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Closing nutrient loops in a maize rotation. Catch crops to reduce nutrient leaching and increase biogas production by anaerobic co-digestion with dairy manure

V. Riau [a], L. Burgos [a], F. Camps [b], F. Domingo [b], M. Torrellas [a], A. Antón [a], A. Bonmatí [a,*]

[a] GIRO, Institute of Agrifood Research and Technology (IRTA), Torre Marimon, 08140 – Caldes de Montbui, Barcelona, Catalonia, Spain
[b] Mas Badia, Sustainable Field Crops, Institute of Agrifood Research and Technology (IRTA), 17134 – La Tallada d'Empordà, Girona, Spain

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ABSTRACT

Three catch crop species, ryegrass, forage rape and black oat, were grown between successive rotations of maize to reduce nitrogen leaching due to maize fertilization with digested dairy manure. Catch crops showed a high nutrient uptake, but with a wide range, depending on the year and the specie. Ensiling was shown to be a feasible storing method increasing catch crop methane production per hectare between 14–36% compared with fresh catch crop. In semi-continuous co-digestion experiments, methane production was increased between 35–48%, in comparison with anaerobic digestion of dairy manure alone. Catch crops were shown to be a good co-substrate, being a sustainable option to prevent leaching of nutrients to the environment, thus closing the loops from production to utilization by optimal recycling measures.

1. Introduction

Livestock production intensification is a current trend of the European dairy sector, which causes the production of large amounts of manure that should be further managed. Manure storage and its subsequent land application are the common management practices because they are simple, cheap, and allow to replace chemical fertilizers, thus reducing crop production costs. However, manure application in agriculture leads to environmental problems due to the lack of enough cropland in many livestock farms, which results in a surplus of nitrogen (N) and phosphorus (P), especially in those areas where intensive farming is concentrated (Rico et al., 2011). Reduction of P and N inputs from agricultural land to water bodies is therefore one of the major challenges for current agriculture. Hence, strategies for an efficient removal of surplus soil nutrients need to be identified.

In this respect, catch crops (ChCps) can be used in order to reduce nutrient losses in the period between two main crops since they are able to efficiently retain soil mineral N and P as well as heavy metals, thus reducing leaching and runoff losses. They also contribute to improve soil quality by reducing its exposure to erosion and adding organic matter. Therefore, by using ChCps a reduction of fertilizer requirements for the following growing season is possible (Molinuevo-Salces et al., 2013). Catch crops can reduce N leaching by 40–50% and 30–38% in conventional (Aronsson and Torstensson, 1998) and organic systems (Askegaard et al., 2005), respectively. Grass or brassica species are the most efficient with an average of 70% reduction whereas legumes only present 20% reduction (Liu et al., 2015), but the extraction efficiency depends, among others, on the initial nutrient amount as well as on soil type, climatic conditions and ChCp management.

Biogas production through anaerobic digestion (AD) has become significant as an efficient manure treatment in the European agricultural sector since, besides the obtention of a renewable fuel, it also improves manure fertilizer quality and reduces odours, pathogens as well as greenhouse gas emissions (Torrellas et al. 2018). One of the main disadvantages of this process is the low energy content of manure that makes necessary to use high energy content co-substrates, such as ChCps, in order to optimize the process and improve the economic feasibility of biogas plants (Pitk et al., 2014).

Moreover, the use of solid–liquid separators after AD of manure is a valued posttreatment since dewatering reduces transport costs, thus allowing the export of nutrients from areas with excess manure and its redistribution to other shortfall areas (Holm-Nielsen et al., 2009). After dewatering, the solid fraction can be also subjected to the composting process therefore producing a by-

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* Corresponding author.
E-mail address: august.bonmati@irta.cat (A. Bonmatí).

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product containing more stable organic matter and nitrogen for cropland. The liquid fraction is usually used as a fertilizer (Mantovi et al., 2010) or is further processed (Bonmatí and Flotats, 2003, Bonmatí et al., 2003, Cerrillo et al., 2015, Laureni et al., 2013).

The use of ChCps biomass as co-substrate in the AD of animal manures notably improves the process performance since, on the one hand, manure provides buffering capacity and essential nutrients for anaerobic microorganisms, while on the other hand, the high carbon content of ChCps balances the carbon to nitrogen (C/N) ratio of the influent, thus reducing the risk of ammonia inhibition.

