forests

MDPI

Article

Establishment of a Laboratory Scale Set-Up with Controlled Temperature and High Humidity to Investigate Dry Matter Losses of Wood Chips from Poplar during Storage

Albert Hernandez-Estrada [1], Ralf Pecenka [1,*], Sabrina Dumfort [2], Judith Ascher-Jenull [3], Hannes Lenz [1], Christine Idler [1] and Thomas Hoffmann [1]

[1] Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Max-Eyth-Allee 100, 14469 Potsdam, Germany; ahernandez@atb-potsdam.de (A.H.-E.); hlenz@atb-potsdam.de (H.L.); cidler@web.de (C.I.); thoffmann@atb-potsdam.de (T.H.)
[2] Department for Environmental, Process and Energy Engineering, Management Center Innsbruck, Maximilianstrasse 2, 6020 Innsbruck, Austria; sabrina.dumfort@mci.edu
[3] Department of Microbiology, University of Innsbruck, Technikerstrasse 25d, 6020 Innsbruck, Austria; judith.ascher@uibk.ac.at
* Correspondence: rpecenka@atb-potsdam.de; Tel.: +49-(0)331-5699-312

Abstract: The aim of this work was to improve the understanding of dry matter losses (DML) that occur in wood chips during the initial phase of storage in outdoor piles. For this purpose, a laboratory scale storage chamber was developed and investigated regarding its ability to recreate the conditions that chips undergo during the initial phase of outdoor storage. Three trials with poplar Max-4 (*Populus maximowiczii* Henry × *Populus nigra* L.) chips were performed for 6–10 weeks in the storage chamber under controlled temperature and assisted humidity. Two different set-ups were investigated to maintain a high relative humidity (RH) inside the storage chamber; one using water containers, and one assisted with a humidifier. Moisture content (MC) and DML of the chips were measured at different storage times to evaluate their storage behaviour in the chamber. Additionally, microbiological analyses of the culturable fraction of saproxylic microbiota were performed, with a focus on mesophilic fungi, but discriminating also xerophilic fungi, and mesophilic bacteria, with focus on actinobacteria, in two trials, to gain a view on the poplar wood chip-inhabiting microorganisms as a function of storage conditions (moisture, temperature) and time. Results show that DML up to 8.8–13.7% occurred in the chips within 6–10 storage weeks. The maximum DML were reached in the trial using the humidifier, which seemed a suitable technique to keep a high RH in the testing chamber, and thus, to analyse the wood chips in conditions comparable to those in outdoor piles during the initial storage phase.

Keywords: dry matter losses; short rotation coppices; poplar wood chips; laboratory scale; cultivable saproxylic microbiota

Citation: Hernandez-Estrada, A.; Pecenka, R.; Dumfort, S.; Ascher-Jenull, J.; Lenz, H.; Idler, C.; Hoffmann, T. Establishment of a Laboratory Scale Set-Up with Controlled Temperature and High Humidity to Investigate Dry Matter Losses of Wood Chips from Poplar during Storage. *Forests* **2022**, *13*, 459. https://doi.org/10.3390/f13030459

Academic Editor: Blas Mola-Yudego

Received: 26 January 2022
Accepted: 12 March 2022
Published: 15 March 2022

1. Introduction

Woody biomass from forestry and agriculture plays an important role in bioenergy technology to fulfill the targets for the global reduction of greenhouse gas emissions [1–3]. As such, to meet the growing demand for woody biomass, an increase of the production of fast-growing trees such as poplar (*Populus* spp. L.), willow (*Salix* spp. L.) and black locust (*Robinia pseudoacacia* L.) in plantations of short-rotation coppices (SRC) or agroforestry systems (AFS) is an important option [4]. A prerequisite for increasing wood production in agriculture, however, is the availability of suitable technologies [5–8] and competitiveness compared to other energy-supplying plants and traditional field crops [9,10]. Key problems interfering with the expansion of SRC cultivation are the high dry matter losses (DML) and quality losses during the storage of wood chips [11–13]. Wood chips from SRC are

harvested in winter with moisture contents (MC) of 50–60% (wet-basis MC; poplar chips). Generally, the wood chips have to be stored and dried for six to nine months until the next heating season starts [14,15]. In order to guarantee low costs and easy handling, wood chips are typically stored to dry outdoors in large piles, and consequently, the wet chips are subjected to microbial attack [16–19]. Depending on the material properties of the wood chips, the design and the location of the storage piles, DML of up to 47% may occur [17,20–25].

1.1. DML and Temperature in Wood Chip Storage Piles

Due to the harvesting of SRC in winter, in some regions like central or northern Europe, the ambient temperature is below 0 °C for some weeks, and therefore the temperature in the storage pile stays, as well, below 0 °C [17,21,26]. This first storage phase is called 'frost phase' in the three-phase model by Idler et al. [19] (Figure 1; phase 0), and it lacks the microbial activity that contributes to DML of the wood chips [17,21,26].

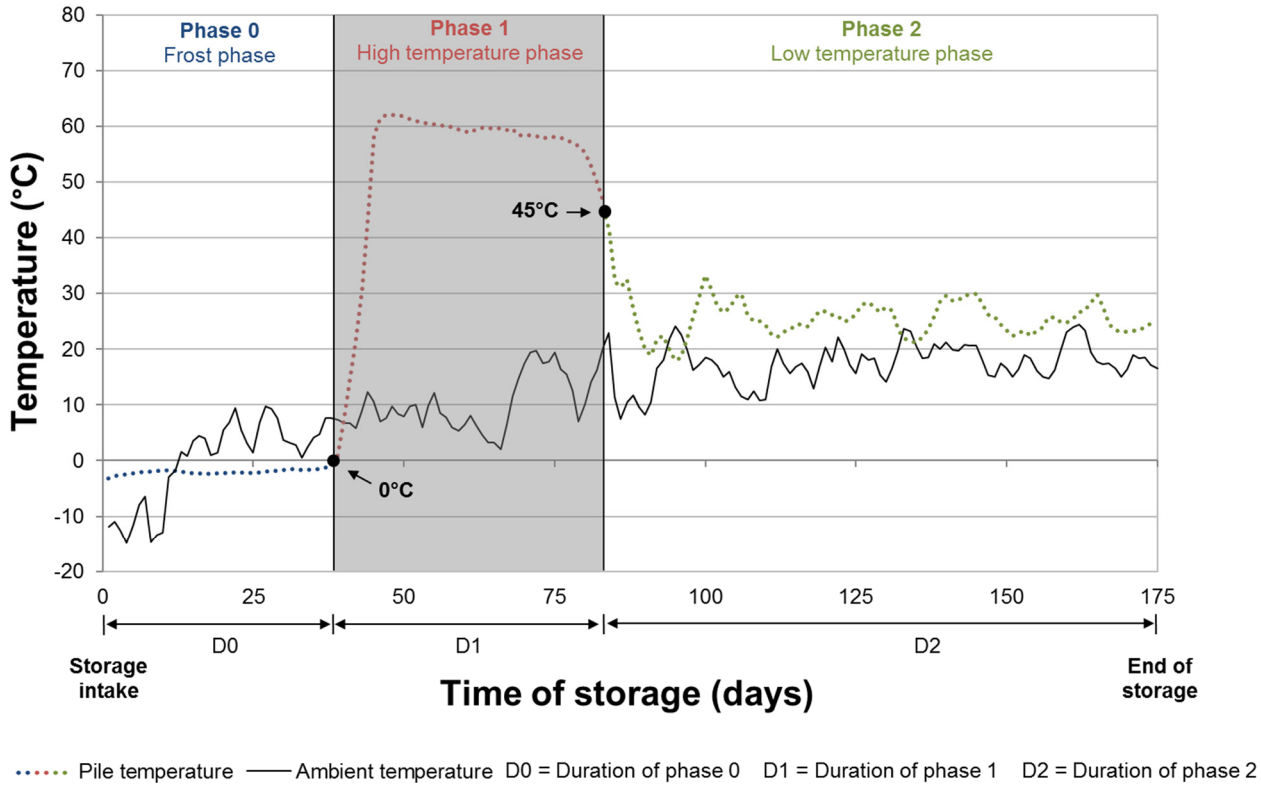


Figure 1. Typical temperature phases of a storage pile of poplar chips harvested in winter, set outdoors in Central Europe. (Modified from Idler et al. [19]).

With the rise of ambient temperature above 0 °C, the temperature inside the wood chips storage pile also increases as a consequence of the occurring microbial activity [16,17,27,28]. This second storage phase (Figure 1; phase 1) is called 'high temperature phase' in Idler et al. [19], and it is characterized by a fast rise of the pile temperature up to 50–60 °C [13,16–18]. This rise of temperature inside the storage pile is the result of both chemical and microbial exothermic reactions that occur in the wood chips when exposed outdoors. However, the temperature progression may differ depending on, e.g., tree variety, chip size and pile set-up [13,16,17,29]. The third storage phase proposed by Idler et al. [19] starts when the pile temperature decreased below 45 °C (Figure 1; phase 2). This 'low temperature phase' was characterized by a progressive decrease of the pile temperature, that gradually approached the ambient temperature, in line with the observations by, e.g., Barontini et al. [13], Manzone et al. [12], and Whittaker et al. [18].

For example, in storage piles of wood chips from eucalyptus with small size, i.e., P16, the high temperature phase can last for five months [29]. On the other hand, storage piles made with larger chips from poplar, i.e., P63 and P45, showed lower increases above ambient temperature in the phase 1 (Figure 1) that lasted for less than two months [17,30]. Similarly, when piles were set with wood chips produced from different parts of the trees (e.g., stem and crown), the pile temperature differed between the storage piles according to the different tree fractions [13]. However, even if this high temperature phase (Figure 1; phase 1) did not last very long, it implied DML of 7–10% for poplar chips stored outdoors [17,30,31]. For example, in the outdoor storage pile of P31 poplar chips done by Pecenka et al. [17], the high temperature phase lasted approximately for nine weeks. In this period, sampling was conducted after five and nine weeks from the storage intake, in order to assess DML at the respective storage times, revealing 5.6% and 9.4% DML, respectively [17].

DML of wood chips stored outdoors in piles can account for up to 47% [17,21–25,29], although there is a large variability, as reviewed, e.g., in Whittaker et al. [22] and Therasme et al. [25]. This variability may depend, among many other parameters, on the size, location and the set-up of the piles [27,29,32,33], and the chip size and the wood properties determined by, e.g., variety and tree age [13,17,18,24,29]. Furthermore, different methods used to determine DML [13,24,31,34] may show different DML values. In this regard, Whittaker et al. [22] provided a comprehensive review of the different parameters used in storage of wood chips and corresponding DML. Likewise, Dimitriou and Rutz [14] and Veste and Böhm [15] offered comprehensive information about SRC and AFS in practice.

1.2. Microorganisms and Degradation of Wood Chips

Wood-inhabiting microorganisms involved in the complex and dynamic process of wood decomposition are bacteria, archaea [35–39], and fungi [36,40,41]. These saproxylic microbiota possess a variety of hydrolytic and oxidative enzymes to degrade the main wood components cellulose, hemicellulose and lignin. The primary degradation of wood is carried out by different fungi, due to their diverse set of enzymes, as well as their ability to colonize wood with the use of hyphae [42]. The primary wood decomposers can be classified into white-(degradation of mainly lignin), soft-(degradation of mainly cellulose) and brown-rot (degradation of mainly cellulose) fungi. Even though bacteria are able to decompose wood, their contribution to wood degradation is negligible with respect to the fungal one [36,41,42], as reflected by so far much less scientific attention. Despite this, there is growing interest on and recent evidence about the role of bacteria within the wood-decomposition and complex bacteria–fungi interactions governing the decomposition of dead-wood [43–45]. Bacteria thrive on nutrients available in the wood cells [35,41] and are able to colonize wood in conditions with low availability of oxygen, or even in the absence of oxygen (anaerobiosis) [46], which are inhibitory to most fungi [35,47]. Saproxylic bacteria can be classified due to their functional role in wood decomposition into (i) bacteria that increase the water permeability of wood without affecting its structural integrity; (ii) bacteria with (albeit limited) decomposition ability; (iii) bacteria that stimulate fungal decomposition; and (iv) bacteria that inhibit fungal decomposition [35,43].

