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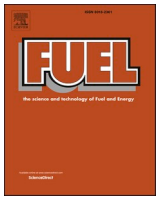

Full Length Article

The potential of calcium hydroxide to reduce storage losses: A four months monitoring study of spruce wood chip piles at industrial scale

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ABSTRACT

The objective of this study was to investigate the effect of an alkaline additive on the storage of wood chips from Norway spruce forest residues. Piles of untreated and calcium hydroxide treated wood chips (250 m^3) were set up and investigated for four months. It was demonstrated that adding $Ca(OH)_2$ to moist wood chips decreased the dry matter loss by 6%. This was attributed to the increase of the pH to a level of 8, rendering the habitat less suitable for fungal colonisation. The results suggest the set-up storage strategy as a potential alternative method for preserving wood chips when long term storage is required.

1. Introduction

By international comparison, Austria is among the leading countries regarding the utilization of biomass with a gross domestic consumption of renewable energy of 33.5% (2016). About 80% of the generated renewable energy in the form of heat and electricity derives from wood [1]. To compensate differences in provision and consumption of wood chips, storage is an important yet problematic step of the wood chip supply chain when considerable losses of the energetic value may occur [2–6]. During the storage of wood chips, destruction and conversion processes take place through the action of a complex microbial wood decomposer community, i.e. primarily fungi and bacteria colonizing the biomass. These microorganisms originate either from the autochthonous microbiota present in the fresh biogenic material and/or from atmospheric deposition. Wood-decaying fungi causing white rot (Ascomycetes and Basidiomycetes) and brown rot (exclusively Basidiomycetes) are the main wood decomposers [7] due to their ability to degrade cellulose, hemicellulose and lignin. These fungi can overcome difficulties in wood decay such as limited nutrient accessibility and the presence of antibiotic compounds (e.g., tannins) [8], also owing to synergistic interactions with bacteria, as recently evidenced [9–12]. Moreover, they can grow over a wide temperature and pH range.

The degradation of the wood components is mainly carried out in aerobic conditions [13]. The biomass loss during storage is influenced by many factors such as tree species, wood chip size, moisture content of the chips at storage intake, weather conditions, storage procedures, duration and design of the storage [5,14]. Dry matter losses of up to 30% can result from the commonly used method of storing and drying wood chips in naturally ventilated outdoor piles [2,14,15]. Although fast drying with heated air is a recommended technical solution, it is not always realisable or economically expedient. Natural drying during storage is less expensive, but its success depends on the environmental conditions during storage [16,17]. Connected to dry matter losses are not only economic losses for the producer but also quality losses, microbiological contamination, greenhouse gas emissions and the risk of self-ignition of wood chips due to the temperature increase in the storage pile caused by biological and chemical degradation processes [18,19].

Besides the temperature, moisture content and oxygen availability [20], the pH-value and nutrient availability are the main microbial growth factors either promoting or inhibiting microbial growth. As it could be demonstrated, especially bark as well as leaves and needles are susceptible to microbial degradation since these parts offer a high surface area and are rich in nutrients [21]. Wood has generally an acidic pH of 3 to 6, depending on the wood species and degree of decay [22,23]. During wood decomposition, microorganisms cause a substantial

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reduction of the pH by producing organic acids such as oxalic acid. When an alkaline environment (pH around 9) is created, the growth of fungi, known to prefer acidic conditions, is restrained [11,24], and consequently, microbial wood chip degradation could be slowed down considerably.

The objective of this study was to investigate the effect of an alkaline additive on the storage of Norway spruce (*Picea abies*) wood chips, derived from forest residues. It is hypothesized that the dry matter loss will be decreased by increasing the pH of the wood chips by the use of calcium hydroxide (slaked lime, $Ca(OH)_2$) as an alkaline additive (pH of a saturated solution of 11–12.6). $Ca(OH)_2$ has not only the potential to increase the pH-value considerably, but can also have a positive effect during biomass combustion since calcium-based additives have the potential of reducing ash slagging in biofuels [25–27]. Besides this, $Ca(OH)_2$ is a cheap raw material and easy to apply. State of the art technology, such as wheel loaders and distributors for fertilizers can be used for mixing the dry additive with moist wood chips.

Three piles of spruce forest residues with different $Ca(OH)_2$ concentrations were set up at practical, industrial scale (250 m^3) and stored outdoor (open-air, no coverage) from April to August 2018 in Güssing (Austria) to monitor the dry matter loss, temperature, moisture content and gas evolution. In addition, aiming at exploring the cultivable members of the autochthonous forest wood chip microbiota (fungi and bacteria) at storage intake, direct- and dilution plating was performed. The focus was given on thermophilic and thermotolerant wood decomposers.

2. Material and methods

2.1. Experimental set-up and sampling

Freshly harvested spruce forest residues (mixture of heartwood, bark and needles) were stored in Güssing, Austria (47° 03′ N, 16° 19′ E) on April 11 and 12, 2018. Directly after chipping three piles of about 250 m^3 each (12 m × 6 m × 3.5 m) were set up on an asphalted area (Fig. 1). The piles differed according to their proportion of added $Ca(OH)_2$ (Table 1). The storage trial was conducted for a total of four months until August 7, 2018.

For periodic recording of the principal storage parameters, i.e., moisture content, ash content and dry matter loss, four stainless steel grit columns were positioned in each pile (Fig. 2).

