

## Article

# Bioenergy Conversion Potential of Decaying Hardwoods

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**Abstract:** Unharvested hardwoods are abundant in eastern Canada, due to the low quality of their fiber and the absence of outlets in conventional wood transformation industries. The objective of this study was to assess the biochemical and thermochemical energy conversion potential of decaying hardwoods and compare their relationships with external and internal indicators of tree degradation. We characterized how wood-decay processes altered the physical and chemical properties of these woods and affected their digestibility yield and their performance according to indexes of stability and efficiency of combustion. DNA analysis on wood samples was also performed to determine the relative abundance of white-rot fungi compared to that of other saprotrophs. All properties stayed within the range of variations allowing the wood to remain suitable for conversion into bioenergy, even with increased decay. We found no significant differences in the physical and chemical properties that are crucial for energy production between wood from externally-assessed live and decayed trees. However, the proportion of wood area affected by rot was significantly associated with increased digestibility yield, and with decreased combustion reactivity. We could not detect any specific effect associated with increased relative abundance of white-rot fungi. These results suggest that the utilization of biomass from decayed hardwoods instead of live trees for bioenergy production should not alter the conversion efficiency and even potentially increase the performance of biochemical pathways, and hence, support their use as feedstock for bioenergy production.

**Keywords:** wood rot; biomass properties; white-rot fungi; biochemical pathway; thermochemical pathway



**Citation:** Dupuis, É.; Thiffault, E.; Barrette, J.; Adjallé, K.; Martineau, C. Bioenergy Conversion Potential of Decaying Hardwoods. *Energies* **2021**, *14*, 93. <https://doi.org/10.3390/en14010093>

Received: 27 November 2020

Accepted: 21 December 2020

Published: 26 December 2020

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## 1. Introduction

The Intergovernmental Panel on Climate Change (IPCC) has identified forest biomass as an important source of renewable energy in the context of GHG mitigation goals [1]. The use of whole trees for energy generation is limited by ecological and carbon balance constraints [2]. However, pulp-quality logs that have no other market have been considered as part of sustainable biomass feedstock for bioenergy [3]. Moreover, the IPCC recognizes that biomass from trees affected by natural disturbances can contribute to the overall technical potential of forest biomass [4]. On the other hand, recent work has shown that the harvesting operations of degraded trees in naturally disturbed stands for wood pellet production were not economically profitable, and suggested that the production of high-value bioenergetic products could raise operational profitability [5].

The conversion of forest biomass to high-value bioenergetic products can follow the biochemical or the thermochemical pathway. The biochemical conversion pathway includes processes that involve natural degradation (that can be induced artificially) through the action of enzymes or bacteria, such as bioethanol production via enzymatic hydrolysis and fermentation or anaerobic digestion [6]. The main obstacle to biochemical conversion

success is biomass recalcitrance, which is defined as the natural resistance of the plant to enzymatic and microbial degradation [7]. Biomass recalcitrance resides in the plant's physical structure (pore size and volume, specific surface area, cellulose crystallinity) and chemical composition (high lignin concentration and acetyl group). Overcoming biomass recalcitrance is possible by the application of pretreatments that alter the physical structure and chemical composition of biomass and enhance the accessibility of cellulose and hemicelluloses to enzymatic digestibility [8]. Pretreatments are responsible for a significant proportion of the conversion costs [9,10], and it is difficult to achieve good bioethanol yield without high energy inputs [11]. The high cost of the conversion process restricts bioethanol production on an industrial scale [12]. Some pretreatment technologies also lead to the denaturation of sugars that will inhibit the fermentation process [13] and therefore lower the conversion efficiency. Furthermore, some pretreatment technologies produce acidic or alkaline wastewater that needs to be treated before being released into the environment [14].

Fungal and microbial treatments have been suggested as an environmentally friendly and cost-competitive alternative to traditional pretreatments in order to enhance enzymatic hydrolysis. The biological pretreatments employ microorganisms such as white-rot fungi to degrade lignin and hemicelluloses extensively, but leave cellulose mostly untouched [15]. The downsides of this method are the long residence period and the loss of carbohydrates (hemicelluloses and, to a lesser extent, cellulose) that reduce the overall conversion efficiency [16]. However, evidence has shown that natural wood degradation processes associated with fungi, insects or fire have the potential to reduce recalcitrance and enhance enzymatic digestibility [17–19].

The thermochemical conversion pathway includes processes that use heat as a vector of decomposition of the raw material. The most studied conversion technologies are combustion, pyrolysis and gasification [6]. The energy content per mass unit, expressed by the calorific value (or higher heating content, HHV), is the most important parameter that influences the quality of thermochemical fuels. Since lignin has a higher calorific value (23.26 to 25.58 MJ/kg) than the other wood components such as cellulose and hemicelluloses (18.60 MJ/kg), the HHV of biomass is strongly correlated to the lignin content of the biomass [20,21]. The presence of lignin-decomposing fungi such as white-rot could negatively affect the HHV of decaying biomass, whereas degradation caused by other types of saprotrophs (whose actions affect other wood components) could have a neutral or a positive impact.

Unharvested volumes of hardwoods are abundant in Quebec (Canada) [22]. Although they are part of the available annual cut (i.e., the sustainable rate of forest harvest), these volumes are often not harvested because of the low quality of their fiber induced by past unsustainable management techniques (e.g., stand high-grading in which only trees of superior quality were harvested) and the attack of fungus and insects. Bioenergy production from unharvested low-quality woods could potentially contribute to meet renewable energy goals as well as the restoration of degraded forests.

The main objective of this study was to assess the biochemical and thermochemical energy conversion potential of decaying and unharvested hardwoods. First, we characterized the impact of wood degradation and fungus type on the expression of the wood's physical and chemical properties. Then, the potential conversion of decaying hardwoods to energy via the biochemical and thermochemical pathways was evaluated using enzymatic hydrolysis and thermogravimetric analysis, respectively. Two indicators were used to describe wood degradation: an external visual indicator of tree degradation based on Hunter's decay classification [23], which could be useful during forest inventory and during tree marking for selection cutting [24], and an internal indicator of wood decay based on the relative wood area affected by rot.

## 2. Materials and Methods

### 2.1. Sampling and Laboratory Analyses

Five different species, indigenous to the province of Quebec, including sugar maple (*Acer saccharum* Marshall), yellow birch (*Betula alleghaniensis* Britt.), white birch (*Betula papyrifera* Marshall), American beech (*Fagus grandifolia* Ehrh.) and trembling aspen (*Populus tremuloides* Michx.), were sampled in different study areas across Quebec's temperate hardwood forest and the southern limit of the boreal forest. Sampling sites were spread across the following bioclimatic domains: sugar maple-basswood, sugar maple-yellow birch and balsam fir-white birch (Figure 1). Trees with a diameter at breast height (1.3 m) ranging from 10 to 40 cm were selected to represent different stages of degradation according to Hunter's visual classification [23]. We modified Hunter's classification and grouped the stages into three primary decay levels: live (Hunter's classes 1–2), dead (Hunter's classes 3–4) and advanced rot (Hunter's classes 5–6–7). These decay levels are therefore based on external visual assessment of tree degradation. The number of sampled trees within each combination of species and the level of decay are specified in Table 1.

