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1 Long term alkaline storage and pretreatment  
2 process of cover crops for anaerobic digestion

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## 7 Abstract

8 The aim of this work was to study an innovative alkaline process on two cover crops. CaO load of  
9  $60\text{g.kgTS}^{-1}$  was implemented to combine the functions of storage and pretreatment. Lab-scale  
10 reactors were monitored for 180 days to assess the effect of this process on the physico-chemical  
11 properties of the biomass. From the first days, pH was not maintained in an alkaline zone and  
12 microbial fermentation activity was observed with the degradation of available carbohydrates and  
13 production of metabolites,  $\text{CO}_2$  and  $\text{H}_2$ . High butyric acid accumulation was observed and mass  
14 losses of 18.1% and 9.0% of initial VS occurred for oat and rye, respectively. However, no methane  
15 potential loss was recorded in the short and long term and the crops were efficiently preserved.  
16 The pretreatment had no major impact on fiber solubilization, and no increase in BMP was  
17 obtained, which was attributed to the short duration of the alkaline conditions.

## 18 Keywords

19 Biomethane; Biogas; Catch crops Storage; Pretreatment; Silage fermentation

## 20 1 Introduction

21 The production of biomethane by anaerobic digestion (AD) is likely to increase greatly in the  
22 coming years, as it is expected to replace part of the natural fossil gas. A large variety of organic  
23 wastes can be valorized such as sewage sludge, biowastes, manure and crop residues.  
24 Additionally, in order to supply a sufficient biomass to meet the demand for biogas, crops are also  
25 grown to feed agricultural biogas plants. These crops are interesting substrates for AD since they  
26 have a high  $\text{CH}_4$  yield and allow a secure feed stock for the biogas plants. However, the cultivation  
27 of energy crops is in competition for arable land with food crops, which questions their

28 sustainability as a substrate for AD (Jury et al., 2010). Therefore, cover crops (CC), also called catch  
29 crops, are receiving an increasing interest as an alternative to energy crops. These crops are not  
30 being grown in place of food/feed crops, but during the intercropping period of their crop rotations.  
31 Thus, they avoid the bare soil period that may happen between two food crops cultivation. The  
32 use of cover crops in farming practices has both agricultural and environmental benefits, since it  
33 allows to avoid erosion and nutrient leaching (Igos et al., 2016; Jian et al., 2020; Sapkota et al.,  
34 2012), improves the accumulation of organic matter in the soil (Jian et al., 2020) and facilitates the  
35 control of undesirable weeds (Büchi et al., 2020). However, the use of CC for biogas production  
36 still faces some difficulties. First, the harvest only takes place once or twice a year, while the  
37 digesters are fed continuously. CC are often grown during short and unfavorable periods, and  
38 consequently their biomass yields are variable and unpredictable from one year to another. These  
39 two aspects make an efficient storage of the harvested crops a mandatory key point in their use  
40 for AD.

41 Currently, the storage step is performed by ensiling, a process widely used for forage storage for  
42 cattle feeding since the end of the 19<sup>th</sup> century (Goffart, 1877) . Ensiling relies on the spontaneous  
43 fermentation of the substrate under anaerobic conditions, allowing the release of organic acids  
44 (mostly lactic acid) and acidification of the medium (Elferink et al., 1999; Weinberg and Ashbell,  
45 2003). The low pH under anaerobic conditions inhibits the microbial activity, thus ensuring the  
46 substrate stability over several months (Driehuis et al., 2003). When executed properly, ensiling  
47 allows a long-term preservation of the biomass energy potential, but various degradations may  
48 occur, depending on a large panel of parameters from the crop characteristics to the silo  
49 conception (Teixeira Franco et al., 2016). If this process has been optimized for animal feeding for

50 decades, its application to anaerobic digestion is still relatively new and may be improved. New  
51 storing methods may also be developed, specifically designed for anaerobic digestion.

52 Another limit in the use of CC for AD is related to their physicochemical characteristics. The cell  
53 walls are composed of a complex lignocellulosic matrix made of cellulose, hemicellulose and lignin.  
54 This structure limits the access of the microorganisms to the highly degradable carbohydrates of  
55 the cellulose and hemicellulose, lowering the yield and the kinetic of the crop conversion into  
56 methane (Monlau et al., 2013b). In order to enhance the anaerobic digestion of such  
57 lignocellulosic matrix, many types of pretreatment processes have been reported in the literature  
58 and their actions on lignocellulose have been well described. Among the different technologies,  
59 alkaline pretreatments are particularly relevant since alkaline conditions are highly effective in  
60 solubilizing lignin (Carrere et al., 2016). High increases in biochemical methane potential (BMP)  
61 were obtained by applying intensive conditions of pretreatments such as high temperature  
62 (Sambusiti et al., 2012) or high chemical concentration (Jiang et al., 2017). In addition, significant  
63 improvements in BMP were also observed in mild conditions, using NaOH, CaO or KOH, allowing  
64 much lower energy consumption (Khor et al., 2015; Thomas et al., 2018). In these conditions, the  
65 extension of the duration had a positive effect on the pretreatment effectiveness, and the  
66 treatment duration appeared, under certain conditions, to be more favorable than the reactive  
67 concentration (Thomas et al., 2018). Despite its lower efficiency when compared to other alkaline  
68 compounds like NaOH and KOH, lime presents the advantage to be less expensive. Additionally,  
69 CaO is already used on agricultural sites for soil liming to control acidity. Consequently, on an  
70 agricultural biogas plant, the use of lime is much more compatible with the use of digestate as a  
71 fertilizer than NaOH that causes an increase in the soil salinity due to sodium.

72 If alkaline pretreatments may constitute an interesting option for lignocellulosic biomass such as  
73 cover crops, applying them on stored silage does not constitute an adequate solution because of  
74 their high concentrations in acidic compounds such as lactic and acetic acids. A large amount of  
75 alkaline chemical would be necessary to neutralize the acids and increase the pH. For this reason,  
76 the implement of the alkaline pretreatment just after harvesting and extension of the  
77 pretreatment reaction time up to several months may constitute an alternative to ensiling for the  
78 storage of cover crops, while maximizing the alkaline treatment action. Compared to silage, which  
79 leads to a drop in pH in acidic regions (pH=4), microbial inhibition, which is necessary to maintain  
80 the methanogenic potential of the substrate, can be ensured by maintaining alkaline conditions.

81 However, as the addition of an alkaline agent is currently mostly used for pre-treatment process  
82 purposes and not for storage purposes, the duration of the treatment reported in the bibliography  
83 usually ranges from a few hours to 1 or 2 days and very few studies investigated longer duration of  
84 up to 7 days (Atelge et al., 2020). Digman et al. (2010) reported an alkaline pretreatment +  
85 anaerobic storage process of switchgrass and reed canarygrass using CaO for ethanol production.  
86 Several CaO loadings from 14.6 to 100g.kg<sup>-1</sup> were applied with storage durations ranging from 30  
87 to 180 days. The authors reported a good preservation of the substrate in all cases, even if no  
88 global energy balance including mass losses was calculated. In this study, the pretreatment  
89 efficiency increased together with lime loading and the highest ethanol yields were obtained with  
90 85 and 100g.kg<sup>-1</sup> CaO. However, the two studied solid content were very high (43 and 66%TS) and  
91 it was observed that moisture content had a positive effect on the pretreatment efficiency. This  
92 may suggest that interesting results with lower CaO loading can be obtained in wetter conditions.  
93 Finally, even if a correlation between the added CaO amount and the final pH of the silos was  
94 reported, no signs of undesirable fermentations were stressed. However, no dynamic monitoring

95 of the variation of the physico-chemical characteristics was made, making difficult the  
96 interpretation of the possible metabolic pathways that took place and fine understanding of the  
97 process.

98 The aim of this study is to evaluate the mechanisms involved during a long-term alkaline storage  
99 (i.e., CaO) of two cover crops, and its impact on the conservation/improvement of the energy  
100 potential in anaerobic digestion. The operating conditions were chosen to limit the pretreatment  
101 cost, following a procedure similar to ensiling, except the CaO addition as a dry powder before  
102 closing the silo. The conditions were set at ambient temperature. The main novelty of this work is  
103 the high solid content of the alkaline pretreatment, and the extended reaction duration until  
104 fulfilling the storage function. The dynamical changes in the physico-chemical characteristics of the  
105 substrate were observed over a six-month period as well as the impact on the methane potential.

## 106 2 Materials and Methods

### 107 2.1 Feedstock

108 Two cover crops were used for this experiment. An oat sample (*Avena sativa*) was collected on an  
109 experimental agricultural site (Arvalis, Montardon 64121, France) and a rye sample (*Secale*  
110 *cereale*) on another agricultural site (Biométharn, Aiguefonde 81200, France). Both crops were  
111 grown as winter cover crops, sowed in mid-October and harvested in the very last days of April at  
112 maturity stage BBCH 60 (beginning of flowering) and BBCH 59 (end of heading) for oat and rye,  
113 respectively. The crops were hand-harvested at approximate cutting height of 10 cm and stored  
114 overnight in sealed plastic bags as whole plants at 4°C before use.

