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C. VAN VLIERBERGHE[1,2], A. CHIBOUBI[1], H. CARRERE[1 *], N. BERNET[1], G. SANTA CATALINA[1], S. FREDERIC[2], R. ESCUDIE[1]

[1] *INRAE, Univ. Montpellier, LBE, 102 Avenue des étangs, F-11100, Narbonne, France.*

[2] *GRDF, 9 rue Condorcet, F-75009, Paris, France*

[*] *Corresponding author: E-mail address: helene.carrere@inrae.fr (Hélène Carrère)*

Abstract

Cover crops harvested at a low maturity stage generally have a high moisture content, which may generate energy losses during silage storage via effluent production and undesirable fermentations. This paper investigates the use of different waste types as absorbent co-substrates to be added before ensiling. The relation between the absorbent water holding capacity and silage effluent volume was first studied to find an effective parameter to prevent effluent production. Effluent retention was found to be proportional to the absorbent loading and water holding capacity (r^2 = 0.98) and up to 90% of effluent production was avoided when compared to control (295 $L.t^{-1}$). The impact of different co-substrates (including bio-waste and manures) on overall ensiling performances was then investigated at an optimized absorbent loading. All co-substrates allowed a total effluent retention while a 76 $L.t^{-1}$ effluent volume was reported for the control. The silage fermentation was modified or mostly unchanged depending on the co-substrate chemical and microbial properties and different metabolic pathways were observed (e.g. homolactic or butyric fermentation). In most conditions, the methane potential of the crop was efficiently preserved over a storage of 60 days. Co-ensiling was shown to be a relevant silage preparation method for biogas production.

1 Introduction

Cover crops, also called catch crops or intermediary crops, are receiving an increasing interest to be used as substrates for anaerobic digestion. These crops are cultivated during the intercultural period of main crops and bring agro-ecological services by limiting soil erosion, nitrate leaching and the need for mineral fertilizers (Molinuevo-Salces et al., 2013). In agricultural biogas sector,

cover crops allow to produce a large amount of biomass for methane production, without competing with the production of food or feed crops. Due to the management of crop rotations, cover crops are usually harvested once or twice a year. Since the biogas digesters are fed continuously, an efficient storage of the crops from their harvest to their final use in anaerobic digesters is mandatory. The storage of fresh fodder or energy crops is commonly performed by ensiling (Teixeira Franco et al., 2016). This storage process relies on the fermentation of the substrate, mostly by lactic acid bacteria. In the anaerobic environment of the storage silos, the water soluble carbohydrates (WSC) are fermented into lactic acid and other metabolites, causing a pH drop to about 4 (Woolford and Pahlow, 1998). The combination of acidic pH and anaerobic environment then ensures the inhibition of microbial activity over the whole storage duration.

The performance of ensiling greatly depends on silo confection practices and crop characteristics (Teixeira Franco et al., 2016). Among the key parameters, the total solids (TS) content of the crop has a major influence on storage performances. For biogas production, a TS range from 26 to 36% has been reported to be optimal (Villa et al., 2020). Silage production from lower TS crops may lead to fermentation pathways dominated by *Clostridia*, characterized by butyric acid and H_2 production (Woolford and Pahlow, 1998). In silage production for animal feeding, clostridial fermentations are highly undesirable because of the possible development of pathogenic microorganisms, poor palatability and decrease in feed value (Woolford and Pahlow, 1998). In silage production for biogas production, such issues are less relevant, but clostridial fermentation is reported to induce energy losses due to the production of H_2 (Kreuger et al., 2011). However, the overall effect of clostridial development in silage for biogas remains to be

investigated, since energy losses may be compensated by an increase in lignocellulose hydrolysis and biomass degradability (Cui et al., 2020). Another issue with the silage of low TS crops is the high production of liquid effluents (Gebrehanna et al., 2014). For TS values lower than 25%, hundreds of liters of effluent can seep per ton of ensiled crop (Bastiman and Altman, 1986; Sutter, 1957). If not collected and valorized properly, silage effluents may cause methane potential losses (Teixeira Franco et al., 2016) and surface water pollution (Holly et al., 2018). In addition, silage effluents can generate significant odor nuisance (Keck et al., 2018) that greatly threatens the local acceptance of biogas plants (Schumacher and Schultmann, 2017). They can also cause corrosion of steel and concrete (Koenig and Dehn, 2016). As a consequence, silage effluent production is reported to be highly undesirable in biogas plants.

Because they are grown during short periods and/or with unfavorable weather conditions, cover crops are often harvested at a low maturity stage which is associated to a low TS. TS of less than 20% are commonly met in cover crops (Molinuevo-Salces et al., 2013) and significant volumes of effluents are expected during the storage of such crops. In order to avoid this issue, various practices can be adopted, such as field wilting or the addition of an absorbent co-substrate. Field wilting consists in a partial open-air drying of the freshly mowed crop prior to its storage and can result in an efficient increase in TS when proper conditions are met (Borreani et al., 2009). This method is however weather-dependent and unsuitable conditions may make wilting inappropriate (Teixeira Franco et al., 2017). The addition of a dry co-substrate presents the advantage of being independent from weather conditions and co-ensiling a wet organic matter with a substrate having absorbent properties allows to immediately increase the TS of the pre-silage mixture and thus avoid the effluent production (Jones and Jones, 1996). In the

literature, diverse co-substrates have been studied like the straw and stalks of different crop species (Haigh, 1998; Razak et al., 2012), rice, barley and wheat bran (Haigh, 1999; Razak et al., 2012), dried beet pulp (Fransen and Strubi, 1998; Haigh, 1999; Razak et al., 2012), ground barley (Khorvash et al., 2006), dry bean hulls (Razak et al., 2012), newspaper (Fransen and Strubi, 1998), sodium bentonite (Fransen and Strubi, 1998; Khorvash et al., 2006; Woolford et al., 1983) and various polymers (Fransen and Strubi, 1998; Healy et al., 1997), with different liquid holding efficiencies. These co-ensiling experiments were mainly applied for silage production for animal feeding and thus most of the tested co-substrates are edible. In the context of anaerobic digestion, the strategy for selecting co-substrates is different, since as an example, high value edible substrates like ground barley or dried beet pulp should be avoided. On the other hand, non-edible substrates compatible with anaerobic digestion can be considered.

This study focuses on the use of waste-like and/or agricultural byproducts as absorbent substrates in ensiling prior to anaerobic digestion. The first objective was to evaluate the behavior of a well-studied absorbent substrate (wheat straw) as a co-ensiling substrate with sunflower, in order to define the adequate co-substrate loading. In a second part, the influence of the addition of various co-substrates (soiled paper and cardboard, wood chips, miscanthus straw, chicken manure and horse manure) on the ensiling of rye was investigated. Production of effluent, metabolites and gas as well as the influence on microbial community structure and the preservation of methane potential were analyzed during two months of storage, in order to propose a global understanding of the addition of co-substrate on silage storage.

2 Material and Methods

The experiments and physico-chemical analyses were performed at the Bio2E platform (Bio2E, 2018).

2.1 Catch crops and co-ensiling substrates

Two catch crops, sunflower and rye, were kindly provided by RAGT Semences and Biométhagri, respectively. Sunflower cover crop was harvested in Cintegabelle (Haute Garonne, France) at BBCH stage 65 (full flowering) in September of 2020. The BBCH maturity scale is described by Meier (2018). Rye cover crop was collected in Florensac (Hérault, France) at BBCH 59 (end of heading). These crops were hand harvested using a sickle, at cutting height of about 10 cm. Sunflower was chopped using a garden shredder (AXT 2550TC, Bosch GmbH) and a knife mill (BB230, BLiK®) and further frozen at -20°C until use. Rye was chopped according the same protocol, but was used directly after for storage experiments.

Concerning the co-substrate, a wheat straw, commercialized as a bedding material, was used (ZOLUX S.A.S., France). Soiled paper and cardboard (named Paper) were separated from kitchen wastes. Wood chips (Wood) were collected in a sawmill that processes different wood species (Ets Guille, Narbonne, Aude, France). Miscanthus stalks (*M. x giganteus Britannique,* Misc) were supplied by INRAE AgroImpact (Estrées Mons experimental unit, Péronne, 80203, France). Chicken manure (Chicken M) was collected on a chicken farm (GAEC d'Empare, Saint Marcel Sur Aude, Aude, France). Horse manure (Horse M) was collected at a horse riding club (Narbonne Equitation, Narbonne, Aude, France). Both manures contained cereal straw as a bedding material.