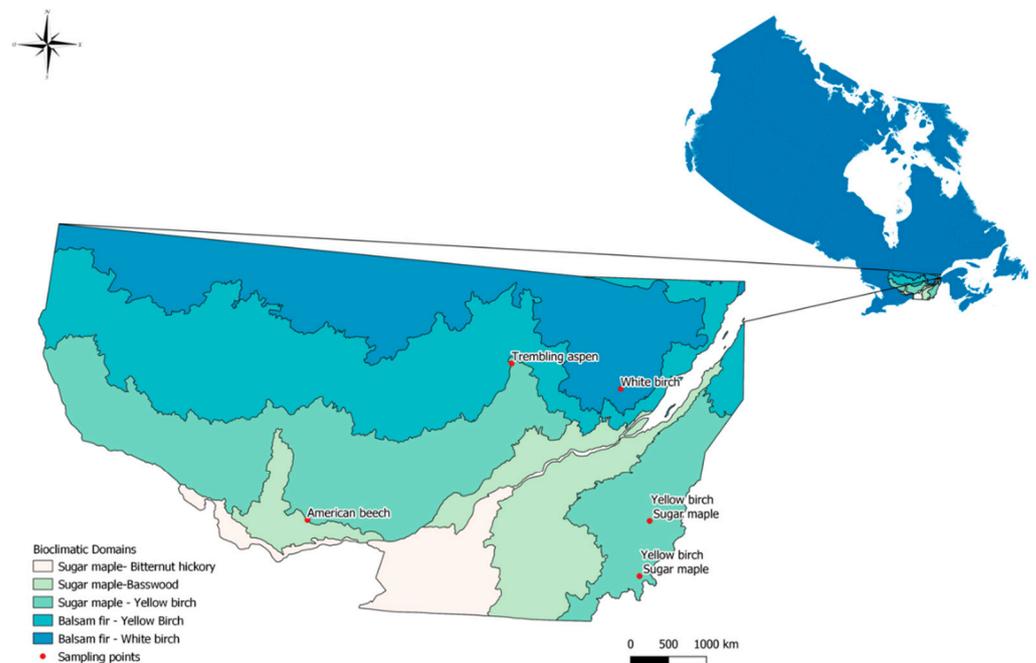
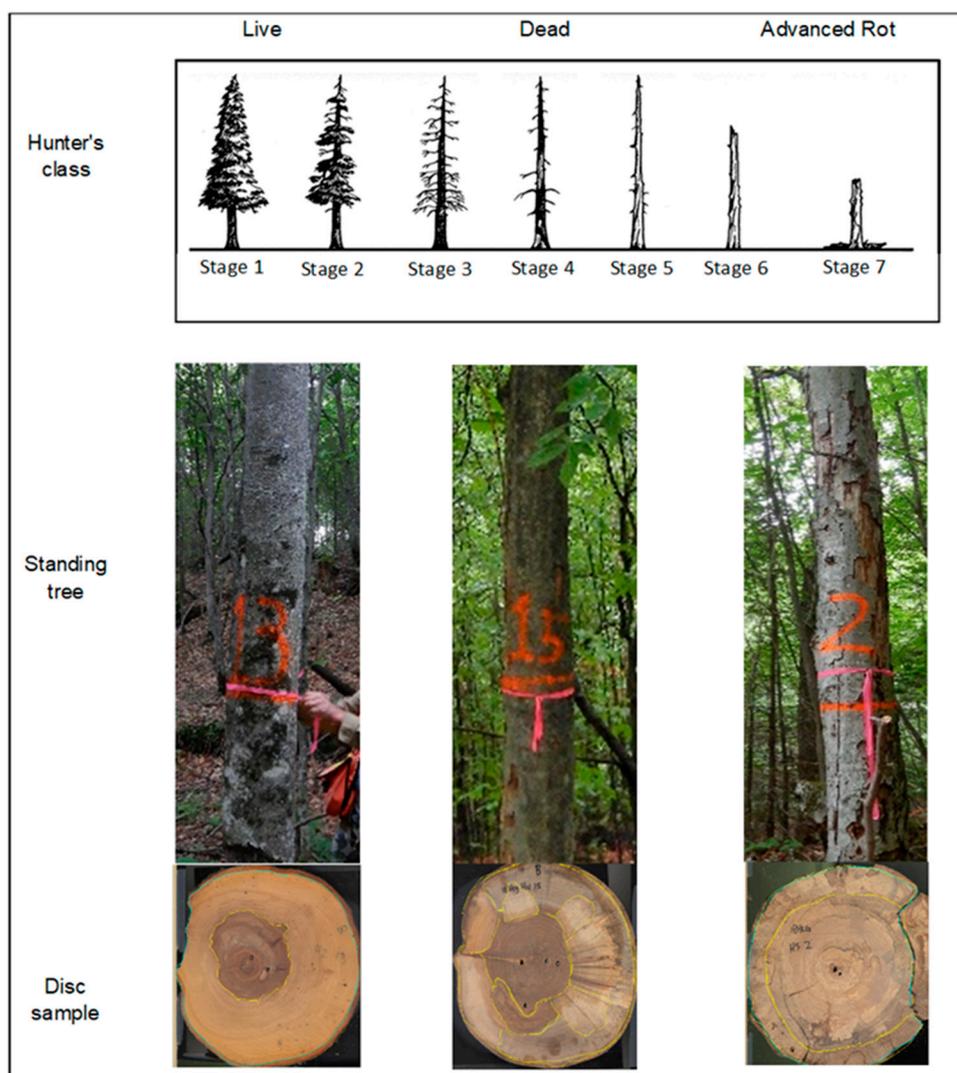


Figure 1. Location of sampling points

Table 1. Description of sampled material.

Decay Level	Species	Number of Samples
Live	White Birch	5
	American Beech	6
	Trembling Aspen	5
Dead	White Birch	8
	American Beech	10
	Trembling Aspen	7
	Sugar Maple	6
	Yellow Birch	7
Advanced rot	White Birch	2
	American Beech	12
	Trembling Aspen	3
	Sugar Maple	17
	Yellow Birch	5

A 30 cm thick disc was collected in each tree at breast height. Figure 2 represents an example of a standing tree and a sampled disc for each decay level and associated Hunter's class of American beech. The discs were stored at  $-5\text{ }^{\circ}\text{C}$  until further analysis.



**Figure 2.** Example of a standing tree and sampled disc for each decay level.

Each wood disc was scanned, and the scans analyzed with the ImageJ software; portions of the disc affected by rot were delineated, and their areas were measured, along with the total area of the disc, so that the proportion of wood area affected by rot could be calculated.

The physical properties of biomass were assessed by the determination of moisture content and basic density, which are relevant to both conversion pathways. Generally, moisture content varies widely because it depends on harvesting and storage methods; moisture content can also be easily manipulated with proper pretreatment and conditioning practices [25]. Basic density is influenced by other factors such as environmental conditions, growth rate, wood defects, and compression wood. We applied the standard methods from ASTM International to determine moisture content [26] and basic density [27].

In order to assess the bioenergy conversion potential for biochemical and thermochemical pathways, we used specific chemical characteristics and conversion tests [28] (Table 2). As an indicator of performance for the biochemical pathway, we tested sugar production by enzymatic hydrolysis. For the thermochemical pathway, we chose to test the combustion process with thermogravimetric analysis.

**Table 2.** Physical and chemical properties, and conversion test responses of interest according to conversion pathways.

	Biochemical Pathway	Thermochemical Pathway
Physical properties	Moisture content (%) (ASTM D4442-16)	
	Basic density $\left(\frac{\text{oven-dry mass (kg)}}{\text{green volume (m}^3\text{)}}\right)$ (ASTM D2395-17)	
Chemical properties	Lignin content (%)	Total ash content (%) (ASTM 1755-01)
	Carbohydrate content (%)	Ash melting behavior ([K])
		High heating value (HHV) (MJ/kg)
Conversion test response	Digestibility yield (%)	Ignition Index
		Combustion characteristic Index
		Flammability Index

Samples were oven-dried at 60 °C for 72 h, debarked, and a section of the discs was milled into 0.5 mm particles and stored in hermetic bags at room temperature until further analysis. The relative content of cellulose, hemicelluloses, lignin and extractives were measured with the method presented in Van Soest, et al. [29].

Ash content was determined by weighting samples in porcelain crucibles, before and after spending 6 h at 600 °C, following the standard procedure of [30]. We used the potassium concentration in ash as a modified alkali index [28]. The concentration of alkali metals in ash indicates the risk of slagging and fouling in the reactor, with a higher index or a higher K concentration representing greater risk. The remaining ashes were then mixed with chloric acid, heated to allow dissolution, and filtered. The resulting liquid was transferred for analysis of major elements using inductively coupled plasma optical emission spectrometry IPC-OES (Agilent 5110).

The higher heating value (HHV), defined as the energy content per mass unit (MJ/kg), was measured with a bomb calorimeter (Parr 6400), which was calibrated with benzoic acid. Approximately 0.7 g of ground wood sample was pressed into a tablet before being burnt in the presence of oxygen. Combustion in the bomb calorimeter was induced by a cotton thread attached to the platinum ignition wire in contact with the sample tablet.

## 2.2. DNA Analysis

The DNA analysis was carried out at the Laurentian Forestry Center (Canadian Forest Service, Quebec City, QC, Canada). DNA analysis was used to identify the different fungal Operational Taxonomic Units (OTUs) colonizing each sample, based on targeted amplicon sequencing of the ITS2 region of the fungal ribosomal DNA. Taxonomic identification of OTUs was used to determine the trophic status of the organisms responsible for wood decay.

