 0.8918$) demonstrates the accuracy with which $C_{SD}$ predicts $C_{mass}$. Forcing the $C_{mass} \sim C_{SD}$ regression through the origin resulted in improved metrics ($R^2 = 0.9930$, Model 2) for which a null hypothesis that $y = x$ (Model 3) could not be rejected ($p < 0.00001$). For each of the study’s 72 trees, two estimates of lower bole carbon mass—$C_{bole}$ accounting for decay, and $C_{bole-ND}$ assuming no decay—were obtained using all three models, with the difference between $C_{bole}$ and $C_{bole-ND}$ used to estimate the proportion of the lower bole’s carbon lost to decay, %$C_{dec}$. Overall, tomography identified decay in 47 of the 72 trees, with %$C_{dec}$ values ranging from 0.13% to 36.7%. No decay was detected by tomography in the remaining 25 trees. The combined uncertainty due to both measurement error and model prediction error was $\pm 2.1\%$ for all three models. These results demonstrate the efficacy of the proposed methodology in non-destructively quantifying the carbon loss associated with internal decay in the boles of living trees, and its applicability to studies aimed at measuring internal decay rates, and more accurately quantifying forest carbon stocks.

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1. Introduction

Forest ecosystems, accounting for 75% of the planet’s stored terrestrial carbon (Pan et al. 2011), constitute an important carbon sink, and therefore are critical to efforts aimed at mitigating rising levels of atmospheric carbon dioxide ($CO_2$) associated with global warming. Policy and management decisions that seek to increase carbon source in forests are needed (McRoberts and Westfall 2014). Allometric equations for estimating carbon stocks have typically been developed from well-formed, presumably non-decaying, trees (Behre et al. 1926), and assume that they are applied to similarly non-decaying trees (Nogueira et al. 2008, Matsuzaki et al. 2013); given the prevalence of internal stem decay (Brazee et al. 2011, Barrette et al. 2013), these assumptions likely lead to an overestimation of tree biomass and carbon. Internal stem decay, a naturally occurring process common to all forests, can be substantial (Heinemann et al. 2015, Hietala et al. 2015), and most prevalent in the lower boles of older trees, given that the likelihood of internal decay increases with tree size. Heterotrophic respiration associated with internal decay represents a critically important countervailing force to carbon sequestration, resulting in the release back into the atmosphere of large amounts of sequestered carbon in the form of both $CO_2$ and methane (Covey et al. 2012, 2016, Hietala et al. 2015).

While recent studies have taken into account the decay patterns of coarse woody debris and its role as a carbon source in forests (Cousins et al. 2015, Forrester et al. 2015), the decades-long process of internal decay in standing, living trees has not been sufficiently addressed quantitatively to date (Frank et al. 2018). The United States Forest Service’s Forest Inventory and Analysis program accounts for biomass lost to decay based on symptoms (Domike et al. 2012) and visual assessments, which may not be accurate: many trees with no visible outward symptoms harbor internal decay (Zillig and Gevorkiantz 1948, Wagener and Davidson 1954); and because most cankering fungi do not decay wood, trees with significant outward symptoms of damage may have perfectly sound wood. Given the absence of data and the challenges of identifying and quantifying internal decay non-destructively (Brazee et al. 2011, Heineman et al. 2015, Frank et al. 2018, Orozco-Aguilar et al. 2018), current biometric studies likely over-estimate net C sequestration as well as C stocks in mature trees (Stephenson et al. 2014) and mature stands (Luyssaert et al. 2008). Further, the absence of quantitative data on internal decay may contribute to the discrepancies found at times between estimates of forest productivity based on biometrics and those based on eddy-covariance methods (e.g., Curtis et al. 2002, Wang et al. 2017).