2.2 Silage storage experiments

2.2.1 Experimental setups

Two different storage protocols were used, depending on the objective.

For the monitoring of silage effluent production, the experiments were conducted in mini-silos made of PVC pipe of 100 mm inner diameter and 800 mm height (6.3 L volume), in a similar configuration to that described by Savoie et al (2002). The filling was carried out by adding, in a successive way, layers of approximately 200 mm of loose catch crop which were compacted using a hydraulic press (20-ton capacity, KS Tools GmbH) until the targeted packing density. The packing density was applied as recommended by Latsch and Sauter (2014) (Fresh matter basis – FM): 800 $kg.m^{-3}$ for 20% TS forage ; 700 $kg.m^{-3}$ for 25% TS forage ; 650 $kg.m^{-3}$ for 30% TS forage. Packing density of intermediate levels of TS were calculated by linear interpolation. The silos were closed, and two tamps allowed to measure the gas volume and composition and to collect the liquid effluent at the bottom. In order to avoid air penetration, liquid effluent drainage was performed at most once a day. The effluent was characterized as described in section 2.2.4. All storage experiments were started on the same day and operated for 2 months. For each storage condition, two replicates were prepared in independent silos. The silos and their content were weighted before and after storage.

For the dynamic monitoring of silage fermentation, 2.6L glass bottles were used, as previously described (Van Vlierberghe et al., 2021). This storage method was used in addition to storage in PVC silos for the co-ensiling of rye, in order to allow a higher number of replicates for dynamic monitoring of storage. For each storage condition, several replicates were prepared to be

sacrificed after 2, 7, 21 and 60 days of storage in order to carry out a complete physico-chemical and microbial analysis of the samples during the ensiling process as described in section 2.2.5.

A 60-day storage period was chosen as sufficient for fine effluent and fermentation characterization. Previous studies of the literature reported that the peak flow of effluent production usually occurs during the first days of storage, with 90% of the total volume being usually released during the 20 to 26 first days of storage (Mayne and Gordon, 1986). Concerning fermentation, most of the transformations that may occur usually happen during the first weeks of storage (Woolford and Pahlow, 1998).

2.2.2 Co-ensiling of sunflower with straw with variable absorbent loading

A first round of experiments was only conducted in tubular PVC silos. Five different ratios of wheat straw were tested: 0, 40, 80, 150 and 250 $g \cdot kg_{sunflower}^{-1}$, on a fresh matter basis. In each reactor, 3 kg_{FM} of sunflower were added, and the amount of wheat straw was adapted in order to match with the desired loading. A first layer of wheat straw was added and packed, and sunflower was then added on top, and packed (protocol called “bottom”). A second protocol, named “mixed”, was also carried out for the ratio of wheat straw of 40 $g \cdot kg^{-1}$; sunflower and straw were homogenized before filling into the silos. 6 different storage conditions were thus tested.

2.2.3 Co-ensiling of rye with diverse co-substrates at a determined absorbent loading.

This storage experiment was conducted both in tubular PVC silos and bottle silos, in order to evaluate the impact of the type of co-substrate on the volume of effluent produced and on the fermentation and microbial community structure, respectively. Five different co-substrates

were used (soiled paper and cardboard, miscanthus stalks, wood chips, chicken manure and horse manure) and rye was used as catch crop. One ensiling condition containing only rye was also prepared as a control. The calculation of the loading of each co-substrate was based on the expected cumulated effluent volume of rye ensiled alone ($mL \cdot kg_{FM}^{-1}$) and the measurement of water holding capacity (WHC) (see section 2.2.6). In each PVC silo, 3 kg_{FM} of rye were added. The amount of absorbent was adapted in order to match with the desired loading. In glass silos, 700g of mixture were added, with the same co-substrate/rye ratio as in the PVC silos.

2.2.4 Silage effluent characterization

Silage effluents from tubular silos were characterized by their volume and their chemical oxygen demand (COD) at most once a day, depending on the flow rate. COD was determined by colorimetric method (Aqualytic® COD Vario 0-1500 $mgO_2.L^{-1}$) after 1/100 dilution.

2.2.5 Physicochemical analysis of solid samples

The cover crops, co-substrates and their mixtures were characterized as follows. Total solids (TS) were determined by oven drying (105°C, 24h). Volatile solids (VS) were measured by calcination of the dry residue (550°C – 3h). A TS correction method for lactic acid, VFA and alcohols volatilization was applied as described by Porter and Murray (2001). For pH, ammonia, WSC, and metabolites analysis, a water extraction of the solid samples was made according to the protocol of Porter and Murray (2001). 30 g of freshly collected sample were soaked in 150 mL of distillated water for 16 h to 20 h at 4°C in sealed plastic pots in triplicate. pH measurement was made directly on the mixture after extraction. The liquid phase was then separated by centrifugation (18750 g, 20 min, 4°C) and stored at -20°C until analysis. The quantification of WSC, VFA and metabolites was performed on the liquid extract by HPLC, and ammonia

172 measurement was made by titration with boric acid using a Gerhardt® Vapodest 50 s® carousel.
173 The total carbon (TC) and total nitrogen (TN) were determined via an elemental analyzer
174 (FlashSmart®, Thermo Fisher Scientific®) on finely grounded freeze dried samples.

175 Biochemical methane potential (BMP) were made according to the recommendations of
176 Holliger et al. (2016). Samples for BMP tests were previously prepared by freezing a certain
177 amount of substrate containing around 2 g_{TS} of sample (exact TS and VS were calculated later).
178 BMP values were calculated following two different methods depending on the objective.
179 Methane potential expressed as the volume of methane per amount of VS added
180 ($NmLCH_4.gVS_{add}^{-1}$) are useful to estimate the substrate degradability before and after storage.
181 BMP reported to the initial amount of VS after taking into account the mass losses that occur
182 during storage ($NmLCH_4.gVS_{init}^{-1}$) are mandatory to assess the global balance of silage storage on
183 energy potential.

184 The buffering capacity (BC) of the fresh cover crops, co-substrates and mixtures was measured
185 following the method of Playne and McDonald (1966). 10 g of finely shredded samples were put
186 in 250 mL of distillated water. This mixture was first titrated under agitation to pH 3 with a
187 solution of 0.1 N HCl in order to release bicarbonate as carbon dioxide, and then titrated to pH 6
188 with a solution 0.1 N NaOH. BC was expressed as mequiv of NaOH per kg of dry matter to
189 elevate the pH from 4 to 6, after correction for the titration of a 250 mL water blank.

190 For microbial community analysis, 300 mg of fresh medium were sampled and stored at -20°C in
191 a 2mL sterile tube. DNA extraction, sequence data analysis and quantitative PCR were performed
192 as described by (Venkiteshwaran et al., 2016). Sequencing was achieved at the technology

platform Genome and Transcriptome (GeT) of the Génopole Toulouse, France. OTUs with a relative abundance of > 1.5% in at least one sample were selected for further analysis.

2.2.6 Water holding capacity and preliminary effluent volume estimation

The water holding capacities of co-ensiling substrates were characterized. WHC was measured following a protocol adapted from Marsac *et al.* (Marsac et al., 2019). 10 g of shredded substrate were introduced into a nylon bag (4 x 12 cm, ~30 µm pore size, FibreBag ref. 10-0127, C. Gerhardt GmbH & Co. KG, Germany). The bags were soaked in tap water for 2 hours and then centrifuged (200 g, 10 min) into a 500 mL bottle with a draining material in the bottom (i.e., glass balls). WHC measurements were performed in triplicate. WHC was calculated as the amount of water that was hold in the sample after centrifugation, as follows:

$$WHC = \frac{m_h - m_r}{m_r} \quad (1)$$

with WHC the Water Holding Capacity ($g.g^{-1}$), m_r the mass of raw sample (g) and m_h the mass of humidified sample after soaking and centrifugation (g).