The material stored at −5 °C was used for this analysis. The procedure for DNA analysis included sample preparation steps to reduce the disc to powder without letting it thaw. A coarse powder was collected from each disc using an electric hand router tool, and it was then crushed into fine particles (about 0.5 mm) with a mortar and pestle in liquid nitrogen. DNA extraction from wood samples was performed on 100 mg of wood particles, with the *DNeasy PowerSoil DNA Isolation Kit* (Qiagen, Valencia, CA, USA), using the manufacturer's instructions ( $n = 92$ ). During the extraction process, cells are broken down mechanically and chemically, and the DNA is isolated and purified. DNA was quantified with the *Qubit dsDNA HS Assay Kit* on a *Qubit 3.0 Fluorometer* (Thermo Fisher Scientific, Waltham, MA, USA). Amplicon library preparation for Illumina sequencing of the ITS2 region of the fungal ribosomal DNA was performed as previously described in Rheault, et al. [31]. Briefly, the polymerization chain reaction (PCR) is first used to amplify the target fungal DNA, and the success of the PCR is validated by gel electrophoresis. After purification of the PCR products with magnetic beads, samples are indexed so that multiple libraries can be pooled and sequenced simultaneously. A second purification step is then performed before samples are normalized, pooled, and sequenced on an *Illumina MiSeq*

system (Illumina, San Diego, CA, USA). Following DNA sequencing, bioinformatic analysis was carried out in QIIME (version 1.9.1 [32]) as previously described [31] to generate alpha and beta diversity metrics and taxa summary tables. The rarefaction was fixed at 3100 sequences per sample, which allowed us to keep 91 of the 92 samples in the analysis. Fungal functional groups were predicted using a homemade Python script based on a published list of fungal genera and their respective functions [33]: the script compares the genus of each OTU to this list to identify the corresponding functional group. OTUs unidentified at the genus level were assigned to the “unidentified function” category. The presence of white-rot fungi was evaluated using the relative abundance of white-rot fungi (as revealed by DNA sequences associated with white-rot fungi) compared to the relative abundance of all saprotrophs.

### 2.3. Enzymatic Hydrolysis and Sugar Recovery

We performed the enzymatic hydrolysis in the Laboratoire de biotechnologie environnementale (LBE) of INRS Centre Eau, Terre et Environnement (Quebec City, QC, Canada). Three samples were selected within each species, including, if available, one live and two decayed ( $n = 15$ ).

### 2.4. Hydrolysis

The samples were subjected to enzymatic digestion: samples were placed in a 250 mL shake flask according to a 10% solid loading, and including a citric acid buffer with a work volume of 125 mL. The ACCELLERASE DUET enzyme (from Dupont Industry Biosciences, CA, USA) was added to the mixture, using a ratio of 0.25 mL per dry g of biomass. The enzymatic hydrolysis was then performed during a 96 h period at 180 rpm, a temperature of 55 °C and a pH of 4.8. To prevent risk of contamination by microorganisms, an antibiotic and a fungicide (tetracycline and cycloheximide) were added at a dosage of 0.5% *v/w* of biomass. Samples were collected after 24, 48, 72 and 96 h of the enzymatic hydrolysis reaction and were stored at 4 °C until analysis. The measurement of the concentration of reducing sugars was performed using dinitrosalicylic acid, according to the method described in Miller [34]. A spectrophotometer (Varian Cary 50 Bio, UV-Visible spectrophotometer) at 540 nm was then used for evaluating total reducing sugars concentrations. The sugar yield was estimated as the ratio of reducing sugars on the sum of carboxylates in wood samples used for enzymatic hydrolysis.

### 2.5. Digestibility Yield Calculation

Total reducing sugars concentration and wood total carbohydrate composition data of each hydrolyzed sample allowed digestibility yield calculation (Equation (1)). Reducing sugar concentration is expressed in mg/mL, and we systematically used the value after 96 h of the reaction process.

$$\text{yield (\%)} = \frac{\left[ \text{Reducing sugar} \left( \frac{\text{mg}}{\text{mL}} \right) \right] * 12.5 \text{g/sample}}{\text{Mass Cellulose (g)} + \text{Mass Hemicelluloses (g)}} \quad (1)$$

### 2.6. Thermogravimetric Analysis

Thermogravimetric analysis (TGA) was used to assess the thermochemical conversion potential. This type of analysis makes it possible to determine the thermal degradation pattern of biomass. The ignition ( $T_i$ ) and burnout ( $T_e$ ) temperatures, along with the ignition index ( $D_i$ ), combustion characteristic index ( $S$ ) and the flammability index ( $C$ ) of biomass, can be deduced from the thermogravimetric results. The comparison of the different parameters expressed by thermal degradation patterns makes it possible to determine which type of biomass has the highest heat transfer and combustion efficiency [35–37]. TGA analysis was performed on a Mettler Toledo TGA.

The crucible was heated to red before and between the analysis of each sample. Approximately 0.15 g of ground material was placed in the crucible. Air was used as a

carrier gas, with a flow rate of 50 mL/min. The samples at room temperature were heated to 105 °C, at a rate of 30 °C/min and kept at 105 °C for 10 min to remove moisture entirely from the samples. In the third heating segment, samples were then heated from 105 to 800 °C, at a rate of 30 °C/min. Thermogram (TGA) curve and its first derivative (DTG) curve, presented in the relative weight scale, were used to determine ignition and burnout temperature using the intersection method (IM) described by [38]. The ignition temperature corresponds to the minimum temperature at which the wood sample ignites spontaneously in the absence of an external source of ignition. For its part, the burnout temperature is an indicator of the reaction degree of the fuel: it corresponds to the temperature at which the wood is almost entirely consumed, or at which 99% of the conversion is completed [38].

In order to have a better overview of the different combustion characteristics, three integrated indexes were computed [39,40]. The ignition index ( $D_i$ ) is calculated using Equation (2):

$$D_i = \frac{(dW/dt)_{\max}}{T_i * T_e} \quad (2)$$

where  $(dW/dt)_{\max}$  is the maximum combustion rate,  $T_i$  is the ignition temperature, and  $T_e$  is the burnout temperature. The larger the ignition index is, the better is the performance of ignition.

The combustion characteristic index (S) [41] arises from Arrhenius Law. The larger the combustion index is, the better is the combustion of the fuel. The combustion characteristic index is expressed by Equation (3).

$$S = \frac{(dW/dt)_{\max} * (dW/dt)_{\text{mean}}}{T_i^2 * T_e} \quad (3)$$

where  $(dW/dt)_{\max}$  is the maximum combustion rate,  $(dW/dt)_{\text{mean}}$  is the average combustion rate,  $T_i$  is the ignition temperature and  $T_e$  the burnout temperature.

The flammability index (C) expresses the combined influence of the maximum combustion rate and ignition temperature. A large flammability index value indicates good combustion stability [39]. The flammability index is expressed by Equation (4).

$$C = \frac{(dW/dt)_{\max}}{T_i^2} \quad (4)$$

## 2.7. Data Analysis

Two-way factorial analysis of variance, using decay level (i.e., external visual assessment of tree degradation) and species as independent variables, and physical and chemical properties, and conversion test results as response variables, were performed with R software version 3.6.1. Multiple comparison tests for determining differences between interaction effects or individual levels were performed using Tukey contrasts. The assumptions of the normality of residuals and homoscedasticity were tested beforehand. In order to keep a balanced experimental design, observations from sugar maple and yellow birch were not taken into consideration in these analyses since there was no live wood sample for these species.

Simple linear regression allowed us to assess the effect of proportion of wood area affected by rot on biomass physical and chemical properties and on biochemical and thermochemical conversion among all samples. The effects of lignin content and relative abundance of white-rot fungi among all saprotrophs present in wood samples on conversion performance were also evaluated using linear regression. Nonmetric multidimensional scaling (NMDS) ordination of fungal community structure based on Bray–Curtis dissimilarity and diversity of the taxonomic data were analyzed with the Metacoder R package [42].

### 3. Results

#### 3.1. Effects of Wood Decay on Physical and Chemical Properties

Results from the ANOVA suggested that the effect of the interaction of species x decay level (the latter being an external indicator of tree degradation) was different between the various physical and chemical properties (Table 3; all data available in the Supplementary materials).

The standard deviation of relative wood area affected by rot (an internal indicator of tree degradation), moisture content and basic density showed a considerable variation within each combination of species x decay level. The percentage of wood affected by rot and moisture content were significantly affected by the interaction of species x decay level ( $p$ -value > 0.001). For trembling aspen, the percentage of wood area affected by rot and moisture content showed somewhat clear distinctions between trees with advanced rot and trees from the same species at lower decay levels (i.e., live and dead trees, based on an external indicator of tree degradation), the former displaying higher % of rot and lower moisture content. American beech trees at an advanced rot decay level also had higher % of wood affected by rot relative to live and dead trees. On the other hand, the effect of species and decay level was not significant for basic density ( $p$ -value: 0.390).

Relative lignin content was significantly affected by the interaction of species and externally-assessed decay level ( $p$ -value: 0.004). However, no clear pattern of the effect of decay level on lignin was detected. The relative carbohydrate content (cellulose + hemicelluloses) was also significantly influenced by species and decay levels ( $p$ -value < 0.001). Trees in the live and dead stages had a higher carbohydrate content than the ones in the advanced rot stage. However, the effect was not detectable on the white birch sample in the advanced rot stage.