Although destructive sampling (i.e., tree felling) has been used in the past to assess the prevalence of internal decay (Arhipova et al. 2012, Heineman et al. 2015, Frank et al. 2018), the many complications associated with this practice—e.g., restrictions in protected and ecologically sensitive areas, cost, and commercial concerns—preclude it as a practical method for assessing the extent of internal decay over large study areas. Non-destructive assessment of internal decay using sonic tomography (SoT), which measures heterogeneity in sonic velocity, has primarily been used for tree risk assessment by commercial arborists in urban and suburban settings (Gilbert and Smiley 2004) and to identify internal decay in forest trees (Gilbert et al. 2016). In addition to being used as a complementary technology to SoT, electrical resistance tomography (ERT), which measures heterogeneity in electrical field, has also been used to address physiological questions about water use and heartwood formation in both conifers and hardwoods (Bieker and Rust 2010, Guyot et al. 2013). An earlier study showed that ERT used in combination with SoT provides the most accurate non-destructive assessment of internal decay and cavities (Brazee et al. 2011). In addition to obviating the many concerns associated with tree felling, the SoT/ERT approach has great promise for the large-scale assessment of the extent of internal decay, thus providing much needed data for forest carbon accounting and modeling.

Our objective in this study was to develop a model using results from SoT and ERT to indirectly estimate carbon mass by taking into account internal stem decay and the loss of carbon it represents. Once validated, this methodology could then be applied to large-scale studies aimed at assessing the role played by internal stem decay as it relates to carbon accounting and carbon dynamics in forest ecosystems.

2. Methods

2.1. Site and tree selection

This study used a single site of approximately 2.8 ha located within the Great Mountain Forest, a private nonprofit research forest in northwestern Connecticut, USA, chosen based on an abundance of the three target species occurring in a range of diameters typical of late-successional forests of the region: Fagus grandifolia (American beech), Acer saccharum (sugar maple), and Betula alleghaniensis (yellow birch). Further details on study site and tree selection are available in supplementary materials: S1, ‘Site and Tree Selection’ (available online at stacks.iop.org/ERL/13/105004/mmedia).
2.2. Sonic and electrical-resistance tomography (SoT and ERT, respectively)
The study used the PICUS® suite of tomographic equipment, which included the Sonic Tomograph (for SoT), TreeTronic Tomograph (for ERT), and the Caliper 3 Geometry Measurement System (Argus Electronic GMBH, Rostock, Germany). The PICUS equipment and ancillary materials are shown in supplementary figure S1. The logistics for set-up and use of the equipment are described in supplementary materials 'S2. Sonic and Electrical-Resistance Tomography,' and demonstrated in supplementary figure S2.

Data from SoT and ERT were interpreted jointly to predict the internal condition at each tree cross-section, based on the following criteria, summarized in table 1: (A) maximum density and the absence of moisture represent sound (non-decayed) wood; (B) maximum density and the presence of moisture represent incipient decay; (C) reduced density and the presence of moisture represent active decay; and (D) reduced density and the absence of moisture represent a cavity. This interpretation is based on an earlier study using a much smaller sample consisting of A. rubra, B. lenta, and F. grandifolia (Brazee et al 2011), as well as guidelines provided by the manufacturer.

2.3. Tree felling and processing
Stem disks corresponding to tomographic cross-sections were necessary for validation of tomographic accuracy (figure 1), and also for quantifying carbon content. For details on tree felling and stem disk processing, see supplementary materials 'S3. Tree Felling and Processing.'

2.4. Carbon density
In order to determine the accuracy with which SoT predicted reductions in density, wood representing each SoT color category was sampled volumetrically from each cross-section and assayed for carbon mass fraction (ω, the proportion of wood mass attributable to carbon) using Gas Chromatographic Elemental Analysis (GC EA). These data were then used with each sample's mass and volume to calculate its carbon density. For details, see supplementary materials 'S4. Carbon Density.'

2.5. Tree size and age effects
Tree ages were determined for all trees whose lowest stem disks had intact pith, in order to study the relationship between decay incidence and individual tree dbh and age. For details on dendrochronology, see supplementary materials 'S5. Tree Size and Age Effects.'