A preliminary estimation of effluent volume to be produced by the cover crop during storage was made as follows. First, effluent volume estimations from Bastiman (1986) (equation(*2*) and Sutter (1957) (equation(3) were calculated.

$$V_{Bastiman} = 767 - 53.4 \times \mathrm{TS} + 0.936 \times \mathrm{TS}^2 \quad (2)$$

$$V_{Sutter} = 672 - 22.40 \times \mathrm{TS} \quad (3)$$

With V = cumulated silage effluent volume per ton of fresh crop ($L.t_{FM}^{-1}$) and TS = Total Solid content ($kg.kg_{FM}^{-1}$). From these values, $V_{effl\ expect}$ was calculated as the average value between $V_{Bastiman}$ and V_{Sutter}, based on previous experiments (not shown). This estimation was used for the calculation of co-substrates loadings in the rye storage experiment following equation(4).

$$Cosub = \frac{V_{effl\ expect}}{WHC} \quad (4)$$

where Cosub represents the co-substrate loading ($g_{FM\ co\text{-}substrate}.kg_{FM\ crop}^{-1}$), $V_{effl\ expect}$ the expected effluent volume ($mL{\cdot}kg_{FM\ crop}^{-1}$) and WHC the Water holding capacity of the co-substrate ($mL{\cdot}g_{FM\ co\text{-}substrate}^{-1}$).

2.3 Statistical analysis and data representation

One-way analysis of variance was made on BMP results after verifying both the normality (Shapiro-Wilk test) and the variance homogeneity (Levene test) with R package “rstatix”. A pair-wise t-test adjusted with Holm method was further realized for assessing the significance of the difference in means between two samples. When data distribution could not be assumed as normal, the Kruskal Wallis test was applied and Wilcoxon test was used with Holm’s p-value correction in the case of pairwise analysis. The matrix of Pearson’s correlation was obtained using “corrplot” package. A linear model was calculated on co-ensiling data of sunflower using lm function of R. The package “ggplot2” was used for graphical representations.

3 Results and discussion

3.1 Sample characterization

The main characteristics of the crops and co-substrates are presented in Table 1. Sunflower and rye were characterized by a low TS content of 14.6 and 21.1%, respectively. The expected volume of effluent produced according to Bastiman and Altman (1986) and Sutter (1957) estimations were 187 and 345 $mL \cdot kg^{-1}$ for sunflower and 57 and 199 $mL \cdot kg^{-1}$ for rye, respectively. The average estimated volume were calculated and corresponded to 138 and 266 $mL \cdot kg^{-1}$ for oat and rye, respectively. Co-ensiling substrates were characterized by higher TS contents, with value ranging from 53 (horse manure) to 92.7 % (miscanthus stalks). The WHC of the different co-substrates ranged from 0.8 (chicken manure) to 2.1 $mL.g^{-1}$ (soiled paper and cardboard), which confirmed their ability to be used as absorbent materials. These values are lower than other found in the literature. For example, WHC of 2.3 – 2.8 $mL.g^{-1}$ were reported for miscanthus stalks (Dennery G., Dezat E., 2012) whereas they were around 3 for wheat straw (Razak et al., 2012). This difference may be explained by the protocol used in the present work (centrifugation with a draining material), ensuring that no water remained in the interstitial spaces of the substrate. The two crops had C/N ratios of 22.2 and 22.1 $g.g^{-1}$ for rye and sunflower, respectively, which corresponds to the medium values for cover crops (Molinuevo-Salces et al., 2014). For the co-ensiling substrates, different patterns can be observed. Wheat straw, soiled paper and cardboard, miscanthus stalks and wood chips had very high C/N ratios ranging from 81 to 447, while horse manure had a similar C/N ratio to that of the crops. Finally, chicken manure presented the highest nitrogen content, with a C/N ratio of 8.2. Both crops presented high WSC of 181 and 131 $g.kg_{TS}^{-1}$ in sunflower and rye, respectively, which are

248 sufficient to initiate the silage fermentation (da Silva et al., 2017). In all co-substrates, a
249 negligible amount of WSC was found.

250 Rye and sunflower had buffering capacities of 248 and 283 mEq·kg_{TS}^{-1}, respectively, which are
251 considered as low values (Kaiser et al., 2004). Wheat straw, soiled paper and cardboard,
252 miscanthus stalks and wood chips had a very low buffering capacity, between 14 to 88 mEq·kg_{TS}^{-1},
253 while that of manure was 148 mEq·kg_{TS}^{-1}, a lower value than for rye. However, chicken
254 manure presented a high buffering capacity of 509 mEq·kg_{TS}^{-1}, which was likely due to its high
255 nitrogen content. A high buffering capacity makes it difficult to lower the pH during silage
256 fermentation and is considered as undesirable (Teixeira Franco et al., 2016).

257 Table 1 *Fresh cover crop and co-substrate characteristics.*
258

259 ## 3.2 Impact of co-substrate loading on co-ensiling

260 The cumulated volumes of effluent measured in the co-ensiling experiment of sunflower with
261 wheat straw are presented in Figure 1. Ensiling of sunflower alone resulted in the production of
262 295 $mL_{effluent}$·$kg_{sunflower}^{-1}$ in average, which is comparable with the average estimations from
263 Bastiman (1986) and Sutter (1957) (266 mL·kg^{-1}). Wheat straw addition was shown to efficiently
264 reduce the production of silage effluent. The volume of effluent produced was inversely
265 proportional to straw loading up to 150 g·kg^{-1}. For higher straw loadings, the effluent is almost
266 totally retaining in the solid medium. A linear model was used to fil the data presented in Figure
267 1, excepted for 250 g·kg^{-1} straw loading, since full retention was considered to be reached over
268 150 g·kg^{-1}. The cumulated effluent volumes from 10 different silos were consequently used for
269 the linear model definition. The regression line was forced to intercept with the effluent volume

value of the control. For a straw loading ranging from 0 to 150 $g \cdot kg^{-1}$, 1.6 mL of effluent were retained per g of wheat straw added, which corresponds to the theoretical WHC capacity of wheat straw calculated previously. The straw addition method also influenced the effluent retention capacity. At a loading of 40 $g \cdot kg^{-1}$, mixing straw with sunflower allowed to decrease the effluent production by 28 $mL \cdot kg_{CC}^{-1}$ compared to adding the same amount of straw as a single layer at the bottom of the silo.

The COD of the effluent produced during the ensiling of sunflower alone was 58 $gO_2.L^{-1}$, which is in the range of COD met in silage effluent in the literature (Gebrehanna et al., 2014). The BMP of pooled effluent samples was measured and a value of 309 ± 18 $NmLCH_4.gCOD^{-1}$ was found, which is close to the theoretical relation of 1g COD = 350 $NmLCH_4$, indicating that the organic matter contained in the effluent is mostly biodegradable. The methane potential of the effluent was consequently estimated to be 17.9 $NLCH_4.L_{effluent}^{-1}$. Considering the initial VS content and cumulated effluent volume of sunflower, a cumulated methane potential of 40.5 $Nm^3.t_{VS\ init}^{-1}$ flowed in the effluent when sunflower was ensiled alone. This represents 18% of fresh sunflower BMP. Co-ensiling allowed to gradually reduce effluent production and thus BMP flow through effluent down to until 4.2 $Nm^3.t_{VS\ init}^{-1}$, i.e. 1.7% of fresh sunflower BMP as the straw loading increased.

Straw addition had little influence on silage fermentation. In fact, very similar chemical properties were found for all conditions at the end of storage, with an average pH of 4.0 ± 0.0 and lactic to acetic acid ratio of 4.6 ± 0.4 (ranging from 3.9 to 5.3). No significant amount of other VFA than acetic acid was produced. All silages were thus considered of “high quality” regarding their fermentation characteristics.

Figure 1: Cumulated effluent volume after 60 days of co-ensiling (sunflower + wheat straw). Dots indicate mean, error bars indicate maximum and minimum

This experiment confirmed the interest of using a dry co-substrate for silage effluent retention. The theoretical WHC of the co-substrate was found to be a relevant parameter for its dosing in a co-ensilage and it was consequently used for the calculation of co-substrate loadings in the following experiment.