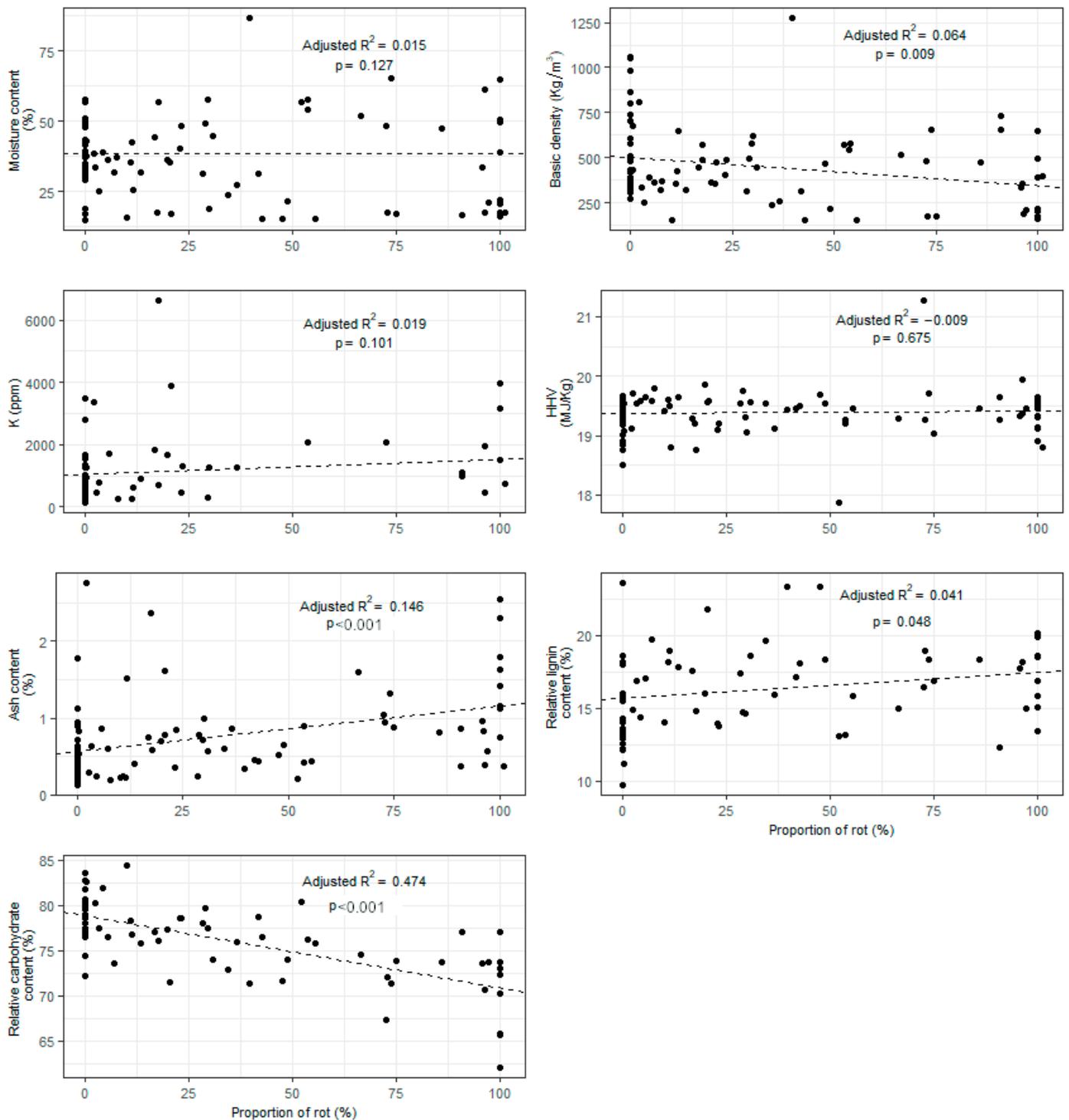
Ash content significantly increased with increasing externally-assessed decay level ( $p$ -value: 0.003). The concentration of K in ash was not affected by decay level but differed between species ( $p$ -value: 0.009): Tukey contrast showed that American beech presented a significantly higher concentration of K than white birch. Higher heating values (HHV) were significantly affected by the interaction effect of species x decay level ( $p$ -value: 0.023), although the variations were maintained within a narrow interval (19.18 for American beech in the advanced rot stage and 20.08 for trembling aspen in advanced rot).

Results from linear regression also showed the significant effects of wood decay when expressed as the proportion of wood area affected by rot, on ash (positive effect;  $p$ -value < 0.001) and carbohydrate (negative effect;  $p$ -value < 0.001) contents (Figure 3). Moreover, this internally-assessed indicator displayed a significant relationship with basic density (negative effect;  $p$ -value = 0.009) and lignin content (positive effect;  $p$ -value = 0.048).

**Table 3.** Physical and chemical properties of hardwoods at different decay levels. n = number of samples. Values in parenthesis show standard deviation.

Decay Level	Species	n	Relative Area Affected by Rot %	Moisture Content %	Basic Density kg/m <sup>3</sup>	[K] * ppm	HHV MJ/kg	Ash %	n	Lignin %	Carbohydrates %
Live	White Birch	5	0.0 <sup>c</sup> (0.0)	75.47 <sup>a,b</sup> (16.44)	426 (54)	478.5 <sup>b</sup> (180.7)	19.55 <sup>a,b</sup> (0.14)	0.26 <sup>b</sup> (0.0008)	5	13.92 <sup>b</sup> (1.19)	79.94 <sup>a</sup> (1.63)
	American Beech	6	0.0 <sup>c</sup> (0.0)	57.02 <sup>a,b,c</sup> (18.45)	609 (322)	1711.5 <sup>a</sup> (1166.5)	19.29 <sup>b</sup> (0.28)	0.78 <sup>a,b</sup> (0.006)	4	15.30 <sup>a,b</sup> (2.94)	79.45 <sup>a</sup> (2.46)
	Trembling Aspen	5	3.5 <sup>c</sup> (7.9)	99.47 <sup>a</sup> (24.12)	493 (64)	600.2 <sup>a,b</sup> (193.0)	19.34 <sup>a,b</sup> (0.34)	0.48 <sup>a,b</sup> (0.001)	5	12.98 <sup>b</sup> (1.98)	79.04 <sup>a</sup> (2.82)
Dead	White Birch	8	17.3 <sup>b,c</sup> (32.7)	65.30 <sup>a,b</sup> (20.51)	485 (236)	670.3 <sup>b</sup> (721.7)	19.59 <sup>a,b</sup> (0.34)	0.34 <sup>b</sup> (0.003)	7	15.56 <sup>a,b</sup> (2.49)	78.32 <sup>a</sup> (4.01)
	American Beech	10	4.1 <sup>c</sup> (6.1)	45.25 <sup>b</sup> (17.39)	499 (210)	1493.2 <sup>a</sup> (774.0)	19.31 <sup>b</sup> (0.27)	0.87 <sup>a,b</sup> (0.007)	6	18.58 <sup>a</sup> (2.54)	76.15 <sup>a</sup> (2.09)
	Trembling Aspen	7	28.2 <sup>b,c</sup> (34.1)	98.58 <sup>a</sup> (34.02)	609 (141)	1132.4 <sup>a,b</sup> (600.6)	19.40 <sup>a,b</sup> (0.19)	0.63 <sup>a,b</sup> (0.002)	6	13.54 <sup>b</sup> (0.85)	77.43 <sup>a</sup> (1.11)
Advanced rot	White Birch	2	11.5 <sup>b,c</sup> (16.3)	69.23 <sup>a,b,c</sup> (3.32)	409 (12)	653.5 <sup>b</sup> (259.2)	19.30 <sup>b</sup> (0.28)	0.44 <sup>a,b</sup> (0.001)	2	13.25 <sup>b</sup> (0.92)	79.45 <sup>a</sup> (1.34)
	American Beech	12	42.1 <sup>a,b</sup> (42.2)	23.70 <sup>b</sup> (11.21)	491 (191)	1883.4 <sup>a</sup> (1916.7)	19.18 <sup>b</sup> (0.26)	1.16 <sup>a</sup> (0.007)	1	15.90 <sup>a,b</sup> (NA)	75.90 <sup>a,b</sup> (NA)
	Trembling Aspen	3	90.9 <sup>a</sup> (15.8)	46.39 <sup>b</sup> (40.26)	286 (170)	2249.1 <sup>a,b</sup> (853.9)	20.08 <sup>a</sup> (1.05)	1.59 <sup>a</sup> (0.008)	3	15.57 <sup>a,b</sup> (1.89)	67.67 <sup>b</sup> (5.86)

For a given property or characteristic, different superscript letters indicate significant differences between interaction effects, according to Tukey's multiple comparisons of means test, with a 95% family-wise confidence interval. Note that means that are not significantly different are not to be judged to be the same. \* [K] Tukey test considers the effect of species only.