The height, diameter and mesh size of the grit columns were 2.5 m, 0.64 m and 20 mm, respectively. Each column was equipped with 18 balance bags (plastic net bags, mesh size 1x1 mm, n = 6 bags per height) filled with 2 kg fresh wood chips. Balance bags were prepared and weighed immediately after taking samples for moisture content measurement and positioned at three levels (0.8 m, 1.6 m, 2.4 m) within the columns. In a four-week interval (t_1, t_2, t_3, t_4) one column of each pile was removed with a truck crane to retrieve the balance bags. The resulting free spaces were filled with wood chips instantly. Wood chips collected immediately before assembling the piles served as control (t_0).

Table 1
Composition and additive concentration of the three wood chip piles.

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	244	76,845	41,596	0	0
2	237	74,750	40,462	595	1.5
3	258	81,157	43,930	1,292	3.0

After removing the last sampling column after four months of storage a moisture profile with 26 sampling points of each pile was determined over the cross-section of each pile.

The dry matter loss (DML) was measured by a method using balance bags previously described by Lenz et al. [13]. Briefly, at each sampling time the balance bags (n = 6 per height of the column) were immediately weighed, opened and samples taken for moisture measurement. Additionally, samples were collected for microbiological analyses; in order to obtain representative samples, aliquots (ca. 300 g) from two balance bags were pooled, resulting in a total of three composite replicates (n = 3) per height of the columns, immediately stored at 4 °C for the transport to the laboratory, and frozen at −20 °C. DML at the sampling time i is calculated using Equation (1), taking the chemical reaction of the additive into account. All used masses at storage intake exclude the mass of added $Ca(OH)_2$. In a wet environment, the applied slaked lime reacts with atmospheric carbon dioxide forming calcium carbonate (calcite), water and heat according to Equation (2).

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

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

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 taken into account when calculating the DML_i. The resulting mass of $CaCO_3$ is calculated by Equation (3).

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

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]

Fig. 1. Construction of the wood chip piles and installation of the measuring columns.

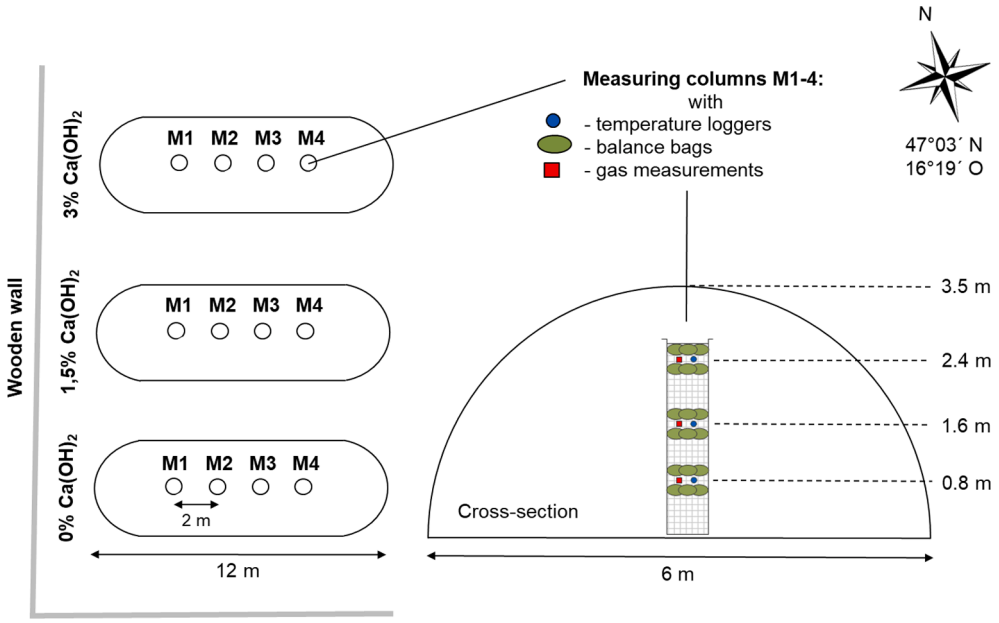

Fig. 2. Schematic structure of the storage piles with measuring columns for the piles containing 0%, 1.5% and 3.0% $Ca(OH)_2$, respectively.

$M_{Ca(OH)2\ M}$ mass of $Ca(OH)_2$ [g mol^{-1}]
M_{CaCO3} molar mass of $CaCO_3$ [g mol^{-1}]

It has to be noted that in this study all moisture contents are defined as the moisture mass fraction on a wet-weight basis.

The development of the pile temperature was measured continuously over the entire storage period with temperature data loggers (Gemini Tinytag TGP-4017). Each column was equipped with three data loggers that were positioned at the same three levels as the balance bags. Additionally, three polyamide pipes were attached to each column for measuring the gas composition at the three levels. The measurement of O_2, CO_2, H_2, H_2S and CH_4 was carried out weekly by a gas monitoring device (Draeger X-am®7000) with an internal pump. All investigated parameters and the respective methodology are given in Table 2.

The results were evaluated by the statistical software SAS 9.4 (SAS Institute Inc., USA). For the evaluation of the data the procedure GLIMMIX was used in order to carry out a two factorial ANOVA. The GLIMMIX procedure adapts statistical models to data with correlations or non-constant variability, homogeneous or non-homogeneous variances where the response is not necessarily normally distributed. Due to the interactions between the two factors, only the twofold effect was considered. Details are given in the section supplementary material, where treatments labelled by different letters are different according to GLIMMIX procedure with $p \leq 0.05$.