3.3 Impact of co-substrate type on co-ensiling

3.3.1 Physicochemical characteristics of fresh mixtures

The characteristics of the different pre-silage mixtures (rye + co-substrates) are presented in Table 2. Due to the loading calculation method based on WHC, the co-substrate loading varied from 58.9 (R + Paper) to 182.3 $g.kg_{rye}^{-1}$ (R + Chicken M) on a fresh matter basis, as an effluent volume of 138 $mL \cdot kg_{rye}^{-1}$ was expected. The TS content of the mixtures ranged from 23.3 (R + Horse M) to 29.6% (R + Chicken M). Whatever the co-substrate, the pH of the mixture was close to that of rye (6.5), excepted for R + Chicken M where it was significantly higher (7.5). Depending on the buffering capacity of co-substrate, those of the mixtures decreased (R+ Misc, R+ Wood), were unchanged (R + Paper, R + Horse M) or increased (R + chicken M). However, due to the low initial buffering capacity of rye, the buffering capacity of all mixtures remained low according to the literature (Kaiser et al., 2004). For the mixtures R + Paper, R + Misc, R + Wood, the C/N ratio was slightly increased, while its value dropped to 14.3 for R + chicken M. The range of C/N ratios was however suitable for further anaerobic digestion, since ratios from 15 to 30 are recommended (Kalač, 2011). WSC content was not significantly different from

314 control in all cases excepted R+Wood whose concentration was lower. WSC content of all
315 mixtures however remained high and suitable for efficient silage fermentation (da Silva et al.,
316 2017).

317 Table 2 : *Fresh co-ensiling mixtures characteristics.*
318

319 As a consequence, the type of co-substrates influenced some key parameters of the mixture
320 that can drive the efficiency of the silage fermentation. However, the main characteristics of the
321 mixture remained in the recommended range for "adequate ensilability", according to the
322 literature.

323 ### 3.3.2 Silage effluent production

324 An effluent was only produced during the ensiling of rye alone. In this condition, an average
325 volume of 76 mL.kg_{CC}^{-1} was measured, which was lower than the previously estimated volume
326 (138 mL·kg^{-1}) but between the estimation of Bastiman and Altman (1986) (57 mL.kg_{CC}^{-1}) and the
327 one Sutter (1957) (199 mL.kg_{CC}^{-1}). In addition, despite of the relatively low TS content of the
328 mixtures R + Paper, R + Wood and R + Horse M that should have led to an effluent production
329 according to Bastiman and Altman (1986) and Sutter (1957) estimations, no effluent was
330 produced. This result shows that, for co-ensiling experiments, the TS content of the mixture is
331 not a reliable parameter to estimate the effluent production, and that it is essential to consider
332 the estimation of the volume of effluent produced by the crop together with the holding
333 capacity of the co-substrate.

334 The average COD concentration in effluent collected during the storage of rye alone was 59
335 $gO_2.L^{-1}$, and a BMP of 332 ± 13 $NmLCH_4.gCOD^{-1}$ (equivalent to 19.6 $NLCH_4.L_{effluent}^{-1}$) was found.

Considering the initial VS content of rye, the cumulated effluent production represented 7.6 $Nm^3.t_{VS\ init}^{-1}$ of fresh rye, i.e 2 % of fresh rye BMP. Co-ensiling of rye allowed to suppress effluent production and thus methane potential losses through effluent.

A significantly lower BMP loss due to effluent production was found during the ensiling of rye alone when compared to sunflower alone, which is due to higher TS content and higher BMP of fresh rye and lower effluent volume. However, the addition of absorbent co-substrates allowed to drastically cut the methane potential losses due to effluent production.

3.3.3 Fermentation pathways during co-ensiling

Figure 2: pH, metabolite concentration and cumulated gas production during storage. Metabolites are expressed in $g.kgVS_{added}^{-1}$ of mix (cover crop + co-substrate). CO_2 and H_2 are expressed in cumulated $L.kgVS_{init}^{-1}$. R stand for rye. Paper, Misc, Wood, Chicken M and Horse M stand for soiled paper and cardboard, miscanthus stalks, wood chips, chicken manure and horse manure, respectively

Figure 3: Evolution of ammonia concentration during storage. Concentrations are expressed in $g.kg_{VSadded}^{-1}$ of mix (cover crop + co-substrate); R stand for rye. Paper, Misc, Wood, Chicken M and Horse M stand for soiled paper and cardboard, miscanthus stalks, wood chips, chicken manure and horse manure, respectively

The fermentation pathways that occurred during the different co-ensiling conditions were monitored by the dynamic analysis of pH, metabolites and gases (Figure 2) and ammonia (Figure 3). Depending on the type of co-substrate and initial properties of the mixture, two different patterns were identified. On the one hand, in all conditions except for the mixture R + Horse M,

362 secondary fermentations occurred from the second week. On the other hand, for the mixture R
363 + Horse M, a more restricted homolactic fermentation took place and led to a fast and efficient
364 acidification. The two fermentation patterns are presented in this section.

365 **Secondary fermentation patterns**

366 In Rye, R + Paper, R + Misc, R + Wood and R + Chicken MIn storage conditions, the fermentation
367 was characterized by limited lactic acid production and acidification during the first days of
368 storage. After one week, a peak in lactic acid concentration of only 30 $g.kg_{VS}^{-1}$ was reached in
369 Rye, R+ Misc, R+ Wood and R+ Chicken M. Due to this low lactic acid production, the pH did not
370 drop below 4.7. In the mixture R + Paper, lactic acid concentration was higher and reached 55
371 $g.kg_{VS}^{-1}$ but the pH value was also higher than 4.7. In the mixture R + Chicken M, the pH was
372 never lower than 6.2, which was likely due to the high ammonia concentration that was
373 measured from the very first days of storage (Figure 3). Whatever the conditions, lactic acid
374 production was also coupled with other metabolites such as acetic acid and ethanol and with
375 CO_2 production, indicating heterolactic fermentation pathways. In silage fermentation,
376 heterolactic metabolisms are mentioned to be less efficient at lowering the pH than homolactic
377 fermentation (Woolford and Pahlow, 1998). Additionally, a significant propionic acid
378 accumulation was found for rye ensiling alone. Propionic acid fermentation may be also
379 considered as undesirable for silage due to its lower acidification capacity compared to lactic
380 acid fermentation. However, acetic and propionic acids have stronger antifungal properties than
381 lactic acid and allow a better aerobic stability when the silo is opened (Pahlow et al., 2003).

382 At the end of this first phase, pH values in all conditions remained above the critical value that is
383 necessary to inhibit clostridial activity and ensure a silage stability (Pahlow et al., 2003).
384 Consequently, from day 7 to 21, clostridial fermentation started. The lactic acid that was
385 previously produced was completely consumed before the end of the 60-day storage and
386 converted into butyric acid. The secondary fermentation was coupled with the production of
387 CO_2 and H_2 which is reported to induce methane potential losses (Kreuger et al., 2011). As lactic
388 acid was converted into butyric acid, the pH increased and reached 5.3 (R + Paper, R + Misc, R +
389 Wood), 5.7 (Rye) and 6.5 (R + Chicken M). Both the pH rise and difference in pH values between
390 the different conditions may also be explained by ammonia production, that mostly took place
391 between days 2 and 21. After three weeks of storage, the highest ammonia concentration was
392 measured in the mixture R + chicken M with a value of 1.6 $g.kg_{VS}^{-1}$, which was equivalent to 36
393 % of initial total nitrogen (TN_{init}). For rye ensiling, 1.3 $g.kg_{VS}^{-1}$ (49 $\%TN_{init}$) were measured, and
394 lower values were found in the other conditions, with 0.7 (33 $\%TN_{init}$), 0.6 (41 $\%TN_{init}$) and 0.8
395 $g.kg_{VS}^{-1}$ (27 $\%TN_{init}$) in R + Paper, R + Wood and R + Misc, respectively. In the literature, it is
396 generally accepted that NH_3-N concentration should be lower than 10% of the total nitrogen in
397 well preserved silages (Bureenok et al., 2016). The high concentration in NH_3-N found here is an
398 indicator of the activity of enterobacteria and/or clostridia (Pahlow et al., 2003). Despite the
399 non-optimal properties of silage with high pH, stabilization was reached from day 30, since gas
400 production stopped. This stable state may be due to the fact that all WSC were consumed
401 during the first weeks of storage (data not shown). The inhibitory effect of the accumulated
402 metabolites, mostly acetic acid and butyric acid, may also contribute to the inhibition of
403 microbial activity (Wang et al., 2008).