Among the most abundant and dominant bacterial phyla generally involved in the complex and dynamic decomposition of dead-wood are the Acidobacteria, Alphaproteobacteria, Gammaproteobacteria, Actinobacteria, and Bacteroidetes [43,44]. The abundance of such microorganisms generally increases with each progressing stage of decomposition. The dynamic microbial succession is due to changing physico-chemical properties during dead-wood decay, with nitrogen content and pH among the master-variables shaping the microbial community in terms of composition and abundance [38,45,48,49]. Actinobacteria (Actinomycetes), commonly found in soil and in wood in contact with soil [41], form mycelia-like filaments [41,42]. This explains—also from a morphologic point of view—their role as wood decomposers, similar to fungi, already in the early stages of decomposition [42]. On the other hand, most of deadwood-decaying bacteria, including Archaea,

thrive on fungal metabolites, thus occurring mainly at later stages of wood decomposition [38]. As already mentioned, among the main driving factors governing the composition and activity of microbial communities in a wood chip storage pile is the chip size [19,21,30]. For example, the work done by Idler et al. [19] in storage piles of 500–1000 m^3 of P31 and P45 poplar chips revealed that mesophilic fungi in small chips (P31) developed in higher concentration when the pile temperature was relatively low, i.e., between 20 °C and 30 °C, and the chips' MC was below 47%. On the other hand, in larger poplar chips (P45), mesophilic fungi developed in its maximum at 19–23 °C and 28–33% MC [19]. Moreover, the maximum content of mesophilic fungi was clearly lower in the chips with the larger size P45 than in the small P31 chips (5.8 $_{lg}$CFU g^{-1} and 6.9 $_{lg}$CFU g^{-1}, respectively; Idler et al. [19]). Furthermore, thermophilic fungi are also present in storage piles of wood chips, as it was reported in, e.g., Idler et al. [19] and Pecenka et al. [30]. In fact, considering the elevated temperatures occurring in outdoor piles during storage, thermotolerant and thermophilic wood-degrading microorganisms play a key role [48,50].

1.3. Aim of This Work

As mentioned before, DML depend on multiple factors such as weather conditions, pile size and wood variety. At ambient temperatures above 0 °C, the fast rise in temperature occurring in wood chip piles and lasting up to 60–90 days is characteristic for wood chips from SRC stored and naturally dried in outdoor piles (high temperature phase; Figure 1). This high temperature phase is of particular interest, because approximately 50% of the total storage losses occur during this relatively short storage time. To what extent this temperature rise caused by microbiological and chemical processes leads to DML is not very well known. Detailed measurements based on frequent sampling at a practice scale are very labour intensive and potentially lead to disturbances of the microbiological and chemical processes in the pile. Furthermore, due to the annually changing weather conditions, it is hardly possible to repeat a storage test under the very same conditions. Therefore, this work aimed at investigating the possibilities and limitations of setting up laboratory-scale trials, in order to provide a better understanding of the DML of poplar chips in outdoor storages.

This work focused on the high temperature storage phase (Figure 1; phase 1), and the trials were set in a storage chamber with controlled temperature and assisted air humidity. The storage conditions in the chamber were chosen based on previous measurements in large-scale outdoor storage trials of poplar chips in central Europe (Figure 1). The aim was to recreate the conditions that wood chips in the inner layers of the pile experience in the high temperature phase when stored in such outdoor piles. In addition to the measurement of DML and chips' MC, microbiological analyses were performed. These analyses aimed to assess the culturable fraction of the autochthonous saproxylic microorganisms present in the poplar chips at storage intake and during the storage, as a function of storage conditions (moisture, temperature) and time. The focus was on xerophilic and mesophilic fungi, and (actino)bacteria. Xerophilic fungi were of particular interest due to the drying of the chips during storage.

2. Materials and Methods

2.1. Raw Material and Chip Preparation

The trees used in this work were poplar Max-4 (*Populus maximowiczii* Henry × *Populus nigra* L.) from a SRC plantation established in March 2016 at the Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB) in Potsdam, Germany (52.49° N, 13.01° E). Trees with a stem diameter at the cutting height of 7–10 cm were selected for each trial and were harvested manually at the times corresponding to each of the trials T1–T3. Table 1 shows the harvesting date and storage times of each trial, as well as the corresponding samplings and sampling frequencies.

Table 1. Storage trials T1–T3 and the corresponding samplings.

Trial No.	Harvest and Storage Intake (Date)	Storage (Weeks)	Samplings		
			MC and DML	Microbiological and pH Analyses	Ash and Elemental Analysis
T1	14 June 2018	6	Storage intake (n = 6; only MC) and after 1, 2, 3, 4, 5, and 6 storage weeks (n = 3; sample bag method) Chips	Storage intake (n = 2) and after 6 storage weeks (n = 3) Chips and wood and bark fractions	Storage intake (n = 2) Chips
T2	20 August 2018	10	Storage intake (n = 6; only MC) and after 2, 4, 6, 8, and 10 storage weeks (n = 3; sample bag method) Chips	Storage intake (n = 2) and after 2, 4, 6, 8 and 10 * storage weeks (n = 3) Chips and wood and bark fractions	Storage intake (n = 2) Chips
T3	16 July 2019	9	Storage intake (n = 6; only MC) and after 3, 6, and 9 storage weeks (n = 3; sample bag method) Chips	Not performed	Storage intake (n = 2) and after 3, 6, and 9 storage weeks (n = 3) Chips

* Microbiological analysis not performed at storage week 10, only pH analysis; MC: moisture content (wet-basis); DML: dry matter loss.

Before chipping the trees, the leaves were removed to avoid any potential effect on DML of the wood chips during the storage. The chips were produced with a stationary woodchipper HE100 500 STA (JENZ GmbH, Petershagen, Germany) equipped with an automatic feeding chain conveyor, which provided a constant feeding rate into the chipping drum. Pecenka et al. [43] offered a detailed overview of the woodchipper; however, the screen used in the outtake by Pecenka et al. [51] was not used in producing the chips of this work. The produced chips were analysed regarding particle size distribution using the oscillating screen method (standard EN ISO 17827-1:2016 [52]) and classified according to the standard EN ISO 17225-1:2014 [53].

The initial MC of the fresh wood chips (MC_{in}) was determined as the mean value of six samples of 250–450 g using a Sartorius TE3102S balance (Göttingen, Germany) with the oven dry method at 105 °C based on EN ISO 18134-2:2017 [54]. The chips undergoing storage used the method based on sample bags, which is often used to calculate DML of wood chips in large outdoor storage trials (e.g., [26,27,30]). Sample bags were prepared right after the chipping process, each one containing about 2 kg of wood chips, and the mass of each sample bag was measured individually (m_{in}; excluding the mass of the net bag) using a Sartorius BP 12000-S balance (Göttingen, Germany).

2.2. Testing Set-Up and Sampling for the DML Calculations

The storage trials were done in a programmable incubation chamber Binder KB-400 (Binder, Tuttlingen, Germany) providing a storage volume of 400 L. The set-ups used are shown schematically in Figure 2. When using such a closed chamber for storage investigations at lab-scale, the conditions that prevail inside a storage pile in the first few weeks after harvest in winter at practice scale should be reproduced as close as possible. This can avoid some of the important known problems of outdoor storage trials with wood chips at a practice scale, such as the large heterogeneity of samples received from a large storage pile or the very labour-intensive sampling. Accordingly, before starting the storage trials, the chamber was pre-conditioned at 5 °C, to start the trials at similar conditions to outdoor storage trials when harvesting in winter. At these conditions, the prepared sample bags were placed in the storage chamber, distributing the three sample bags corresponding

to each sampling time in different shelves, and left overnight (about 15–18 h) to condition the poplar chips at 5 °C.

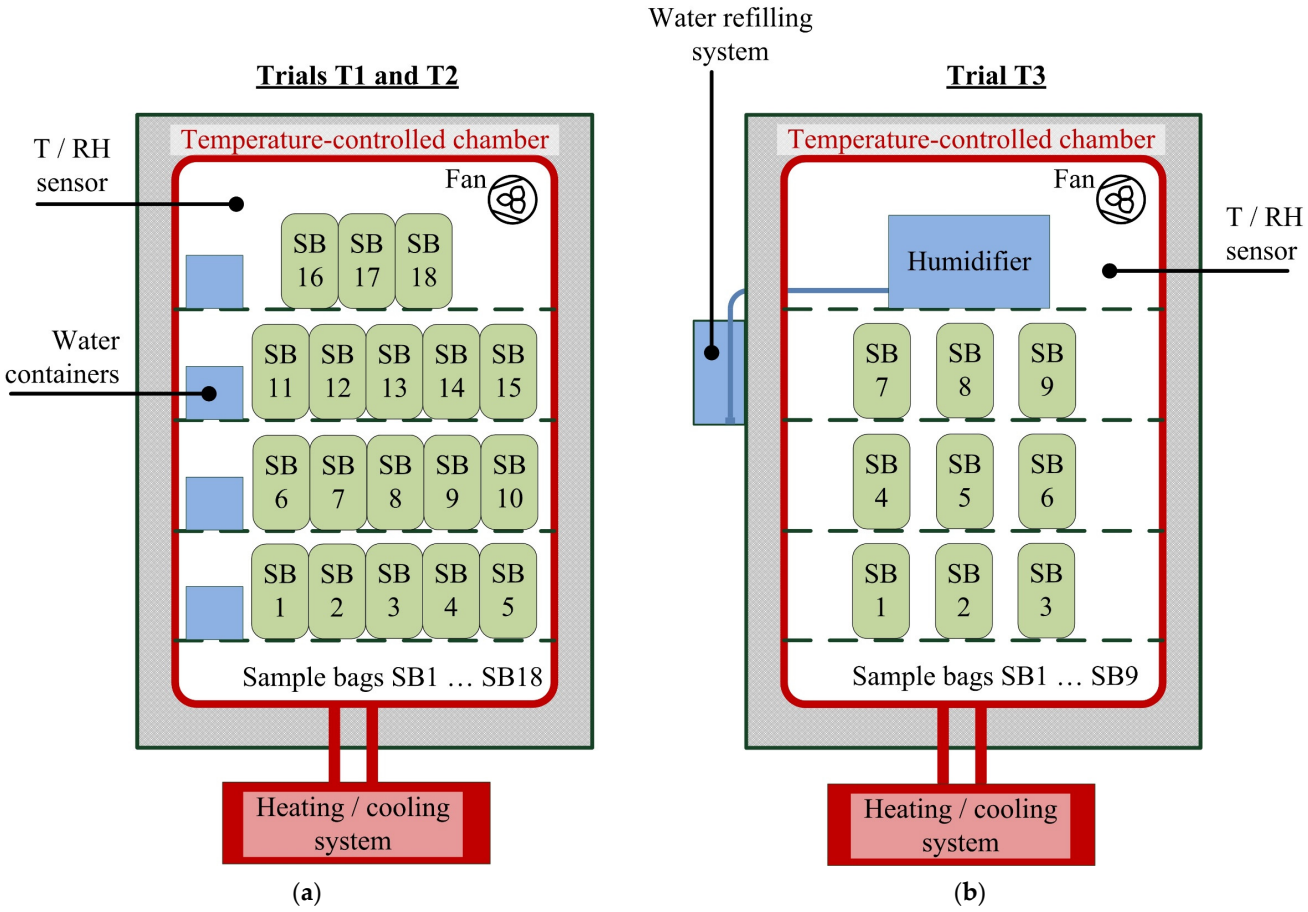


Figure 2. Storage of sample bags in a chamber under controlled temperature and assisted humidity. (**a**) Set-up used in trials T1 and T2 with water containers placed in the chamber, and (**b**) set-up using a humidifier in trial T3. T: temperature; RH: relative humidity; SB: sample bag.

The programmed storage plan started by progressively rising the chamber temperature from 5 °C to 55 °C during the first 48 h. Afterwards, the chamber temperature was kept constant at 55 °C for the rest of the storage experiment, i.e., six weeks (Trial T1 in Table 1), nine weeks (Trial T2 in Table 1) or ten weeks (Trial T3 in Table 1). The internal fan of the incubation chamber was set at the minimum speed of the chamber control, which was 620 rpm (measured with a hand-held tachometer, model 6611, from Veeder-Root GmbH, Neuhausen, Germany). This configuration allowed minimized air circulation inside the chamber and, on the other hand, contributed to having comparable storage conditions for all sample bags in the chamber.

Two storage set-ups were evaluated in order to assist in having a high humidity atmosphere inside the chamber, comparable to the moisture conditions in outdoor piles. In the trials T1 and T2 (Table 1), four containers with distilled water placed inside the chamber were used (Figure 2a). In the alternative set-up, trial T3, we used an in-house developed humidifier (Figure 2b). This humidifier was actively transferring moisture to the chamber atmosphere, and was equipped with a water refilling system to assure a sufficient water supply (Figure 3). An Almemo ZA 9020-FS Thermo R2E4 sensor was used in the trials to measure the temperature inside the storage chamber, and the relative humidity (RH) was measured with Almemo FHAD36RAS (trials T1 and T2) and Almemo FH0D46C sensors (trial T3), together with an Almemo 3290-8 datalogger. The sensors and the datalogger were manufactured by Ahlborn Mess-und Regelungstechnik (Holzkirchen, Germany).

