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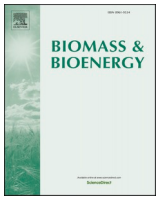

The effect of calcium hydroxide on the storage behaviour of poplar wood chips in open-air piles

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ABSTRACT

Biomass degradation by microorganisms may cause major losses during the storage of wood chips for energy production. Poplar wood chips from short rotation coppices are especially prone to degradation with dry matter losses (DML) of up to 25% within a storage period, emphasizing the need for countermeasures. Therefore, we investigated the potential of the addition of alkaline $Ca(OH)_2$ to the wet biomass of poplar wood chips and hypothesised that the establishment of an alkaline environment would reduce the activity of fungi, the primary wood degraders. Three industrial-scale piles (250 m^3) with 0, 1.5 and 3% $Ca(OH)_2$ were installed in Güssing, Austria and for four months (April–August 2019) the pile temperature, pH, moisture content, gas evolution (O_2, CO_2, H_2, H_2S, CH_4) as well as DML were monitored. $Ca(OH)_2$ altered the physicochemical properties of the wood chips but did not prevent biomass losses. However, as compared to literature, the DML were, compared to earlier investigations, also low in the control. In addition, cultivation methods were performed to evaluate the diversity of thermophilic microbes throughout the storage. Numerous filamentous fungi belonging to the phyla Ascomycota and Mucoromycota were isolated, being *Rhizomucor pusillus, Aspergillus fumigatus*, *Thermomyces lanuginosa* and *Thermoascus aurantiacus* the dominant species. Only minor differences in the fungal composition were detected as a result of $Ca(OH)_2$ addition. Instead, clear shifts in colony forming units (CFUs) were detected as a function of progressing storage time, with a decrease of the number of propagules after four months.

1. Introduction

Wood chip storage is an important yet problematic step of the wood chip supply chain as considerable losses of biomass, i.e. dry matter, may occur [1–5]. This dry matter loss is mainly caused by a consortium of different wood-decaying microorganisms. Even though bacteria are capable of degrading woody biomass, fungi seem to be the main decomposers, especially in aerobic environments [6–8]. Amongst them, brown-, white- and soft-rot fungi can be distinguished, depending on their ability to degrade the major polymers of the woody biomass, i.e. cellulose, hemicellulose and lignin [6,9]. Besides the composition of the autochthonous microbiota present in the fresh biomass, the overall level of wood decay is determined by environmental conditions (temperature, moisture content, oxygen availability, pH, chip size, storage procedure, weather conditions) as well as by the biomass composition (tree species) [2,10,11]. For example, during storage periods of seven to ten months, recent studies reported dry matter losses (DML) of up to 11% and 32% for wood chips originating either from coniferous trees (spruce, pine) [2,12] or short rotation coppice (poplar, willow) [5,12,13], respectively. However, the comparison of different studies is difficult due to variations in experimental designs and storage conditions. Furthermore, the wood degradation process is very complex and dynamic due to co-dependencies among temperature, atmospheric conditions (oxygen concentration), moisture content, substrate composition as well as the microbiota present in the substrate. Nevertheless, numerous studies have contributed to a better understanding of the overall characteristics of wood degradation. Highest DML were proven within the first four storage months, where the temperature inside storage piles rises erratic

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to values of 30 °C or more for coarse wood chips and up to 60 °C for fine chipped biomass [2,4,10,14]. This initial temperature increase bears the risk for partial pyrolytic degradation of wood chips inside the areas with low oxygen concentrations that can subsequently result in self-ignition [15–17].

In the above context, microbially-driven exothermic reactions during wood decomposition play a significant role in the heating of wood chip piles [18]. However, the increase in temperature is also associated with a certain sanitization of the autochthonous microbiota in favour of thermophilic and thermotolerant microbes. This selective process is commonly known as 'microbial suicide' and has been described for similar environments such as compost [18–20]. The microbiota which survives (or even gets activated by) high temperatures is therefore supposed to establish communities which are adapted to degrade the woody biomass [21]. Thus, countermeasures focus especially on the inhibition of the microbiota by withdrawing water, which is essential for their activity. Measures to minimize DML include optimized storage concepts (smaller piles, natural drying, cover to prevent rewetting) as well as technical drying of the biomass [12,22,23].

In our previous study [14], we tested a promising alternative approach by using an alkaline additive, namely calcium hydroxide ($Ca(OH)_2$) that has been mixed with freshly chipped forest residues from Norway spruce (*Picea abies*). The induced shifts in wood pH from its natural acidic value to an alkaline environment led to a significant decrease of DML by 6% points compared to untreated wood chips. Generally, the pH of wood varies between 3 and 6, depending on the wood species and degree of decay [24,25]. Especially fungi are known for their optimal growth under acidic conditions and consequently, the wood degradation could be slowed down under alkaline conditions. A similar approach was reported by [26] where the mold degree was reduced effectively applying calcium oxide (CaO). Additionally, slagging tendencies of fuel ash during the combustion of these amended wood chips were reduced. However, CaO reacts with water in a strong exothermic reaction to $Ca(OH)_2$ leading to an increase in the pile temperature. Additionally, due to this reaction, pH drops within the first storage month, weakening the effect. To avoid this, $Ca(OH)_2$ was selected as a suitable additive since the hydroxide ion serves as a strong base, increasing the pH of wood significantly.

In this study, the influence of $Ca(OH)_2$ on the storage of poplar wood chips was investigated at industrial scale (250 m^3) in Güssing (Austria) aiming to determine and monitor i) the overall storage behaviour (temperature, pH, moisture development, and gas evolution), ii) DML as well as iii) the cultivable fraction of the thermotolerant/thermophilic and saproxylic mycobiota. We hypothesised a significant reduction of DML for wood chip piles treated with $Ca(OH)_2$ and, based on previous studies with spruce forest residues, no adverse effects on the storage behaviour. As our first storage experiment at industrial-scale was run with spruce forest residues, the present study was conducted with poplar wood chips to further investigate the hypothesised quality maintaining potential of slaked lime on wood chips as a function of three species (soft vs. hard wood). Given that the chemical composition as well as the autochthonous microbiota of poplar wood chips differ from spruce wood chips, differences are to be expected regarding DML, temperature development as well as in the cultivable fraction of the thermotolerant/thermophilic wood chip mycobiota as a function of treatment (additive) and storage time.

Molecular analysis such as amplicon sequencing [27] and quantitative real-time PCR [21] have already been conducted for this storage experiment to gain profound insights into the composition and abundance of the saproxylic microbiota. However, these analyses were based on the whole community DNA which does not allow the distinction between living and dead and/or active and inactive members of microbial communities. Even though just a small portion of the entire microbiota can be cultivated (1–10% [20]), we focused on the culturable fraction of thermophilic/thermotolerant fungi as they are supposed to be among the main wood decomposers affected by the alkaline additive and by the elevated temperatures occurring during the initial stages of storage.

2. Material and methods

2.1. Experimental set-up and sampling

The experiment was carried out from April to August 2019 in Güssing, Austria (47° 03′ N, 16° 19' E) with wood chips from *Populus canadensis*. Ten years old poplar trees were harvested in the end of January 2019 and trunks were stored on the field until the end of March 2019. Wood chips were produced by a commercial mobile chipper (Mus-Max WT12, MUS MAX GmbH, Austria, 100 × 100 mm screen; 1st of April 2019) and three piles of 250 m^3 (12 m × 6 m x 3.5 m) were installed on an asphalted area (Fig. 1). To investigate the influence of $Ca(OH)_2$ on the storage behaviour, freshly chipped poplar wood chips were mixed with $Ca(OH)_2$ with the help of a wheel loader. To achieve a good homogenization, a full bucket (of the wheel loader) of wood chips was spread on the ground and the weighed amount of additive was spread on the wood chips. The mixture was homogenized by turning with the wheel loader and piling it inside the storage hall of the heating plant. The designed concentrations of $Ca(OH)_2$ in the dry wood chips were 0, 1.5 and 3.0%. The number of full buckets of the wheel loader set the volume of each pile and the resulting fresh mass of the wood chips was calculated by means of bulk density. After determining the initial moisture content, the actual set additive concentrations were calculated, resulting in values of 1.5% and 2.9%. Table 1 gives an overview of the volume and composition of each pile.

For periodic recording of the principal storage parameters, four stainless steel grit columns (height 2.5 m, diameter 0.64 m, mesh size 20 mm) were placed within each pile (Fig. 2). All columns were equipped with a Gemini Tinytag TGP-4017 temperature data logger at a height of 1.6 m for continuous temperature monitoring. The last column was equipped with in total six temperature data logger at 0.8 m, 1.6 m and 2.4 m (three per height) to measure temperature at different heights as well as to generate redundancy in case a temperature data logger fails. Polyamide pipes were attached to each column at 0.8 m, 1.6 m and 2.4 m, to measure the gas composition in regard of O_2, CO_2, H_2, H_2S and CH_4. Gas measurement was carried out weekly by a gas monitoring device (Draeger X-am®7000, Dräger AG, Germany) with an internal pump. Before entering the Draeger device, the gas stream was dried by attaching the pipe to a gas washing bottle filled with silica gel, as well as to a syringe filter (ReZist, PTFE, 50 mm, 0,20 μm) to filter any particles left in the gas stream. After ~5 min of measuring a constant value has set in, was noted and the pipe removed. Per height, one measurement has been performed.