Homolactic fermentation pattern

The addition of horse manure to rye had a different impact on silage fermentation. This storage condition was characterized by a fast and high lactic acid production. After 7 days of storage, 92 $g.kg_{VS}^{-1}$ of lactic acid had accumulated in the medium, i.e. nearly 2 to 3 times more than in the other storage conditions. This accumulation induced a significant acidification as pH dropped to 4.4 after 2 days and stabilized at 4.0 after one week. The fermentation was coupled to a limited CO_2 production, since only 5 $NL.kg_{VS}^{-1}$ were measured and no H_2 was detected. The fermentation characteristics suggested that mostly homolactic fermentations occurred, since lactic acid was largely dominant among the other metabolites produced. Since the pH was below the critical value that ensures anaerobic stability (Pahlow et al., 2003), secondary fermentations did not occur. Production of NH_3-N was also lower than in the other conditions (0.4 $g.kg_{VS}^{-1}$, 21 $\%TN_{init}$). The silage obtained by co-ensiling rye with horse manure fulfilled the criteria defining a "well preserved silage" according to the literature, with pH <4.5, lactic acid > 30 $g.kg_{TS}^{-1}$ and butyric acid < 10% of total volatile fatty acids. Only NH_3-N overpassed 10% of total Nitrogen, however no other signs of undesirable microbial activity were recorded.

3.3.4 Microbial development during ensiling and impact of co-substrate addition

Figure 4: (A) Microbial characterization of raw substrates (relative abundance of major orders). Values on top represent the microbial population quantification (16S rRNA gene copy number .

g_{FM}^{-1}); (B.1) Variation of total microbial abundance during storage (16S rRNA gene copy number . g_{FM}^{-1}); (B.2) Relative composition of microbial community at the order level

In addition to modifying the physico-chemical characteristics of the mixture, the co-substrates had different microbial communities that can influence the silage fermentation. Microbial communities and total microbial abundance of raw substrates and their change during co-ensiling storage are presented in Figure 4.

Among the microorganisms that are likely to play a role in silage fermentation, the microbial community of fresh rye was largely dominated by enterobacteriales order, mostly from enterobacteria family. Enterobacteria are commonly found on crops as epiphytic bacteria (Pahlow et al., 2003). In silage fermentation, enterobacteria compete with lactic acid bacteria for WSC, produce a mixture of acids and ethanol and have thus a detrimental action on silage acidification. In paper, Misc and Wood, Lactobacillales, Enterobacteriales and Clostridiales were found. However, the total microbial abundance of these substrates was much lower. The total specific abundance of these three orders was thus lower than in rye and few modifications of the initial microbial community of the related mixtures were noted (Figure 4). Paper, Misc and Wood had also few influence on microbial community changes during storage when compared to rye alone, which is consistent with the observations made previously on fermentation metabolites. In these four storage conditions, Lactobacillales grew fast from the very first days of ensiling and likely competed with Enterobacteriales, whose abundance remained high all over the storage duration. From the second week of storage, the amount of Clostridiales increased, which is consistent with the butyric acid and H_2 production that was observed.

447 The microbial abundances of chicken and horse manures were much higher than the other co-
448 substrates, with $2.7 \cdot 10^8$ and $1.1 \cdot 10^8$ rRNA gene copy number$\cdot g_{FM}^{-1}$, respectively. Within the
449 bacteria orders that are expected to drive silage fermentations, a high amount of
450 Enterobacteriales, Lactobacilles and Clostridiales was present. Consequently, the microbial
451 communities of the pre-silage mixtures were strongly impacted by the addition of these co-
452 substrates. The initial abundances of Enterobacteriales, Lactobacilles and Clostridiales were
453 $8.5 \cdot 10^5$, $3.9 \cdot 10^6$ and $2.4 \cdot 10^6$ in R + Chicken Manure and $9.5 \cdot 10^5$, $1.2 \cdot 10^7$ and $5.9 \cdot 10^5$ in R + Horse
454 M, respectively. The ratio between Lactobacillales and Enterobacteriales was thus reversed
455 after adding manures. Clostridiales abundance was also increased. The initial Lactobacillales to
456 Enterobacteriales seemed to still play a role all over the storage duration, since the proportion
457 in Enterobacteriales remained much lower in R + Chicken M and R + Horse M than in the 4 other
458 co-ensiling experiments. However, due to the difference in acidification kinetics, the
459 development of Clostridiales could not be avoided in R + Chicken M, which led to the
460 dominance of this bacterial order after 60 days of storage. On the contrary, Lactobacillales
461 largely dominated the microbial community of R + Horse M, as the low pH prevented the
462 development of other bacteria (Gharechahi et al., 2017). Due to the relatively similar microbial
463 communities at the order level in R + Chicken M and R + Horse M, the difference in
464 fermentation mechanisms that occurred during storage may therefore be due to different
465 chemical properties between these two co-ensiling conditions. In particular, the difference in
466 buffering capacity and nitrogen content may have played a critical role.

3.3.5 Impact of fresh mixture properties on fermentation and microbial populations

*Figure 5: Matrix of Pearson's correlation with the main bacterial orders for all ensiling and co-ensiling experiments of rye. **: Significant with p-value <0.01, *: Significant with p-value < 0.05*

In order to assess the impact of the initial properties of the co-ensiling mixtures on fermentation, a matrix of Pearson's correlations was calculated and is presented in Figure 5. The impact of physico-chemical properties (TS, WSC, BC and C/N) had a weak influence on the fermentations contrary to what could be expected. This may be explained by the weak heterogeneity of these parameters in the various tested conditions. Interestingly, despite the results the literature for the ensiling of wilted and unwilted crops, initial TS was not negatively correlated with butyric fermentation nor Clostridiales growth (Borreani et al., 2009). This result points out that increasing TS by adding a co-substrate may have a different impact on fermentation than harvesting a crop at a high TS or applying field-wilting. Since the inhibition of clostridial growth is related to the water activity (a_W) of the medium, the influence of a co-substrate addition on a_W should be further studied.

About initial microbial properties of samples, it can be seen that the initial amount of Lactobacillales was strongly correlated with lactic acid production, and a negative correlation was established between initial content of Lactobacillales and the production of butyric acid and CO_2. If a silage fermentation dominated by lactic acid production is expected, a co-substrate with a high amount of lactic acid bacteria is thus recommended. The presence of a high quantity of Enterobacteriales at the end of storage was correlated with CO_2 and H_2 production, while Clostridiales were correlated with a higher concentration in acetic acid. However, the study of the fermentation kinetics and microbial populations variations presented in Figure 2

and Figure 4 clearly shows that H_2 production coincided with the development of Clostridiales. However, a large amount of Enterobacteriales was still present at the end of the storage period in Rye, R + Paper, R + Misc and R + Wood where a significant H_2 production occurred, which may have influenced the output of the correlation matrix.

3.3.6 Impact of fermentation on mass and methane potential preservation during co-ensiling

The gas production that occurred during silage fermentation caused mass losses in all conditions. For Rye, R + Paper, R + Misc, R + Wood, R + Chicken M and R + Horse M, 11.3, 7.4, 6.4, 8.0, 9.1 and 1.9 % of initial VS of each mixture were lost during storage, respectively, mostly under the form of CO_2. Since different absorbent loading were applied, the VS losses were also reported to the initial VS of rye. Following this calculation method, 11.3, 9.0, 9.2, 11.2, 11.9 and 2.3 % of initial VS of Rye were lost. It is important to stress that mixtures with similar fermentation pathways (Rye, R + Paper, R + Misc, R + Wood and R + Chicken M) led to close VS losses (i.e., 9.0 – 11.9%) reported to the initial VS of rye despite different co-substrate loadings, suggesting that VS from rye were mostly lost. This hypothesis is consistent with the fact that the easily degradable compounds, e.g. WSC, were mainly originated from rye. For the mixture R + Horse, VS losses were much lower than in any other co-ensiling condition, due to the homolactic fermentation pathway that prevailed (Kreuger et al., 2011).

BMP tests were carried out on raw substrates and after 60 days of storage to evaluate the impact of storage on the methane potential (Figure 6). In most conditions, no significant BMP variations were found to take place during storage, except in R + Wood where a significant

511 decrease in BMP of 18 and 24% was observed when reporting BMP to added VS or initial VS,
512 respectively. This difference in BMP preservation between R + Wood and all other co-ensiling
513 conditions is surprising, since no difference in mass losses, fermentation properties nor gas
514 production was observed. Consequently, this difference may be due to sampling issues due to
515 the heterogeneity of the co-ensiling mixture. Since wood chips presented the lowest BMP
516 among all co-substrates, a small difference in the catch crop/co-substrate ratio in sampling for
517 BMP measurement may influence greatly the measured BMP value. Indeed, the BMP measured
518 for fresh R + Wood (330 ± 36 $NmLCH_4 \cdot g_{VS}^{-1}$) is higher than the theoretical BMP calculated as the
519 weighted average between rye and wood chips (267 $NmLCH_4 \cdot g_{VS}^{-1}$). The BMP of fresh R+ Wood
520 was thus probably overestimated. Even if butyric fermentation and the related hydrogen
521 production occurred during most of storage conditions, hydrogen production did not exceed 15
522 $NL.kg_{VS}^{-1}$, which theoretically induced BMP losses of about 4 $NmL.g_{VS}^{-1}$. However, this value is
523 very low compared to those of the raw substrates, which explains the small difference between
524 the methane potential before and after silage.