## 115 2.2 Alkaline storage conditions

116 Prior to the experiment, the crops were chopped into 1-2 cm pieces using a garden shredder (AXT  
117 2550TC, Bosch GmbH). Then, the alkaline reagent CaO was added as a dry powder and was  
118 homogenized manually. A CaO loading of  $60\text{g.kg}_{\text{TS}}^{-1}$  was chosen as a compromise between  
119 pretreatment efficiency and reactive cost according to the literature. Jiang et al. (2017) found the  
120 most interesting lime load for  $70\text{g.kg}_{\text{TS}}^{-1}$   $\text{Ca}(\text{OH})_2$  (equivalent to  $53\text{g.kg}_{\text{TS}}^{-1}$  CaO) while Thomas et al.  
121 (2018) and Khor et al. (2015) obtained significant BMP improvement for  $50\text{g.kg}_{\text{TS}}^{-1}$  CaO and  $75$   
122  $\text{g.kg}_{\text{TS}}^{-1}$   $\text{Ca}(\text{OH})_2$  (equivalent to  $57\text{g.kg}_{\text{TS}}^{-1}$  CaO) respectively. The storage experiments were  
123 conducted in 2.6 L glass flasks sealed with air-tight lids equipped with a rubber septum that allows  
124 gas sampling, pressure measurement and pressure release. The experimental setup was inspired  
125 by the one used by Hillion (2017), which provided a high repeatability. For each crop, 5 replicates  
126 were prepared to be sacrificed after 2, 7, 21, 60 and 180 days to monitor the impact of the  
127 pretreatment on biomass conservation and the variation of the physico-chemical properties. 700 g  
128 of samples were introduced in each flask just after mixing with CaO, and packed manually until a  
129 density of  $500\text{gFM.L}^{-1}$ . The flasks were finally flushed with  $\text{N}_2$ , sealed and stored in a dark place at  
130  $22^\circ\text{C}$  until their opening.

## 131 2.3 Silo monitoring and sampling

132 Gas production and mass losses were monitored regularly all over the experiment. The volumetric  
133 gas production was measured by a pressure difference method. Gas sampling and pressure release  
134 were made as often as necessary depending on the gas production kinetics. The pressure was  
135 measured through the septum with a manometer (Keller LEO 2). Gas was released when pressure  
136 exceeded 1.2 bar, and the flask was weighted. The volume of gas inside the flasks (headspace +



137 pore space) was calculated by subtracting the volume of added substrate to the flask volume. The  
138 volume of the substrate was calculated using the theoretical density equation adapted from  
139 McNulty *et al.* (1982) (Eq.(1)):

$$140 \quad \rho_t = (1 - C) [(M/\rho_l) + (1 - M)/\rho_s] + C/\rho_{CaO} \quad (1)$$

141 where M = fractional moisture content related to fresh matter (FM); C = CaO content related to  
142 fresh matter;  $\rho_l$  = water density = 1000 kg.m<sup>-3</sup>;  $\rho_s$  = dry matter density = 1421 kg.m<sup>-3</sup> and  $\rho_{CaO}$  = CaO  
143 density = 3345 kg.m<sup>-3</sup>. The gas composition was analyzed using a gas chromatography (Perkin  
144 Elmer Clarus 580) as described in section 2.4.

145 For each sampling date, one flask was opened after measuring final gas volume and composition  
146 and weight. The whole sample was homogenized and mixed using a knife mill (Pulverisette 11,  
147 Fritsch). Samples were prepared and stored for BMP test by freezing around 2 g<sub>TS</sub> of matter at -  
148 20°C. Total solids (TS) and volatile solids (VS) were measured directly on the samples in triplicate.  
149 Water extraction was performed for the measurement of pH, water-soluble carbohydrates (WSC),  
150 volatile fatty acids (VFA) and other metabolites. 30 gFM of sample were steeped in 150 mL of  
151 deionized water for 18 h at 4°C in sealed plastic pots in triplicate, as suggested by Porter & Murray  
152 (2001). Each liquid extract was used separately for pH and soluble compounds analysis. pH was  
153 measured directly after extraction on the mixture. Then, the liquid phase was separated by  
154 centrifugation (18750 g, 20 min, 4°C) and frozen in air-tightly closed tubes for further WSC, VFA  
155 and metabolites analysis.

## 156 2.4 Physicochemical analysis

157 TS were measured in triplicate by drying 30 g of sample at 105°C for 24 h. Volatile Solids (VS) were  
158 then measured by calcination of the dry residue (550°C, 3 h). Pretreated substrates can contain

159 volatile fatty acids (VFA), lactic acid (LA) and some alcohols that may evaporate during oven  
160 drying, causing underestimation of TS and VS. For this reason, the TS content value measured by  
161 oven drying was corrected using the equation proposed by Porter & Murray (2001) (Eq. (2)):

$$162 \quad TS_C = TS_M + 0.375 LA + 0.892 VFA + 0.975 Alcohols \quad (2)$$

163 where  $TS_C$  = corrected TS;  $TS_M$  = measured TS; LA = lactic acid concentration; VFA = total VFA  
164 concentration; Alcohols = total alcohols concentration. All concentrations are in  $\text{g}\cdot\text{gFM}^{-1}$ .

165 pH was measured in triplicate directly on the extraction mixture using a WTW® SenTix® 41 probe  
166 on a WTW® inoLab® pH7110. WSC and concentration of metabolites (i.e., lactic acid (LA), acetic  
167 acid (AA), propionic acid (PA), butyric acid (BA) and ethanol (EtOH)) were measured from the  
168 centrifuged liquid phase after filtering (0,2  $\mu\text{m}$  nylon filter) by High Performance Liquid  
169 Chromatography on Aminex 4PX-87H column (Bio-Rad) at 45°C. Sulfuric acid (0,005 M; 0,3  $\text{mL}\cdot\text{min}^{-1}$ )  
170 <sup>1</sup>) was used as mobile phase. WSC content was calculated as the sum of glucose, fructose, xylose  
171 and arabinose. Ammonia concentration was measured on liquid extract by titration with boric acid  
172 using a Gerhardt® Vapodest 50s® carousel. The gas composition was analyzed by gas  
173 chromatography (Perkin Elmer Clarus® 580). The volume of the gas sample was 200  $\mu\text{L}$  and argon  
174 was used as carrier gas (350 kPa at 34  $\text{mL}\cdot\text{min}^{-1}$ ). After injection,  $\text{CO}_2$  was separated from other gas  
175 by a capillary R-Q-bond column (30 m x 0.32 mm).  $\text{H}_2$ ,  $\text{O}_2$ ,  $\text{N}_2$  and  $\text{CH}_4$  were separated on a Rt-  
176 Molsieve 5 Å capillary column (30 m x 0.32 mm). Injector and thermal conductivity temperatures  
177 were set at 250 °C and 150 °C, respectively.

178 Fiber distribution was analyzed in triplicate using the Van Soest and Wine method (1967) from  
179 freeze-dried samples crushed to 1mm theoretical particle size. Water extract (W.EX), neutral  
180 detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and calcination

181 residue (CAL) content were determined. Water-soluble compounds (W.SOLU), neutral detergent  
182 soluble compounds (SOLU), hemicellulose (HEMI), cellulose (CELL) and lignin content (LIGN) were  
183 calculated as follow :  $W.SOLU = 1 - W.EX$ ;  $SOLU = WEX - NDF$ ;  $HEMI = NDF - ADF$ ;  $CELL = ADF -$   
184  $ADL$ ;  $LIGN = ADL - CAL$ . One-way analysis of variance (ANOVA) was used to test the difference in  
185 mean value between the different fiber fractions.

186 The total carbon (TC) and total nitrogen (TN) were determined via an elemental analyzer  
187 (FlashSmart®, Thermo Fisher Scientific®) on finely grounded freeze dried samples. TC and TN  
188 analysis were not replicated.

189

## 190 2.5 Biochemical Methane Potential Test

191 BMP was measured in batch assay. Samples were digested in 550 mL flasks with a working volume  
192 of 400 mL. Each flask is filled with 5 gTS.L<sup>-1</sup> of substrate, 5 gVS of anaerobic inoculum (UASB  
193 granular sludge), NaHCO<sub>3</sub> buffer (50g.L<sup>-1</sup>), and macro- and microelement solutions as described by  
194 Monlau (Monlau et al., 2012). Before being closed, the flasks were flushed with N<sub>2</sub> to obtain  
195 anaerobic conditions. Triplicate bottles were incubated at 35°C. Controls containing only inoculum,  
196 buffer and macro- and microelement solutions were prepared in order to subtract the  
197 endogenous methane production of the inoculum from the one due to the samples digestion. The  
198 methane production was measured using an automatic batch test system (AMPTS® II, Bioprocess  
199 Control®, Sweden). Methane potentials are expressed as the volume of methane produced per  
200 amount of VS added for the BMP test, or per initial amount of VS estimated after taking into  
201 account the mass losses. BMP expressed in NmLCH<sub>4</sub>.gVS<sub>added</sub> is useful to estimate substrate  
202 degradability after storage, while BMP expressed in NmLCH<sub>4</sub>.gVS<sub>initial</sub> is mandatory to evaluate the

203 global balance of the alkaline pretreatment process. One-way analysis of variance (ANOVA) was  
204 used to test the difference in mean value between BMP of fresh and stored samples.