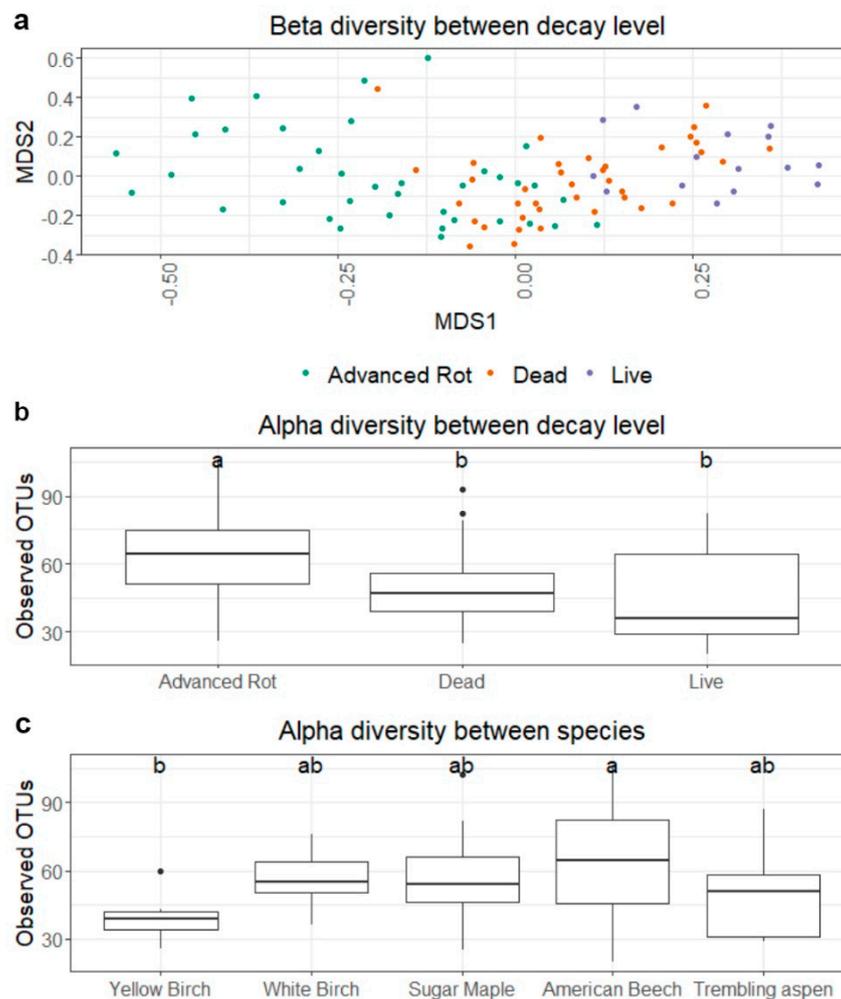


**Figure 3.** Relationships between proportion of wood area affected by rot and biomass properties.

### 3.2. Decaying Fungal Organisms and Effect on the Wood Composition

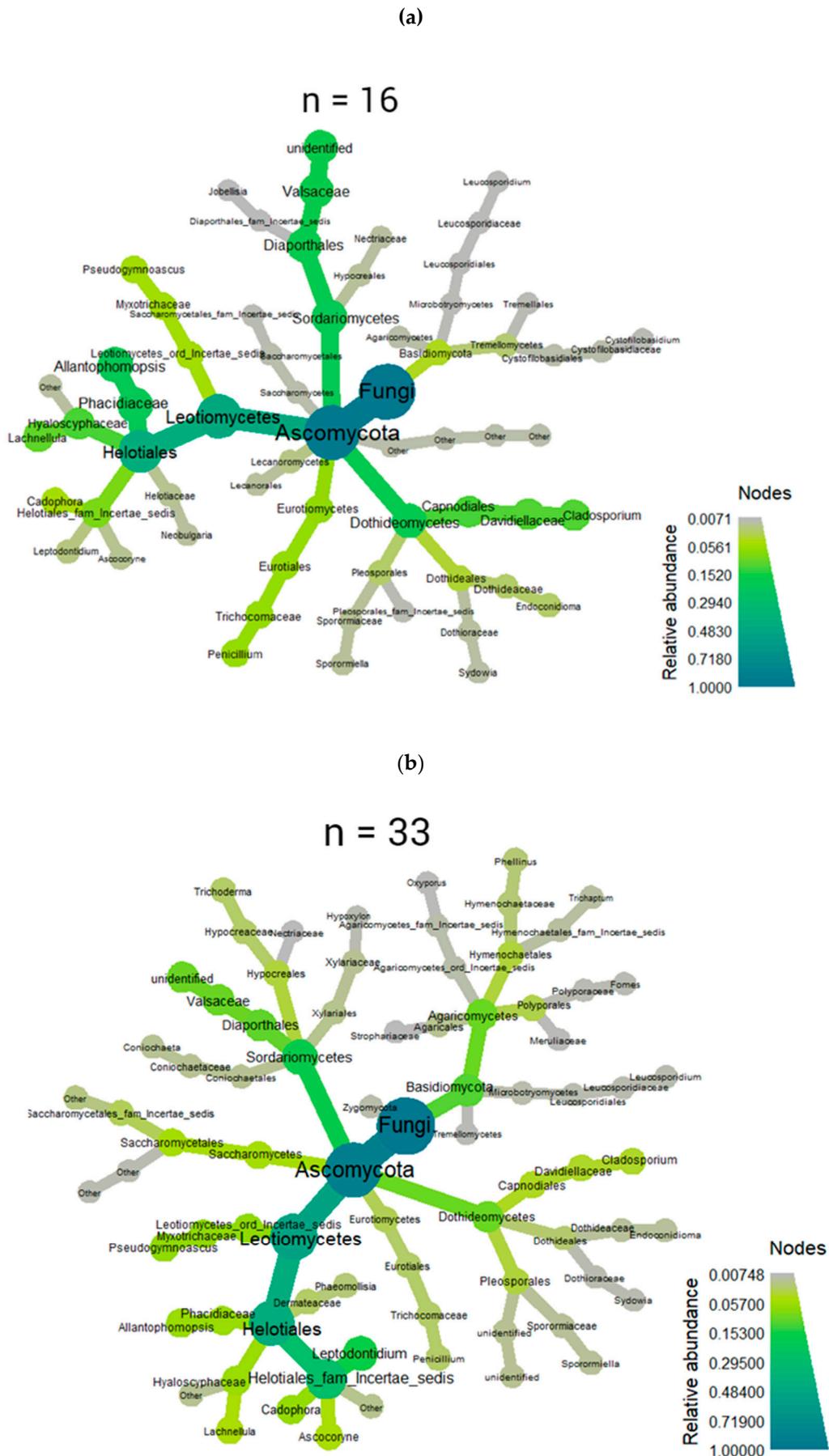
Results from DNA analysis suggested that fungal communities (beta diversity) were different between decay levels (Figure 4a), but not between tree species. The number of OTUs (alpha diversity) detected in each tree increased with the external signs of decay (Figure 4b). The number of different OTUs also differed between tree species (Figure 4c).