Dry matter loss (DML) was determined according to [4,14]. For the assessment of DML, 18 balance bags (plastic net bags, mesh size 1 × 1 mm) were filled with 2 kg fresh wood-chip-additive-blends, weighed and placed at three heights (0.8 m, 1.6 m, 2.4 m) within the measuring columns. In a four-week interval (t1, t2, t3, t4), one column of each pile was removed with a truck crane (destructive sampling) to retrieve the balance bags, which were immediately weighed and dried to determine the moisture content. 20 wood chip samples (~300 g fresh mass) collected just before assembling the piles served as control (t0). These samples were combined to receive 3 samples that served for the determination of the physicochemical properties (ash content, pH, EC, C, N) and the microbiological analyses. MC of the sample bags represent the larger inner part of the storage pile and not the peripheral layers which are influenced by the weather. Therefore, a moisture profile with 26 sampling points (Figure A1) of each pile was determined over the cross-section of each pile at the end of the storage experiment. These 26 samples, evenly distributed across the cross-section, were then used to create a moisture profile across the entire cross-section for every pile by means of interpolation.

DML at the sampling time i is calculated using Equation (1). All used

Fig. 1. Installation of the measuring columns (left) and construction of the wood chip piles (right).

Table 1
Composition and calculated additive concentration of the three wood chip piles at storage intake. Dry mass of wood chips was calculated for a measured moisture content of fresh biomass of 52.4%.

Pile number	Volume wood chips	Fresh mass wood chips	Dry mass wood chips	Mass Ca $(OH)_2$	Concentration $Ca(OH)_2$
	m^3	kg	kg	kg	%
1	294	83,496	39,744	0	0
2	245	69,580	33,120	496	1.5
3	252	71,568	34,066	970	2.9

masses at storage intake exclude the mass of added $Ca(OH)_2$. In a wet environment, the applied slaked lime reacts with atmospheric CO_2 forming calcium carbonate (calcite), water and heat according to Equation (2).

$$DML_i = \left(1 - \frac{m_{out,i}(100 - x_{out,i}) - m_{Ca(OH)_2}}{m_{in}(100 - x_{in}) - m_{CaCO_3,i}}\right) \bullet 100 \quad (1)$$

$$Ca(OH)_2 + CO_2 \leftrightarrows CaCO_3 + H_2O \quad (2)$$

This chemical reaction leads to the absorption of CO_2, thus increasing the mass of the applied additive. This increase in additive mass has to be considered when calculating the DML_i. The resulting mass of $CaCO_3$ is calculated by Equation (3).

$$m_{CaCO_3} = \frac{m_{Ca(OH)_2} \bullet M_{CaCO_3}}{M_{Ca(OH)_2}} \quad (3)$$

DML_i dry matter loss at time i [%]
m_{in} wet mass at storage intake [kg]
$m_{out,i}$ wet mass at storage outtake at time i [kg]
x_{in} moisture content at storage intake [%]
$x_{out,i}$ moisture content at storage outtake at time i [%]
$m_{Ca(OH)2}$ dry mass of added $Ca(OH)_2$ at storage intake [kg]
$m_{CaCO3,i}$ dry mass of formed $CaCO_3$ at time i [kg]
$M_{Ca(OH)2}$ M mass of $Ca(OH)_2$ [g mol^{-1}]
M_{CaCO3} molar mass of $CaCO_3$ [g mol^{-1}]

Additionally, samples were collected for microbiological analyses; to obtain representative samples, aliquots (ca. 300 g) from two balance bags were pooled, resulting in a total of three composite replicates (n = 3) of each column at the middle of the test piles (at 1.6 m), being the most representative and thus, the most relevant portion of the entire

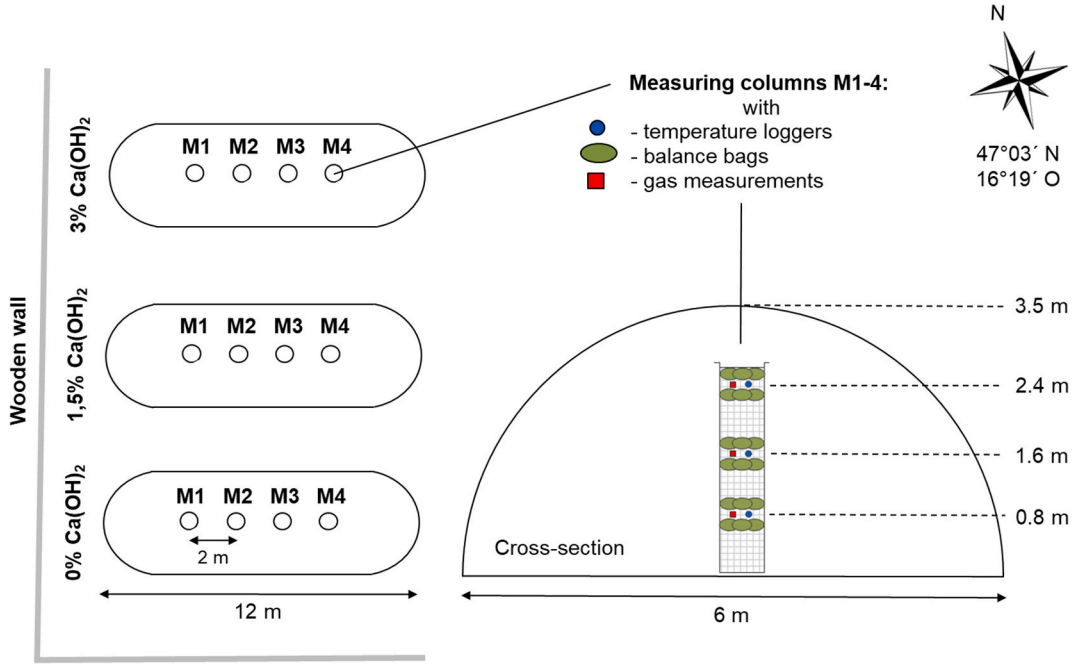

Fig. 2. Schematic structure of the storage piles with measuring columns (M1 to M4).

piles for assessing its cultivable mycobiota. Samples were immediately transported to the laboratory and stored at 4 °C until the isolation of thermophilic and thermotolerant fungi.

It must be noted that in this study, all moisture contents are defined as the moisture mass fraction on a wet-weight basis. All investigated parameters and the respective methodology are given in Table 2.

2.2. Microbiological analyses: dilution vs. direct plating of the thermophilic and thermotolerant saproxylic mycobiota

Cultivation experiments were carried out to evaluate the species diversity of thermophilic fungi associated to poplar wood chips amended with specific amounts (0%, 1.5% and 2.9%) of the alkaline additive $Ca(OH)_2$ after one month (t1, short-term) and four months (t4, long-term) of storage.

Fungal isolation was carried out by the serial dilution method modified by [34]. To estimate the fungal viable load which was formed after the initial sanitization due to elevated temperatures at storage intake, samples of short- and long-term stored wood chips were pre-treated at a temperature of 45 °C in an incubator for 2 days, to enhance the thermophiles. Aliquots (20 g) of the preheated material were ground and homogenized using a mixer-mill disruptor (MM 400, Retsch, Germany) at 25Hz for 25s. Serial dilutions were made by suspending 1 g of homogenized sample in 10 mL of PBS buffer and shaken at 3000 rpm for 1 min. Dilutions of 10^{-3} and 10^{-4} were used to isolate fungi in order to avoid over-crowding of the plates. 100 μL of the suspension of each concentration were spread on sterile Petri dishes, in triplicates of each dilution, containing sterile yeast extract soluble starch agar (YpSs) medium with chloramphenicol (50 mg mL^{-1}) to prevent bacterial growth. The plates were then incubated at 45 ± 1 °C for a total of 10 days and monitored at regular time intervals to detect the appearance of colonies. Fungal CFUs were counted and transferred for identification. Isolates were divided into different 'morphotypes' based on morphological features such as obverse and reverse colony colors, colony texture, degree of sporulation, production of soluble pigments and exudates. Sporulating fungi were identified based on the morphological characteristics of the conidiophores and conidia observed under microscope (Eclipse 80i Nikon, USA). The fungal isolates were identified through their cultural and morphological profiles compared with available literature.

Besides the dilution plating, fungal isolation was carried out using a modified direct plating method [35] to increase the possibility of isolating fungal species present in the samples. Randomly selected wood chips were therefore surface-flamed and small wood fragments (about 0.5 × 0.5 cm) were removed and placed into petri dishes containing selective YpSs medium with chloramphenicol as described before. Likewise, the plates were then incubated at 45 ± 1 °C for a total of 10 days and monitored at regular time intervals for fungal growth. Fungal strains that have grown on the surface of the wood chip surface were isolated and identified as described above. After ensuring purity, fungal isolates were sub-cultured on YpSs agar slants for 5–7 days and subsequently stored at 4 °C for morpho-taxonomical characterisation.

Table 2
Investigated parameters including the methodology and devices. The sample numbers per wood chip pile and sampling time are also given.