It is important to note that since catch crops are seasonally sown (autumn) and harvested (spring), it is necessary to find a storage method that guarantees the availability of this co-substrate for biogas production throughout the whole year. Silage has been proved to preserve over 90% of the energy content of crops, which ensures a good nutritional value when used as animal feed (Pakarinen et al., 2008). The advantages of ensiling include its low cost, its small energy footprint, the small amount of waste produced and the fact that it is a particularly environmentally friendly method, because no chemical additives are required. However, the chemical composition of the crop or its dry matter content are important factors for a successful ensilage and also for the subsequent anaerobic digestion, it might therefore be expected that methane yield is affected if previous ensilage of the substrate is carried out (Kafle and Kim, 2013). Nevertheless, the relation between ensiling and methane production is still little known (Herrmann et al., 2011). Anaerobic co-digestion of animal manure with various agro-industrial waste has been previously reported (Ferrer et al., 2014; Søndergaard et al., 2015; Aboudi et al., 2016). However, there is no data published on the co-digestion of dairy manure and ensiled catch crops.

The main objective of this study was to investigate the nutrient extraction efficiency from soil of three different catch crop species, grown between successive maize crops, as well as the viability of using them as co-substrates for the anaerobic co-digestion with dairy manure, with emphasis on the effect of ensiling on the anaerobic biodegradability and biogas potential. Digestate from the anaerobic co-digestion was used to fertilize the main crop, maize, thus closing the nutrient cycles.

2. Material and methods

2.1. Agricultural practices

A field trial on the implementation of different ChCp species, after maize as a main crop, was carried out at the Mas Badia Experimental Station (NE Catalonia). This area has a Mediterranean climate with an annual average temperature of 14.9 °C; min. 9.0 °C, max. 20.8 °C; and an annual average accumulated rainfall of 658 mm. The trial lasted three years (April 2014- March 2017) and had a randomized block design with four treatments and three replicates, with elementary plots of 131 m2 each. Soil in the trial is calcareous (pH water: 8.4), non– saline (EC 1:5 water: 0.142 dS m-1), very deep (>1.20 m), well drained, medium textured (loamy to sandy-loam) without coarse elements and a low content of organic matter on the topsoil (1.58%). It is classified as a Typic xerofluvent (Soil Taxonomy, 1999). Maize was fertilized, at a rate of 170 kg N ha^{-1}, with the liquid fraction of digested dairy manure coming from a nearby biogas plant. The ChCp species used, were *Lolium multiflorum* (Ryegrass), *Brassica napus* (Forage rape) and *Avena strigosa* (Black oat). An additional treatment with spontaneous herbage was also included. ChCps were sown once the main crop had been harvested, by the end of September-beginning of October, and were grown with minimum effort in care and cultivation as well as without use of fertilizers and irrigation water. Finally, ChCps were harvested in March and biomass exported from the field. Treatments were repeated on the same plots for the three years. The efficiency of catch crops included after a maize crop was evaluated in terms of biomass yield and nutrient uptake.

2.2. Substrates and inoculum

Raw manure (TS = 41 ± 0.3 g Kg^{-1}; VS = 31 ± 0.2 g Kg^{-1}; COD = 55.9 ± 8.1 g Kg^{-1}) used in the semi-continuous experiments and digested manure (VSS = 16.12 ± 3.01 g Kg^{-1}) used as inoculum in all the experiments were sampled from a nearby biogas plant before and after anaerobic digestion, respectively. Once collected, inoculum acclimation to laboratory conditions was carried out before starting the experiments in order to minimize the effects of sampling, transport and sample conservation on the anaerobic microbial activity. Two CSTR reactors were filled with the inoculum and fed with the same dairy manure. Both reactors operated under the same working conditions (HRT = 20 d; 37 °C) until reaching the steady state, after 20 days. The characterization of fresh and ensiled ChCps used for the semi-continuous experiments is summarized in the Results and Discussion section (Table 1).

2.3. Ensilage

In order to accurately control the ensiling conditions, ChCps silage was carried out, without the adding of additives, at the laboratory. Once harvested, the ChCps were chopped to 1–3 cm particle size and introduced into 30 L-tanks, pressed to remove interstitial air and tightly sealed. Then, the remaining oxygen was extracted using a vacuum pump to ensure anaerobic conditions in the medium from the beginning. A sampling bag was coupled in each tank in order to sample the gas (mainly CO_2) produced during the lactic fermentation. The fermentation period lasted for 3 months, after which the tanks were opened, and the content was characterized. Besides this, in order to elucidate the influence of the storage time on ChCps characteristics, ensiling was also carried out at 6 months for the ChCps harvested during the second season, and at 9 and 12 months for the ChCps harvested during the third season. The effect of the fermentation on ChCps composition and its methane potential is discussed in the Results and Discussion section.