2.6. Development of a tomography-based model for indirect estimation of carbon mass and its application to boles
Indirect tomography-based estimates of stem disk carbon mass, accounting for decay predicted by tomography, were validated with direct estimates based on actual stem disk mass, obtained by multiplying the stem disk's dry mass by the mean carbon mass fraction for that stem disk's species. These direct and indirect estimates of stem disk carbon mass were used to generate allometric equations for the prediction of carbon mass-based on tomographic data alone, which were then used to estimate the lower bole carbon mass of the study's trees. For details on the development and application of this model, please see supplementary materials 'S6. Development of a Tomography-based Model for Indirect Estimation of Carbon Mass,' and 'S7. Estimating Carbon Mass of Boles.'

2.7. Uncertainty analyses
Uncertainty analyses for this study, using an approach described by Yanai et al (2010), focused on measurement error, for which we identified two sources, and model prediction error. For a detailed description of this approach, please see supplementary materials 'S8. Uncertainty Analyses.'

Table 1. Decision rules for predicting internal condition based on tomographic data.

| Decay category | SoT Color | Density | ERT Color | Moisture | Predicted internal condition |
|----------------|-----------|---------|-----------|----------|------------------------------|
| A              | Brown     | Maximum | Red       | None     | No decay                     |
| B              | Brown     | Maximum | Non-red   | Present  | Incipient decay              |
| C              | Non-brown | Reduced | Non-red   | Present  | Active decay                 |
| D              | Non-brown | Reduced | Red       | None     | Cavity                       |

3. Results

3.1. Tree age effects
Of the 32 trees for which reliable ages could be determined, 19 had measurable decay present (mean age = 133 years old) while 13 had no decay (mean age = 127 years old). Results from the logistic regressions revealed that decay incidence (i.e., presence or absence of decay) was unrelated to dbh, age, species, or any of the interactions tested (all p-values > 0.202). Similarly, regression results revealed that the area, and by extension, volume of decay were unrelated to dbh or age (all p-values > 0.59).
3.2. Qualitative assessments of tomography

The accuracy with which tomography (SoT and ERT combined) predicted the internal condition of each of the 105 stem disks was assessed qualitatively through comparison of stem disk photographs to the tomo-grams of the corresponding cross-sections. These assessments, based on the rules criteria in table 1, showed that tomography correctly predicted the internal condition of 95 cross-sections (table 2, supplementary table 3). Tomography misidentified cavities as active decay (category ‘C’, table 1) in ten cross-sections; of these, seven were small cavities, <10 cm in diameter. Tomography correctly predicted cavities in the remaining 26 stem disks that had cavities. In no

Figure 1 Stem disk SoT and ERT tomograms and photographs corresponding to each of four decay categories, according to table 1. (a) Category A: No Decay; (b) Category B: Incipient Decay; (c) Category C: Active Decay; (d) Category D: Cavity. The five images within each set are, clockwise from top right: SoT tomogram—stem disk photograph—ERT tomogram—ERT tomogram with photograph overlay—SoT tomogram with photograph overlay.

Table 2. Qualitative assessment of predictive accuracy of tomography, based on comparison of tomograms with corresponding stem disk photographs. See supplementary table 4 for complete dataset.

| Decayed category | Accurate | Inaccurate | Over | Equal | Under |
|------------------|----------|------------|------|-------|-------|
| A                | 10       | 1          | n/a  | n/a   | n/a   |
| B                | 31       | 1          | n/a  | n/a   | n/a   |
| C                | 28       | 8          | 2    | 8     | 19    |
| D                | 26       | 0          | 5    | 12    | 9     |
| Total            | 95       | 10         | 7    | 20    | 28    |

* All ten mischaracterized cross-sections had cavities (category ‘D’).
case did tomography falsely predict a cavity. Examples of each of the four decay categories in table 1 are presented in figure 1.

Using the criteria for cavity prediction (‘D’, table 1), it was possible to make qualitative assessments of the accuracy with which tomography predicted cavity size. Under these criteria, SoT made a fairly accurate prediction of cavity size for 12 cross-sections; over-predicted cavity size for five cross-sections; and under-predicted cavity size for nine cross-sections. Similarly, tomography accurately identified the relative locations of active decay (‘C’, table 1) for all 29 stem disks for which active decay was accurately predicted; of these, SoT made a fairly accurate estimate of the area of decay for eight stem disks, underestimated the area of decay for 19 stem disks, and overestimated the area of decay for two stem disks (table 2, supplementary table 3).