Parameter	Unit	Samples per pile and sampling time	Methodology
[a]Ash content	%	n = 3	ISO 18122:2022 [28]
[a]pH	–	n_{t0} = 3 n_{t1} - n_{t4} = 9	wood:water extracts (1:20, w:v), pH-Meter Metrohm 826 (Metrohm, Switzerland) [29]
[a]Electrical conductivity (EC)	μS cm^{-1}	n = 3	wood:water extracts (1:20, w:v), conductivity Meter LF 330 WTW (Weilheim, Germany) [29]
[a]C:N	%	n = 3	CN analyser, TruSpec (Leco, USA) [29]
[a]SOM (volatile solids, VS)	%	n = 3	mass loss following ignition in a muffle furnace, Carbolite CWF 1000 (Carbolite Gero, Germany) [29]
Moisture Content	%		EN ISO 18134-2 [30]
at storage intake		n = 20	
at storage outtake		n = 18	
Cross-section		n = 26	
Dry matter loss	%	n = 18	Equations (2) and (3)
Particle size distribution	%	n = 3	EN 15149-1 [31]
Class characterization	–	–	EN ISO 17225-4 [32]
Bulk density	kg m^{-3}	n = 1	ÖNORM EN ISO 17828 [33]
CO_2 concentration	%	n = 3	IR- sensor, Draeger X-am®7000
CH_4 concentration	%	n = 3	IR-sensor, Draeger X-am®7000
O_2 concentration	%	n = 3	Electrochemical sensor, Draeger X-am®7000
H_2S concentration	ppm	n = 3	Electrochemical sensor, Draeger X-am®7000
H_2 concentration	ppm	n = 3	Electrochemical sensor, Draeger X-am®7000
Weather conditions			ZAMG weather station
Ambient temperature	°C	–	Güssing
Precipitation	mm	–	
Relative humidity	%	–	

[a] Analyses were performed with cut-milled samples (Fritsch Pulverisette 19, Ø 2 mm).

2.3. Statistical analyses

The effect of categorical variables ($Ca(OH)_2$ addition, storage time and sampling height) on physicochemical properties including moisture content, pH, EC, C:N and ash content was analysed by non-parametric Kruskal-Wallis tests, which were executed in R v.3.6.2 [36]. Prior to this, data were tested for normal distribution (Shapiro-Wilk-Test) and homoscedasticity (Levene-Test) which was rejected by many cases which is why a non-parametric test has been chosen. Post-hoc tests according to Dunn were used to perform pairwise comparisons (package PMCMR v.4.3) [37]. Results were considered significant if $p \leq 5\%$.

3. Results

3.1. Raw material properties

The mean material-descriptive parameters at the time of storage intake (t0) are given in Table 3. The initial moisture content of the different wood chip-additive blends varied between 50 and 52%, which is typical for freshly harvested poplar wood chips [4,10]. pH ($p = 0.027$), EC ($p = 0.027$) and ash content ($p = 0.039$) were higher in both biomass-additive blends (1.5% and 2.9%) compared to the unamended wood chips. No immediate effect was observed for the other variables. The addition of $Ca(OH)_2$ raised the pH from 5.2 to 6.5 (1.5% additive)

Table 3
Overview of the mean material-descriptive parameters (±standard deviation) at storage intake (t0).

Wood chip-additive blend	Moisture content	Ash content	pH	EC	C	N
	%	%	–	μS cm^{-1}	%	%
0% $Ca(OH)_2$	52.4 ± 2.60	2.0 ± 0.32	5.2 ± 0.22	273 ± 34.0	46.8 ± 0.34	0.21 ± 0.151
1.5% $Ca(OH)_2$	50.5 ± 1.96	3.7 ± 0.17	6.5 ± 0.13	687 ± 17.0	46.4 ± 1.50	0.32 ± 0.127
2.9% $Ca(OH)_2$	50.2 ± 1.49	5.0 ± 1.03	7.2 ± 0.37	853 ± 57.3	45.0 ± 0.60	0.41 ± 0.046

and 7.2 (2.9% additive). The particle size distribution of all three wood-chip-additive blends corresponded to class P45 (Fig. 3).

3.2. Weather conditions

The long-term median value of the average daily ambient temperature (16.2 °C) and precipitation sum (322 mm) were calculated for the period from April to July (1990–2019) based on the data provided by the ZAMG (Zentralanstalt für Meteorologie und Geodynamik, Austria). During the storage experiment in 2019, an average daily temperature of 17.6 °C and a total precipitation of 265 mm were recorded (Fig. 4). Compared to the long-term median value (322 mm), the precipitation for the summer months 2019 was considerably lower and rain events differed strongly between each storage month with 33 mm (April), 122 mm (May), 30 mm (June) and 80 mm (July).

3.3. Pile temperature

Pile temperatures (Fig. 5) raised to their maximum of 61 °C (0% additive), 63 °C (1.5% additive) and 66 °C (2.9% additive) at day six after storage intake. The temperature at different height within each pile differed by 7–10 °C during the first 24 days, with the highest temperatures for the top levels. With increasing additive concentration higher maximum temperatures were reached, however, all piles subsequently cooled down to <40 °C after 23 days (0% additive) and 27 days (1.5% and 2.9% additive). After 60 days, all pile temperatures stabilized at approximately 30 °C. Apart from the first storage month, the temperature development within each pile was comparable and no difference was measured in average temperatures among treatments.

3.4. Moisture content, pH and dry matter losses

Adding dry $Ca(OH)_2$ to freshly chipped poplar wood chips reduced the initial moisture content (MC) significantly to 50.5% ($p = 0.0073$, 1.5% additive) and 50.2% ($p = 0.0026$, 2.9% additive). In the first storage month, MC decreased sharply to 31% (0% additive), 41% (1.5% additive) and 33% (2.9% additive) and stayed on a level between 30 and 40% for the rest of the storage time (Fig. 6a). MC within each measuring column showed a comparable, uniform distribution with slightly higher MCs at the top height (Appendix, Table A1).

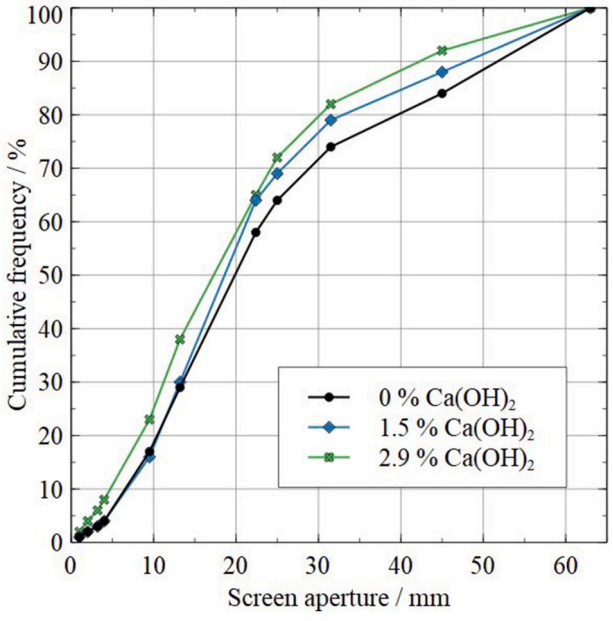

Fig. 3. Particle size distribution of poplar wood chips amended with 0, 1.5 and 2.9% $Ca(OH)_2$, respectively.

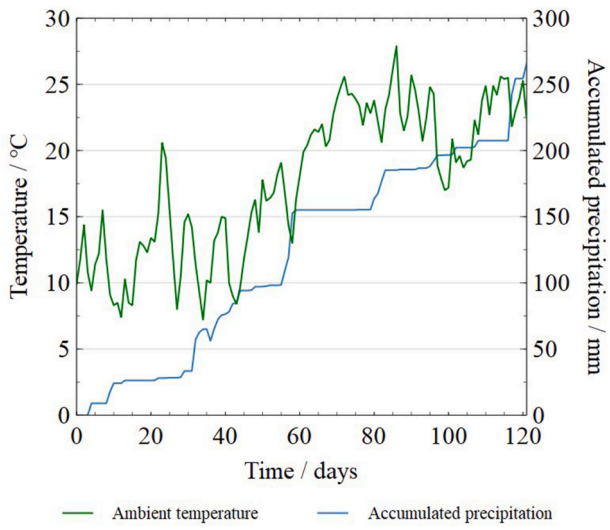

Fig. 4. Weather conditions from April to August 2019 in Güssing (Austria).

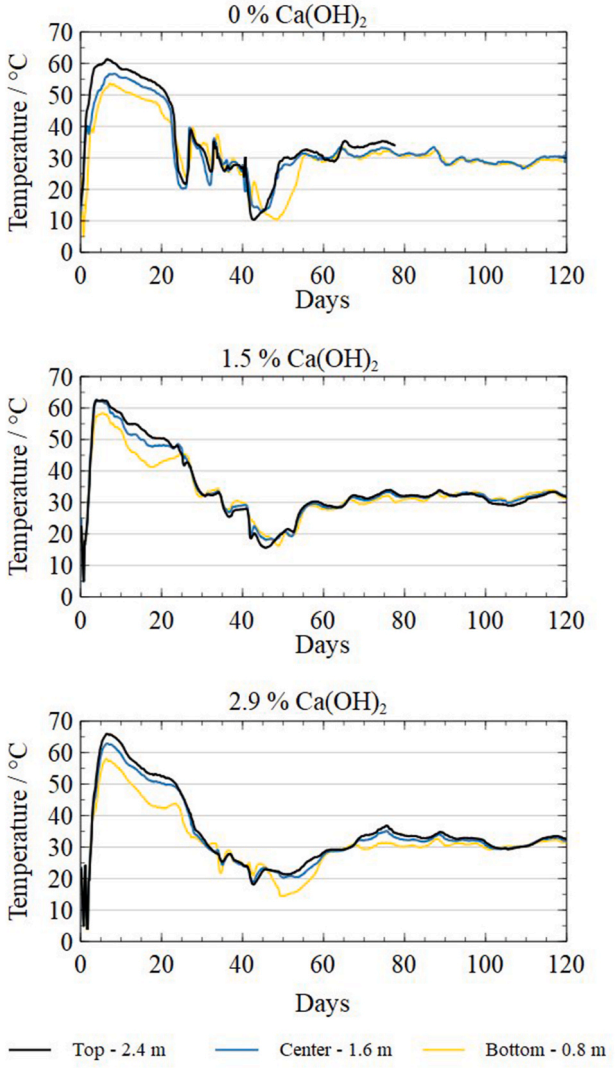

Fig. 5. Pile temperatures during the four months storage trial at three layers (top, center and bottom). The top temperature data logger of the 0% $Ca(OH)_2$ pile failed after 79 days.