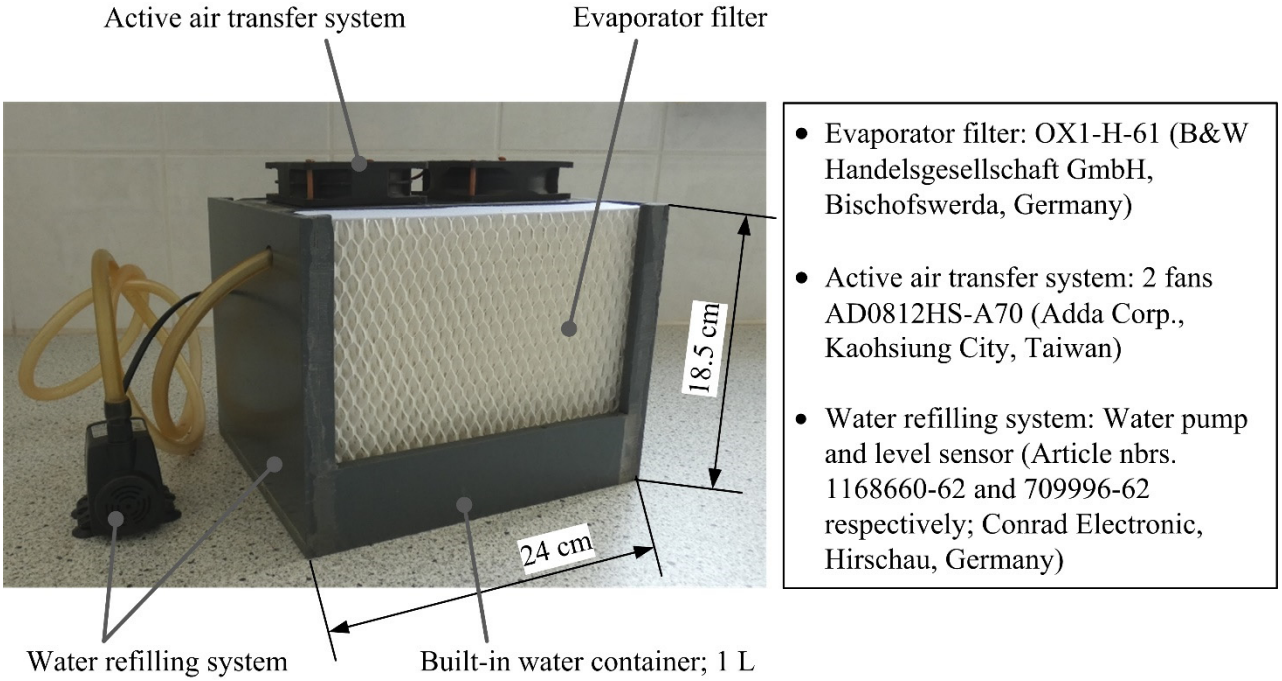


Figure 3. Humidifier used in the set-up of trial T3 and details of the components.

The method to calculate DML was done according to the Equation (1), with the MC and the masses of the sample bags at the storage intake and at the sampling time (after storage). The mass of the net bag was subtracted from the mass measurements, and the calculations were done only considering the mass of the chips contained in each sample bag. At each sampling time (see Table 1), the corresponding sample bags were unloaded from the chamber and the mass of each sample bag was measured ($m_{out,i}$; excluding the mass of the net bag). Right after opening the sample bags and before any further manipulation, the sampling for the microbiological and pH analyses was done (see Section 2.3). With the remaining material, the MC of the chips ($MC_{out,i}$ in the Equation (1)) was determined for each sample bag and expressed as the mean value (n = 4), using the oven dry method at 105 °C.

$$DML_i = \left[1 - \frac{m_{out,i}\,(100 - MC_{out,i})}{m_{in}(100 - MC_{in})}\right] \times 100 \quad (1)$$

where
DML_i, dry matter loss of the chips in the sample bag at the storage time i [%];
m_{in}, wet mass of the chips at the storage intake (excluding the net bag) [kg];
$m_{out,i}$, wet mass of the chips after storage at the time i (excluding the net bag) [kg];
MC_{in}, MC (wet-basis) of the chips at the storage intake [%]; and
$MC_{out,i}$, MC (wet-basis) of the chips in the sample bag after storage, at the time i [%].

2.3. Microbiological and pH Analyses

The sampling for the microbiological and pH analyses was done in the trials T1 and T2. For these analyses, not only the wood chips, but also wood and bark fractions of the same sample bags were investigated, due to the different nature and chemical composition of both materials. Therefore, herein, the terms 'wood chips' or 'chips' refer to the entire material as obtained after the chipping process (Section 2.1), i.e., containing wood and bark. On the other hand, the term 'wood' refers to the wood fraction of the chips, i.e., resulting after removing the bark from the chips; likewise, the term 'bark' refers to the bark fraction prepared from the chips after removing the woody parts, and so containing only bark.

The sampling at the storage intake was done preparing two sets (n = 2) of the analysed samples (i.e., chips, wood, and bark; Table 1) and the mean is reported herein. The sampling

of the bags undergoing storage was done right after opening the sample bags and before any further manipulation of the chips; from each sample bag, the sampling of the chips, wood and bark was prepared (Table 1) separately. Each sampling time included three sample bags (n = 3; Table 1), in order to report the means for the chips, and the fractions of wood and bark, separately (Table 1). The chips, wood and bark pieces were cut manually into smaller pieces (maximum size of 1 cm × 1 cm × 1 cm), avoiding any contamination.

Microbiological analysis consisted of dilution plating to assess the culturable fraction of the wood chip-inhabiting microbiota (saproxylic microbiota) as a function of storage conditions (temperature, moisture) and time. We used 20 g each of the cut chips, wood and bark, which were analysed separately. The samples were transferred to Erlenmeyer flasks containing 180 mL of sterile Ringer's solution (Merck, Darmstadt, Germany) and shaken at room temperature for 30 min in an orbital shaker TR-125 (Infors AG, Bottmingen, Switzerland) at 180 rpm. Afterwards, a 1:10 dilution series was prepared in Ringer's solution and, from the dilution series, aliquots of 100 µL were plated on the respective nutrient agars.

The wood chip samples were screened for their saproxylic microbiota discriminating fungi (mesophilic; xerophilic) and bacteria. Mesophilic fungi were isolated using selective malt extract agar (MEA; Merck, Darmstadt, Germany) containing 0.01% chloramphenicol (Fluka, Ulm, Germany), and incubated aerobically for seven days, at 25 °C in dark conditions. Dicloran glycerol agar (DG18; Oxoid, Hampshire, UK), a low water activity medium [55], was used for isolation and enumeration (CFUs) of xerophilic fungi under the conditions described before. The agar used to investigate mesophilic bacteria was plate count agar (PCA; Merck, Darmstadt, Germany), and the plates were incubated aerobically at 25 °C in dark conditions for four days. Under the same conditions, the presence of actinobacteria was investigated using casein-soja-pepton-agar (CASO; Merck, Darmstadt, Germany).

After the incubation period, all colonies were counted as colony forming units (CFU) per gram of sample (CFU g^{-1}; wet basis) and expressed as logarithmic values to base 10 ($_{lg}$CFU g^{-1}). Plates prepared for mesophilic and xerophilic fungi with fewer than 10 colonies or exceeding 150 colonies per plate were excluded from the calculations. Likewise, for mesophilic bacteria and actinobacteria, the plates containing less than 10 or more than 300 CFUs were not considered for the quantitative assessment. The detection limit was 100 CFU g^{-1}, and in case the plate of the first dilution did not present any colony of the target microorganism, the value of 99 CFU g^{-1} (i.e., 2 $_{lg}$CFU g^{-1}) was assigned. This is the minimum content of microorganisms that is possible to detect in a sample according to our methods applied for the sample preparation [19].

The pH analysis used 10 g of the cut materials mentioned above, i.e., chips, wood and bark, and the analysis was done separately for each prepared sample (see Table 1) according to DIN 38404-5:2009 [56]. The material was placed in an Erlenmeyer flask containing 100 mL of distilled water, and the mixture (1:10 wt/v) was shaken at 180 rpm for 15 min at room temperature in the orbital shaker mentioned before. Afterwards, the pH was measured with a WTW 3210 pH meter (Xylem Analytics, Weilheim, Germany).

2.4. Elemental and Ash Analyses

Ash and elemental analyses were performed to have a basic information about the material properties. Chips used previously to calculate the MC, at the storage intake and at the different storage times, were used, afterwards, in the elemental and ash analyses (see Table 1). For these analyses, the chips were ground to the maximum size of 1 mm using a Pulverisette 15 cutting mill (Fritsch, Oberstein, Germany).

The elemental analyser Vario EL III (Elementar Analysensysteme GmbH, Langenselbold, Germany) was used to perform the elemental analysis according to VDLUFA:1997 [57]. For each sample (see Table 1), two tin boats of 6 mm × 6 mm × 12 mm (article S22 137 419, Elementar Analysensysteme GmbH, Langenselbold, Germany) were prepared, each one

containing 10 mg of the ground chips; therefore, the percentage of C, H, N and S is herein reported as a mean value (n = 2).

The ash content analysis was done with 2.5–5 g of the ground chips according to EN ISO 14775:2009 [58]. One measure was done for each prepared sample of the ground chips at the storage intake and the different storage times (Table 1).

2.5. Statistical Analysis

It was investigated for MC, DML, microbiological parameters and pH value whether the mean values of the measurement series differ significantly over storage time ($p < 0.05$). The mean value comparisons between weeks and trials, as well as between weeks and materials (chips, wood, bark), were carried out using the single values. The interactions week–trial and week–material were considered. For the evaluation, the procedure GLIMMIX of the statistical software SAS (Version 9.4, SAS Institute, Cary, NC, USA) was used in order to carry out a two factorial ANOVA.

3. Results and Discussion

3.1. Particle Size Distribution

Figure 4 shows the particle size distribution of the poplar chips used in each of the storage trials T1, T2 and T3 (Table 1), which complied with the wood chip class P31 according to EN ISO 17225-1:2014 [53]. The analysis of the particle size distribution showed no significant differences for the wood chips used for the trials T1–T3.

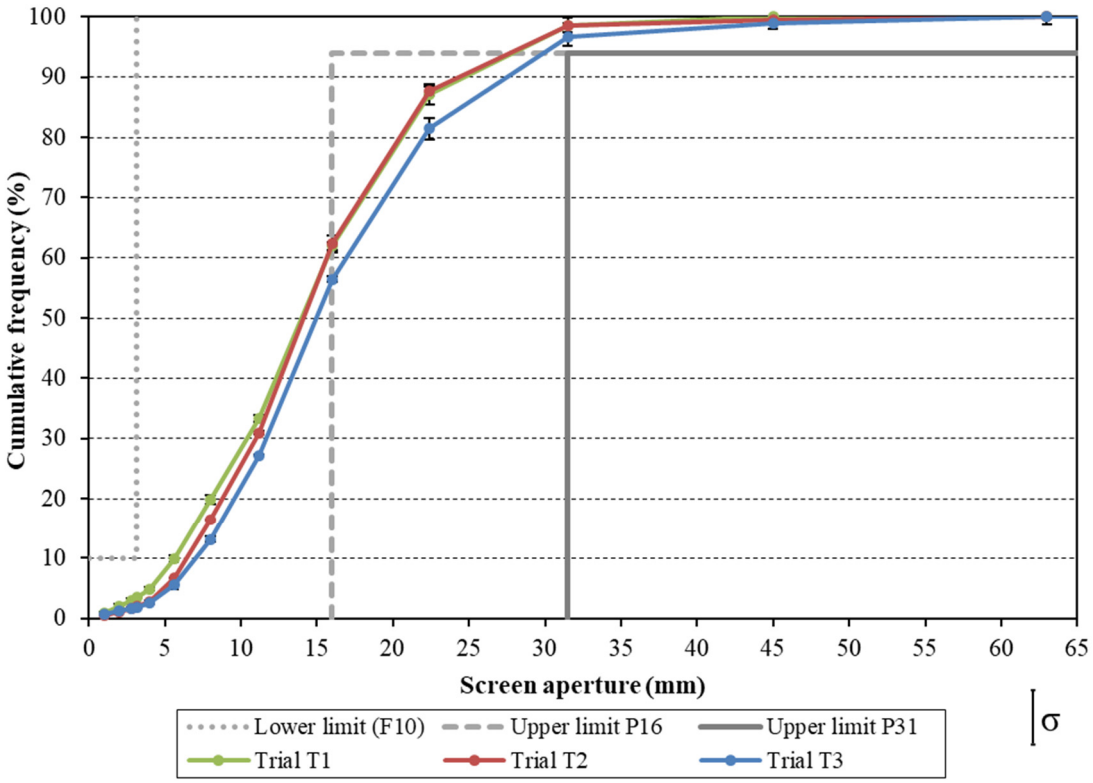


Figure 4. Particle size distribution of wood chips used in the storage trials T1–T3.

3.2. MC and DML of Poplar Chips, and RH Values, in the Storage Trials

Table 2 shows MC and DML values of the three storage trials T1, T2 and T3, as well as the RH measured during the storage trials. As supplementary information, detailed MC and DML obtained in the trials T1–T3 are reported in Appendix A (Tables A1–A3). The sample bags yielding a negative DML were considered as an experimental error and, consequently, excluded from the calculations. This was the case for one sample bag at

the storage week 2 in the trial T2, that resulted in −2.1% DML (Table A2; Appendix A); therefore, the MC and DML of this storage week 2 in trial T2 is the mean of two sample bags (n = 2).

Table 2. MC, DML and RH of poplar chips of the storage trials T1–T3. MC and DML expressed as mean (n = 6 and n = 3 at the storage intake and storage times, respectively) ± standard deviation (SD).