It was not possible to assess the accuracy with which tomography predicted areas of incipient decay (‘B’, table 2); by definition these cross-sections had areas of moisture, as indicated by ERT, that corresponded to uniform density according to SoT. However, in some cases, stem disks had areas of internal wood discoloration (e.g., figure 1(b)) that aligned, in tomogram/photograph overlays, with areas where ERT indicated moisture.

### 3.3.3. Quantitative assessments of tomography

#### 3.3.1. Carbon mass fraction and density

A total of 508 wood samples (229 from *F. grandifolia*, 170 from *A. saccharum*, and 109 from *B. alleghaniensis*) were analyzed by GCEA for carbon mass fraction (ω) (table 3). Fifty-six samples identified as outliers, either due to errors in sample acquisition or because the sample was mistakenly taken from a different SoT color category after re-inspection of tomogram/photograph overlays, were removed from the dataset.

We note that our carbon mass fractions were within 2% of those reported by Lamplom and Savidge (2003) for these same species. Sample volumes of the remaining 452 samples (220 from *F. grandifolia*, 127 from *A. saccharum* and 105 from *B. alleghaniensis*) were used to convert carbon mass fraction to carbon density (g cm$^{-3}$), which was then used to test for significant differences in mean carbon density by SoT color category. Initial results of the ANOVA determined that there were no statistically significant differences in densities between samples taken from areas corresponding to green (IC$_{gr}$) and magenta (IC$_{mg}$) SoT density categories. Therefore, these data were grouped into a single non-brown (IC$_{nb}$) category for comparison against brown (IC$_{br}$) (table 4(a)). Based on this analysis, brown samples exhibited significantly higher carbon densities compared to non-brown samples, both among and within species ($p < 0.001$) (table 4(a)). Carbon density values for brown and non-brown samples fell within the range of dry mass densities reported by Ketterings et al (2001) and compiled into a global wood density database for gymnosperms and angiosperms by Swenson and Enquist (2007). The proportional reduction in carbon density between sound and actively decaying wood was 34% for American beech, 22% for sugar maple, 19% for yellow birch, and 27% for the three species combined. No significant difference in carbon densities was observed in an ANOVA comparing SoT-Brown/ERT-Red samples to SoT-Brown/ERT-Blue samples ($p = 0.60$; table 4(b)), suggesting that the minimal resistivity associated with ERT-Blue, presumed to be incipient decay, is not associated with a detectable loss of structural carbon.

#### 3.3.2. Carbon mass of stem disks

Data from stem disks were used to derive regression equations for the estimation of carbon mass of lower boles using tomographic data. To that end, direct mass-based estimates of carbon mass of stem disks, $C_{mass}$, were regressed on indirect tomography-based estimates, $C_{SD}$ (supplementary table 4). Data from one *F. grandifolia* stem disk was not included in this analysis due to loss of a portion of the stem disk during moving and storage.

The resulting equation for the $C_{mass} - C_{SD}$ linear regression was $y = -95.39 + 1.0138x$, with a correlation coefficient, $R^2 = 0.9733$ (Model 1, figure 2(a)). The null hypothesis that the $y$-intercept, $b_0 = 0$ was rejected at the 0.05 significance level ($t = −2.0349, p = 0.0455$). The null hypothesis that the slope is 1 could not be rejected ($t = −1.6822, p = 0.0956$).