MC of the wood chip samples collected over the cross-sections of the storage piles at the end of the experiment provided information about the MC distribution in the edge areas (Fig. 7). In general, the centre and lower parts of all three piles were comparably dry with MCs between 24 and 40%, whereas edges and the top showed MCs of 60–70%, reflecting the weather conditions and condensation effects. No significant differences were observed for the mean MC of the cross-sections amounting 42.8% (±13.88, 0% $Ca(OH)_2$), 41.9% (±13.89, 1.5% $Ca(OH)_2$) and 44.7% (±12.46, 2.9% $Ca(OH)_2$).

The pH of untreated poplar wood chips amounted 5.2 (±0.27). The additive led to an increase to 6.5 (±0.16) and 7.2 (±0.5) for 1.5% and 2.9% $Ca(OH)_2$, respectively. The pH of all three piles increased steadily during storage resulting in 6.7 (±0.18) for untreated wood chips, 7.6 (±0.11) for wood chips with 1.5% additive and 8.0 (±0.20) for wood chips with 2.9% additive (Fig. 6b).

The monthly dry matter losses (DMLs) are given in Fig. 8. The boxes represent the interquartile range (IQR, middle 50% of the data) whereby the central lines represent the median value and crosses the mean value. Whiskers represent the variability of the data outside the interquartile range, extending to 1.5 times the IQR. The DML of untreated poplar wood chips resulted in negative losses in the first two months, and a DML of close to zero after the third month (Fig. 8a). After the fourth storage month, the DML amounted 6.9% (±4.5). In general, a characteristic trend with a continuous increase of DMLs over time was observed. For the pile treated with 1.5% additive, DML increased within the first storage month to 7.8% (Fig. 8b), however, there was no significant change in DML in the following storage months ($p = 0.637$). DML of poplar wood chips treated with 2.9% additive showed a typical trend with a continuous increase over time (Fig. 8c) and amounted 7.7% (±2.9) after four months ($p < 0.001$). The heterogenic nature of poplar wood chips resulted in comparably high standard deviations (Fig. 8).

3.5. Gaseous emissions

After storage intake, O_2-content decreased slightly to 18% (all piles) and simultaneously, CO_2 increased to a maximum value of 2.6% (0% additive), 2.6% (1.5% additive) and 2.4% (2.9% additive) at day 8 (Fig. 9). After this peak, CO_2 concentrations decreased and fluctuated around 1.0%, showing no difference among treatments. Analogous, hydrogen concentration increased within the first week reaching maximum values of 40 ppm (0% additive), 33 ppm (1.5% additive) and 57 ppm (2.9% additive). Subsequently, the hydrogen concentration decreased, and no hydrogen could be measured after 30 days of storage. No methane or hydrogen sulphide was detected over the whole storage period.

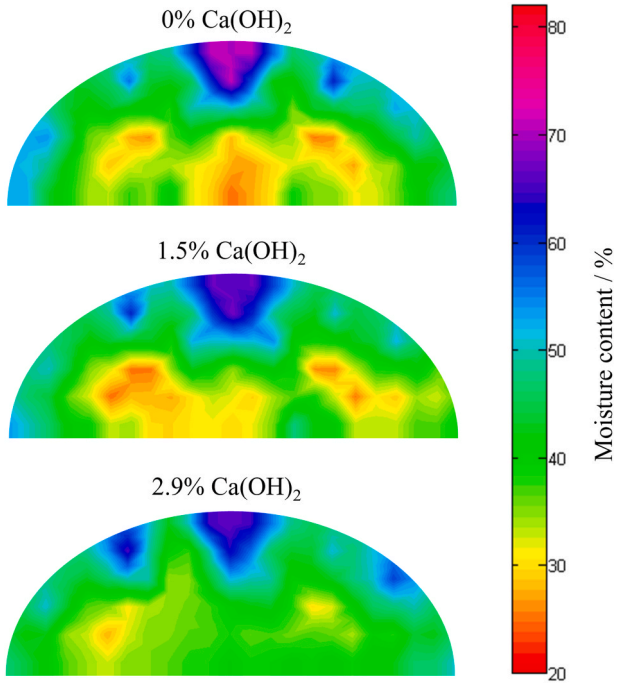

Fig. 7. Moisture content (MC) distribution along a cross-section of the three test piles after four months of storage (t4).

3.6. Microbiological analyses: dilution vs. direct plating of the thermophilic and thermotolerant saproxylic mycobiota (t1 vs. t4)

The fungal community composition was determined based on the identification of morphologically distinct colonies isolated by using both the dilution and direct plating method (Table 4). Isolations of serial dilution led to 55 and 33 single cultures from samples collected after one and four months of storage, respectively. In contrast, 55 and 42 purified cultures were isolated by the direct plating method after one and four months of storage, respectively. In total, these 193 purified cultures capable of growing at 45 °C were grouped in 9 fungal types.

Differences in fungal composition were found as a function of storage time and $Ca(OH)_2$ treatment in direct plate method. Colonies of *A. fumigatus* were the most representative in samples without additive

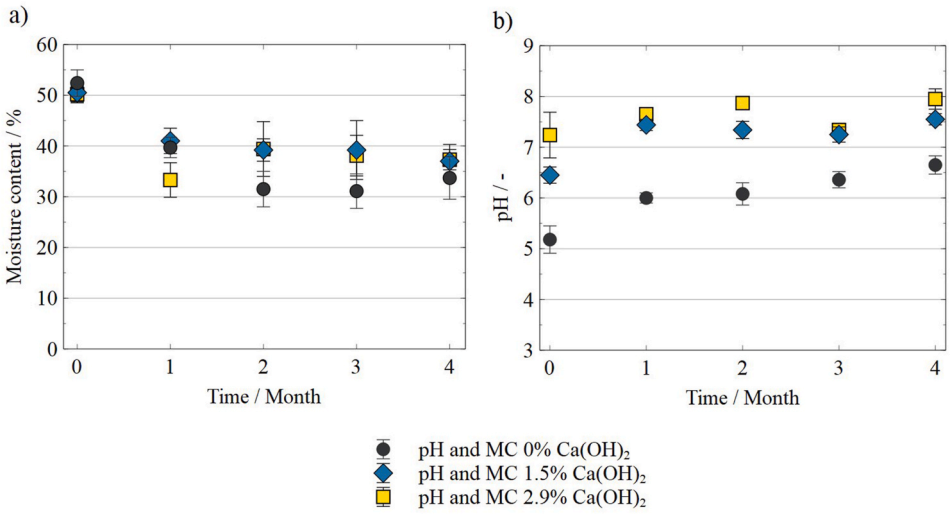

Fig. 6. Development of the (a) moisture content (MC) (n = 18) and (b) pH (n = 9) during the four months of storage. Whiskers indicate the standard deviation.

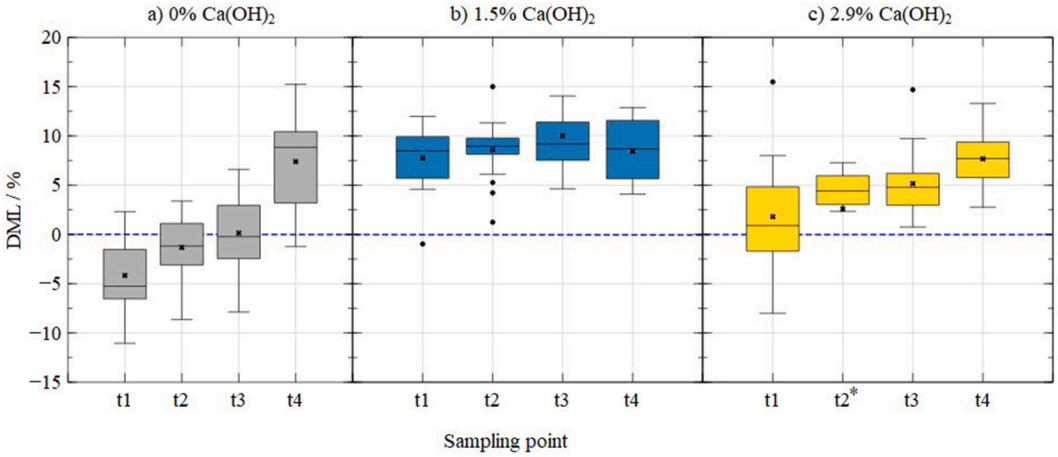

Fig. 8. Dry matter losses (DMLs) of poplar wood chips without alkaline amendment (a), and supplemented with 1.5% $Ca(OH)_2$ (b) and 2.9% $Ca(OH)_2$ (c) during four storage months (t1-t4). Box = IQR (25–75%); whisker = distance of 1.5 times the IQR; central line = median value, cross = mean value. * indicates a not shown value of −33.5% (t2, 2.9% $Ca(OH)_2$).