## 205 2.6 Theoretical gas production calculation

206 In addition to the measured volume by pressure difference and gas chromatography, the  
207 cumulated gas production at 180 days of CO<sub>2</sub> and H<sub>2</sub> was estimated from the associated  
208 metabolites. Since most of the microbial fermentations are accompanied by CO<sub>2</sub> and H<sub>2</sub>  
209 production, their respective stoichiometric reactions were used in the following equations,  
210 adapted from Hillion et al. (2018):

211

$$\begin{aligned} 212 \text{ Cumulated CO}_2 = & \text{ Acetic acid + Ethanol + Propionic acid} \\ 213 & + 2 \text{ Butyric acid + Isobutyric acid} \\ 214 & + \text{ Valeric acid + Isovaleric acid + Caproic acid} \quad (3) \end{aligned}$$

$$\begin{aligned} 215 \text{ Cumulated H}_2 = & 2 \times [\text{Acetic acid + Butyric acid + Isobutyric acid} \\ 216 & + \text{ Valeric acid + Isovaleric acid}] \quad (4) \end{aligned}$$

217 where cumulated CO<sub>2</sub> and H<sub>2</sub> are expressed in mmol.kgVS<sub>init</sub><sup>-1</sup> and all metabolites concentrations  
218 are expressed in mmol.kgVS<sub>add</sub><sup>-1</sup>.

219

## 220 3 Results and Discussion

### 221 3.1 Raw material characterization

222 The main characteristics of the oat and rye fresh samples are summarized in Table 1. Both crops  
223 show a relatively low TS content when compared to other forage crops for silage making, whose  
224 optimal TS at harvest is around 30% (Teixeira Franco et al., 2016). These values are representative  
225 of what is expected for cover crops. Molinuevo-Salces et al. (2013) reported an average TS value of  
226  $17 \pm 5 \%$  and a variation from 10 to 29%TS for 20 different experimental cover crop assays. Cover  
227 crops usually have a low TS at harvest since they are cut before the optimal growth stage.  
228 However, since no water was added for the alkaline storage process, the TS value is high when  
229 compared to other alkaline pretreatment conditions in the literature, where TS is usually less than  
230 or equal to 10% (Khor et al., 2015; Monlau et al., 2013a, 2012; Thomas et al., 2018).

231 Both crops had a low nitrogen content, with C/N ratios of 59 and 38 for oat and rye, respectively.  
232 Cereals are reported to have a lower nitrogen content than legumes or grasses in the literature  
233 (Kaiser and Piltz, 2004).

234 Water-soluble carbohydrates (WSC) were mostly composed of fructose and glucose, which are the  
235 principal primary soluble sugars found in temperate forage grass (Downing et al., 2008). Initial  
236 concentrations in WSC of  $131 \text{ g.kgVS}^{-1}$  and  $66 \text{ g.kgVS}^{-1}$  were measured for oat and rye, respectively.  
237 These are considered as medium (oat) to low (rye) when compared to other crops forages in  
238 temperate climate, whose WSC content varies from 50 to more than  $300 \text{ g.kgVS}^{-1}$  (Kaiser and Piltz,  
239 2004). However, if a high WSC amount is essential to ensure a good quality of lactic fermentation  
240 in silage making, these soluble sugars can be used as easily accessible and biodegradable  
241 substrates for undesired fermentations in the present process. Fermentative metabolites such as

242 VFA, lactic acid or ethanol were not detected in the raw samples, indicating the absence of  
243 fermentative activity during harvesting and storage prior to the start-up of the experiments.

244 Both crops present similar composition in fiber constituents in the range of commonly  
245 encountered values in cover crops, with relatively high hemicellulose content (Molinuevo-Salces  
246 et al., 2013)

### 247 3.2 Evolution of the chemical characteristics

248 The parameters allowing to follow the possible microbial activity and fermentative pathways  
249 involved during storage were monitored all along the experiments. Table 2 presents the variation  
250 of pH and ammonia concentration over storage period. Figure 1 shows the profile of the main  
251 analyzed components at different studied storage times and the cumulated gas production. Figure  
252 2 shows the distribution of the energy potential of the main analyzed compounds (WSC,  
253 metabolites and gases) expressed in COD equivalent. Unexpectedly, the pH dropped very quickly  
254 after closing the flasks and reached the neutrality in less than one week. At the same time,  
255 increasing concentrations of organic acids were recorded. The loss of the alkaline conditions was  
256 coupled to a quick WSC fermentation to VFA, lactic acid, CO<sub>2</sub> and H<sub>2</sub> (Figures 1 et 2). From the very  
257 first days of the storage, different metabolic pathways occurred one after the other and were  
258 associated with the accumulation and/or consumption of different kinds of metabolites (Figure 1).  
259 Depending on the type of crop, even if some differences in the profile of metabolite  
260 concentrations were observed, four main phases can be identified.

261 During the first two days, a fast and strong accumulation of acetic acid was measured, up to  
262 concentration as high as 20.7 g.kgVS<sub>add</sub><sup>-1</sup> and 25.8 g.kgVS<sub>add</sub><sup>-1</sup> for oat and rye, respectively.

263 Interestingly, this production of acetic acid was not accompanied by any significant gas

264 production, and occurred at a high pH (between 12 and around 10). If this pH range is not  
265 favorable to most of fermentative bacteria, examples of the literature reported high acetic acid  
266 fermentation rates at elevated pH of 10 in mesophilic conditions (Dai et al., 2018; Ma et al., 2017)  
267 and even 11 at 22°C (Yuan et al., 2006). It was shown that most bacteria could not survive to the  
268 alkaline environment (Ma et al., 2017) and the cited works used inoculum from waste water  
269 treatment plants or anaerobic reactors with a high initial microbial diversity, which was not the  
270 case in the present work. A well-known abiotic chemical reaction, the deacetylation of  
271 hemicellulose, could thus be implicated in alkaline conditions. Alkaline deacetylation of  
272 lignocellulosic biomasses such as rice straw (Castro et al., 2017) and corn stover (Chen et al., 2014)  
273 using a dilute NaOH pretreatment resulted in the release of up to 24 g.kgVS<sup>-1</sup> acetic acid by  
274 solubilizing the acetyl groups from xylans of hemicellulose. This deacetylation reaction was carried  
275 out at high temperature (50 to 80°C), with medium to high reagent concentration (20 to 80g.kgTS<sup>-1</sup>  
276 NaOH), with short reaction time of less than two hours, making obvious the absence of  
277 microorganism action in the process. The acetic acid release is strongly associated to the fast pH  
278 drop during the first two days of the storage.

279 From day 2 to day 7, whatever the substrate, different soluble fermentative metabolites  
280 accumulated, and in particular lactic and propionic acids (oat and rye), acetic acid (oat) and  
281 ethanol (rye). The evidence of fermentative activities is also confirmed by the degradation of  
282 glucose and fructose with a major decrease of their initial concentration. An increase in CO<sub>2</sub> and H<sub>2</sub>  
283 production rates was observed. The nature of the consumed sugars and produced metabolites,  
284 including gases, indicate that heterolactic fermentation occurred, following one or the other of the  
285 reactions below (Eq. (5) and (6)) (Hillion et al., 2018):





288      Heterolactic fermentations are commonly met in silage fermentation. However, the optimal pH  
289      range for the growth of the involved lactic acid bacteria is in the range of 5 to 6, and their growth  
290      rate is often reduced if the pH of the crop to be ensiled is equal to or greater than 7 (Driehuis et  
291      al., 2003) even if some lactic fermentations were reported with a starting pH of 9.5 using selected  
292      *Lactobacillus* strains as inoculum. This may evidence the existence of heterogeneous zones in the  
293      substrate with a lower pH than the measured value, allowing the development of lactic acid  
294      bacteria. The pH heterogeneity was probably due to the application of CaO as a dry powder  
295      and/or the acetic acid release during the deacetylation phase.

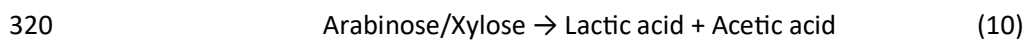
296      From the second week of storage until day 60 (oat) or 180 (rye), acetic acid concentrations were  
297      increasing, while certain metabolites accumulated after one week of storage were completely  
298      (lactic acid, ethanol) or partially (acetic acid, propionic acid) converted into butyric acid. During  
299      this period, residual glucose and fructose were completely consumed. High gas production was  
300      observed during this phase, and the ratio between CO<sub>2</sub> and H<sub>2</sub> production flow rates remained  
301      stable at CO<sub>2</sub>/H<sub>2</sub> = 2.5. Butyric fermentation is due to bacteria of the *Clostridium* genus, which  
302      commonly grow in insufficiently acidified silages. They grow on soluble carbohydrates (Eq. (7),  
303      (Hillion et al., 2018)) and organic acids by a fermentation pathway that can be considered as the  
304      sum of Eq. (8) and (9) (Driehuis et al., 2003).