	Trial T1			Trial T2			Trial T3		
Storage Time (Weeks)	**MC (%)**	**DML (%)**	**RH (%)**	**MC (%)**	**DML (%)**	**RH (%)**	**MC (%)**	**DML (%)**	**RH (%)**
Storage intake, 0	56.8 ± 0.7^{ab}	0.0^{A}	92.7%	52.3 ± 0.7^{ab}	0.0^{a}	88.0%	58.3 ± 0.7^{a}	0.0^{A}	
1	52.7 ± 1.0	4.2 ± 0.4	95.6%	-	-	95.0%	-	-	
2	47.8 ± 1.7^{abc}	6.4 ± 0.9^{BC}	93.9%	44.4 ± 0.5^{bcd}	0.6 ± 0.5^{A}	93.3%	-	-	
3	39.5 ± 1.3	7.5 ± 0.6	93.9%			91.6%	44.3 ± 1.3	11.0 ± 1.3	
4	32.4 ± 3.9^{de}	9.8 ± 0.9^{CD}	94.2%	37.8 ± 6.2^{dc}	5.0 ± 0.8^{B}	91.2%	-	-	98.5 to
5	25.1 ± 2.5	8.3 ± 0.8	93.6%	-	-	90.4%	-	-	100%
6	21.3 ± 1.6^{efg}	8.8 ± 0.8^{CD}	92.7% *	23.5 ± 3.2^{ef}	8.1 ± 1.4^{BC}	90.3%	31.9 ± 8.6^{ed}	11.6 ± 2.1^{DE}	
7	-	-	-	-	-	89.0%	-	-	
8	-	-	-	17.0 ± 0.8^{fg}	9.4 ± 0.6^{CD}	85.6%	-	-	
9	-	-	-	-	-	70.4%	24.5 ± 4.7^{ef}	13.7 ± 0.3^{E}	
10	-	-	-	9.9 ± 0.3^{g}	9.3 ± 1.2^{CD}	64.2%	-	-	

* Due to sensor failure, this RH value corresponds to two days before the end of the storage (week 6); MC: moisture content (wet-basis); DML: dry matter loss; RH: relative humidity; significant differences between trials and over storage are marked by different small letters for MC and different capital letters for DML (GLIMMIX, SAS 9.4).

The initial MC_{in} of the poplar chips were 56.8%, 52.3% and 58.3% (Table 2), respectively, for the trials T1, T2 and T3 (Table 1), which are in line with the wet-basis MC of poplar chips at harvest reported by, e.g., Barontini et al. [13], Pecenka et al. [30] and Pari et al. [24]. At the end of the respective six, ten and nine storage weeks, the wood chips showed a significantly reduced MC of 21.3%, 9.9% and 24.5% ($p < 0.05$; trials T1, T2 and T3 in Table 2), which represented a decrease of 5.9%, 4.2% and 3.8% MC per week on average (MC_{PWAV}), respectively. The lowest MC_{PWAV} obtained in the trial T3 (i.e., 3.8% MC_{PWAV}) suggested the impact of assisting the storage trial with a humidifier (Figure 2b).

Focusing on the six initial storage weeks allowed a better comparison of the results. Table 2 shows that chips' MC decreased by 5.9% and 4.8% MC_{PWAV} in the initial six storage weeks (trials T1 and T2 in Table 2). The lowest value, however, was obtained in trial T3, i.e., 4.4% MC_{PWAV} (from storage intake to storage week 6; Table 2), which indicated the impact of using the humidifier on lowering the rate at which chips lose MC. However, no significant differences could be detected for MC among all trials T1–T3 at storage week 6 ($p < 0.05$). Moreover, all these MC_{PWAV} of 4.4% to 5.9% in the initial 6–10 weeks showed a faster reduction in MC compared to practice storage trials of poplar chips in outdoor piles. Lenz et al. [26] measured 1.7% MC_{PWAV} in the initial six storage weeks of fine P31 poplar chips, and 2.2% MC_{PWAV} was reported by Pecenka et al. [17] in the initial nine storage weeks at practice conditions. This shows the slower drying rate of wood chips in outdoor storage piles at practice scale compared to chips investigated in the storage chamber trials T1–T3 at laboratory scale, and it highlights a limitation of the set-up used in the trials T1–T3 (Table 1).

After six storage weeks, the chips' wet-based MC in trials T1 and T2 were 21.3% and 23.5%, respectively (Table 2). These values correspond to 27.1% and 30.7% dry-basis MC (MC_{DB}), which are close to the fibre saturation point (FSP) of poplar wood, i.e., 32–35% MC_{DB} [59], and in agreement to the range of FSP reported in literature for wood, i.e., 21–35% MC_{DB} [59–61]. FSP in wood is the MC point at which the wood cell lumen is empty of water while the wood cell wall is water saturated [59,60], both named respectively 'free water' and 'bound water' [59,60]. The total value of FSP varies depending on wood species [59,61]. Moreover, FSP values reported in literature refer to 'only wood' specimens, whereas the MC values reported in Table 2 refer to the 'whole chip', i.e., containing wood

and bark. However, the chips' MC in trials T1 and T2 after six weeks of storage, were in the range of the FSP data reported in literature. This might indicate that poplar chips in the trials T1 and T2 had very little free water in the cell lumens at this storage time.

On the other hand, at the storage week 6, chips in trial T3 showed a MC of 31.9% (Table 2), which equals to 46.8% MC_{DB}. This value is clearly higher than the FSP of 32–35% MC_{DB} for poplar wood [59], and it might indicate that chips in trial T3, at the storage week 6, still contained free water in the cell lumen. As long as the chips' MC is above the FSP, it can be assumed that a reduced availability of water is not a limiting factor for microbiological degradation processes in the chips [19,41]. Furthermore, these results suggest the suitability of the set-up assisted with the humidifier, in order to resemble the conditions that wood chips undergo during the initial storage weeks in outdoor piles.

DML of Poplar Chips

Focusing on DML, the three trials T1–T3, despite having some similitudes, did not follow a similar progression. For example, the DML of both trials T1 and T2 (Table 2) stabilized between 8.1% and 9.8%, regardless of the storage time. The stabilisation of DML in both trials T1 and T2 occurred approximately at the same time as the MC decreased to values near the FSP (Table 2). On the other hand, chips in the trial T3 showed 11.0% DML (Table 2) already at the storage week 3, which was higher than the DML measured in the storage trials T1 and T2 (see Table 2), and increased up to 13.7% at the end of the nine-week storage (Trial T3; Table 2). Results presented in Table 2 suggest that the high RH provided by the humidifier used in the trial T3 (Figure 2b) might contribute to higher DML in the wood chips stored in the trial T3 when compared to the trials T1 and T2. The statistical analysis of the results has shown that DML increased for all trials with storage time. While significant differences were found for the DML between trial T2 and T3 at the storage week 6, these were not visible comparing trial T1 and T3, as well as T1 and T2 (Table 2). However, the number of repetitions (n = 3) in every trial was limited due to the limited volume of the storage chamber, and a higher number of samples would have provided statistically more robust results.

The DML results obtained in the trial T3 (Table 2) were closer to the DML reported for outdoor pile storages, e.g., approximately 10% for the initial storage phase of 9–12 weeks in 500 m^3 storage piles of P31 poplar chips [17,30]. This would suggest that the use of the humidifier in the storage set-up of trial T3 (Figure 2b) is more suitable for storage trials at a laboratory scale.

3.3. Microbiological and pH Analysis

The results of the microbiological analyses done in the storage trials T1 and T2 (Table 1) are shown in Figure 5a–d, corresponding to mesophilic fungi, xerophilic fungi, mesophilic bacteria and actinobacteria, respectively. Detailed numerical values are presented in Appendix B (Tables A4 and A5).

Our isolation/cultivation experiments revealed that xerophilic and mesophilic fungi, and mesophilic bacteria and actinobacteria, were present in the fresh poplar chips at the storage intake, for both trials T1 and T2 (Figure 5a–d). The statistical analysis showed no significant differences between the trials ($p < 0.05$). However, the number of mesophilic bacteria and actinobacteria (Figure 5c,d, respectively) present in the chips at the storage intake were higher than the number of mesophilic fungi and xerophilic fungi (Figure 5a,b, respectively). Whilst the number of mesophilic fungi and xerophilic fungi were in the range of 3–5 $_{lg}$CFU g^{-1}, mesophilic bacteria and actinobacteria showed numbers between 4 $_{lg}$CFU g^{-1} and 7 $_{lg}$CFU g^{-1} (Figure 5).

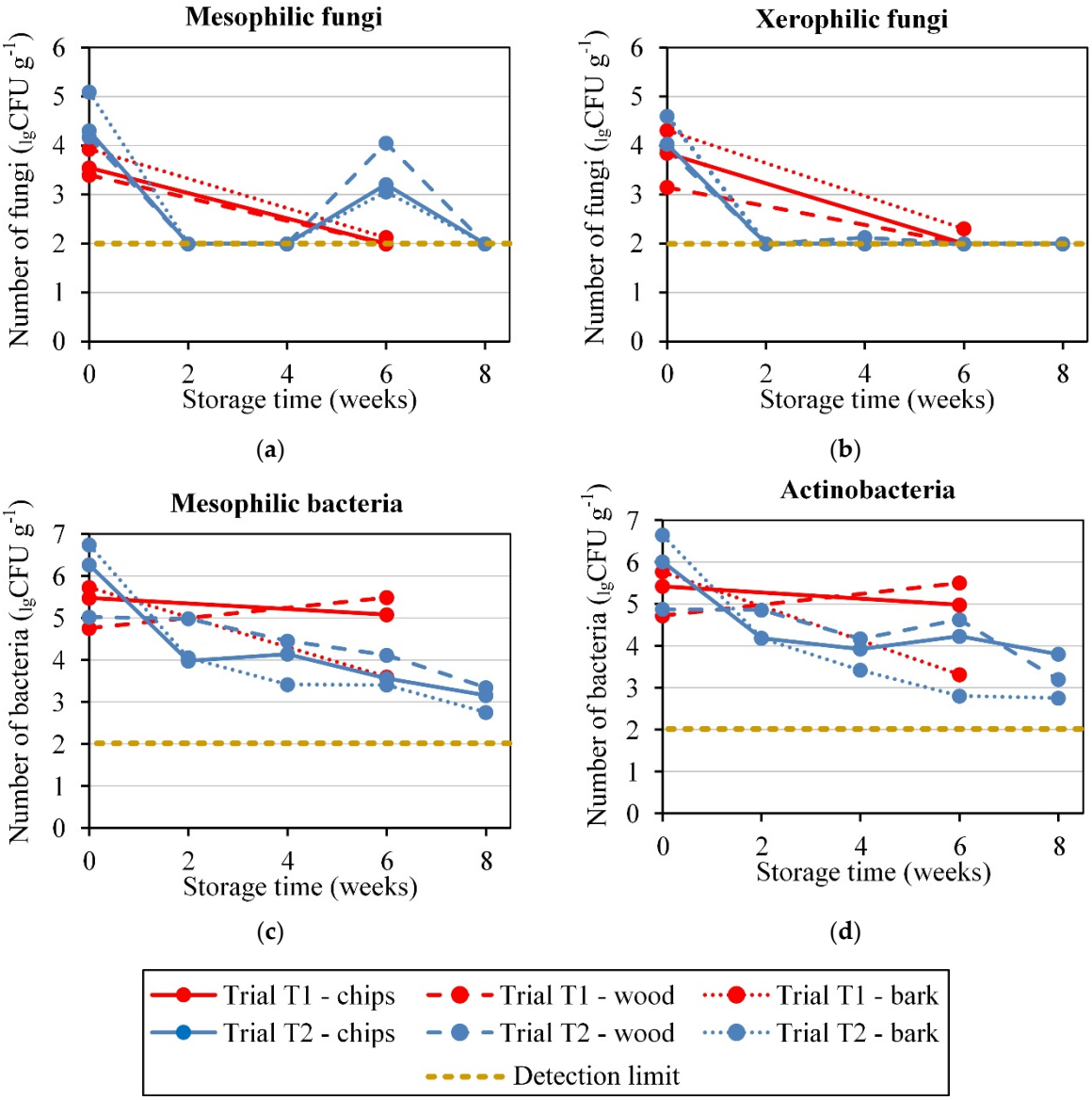


Figure 5. Results of the microbiological analyses of poplar chips, wood and bark of trials T1 and T2.