2.2. Preparation of different wood chip and $Ca(OH)_2$ blends

For investigating the influence of $Ca(OH)_2$ on the storage behaviour as function of i) storage time and ii) admixture of alkaline additive, freshly chipped forest residues were mixed with $Ca(OH)_2$ with the help of a wheel loader. The designed concentrations of $Ca(OH)_2$ to dry wood chips were 0, 1.5 and 3.0%. The volume of each pile was set by the number of full buckets of the wheel loader and the resulting fresh mass of the wood chips was calculated by means of the bulk density. Every bucket (8 m^3) of wood chips was mixed with additive by turning the blend for several times with the wheel loader (Fig. 3). After mixing the biomass-additive blend, samples for moisture and dry matter loss determination were taken immediately (t_0). Table 1 gives an overview of the volume and composition of each pile.

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
*Ash content	%	n = 12	EN 14,775[28]
*pH	–	n_{t0} = 3 n_{t4} = 9	wood:water extracts (1:10), w: v), pH-Meter Metrohm 744 [9]
*Electrical conductivity (EC)	µS cm^{-1}	n = 3	wood:water extracts (1:10), w: v), conductivity Meter LF 330 WTW (Weilheim, Germany) [9]
*C:N	%	n = 3	CN analyser (Vario Macro CN, Elementar, Hanau, Germany–combustion analysis) [9]
*SOM (volatile solids, VS)	%	n = 3	mass loss following ignition in a muffle furnace (Carbolite, CWF 1000) [9]
Moisture Content	%		EN 18134-2 [29]
at storage intake		n = 20	
at storage outtake		n = 12	
Cross-section		n = 26	
Dry matter loss	%	n = 12	Equation (2) and (3)
Particle size distribution	%	n = 3	EN 15149-1[30]
Class characterization	–	–	EN ISO 17225-1[31]
Bulk density	kg m^{-3}	n = 1	ÖNORM EN ISO 17,828 [32]
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	%	–	

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

Fig. 3. Preparation of the wood chip additive blends.

2.3. Microbial screening of the thermotolerant autochthonous wood chip microbiota

The cultivable fraction of the freshly chipped forest wood material at storage intake (t_0) was assessed by direct plating and dilution plating. Direct plating was performed with untreated wood chips; small pieces (ca. 0.5 × 0.5 × 0.5 cm) were cut off from representative wood chips and three pieces each were placed on a petri dish with PDA Agar (Potato Extract Glucose Agar, Roth) [33].

For the dilution plating, 5 g of cut milled (2 mm) wood chips were first extracted 1:5 (w:v) in sterile deionized water containing 0.02% Tween (Polysorbate 80) by horizontal shaking (30 min., 100 rpm, 20 °C). The suspension was filtered and 200 µL of the obtained extract (10^{-1}-10^{-5}) were plated on PDA Agar. Isolation was performed at 37 °C and 50 °C. All cultivation assays were run in three field replicates with five technical replicates each. From the different morphotypes, pure cultures were generated and incubated at 20 °C (control), 37 °C, and 55 °C, and analysed in terms of growth performance for a total period of 7 days.

Morphological identification was combined with molecular identification by colony PCR [34] and subsequent Sanger sequencing (Eurofins-Genomics) of the ITS1-2 region for fungal- and the V3-V4 region of the 16S rRNA gene for bacterial isolates. Primers and PCR conditions were those as described in [34] and [9], respectively. The generated sequences were processed by BLAST analysis.

3. Results

3.1. Properties of the harvested wood chips

Table 3 shows the mean material-descriptive parameters at the time of storage intake (t_0). The initial moisture content of the different wood chip-additive blends at the start was between 50 and 55%, which is typical for freshly harvested spruce forest residues. The addition of Ca$(OH)_2$ raised the pH significantly from 5.1 to 7.7 (1.5% additive) and 7.9 (3.0% additive). The particle size distribution of the wood chips with 0 and 1.5% Ca$(OH)_2$ corresponded to class P31 (Fig. 4). In contrast, wood chips with 3.0% additive corresponded to class P16, due to a very high content of fine particles. 25% of the wood chips had a particle size of < 3.15 mm. In general, the harvested wood chips contained a high portion of fine particles, which was due to the high content of spruce needles. In fact, the used wood chip material was a mixture of heart wood, bark and needles leading to a high heterogeneity which is also reflected by the varying particle size distribution. According to the screen analysis, the median (x_{50}) for the different wood chip-additive blends was 12 mm (0% additive), 17 mm (1.5% additive) and 8 mm (3.0% additive).

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

Wood chip-additive blend	Moisture content %	Ash content %	pH -	EC µS cm^{-1}	C %	N %
0% Ca$(OH)_2$	55.5 ± 1.2	10.3 ± 0.9	5.1 ± 0.6	613 ± 137	44.6 ± 0.2	0.60 ± 0.10
1.5% Ca$(OH)_2$	49.6 ± 0.8	6.0 ± 0.7	7.7 ± 0.2	1141 ± 81	45.1 ± 0.6	0.68 ± 0.09
3.0% Ca$(OH)_2$	50.4 ± 0.7	6.9 ± 1.2	7.9 ± 0.1	1371 ± 141	45.1 ± 0.4	0.53 ± 0.05