308 In storage processes for anaerobic digestion, clostridial fermentations are considered as  
309 undesired, since they lead to the production of H<sub>2</sub> that escapes from the silo and induces losses of  
310 methane potential (Kreuger et al., 2011; Teixeira Franco et al., 2016).

311 In the present case, the total consumption of the previously produced lactic acid indicates that, at  
312 least, clostridial fermentations of lactic acid occurred. However, regarding the balance between  
313 degraded COD from initial WSC and cumulated metabolites (Figure 2), the observed sugar  
314 consumption could not explain alone the accumulation level of primary metabolites (LA, AA, PA,  
315 EtOH) and their further conversion into BA. This indicates that more complex carbohydrates were  
316 constantly degraded and released soluble sugars. These released WSC, mostly xylose, glucose and  
317 arabinose from the hemicellulose, may have been continuously converted into lactic acid (Eq. (5)  
318 or (10) (Hillion et al., 2018)) and then into butyric acid (Eq. (8) and (9)), or directly degraded into  
319 butyric acid following Eq. (7).



321 The clostridial activity may also be evidenced by the fermentation of amino acids and amines into  
322 ammonia (Hillion et al., 2018). Table 2 shows a constant increase in ammonia concentration in rye,  
323 reaching 22% of initial TN after 180 days of storage. Surprisingly, a much lower ammonia  
324 accumulation was observed in oat, despite an important butyric fermentation. This could be  
325 explained by the fact that butyric fermentation started earlier in oat, with BA apparition before 7  
326 days of storage, when pH was still lowering from 10.5 to 6.6. In these conditions of high pH, a large  
327 fraction of the ammonia may have undergone a faster volatilization in the form of NH<sub>3</sub>. Total  
328 Nitrogen analysis could help to conclude on this hypothesis, but no such measurements were  
329 performed on stored samples in the present work.

330 During the last phase of the process, the microbial activity was considerably reduced, except an  
331 acetic acid production that continued following a slow kinetic. Oat samples reached this relative  
332 steady state 3 times faster than rye samples after only 60 days and the final concentration in BA  
333 was twice higher, what may be explained by the higher initial amount of WSC in oat. Interestingly,  
334 this state of relative stability began after BA reached a threshold value of  $100\text{g}\cdot\text{gVS}_{\text{add}}^{-1}$ , after what  
335 no further BA production was observed. BA may therefore have an inhibitory effect. However, the  
336 fact that rye samples also seem to reach a similar stable state at the end of the experiment from  
337 day 108, even with a 2.7 times lower BA amount, indicates that the decrease of the microbial  
338 activity is more probably due to the depletion of the accessible fermentation substrates.

### 339 3.3 Process impact on Fiber distribution, VS and BMP preservation.

340 The pretreatment effect on the fiber distribution of the crops was studied, and the results are  
341 presented in Figure 3. In Oat samples, the three most accessible fractions significantly varied, from  
342  $39.6$  to  $31.6\%TS_{\text{init}}$  (W.SOLU),  $1.1$  to  $4.3\%TS_{\text{init}}$  (SOLU) and  $26.3$  to  $11.8\%TS_{\text{init}}$  (HEMI) fractions  
343 significantly varied ( $p < 0.05$ ). Rye samples undergone a lower transformation, with significant  
344 changes ( $p < 0.05$ ) for SOLU ( $27.0$  to  $25.3\%TS_{\text{init}}$ ) and HEMI ( $29.4$  to  $17.7\%TS_{\text{init}}$ ) fractions only. In  
345 both cases, no solubilization of the cellulose and lignin fractions were observed. The hemicellulose  
346 was greatly reduced, and easily accessible carbohydrates were released, mostly xylose, glucose  
347 and arabinose. This WSC release explains the difference in the balance between the consumed  
348 COD of the initial WSC and the accumulated COD of the metabolites (section 3.2 and Figure 2).  
349 Nonetheless, since no effect on cellulose and lignin were observed, the pretreatment action of  
350 CaO was seemingly limited, and the long-term effect of the pretreatment on lignin wasn't  
351 obtained.

352 Figure 4 shows the dynamics of mass losses for both crops over the whole storage period. The gas  
353 production related to microbial fermentations was responsible for significant VS losses of 18.1%  
354 and 9.0% respectively for oat and rye during the experiment. The measured values were higher  
355 (oat) or comparable (rye) to the ones that are commonly observed in ensiling, where 5 to 12% TS  
356 losses occur during the fermentation of properly managed silages (Kaiser and Piltz, 2004).

357 The VS losses were mostly due to CO<sub>2</sub> production. Mass losses and cumulated CO<sub>2</sub> production  
358 followed the same behavior (Figures 1 et 3). Considering mass balance during alkaline  
359 pretreatments, mass losses computed on the base of the mass of gas release were 123 and 40  
360 g.kgVS<sub>init</sub><sup>-1</sup> for oat and rye respectively and were significantly lower than direct mass measurement  
361 of the jars (181 and 90 g.kgVS<sub>init</sub><sup>-1</sup>) respectively. This may evidence the presence of gas leaches on  
362 the experimental setup over the 6-month period, as direct weighting of jar is a more reliable  
363 method. Considering the theoretical gas production based on the accumulated metabolites  
364 calculated as presented in section 2.6, cumulated CO<sub>2</sub> and H<sub>2</sub> should theoretically be of 3572 and  
365 4341 mmol.kgVS<sub>init</sub><sup>-1</sup> for oat, 2207 and 3158.4 mmol.kgVS<sub>init</sub><sup>-1</sup> for rye.

366 The significant difference between these values and the measured ones presented in Figure 1,  
367 particularly considering the CO<sub>2</sub>/H<sub>2</sub> ratio, may also indicates that in-situ gas consumption like  
368 homoacetogenesis took place, converting H<sub>2</sub> and CO<sub>2</sub> into acetic acid (Eq. (11), (Hillion et al.,  
369 2018)).



371 This phenomenon occurs in dark fermentation, where in-situ H<sub>2</sub> consumption by homoacetogenic  
372 bacteria is reported, particularly at pH close to 7 (Saady, 2013). Consequently, the effective energy  
373 losses due to H<sub>2</sub> production were low.

374 BMP tests were carried out on the raw substrates and on substrates stored for 7, 60 and 180 days.  
375 Figure 5 summarizes the methane potential expressed in  $\text{NmLCH}_4 \cdot \text{gVS}_{\text{add}}^{-1}$  and  $\text{NmLCH}_4 \cdot \text{gVS}_{\text{init}}^{-1}$ , as  
376 detailed in section 2.5. The raw oat and rye samples BMP were respectively  $294 \pm 3$  and  $348 \pm 11$   
377  $\text{NmLCH}_4 \cdot \text{gVS}_{\text{init}}^{-1}$ . These values are comparable to other BMP of cereal cover crops that commonly  
378 vary from 200 to 400  $\text{NmLCH}_4 \cdot \text{gVS}^{-1}$  in the literature (Molinuevo-Salces et al., 2013).

379 Whatever the cover crop, the degradability of the substrate increased during the alkaline  
380 pretreatment storage by 29% (oat) and 8.5% (rye) after 180 days, suggesting at first glance a  
381 significant ( $p = 0.015$  and  $0.056$ , respectively) action of the pretreatment on the lignocellulosic  
382 biomass. However, after taking into account the mass losses during the storage, the methane  
383 potential only varied by +6% and -1% for oat and rye, respectively, which indicates no significant  
384 difference from the BMP of the fresh substrates ( $p = 0.355$  and  $0.676$ , respectively). During the  
385 storage period, the production of  $\text{H}_2$  can be the cause of an energy loss, and thus of the reduction  
386 of the methane potential. This loss was estimated by calculating the COD of  $\text{H}_2$  produced (Figure 2)  
387 and it represented 5.4 and 2.2  $\text{NmLCH}_4 \cdot \text{gVS}^{-1}$  for oat and rye, respectively 1.8 and 0.6% of initial  
388 BMP. This theoretical value is very low compared to that of the BMP of the fresh substrate, which  
389 may explain why no loss of methane potential was experimentally observed during storage. In  
390 well-preserved silages, slightly higher energy losses from 2 to 4% happen during fermentation  
391 (Kaiser and Piltz, 2004). Consequently, during the alkaline storage, because of the loss of mass of  
392 the substrate in the form of  $\text{CO}_2$  and the conversion of the initial soluble carbohydrates ( $373 \text{ NmL}$   
393  $\text{CH}_4 \cdot \text{g}^{-1}$ ) into more energetic compounds such as butyric acid that were released ( $636 \text{ NmLCH}_4 \cdot \text{g}^{-1}$ ),  
394 the methane potential was concentrated in the remaining dry matter. Figure 5 presents the  
395 estimated BMP of the present metabolites in the substrates, in  $\text{NmLCH}_4 \cdot \text{gVS}_{\text{add}}^{-1}$ . The increase of  
396 the BMP of the metabolites was strongly linked to the increase of the substrate degradability and

397 mass losses, showing the concentration of the BMP of the crop in the remaining biomass after  
398 storage.