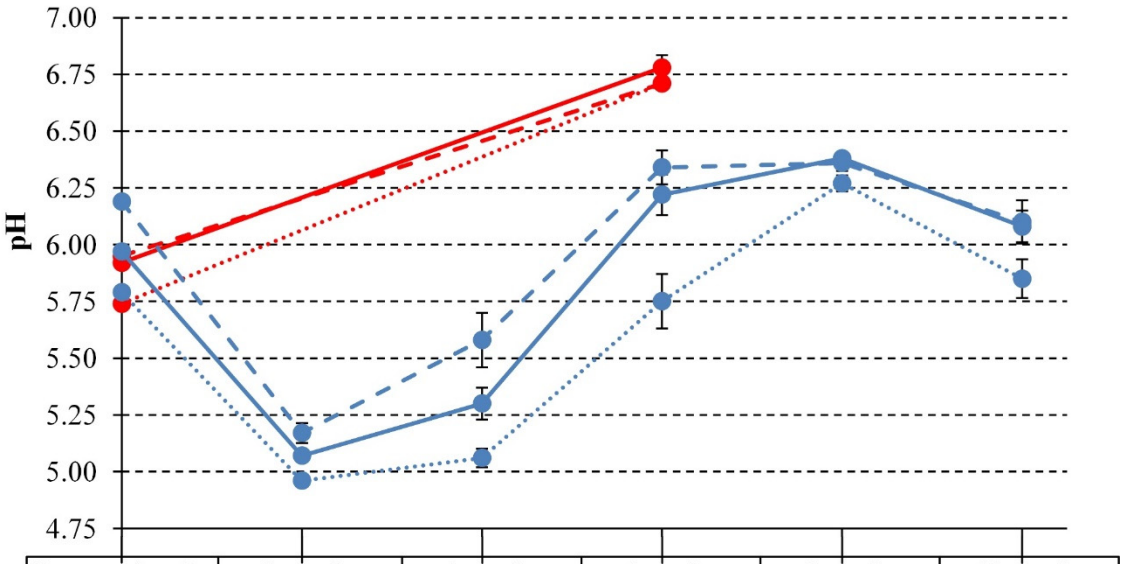
As a function of progressing wood decomposition during storage, bacteria and fungi trends became evident (Figure 5). Mesophilic bacteria and actinobacteria, despite showing a decrease over the course of storage, remained detectable until the end of storage, in both trials T1 and T2 (Figure 5c,d, respectively). This decrease over storage time proved to be significant for trial T1, but was not significant if all results from T1 and T2 were analysed together. On the other hand, xerophilic and mesophilic fungi (Figure 5a,b, respectively) dropped to the detection limit (i.e., 2 $_{lg}$CFU g^{-1}) during storage. One exception was at the storage week 6 in trial T2, where chips, wood and bark samples showed a presence of mesophilic fungi (Figure 5a) between 3 $_{lg}$CFU g^{-1} and 4 $_{lg}$CFU g^{-1}. This trend proved to be significant for both trials and all investigated fungi, if the outlier for mesophilic fungi in trial T2 at storage week 6 was excluded from the statistical analysis. However, this peak could be attributed to spores present in the sample at the sampling time, that started to grow during the lab analyses when the optimal conditions were provided. Furthermore, in line with our findings, the results of Idler et al. [19] for trials with P45 poplar chips stored outdoors demonstrated the presence of mesophilic fungi of around 3 $_{lg}$CFU g^{-1} during the initial 50 storage days at pile temperatures of 45–55 °C. This might explain the peak of mesophilic fungi observed at the storage week 6 in trial T2 (Figure 5a).

The decrease in the observed number of bacteria and fungi is a consequence of the test temperature being 55 °C. This temperature is too elevated for mesophilic microorganisms growing at temperatures between 20 °C and 50 °C and it is selective for thermotolerant and thermophilic microorganisms. The thermophilic phase is characterized by temperatures up to 50 °C or even 75 °C [62]. In fact, the temperature is a major abiotic factor selective for the majority of environmental microorganisms. Zöhrer et al. [48] investigated the fungal and bacterial community of poplar wood chips stored in 2019 in Austria by high throughput next-generation sequencing over a storage period of 120 days. Their research revealed a dynamic shift of the bacterial and fungal community over time being mainly a result of the storage temperature, pH and nutrient availability. After storage intake, and long-term storage relative abundancy of the predominant species decreased. However, it must be taken into account that molecular analyses based on the extraction of microbial DNA are culture independent. Furthermore, these techniques reveal all microorganisms, independent of their physiological status, not discriminating between living, dormant (e.g., spores) and dead microbial cells. In contrast, culture dependent methods, such as agar plate cultivation techniques which have been used here, are restricted to the cultivable fraction of the microbiota, embracing only a small fraction (1–10%) of the overall (soil) microbiota [63,64].

Comparing the results of the microorganisms isolated from the wood and bark fractions (Figure 5) at the storage intake, the presence of mesophilic fungi, xerophilic fungi, mesophilic bacteria and actinobacteria was generally significantly higher in bark than in wood. However, at the end of the storage trials, the wood fraction showed a higher presence of mesophilic bacteria and actinobacteria when compared to bark (Figure 5c,d, respectively), for both trials T1 and T2. On the other hand, mesophilic and xerophilic fungi (Figure 5a,b, respectively) decreased to the detection limit. However, at the end of the storage trial T1 (i.e., six weeks), xerophilic fungi showed a presence of 2.3 $_{lg}$CFU g^{-1} in the bark fraction (Table A4; Appendix B), while they decreased to the detection limit in the respective wood fraction (Figure 5b). The varying presence of microorganisms in wood and bark fractions can be attributed to differences in environmental conditions of the different wood fractions being already apparent at storage intake. In particular, the tree characteristics such as wood density, wood composition (regarding the different carbohydrates and lignin), as well as pH, shape the microbial communities and in turn determine the decomposition rates of different wood fractions [65–67]. Due to the complex structure of bark with a higher content of sugars and nutrients compared to heart- and sapwood, it hosts a diverse microbiological community with an especially high fungal species richness and higher abundancies [68]. However, in order to further interpret our complex and changing storage-condition dependent findings revealed by cultivation techniques, molecular analysis is needed.

pH Results

The results of the pH analysis showed that, at the storage intake, the pH of the chips in trials T1 and T2 were similar (i.e., 5.9 and 6.0, respectively; Figure 6). Moreover, wood fractions in both trials T1 and T2 showed slightly higher pH than bark fractions (6.0–6.2 and 5.7–5.8, respectively; Figure 6). However, the statistical analysis has demonstrated that differences between the fractions were not significant at storage intake (Table A6, Appendix B). Whereas, for trial T1, the pH values were measured at the storage intake and at the end of the trial only, the results presented for the trial T2 (Figure 6) allowed a more detailed analysis, due to the two-week sampling frequency used in trial T2 (Table 1).



		Storage intake	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks
T1	Chips	5.9 ± 0.0	-	-	6.8 ± 0.1	-	-
	Wood	6.0 ± 0.0	-	-	6.7 ± 0.1	-	-
	Bark	5.7 ± 0.0	-	-	6.7 ± 0.1	-	-
T2	Chips	6.0 ± 0.0	5.1 ± 0.0	5.3 ± 0.1	6.2 ± 0.2	6.4 ± 0.0	6.1 ± 0.1
	Wood	6.2 ± 0.0	5.2 ± 0.1	5.6 ± 0.2	6.3 ± 0.2	6.4 ± 0.1	6.1 ± 0.2
	Bark	5.8 ± 0.0	5.0 ± 0.0	5.1 ± 0.1	5.8 ± 0.2	6.3 ± 0.1	5.9 ± 0.2

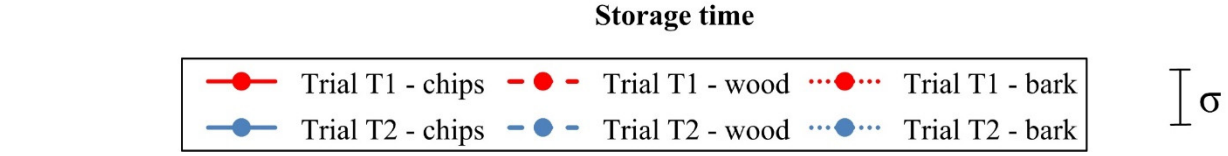


Figure 6. The pH data of wood chips as a function of storage trials (T1 and T2) and time (0–10 weeks); presented as mean (n = 2 and n = 3 for the storage intake and storage times, respectively) ± standard deviation.

The pH values of the wood chips in the trial T2 increased between storage intake and storage week 8 (from 6.0 to 6.4; Figure 6), similarly to trial T1, where a pH of 6.8 was reached after six weeks of storage. However, the two-week sampling frequency used in the trial T2 allowed the observation that, in the initial two storage weeks, the pH decreased significantly to 5.0–5.2 for all the analysed samples, i.e., chips, wood and bark (Figure 6 and Table A6, Appendix B). This can be attributed to acidification by microorganism, especially brown-rot fungi, producing organic acids [69]. In their work, Humar et al. [69] compared pH values of wood with the rate of decay by different fungi, showing a significant pH drop before mass loss was exhibited. The pH reduction was caused by the production of oxalic acid by brown-rot fungi, playing an important role in the non-enzymatic stage of decay. After the storage week 2, the pH of chips, wood and bark in trial T2 increased up to values of 6.3–6.4 (Figure 6); however, although wood and bark followed a similar progression, they slightly differed in pH. The decrease of pH in the initial two storage weeks in trial T2 resembled the results reported in Pecenka et al. [17]. This study observed a similar decrease of the pH from 6.37 to 5.06 in the initial five storage weeks of P31 poplar chips stored in outdoor piles, followed by an increase to 7.48 in the following two months. Additionally, Humar et al. [69] showed that some wood-degrading fungal species, such as *Schizophyllum commune*, caused an increase in wood pH, since this fungi prefer pH values in the range of pH 5 and 7 [69]. Based on the fact that pH is among the most prominent factors shaping microbial communities, the observed changes in pH during the storage

could have induced the observed shifts in the microbial community during the storage trial (Figure 6). During the initial stage with high dry matter losses, brown-rot fungi in particular produce an acidic environment for degrading wood cellulose; whereas, in later stages of lower degradation rates, fungi preferring higher pH values take over.

3.4. Ash and Elemental Analyses

Table 3 shows the results of the ash content and elemental analyses of the poplar chips of storage trials T1–T3, and the complete dataset of the elemental and ash analyses is shown in Appendix C (Table A7).

Table 3. Results of the elemental and ash analyses of poplar chips. (Mean ± standard deviation).

Trial	Storage Time (Weeks)	*n*	Ash (%) *	C (%) *	H (%) *	N (%) *	S (%) *	C:N Ratio **
T1	Storage intake, 0	2	2.34 ± 0.05	52.07 ± 0.03	4.79 ± 0.01	0.305 ± 0.001	0.119 ± 0.004	171:1 ± 0
T2	Storage intake, 0	2	2.22 ± 0.05	52.32 ± 0.18	4.85 ± 0.05	0.294 ± 0.043	0.113 ± 0.000	182:1 ± 27:1
	Storage intake, 0	2	3.12 ± 0.16	51.36 ± 0.08	4.65 ± 0.04	0.274 ± 0.008	0.107 ± 0.004	187:1 ± 5:1
T3	3	3	2.71 ± 0.14	51.91 ± 0.06	4.76 ± 0.06	0.328 ± 0.047	0.111 ± 0.006	161:1 ± 21:1
	6	3	3.19 ± 0.21	51.68 ± 0.15	4.65 ± 0.03	0.272 ± 0.035	0.105 ± 0.007	194:1 ± 28:1
	9	3	2.97 ± 0.47	51.50 ± 0.12	4.71 ± 0.04	0.326 ± 0.022	0.107 ± 0.004	159:1 ± 12:1

* Expressed as percentage of the dry matter fraction; ** C:N ratios calculated as the mean value of the C:N ratios of the single measures.

The ash contents at the storage intake of the trials T1–T3 (Table 3) are within the range of literature data for poplar chips, i.e., 2–3.02% [13,17,24,31]. Moreover, the analysis at different storage times in the storage trial T3 showed that the progression of the ash content oscillated between 2.71% and 3.19% (Table 3). These results differ from the trend reported in literature for chips stored in piles outdoors, that showed that ash content increased with increasing storage time [13,24,31]. For example, Pari et al. [24] investigated storage of poplar chips produced from the stem showing an initial ash content of 3.02%, whereas, after 18 months of storage, this value increased to 3.36%. Similarly, Lenz et al. [34] analysed the ash content of P31 poplar chips stored outdoors for nine months and found that the ash content increased from 2.07% at the storage intake to 2.81% at the end of the storage. However, our reported ash contents (Table 3) are still in the typical range for poplar wood from SRC.

Results from the elemental analysis of the trials T1–T3 (Table 3) showed that C:N ratios at the storage intake were between 171:1 and 187:1, being in the typical range for C:N ratios reported in literature for poplar chips, which range from 113:1 to 224:1 [17,24,26,70]. Moreover, C:N ratios at different storage times in trial T3 (Table 3) did not show a clear trend and oscillated in the same range of the above reported literature.