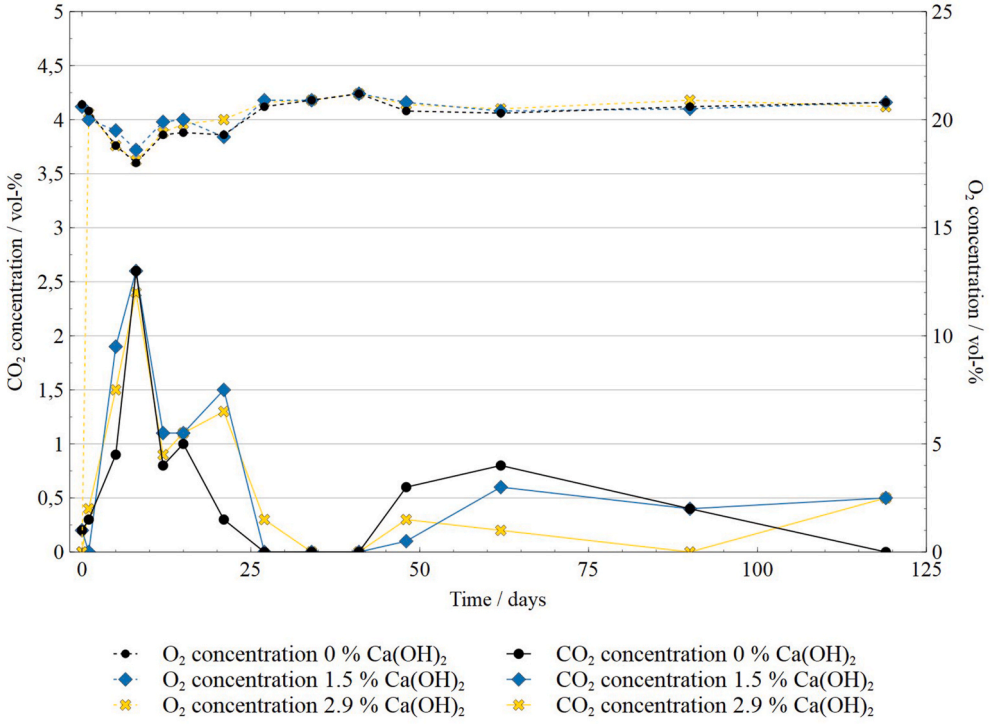

Fig. 9. O_2 and CO_2 concentrations inside the different piles.

(0% Ca $(OH)_2$) at short-term storage (t1), whereas the number of colonies was smaller in the samples with additive (1.5 and 2.9%), indicating a potential inhibitory effect of the alkaline additive on the growth of this fungus. Likewise, *R. pusillus* was the dominant species in samples without additive at t4 while a lower number of colonies was isolated in samples supplemented with 2.9% of the additive. Even though few isolates of *A. fumigatus* were obtained at t4 in samples without additive, this fungus has mainly been replaced by *R. pusillus*, which was the dominant species in this sample group. The colonies of *T. lanuginosa* as well as the total number of colonies were slightly higher from t1 to t4 in all samples.

Other taxa, such as *Thermoascus thermophilus, Paecilomyces* sp., *Chaetomium* sp. And sterile mycelia, were unevenly distributed in the samples and *Rasamsonia emersonii* was rarely isolated. However, the presence of these fungi may have been masked by some fast-growing fungi like *A. fumigatus* and *R. pusillus* which outperformed the slow-growers.

4. Discussion

The objective of this study was to investigate the quality maintaining potential of $Ca(OH)_2$ during a four months storage trial of poplar wood chips. By simulating outdoor storage scenarios at industrial scale, its effectiveness was evaluated in terms of DML. Additionally, the experimental set-up allowed the monitoring of principal pile characteristics such as MC, pH, temperature and gas emissions. Furthermore, since fungi are the primary microbial wood degraders and high temperatures (>40–60 °C) are reached immediately after storage intake, focus was

Table 4
Fungal community composition of poplar wood chips with addition of 0, 1.5 and 2.9% $Ca(OH)_2$ after one and four months of storage at height level 1.6 m.

Method	Dilution plate (10^3 cfu g^{-1})[a]						Direct plate[b]					
$Ca(OH)_2$% Addition	0		1.5		2.9		0		1.5		2.9	
Storage (month)	1	4	1	4	1	4	1	4	1	4	1	4
Aspergillus fumigatus	5	3	1	1	1	1	13	2	0	1	0	1
Chaetomium sp.	1	1	3	2	1	1	4	0	8	0	4	0
Thermoascus thermophilus	1	0	2	0	0	0	0	1	1	3	2	1
Thermomyces lanuginosus	5	3	6	3	6	3	4	0	8	0	4	0
Paecilomyces sp.	1	1	0	0	2	1	2	2	0	0	5	0
Rhizomucor pusillus	3	1	2	1	1	1	1	11	4	10	0	5
Rasamsonia emersonii	0	0	3	1	0	0	0	0	0	0	0	0
Thermoascus aurantiacus	4	1	1	1	2	2	0	2	0	0	4	1
Non identified (micelia sterile)	2	2	0	2	2	1	0	1	0	0	0	0
Number of identified colonies	**22 (± 3.08)**	**12 (± 1.01)**	**18 (± 2.93)**	**11 (± 0.72)**	**15 (± 3.26)**	**10 (± 1.10)**	**24**	**19**	**21**	**14**	**19**	**8**

[a] Mean of three replicates.
[b] Sum of colonies isolated from the wood fragments Overall, both methods revealed fungal taxa mainly affiliated to the phyla Ascomycota and Mucoromycota. The most abundant taxa were classified as *Rhizomucor pusillus* (Mucoromycota, 22.2% of purified cultures), *Thermomyces lanuginosus* (Ascomycota, 22.7%), *Aspergillus fumigatus* (Ascomycota, 15.7%) and *Thermoascus aurantiacus* (Ascomycota, 9.7%). Besides, *Paecilomyces* sp., *Thermoascus thermophilus*, *Chaetomium* sp. And *Rasamsonia emersonii* (all Ascomycota) as well as sterile (non-sporulating) mycelia were isolated.

further given on the cultivable fraction of the thermophilic/thermotolerant mycobiota as a function of additive-amendment (0% vs. 1.5% vs. 2.9% $Ca(OH)_2$) and storage time (t1 vs. t4).

The expected effect of adding $Ca(OH)_2$ is a rise in the surface pH of wood from an acidic to an alkaline pH, which is supposed to reduce the activity of wood-degrading fungi. In the present study, the addition of 1.5% and 2.9% $Ca(OH)_2$ significantly increased the pH of poplar wood chips from 5.2 to 6.5 and 7.2 ($p = 0.027$) directly after its amendment. In addition, during the storage period, the pH of all piles increased significantly ($p < 0.001$). However, the addition of $Ca(OH)_2$ did not exceed a pH of 9, which is a critical value for inhibiting microbial growth [26]. In fact, elevating pH to 12 is sometimes even suggested to suppress microorganisms of biosolids [38,39]. Preliminary investigations with different poplar and spruce wood chips at laboratory scale indicated the establishment of an alkaline environment (pH > 8), however, this was not the case for poplar wood chips that were used in this study. Our findings support that the achieved surface pH through adding Ca $(OH)_2$ strongly depends on different factors such as the wood type, age, degree of degradation, the initial wood pH and moisture content. For future studies, wood chips of different origin and age should be mixed with different amounts of additive and pH measured to gain more information on how the wood chip properties affect the pH. A higher additive dose would have led to a higher pH. Based on our measurements an additive concentration of at least 4% might be enough for increasing wood pH appropriately to an alkaline environment. However, for the practicability of this procedure, the additive dosage should be kept to a minimum since it increases the ash content of the biomass significantly. This results in higher disposal costs. Increased ash amount can influence the combustion behaviour by affecting the combustion rate, temperature distribution, and emissions. On the other hand, as an advantage, the addition of calcium-based additives can reduce slagging of ash [26]. To reliably evaluate the practicability of the here presented method, the effects of $Ca(OH)_2$ on biomass ash composition and the possible effects on combustion have to be studied in more detail for different combustion units.

The results regarding the dry matter loss (DML) of the untreated pile impedes a clear interpretation and comparison to the treated wood chip piles. In the first, second and third storage month, DML of ≤0% were measured, meaning a mass increase. This phenomenon has been reported previously, where negative DMLs occurred within the first four weeks of storage and authors suggested that DML occurring during this time is inaccurate [10,13]. However, the development of DML over time, following an already well-described trend [2,10,13,22], supports that absolute DML values have been underestimated due to measurement uncertainties at the beginning of the storage trial that might have happen in previous work too. To be more specific, the calculation of the initial dry matter (inside the sample bags) was based on the measurement of the initial MC, which has been determined by the collection of 20 samples across the chipped raw material. Due to the conscientious mixing of the raw material with additive and the subsequent sample collection, the initial MC values of the additivated piles seem to be more accurate, representing the entire wood chip pile of almost 250 m^3 compared to the untreated pile. This explanation can also be supported by a comparably higher standard deviation of the initial MC of the untreated pile. However, the negative DML might indicate a weakness of the here used method, which should be adapted and tested since the calculated initial MC has a huge impact on the determined DML. Calculating the DML for an initial MC of just 50% (instead of 52.4%), positive DML would result. It is suggested to adapt the procedure for determining the initial MC, whereby, instead of collecting random samples, MC samples should be taken directly from the sample bags. In detail, a certain amount of wood chips (e. g. 4 kg) should be prepared for filling one sample bag and split in half. One half serves for MC determination, the other half for filling the sample bag. This procedure can overcome issues due to inhomogeneity as well as it could avoid a possible drying of the wood chips during pile build-up and sample preparation.