399 Thus, negligible BMP losses occurred considering the whole process, and the initial methane  
400 potential of the harvest was efficiently preserved, despite microbial reactions considered as highly  
401 undesirable in crop storage processes such as butyric fermentation. By extension, this work brings  
402 interesting data for understanding the impact of ensiling on crops for biogas production. In fact,  
403 the substrate characteristics along with the microbial fermentation pathways that took place after  
404 the first days (pH close to neutrality and important butyric fermentation) is highly comparable to a  
405 silage fermentation considered as of a very poor quality. The quality of silage for animal feeding  
406 fulfills the following criteria: pH < 4.5, lactic acid > 30g.kgTS<sup>-1</sup>, butyric acid < 10% of total VFA  
407 (Bureenok et al., 2016), while the present stored substrate is far from these characteristics,  
408 showing that ensiling for biogas production has very different purposes than for animal feed. This  
409 suggests that the ideal silage characteristics for anaerobic digestion are not well defined yet, and  
410 future researches may address this subject.

411 Considering the initial pretreatment objective of the process, it can be concluded that the alkaline  
412 conditions were not maintained long enough to obtain the expected long-term pretreatment and  
413 increase the BMP. However, even if a significant increase is not already reached, the elevated pH  
414 (pH 7) of the stored crop could lower the detrimental effect of organic acids on the concrete walls  
415 of the silo, which is a reported problem with acid silages (pH 4) (Koenig and Dehn, 2016). Research  
416 needs to be undertaken in order to reach the optimal conditions of operation by focusing on the  
417 initial characteristics like the alkaline reactive nature and load, global TS and the presence of  
418 available substrates for microbial fermentation, with the purpose of maintaining the alkaline  
419 conditions for a sufficient time in order to increase the pretreatment efficiency.

## 420 4 Conclusions

421 A succession of abiotic and biotic reactions induced a fast and sharp pH drop from 12 to 7, and  
422 thus compromised the long-term action of the alkaline agent. Despite undesirable fermentations  
423 and high mass losses, no significant effect on the methane potential was observed during the  
424 storage process. Alkaline storage, however, induced an increase in specific biodegradability which  
425 is associated with mass loss. This study clearly demonstrates that the methanogenic potential of  
426 the two selected cover crops was maintained for more than 6 months. An optimized process under  
427 stable alkaline conditions could therefore allow a better pretreatment efficiency.

428

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434 (doi:10.5454/1.557234103446854E12)

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- 437 Atelge, M.R., Atabani, A.E., Banu, J.R., Krisa, D., Kaya, M., Eskicioglu, C., Kumar, G., Lee, C.,  
438 Yildiz, Y., Unalan, S., Mohanasundaram, R., Duman, F., 2020. A critical review of  
439 pretreatment technologies to enhance anaerobic digestion and energy recovery. *Fuel*  
440 270. <https://doi.org/10.1016/j.fuel.2020.117494>
- 441 Büchi, L., Wendling, M., Amossé, C., Jeangros, B., Charles, R., 2020. *Field Crops Research*  
442 Cover crops to secure weed control strategies in a maize crop with reduced tillage. *F.*  
443 *Crop. Res.* 247, 107583. <https://doi.org/10.1016/j.fcr.2019.107583>
- 444 Bureenok, S., Sisaath, K., Yuangklang, C., Vasupen, K., Schonewille, J.T., 2016. Ensiling  
445 characteristics of silages of Stylo legume (*Stylosanthes guianensis*), Guinea grass  
446 (*Panicum maximum*) and their mixture, treated with fermented juice of lactic  
447 bacteria, and feed intake and digestibility in goats of rations based on these silages.  
448 *Small Rumin. Res.* 134, 84–89. <https://doi.org/10.1016/j.smallrumres.2015.12.006>
- 449 Carrere, H., Antonopoulou, G., Affes, R., Passos, F., Battimelli, A., Lyberatos, G., Ferrer, I.,  
450 2016. Review of feedstock pretreatment strategies for improved anaerobic digestion:  
451 From lab-scale research to full-scale application. *Bioresour. Technol.* 199, 386–397.  
452 <https://doi.org/10.1016/J.BIORTECH.2015.09.007>
- 453 Castro, R.C. de A., Fonseca, B.G., dos Santos, H.T.L., Ferreira, I.S., Mussatto, S.I., Roberto,  
454 I.C., 2017. Alkaline deacetylation as a strategy to improve sugars recovery and  
455 ethanol production from rice straw hemicellulose and cellulose. *Ind. Crops Prod.* 106,  
456 65–73. <https://doi.org/10.1016/j.indcrop.2016.08.053>
- 457 Chen, X., Shekiro, J., Pschorn, T., Sabourin, M., Tao, L., Elander, R., Park, S., Jennings, E.,  
458 Nelson, R., Trass, O., Flanagan, K., Wang, W., Himmel, M.E., Johnson, D., Tucker,  
459 M.P., 2014. A highly efficient dilute alkali deacetylation and mechanical (disc) refining  
460 process for the conversion of renewable biomass to lower cost sugars. *Biotechnol.*  
461 *Biofuels* 7. <https://doi.org/10.1186/1754-6834-7-98>
- 462 Dai, K., Zhang, F., Zhang, Y., Zeng, R.J., 2018. The chemostat metabolite spectra of alkaline  
463 mixed culture fermentation under mesophilic, thermophilic, and extreme-  
464 thermophilic conditions. *Bioresour. Technol.* 249, 322–327.  
465 <https://doi.org/10.1016/j.biortech.2017.10.035>
- 466 Digman, M.F., Shinnars, K.J., Casler, M.D., Dien, B.S., Hatfield, R.D., Jung, H.-J.G., Muck,  
467 R.E., Weimer, P.J., 2010. Optimizing on-farm pretreatment of perennial grasses for  
468 fuel ethanol production. *Bioresour. Technol.* 101, 5305–5314.  
469 <https://doi.org/10.1016/J.BIORTECH.2010.02.014>
- 470 Downing, T.W., Buyserie, A., Gamroth, M., French, P., 2008. Effect of Water Soluble  
471 Carbohydrates on Fermentation Characteristics of Ensiled Perennial Ryegrass. *Prof.*

472 Anim. Sci. 24, 35–39. [https://doi.org/10.15232/S1080-7446\(15\)30807-X](https://doi.org/10.15232/S1080-7446(15)30807-X)

473 Driehuis, F., Elferink, S.O., Spoelstra, S.F., 2003. Microbiology of Ensiling.  
474 <https://doi.org/10.2134/agronmonogr42.c2>

475 Elferink, S.J.W.H., Driehuis, F., Gottschal, J.C., Spoelstra, S.F., 1999. Silage fermentation  
476 processes and their manipulation. FAO Plant Prod. Prot. Pap. 17–30.

477 Goffart, A., 1877. Manuel de la culture et de l'ensilage de maïs et autres fourrages verts  
478 [Manual of the cultivation and siloing of maize and other green fodders]. Paris.

479 Hillion, M. Lou, Moscoviz, R., Trably, E., Leblanc, Y., Bernet, N., Torrijos, M., Escudié, R.,  
480 2018. Co-ensiling as a new technique for long-term storage of agro-industrial waste  
481 with low sugar content prior to anaerobic digestion. Waste Manag. 71, 147–155.  
482 <https://doi.org/10.1016/j.wasman.2017.10.024>

483 Hillion, M., 2017. Optimisation de la digestion anaérobie en voie sèche continue de  
484 résidus lignocellulosiques. PhD thesis, Montpellier Supagro.

485 Igos, E., Golkowska, K., Koster, D., Vervisch, B., Benetto, E., 2016. Using rye as cover crop  
486 for bioenergy production: An environmental and economic assessment. Biomass and  
487 Bioenergy 95, 116–123. <https://doi.org/10.1016/j.biombioe.2016.09.023>

488 Jian, J., Du, X., Reiter, M.S., Stewart, R.D., 2020. A meta-analysis of global cropland soil  
489 carbon changes due to cover cropping. Soil Biol. Biochem. 143, 107735.  
490 <https://doi.org/10.1016/j.soilbio.2020.107735>

491 Jiang, D., Ge, X., Zhang, Q., Zhou, X., Chen, Z., Keener, H., Li, Y., 2017. Comparison of  
492 sodium hydroxide and calcium hydroxide pretreatments of giant reed for enhanced  
493 enzymatic digestibility and methane production. Bioresour. Technol. 244, 1150–1157.  
494 <https://doi.org/10.1016/J.BIORTECH.2017.08.067>

495 Jury, C., Benetto, E., Koster, D., Schmitt, B., Welfring, J., 2010. Life Cycle Assessment of  
496 biogas production by monofermentation of energy crops and injection into the  
497 natural gas grid. Biomass and Bioenergy 34, 54–66.  
498 <https://doi.org/10.1016/j.biombioe.2009.09.011>

499 Kaiser, A.G., Piltz, J.W., 2004. Successful silage.

500 Khor, W.C., Rabaey, K., Vervaeren, H., 2015. Low temperature calcium hydroxide  
501 treatment enhances anaerobic methane production from (extruded) biomass.  
502 Bioresour. Technol. 176, 181–188. <https://doi.org/10.1016/J.BIORTECH.2014.11.037>

503 Koenig, A., Dehn, F., 2016. Biogenic acid attack on concretes in biogas plants. Biosyst. Eng.  
504 147, 226–237. <https://doi.org/10.1016/j.biosystemseng.2016.03.007>

505 Kreuger, E., Nges, I., Björnsson, L., 2011. Ensiling of crops for biogas production: Effects on  
506 methane yield and total solids determination. Biotechnol. Biofuels 4, 1–8.