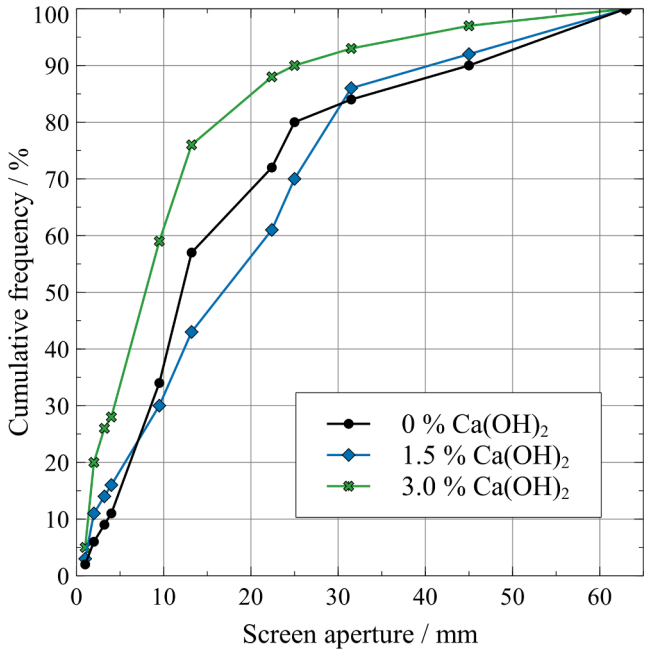

Fig. 4. Particle size distribution of spruce forest residues with 0, 1.5 and 3.0% Ca$(OH)_2$, respectively.

3.2. Weather conditions

Based on the weather data provided by the ZAMG (Zentralanstalt für Meteorologie und Geodynamik, Austria) the long-term median value (1990–2019) of the average daily ambient temperature (16.2 °C) and precipitation sum (322 mm) for the months April to July were calculated. During the time of storage, an average daily temperature of 18.7 °C and a precipitation sum of 260 mm were recorded. Due to this significantly lower precipitation (compared to the long-term median value), a better drying effect of the wood chips during storage can be concluded. The mean daily ambient temperature and precipitation sums are given in Fig. 5.

3.3. Pile temperature

The mean pile temperatures (n = 3) over storage time show an immediate temperature rise after storage intake (Fig. 6). In detail, a maximum temperature of 66.5 °C has been reached after 9 days (0%

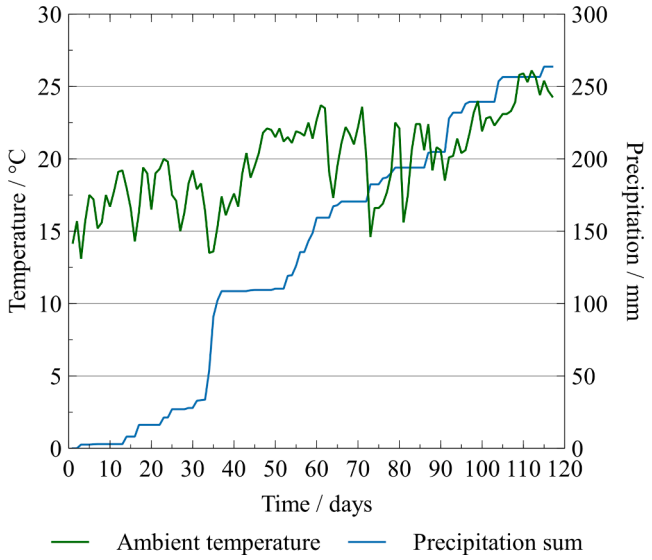

Fig. 5. Weather conditions during the wood chip storage trial from April to August 2018 in Güssing (Austria).

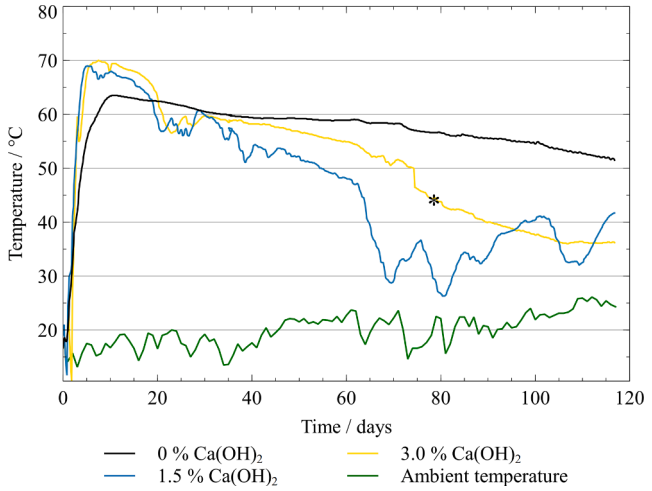

Fig. 6. Pile and ambient temperature during the four months storage trial. Pile temperatures are given as the mean value of the three temperature data loggers ($CV_{0\ \%} = 6.0\%$, $CV_{1.5\ \%} = 8.9\%$, $CV_{3\ \%} = 8.5\%$). *top temperature data logger failed after 79 days.

additive, top level), 70.3 °C after 7 days (1.5% additive, centre level) and 71.9 °C after six days (3.0% additive, top level), respectively. Even though the heating-up velocity and the maximum temperature increased with increasing additive concentration, also a subsequent cooling was observed for the two piles with additive, only. In the top and centre part of the pile without additive, the temperature remained at around 60 °C for all four months. Only the lower part showed a significant cooling effect after 75 days. Apart from some fluctuations, the temperature development at the three height levels was very similar for the pile with 1.5% additive. For the pile with 3.0% additive, the cooling started at the bottom. Due to a technical defect, the temperature data logger at the top failed after 79 days (Fig. 6 indicated by *). Therefore, only the mean temperature of the centre and bottom are given between days 79 and 120. At the end of the storage trial, the temperature in all piles still exceeded 30 °C.