Even though a clear scientific statement regarding the effect of Ca $(OH)_2$ on the DML of poplar wood chips cannot be made, it is worth comparing the measured DML of the two treated piles (1.5% and 2.9%) with literature values. For example, Lenz [4,13] and Pecenka [10] investigated the DML of poplar wood chips of varying chip size and under varying storage conditions. The experimental design and set-up were identical to the work presented here, however, storage experiments were conducted in Northern Germany starting in February. For poplar wood chips of the same wood chip class (P45) and a comparable initial MC of 56–58%, DML was 17% [4], 16% [13] and 16% [10] after four months of open-air storage which is twice as high as the DML found here. Other experiments with poplar wood chips of class P31 indicated monthly DMLs of 2.3–4.2% per month [4,40–42]. A certain variance in DML can be ascribed to the differences in the investigated poplar wood chips (especially differences in tree age), the storage period (winter versus spring) and weather conditions. Poplar wood chips investigated in the mentioned studies originated from 3–5-year-old trees, whereas the age of poplar trees used in this study was 10–15 years. As a result, especially the ratio of wood to bark changes with increasing tree diameter, thus presumably affecting decomposition processes. However, ash content of the mentioned poplar wood chips amounted 1.6–2.0% (versus 2.0% in this study) indicating a very similar bark content of the used wood chips. Beginning in February [4,10,13], also the storage

period differed from our study, whereby MC of the first 3 months stayed on a high level of >50% [4,13]. Interestingly, this was not the case for storage experiments by [10] where MC dropped within the first 2 months to about 40% and still DML amounted 16% after 4 months. Assuming the additive dosage did not affect the degradation processes, DML of 10–15% would have to be expected for all experimental piles supporting our hypothesis.

Within the first storage month, MC of all piles dropped significantly below 40% but the drying effect over the course of the following storage months was very low. Due to stronger rain events in May and July, MC increased especially in the upper and middle part of all piles. Therefore, MC never fell below 30%, conditions supporting microbial activities over the whole storage time [43]. The highest decrease was observed for the reference pile without any additive. Likewise, the distribution of the MC over the cross-section indicates dryer central parts of the reference pile. As described previously [10,14], the drying process started at the bottom level and central parts, with low values between 25 and 30%, whereas the outer and top layer showed values between 50 and 73%. On the contrary, this drying process could not be observed for the pile with 2.9% additive, where the bottom and centre of the pile revealed values between 30 and 41%. During our previous study [14] with spruce forest residues one year earlier (same location) the MC development showed a fast and continuous drying process of all piles even though rain events and total precipitation were higher (total precipitation of 322 mm). This indicates that the effect of wood chip origin (tree species) and particle size distribution on the drying process might be higher than the influence of seasonal weather conditions. Even though special care was taken during built-up of the experimental piles, effects by the pile structure which may occur can't be neglected. External effects due to the monthly removal of the columns only minimally affects the surrounding wood chip pile. Due to the rapidly compacting pile structure during storage, the woodchips interlock with each other and no woodchips slip into the tubular opening created when the columns are pulled out. After removing the samples from the column, the openings are filled again with the residual material from the columns. Due to the distance between the columns of 2 m, the measurement in the neighbouring columns is only minimally influenced. Thus, in other tests, even under winter conditions, there was no change in temperature during sampling in neighbouring columns.

The dynamics of temperature and CO_2 release within the piles followed a very similar trend peaking after 6 days of storage. After approximately 30 days the temperature and CO_2 concentration dropped to low levels (20 °C), increasing again after storage day 50. Within this time, the strongest rain events could be observed presumably promoting the microbial activity inside the piles. However, a decrease and following increase of the MC around this time (storage month 1–2) could only be demonstrated for the wood chip pile with 1.5% additive. More measurements of the MC would have been necessary to prove this effect.

In all treatments, the maximum pile temperature reached levels exceeding 60 °C. As it has already been shown [14], the additive dosage increased the maximum pile temperatures by up to 5 °C (2.9% additive) as a result of a chemical exothermic process of the $Ca(OH)_2$ with CO_2 forming $CaCO_3$ and water.

Microbiological analyses revealed that thermophilic fungi inhabit the core of the wood pile. Whereas the direct plating method generally revealed a higher number of cultivable fungi, the diversity of obtained colonies was higher for the dilution plating method. Moreover, the composition of fungal isolates was similar between both methods, even though at least one species (*Rasamsonia emersonii*) was individuated exclusively in the dilution plating method. However, integrating the results of both methods clearly indicated the occurrence of a thermophilic mycobiota during the storage and self-heating of the chip piles. Possibly, the lower pile temperatures after four storage months could have a negative effect on the thermophilic fungal communities with a decrease of CFUs number in all samples at long-term storage independent of cultivation method. In addition, the decreasing moisture and increasing pH of the piles with progressing storage time could also have contributed to the overall decrease of the thermophilic members of the saproxylic mycobiota.

The isolated fungal species from poplar wood chips were classified as known thermophilic taxa, comprising members from Ascomycota, Mucoromycota and *mycelia sterilia*. The most abundant species, *A. fumigatus*, *R. pusillus and T. lanuginosus,* are ubiquitous and common species of thermophilic and thermotolerant fungi described in precedent studies in different wood chip types, diverse substrates (compost, mulch, soil) and different locations [14,34,44–46]. In addition, most of the cultivated taxa are known to produce (ligno)cellulolytic enzymes, e.g. cellulases and xylanases [47–49] emphasizing the establishment of a mycobiota with both thermophilic and lignocellulolytic traits.

Although the cultivable fraction of the saproxylic mycobiota was lower than the fungal ASVs (amplicon sequence variants) detected by culture-independent sequencing, the community profiles and composition of the dominant culturable fungi were close to previous metabarcoding analysis performed with the same poplar wood chips [27]. In fact, the five most abundant ASVs detected in the same wood piles using amplicon sequencing (*A. fumigatus*, *T. lanuginosus*, *R. emersonii*, Chaetomiaceae) were also captured by cultivation. Furthermore, both classical culture- and molecular methods revealed shifts in the fungal composition of all samples as a consequence of the overall storage effect, in terms of storage time and related alterations of the environmental conditions (e.g., moisture, pH). For example, the mean relative abundance of *A. fumigatus* was highest in the short-term storage pile without amendment, supporting at least the beneficial short-term effect of the tested amendment strategy.

The lower number of fungal colonies detected in the amended than in the unamended wood chips indicates an additive effect. Specifically, a certain inhibitory effect of $Ca(OH)_2$ on *A. fumigatus*, after one month of storage, and *R. pusillus*, after four months of storage, was revealed by the decreased colony numbers in the samples with additive. Likewise, according to metabarcoding analyses [27], fungal communities of short- and long-term stored wood chips were similar as the effects of both storage time and $Ca(OH)_2$ addition appeared to be minor. Basidiomycota were not isolated in the present study. Similarly, metabarcoding analysis [23] found them mainly at storage intake, but only occasionally (mean relative abundance <1%) in short- and long-term stored wood chips. This emphasizes their susceptibility to high temperatures. Indeed, most thermophilic fungi have been affiliated to the Sordariales, Eurotiales and Onygenales within the Ascomycota as well as the Mucorales within the Mucoromycota [50].

Some of the identified isolated species also represent a potential risk to human health. The species belonging to *Aspergillus* and *Rhizopus* may cause mycotic infections with a high mortality rate in immunosuppressed patients. For example, *A. fumigatus* can cause allergic disease and the pulmonary disease Aspergillosis [51]. Our findings support that the proposed Ca $(OH)_2$ application strategy warrants further investigation about its potential as a simple and low cost improvement for the outdoor storage of uncovered wood chip piles to control potentially harmful fungi, thus protecting the health of employees' of heating plants.

The ability of thermophilic fungi to secrete a broad spectrum of enzymes involved in the biochemical conversion of lignocellulosic biomass makes these microorganisms a promising biotechnological resource for industrial application [48,52,53]. For example, previous studies identified the secretion of large amounts of cellulases and xylanases from *T. aurantiacus* and *T. lanuginosus*, respectively, suggesting their potential use in industrial production [48,53,54].

As it could be demonstrated, the addition of $Ca(OH)_2$ to wet biomass might be a promising countermeasure for lowering DML. In order to be feasible and realizable for biomass power plants, an easy and fast application of the additive is necessary. Essentially, the application and mixing can be done with existing equipment (shovel, wheel loader). The mixing and build-up however will take some additional time and fuel for

the wheel loader. The ultimate additional effort has to be tested since for the experiment, the mixing and pile build-up have been done as accurately as possible which took more time and machine hours as potentially needed. The additional expenditure has to be estimated and compared to the savings of biomass. However, also other effects regarding the combustion unit (ash slagging behaviour, higher ash content, particle emissions) as well as the observed reduction of potential pathogenic microorganism and products of them, have to be included in this overall evaluation. Moreover, the usage of additives to wood chips has to be compared to other potential countermeasures and storage methods such as drying and pile covering and economically evaluated.

5. Conclusions and outlook

The effect of the alkaline additive $Ca(OH)_2$ on the storage behaviour of poplar wood chips was investigated by outdoor storage experiments at industrial scale (250 m^3) in Güssing, Austria. The $Ca(OH)_2$ amendment increased the wood pH significantly and inhibitory effects on thermophilic fungi could be observed, even though storage time had a greater influence on the observed shift in the fungal composition. Further research on variations in pH values with biomass origin and interactions between moisture content, initial pH and additive dosage are recommended. There was a minor additive effect on overall storage conditions such as drying characteristics, gas evolution and pile temperature development over storage time. The additive dosage led to up to 5 °C higher maximum pile temperatures being relevant in terms of microbial sanitization. The effect on the biomass degradation cannot be clearly interpreted since negative storage losses were measured for the untreated pile, indicating measurement uncertainties at the beginning of the storage experiment. To avoid this bias in future studies, accurate mixing of the wood chips prior to storage intake and sample collection is recommended. Additionally, the procedure of determining the initial MC must be changed and adapted as shown in the discussion section to avoid future issues. Still, our findings provide evidence of the tendential quality maintaining effect of the tested additive on the stored wood chips, since, compared to literature, low dry matter losses were measured for the two amended piles during the 4 months storage.