An estimate of carbon mass that assumed no decay, $\overline{C}_{ND}$, was similarly compared to $C_{mass}$ (figure 2(b)), yielding the $C_{mass} - \overline{C}_{ND}$ regression equation, $y = 305.76 + 0.7739x$, $R^2 = 0.8918$. The
Figure 2. Linear regressions of direct mass-based carbon estimates ($C_{mass}$) on indirect tomography-based carbon estimates of stem disks, based on data from table 4, with 95% confidence intervals (magenta) and prediction intervals (green). $C_{SD}$ represents tomography-based carbon estimates accounting for internal decay, whereas $C_{ND}$ represents tomography-based carbon estimates assuming no decay (see text). (a) $C_{mass} \sim C_{SD}$, Model 1; (b) $C_{mass} \sim C_{ND}$, Model 1; (c) $C_{mass} \sim C_{SD}$, Model 2, regression through origin; (d) $C_{mass} \sim C_{ND}$, Model 2, regression through origin.

Table 4. (a) C density, [C], (g cm$^{-3}$), for each SoT color category. (b) C density, [C]$_{br}$, (g cm$^{-3}$), for SoT-brown corresponding to ERT-red and ERT-non-red.

| SoT color | F. grandifolia | A. saccharum | B. alleghaniensis | All trees |
|-----------|----------------|--------------|-------------------|-----------|
|           | n   | $\bar{X}$ (sd) | n   | $\bar{X}$ (sd) | n   | $\bar{X}$ (sd) | n   | $\bar{X}$ (sd) |
| Brown, [C]$_{br}$ | 158  | 0.35 (0.04) | 96   | 0.32 (0.04) | 84   | 0.32 (0.03) | 338  | 0.33 (0.04) |
| Green, [C]$_{gr}$  | 38   | 0.24 (0.09) | 16   | 0.24 (0.10) | 13   | 0.27 (0.07) | 67   | 0.25 (0.09) |
| Magenta, [C]$_{mg}$ | 23   | 0.22 (0.09) | 15   | 0.26 (0.11) | 8    | 0.23 (0.06) | 46   | 0.24 (0.09) |
| Non-brown*, [C]$_{nbr}$ | 61   | 0.23 (0.09) | 31   | 0.25 (0.10) | 21   | 0.26 (0.07) | 113  | 0.24 (0.09) |
| ANOVA$^b$ (F, p)  | 96.8, <0.001 | 17.4, <0.001 | 20.1, <0.001 | 127.4, <0.001 |

| ERT Color | F. grandifolia | A. saccharum | B. alleghaniensis | All trees |
|-----------|----------------|--------------|-------------------|-----------|
|           | n   | $\bar{X}$ (sd) | n   | $\bar{X}$ (sd) | n   | $\bar{X}$ (sd) | n   | $\bar{X}$ (sd) |
| Red       | 40   | 0.35 (0.03) | 15   | 0.32 (0.04) | 28   | 0.32 (0.03) | 83   | 0.33 (0.03) |
| Non-red   | 116  | 0.35 (0.04) | 78   | 0.32 (0.04) | 56   | 0.32 (0.04) | 250  | 0.34 (0.04) |
| ANOVA (F, p) | 0.23, 0.63 | 0.03, 0.87 | 0.23, 0.63 | 0.05, 0.83 |

* Non-brown: Green and Magenta samples pooled together.
$^b$ ANOVA conducted using Brown and Non-brown C densities only.
null hypothesis that the $y$-intercept = 0 was rejected ($t = 3.2615, p = 0.0008$), as was a null hypothesis that the slope = 1 ($t = 16.3252, p < 0.0001$).

Forcing the $C_{mass} \sim C_{ND}$ regression through the origin ($b_0 = 0$) yielded the regression equation $y = 0.9945x$, $R^2 = 0.9930$ (Model 2, figure 2(c)). The null hypothesis that the slope = 1 could not be rejected ($t = -0.66241, p = 0.5095$). Forcing the $C_{mass} \sim C_{ND}$ regression through the origin yielded the regression equation $y = 0.8247x$, $R^2 = 0.9704$ (figure 2(d)). The null hypothesis that the slope = 1 was rejected ($t = -12.4202, p < 0.0001$).

Based on the results for Models 1 and 2, the equation $y = x$ ($b_0 = 0, b_1 = 1.0$) (Model 3) was also used for estimating carbon mass of lower boles (supplementary table 2(b), and see below).