525 Limited differences were found between BMP reported to added and initial VS in all conditions,
526 which was due to the fact that VS loss were relatively small. When higher VS losses occurred, a
527 larger difference between these two BMP values was found, as BMP was concentrated in the
528 remaining matter as WSC were converted into more energetically dense compounds like butyric
529 acid during fermentation while CO_2 was released (Kreuger et al., 2011; Van Vlierberghe et al.,
530 2021).

*Figure 6: Impact of storage under the different co-ensiling conditions on methane production per unit of VS of mix (cover crop + co-substrate). BMP reported to added VS represent the crop biodegradability after storage, and BMP reported to initial VS reflects the global impact of storage on methane potential. *: significant difference two BMP values for a given storage condition ($p<0.05$)*

As a consequence, this study shows that the co-silage of a wet cover crop with an absorbent co-substrate allows an efficient storage before anaerobic digestion, avoiding also the production of a liquid effluent. In addition to the preservation of catch crops itself, co-ensiling may be considered as a conservation method of manures, since this type of substrate is known to lose methane potential during storage (Teixeira Franco et al., 2018). A synergistic effect can thus be achieved, with the crop providing the necessary WSC for silage fermentations and the manure avoiding effluent production. Teixeira Franco et al. (2018) recommended an initial WSC content of 166 $g.kg_{VS}^{-1}$ for an efficient ensiling of cattle manure , which is a higher but close to initial value of the present experiment (126 ± 21 and 115 ± 5 $g.kg_{VS}^{-1}$, for R + Horse M and R + Chicken M, respectively).

4 Conclusion

Co-ensiling with dryer co-substrates was shown to be an appropriate method for the ensiling of wet cover crops and allowed a drastic volume reduction of energetically dense silage effluent. It was found that the specific water holding capacity was an appropriate parameter for absorbent dosing. Absorbent addition was shown to influence the chemical and microbial properties of silage depending on whether butyric or homolactic fermentation was promoted, though neither of them significantly impacted the preservation of methane potential. Co-substrate addition

was thus found to increase the methane potential preservation of wet crops through effluent retention more than through the control of fermentation. Co-ensiling crops with agricultural waste such as manures may constitute an interesting option for increasing the storage of these two substrates.

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693

694

695 *Table 1: Fresh cover crop and co-substrate characteristics.*

Fresh substrates	**Sunflower**	**Rye**	**Wheat straw**	**Soiled paper and cardboard**	**Miscanthus stalks**	**Wood chips**	**Horse manure**	**Chicken manure**
TS (%FM)	14.6 ± 0.7	21.1 ± 0.2	90.4 ± 0.1	88.4 ± 0.4	92.7 ± 0.1	88.3 ± 0.1	53.0 ± 0.1	75.5 ± 0.0
VS (%FM)	12.9 ± 0.6	19.5 ± 0.1	84.2 ± 0.1	73.2 ± 1.2	69.2 ± 6.5	87.6 ± 0.8	44.7 ± 0.1	31.8 ± 0.0
pH	7.1 ± 0.0	6.5 ± 0.0	6.4 ± 0	8.2 ± 0.1	6.27 ± 0.1	5.42 ± 0.0	7.53 ± 0.1	8.59 ± 0.1
BC ($mEq.kg_{TS}^{-1}$)	283	248	88	73	14	23	143	509
WHC ($g_{water}.g^{-1}_{FM}$)	n.d.	n.d.	1.6 ± 0.2	2.1 ± 0.19	1.0 ± 0.13	1.4 ± 0.09	1.4 ± 0.18	0.8 ± 0.14
C (%TS)	40.7	46.8	42.6	47.5	49.2	52.5	42.1	19.8
N (%TS)	1.84	2.11	0.52	0.21	0.23	0.11	1.43	2.43
C/N ($g.g^{-1}$)	22.1	22.2	81.4	223.0	216.8	477.1	29.4	8.2
VFA ($g.kg^{-1}_{VS}$)	< d.l.	3.6 ± 0.2	4.0 ± 0.3	<d.l	<d.l	<d.l	5.7 ± 1.4	19.9 ± 0.9
WSC ($g.kg^{-1}_{VS}$)	181 ± 13	141 ± 15	14.8 ± 0.7	<d.l	<d.l	<d.l	2.0 ± 0.3	<d.l
BMP ($NmLCH_4.g_{VS}^{-1}$)	n.d.	360 ± 31	n.d.	354 ± 25	181 ± 22	26 ± 16	134 ± 65	172 ± 10

696 TS: total solids; FM: fresh matter; VS: volatile solids; BC: buffering capacity; WHC: water holding capacity; VFA: volatile fatty acids;
697 WSC: water soluble carbohydrates; BMP: biochemical methane potential. Measurements that were performed in triplicates are
698 expressed as mean ± standard deviation (sd); n.d. : not determined; <d.l. : below the detection limit.