- 507 <https://doi.org/10.1186/1754-6834-4-44>
- 508 Ma, H., Liu, He, Zhang, L., Yang, M., Fu, B., Liu, Hongbo, 2017. Novel insight into the  
509 relationship between organic substrate composition and volatile fatty acids  
510 distribution in acidogenic co-fermentation. *Biotechnol. Biofuels*.  
511 <https://doi.org/10.1186/s13068-017-0821-1>
- 512 McNulty, P.B., Kennedy, S., 1982. Density Measurements of Grass by Toluene  
513 Displacement and Air Comparison Pycnometry. *Irish J. Agric. Reserach* 21, 75–83.
- 514 Molinuevo-Salces, B., Larsen, S.U., Ahring, B.K., Uellendahl, H., 2013. Biogas production  
515 from catch crops: Evaluation of biomass yield and methane potential of catch crops  
516 in organic crop rotations. *Biomass and Bioenergy* 59, 285–292.  
517 <https://doi.org/10.1016/j.biombioe.2013.10.008>
- 518 Monlau, F., Aemig, Q., Barakat, A., Steyer, J.P., Carrère, H., 2013a. Application of  
519 optimized alkaline pretreatment for enhancing the anaerobic digestion of different  
520 sunflower stalks varieties. *Environ. Technol. (United Kingdom)* 34, 2155–2162.  
521 <https://doi.org/10.1080/09593330.2013.808247>
- 522 Monlau, F., Barakat, A., Steyer, J.P., Carrere, H., 2012. Comparison of seven types of  
523 thermo-chemical pretreatments on the structural features and anaerobic digestion of  
524 sunflower stalks. *Bioresour. Technol.* 120, 241–247.  
525 <https://doi.org/10.1016/j.biortech.2012.06.040>
- 526 Monlau, F., Barakat, A., Trably, E., Dumas, C., Steyer, J.-P., Carrère, H., 2013b.  
527 Lignocellulosic Materials Into Biohydrogen and Biomethane: Impact of Structural  
528 Features and Pretreatment. *Crit. Rev. Environ. Sci. Technol.* 43, 260–322.  
529 <https://doi.org/10.1080/10643389.2011.604258>
- 530 Porter, M.G., Murray, R.S., 2001. The volatility of components of grass silage on oven  
531 drying and the inter-relationship between dry-matter content estimated by different  
532 analytical methods. *Grass Forage Sci.* 56, 405–11. <https://doi.org/10.1046/j.1365-2494.2001.00292.x>
- 534 Saady, N.M.C., 2013. Homoacetogenesis during hydrogen production by mixed cultures  
535 dark fermentation: Unresolved challenge. *Int. J. Hydrogen Energy* 38, 13172–13191.  
536 <https://doi.org/10.1016/j.ijhydene.2013.07.122>
- 537 Sambusiti, C., Ficara, E., Malpei, F., Steyer, J.P., Carrère, H., 2012. Influence of alkaline pre-  
538 treatment conditions on structural features and methane production from ensiled  
539 sorghum forage. *Chem. Eng. J.* 211–212, 488–492.  
540 <https://doi.org/10.1016/j.cej.2012.09.103>
- 541 Sapkota, T.B., Askegaard, M., Lægdsmand, M., Olesen, J.E., 2012. Effects of catch crop  
542 type and root depth on nitrogen leaching and yield of spring barley. *F. Crop. Res.* 125,

543 129–138. <https://doi.org/10.1016/j.fcr.2011.09.009>

544 Teixeira Franco, R., Buffière, P., Bayard, R., 2016. Ensiling for biogas production: Critical  
545 parameters. A review. *Biomass and Bioenergy* 94, 94–104.  
546 <https://doi.org/10.1016/j.biombioe.2016.08.014>

547 Thomas, H., Seira, J., Escudié, R., Carrère, H., 2018. Lime Pretreatment of Miscanthus:  
548 Impact on BMP and Batch Dry Co-Digestion with Cattle Manure. *Molecules* 23, 1608.  
549 <https://doi.org/10.3390/molecules23071608>

550 Van Soest, P.J., Wine, R.H., 1967. Use of Detergents in the Analysis of Fibrous Feeds . IV .  
551 Determination of Plant Cell-Wall Constituents. *J. Assoc. Off. Anal. Chem.* 50, 50–55.

552 Weinberg, Z., Ashbell, G., 2003. Engineering aspects of ensiling. *Biochem. Eng. J.* 13, 181–  
553 188. [https://doi.org/10.1016/S1369-703X\(02\)00130-4](https://doi.org/10.1016/S1369-703X(02)00130-4)

554 Yuan, H., Chen, Y., Zhang, H., Jiang, S., Zhou, Q., Gu, G., 2006. Improved bioproduction of  
555 short-chain fatty acids (SCFAs) from excess sludge under alkaline conditions. *Environ.*  
556 *Sci. Technol.* 40, 2025–2029. <https://doi.org/10.1021/es052252b>

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## 560 Table and Figure Captions

561 Table 1: Physicochemical characteristics of the catch crops

562 Table 2: pH and NH<sub>4</sub>-N variation during storage.

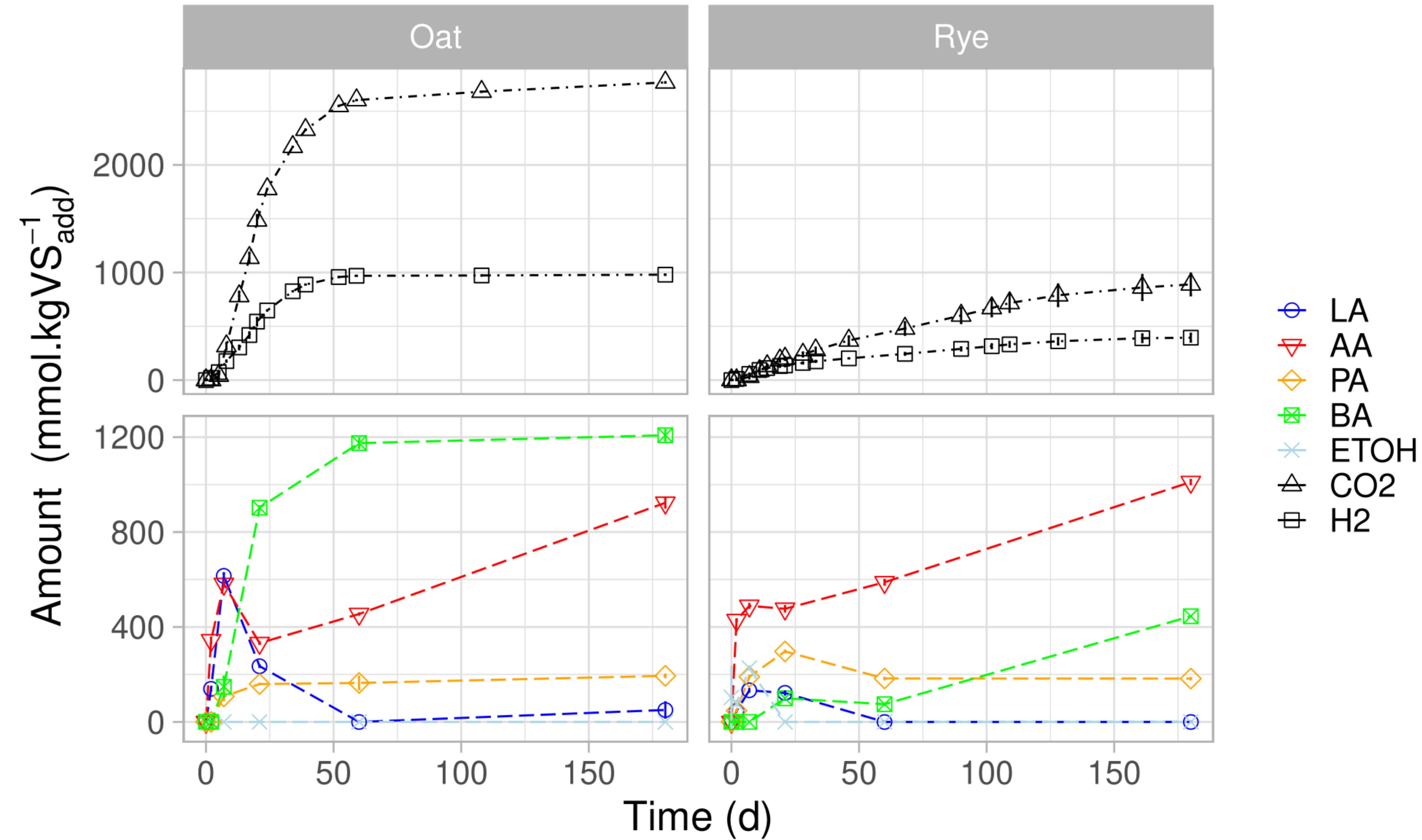
563 Figure 1: WSC, gas and metabolites variation during storage. Lactic acid (LA), Acetic acid (AA),  
564 Propionic Acid (PA), Butyric acid (BA) and Ethanol (ETOH) are expressed in mmol.kgVS<sub>add</sub><sup>-1</sup>. CO<sub>2</sub> and  
565 H<sub>2</sub> are expressed in cumulated mmol/kgVS<sub>init</sub><sup>-1</sup>. The error bars indicate standard deviation.