It could be confirmed that such self-heating environments are a source of thermoresistant species involved in the degradation of woody biomass, containing potential human pathogenic fungal species that might be suppressed by the proposed alkaline amendment strategy.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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A Appendix

Table A.1
Overview of the monthly moisture content and dry matter loss (± standard deviation, n = 6) for each height level (top = 2.4 m, centre = 1.6 m, bottom = 0.8 m).

Month	Moisture content%				Dry matter loss%				
	1	2	3	4	1	2	3	4	
0%$Ca(OH)_2$	top	33.6 (±4.27)	40.1 (±1.77)	32.6 (±4.16)	37.8 (±1.41)	−4.4 (±4.0)	−2.4 (±3.5)	0.7 (±3.6)	10.1 (±1.4)
	centre	31.3 (±3.34)	40.8 (±1.92)	32.0 (±2.88)	34.4 (±2.89)	−2.7 (±4.2)	0.2 (±3.5)	1.6 (±3.6)	9.4 (±3.1)
	bottom	29.6 (±1.65)	38.1 (±1.64)	28.6 (±1.77)	28.8 (±0.51)	−4.4 (±2.2)	−1.8 (±3.3)	−1.8 (±3.4)	1.7 (±2.2)
1.5% $Ca(OH)_2$	top	42.7 (±2.03)	39.1 (±1.34)	43.3 (±8.50)	39.8 (±1.75)	7.8 (±2.4)	8.3 (±2.3)	9.1 (±3.5)	8.0 (±3.3)
	centre	39.3 (±2.99)	40.1 (±1.24)	38.2 (±2.03)	37.2 (±2.58)	7.9 (±2.3)	7.8 (±3.6)	8.3 (±1.9)	7.6 (±3.2)
	bottom	41.2 (±1.38)	38.5 (±3.37)	36.2 (±2.48)	33.9 (±2.42)	9.1 (±2.4)	9.6 (±3.2)	9.9 (±2.9)	9.7 (±3.3)
2.9% $Ca(OH)_2$	top	34.1 (±2.05)	38.8 (±9.39)	40.7 (±5.21)	38.3 (±1.66)	0.8 (±2.5)	4.9 (±1.4)	4.6 (±2.6)	6.8 (±2.4)
	centre	31.2 (±2.23)	41.2 (±1.44)	38.4 (±2.19)	36.9 (±1.97)	−2.2 (±4.1)	4.0 (±1.8)	4.7 (±3.1)	6.3 (±2.5)
	bottom	34.7 (±4.67)	38.2 (±1.59)	35.1 (±1.81)	36.9 (±2.28)	6.8 (±5.1)	5.2 (±1.5)	4.5 (±2.2)	9.9 (±2.8)

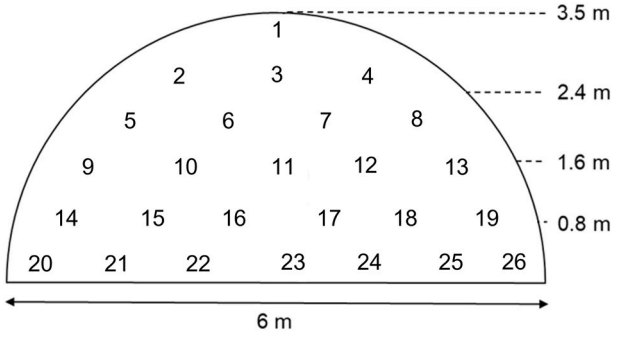

Fig. A.1. Distribution of the 26 samples of the cross-section for MC determination at the end of the storage experiment.

References

[1] X. He, A.K. Lau, S. Sokhansanj, C. Jim Lim, X.T. Bi, S. Melin, Dry matter losses in combination with gaseous emissions during the storage of forest residues, Fuel 95 (2012) 662–664, https://doi.org/10.1016/j.fuel.2011.12.027.

[2] N. Hofmann, T. Mendel, F. Schulmeyer, D. Kuptz, H. Borchert, H. Hartmann, Drying effects and dry matter losses during seasonal storage of spruce wood chips under practical conditions, Biomass Bioenergy 111 (2018) 196–205, https://doi.org/10.1016/j.biombioe.2017.03.022.

[3] R. Jirjis, O. Theander, The effect of seasonal storage on the chemical composition of forest residue chips, Scand. J. For. Res. 5 (1–4) (1990) 437–448, https://doi.org/10.1080/02827589009382626.

[4] H. Lenz, C. Idler, E. Hartung, R. Pecenka, Open-air storage of fine and coarse wood chips of poplar from short rotation coppice in covered piles, Biomass Bioenergy 83 (2015) 269–277, https://doi.org/10.1016/j.biombioe.2015.09.018.

[5] R. Jirjis, L. Pari, F. sissot, Storage of Poplar Wood Chips in Northern Italy, Swedish Bioenergy Association, Stockholm, Sweden, 2008.

[6] W.d. Boer, L.B. Folman, R.C. Summerbell, L. Boddy, Living in a fungal world: impact of fungi on soil bacterial niche development, FEMS Microbiol. Rev. 29 (4) (2005) 795–811, https://doi.org/10.1016/j.femsre.2004.11.005.

[7] M. Noll, R. Jirjis, Microbial communities in large-scale wood piles and their effects on wood quality and the environment, Appl. Microbiol. Biotechnol. 95 (3) (2012) 551–563, https://doi.org/10.1007/s00253-012-4164-3.

[8] E.M. Owens, C.A. Reddy, H.E. Grethlein, Outcome of interspecific interactions among brown-rot and white-rot wood decay fungi, FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 14 (1) (1994) 19–24, https://doi.org/10.1111/j.1574-6941.1994.tb00086.x.

[9] E. Bari, G. Daniel, N. Yilgor, J.S. Kim, M.A. Tajick-Ghanbary, A.P. Singh, J. Ribera, Comparison of the decay behavior of two white-rot fungi in relation to wood type and exposure conditions, Microorganisms 8 (12) (2020), https://doi.org/10.3390/microorganisms8121931.

[10] R. Pecenka, H. Lenz, C. Idler, 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 116 (2018) 140–150, https://doi.org/10.1016/j.biombioe.2018.06.005.

[11] N. Hofmann, T. Mendel, D. Kuptz, F. Schulmeyer, H. Borchert, H. Hartmann, in: Lagerung von Holzhackschnitzeln - Trockenmasseverluste, Änderungen der Brennstoffqualität und Kosten, Berichte aus dem TFZ, 2017. Straubing.

[12] E. Anerud, R. Jirjis, G. Larsson, L. Eliasson, Fuel quality of stored wood chips – influence of semi-permeable covering material, Appl. Energy 231 (2018) 628–634, https://doi.org/10.1016/j.apenergy.2018.09.157.

[13] H. Lenz, R. Pecenka, E. Hartung, C. Idler, Development and test of a simplified method to calculate dry matter loss during open-air storage of poplar wood chips by analyzing ash contents, Biomass Bioenergy 94 (2016) 258–267, https://doi.org/10.1016/j.biombioe.2016.09.011.

[14] S. Dumfort, R. Pecenka, J. Ascher-Jenull, U. Peintner, H. Insam, H. Lenz, The potential of calcium hydroxide to reduce storage losses: a four months monitoring study of spruce wood chip piles at industrial scale, Fuel 298 (2021), 120738, https://doi.org/10.1016/j.fuel.2021.120738.

[15] M. Noll, A. Naumann, F. Ferrero, M. Malow, Exothermic processes in industrial-scale piles of chipped pine-wood are linked to shifts in gamma-, alphaproteobacterial and fungal ascomycete communities, Int. Biodeterior. Biodegrad. 64 (7) (2010) 629–637, https://doi.org/10.1016/j.ibiod.2010.07.002.

[16] F. Ferrero, C. Lohrer, B.M. Schmidt, M. Noll, M. Malow, A mathematical model to predict the heating-up of large-scale wood piles, J. Loss Prev. Process. Ind. 22 (4) (2009) 439–448, https://doi.org/10.1016/j.jlp.2009.02.009.

[17] F. Ferrero, M. Malow, M. Noll, Temperature and gas evolution during large scale outside storage of wood chips, Eur. J. Wood Prod. 69 (4) (2011) 587–595, https://doi.org/10.1007/s00107-010-0512-0.

[18] M.S. Finstein, J. Cirello, S.T. MacGregor, F.C. Miller, D.J. Suler, P.F. Strom, Engineering principles of sludge composting, J. (Water Pollut. Control Fed.) 52 (7) (1980) 2037–2044.

[19] S.T. MacGregor, F.C. Miller, K.M. Psarianos, M.S. Finstein, Composting process control based on interaction between microbial heat output and temperature, Appl. Environ. Microbiol. 41 (6) (1981) 1321–1330, https://doi.org/10.1128/aem.41.6.1321-1330.1981.

[20] M. de Bertoldi, G. Vallini, A. Pera, The biology of composting: a review, Waste Manag. Res. 1 (1) (1983) 157–176, https://doi.org/10.1177/0734242X8300100118.