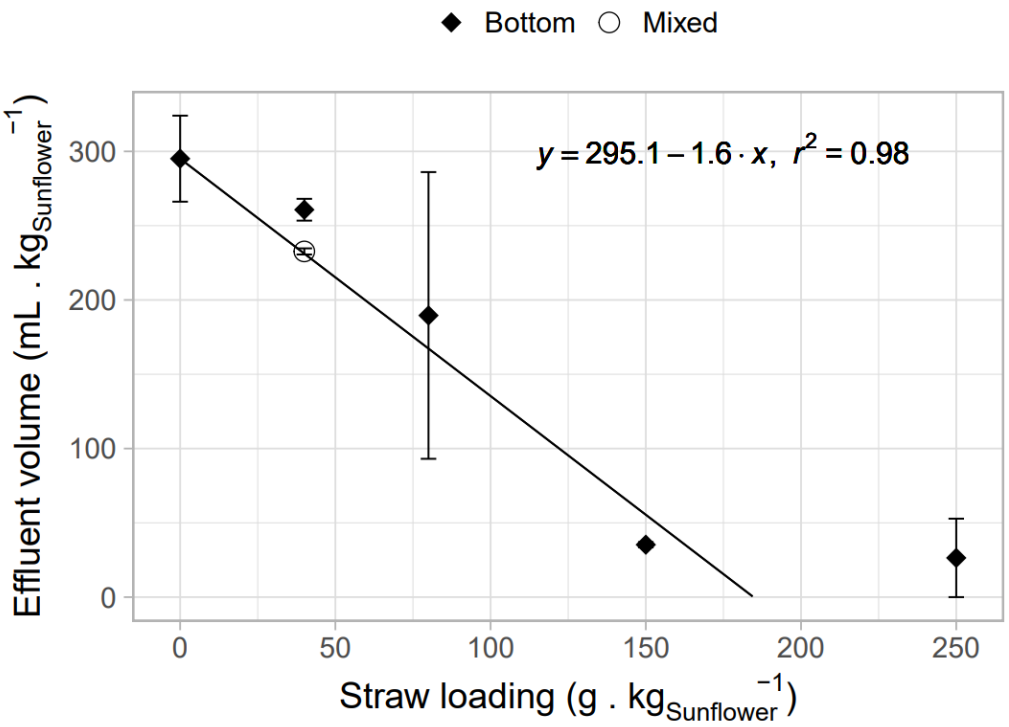
699

700 *Table 2: Fresh co-ensiling mixtures characteristics.*

Fresh co-ensiling mixtures	Rye	R + Paper	R + Misc	R + Wood	R + Horse M	R + Chicken M	Pooled sd.
Co-substrate loading ($g.kg^{-1}$)	0	58.9	124.3	85.6	92.5	182.3	
TS (%FM)	21.1 ± 0.2 [a]	25.1 ± 0.1 [b]	29.1 ± 1.1 [c]	25.1 ± 0.3 [b]	23.3 ± 0.2 [d]	29.6 ± 0.4 [c]	0.4
VS (%FM)	19.5 ± 0.1 [a]	23.4 ± 0.1 [b]	26.4 ± 1.1 [c]	23.5 ± 0.6 [b]	20.9 ± 0.1 [d]	20.4 ± 0.6[ad]	0.5
pH	6.5 ± 0.0 [a]	6.8 ± 0.0 [b]	6.3 ± 0.0 [c]	6.4 ± 0.0 [d]	6.4 ± 0.1 [d]	7.5 ± 0.0 [e]	0.0
BC ($mEq.kg_{TS}^{-1}$)	248	246	179	214	268	314	
C (%TS)	46.8	46.1	46.7	48.3	45.7	37.6	
N (%TS)	2.1	1.7	1.9	1.9	1.9	2.6	
C/N	22.2	26.7	25.2	26.1	23.5	14.3	
VFA ($g.kg^{-1}_{VS}$)	3.6 ± 0.2 [a]	3.4 ± 0.2 [a]	3.6 ± 0.6 [a]	3.3 ± 0.4 [a]	5.5 ± 0.3 [b]	6.5 ± 0.5 [b]	0.4
WSC ($g.kg^{-1}_{VS}$)	141 ± 15[a]	117 ± 4 [ab]	109 ± 7 [ab]	102 ± 17 [b]	126 ± 21 [ab]	115 ± 5 [ab]	11

701 Within a row, different letters express a statistical difference ($p < 0.05$) between two samples.
702 Measurements that were performed in triplicates are expressed as mean ± sd.

703



704

705 *Figure 1: Cumulated effluent volume after 60 days of co-ensiling (sunflower + wheat straw). Dots*
706 *indicate mean, error bars indicate maximum and minimum.*

707

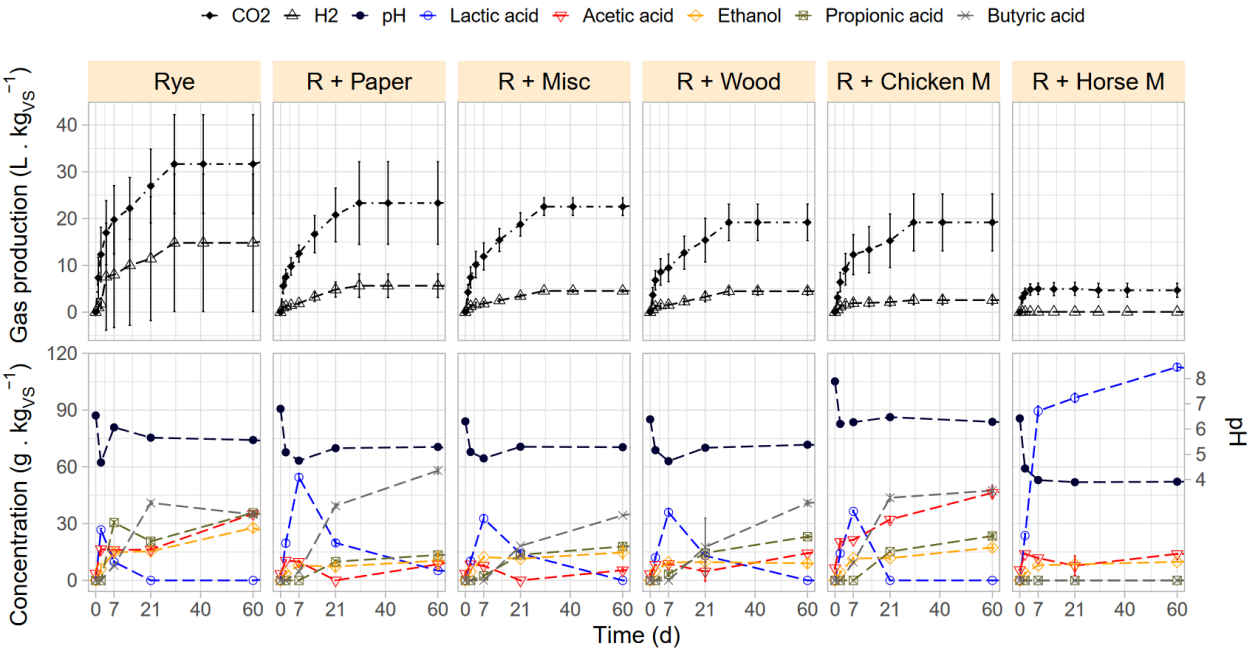


Figure 2 : pH, metabolite concentration and cumulated gas production during storage. Metabolites are expressed in g.kgVS$_{added}^{-1}$ of mix (cover crop + co-substrate). CO_2 and H_2 are expressed in cumulated L.kgVS$_{init}^{-1}$. R stand for rye. Paper, Misc, Wood, Chicken M and Horse M stand for soiled paper and cardboard, miscanthus stalks, wood chips, chicken manure and horse manure, respectively.

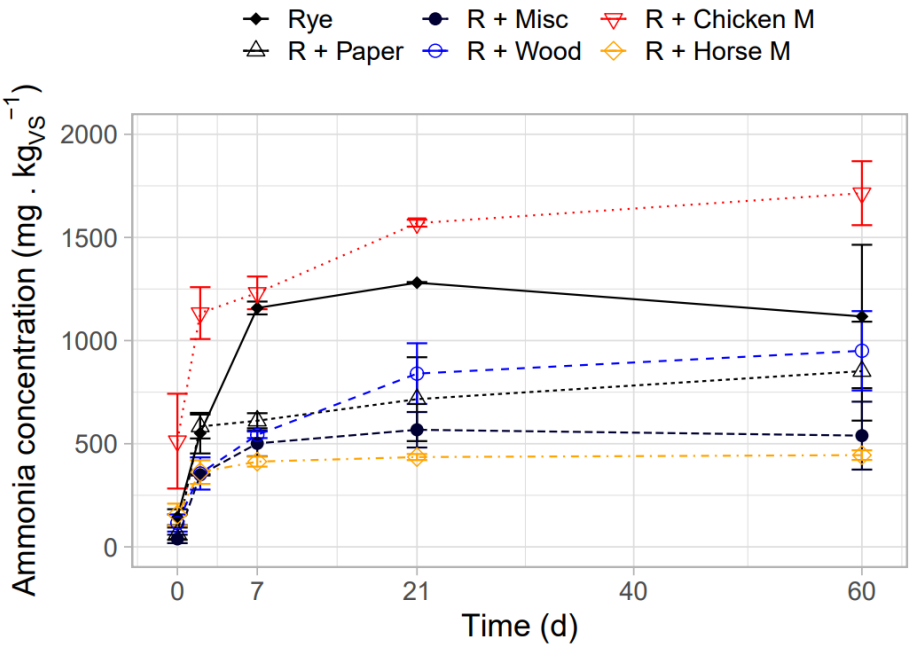


Figure 3: Evolution of ammonia concentration during storage. Concentrations are expressed in g.kg$_{VSadded}^{-1}$ of mix (cover crop + co-substrate); R stand for rye. Paper, Misc, Wood, Chicken M and Horse M stand for soiled paper and cardboard, miscanthus stalks, wood chips, chicken manure and horse manure, respectively.