3.4. Moisture content and dry matter losses

The initial moisture content of the freshly chipped forest residues was 55.5%. Adding 1.5% and 3.0% $Ca(OH)_2$ resulted in a reduction to a moisture content of around 50.0% (Table 4). After storage intake, the moisture content of all piles decreased steadily (Fig. 8) to a final value of 32.1% (±8.1%, 0% additive), 25.9% (±6.1%, 1.5% additive) and 23.1% (±6.5%, 3.0% additive) after four months of storage. Taking a closer look at the development of the moisture content at the three different pile levels (Table 4), a clear influence of the ambient weather conditions became evident for the top level. After an initial drying process (first two months), the moisture content of all top levels increased due to heavy rainfalls. The centre and bottom parts were unaffected and the drying process continued over the whole storage period. Due to this different behaviour of the top level, only the sample bags of the bottom and centre parts were considered for calculating both the mean moisture content and the DML (twelve balance bags per sampling date and additive concentration), since the conditions were not comparable and the resulting variances high.

The heterogeneous moisture content reflects the weather conditions resulting in higher moisture contents in the outer layers (Fig. 7). In the case of 0% additive, the moisture content fluctuated between 13% in the centre and > 60% in the peripheral areas. The mean moisture content of the cross-section was 40.9% (±18.9%, 0% additive), 27.8% (±14.4%, 1.5% additive) and 25.2% (±10.1%, 3.0% additive).

The DML after 1, 2, 3 and 4 months of storage are given in Fig. 8. Except for the third storage month, the DML decreased with increasing additive concentration resulting in a dry matter loss of 15.1% (0% additive), 10.1% (1.5% additive) and 9.0% (3.0% additive), respectively, after 117 days of storage. The comparable high standard deviations are due to the inhomogeneity of the used forest residues. Statistical analyses showed significant differences between 0% and 3% additive ($p = 0.0290$). No significant differences of the dry matter loss were found between 0% and 1.5% additive ($p = 0.2597$) as well as between 1.5% and 3.0% additive ($p = 0.5667$). Analysing the dry matter loss development over storage time, significant differences can only be postulated for the first 2 months (details in supplementary material).

The dynamics of pH over the storage time varied by additive treatment. The pile without additive had an initial pH of 5.1 (±0.6) and 4.9 (±0.9) after four months. With 1.5% additive the initial pH of 7.7 (±0.2) decreased slightly to 7.2 (±0.1), while the pH of wood chips with 3.0% additive decreased from 7.9 (±0.1) to 7.8 (±0.8). The electrical conductivity (EC) of the wood chips without additive increased from 613 µS cm^{-1} (±137) to 660 µS cm^{-1} (±231). The EC of the wood chips with 1.5% additive decreased from 1141 µS cm^{-1} (±81) to 510 µS cm^{-1} (±56) and that of wood chips with 3% additive decreased from 1371 µS cm^{-1} (±141) to 793 µS/cm (±126).

During the storage the C:N ratio changed from 76:1 to 73:1 (0% additive), 67:1 to 72:1 (1.5% additive) and from 85:1 to 77:1 (3.0% additive).

3.5. Gaseous emissions

The gas measurements revealed a drop of the O_2 as well as a distinct peak of the CO_2 and H_2 concentration within the first two weeks of storage (Fig. 9). Maximum CO_2 values were 10.0% after 11 days (0% additive, bottom), 8.0% after 11 days (1.5% additive, bottom) and 15.5% after 7 days (3.0% additive, bottom). Hydrogen concentration peaked after seven days of storage and reached 480 ppm (0% additive, bottom), 330 ppm (1.5% additive, bottom) and 605 ppm (3.0% additive, bottom). Methane was only found at the fifth storage day with a concentration of 0.5% (0% additive) and 0.4% (1.5% and 3.0% additive).

Table 4
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	46.5 (±2.7)	38.3 (±3.6)	50.0 (±3.1)	53.0 (±7.9)	6.9 (±5.5)	15.1 (±3.8)	8.9 (±5.0)	−3.9 (±4.3)
	centre	47.5 (±2.1)	36.2 (±2.5)	42.6 (±7.2)	36.8 (±8.8)	7.1 (±2.7)	10.8 (±4.2)	7.9 (±5.6)	13.0 (±3.6)
	bottom	45.9 (±2.7)	41.4 (±4.5)	32.4 (±5.1)	27.2 (±3.5)	7.5 (±4.7)	13.7 (±2.9)	14.8 (±4.2)	17.1 (±4.7)
1.5% $Ca(OH)_2$	top	38.9 (±1.1)	36.5 (±5.2)	52.5 (±10.1)	27.9 (±1.2)	5.2 (±5.9)	8.4 (±2.3)	6.6 (±1)	11.4 (±4.5)
	centre	41.3 (±1.5)	32.0 (±0.8)	28.7 (±1.1)	24.7 (±0.9)	5.9 (±2.3)	9.3 (±3.5)	10.6 (±1.0)	9.5 (±0.9)
	bottom	37.3 (±1.3)	31.4 (±2.4)	28.9 (±2.0)	27.0 (±8.9)	7.8 (±3.8)	10.9 (±1.2)	12.4 (±1.4)	10.9 (±3.2)
3.0% $Ca(OH)_2$	top	50.6 (±6.2)	42.6 (±7.9)	43.9 (±12.0)	47.7 (±8.5)	3.9 (±3.2)	8.2 (±6.1)	10.9 (±5.3)	−2.3 (±1)
	centre	46.7 (±1.5)	35.2 (±2.0)	31.0 (±2.5)	28.8 (±3.7)	4.0 (±2.6)	8.6 (±3.2)	10.7 (±1.8)	9.1 (±2.0)
	bottom	44.0 (±1.8)	29.3 (±3.3)	23.9 (±2.1)	17.5 (±1.8)	7.5 (±4.0)	11.9 (±3.0)	13.7 (±4.3)	8.8 (±4.6)