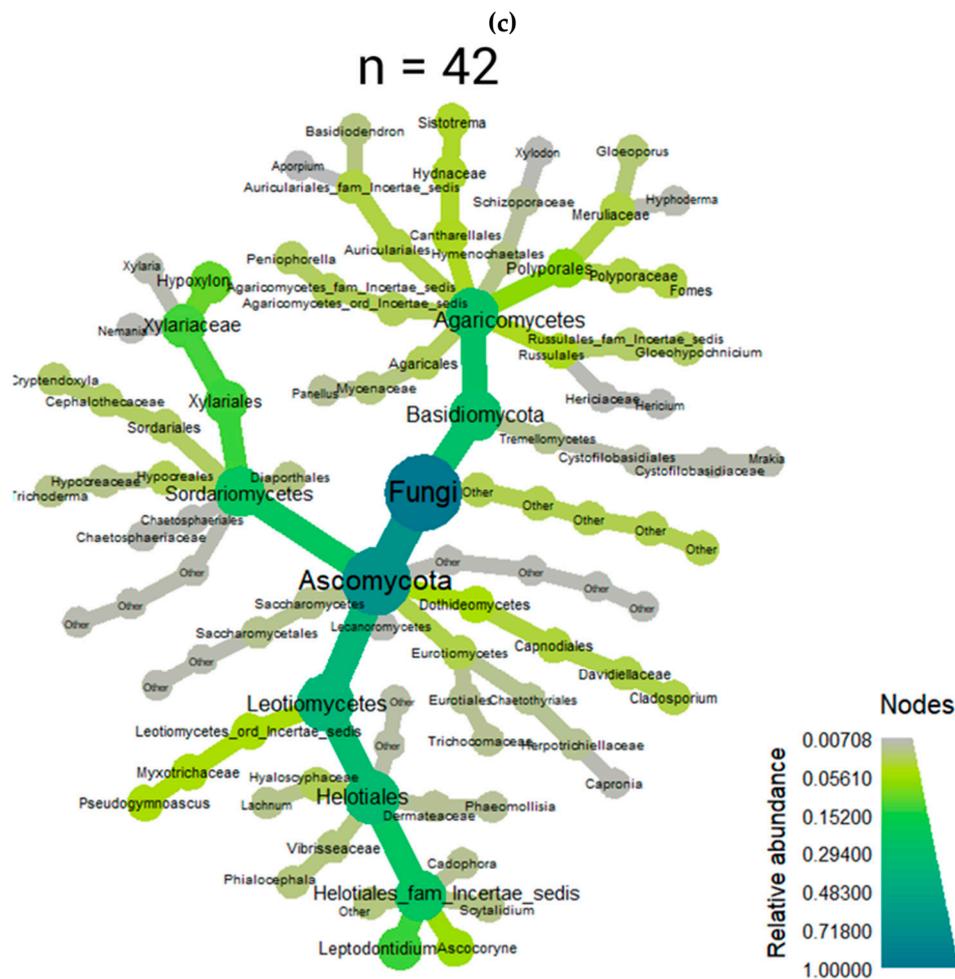
Using ANOVA and Tukey contrasts, we observed a significant difference between the alpha diversity of yellow birch and American beech ( $p$ -value: 0.002).



**Figure 4.** Beta diversity (nonmetric multidimensional scaling ordination of fungal community structure based on Bray-Curtis dissimilarity) between decay levels (a) and alpha diversity (expressed by the number of observed OTUs) between decay level (b) and species (c). Different letters indicate significant means difference, according to Tukey's multiple comparisons of means test, with a 95% family-wise confidence interval.

A total of 286 different taxonomic units (OTUs) were identified among the 91 samples tested. Over 20% of the overall fungal diversity (in terms of the total number of OTUs) was represented by organisms belonging to the Agaricomycetes class, which are thought to be the most efficient decaying fungi. Agaricomycetes exhibit two main modes of plant cell wall decomposition: (i) white-rot, which will digest lignin first and; (ii) brown-rot, which will digest carbohydrates first [43]. Our results showed that the relative abundance of Agaricomycetes (based on the relative abundance of DNA sequences associated with this fungi class) was higher within advanced rot samples than within dead and live samples (Figure 5). The relative abundance of white-rot fungi (also based on the relative abundance of DNA sequences associated with these fungi) was also significantly higher in advanced rot samples than in live and dead samples ( $p$ -value < 0.001).





**Figure 5.** Distribution of the fungal diversity and relative abundance of fungi in wood from (a) live trees; (b) dead trees; (c) trees with advanced rot.

### 3.3. Biochemical Conversion Test

Digestibility yield of cellulose and hemicelluloses varied among the different samples that we selected for enzymatic hydrolysis (Figure 6). There was no detectable pattern, among all species, of the influence of decay level on digestibility yield. Nevertheless, the digestibility yield of trembling aspen increased with the severity of rot.

However, when tree degradation was based on the internal indicator of the proportion of wood area affected by rot, a significant ( $p$ -value = 0.002) positive relationship could be found among all samples with digestibility yield (Figure 7). On the other hand, our results did not show that lignin content ( $p$ -value: 0.633) or the relative abundance of white-rot fungi relative to all saprotrophs ( $p$ -value = 0.213) could drive the digestibility yield of carbohydrates (Figure 7).

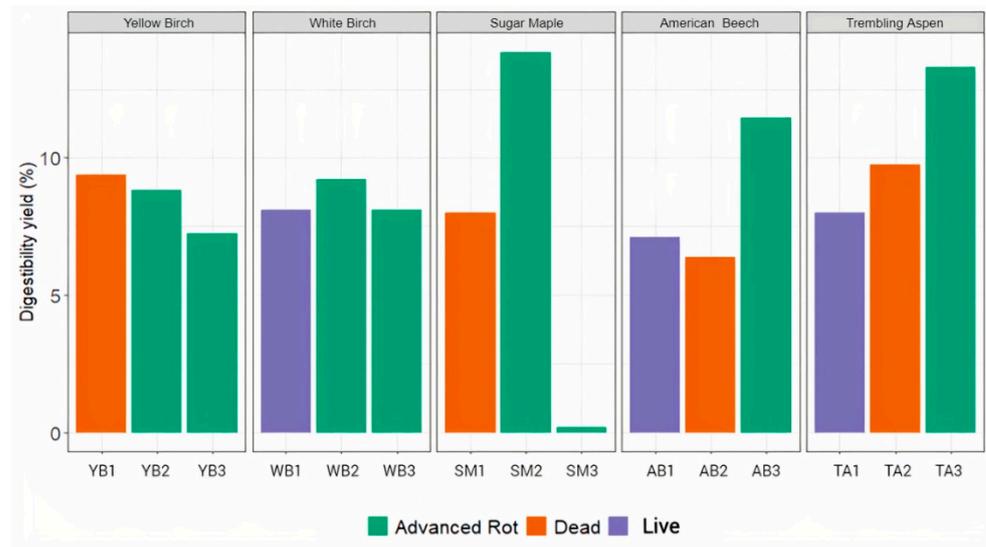


Figure 6. Digestibility yield attained by each analyzed sample.

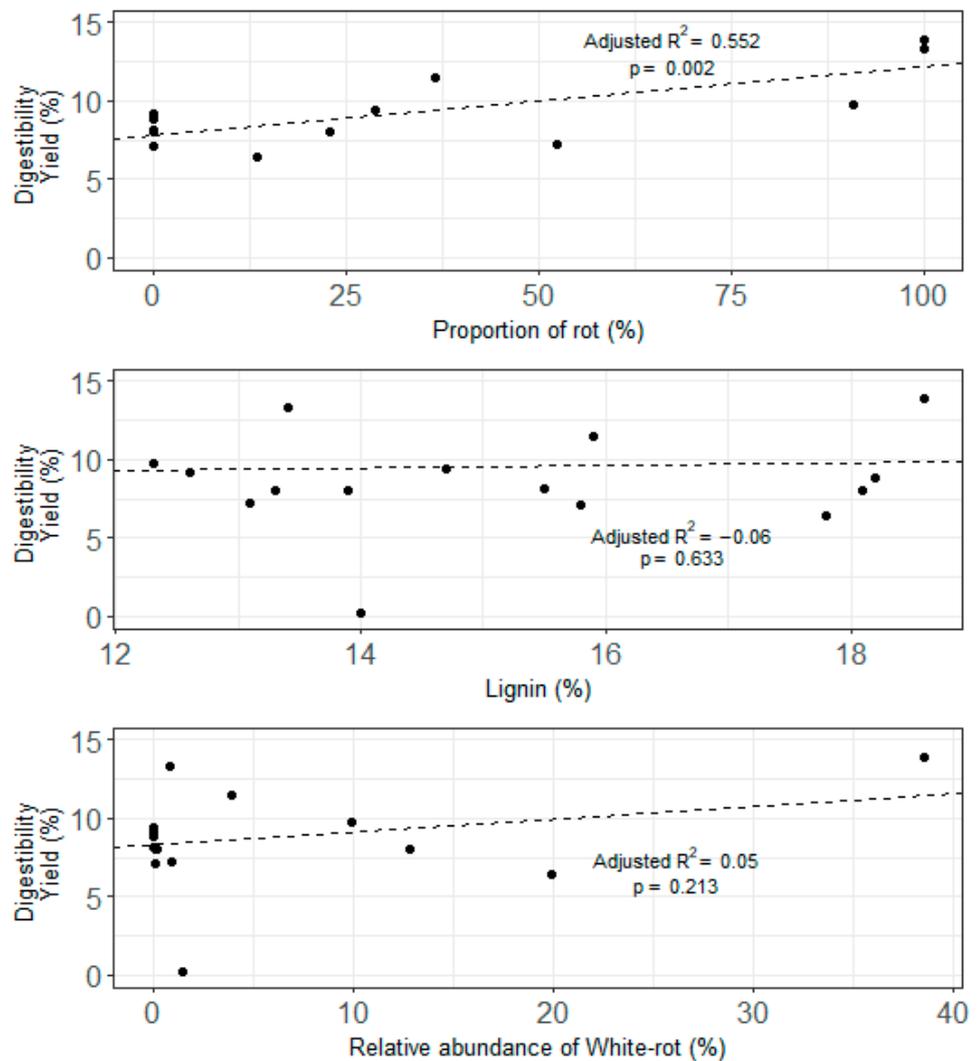


Figure 7. Relationships between proportion of wood area affected by rot, lignin content and relative abundance of white-rot fungi compared to all saprotrophs, and digestibility yield of carbohydrates.