### 3.3.3. Carbon mass of tree boles

For each of the 72 trees used in this study, two estimates of lower bole carbon mass, $C_{bole}$ and $C_{bole-ND}$, were obtained indirectly using equations for Models 1–3 as described above. For each tree, under each model, the difference between $C_{bole}$ and $C_{bole-ND}$ was used to estimate the proportion of the lower bole’s carbon lost to decay, $\%C_{dec}$ (supplementary table 2(b)). The three models differed in $\%C_{dec}$ by less than 0.01%.

No loss of carbon in the lower bole was measured for seven of the 28 F. grandifolia trees analyzed, nine of the 23 A. saccharum trees analyzed, and nine of the 17 B. alleghaniensis trees analyzed.

Of the 28 trees predicted to have active decay but no cavities, the proportion of carbon lost to decay in the lower bole averaged 3.8%, ranging from: 0.13% to 8.5% in 15 F. grandifolia trees; 0.35% to 18.7% for ten A. saccharum trees; and 0.35% to 23.6% for seven B. alleghaniensis trees.

Of the 14 trees predicted to have cavities, the proportion of carbon lost to both active decay and cavities in the lower bole averaged 18.7%, ranging from: 4.8% to 21.9% for six F. grandifolia trees; 6.3% to 36.7% for four A. saccharum trees; and 9.7% to 34.4% for five B. alleghaniensis trees.

### 3.4. Uncertainty analyses

Uncertainty in tomography-based estimates of carbon mass of lower boles was attributed to error in the regression models and to two sources of measurement error (table 5). Uncertainty due to error in estimates of cross-sectional area was ±1.05% for Model 1, ±1.04% for Model 2, and ±1.04% for Model 3. Uncertainty due to error in carbon density estimates was ±0.0002% for all three models, and was therefore excluded from subsequent Monte Carlo simulations. Uncertainty in the regression models was ±1.78% for Model 1, ±1.77% for Model 2, and ±1.81% for Model 3.

The combined uncertainty for all three sources of error (note that they are not additive) was ±2.10% for Models 1 and 2, and ±2.11% for Model 3. Differences among the three regression models were minimal (< 0.1%).

### 4. Discussion

While our study site was representative of a mature northern temperate hardwood forest, our sampling design was not meant to produce a valid sample of trees from which data could be incorporated into existing carbon accounting models. Nonetheless, the application of the methodology described here demonstrates that tomographic data, by taking into account the reduction in carbon mass associated with internal decay, has the potential to significantly improve the accuracy with which forest carbon mass is estimated from field inventory data. An important conclusion from this study is that when carbon loss from internal decay is not considered, carbon accounting models significantly over-estimate the mass of carbon stored in forest trees.

The qualitative assessment of tomographic predictions corresponding to stem disk photographs underscored the importance of combining SoT and ERT, which facilitates distinguishing among the four ‘decay’ categories identified for this study (table 1). While this corroborates the manufacturer’s guidelines, to our
knowledge this is the first published report to experimentally demonstrate the efficacy of combined SoT/ERT interpretation as described in table 1, which is essential for distinguishing between active decay and cavity, and therefore critical to accurate carbon mass estimation, the overriding objective of this study. Tomography correctly identified all but the smallest (<10 cm diameter) cavities and in no instance predicted a cavity where none existed. Further, most of the tomographically identified cavities were not suspected through external visual assessments of the trees. Despite a tendency for tomography to slightly under-predict the area, and therefore volume, of decay or cavity—and also to mischaracterize small cavities as active decay for ten stem disks (table 2)—we nonetheless observed strong concordance in the linear regressions of direct mass-based versus indirect tomography-based estimates of carbon content (figure 2).

Our results did not concur with several recent studies that demonstrated a positive relationship between tree diameter and decay incidence, or tree age (inferred from stand age) and decay incidence (Barrette et al 2013). This finding may result from a limited range of diameters (ca 33–71 cm) and ages (68–192 years) with which to fully evaluate the effects of diameter and age.