3.5. Limitations of the Storage Chamber Trials

The experiments have shown that the storage conditions which occur inside of a large wood chips pile under practice conditions after harvesting in winter in central Europe can be reproduced well with the investigated storage chamber set-up, particularly when using the humidifier (Figure 3). As shown, DML, pH and the development of microorganisms are within the ranges that are known from other outdoor storage experiments with poplar wood chips under comparable temperature–humidity conditions [13,17–19,26]. However, as mentioned before, the MC in the chamber set-ups decreased slightly faster than in outdoor storage trials. In addition, not all influencing variables that are effective in practice could be recreated accordingly. These are, in particular, the influence of changing weather conditions, such as heat transfer due to solar radiation, rewetting from rain, changing air flow conditions due to pile compaction as a result of large pile sizes, or changing gas concentrations in the pile (e.g., CO_2 and O_2) due to microbiological–chemical degradation processes [22,26]. However, the influence of changing weather conditions in particular is limited to the outer pile layers and has only little influence on the processes inside large

wood chip pile which have been the focus of the experiments presented here [18,22,34]. For reliable statistical evaluation, a larger volume of storage chamber should be used, in order to be able to increase the number of samples per each sampling date. Furthermore, even if no negative effects of opening the door during sampling could be determined, additional measures to reduce the air exchange during sampling and to monitor the testing atmosphere inside the chamber appear to be advisable as well.

4. Conclusions

The three storage trials presented in this work were conducted to investigate DML occurring in poplar chips in the early (high temperature) storage phase, using a laboratory set-up in a chamber under controlled temperature. This experimental set-up enabled regular sampling at short intervals during wood chip storage, and thus contributed to a better understanding of processes occurring during storage of poplar chips. The use of an incubation chamber seemed a good starting method for this purpose. In addition, the assistance of a humidifier proved to be a good system to keep a high RH, i.e., resembling the conditions that wood chips in outdoor storage piles undergo during the early storage phase (phase 1). Results showed that DML of 8.8–13.7% occurred in the poplar chips stored in the chamber for 6 to 10 weeks, which is a substantial portion of the total DML reported for outdoor storages of wood chips, that may account for up to 47% DML in storage piles set for 6–12 months. Microbiological results showed good correlation with results from a practice scale for the aspects considered in this work. Thus, overall, the storage chamber set-up has the potential to gain new and much more detailed information about the microbiological and chemical processes during storage. However, further research coupling isolation-cultivation techniques and cultivation independent molecular techniques (high throughput next generation sequencing) is needed, in order to unravel the complex and dynamic microbial processes (microbial succession) during storage. In conclusion, this work highlighted the possibilities of setting up such laboratory scale storage trials, in order to investigate the DML occurring in wood chips when drying in outdoor storage piles. Compared to large practice scale experiments, the proposed setting enables a better monitoring of the overall system under controlled conditions, and a more accurate sampling at shorter time intervals with a significantly reduced workload. The potential of the proposed laboratory scale set-up could be further improved by implementing additional storage parameters into the monitoring. For example, monitoring and control of CO_2 and O_2 concentrations would be suitable in order to simulate storage conditions even more accurately.

Author Contributions: Conceptualization, A.H.-E., R.P., S.D., H.L., C.I. and T.H.; methodology, investigation and validation, A.H.-E., R.P., S.D., J.A.-J., H.L., C.I. and T.H.; writing—original draft preparation, A.H.-E. and R.P.; writing—review and editing, A.H.-E., R.P., S.D., J.A.-J., H.L., C.I. and T.H.; supervision, R.P. and C.I.; funding acquisition, S.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Austrian Research Promotion Agency (FFG), Energieforschung program, project number 858837. The publication of this article was funded by the Open Access Fund of the Leibniz Association, Germany.

Institutional Review Board Statement: Not applicable.

Acknowledgments: Teodor Teodorov, Peter Kaulfuß and Helmuth Carl (ATB) are thanked for the technical assistance in performing the storage trials. Katrin Busse (ATB) is thanked for performing the microbiological analyses. Miriam Felgentreu, Mandy Jäkel and Giovanna Rehde (ATB) are thanked for conducting the ash and elemental analyses.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A

Table A1. Moisture content and dry matter losses of the sample bags in trial T1.

MC_{in} = 56.8% ± 0.7%			
Storage Time (Weeks)	**Sample Bag**	MC_{out} **(%)**	DML_i **(%)**
1	1	53.8	4.1
	2	51.3	3.9
	3	53.1	4.7
	Mean ± SD	52.7 ± 1.0	4.2 ± 0.4
2	1	50.1	7.4
	2	46.6	5.3
	3	46.6	6.6
	Mean ± SD	47.8 ± 1.7	6.4 ± 0.9
3	1	40.6	8.0
	2	40.2	7.6
	3	37.7	6.7
	Mean ± SD	39.5 ± 1.3	7.5 ± 0.6
4	1	35.7	10.7
	2	34.7	10.0
	3	27.0	8.6
	Mean ± SD	32.4 ± 3.9	9.8 ± 0.9
5	1	21.6	7.8
	2	27.4	9.4
	3	26.4	7.7
	Mean ± SD	25.1 ± 2.5	8.3 ± 0.8
6	1	21.8	8.5
	2	19.1	8.1
	3	22.9	9.9
	Mean ± SD	21.3 ± 1.6	8.8 ± 0.8

MC_{in}: moisture content (wet-basis) of poplar wood chips at the storage intake; MC_{out}: moisture content (wet-basis) of poplar wood chips at the sampling time; DML: dry matter loss; SD: standard deviation.

Table A2. Moisture content and dry matter losses of the sample bags in trial T2.

MC_{in} = 52.3% ± 0.7%			
Storage Time (Weeks)	**Sample Bag**	MC_{out} **(%)**	DML_i **(%)**
2	1	45.0	1.1
	2	43.9	0.1
	3	39.9 *	−2.1 *
	Mean ± SD	44.4 ± 0.5	0.6 ± 0.5
4	1	43.1	3.8
	2	41.3	5.8
	3	29.1	5.2
	Mean ± SD	37.8 ± 6.2	5.0 ± 0.8
6	1	25.0	10.0
	2	26.4	7.6
	3	19.1	6.7
	Mean ± SD	23.5 ± 3.2	8.1 ± 1.4

Table A2. *Cont.*

MC_{in} = 52.3% ± 0.7%			
Storage Time (Weeks)	**Sample Bag**	MC_{out} **(%)**	DML_i **(%)**
8	1	17.6	9.2
	2	15.9	8.9
	3	17.6	10.2
	Mean ± SD	17.0 ± 0.8	9.4 ± 0.6
10	1	10.3	9.7
	2	9.7	7.7
	3	9.8	10.6
	Mean ± SD	9.9 ± 0.3	9.3 ± 1.2

* Experimental error; results excluded from the analysis; MC_{in}: moisture content (wet-basis) of poplar wood chips at the storage intake; MC_{out}: moisture content (wet-basis) of poplar wood chips at the sampling time; DML: dry matter loss; SD: standard deviation.

Table A3. Moisture content and dry matter losses of the sample bags in trial T3.

MC_{in} = 58.3% ± 0.7%			
Storage Time (Weeks)	**Sample Bag**	MC_{out} **(%)**	DML_i **(%)**
3	1	44.5	12.0
	2	45.8	11.8
	3	42.5	9.3
	Mean ± SD	44.3 ± 1.3	11.0 ± 1.3
6	1	28.0	13.6
	2	43.9	12.4
	3	23.9	8.7
	Mean ± SD	31.9 ± 8.6	11.6 ± 2.1
9	1	21.1	13.9
	2	31.1	13.2
	3	21.1	13.9
	Mean ± SD	24.5 ± 4.7	13.7 ± 0.3

MC_{in}: moisture content (wet-basis) of poplar wood chips at the storage intake; MC_{out}: moisture content (wet-basis) of poplar wood chips at the sampling time; DML: dry matter loss; SD: standard deviation.

Appendix B

Table A4. Results of the microbiological analysis of chips, wood and bark in trial T1; presented as mean ± standard deviation, both in colony forming units per gram of sample (CFU g^{-1}). In brackets, the mean is expressed as logarithmic to base 10 ($_{lg}$CFU g^{-1}). Different small letters indicate significant differences between storage intake and end of storage ($p < 0.05$). Different capital letters indicate significant differences between wood fractions at storage intake respectively at week 6 ($p < 0.05$).

Storage Time (Weeks)	**Sample**	**Mesophilic Fungi (CFU g^{-1} ± CFU g^{-1}) [$_{lg}$CFU g^{-1}]**	**Xerophilic Fungi (CFU g^{-1} ± CFU g^{-1}) [$_{lg}$CFU g^{-1}]**	**Mesophilic Bacteria (CFU g^{-1} ± CFU g^{-1}) [$_{lg}$CFU g^{-1}]**	**Actinobacteria (CFU g^{-1} ± CFU g^{-1}) [$_{lg}$CFU g^{-1}]**
Storage intake, 0	Chips	$3.5 \times 10^3 \pm 5.0 \times 10^2$ [3.5] $^{a\,A}$	$7.0 \times 10^3 \pm 2.0 \times 10^3$ [3.8] $^{a\,A}$	$3.0 \times 10^5 \pm 9.0 \times 10^4$ [5.5] $^{a\,A}$	$2.7 \times 10^5 \pm 7.5 \times 10^4$ [5.4] $^{a\,A}$
	Wood	$2.5 \times 10^3 \pm 5.0 \times 10^2$ [3.4] $^{a\,A}$	$1.4 \times 10^3 \pm 4.0 \times 10^2$ [3.1] $^{a\,A}$	$5.7 \times 10^4 \pm 1.1 \times 10^4$ [4.8] $^{a\,A}$	$5.3 \times 10^4 \pm 1.2 \times 10^4$ [4.7] $^{a\,A}$
	Bark	$8.5 \times 10^3 \pm 4.5 \times 10^3$ [3.9] $^{a\,A}$	$2.1 \times 10^4 \pm 4.5 \times 10^3$ [4.3] $^{a\,A}$	$5.4 \times 10^5 \pm 1.5 \times 10^5$ [5.7] $^{a\,A}$	$5.9 \times 10^5 \pm 1.7 \times 10^5$ [5.8] $^{a\,A}$

Table A4. *Cont.*

Storage Time (Weeks)	Sample	Mesophilic Fungi (CFU $g^{-1} \pm$ CFU g^{-1}) [$_{lg}$CFU g^{-1}]	Xerophilic Fungi (CFU $g^{-1} \pm$ CFU g^{-1}) [$_{lg}$CFU g^{-1}]	Mesophilic Bacteria (CFU $g^{-1} \pm$ CFU g^{-1}) [$_{lg}$CFU g^{-1}]	Actinobacteria (CFU $g^{-1} \pm$ CFU g^{-1}) [$_{lg}$CFU g^{-1}]
6	Chips	ND $^{b\,A}$	ND $^{a\,A}$	$1.2 \times 10^5 \pm 3.2 \times 10^4$ [5.1] $^{a\,A}$	$9.5 \times 10^4 \pm 6.3 \times 10^4$ [5.0] $^{a\,AB}$
	Wood	ND $^{b\,A}$	ND $^{a\,A}$	$3.1 \times 10^5 \pm 8.1 \times 10^4$ [5.5] $^{a\,B}$	$3.2 \times 10^5 \pm 1.1 \times 10^5$ [5.5] $^{a\,B}$
	Bark	$1.3 \times 10^2 \pm 4.8 \times 10^1$ [2.1] $^{a\,A}$	$2.0 \times 10^2 \pm 8.2 \times 10^1$ [2.3] $^{a\,A}$	$3.9 \times 10^3 \pm 3.3 \times 10^2$ [3.6] $^{a\,C}$	$2.1 \times 10^3 \pm 2.4 \times 10^2$ [3.3] $^{a\,AC}$

ND: Not detected; detection limit 2.0 $_{lg}$CFU g^{-1}.

Table A5. Results of the microbiological analysis of the chips, wood and bark in trial T2; presented as mean ± standard deviation, both in colony forming units per gram of sample (CFU g^{-1}). In brackets, the mean is expressed as logarithmic to base 10 ($_{lg}$CFU g^{-1}). Different small letters indicate significant differences between storage intake and end of storage ($p < 0.05$). Different capital letters indicate significant differences between wood fractions at storage intake respectively at week 6 or 8 ($p < 0.05$).