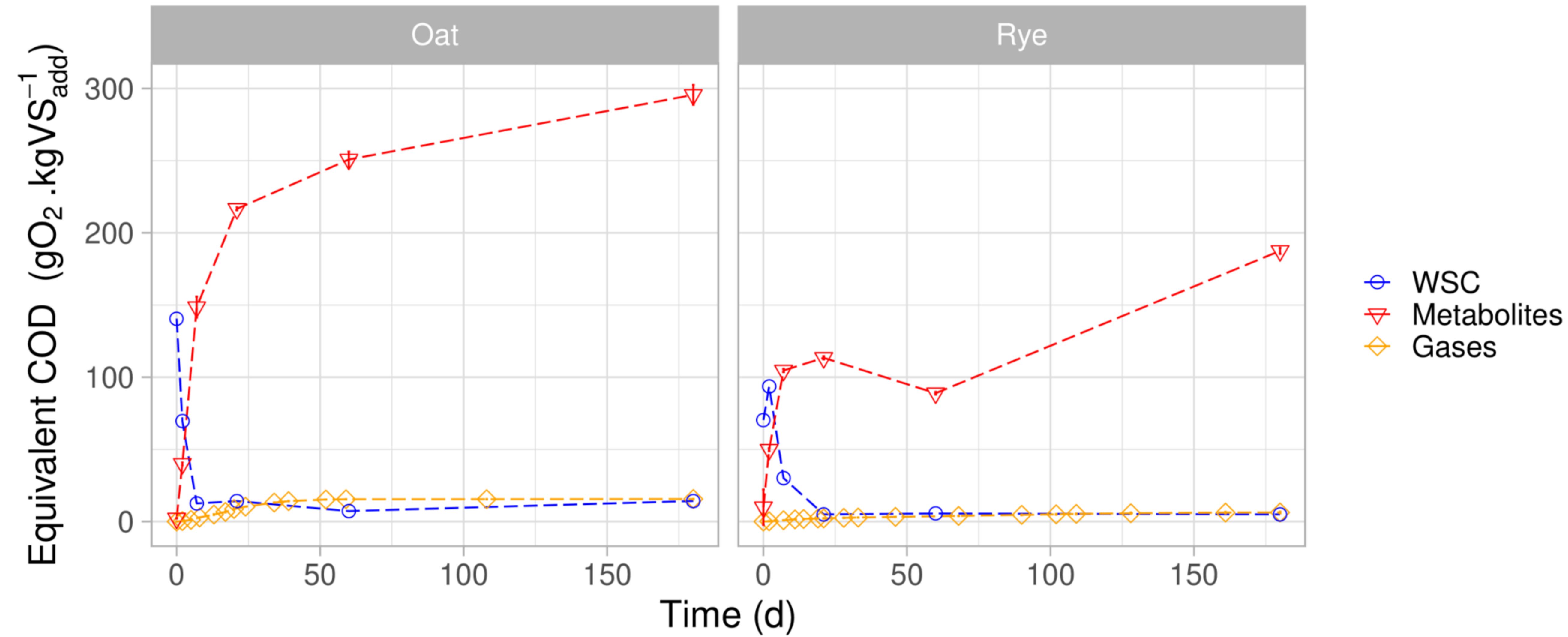
566 Figure 2: Variation of the COD repartition between WSC, soluble metabolites and gases. The error  
567 bars indicate standard deviation.

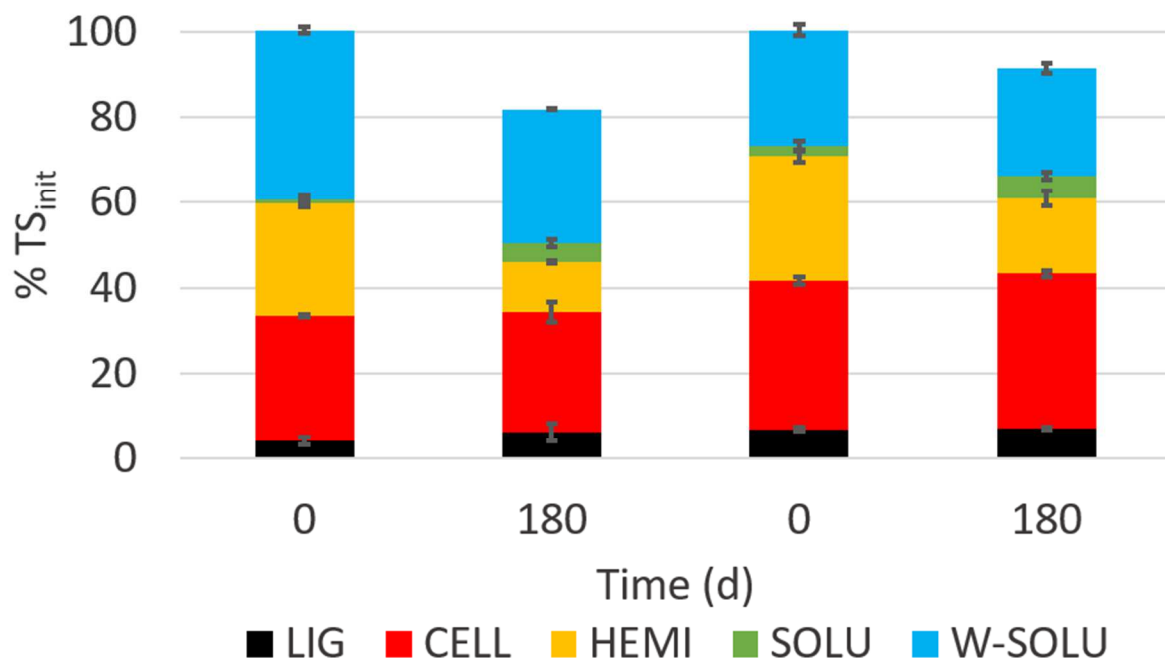
568 Figure 3: Alkaline storage effect on fiber distribution. The error bars indicate standard deviation.

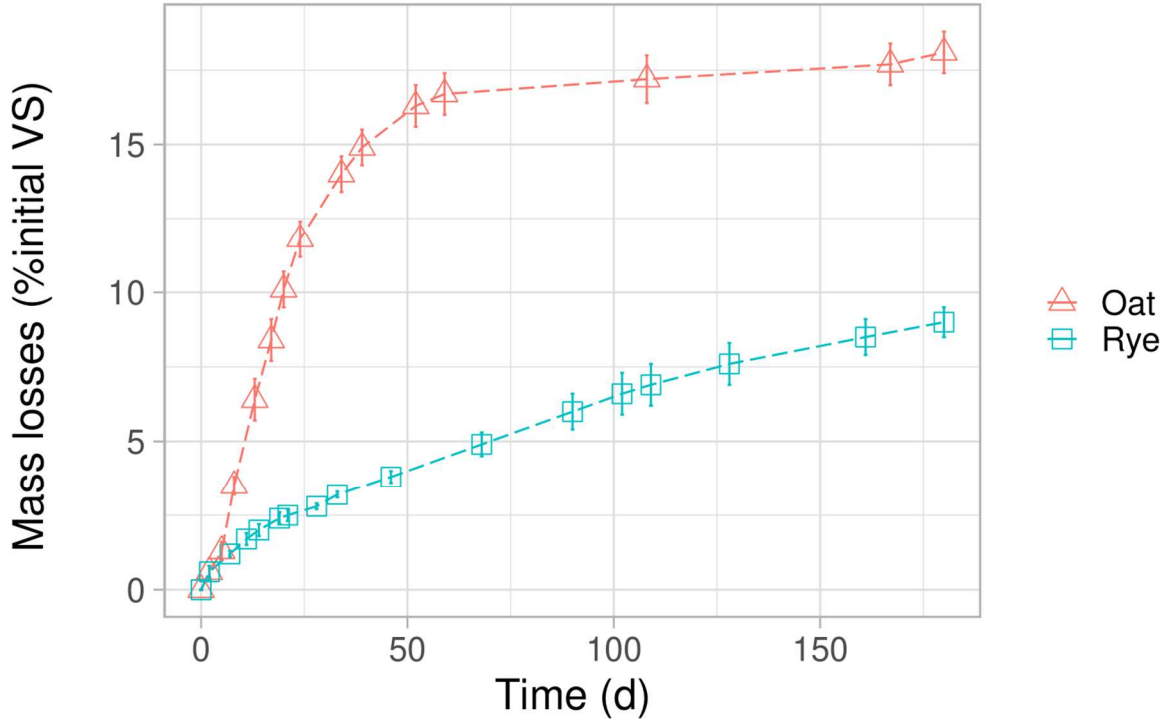
569 Figure 4: Dynamics of mass losses. The error bars indicate standard deviation.