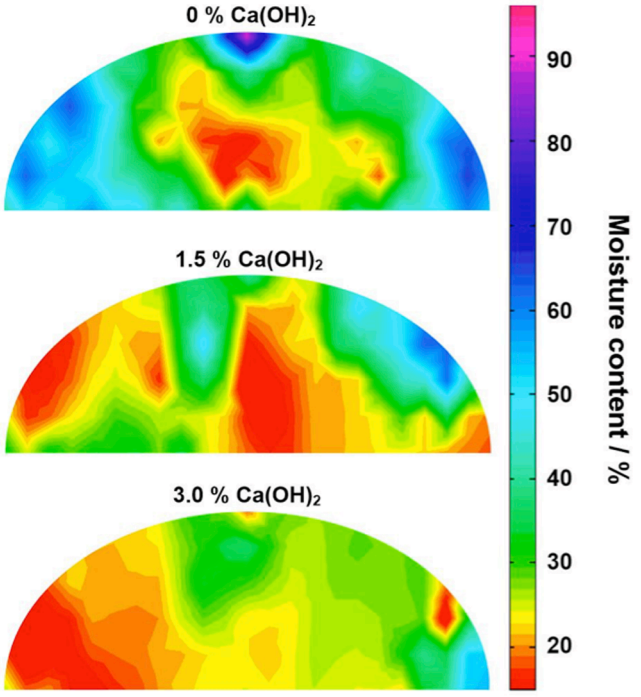

Fig. 7. Moisture content of the wood chips along a cross-section of the three testing piles at the end of the four months storage trial.

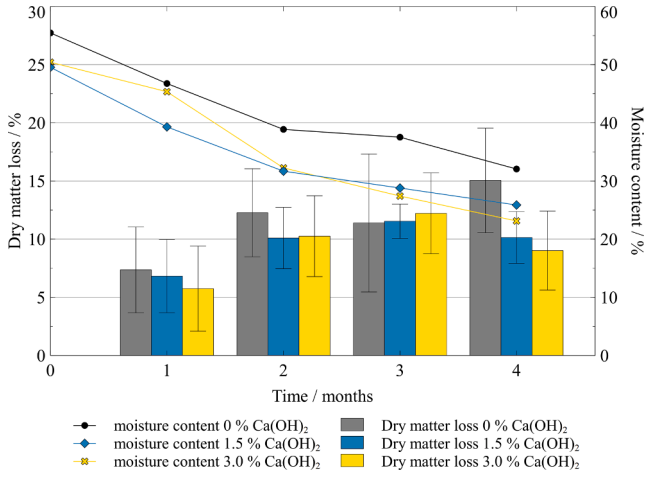

Fig. 8. Development of the moisture content (n = 12) and the dry matter loss (n = 12) during the four months of storage. Only samples of the bottom and centre were considered.

3.6. Microbial screening of the thermotolerant autochthonous wood chip microbiota

Direct plating was more effective for the isolation of fungi, while the dilution plating technique was more selective for bacteria. Overall, the forest wood chips – prior to storage (t_0) - showed a high diversity (5.25 ± 0.37 species/wood chip; n = 15) of cultivable mesophilic fungi (20 °C). Among the isolates obtained from wood chips at the storage intake (t_0), there were several wood decomposers that revealed to be thermo-activated, showing best growth performances *in vitro* at 50 °C: *Rhizomucor miehei* and *Streptomyces spp.*. Five thermotolerant microorganisms isolated from freshly chipped forest residues were able to grow even at 60 °C: four bacteria (*Bacillus licheniformis, Bacillus subtilis* (thermostable strain), *Streptomyces thermodiastaticus, Streptomyces* sp., and the fungus *Rhizomucor miehei.*

4. Discussion

The results of this study show the potential of adding $Ca(OH)_2$ to moist wood chips for preserving the wood biomass during storage. After four months of storage the dry matter loss (DML) decreased significantly by 6.1 percentage points (3.0% additive) compared to wood chips without additive. The difference between the addition of 0% and 1.5% was not significant. The main effect of adding $Ca(OH)_2$ was a rise of the surface pH of wood from an acidic to an alkaline environment with a pH of about 8. Interestingly, the pH of all wood chip piles did not change significantly over time and still after four months of storage, the additive kept the pH high. The selected additive concentrations seem in a suitable and reasonable range, but necessary additive dosage depends strongly on the initial pH and moisture content of the wood chips and has to be taken into account.

The DML curve followed an already well-known trend with the highest degradation rates within the first and second month. As other studies [5,35] have shown, the marked increase of the DML ended after around four months of storage with only little further degradation. Even though the storage trial was only conducted for four months, it can be assumed that further degradation processes would be rather low due to the low moisture contents of < 35% (t_4).