### 3.4. Thermochemical Conversion Test

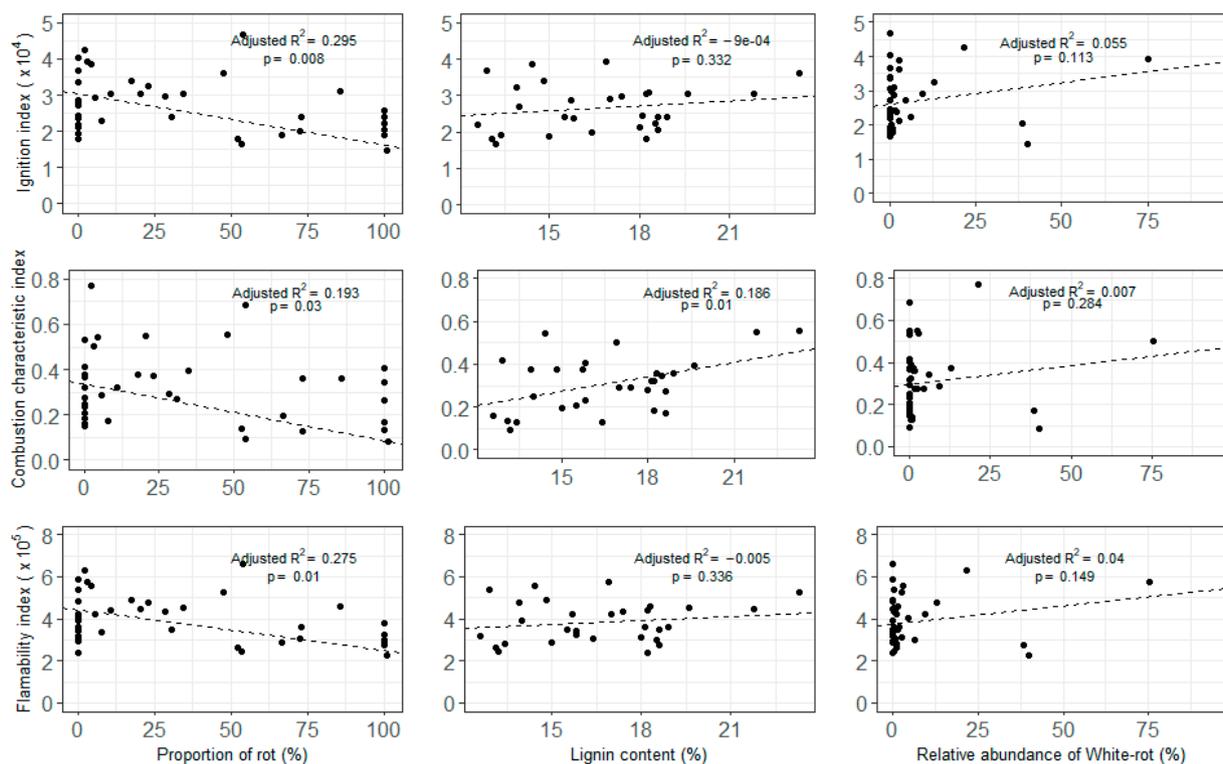
The variations in the combustion characteristic parameters of white birch, American beech and trembling aspen suggested that they cannot directly be explained by externally-assessed decay level (Table 4). The interaction between species and decay level was not significant for burnout temperature ( $T_e$ ), ignition index ( $D_i$ ), combustion characteristic index ( $S$ ) and flammability index ( $C$ ). The ANOVA detected a significant effect of , level on the ignition temperature ( $T_i$ ) ( $p$ -value: 0.004). Therefore, a multiple comparison test was performed on  $T_i$ , which did not reveal a clear pattern among decay levels or species, although  $T_i$  tended to be lower for American beech and trembling aspen at a more advanced decay level.

**Table 4.** Combustion characteristic parameters of hardwoods at different decay level.  $n$  = number of samples. Values in parentheses show standard deviation.

Decay Level	Species	$n$	$T_i$ °C	$T_e$ °C	$D_i \times 10^4$	$S \times 10^7$	$C \times 10^5$
Live	White Birch	2	273.90 <sup>a</sup> (0.44)	396.59 (0.57)	2.56 (0.21)	3.71 (0.30)	0.23 (0.03)
	American Beech	2	269.70 <sup>a,b,c</sup> (1.48)	391.50 (1.35)	3.70 (0.50)	5.36 (0.71)	0.45 (0.12)
	Trembling Aspen	2	273.35 <sup>a</sup> (4.21)	396.80 (1.58)	3.55 (0.19)	5.15 (0.38)	0.39 (0.03)
Dead	White Birch	2	273.98 <sup>a</sup> (0.57)	398.25 (0.24)	2.34 (0.05)	3.40 (0.06)	0.20 (0.04)
	American Beech	4	270.38 <sup>a,b</sup> (1.56)	396.48 (3.80)	3.46 (0.76)	5.08 (1.13)	0.46 (0.23)
	Trembling Aspen	2	267.84 <sup>a,b,c</sup> (4.92)	394.75 (3.32)	2.23 (0.80)	3.28 (1.14)	0.17 (0.11)
Advanced rot	White Birch	2	272.46 <sup>a</sup> (1.53)	396.09 (2.60)	2.71 (0.74)	3.95 (1.13)	0.27 (0.15)
	American Beech	2	260.41 <sup>b,c</sup> (2.56)	397.5 (1.45)	1.70 (0.33)	2.59 (0.49)	0.12 (0.04)
	Trembling Aspen	2	260.08 <sup>c</sup> (6.71)	392.24 (3.29)	1.95 (0.07)	2.95 (0.15)	0.13 (0.003)

$T_i$  = ignition temperature;  $T_e$  = burnout temperature;  $D_i$  = ignition index;  $S$  = combustion characteristic index;  $C$  = flammability index. For  $T_i$ , different superscript letters indicate significant differences between interaction effects, according to Tukey's multiple comparisons of means test, with a 95% family-wise confidence interval. Note that means that are not significantly different are not to be judged to be the same.

On the other hand, the combustion characteristic parameters were all significantly, and negatively, related to the % of wood area affected by rot (Figure 8). The amount of variability explained by these relationships (i.e., R-squared) was however low, ranging from 0.193 to 0.295. Moreover, a higher lignin content significantly increased (i.e., improved) the characteristic combustion index, which is an indicator of good fuel combustion. The linear relationships between the relative abundance of white-rot fungi compared to all saprotrophs and the thermochemical indexes were all nonsignificant.



**Figure 8.** Variation of combustion characteristic parameters with proportion of rot, lignin content and relative abundance of white-rot fungi compared to all saprotrophs.

#### 4. Discussion

We found evidence that decaying processes, as expressed by the proportion of internal wood area affected by rot, can significantly influence physical and chemical properties of forest biomass related to their bioenergy conversion performance. On the other hand, the visual indicator of tree degradation based on Hunter's decomposition class [23] was not appropriate to infer relationships with most physical properties and performance indexes for bioenergy conversion. The rationale of this indicator was to assess whether a visual index relating to tree suitability for bioenergy conversion could be developed and then used in uncut stands during forest inventories and tree marking. Although Hunter's decay classification, assessed during forest inventories, has been proven to be useful for predicting tree lumber volume [44], the decay categories used in our study (adapted from Hunter's classes) appear to be too imprecise to adequately capture the relevant wood physical and chemical properties for bioenergy. A larger number of replicates covering a larger gradient of Hunter's classes could have helped to provide a better assessment of external indicators of tree degradation. For its part, the proportion of wood area affected by rot can be assessed during harvesting; while operators handle a freshly cut stem with the processing head of a harvester, they already routinely assess wood rot to determine suitability for conventional wood products.

Moisture content can easily be manipulated with simple conditioning of biomass [25]. Still, our results showed that hardwoods in an advanced stage of decay have a lower moisture content, which is generally an advantage for thermochemical conversion technologies. For example, combustion cannot be carried out on feedstock with over 50% of moisture content [6]. The fast pyrolysis process requires a maximum moisture content of 10% to be efficient [45]. The use of decaying trees with naturally lower moisture content is, therefore, a non-negligible advantage in comparison with live trees, because it makes it possible to reduce or eliminate the drying stage.