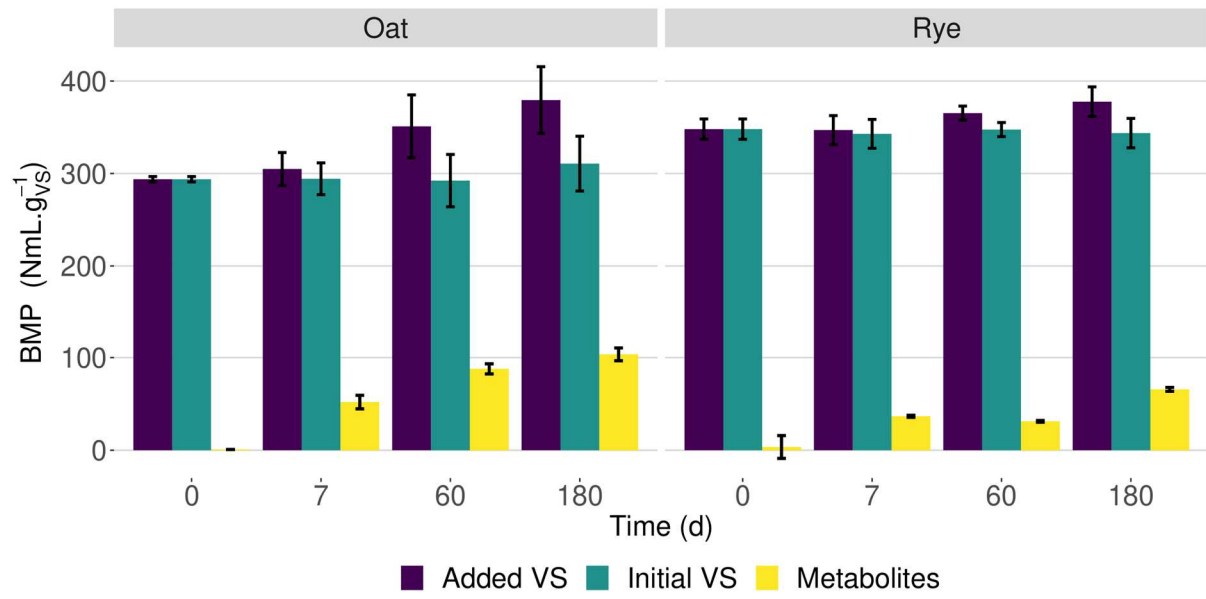
570 Figure 5: Impact of storage duration on methane potential. BMP (NmLCH<sub>4</sub>.gVS<sub>added</sub><sup>-1</sup>) and BMP  
571 (NmLCH<sub>4</sub>.gVS<sub>initial</sub><sup>-1</sup>) represent pretreated crop biodegradability and methane potential reported to  
572 the VS amount before pretreatment, respectively. BMP<sub>metabolites</sub> represent the contribution of  
573 soluble metabolites in BMP (NmLCH<sub>4</sub>.gVS<sub>initial</sub><sup>-1</sup>). The error bars indicate standard deviation.







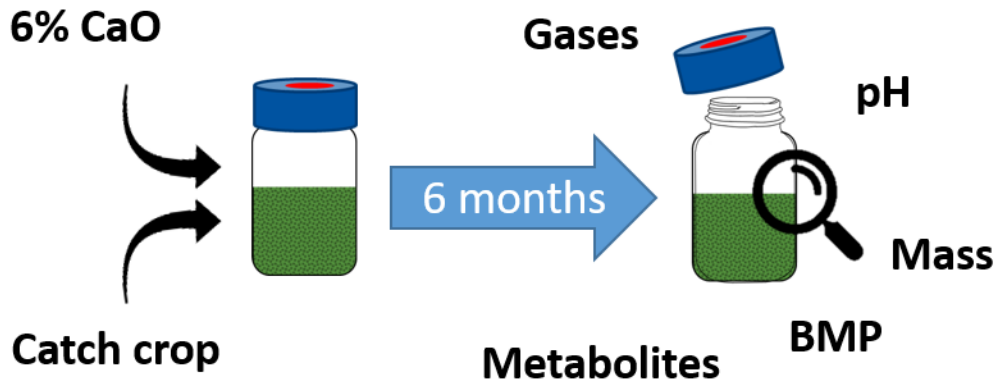
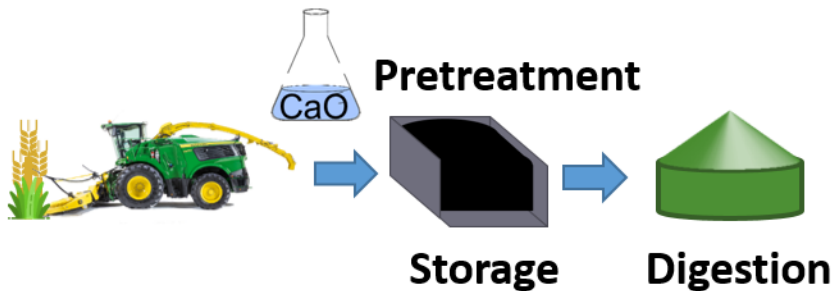






|  | <i>Oat</i>  | <i>Rye</i>  |
|--|-------------|-------------|
| <i>pH</i>  | 6.0 ± 0.0   | 6.0 ± 0.0   |
| <i>TS (%FM)</i>                                  | 21.8 ± 0.01 | 24.9 ± 0.03 |
| <i>VS (%FM)</i>                                  | 20.8 ± 0.01 | 23.7 ± 0.03 |
| <i>TN (%VS)</i>                                  | 0.7         | 1.1         |
| <i>TC (%VS)</i>                                  | 41.4        | 42.1        |
| <i>VFA (g.kg<sub>vs</sub><sup>-1</sup>)</i>      | < d. l.     | < d. l.     |
| <i>Fructose (g.kg<sub>vs</sub><sup>-1</sup>)</i> | 76.6 ± 0.8  | 34.0 ± 0.4  |
| <i>Glucose (g.kg<sub>vs</sub><sup>-1</sup>)</i>  | 54.7 ± 0.7  | 31.4 ± 0.1  |
| <i>WSC (g.kg<sub>vs</sub><sup>-1</sup>)</i>      | 131 ± 1     | 66 ± 0      |
| <i>EtOH (g.kg<sub>vs</sub><sup>-1</sup>)</i>     | < d. l.     | < d. l.     |
| <i>W.SOLU (%TS)</i>                              | 39.6 ± 0.7  | 27 ± 1.4    |
| <i>SOLU (%TS)</i>                                | 1.1 ± 0.8   | 2.4 ± 1.2   |
| <i>HEMI (%TS)</i>                                | 26.3 ± 0.7  | 29.4 ± 1.5  |
| <i>CELL (%TS)</i>                                | 29.3 ± 0.2  | 34.9 ± 0.9  |
| <i>LIG (%TS)</i>                                 | 4.1 ± 0.8   | 6.8 ± 0.5   |

| <i>Time (d)</i> |  | <b>0</b>   | <b>2</b>   | <b>7</b>  | <b>21</b> | <b>60</b> | <b>180</b> |
|-----------------|--|------------|------------|-----------|-----------|-----------|------------|
| <i>Oat</i>      | <i>pH</i>  | 11.9 ± 0.1 | 10.5 ± 0.0 | 6.6 ± 0.1 | 7.1 ± 0.1 | 7.5 ± 0.0 | 6.9 ± 0.0  |
|                 | <i>NH4-N (g.kgVS<sub>add</sub><sup>-1</sup>)</i> | 0.2 ± 0    | 0.8 ± 0.1  | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.4 ± 0.1 | 0.3 ± 0.2  |
| <i>Rye</i>      | <i>pH</i>  | 12 ± 0.1   | 9.8 ± 0.0  | 7.2 ± 0.0 | 7.2 ± 0.1 | 7.9 ± 0.1 | 7.7 ± 0.1  |
|                 | <i>NH4-N (g.kgVS<sub>add</sub><sup>-1</sup>)</i> | 0.3 ± 0    | 0.6 ± 0.1  | 1.1 ± 0   | 1.7 ± 0.1 | 2.3 ± 0   | 2.7 ± 0.1  |



No alkaline pH stability



Microbial activity



High mass losses



No impact on CH<sub>4</sub> potential