During the four months of storage a continuous and significant drying effect was seen in all piles. Due to the addition of $Ca(OH)_2$ in dry form, the initial moisture content of the piles with additive was approx. 5 percentage points lower than the moisture content of the piles without additive. The piles did not differ in their drying dynamics. The drying process of the wood chips started at the bottom level and was further on slowly migrating to the centre and top part of the piles. The distribution of the moisture contents over the cross-section indicated heterogeneous drying. Apparently, the cross-section moisture content of the reference pile (0% additive) differed considerably with a particularly wetter outer layer, as the other two piles that seemed to dry uniformly. Unexpectedly, the top level revealed a moisture content of 89% (0% additive). As seen in other studies, common moisture contents of the top level are within the range of 65 to 70% [5,36]. However, comparing the mean moisture

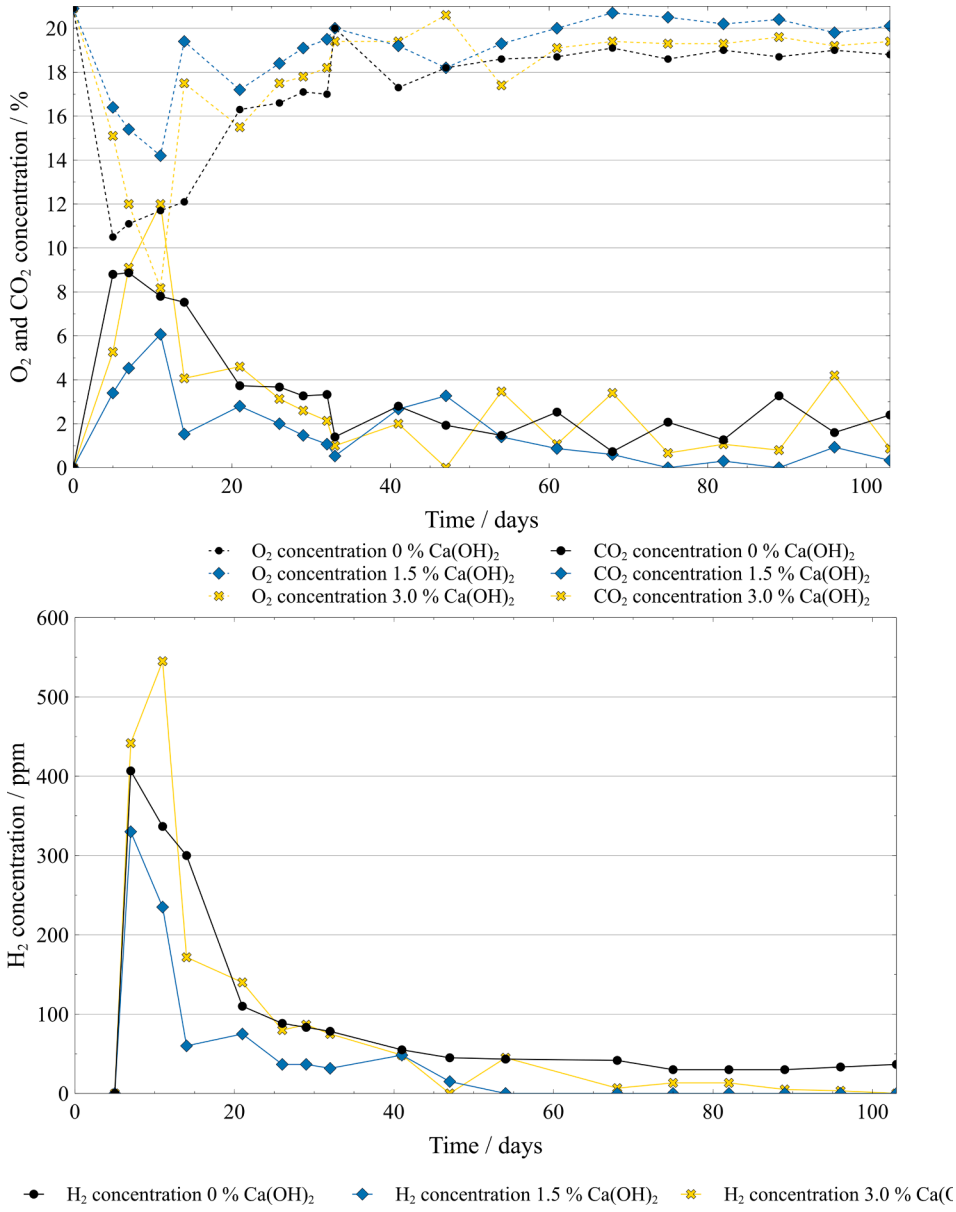

Fig. 9. Mean CO_2, O_2 and H_2 concentrations in the piles during the storage trial.

content of the cross-sections and of the sampling columns, the latter provides a good portrayal of the overall pile conditions. Since the moist outer layer is included in the mean values of the cross-section moisture contents, the values of the sampling columns are lower by two to nine percentage points. As recently reported by [37], high moisture content is a good indicator of microbial, especially fungal activity.

The pile temperature is a reliable indicator representing microbial respiratory activity, as shown by CO_2 concentrations. However, discrimination between CO_2 deriving from microbial activity and chemical oxidation of the wood chips was not possible. Due to optimal conditions at the beginning of the storage (high amount of easily available nutrients, water and oxygen), the maximum CO_2 production can be evidenced within the first two weeks. However, as other studies showed [36,38,39], besides heat generated by microbial respiration, a significant amount of the generated heat results from residual respiration by wood parenchyma cells. As the results show, the pile temperatures were comparably high reaching > 65 °C within the first week and the addition of $Ca(OH)_2$ further increased the pile temperature. The larger the additive dosage, the higher was the pile temperature. This temperature increase results from the exothermic reaction of $Ca(OH)_2$ with CO_2 from the atmosphere, generating $CaCO_3$, water and heat. After a distinct peak, the temperatures of the piles with additive decreased steadily to < 40 °C; on the other hand, the temperature of the reference pile without additive never fell below 50 °C within the four months of storage. This very slow temperature decline can either be attributed to the porosity and structure of the wood chip piles itself, or to a high microbial activity. Microorganisms responsible for the degradation of the wood chips must be able to survive temperatures of 65 °C for at least one week, and be able to grow at 50–60 °C. Survival of high temperatures can be due to the production of thermo-resistant reproductive structures like endospores, ascospores, or chlamydospores. Only a few species of microorganisms with this property were isolated in this study, but they can occur in high abundances in this special substrate. The fungus *Rhizomucor miehei* and the endospore-forming bacterium *B. subtilis* (thermostable strain), both characterized by a good ligno-cellulolytic potential, are such typical thermotolerant wood decomposers [40].