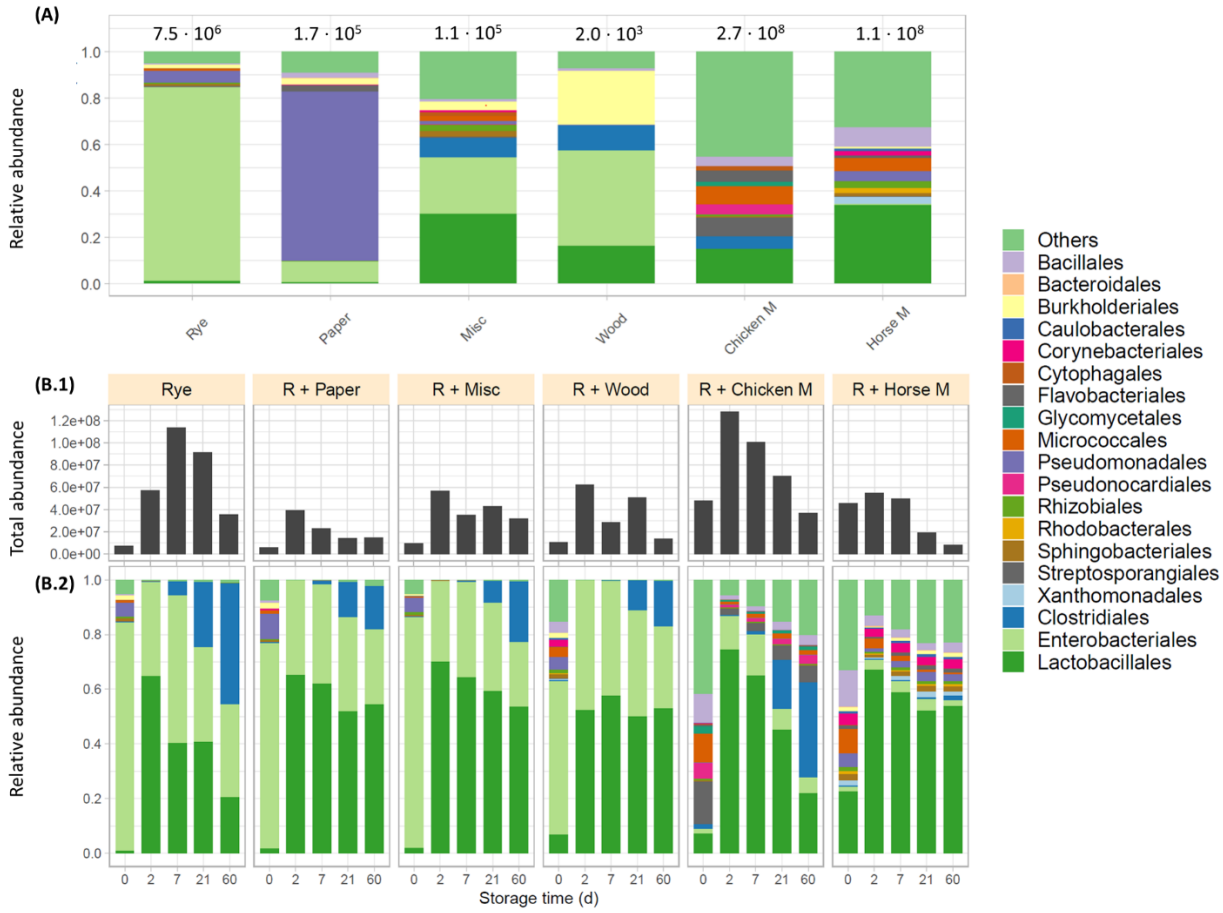
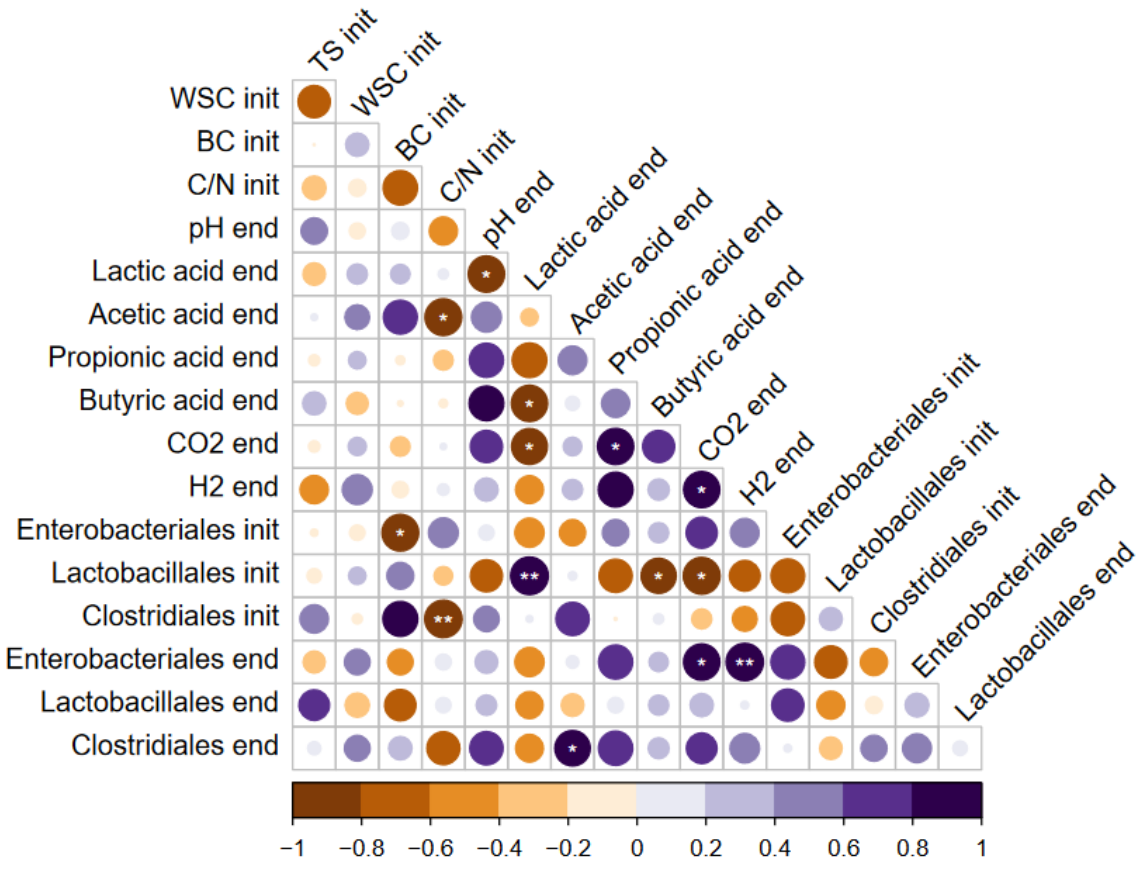


Figure 4: (A) Microbial characterization of raw substrates (relative abundance of major orders). Values on top represent the microbial population quantification (16S rRNA gene copy number . g_{FM}^{-1}); (B.1) Variation of total microbial abundance during storage (16S rRNA gene copy number . g_{FM}^{-1}); (B.2) Relative composition of microbial community at the order level.



729

730 *Figure 5 : Matrix of Pearson's correlation with the main bacterial orders for all ensiling and co-*
731 *ensiling experiments of rye. **: Significant with p-value <0.01, *: Significant with p-value <0.05*

732

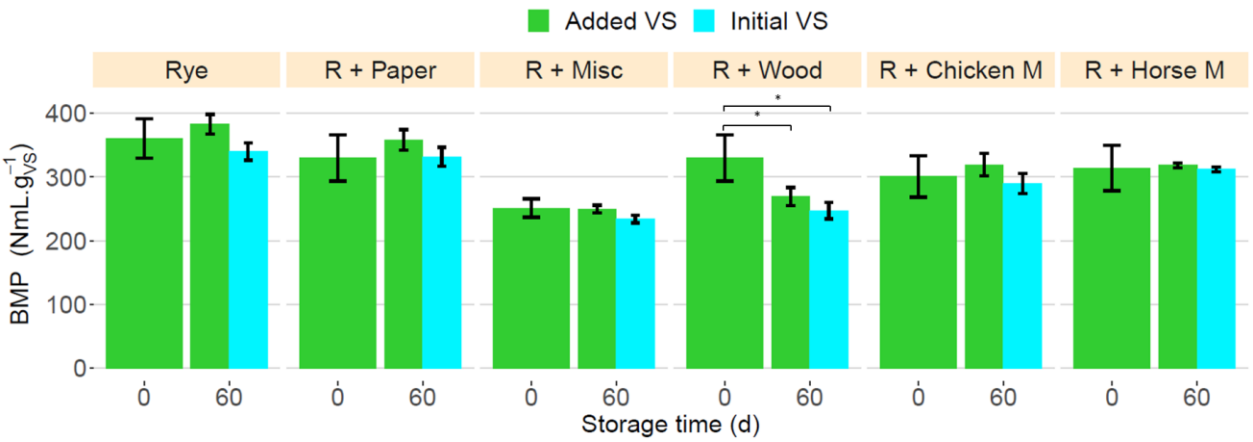


*Figure 6: Impact of storage under the different co-ensiling conditions on methane production per unit of VS of mix (cover crop + co-substrate). BMP reported to added VS represent the crop biodegradability after storage, and BMP reported to initial VS reflects the global impact of storage on methane potential. *: significant difference two BMP values for a given storage condition ($p<0.05$).*