[21] M. Nagler, M. Probst, J. Zöhrer, S. Dumfort, F. Fornasier, R. Pecenka, H. Lenz, H. Insam, J. Ascher-Jenull, Microbial community dynamics during the storage of industrial-scale wood chip piles of Picea abies and Populus canadensis and the impact of an alkaline stabilization agent, Biomass Bioenergy 165 (2022), 106560, https://doi.org/10.1016/j.biombioe.2022.106560.

[22] R. Jirjis, Effects of particle size and pile height on storage and fuel quality of comminuted Salix viminalis, Biomass Bioenergy 28 (2) (2005) 193–201, https://doi.org/10.1016/j.biombioe.2004.08.014.

[23] M.A. Brand, Bolzon de Muñiz, Graciela Inês, W.F. Quirino, J.O. Brito, Storage as a tool to improve wood fuel quality, Biomass Bioenergy 35 (7) (2011) 2581–2588, https://doi.org/10.1016/j.biombioe.2011.02.005.

[24] M. Humar, M. Petrič, F. Pohleven, Changes of the pH value of impregnated wood during exposure to wood-rotting fungi, Holz als Roh- Werkst. 59 (4) (2001) 288–293, https://doi.org/10.1007/s001070100207.

[25] J. Jellison, J. Connolly, B. Goodell, B. Doyle, B. Illman, F. Fekete, A. Ostrofsky, The role of cations in the biodegradation of wood by the brown rot fungi, Int. Biodeterior. Biodegrad. 39 (2) (1997) 165–179, https://doi.org/10.1016/S0964-8305(97)00018-8.

[26] S. Xiong, M. Bozaghian, T.A. Lestander, R. Samuelsson, S. Hellqvist, M. Öhman, Calcium oxide as an additive for both conservation and improvement of the combustion properties of energy grass: a preliminary study, Biomass Bioenergy 99 (2017) 1–10, https://doi.org/10.1016/j.biombioe.2017.02.010.

[27] J. Zöhrer, M. Probst, S. Dumfort, H. Lenz, R. Pecenka, H. Insam, J. Ascher-Jenull, Molecular monitoring of the poplar wood chip microbiome as a function of storage strategy, Int. Biodeterior. Biodegrad. 156 (2021), 105133, https://doi.org/10.1016/j.ibiod.2020.105133.

[28] DIN EN ISO 18122:2022, Solid Biofuels - Determination of Ash Content, 2022.

[29] M. Gómez-Brandón, J. Ascher-Jenull, T. Bardelli, F. Fornasier, G. Fravolini, P. Arfaioli, M.T. Ceccherini, G. Pietramellara, K. Lamorski, C. Sławiński, D. Bertoldi, M. Egli, P. Cherubini, H. Insam, Physico-chemical and microbiological evidence of exposure effects on Picea abies – coarse woody debris at different stages of decay, For. Ecol. Manag. 391 (2017) 376–389, https://doi.org/10.1016/j.foreco.2017.02.033.

[30] ISO 18134-2:2017 Solid biofuels, Determination of moisture content — oven dry method — Part 2: total moisture, Simplified method (2017) (ISO 18134-2:2017).

[31] EN 15149-1, Solid Biofuels - Determination of Particle Size Distribution - Part 1: Oscillating Screen Method Using Sieve Apertures of 1 Mm and above, 2011.

[32] DIN EN ISO 17225-4: Biogene Festbrennstoffe - Brennstoffspezifikationen und -klassen - Teil 4, Klassifizierung von Holzhackschnitzeln, 2014.

[33] I.S.O. ÖNORM EN, Biogene Festbrennstoffe - Bestimmung der Schüttdichte, 2016, 17828.

[34] S. Ahirwar, H. Soni, B.P. Prajapati, N. Kango, Isolation and screening of thermophilic and thermotolerant fungi for production of hemicellulases from heated environments, Mycology 8 (3) (2017) 125–134, https://doi.org/10.1080/21501203.2017.1337657.

[35] A. Langarica-Fuentes, U. Zafar, A. Heyworth, T. Brown, G. Fox, G.D. Robson, Fungal succession in an in-vessel composting system characterized using 454 pyrosequencing, FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 88 (2) (2014) 296–308, https://doi.org/10.1111/1574-6941.12293.

[36] R. Team, A language and environment for statistical computing, Computing 1 (2006), https://doi.org/10.1890/0012-9658, 2002)083[3097:CFHIWS]2.0.CO;2.

[37] Thorsten Pohlert, The Pairwise Multiple Comparison of Mean Ranks Package, PMCMR), 2016.

[38] C.L. Bean, J.J. Hansen, A.B. Margolin, H. Balkin, G. Batzer, G. Widmer, Class B alkaline stabilization to achieve pathogen inactivation, Int. J. Environ. Res. Publ. Health 4 (1) (2007) 53–60, https://doi.org/10.3390/ijerph2007010009.

[39] C. Williford, W.-Y. Chen, N.K. Shamas, L.K. Wang, Lime stabilization, in: L. K. Wang, N.K. Shammas, Y.-T. Hung (Eds.), Biosolids Treatment Processes, Humana Press, Totowa, NJ, 2007, pp. 207–241.

[40] L. Pari, M. Brambilla, C. Bisaglia, A. Del Giudice, S. Croce, M. Salerno, F. Gallucci, Poplar wood chip storage: effect of particle size and breathable covering on drying dynamics and biofuel quality, Biomass Bioenergy 81 (2015) 282–287, https://doi.org/10.1016/j.biombioe.2015.07.001.

[41] M. Barontini, A. Scarfone, R. Spinelli, F. Gallucci, E. Santangelo, A. Acampora, R. Jirjis, V. Civitarese, L. Pari, Storage dynamics and fuel quality of poplar chips, Biomass Bioenergy 62 (2014) 17–25, https://doi.org/10.1016/j.biombioe.2014.01.022.

[42] R. Pecenka, H. Lenz, C. Idler, W. Daries, D. Ehlert, Development of bio-physical properties during storage of poplar chips from 15 ha test fields, Biomass Bioenergy 65 (2014) 13–19, https://doi.org/10.1016/j.biombioe.2014.04.017.

[43] C. Brischke, S. Stricker, L. Meyer-Veltrup, L. Emmerich, Changes in sorption and electrical properties of wood caused by fungal decay, Holzforschung 73 (5) (2019) 445–455, https://doi.org/10.1515/hf-2018-0171.

[44] F. Witfeld, D. Begerow, M.A. Guerreiro, Improved strategies to efficiently isolate thermophilic, thermotolerant, and heat-resistant fungi from compost and soil, Mycol. Prog. 20 (3) (2021) 325–339, https://doi.org/10.1007/s11557-021-01674-z.

[45] R. Michael, Tansey, isolation of thermophilic fungi from self-heated, industrial wood chip piles, Mycologia 63 (3) (1971) 537–547, https://doi.org/10.1080/00275514.1971.12019133.

[46] Sally Krigstin, Suzanne Wetzel, A review of mechanisms responsible for changes to stored woody biomass fuels, Fuel 175 (2016) 75–86, https://doi.org/10.1016/j.fuel.2016.02.014.

[47] J. Houbraken, P.E. Verweij, A.J.M.M. Rijs, A.M. Borman, R.A. Samson, Identification of Paecilomyces variotii in clinical samples and settings, J. Clin. Microbiol. 48 (8) (2010) 2754–2761, https://doi.org/10.1128/JCM.00764-10.

[48] R. Maheshwari, G. Bharadwaj, M.K. Bhat, Thermophilic fungi: their physiology and enzymes, Microbiol. Mol. Biol. Rev. 64 (3) (2000) 461–488, https://doi.org/10.1128/MMBR.64.3.461-488.2000.

[49] F. Tekaia, J.P. Latgé, Aspergillus fumigatus: saprophyte or pathogen? Curr. Opin. Microbiol. 8 (4) (2005) 385–392, https://doi.org/10.1016/j.mib.2005.06.017.

[50] I. Morgenstern, J. Powlowski, N. Ishmael, C. Darmond, S. Marqueteau, M.-C. Moisan, G. Quenneville, A. Tsang, A molecular phylogeny of thermophilic fungi, Fungal Biol. 116 (4) (2012) 489–502, https://doi.org/10.1016/j.funbio.2012.01.010.

[51] J. Suchomel, K. Belanová, M. Gejdoš, M. Němec, A. Danihelová, Z. Mašková, Analysis of fungi in wood chip storage piles, Bioresources 9 (2014) 4410–4420, https://doi.org/10.15376/biores.9.3.4410-4420.

[52] H. El-Gendi, A.K. Saleh, R. Badierah, E.M. Redwan, Y.A. El-Maradny, E.M. El-Fakharany, A comprehensive insight into fungal enzymes: structure, classification, and their role in mankind's challenges, J. Fungi 8 (1) (2022), https://doi.org/10.3390/jof8010023.

[53] S.D. McClendon, T. Batth, C.J. Petzold, P.D. Adams, B.A. Simmons, S.W. Singer, Thermoascus aurantiacus is a promising source of enzymes for biomass deconstruction under thermophilic conditions, Biotechnol. Biofuels 5 (1) (2012) 54, https://doi.org/10.1186/1754-6834-5-54.

[54] S. Singh, A.M. Madlala, B.A. Prior, Thermomyces lanuginosus: properties of strains and their hemicellulases, FEMS Microbiol. Rev. 27 (1) (2003) 3–16, https://doi.org/10.1016/S0168-6445(03)00018-4.