The development of a wood colonizing microbiota, especially fungi, and consequently the process of wood decomposition within wood chip piles, is a very complex and dynamic successional process governed by

the rising pile temperatures and the relative moisture content [37,41], nutrient availability, and pH. It also depends on further multiple factors, e.g. wood species, chip particle size (size of surface as reactive part for microbes [42]), pile size [43], bulk density, and storage strategy (indoor vs. outdoor; coverage vs. no coverage). In contrast to earlier assumptions, this study demonstrated that most of the thermotolerant microorganisms responsible for wood degradation are already present in the natural inoculum of the wood substrate. This enables a fast and comparatively uniform colonization of the substrate: temperatures rise quickly to 65 °C within a few days, thus positively selecting for thermotolerant microorganisms present in the substrate. Elevated temperatures can be maintained for weeks and it can take months before piles return to ambient temperature.

The dry matter loss (DML) of the two piles with additive fluctuated at a level of around 10% after the second month; in contrast, the DML of the reference pile was constantly rising until the fourth month, suggesting a higher microbial activity until the end of storage. Again, this phenomenon might be ascribed to thermotolerant wood decomposers [44]. Taking a look at the gaseous emissions, there was a clear peak of CO_2 that correlates with the maximum temperature within the first two weeks of storage, but after this peak, the CO_2 concentration within all three piles was fluctuating between 0.5 and 4%. There was no significant difference in the CO_2 concentration between the piles. On the contrary, the H_2 evolution of the reference pile, first following the course of the CO_2 evolution, remained at a level of ~ 400 ppm for the whole storage time. No H_2 was released in the piles with additive after 50 days. The CO_2 emissions during wood chip storage are already well known [45–47]. Resulting from the decomposition of the wood components by bacteria and fungi, CO_2 is released under aerobic and anaerobic conditions. The magnitude of CO_2 emissions is directly linked to available nutrients, especially bark and needles have a high nutrient content leading to higher CO_2 emissions, as well as to a higher DML [45]. On the other hand, the role of hydrogen emissions during wood chip storage has not been studied extensively yet. Hydrogen may be produced microbially from cellulosic material under anaerobic conditions [48,49]. In fact, fungal and bacterial primary and secondary decomposers produce carbon dioxide, hydrogen and volatile organic acids during cellulose degradation [49].

The slower cooling down observed for the reference pile could be ascribed to both a higher microbial activity and a high content of small particles leading to a limited air circulation. Due to the heterogeneous nature of the spruce forest residues, differences in the porosity of the three piles cannot be excluded. In fact, the development of the temperature and moisture content of the two piles with additive was more uniform compared to the reference pile without additive. This is corroborated by smaller standard deviations of the moisture content (both from the sampling columns and the cross-section), dry matter loss and temperature.

The microbial screening showed that thermotolerant fungi and bacteria were already part of the autochthonous wood-chip microbiota at the storage intake (t_0). Thermo-resistance cultivation tests (at 37, 50, 55, 60, and 65 °C) revealed that only five species were able to grow at temperatures > 55 °C. Thus, the overall number of wood colonizing microorganisms was strongly reduced at elevated temperatures, indicating a good sanitization effect during storage at industrial scale.

5. Conclusions and outlook

In this first study investigating the effect of an alkaline additive on the storage behaviour of wood chips at industrial scale, $Ca(OH)_2$ was able to decrease the dry matter loss of moist wood chips from spruce significantly. The additive admixture led to up to 5 °C higher maximum pile temperatures, relevant also in terms of microbial sanitization, and a constant and fast drying process occurred. No change in the drying velocity could be demonstrated. The microbial colonization mainly occurs from autochthonous thermotolerant microorganisms already present in fresh wood chips.

It can be concluded that adding $Ca(OH)_2$ to moist wood chips can be considered as a simple, low-cost yet powerful method for conserving biomass and may have additional implications, such as reducing fungal and bacterial pathogens, reducing ash slagging and repurposing wood ash as a fertilizer for the agriculture and forestry sector. In order to develop a cost-effective concept for using $Ca(OH)_2$ as a conservation agent in full-scale, especially the preparation of the biomass-additive-blend has to be optimized. Mixing the dry additive with moist wood chips with a wheel loader was easy to perform and led to a good homogenization, however, is time-consuming. Using commercially available technology from agriculture (e.g. distributors for fertilizers) could be an alternative. For further reducing time and costs, the additive could be mixed with wood chips directly at the chipping site.

Further storage trials have to be conducted to proof the positive effect of the additive, as well as to investigate how a full-scale system can work economically feasible. For performing a profitability analysis, the method of how the wood chip and additive mixture is prepared has to be evaluated and tests performed. Additionally, the impact of $Ca(OH)_2$ on the combustion as well as the gasification of the stored biomass will be investigated in order to highlight possible positive effects during conversion.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fuel.2021.120738.

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