The basic density of decaying hardwoods, averaging  $479 \text{ kg/m}^3$ , is slightly lower than the typical value from live stem wood of broadleaved species, which has an average of  $54 \text{ kg/m}^3$  [46]. A higher basic density means less volume for the same mass of feedstock. It is advantageous for the different steps of handling and transportation to have a material that is as dense as possible. In that way, decaying trees might incur higher supply costs than regular wood feedstocks due to their lower density.

For their part, feedstock relative contents of lignin and carbohydrates are characteristics of interest for biochemical conversion technologies, and they cannot be easily manipulated with conditioning. They were both influenced by the proportion of wood rot, which tended to decrease carbohydrate content and increase that of lignin. However, contrary to our hypothesis based on the literature [47,48], there was no significant specific influence of white-rot fungi on biomass characteristics and conversion performance.

In our experiment, the average value for the relative lignin content of hardwoods in the dead and advanced rot stages of decay was 15.4%. This is still lower than the average value of 20.8% observed by Elbersen, et al. [46] in broadleaved species. According to the indicators established by the S2Biom project, the proportion of lignin that we measured in this study was within the “Standard” range of suitability for biochemical conversion. Moreover, the carbohydrate content of our decaying hardwoods tended to be lowered by the higher proportion of rot. Still, the average carbohydrate content of our decaying wood samples can be classified as “Highly desirable” [28] for biochemical conversion and is higher than the average value generally observed in live wood [46].

The ash content was significantly increased by decay, perhaps due to the degradation of other wood components by fungi, causing a relative increase of mineral elements. Higher ash content is less profitable for both biochemical and thermochemical conversion because it cannot be converted to energy; feedstock with high ash content also tends to foul reactors, and energy has to be consumed to dispose of them. However, in comparison with agricultural biomass, forest biomass typically has a low ash content. Different parts of the tree have different ash contents. It has been reported that the ash content in the bark was higher in comparison to stem wood, i.e., 1.6–2.8% vs. 0.3–1.0%, respectively (Annevelink et al. 2016). Since decaying trees tend to lose their bark, it might be beneficial for high-value bioenergy production [49]. Nevertheless, the ash contents that we measured still fall in the range of the “Ideal” and “Desirable” categories, for both thermochemical and biochemical conversion, according to Elbersen, et al. [28].

Ash agglomeration is a potential problem that could lead to slagging and fouling in thermal conversion reactors. The higher the K concentration, the higher is the risk of slagging and fouling. The K concentration that we measured was not influenced by the level of decay; hence there is no evidence that using decaying trees would increase the risk of fouling. Nevertheless, other studies have observed variations in K with increased wood decay, for example a decrease in concentration between living and dead standing trees [50], and various patterns with increasing decay of debris in contact with soil [51]. The high solubility of K relative to other ash-forming elements of wood, such as Ca and Mg, might explain the difference of patterns we observed between total ash and K [51].

Knoll, et al. [17] observed that decayed aspen had a calorific value that was 40% higher than sound aspen. However, they attributed this variation to the extractives (wood degradation products) and not to the lignin content. On the other hand, Nguyen, et al. [52] did not detect any significant differences in HHV between sugar maple and yellow birch trees of different vigor status (including dying trees). Our results showed that the variations of the calorific value of hardwoods at different decay levels are maintained in a narrow interval, which is within the interval observed in live wood by Elbersen, et al. [46].

The restrictive impact of high lignin content on enzymatic digestibility is well known and documented [7,53,54]. In our study, the low number of observations for biochemical conversion tests ( $n = 15$ ) somehow restricts the inference that can be made from the data. Yet, the exploratory results that we produced do not confirm our hypothesis of an increase of digestibility with the decrease of lignin and with an increase of the relative abundance

of white-rot fungi, although there was a slight trend that will have to be validated by further observation. Nevertheless, the proportion of wood affected by rot (including all saprotrophs) was significantly and positively related with digestibility (despite the positive effect of rot on lignin, and its negative effect on carbohydrates). Our results are aligned with previous research that showed that the natural wood degradation process has the potential to reduce recalcitrance and enhance enzymatic digestibility. Knoll, et al. [17] discovered that the disrupted structure of decaying aspen wood would allow enzymatic hydrolysis of cellulose and hemicelluloses to yield a higher percentage of sugars, without pretreatment. Similarly, the subsequent fungal degradation that followed mountain pine beetle (*Dendroctonus ponderosae*) attack on lodgepole pine (*Pinus contorta* Douglas ex Loudon) resulted in a higher enzymatic digestibility and ethanol yield than sound wood, after sulfite or organosolv process pretreatment [18,19]. Although we cannot confirm the specific role of white-rot fungi, other saprotrophs may play a role in facilitating the biochemical conversion of wood into energy.

The conversion test that we carried out in this study was more adapted for combustion conversion technologies since it was performed in an air atmosphere. We observed that the ignition temperature of decaying hardwoods would vary from 260 to 274 °C. The values of ignition temperature for hardwoods are typically between 250 and 310 °C [55], and 235 °C for poplar wood [56]. We found out that more advanced stages of decay in American beech and trembling aspen were associated with lower ignition temperatures. When the fuel has a lower ignition temperature, less energy has to be supplied by an external source before the activation energy needed for combustion is attained. On the other hand, based on the tested combustion parameters, we could see that increased wood rot negatively influenced overall feedstock reactivity to combustion, despite its positive effect on lignin content. Rot might thus negatively influence wood thermal properties through another mechanism that was not captured in our study.

One of the limits of our study was the small number of observations available for each different analysis that we carried out, which restricts the conclusions that can be drawn from our experience. The unbalanced experimental design that we used might have altered the precision of our estimations and is the major limitation of this study. Another limit is the chosen method to identify the presence of white-rot fungi. Even though a DNA analysis makes it possible to identify most taxa present in wood, we had access to the relative abundance of each taxon, such as those associated with white-rot fungi, and not their quantitative abundance. Quantitative PCR analyses would allow further exploration of the exact role of white-rot fungi. In the absence of DNA analysis, other techniques might be used, such as visual identification of species in laboratory cultures, or identification of white filaments characterizing white-rot fungi on the disc samples. A sampling method of decaying trees that considers visual identification of sporophores from white-rot would also ensure the selection of more trees with a higher proportion of these specific fungi. There is also the possibility that white-rot fungi are not the primary organism type that drives biomass conversion efficiency. Other types of fungi might also have an influence on digestibility yield and combustion performance parameters.

## 5. Conclusions

Our study gives an overview of the decay process of hardwoods in the eastern Canadian context, and its impact on bioenergy conversion. It explored the effect of decay processes on the bioenergy conversion of hardwoods, by analyzing the impact on physical and chemical properties on the biochemical and thermochemical responses of decaying biomass. We found out that biochemical conversion can be improved by a higher proportion of wood rot. Nonetheless, natural wood decay, even by white-rot organisms, is apparently not sufficient to act as a biomass pretreatment, as enzymatic hydrolysis of decaying hardwoods yielded overall poor digestibility. Results however showed that indicators of combustion performance drastically dropped with the advance of decay. Our study also suggests that tree decay, as visually assessed with Hunter classification on standing trees

during forest inventories, is not precise enough to serve as an indicator of wood suitability for bioenergy conversion. However, the proportion of wood area affected by rot, which can be assessed on cut logs, can be a relevant index for bioenergy conversion potential. As such, this index could be used during log sorting at the roadside or in a mill yard to identify adequate feedstocks for conventional wood products (lumber, pulp) and bioenergy.

**Supplementary Materials:** All data are available online at <https://www.mdpi.com/1996-1073/14/1/93/s1>.

**Author Contributions:** Conceptualization, É.D. and E.T.; formal analysis, É.D.; funding acquisition, E.T.; methodology, É.D., J.B., K.A. and C.M.; project administration, E.T.; writing—original draft, É.D.; writing—review and editing, E.T., J.B., K.A. and C.M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was funded by Fonds de recherche du Québec—Nature et Technologies (New researcher start-up grant to E. Thiffault; 2018-NC-204627) and the Natural Science and Engineering Research Council of Canada (Discovery grant to E. Thiffault, RGPIN-2018-05755).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** All data from this study are available in the Supplementary Materials.

**Acknowledgments:** Authors wish to acknowledge the technical support of the Research Centre on Renewable Materials, the Canadian Forest Service and the Institut National de la Recherche Scientifique du Québec. Warm thanks to Karelle Rheault, Mathieu Béland, Yves Bédard, Alain Brousseau and Paul Desaulniers for their help in the lab and in the field.

**Conflicts of Interest:** The authors declare no conflict of interest.

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