Storage Time (Weeks)	Sample	Mesophilic Fungi (CFU $g^{-1} \pm$ CFU g^{-1}) [$_{lg}$CFU g^{-1}]	Xerophilic Fungi (CFU $g^{-1} \pm$ CFU g^{-1}) [$_{lg}$CFU g^{-1}]	Mesophilic Bacteria (CFU $g^{-1} \pm$ CFU g^{-1}) [$_{lg}$CFU g^{-1}]	Actinobacteria (CFU $g^{-1} \pm$ CFU g^{-1}) [$_{lg}$CFU g^{-1}]
Storage intake, 0	Chips	$2.0 \times 10^4 \pm 1.0 \times 10^4$ [4.3] $^{a\,A}$	$1.1 \times 10^4 \pm 2.3 \times 10^3$ [4.0] $^{a\,A}$	$1.9 \times 10^6 \pm 1.2 \times 10^6$ [6.3] $^{a\,A}$	$1.0 \times 10^6 \pm 1.8 \times 10^5$ [6.0] $^{a\,A}$
	Wood	$1.5 \times 10^4 \pm 5.0 \times 10^3$ [4.2] $^{a\,A}$	$8.5 \times 10^3 \pm 5.0 \times 10^2$ [3.9] $^{a\,A}$	$1.1 \times 10^5 \pm 2.5 \times 10^4$ [5.0] $^{a\,A}$	$7.5 \times 10^4 \pm 2.5 \times 10^4$ [4.9] $^{a\,AB}$
	Bark	$1.3 \times 10^5 \pm 7.5 \times 10^4$ [5.1] $^{a\,A}$	$4.0 \times 10^4 \pm 1.0 \times 10^4$ [4.6] $^{a\,A}$	$5.6 \times 10^6 \pm 1.1 \times 10^6$ [6.7] $^{a\,A}$	$4.5 \times 10^6 \pm 5.5 \times 10^5$ [6.6] $^{a\,C}$
2	Chips	ND	ND	$9.5 \times 10^3 \pm 4.1 \times 10^3$ [4.0]	$1.5 \times 10^4 \pm 5.0 \times 10^3$ [4.2]
	Wood	ND	ND	$9.6 \times 10^4 \pm 4.9 \times 10^4$ [5.0]	$7.2 \times 10^4 \pm 5.0 \times 10^4$ [4.9]
	Bark	ND	ND	$1.1 \times 10^4 \pm 5.8 \times 10^3$ [4.1]	$1.5 \times 10^4 \pm 5.9 \times 10^3$ [4.2]
4	Chips	ND	ND	$1.4 \times 10^4 \pm 9.6 \times 10^3$ [4.1]	$8.4 \times 10^3 \pm 5.5 \times 10^3$ [3.9]
	Wood	ND	$1.3 \times 10^2 \pm 4.8 \times 10^1$ [2.1]	$2.8 \times 10^4 \pm 3.5 \times 10^4$ [4.5]	$1.5 \times 10^4 \pm 1.0 \times 10^4$ [4.2]
	Bark	ND	ND	$2.6 \times 10^3 \pm 2.3 \times 10^3$ [3.4]	$2.6 \times 10^3 \pm 1.0 \times 10^3$ [3.4]
6	Chips	$1.6 \times 10^3 \pm 5.1 \times 10^2$ [3.2] $^{a\,A}$	ND $^{a\,A}$	$3.6 \times 10^3 \pm 3.1 \times 10^3$ [3.6] $^{a\,A}$	$1.7 \times 10^4 \pm 1.3 \times 10^4$ [4.2] $^{a\,A}$
	Wood	$1.1 \times 10^4 \pm 1.5 \times 10^4$ [4.1] $^{a\,A}$	ND $^{b\,A}$	$1.3 \times 10^4 \pm 1.5 \times 10^4$ [4.1] $^{a\,A}$	$4.2 \times 10^4 \pm 5.5 \times 10^4$ [4.6] $^{a\,A}$
	Bark	$1.1 \times 10^3 \pm 1.1 \times 10^3$ [3.1] $^{a\,A}$	ND $^{a\,A}$	$2.5 \times 10^3 \pm 3.2 \times 10^3$ [3.4] $^{a\,A}$	$6.3 \times 10^2 \pm 2.6 \times 10^2$ [2.8] $^{a\,A}$
8	Chips	ND $^{a\,A}$	ND $^{a\,A}$	$1.4 \times 10^3 \pm 9.4 \times 10^2$ [3.2] $^{a\,A}$	$6.3 \times 10^3 \pm 6.3 \times 10^3$ [3.8] $^{a\,A}$
	Wood	ND $^{a\,A}$	ND $^{b\,A}$	$2.2 \times 10^3 \pm 2.8 \times 10^3$ [3.3] $^{a\,A}$	$1.6 \times 10^3 \pm 1.5 \times 10^3$ [3.2] $^{a\,A}$
	Bark	ND $^{a\,A}$	ND $^{a\,A}$	$5.7 \times 10^2 \pm 4.7 \times 10^1$ [2.8] $^{a\,A}$	$5.7 \times 10^2 \pm 1.2 \times 10^2$ [2.8] $^{a\,A}$

ND: Not detected; detection limit 2.0 $_{lg}$CFU g^{-1}.

Table A6. Results of the pH analysis of the chips, wood and bark in trial T1 and T2; presented as mean ± standard deviation. Different letters indicate significant differences within one trial over storage time ($p < 0.05$).

Storage Time (Weeks)	Sample	Trial T1	Trial T2
Storage intake, 0	Chips	5.9 ± 0.0^{a}	6.0 ± 0.0^{abcde}
	Wood	6.0 ± 0.0^{a}	6.2 ± 0.0^{abcd}
	Bark	5.7 ± 0.0^{a}	5.8 ± 0.0^{cdef}
2	Chips	-	5.1 ± 0.0^{gh}
	Wood	-	5.2 ± 0.2^{gh}
	Bark	-	5.0 ± 0.0^{h}
4	Chips	-	5.3 ± 0.1^{gfh}
	Wood	-	5.6 ± 0.2^{egf}
	Bark	-	5.1 ± 0.1^{h}
6	Chips	6.8 ± 0.1^{b}	6.2 ± 0.2^{abcd}
	Wood	6.7 ± 0.1^{b}	6.3 ± 0.2^{abc}
	Bark	6.7 ± 0.1^{b}	5.8 ± 0.2^{def}
8	Chips	-	6.4 ± 0.0^{a}
	Wood	-	6.4 ± 0.1^{ab}
	Bark	-	6.3 ± 0.1^{abc}
10	Chips	-	6.1 ± 0.1^{abcde}
	Wood	-	6.1 ± 0.2^{abcd}
	Bark	-	5.9 ± 0.2^{bcde}

Appendix C

Table A7. Results of the elemental analysis and ash analysis.

Trial	Storage Time (Weeks)	Sample	Ash (%) *	C (%) *	H (%) *	N (%) *	S (%) *	C:N Ratio **
T1	Storage intake, 0	1	2.39	52.04	4.80	0.306	0.123	170
		2	2.29	52.10	4.77	0.304	0.116	171
		Mean ± SD	2.34 ± 0.05	52.07 ± 0.03	4.79 ± 0.01	0.305 ± 0.001	0.119 ± 0.004	171:1 ± 0
T2	Storage intake, 0	1	2.27	52.14	4.89	0.337	0.113	155
		2	2.16	52.50	4.80	0.251	0.113	210
		Mean ± SD	2.22 ± 0.05	52.32 ± 0.18	4.85 ± 0.05	0.294 ± 0.043	0.113 ± 0.000	182:1 ± 27:1
T3	Storage intake, 0	1	3.28	51.44	4.62	0.282	0.111	183
		2	2.96	51.28	4.69	0.267	0.102	192
		Mean ± SD	3.12 ± 0.16	51.36 ± 0.08	4.65 ± 0.04	0.274 ± 0.008	0.107 ± 0.004	187:1 ± 5:1
	3	1	2.89	51.85	4.68	0.395	0.119	131
		2	2.55	51.88	4.78	0.289	0.110	180
		3	2.70	52.00	4.82	0.302	0.104	172
		Mean ± SD	2.71 ± 0.14	51.91 ± 0.06	4.76 ± 0.06	0.328 ± 0.047	0.111 ± 0.006	161:1 ± 21:1
	6	1	2.97	51.86	4.69	0.223	0.095	233
		2	3.46	51.68	4.61	0.297	0.111	174
		3	3.12	51.50	4.65	0.296	0.111	174
		Mean ± SD	3.19 ± 0.21	51.68 ± 0.15	4.65 ± 0.03	0.272 ± 0.035	0.105 ± 0.007	194:1 ± 28:1
	9	1	2.52	51.46	4.70	0.339	0.106	152
		2	3.61	51.39	4.66	0.344	0.111	149
		3	2.76	51.66	4.75	0.294	0.103	176
		Mean ± SD	2.97 ± 0.47	51.50 ± 0.12	4.71 ± 0.04	0.326 ± 0.022	0.107 ± 0.004	159:1 ± 12:1

* Expressed as percentage of the dry matter fraction; ** C:N ratios calculated as the mean value of the C:N ratios of the single measures; SD: standard deviation.

References

1. Kriegler, E.; Weyant, J.P.; Blanford, G.J.; Krey, V.; Clarke, L.; Edmonds, J.; Fawcett, A.; Luderer, G.; Riahi, K.; Richels, R.; et al. The role of technology for achieving climate policy objectives: Overview of the EMF 27 study on global technology and climate policy strategies. *Clim. Chang.* **2014**, *123*, 353–367. [CrossRef]
2. Rose, S.K.; Kriegler, E.; Bibas, R.; Calvin, K.; Popp, A.; van Vuuren, D.P.; Weyant, J. Bioenergy in energy transformation and climate management. *Clim. Chang.* **2014**, *123*, 477–493. [CrossRef]
3. Amanatidis, G. *European Policies on Climate and Energy towards 2020, 2030 and 2050*; European Parliament, Policy Department for Economic, Scientific and Quality of Life Policies: Brussels, Belgium, 2019.
4. European Commission. *Impact Assessment—A Policy Framework for Climate and Energy in the Period from 2020 up to 2030*; European Commission: Brussels, Belgium, 2014.
5. Berhongaray, G.; El Kasmioui, O.; Ceulemans, R. Comparative analysis of harvesting machines on an operational high-density short rotation woody crop (SRWC) culture: One-process versus two-process harvest operation. *Biomass Bioenergy* **2013**, *58*, 333–342. [CrossRef]
6. Landgraf, D.; Carl, C.; Neupert, M. Biomass yield of 37 different SRC poplar varieties grown on a typical site in North Eastern Germany. *Forests* **2020**, *11*, 1048. [CrossRef]
7. Pecenka, R.; Hoffmann, T. Harvest technology for short rotation coppices and costs of harvest, transport and storage. *Agron. Res.* **2015**, *13*, 361–371.
8. Stolarski, M.J.; Niksa, D.; Krzyzaniak, M.; Tworkowski, J.; Szczukowski, S. Willow productivity from small- and large-scale experimental plantations in Poland from 2000 to 2017. *Renew. Sustain. Energy Rev.* **2019**, *101*, 461–475. [CrossRef]
9. Manzone, M.; Bergante, S.; Facciotto, G. Energy and economic sustainability of woodchip production by black locust (*Robinia pseudoacacia* L.) plantations in Italy. *Fuel* **2015**, *140*, 555–560. [CrossRef]
10. Rosenqvist, H.; Berndes, G.; Börjesson, P. The prospects of cost reductions in willow production in Sweden. *Biomass Bioenergy* **2013**, *48*, 139–147. [CrossRef]
11. Noll, M.; Jirjis, R. Microbial communities in large-scale wood piles and their effects on wood quality and the environment. *Appl. Microbiol. Biotechnol.* **2012**, *95*, 551–563. [CrossRef] [PubMed]
12. Manzone, M.; Balsari, P.; Spinelli, R. Small-scale storage techniques for fuel chips from short rotation forestry. *Fuel* **2013**, *109*, 687–692. [CrossRef]
13. Barontini, M.; Scarfone, A.; Spinelli, R.; Gallucci, F.; Santangelo, E.; Acampora, A.; Jirjis, R.; Civitarese, V.; Pari, L. Storage dynamics and fuel quality of poplar chips. *Biomass Bioenergy* **2014**, *62*, 17–25. [CrossRef]
14. Dimitriou, I.; Rutz, D. *Sustainable Short Rotation Coppice—A Handbook*; SRC+: Munich, Germany, 2015.
15. Veste, M.; Böhm, C. *Agrarholz—Schnellwachsende Bäume in der Landwirtschaft*; Springer Spektrum: Berlin/Heidelberg, Germany, 2018.
16. Scholz, V. *Lagerung von Holzhackschnitzeln—Experten-Workshop*; Bornimer Agrartechnische Berichte-Heft 63, Leibniz-Institut für Agrartechnik Potsdam-Bornim e.V. (ATB): Potsdam, Germany, 2008.
17. Pecenka, R.; Lenz, H.; Idler, C. Influence of the chip format on the development of mass loss, moisture content and chemical composition of poplar chips during storage and drying in open-air piles. *Biomass Bioenergy* **2018**, *116*, 140–150. [CrossRef]
18. Whittaker, C.; Yates, N.E.; Powers, S.J.; Misselbrook, T.; Shield, I. Dry matter losses and quality changes during short rotation coppice willow storage in chip or rod form. *Biomass Bioenergy* **2018**, *112*, 29–36. [CrossRef]
19. Idler, C.; Pecenka, R.; Lenz, H. Influence of the particle size of poplar wood chips on the development of mesophilic and thermotolerant mould during storage and their potential impact on dry matter losses in piles in practice. *Biomass Bioenergy* **2019**, *127*, 105273. [CrossRef]
20. Gigler, J.K.; van Loon, W.K.P.; Vissers, M.M.; Bot, G.P.A. Forced convective drying of willow chips. *Biomass Bioenergy* **2000**, *19*, 259–270. [CrossRef]
21. Jirjis, R. Effects of particle size and pile height on storage and fuel quality of comminuted Salix viminalis. *Biomass Bioenergy* **2005**, *28*, 193–201. [CrossRef]
22. Whittaker, C.; Macalpine, W.; Yates, N.E.; Shield, I. Dry matter losses and methane emissions during wood chip storage: The impact on full life cycle greenhouse gas savings of short rotation coppice willow for heat. *Bioenergy Res.* **2016**, *9*, 820–835. [CrossRef] [PubMed]
23. Krzyzaniak, M.; Stolarski, M.J.; Niksa, D.; Tworkowski, J.; Szczukowski, S. Effect of storage methods on willow chips quality. *Biomass Bioenergy* **2016**, *92*, 61–69. [CrossRef]
24. Pari, L.; Scarfone, A.; Santangelo, E.; Gallucci, F.; Spinelli, R.; Jirjis, R.; Del Giudice, A.; Barontini, M. Long term storage of poplar chips in mediterranean environment. *Biomass Bioenergy* **2017**, *107*, 1–7. [CrossRef]
25. Therasme, O.; Volk, T.A.; Eisenbies, M.H.; San, H.; Usman, N. Hot water extracted and non-extracted willow biomass storage performance: Fuel quality changes and dry matter losses. *Front. Energy Res.* **2020**, *7*, 165. [CrossRef]
26. Lenz, H.; Idler, C.; Hartung, E.; Pecenka, R. Open-air storage of fine and coarse wood chips of poplar from short rotation coppice in covered piles. *Biomass Bioenergy* **2015**, *83*, 269–277. [CrossRef]
27. Hofmann, N.; Mendel, T.; Schulmeyer, F.; Kuptz, D.; Borchert, H.; Hartmann, H. Drying effects and dry matter losses during seasonal storage of spruce wood chips under practical conditions. *Biomass Bioenergy* **2018**, *111*, 196–205. [CrossRef]