As expected, carbon densities of wood samples (table 4(a)) were significantly higher for brown samples than for those sampled from either green or magenta, corroborating the correlation between sonic velocity and wood density (Bucur 2005). Green signifies higher velocities than magenta, and analysis of the associated wood densities showed slight but statistically insignificant trends in that direction for American beech and yellow birch, but the opposite trend for sugar maple. At least two factors may have influenced these unexpected results: first, much smaller green and magenta sample sizes resulted in higher standard deviations; second, there was unavoidable imprecision in locating areas on stem disks corresponding to green and magenta on the tomograms, errors which, given the small sample sizes, would contribute disproportionately to the averages. Nonetheless, for all three species the average density for pooled green and magenta samples (non-brown) was significantly lower than for brown, and the resulting density metrics yielded estimates of stem disk carbon mass that strongly correlated to direct mass-based estimate (figure 2).

The aforementioned highlights the pivotal role that tomography, given its ability to identify all but the smallest cavities, and to distinguish them from active decay or the absence of decay, played in achieving the high level of accuracy in the carbon mass estimation methodology presented here. The proportional reduction in carbon density between sound and actively decaying wood contributed significantly to the accuracy of carbon mass estimates, but even more critical was the ability to identify and estimate the volumes of cavities, which represent a complete loss of carbon to decay. This level of accuracy exceeded expectation, especially considering two possible sources of imprecision underlying the indirect tomography-based estimates. First, there was likely error, albeit immeasurable, in ‘percent brown’ metrics, given the tendency, as noted above, for tomography to under-predict the areas of decay and cavity. More importantly, tomography-based carbon mass estimates of stem disks were subject to error in stem disk volume estimates, given the high degree of unevenness in stem disk thicknesses; these volume-estimate errors likely explain much of the scatter around the regression lines in figure 2. Estimating the volumes of the stem disks by taking the average of many thickness measurements was imperfect, but not necessarily less so than alternatives such as measuring volume displacement in water. No other error associated with stem disk volume estimates, other than that associated with caliper measurements and their impact on area estimates, was accounted for in uncertainty analysis, given that it would have no relevance to carbon mass estimates of lower boles. Furthermore, stem disks served additional utility for both photography and for wood sampling for carbon density.

The slope of the original linear regression (Model 1, \( y = -95.39 + 1.0138x \)) was not significantly different than 1, with a small y-intercept and very high correlation coefficient. Nonetheless, forcing the regression through the origin (Model 2)—an intuitively justifiable assumption—effectively improved in both slope and correlation coefficient, leading to Model 3, which postulates that the best estimate of carbon mass is the indirect estimate itself; i.e., \( Y = X \).

Sources of uncertainty in ecological studies fall into four general classes: measurement, sampling, model prediction, and model-selection (Yanai et al 2012, Harmon et al 2015). Sampling error as a source of uncertainty was not relevant in this study, but will be in future anticipated studies utilizing our methodology, in which multiple plots are sampled. We also ignored uncertainty due to model-selection error, given the near-identical results from the three regression models. In applying uncertainty analysis to lower bole carbon mass estimates, we focused on two sources of measurement error. Although standard deviations around the carbon density metrics for brown and non-brown samples were large (table 4(a)), they manifested in an insignificant amount (±0.0002%) of uncertainty (table 5). By contrast, the ‘play’ we measured in the PiCUS calipers introduced error in estimates of cross-sectional area, which in turn impacted volume estimates, manifesting in uncertainty of ±1.04% (table 5). For practical reasons, we did not address the additional uncertainty caused by error associated with manually applying MP1-MP5 scalars to cross-sectional images in ImageJ; however, this issue is now obviated with the most recent version of the PiCUS software, which provides an area estimate for each cross-section. Model prediction
uncertainty—the uncertainty in predicting the carbon mass of an individual tree—is depicted as the prediction interval in figure 2 regressions, and should be distinguished from uncertainty in estimating a population mean, depicted as the confidence interval in these regressions. We focused here exclusively on the models’ ability to predict a new response, which is also relevant to the potential future use of this methodology, rather than the models’ ability to estimate a population mean. However, it should be noted that prediction uncertainty incorporates uncertainty in knowing the true population mean. Prediction uncertainty was essentially the same (±1.8%) for each of the three models (table 5), providing further support for the use of Model 3. The composite uncertainty associated with both measurement error and model prediction error was ±2.11%, which illustrates that uncertainties do not necessarily combine additively (Harmon et al 2007).