28. Barontini, M.; Crognale, S.; Scarfone, A.; Gallo, P.; Gallucci, F.; Petruccioli, M.; Pesciaroli, L.; Pari, L. Airborne fungi in biofuel wood chip storage sites. *Int. Biodeterior. Biodegrad.* **2014**, *90*, 17–22. [CrossRef]
29. Pari, L.; Bergonzoli, S.; Cetera, P.; Mattei, P.; Alfano, V.; Rezaei, N.; Suardi, A.; Toscano, G.; Scarfone, A. Storage of fine woodchips from a medium rotation coppice eucalyptus plantation in central Italy. *Energies* **2020**, *13*, 2355. [CrossRef]
30. Pecenka, R.; Lenz, H.; Idler, C.; Daries, W.; Ehlert, D. Development of bio-physical properties during storage of poplar chips from 15 ha test fields. *Biomass Bioenergy* **2014**, *65*, 13–19. [CrossRef]
31. Lenz, H.; Pecenka, R.; Hartung, E.; Idler, C. Development and test of a simplified method to calculate dry matter loss during open-air storage of poplar wood chips by analysing ash contents. *Biomass Bioenergy* **2016**, *94*, 258–267. [CrossRef]
32. Anerud, E.; Bergstrom, D.; Routa, J.; Eliasson, L. Fuel quality and dry matter losses of stored wood chips-influence of cover material. *Biomass Bioenergy* **2021**, *150*, 106109. [CrossRef]
33. Manzone, M.; Balsari, P. Poplar woodchip storage in small and medium piles with different forms, densities and volumes. *Biomass Bioenergy* **2016**, *87*, 162–168. [CrossRef]
34. Lenz, H.; Pecenka, R.; Idler, C.; Dumfort, S.; Whittaker, C.; Ammon, C.; Hartung, E. Continuous weighing of a pile of poplar wood chips—A comparison of methods to determine the dry matter losses during storage. *Biomass Bioenergy* **2017**, *96*, 119–129. [CrossRef]
35. Greaves, H. The bacterial factor in wood decay. *Wood Sci. Technol.* **1971**, *5*, 6–16. [CrossRef]
36. Blanchette, R.A.; Nilsson, T.; Daniel, G.; Abad, A. Biological degradation of wood. In *Archaeological Wood*; American Chemical Society: Washington, DC, USA, 1990; Volume 225, pp. 141–174.
37. Singh, A.P.; Kim, Y.S.; Singh, T. Chapter 9—Bacterial degradation of wood. In *Secondary Xylem Biology*; Kim, Y.S., Funada, R., Singh, A.P., Eds.; Academic Press: Boston, MA, USA, 2016; pp. 169–190.
38. Pastorelli, R.; Paletto, A.; Agnelli, A.E.; Lagomarsino, A.; De Meo, I. Microbial communities associated with decomposing deadwood of downy birch in a natural forest in Khibiny Mountains (Kola Peninsula, Russian Federation). *For. Ecol. Manag.* **2020**, *455*, 117643. [CrossRef]
39. Moll, J.; Kellner, H.; Leonhardt, S.; Stengel, E.; Dahl, A.; Bassler, C.; Buscot, F.; Hofrichter, M.; Hoppe, B. Bacteria inhabiting deadwood of 13 tree species are heterogeneously distributed between sapwood and heartwood. *Environ. Microbiol.* **2018**, *20*, 3744–3756. [CrossRef] [PubMed]
40. Daniel, G. Chapter 8—Fungal degradation of wood cell walls. In *Secondary Xylem Biology*; Kim, Y.S., Funada, R., Singh, A.P., Eds.; Academic Press: Boston, MA, USA, 2016; pp. 131–167.
41. Schmidt, O. *Wood and Tree Fungi*; Springer: Berlin/Heidelberg, Germany, 2006.
42. Eriksson, K.-E.L.; Blanchette, R.; Ander, P. *Microbial and Enzymatic Degradation of Wood and Wood Components*; Springer: Berlin/Heidelberg, Germany, 1990.
43. Johnston, S.R.; Boddy, L.; Weightman, A.J. Bacteria in decomposing wood and their interactions with wood-decay fungi. *FEMS Microbiol. Ecol.* **2016**, *92*, fiw179. [CrossRef] [PubMed]
44. Tlaskal, V.; Baldrian, P. Deadwood-inhabiting bacteria show adaptations to changing carbon and nitrogen availability during decomposition. *Front. Microbiol.* **2021**, *12*, 685303. [CrossRef] [PubMed]
45. Gómez-Brandón, M.; Probst, M.; Siles, J.A.; Peintner, U.; Bardelli, T.; Egli, M.; Insam, H.; Ascher-Jenull, J. Fungal communities and their association with nitrogen-fixing bacteria affect early decomposition of Norway spruce deadwood. *Sci. Rep.* **2020**, *10*, 8025. [CrossRef] [PubMed]
46. Van der Lelie, D.; Taghavi, S.; McCorkle, S.M.; Li, L.L.; Malfatti, S.A.; Monteleone, D.; Donohoe, B.S.; Ding, S.Y.; Adney, W.S.; Himmel, M.E.; et al. The metagenome of an anaerobic microbial community decomposing poplar wood chips. *PLoS ONE* **2012**, *7*, e36740. [CrossRef]
47. Kretschmar, E.I.; Gelbrich, J.; Militz, H.; Lamersdorf, N. Studying bacterial wood decay under low oxygen conditions—Results of microcosm experiments. *Int. Biodeterior. Biodegrad.* **2008**, *61*, 69–84. [CrossRef]
48. Zöhrer, J.; Probst, M.; Dumfort, S.; Lenz, H.; Pecenka, R.; Insam, H.; Ascher-Jenull, J. Molecular monitoring of the poplar wood chip microbiome as a function of storage strategy. *Int. Biodeterior. Biodegrad.* **2021**, *156*, 105133. [CrossRef]
49. Probst, M.; Gómez-Brandón, M.; Bardelli, T.; Egli, M.; Insam, H.; Ascher-Jenull, J. Bacterial communities of decaying Norway spruce follow distinct slope exposure and time-dependent trajectories. *Environ. Microbiol.* **2018**, *20*, 3657–3670. [CrossRef] [PubMed]
50. Dumfort, S.; Pecenka, R.; Ascher-Jenull, J.; Peintner, U.; Insam, H.; Lenz, H. The potential of calcium hydroxide to reduce storage losses: A four months monitoring study of spruce wood chip piles at industrial scale. *Fuel* **2021**, *298*, 120738. [CrossRef]
51. Pecenka, R.; Lenz, H.; Jekayinfa, S.O.; Hoffmann, T. Influence of tree species, harvesting method and storage on energy demand and wood chip quality when chipping poplar, willow and black locust. *Agriculture* **2020**, *10*, 116. [CrossRef]
52. *EN ISO 17827-1*; Solid Biofuels—Determination of Particle Size Distribution for Uncompressed Fuels—Part 1: Oscillating Screen Method Using Sieves with Apertures of 3.15 mm and above. ISO: Geneva, Switzerland, 2016.
53. *EN ISO 17225-1*; Solid Biofuels—Fuel Specifications and Classes—Part 1: General Requirements. ISO: Geneva, Switzerland, 2014.
54. *EN ISO 18134-2*; Solid Biofuels—Determination of Moisture Content—Oven Dry Method—Part 2: Total Moisture—Simplified Method. ISO: Geneva, Switzerland, 2017.
55. Hocking, A.D.; Pitt, J.I. Dichloran-glycerol medium for enumeration of xerophilic fungi from low-moisture foods. *Appl. Environ. Microb.* **1980**, *39*, 488–492. [CrossRef] [PubMed]

56. *DIN 38404-5:2009*; Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung—Physikalische und Physikalisch-Chemische Kenngrößen (Gruppe c)—Teil 5: Bestimmung des ph-Werts (c 5). DIN: Berlin, Germany, 2009.
57. VDLUFA. *Methodenbuch Band iii "Die Chemische Untersuchung von Futtermitteln" (the Chemical Inspection of Feedstuffs)*, 3rd ed.; Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten: Speyer, Germany, 1997; Chapter 4.1.2.
58. *EN ISO 14775*; Solid Biofuels—Determination of Ash Content. ISO: Geneva, Switzerland, 2009.
59. Lohmann, U. *Holz Handbuch*; DRW-Verlag: Leinfelden-Echterdingen, Germany, 2001.
60. Hill, C.A.S. *Wood Modification*; Wiley: West Sussex, UK, 2006.
61. Panshin, A.J.; de Zeeuw, C. *Textbook of Wood Technology*, 4th ed.; McGraw-Hill: New York, NY, USA, 1980.
62. Biyada, S.; Merzouki, M.; Demcenko, T.; Vasiliauskiene, D.; Ivanec-Goranina, R.; Urbonavicius, J.; Marciulaitiene, E.; Vasarevicius, S.; Benlemlih, M. Microbial community dynamics in the mesophilic and thermophilic phases of textile waste composting identified through next-generation sequencing. *Sci. Rep.* **2021**, *11*, 23624. [CrossRef] [PubMed]
63. Nannipieri, P.; Ascher-Jenull, J.; Ceccherini, M.T.; Pietramellara, G.; Renella, G.; Schloter, M. Beyond microbial diversity for predicting soil functions: A mini review. *Pedosphere* **2020**, *30*, 5–17. [CrossRef]
64. Nannipieri, P.; Ascher, J.; Ceccherini, M.T.; Landi, L.; Pietramellara, G.; Renella, G. Microbial diversity and soil functions. *Eur. J. Soil Sci.* **2003**, *54*, 655–670. [CrossRef]
65. Hoppe, B.; Purahong, W.; Wubet, T.; Kahl, T.; Bauhus, J.; Arnstadt, T.; Hofrichter, M.; Buscot, F.; Kruger, D. Linking molecular deadwood-inhabiting fungal diversity and community dynamics to ecosystem functions and processes in Central European forests. *Fungal Divers.* **2016**, *77*, 367–379. [CrossRef]
66. Hoppe, B.; Kruger, D.; Kahl, T.; Arnstadt, T.; Buscat, F.; Bauhus, J.; Wubet, T. A pyrosequencing insight into sprawling bacterial diversity and community dynamics in decaying deadwood logs of Fagus sylvatica and Picea abies. *Sci. Rep.* **2015**, *5*, 9456. [CrossRef] [PubMed]
67. Kahl, T.; Arnstadt, T.; Baber, K.; Bassler, C.; Bauhus, J.; Borken, W.; Buscot, F.; Floren, A.; Heibl, C.; Hessenmoller, D.; et al. Wood decay rates of 13 temperate tree species in relation to wood properties, enzyme activities and organismic diversities. *For. Ecol. Manag.* **2017**, *391*, 86–95. [CrossRef]
68. Krah, F.S.; Seibold, S.; Brandl, R.; Baldrian, P.; Muller, J.; Bassler, C. Independent effects of host and environment on the diversity of wood-inhabiting fungi. *J. Ecol.* **2018**, *106*, 1428–1442. [CrossRef]
69. Humar, M.; Petric, M.; Pohleven, F. Changes of the pH value of impregnated wood during exposure to wood-rotting fungi. *Holz Roh Werkst.* **2001**, *59*, 288–293. [CrossRef]
70. Hartmann, H.; Reisinger, K.; Turowski, P.; Roßmann, P. *Handbuch Bioenergie Kleinanlagen*; FNR: Guelzow, Germany, 2013.