‘Incipient decay’ here refers to the co-occurrence of high density (high sonic velocities) with moisture (low electrical resistance). This contrasts with ‘no decay,’ where high density is accompanied by low or no moisture. Interestingly, incipient decay does not appear to be associated with measurable density reduction; there were no differences in carbon density between ‘incipient decay’ and ‘no decay’ wood samples (table 4(b)). ‘Incipient decay’ may be indicative of bacterial wetwood (Sinclair and Lyon 2005), but it also corroborates earlier studies associating water-soaked heartwood with the presence of anaerobic methanogenic microbes (Zeikus and Ward 1974, Shortle et al 1978). More recently, Covey et al (2016) observed a negative correlation between decay stage and methane concentration in hardwoods, with the highest levels of methane associated with the earliest stages of decay; from these observations they hypothesized that methanogenesis may be driven by archaeal metabolism of non-structural carbohydrates, which do not contribute to wood density. The ‘incipient decay’ category described here, showing no evidence of structural carbohydrate metabolism, provides compelling support for this hypothesis.

5. Conclusion

In this study we demonstrate how SoT and ERT, used in combination, can be used to non-destructively quantify the extent of internal decay, and the associated carbon loss, in the boles of living trees. The methodology presented here can also be applied to long-term studies aimed at measuring rates of decay in living trees, tracking temporal changes in stored carbon over 5- and 10-year intervals. The PiCUS equipment is relatively compact and light-weight, and the entire set-up can easily be managed by teams of two individuals, even in very rugged forested settings (supplemental figure 1).

Given that tree carbon mass is typically predicted from allometric equations that either assume non-decayed stems or use only visual symptoms of decay to deduct biomass (Nogueira et al 2008, Domke et al 2012, Matsuzaki et al 2013), our results highlight the likelihood that these methods over-estimate carbon mass in individual trees and hence larger-scale forest inventories. For example, based on a study of trunk rot from mixed dipterocarp forests of Borneo, Heineman et al (2015) concluded that studies that assume that all trees consist of intact non-decayed wood may be over-estimating above-ground biomass by as much as 7%. The growing emphasis on accurate forest carbon accounting, particularly in the context of climate change adaptation and mitigation, calls for improvements in inventory methods and allometric models (Woodall et al 2011, Weiskittel et al 2015). While the work presented here focused on individual trees, more importantly it creates the framework for such improvements across larger spatial extents, with the ultimate goal of more accurate forest carbon accounting.

Our findings illustrate the dynamic nature of internal decay in living trees, and its integral role in the forest carbon cycle. As the decay process, driven by microbial metabolism, advances and gives way to cavities that expand over time, stored carbon cycles back into the atmosphere as CO₂, even as photosynthesis drives the reverse process through annual increment growth, accumulating and converting atmospheric CO₂ into wood. Whether the latter process outpaces and offsets the former, and what factors drive the dynamic, are matters for ongoing research, discussion and debate. While the likelihood of decay and cavities may increase with the age and/or size of a tree (Boddy 2001, Heineman et al 2015, Frank et al 2018), there is also some evidence that the rate of carbon sequestration increases with tree size (Stephenson et al 2014). Old-growth forests are known to store more carbon than younger, managed forests (Luysaert et al 2008), however this difference may be less than typically thought, considering that large and old trees can lose significant stem volume to internal decay.

Acknowledgments

This work was supported by the National Science Foundation EARly-concept Grants for Exploratory Research (EAGER) Program, Grant #DEB-1346258. We wish to acknowledge Joel E Bronson and Jean Bronson of GMF for assistance with site selection and logistical support; Kelly Allen and Wesley Gomez for assistance with field data collection; Michael Ammirata and Adam Argraves for laboratory assistance; and Dr Ruth Yanai for helpful guidance in uncertainty analysis.
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