2.4. Biochemical methane potential assays

Biochemical methane potential (BMP) tests were carried out at 37 °C (per duplicate) according to Campos et al. (2008) and anaerobic biodegradability (%) was calculated as described elsewhere (Soto et al. 1993). 1.2 L-glass vials were filled with 0.5 L of a mixture of digested cow manure as inoculum (1.5 gVSS/L), ground silage and fresh catch crops as substrates (2.5 gCOD/L) as well as deionized water. The mixture was supplemented with macro/micronutrients and bicarbonate following Ferrer et al. (2010). A control vial without substrate was included to assess the residual methane (CH_4) potential of the inoculum. The glass vials were shaken and O_2 was removed injecting a mixture of N_2/CO_2 (80/20 v/v), and then closed with rubber stoppers. The tests lasted an average of 40 days.

2.5. Semi-continuous co-digestion experiments

Semi-continuous co-digestion experiments were carried out in two CSTRs with a working volume of 5.5 L. The CSTRs were operated at mesophilic range (37 °C), with a hydraulic retention time (HRT) of 40 days. RC1, RC2 and RC3 were the control digesters in

Table 1
Biomass yield and nutrient (N, P, Cu and Zn) uptake for ryegrass, forage rape, black oat and spontaneous herbage during crop rotations. Three-year mean value ± standard deviation

	Ryegrass	Forage rape	Black oat	Spontaneous herbage
Biomass yield (t_{DM} ha^{-1})	6,5 ± 1,4	7,1 ± 2,5	5,5 ± 1,8	2,9 ± 2,1
N uptake (kg ha^{-1})	115,5 ± 64,2	154,1 ± 72,9	88,9 ± 43,4	67,7 ± 57,5
P uptake (kg ha^{-1})	13,8 ± 3,7	18,9 ± 4,8	11,0 ± 3,3	8,3 ± 5,8
Zn uptake (g ha^{-1})	150 ± 58	170 ± 72	180 ± 74	88 ± 47
Cu uptake (g ha^{-1})	34 ± 17	26 ± 13	45 ± 24	19 ± 12

which only raw dairy manure was used as substrate, whereas the co-digestion reactors were fed with a mixture of raw dairy manure and ensiled catch crop (10% on a wet weight (w/w) basis): ryegrass (RCO1), forage rape (RCO2) and black oat (RCO3). The co-digestion assays (including control reactors) were not carried out simultaneously but successively. Thus, RCO1 was started-up and run at the same time as its control RC1, and so on for RCO2/RC2 and RCO3/RC3, with a working time of 170 days each. Hence, the experimental procedure was composed of three independent and comparative sub-studies: RC1 vs RCO1, RC2 vs RCO2 and RC3 vs RCO3. Each reactor was fed once a day and the effluent characteristics and biogas composition were measured once a week. The attainment of the steady state for all the experiments was verified by checking if the effluent composition remained constant (total solids (TS), volatile solids (VS), biogas production and composition, and volatile fatty acids (VFA) levels), after a period equivalent to three times the HRT.

2.6. Analytical methods

In the agronomical part of the study, aboveground biomass of ChCp was determined by collecting plants produced on 12 m^2 in each elementary plot at the moment of harvesting, just like fresh matter produced, whereas a representative sample of the plants for each elementary plot was oven-dried at 60 °C. Both the dried samples and the liquid fraction of the digested manure applied to maize fields (sampled every year) were analysed by external laboratories following the UNE standard methods on dry matter content (UNE-EN 12880), N (UNE-ES 13342), P (UNE-EN 16170), Cu (UNE-EN 16170), Zn (UNE-EN 16170) and pH (UNE-ES 15933).

With respect to the ensiling and anaerobic digestion studies, samples were all characterised following the Standard methods for the examination of water and wastewater (AWWA, APHA, WEF, 2005): TS and VS (2540G), ammonia nitrogen (NH_4^+-N) (4500-NH3-C), and total alkalinity (TA) (2320B). Since silage biomass contains large amounts of volatile compounds, the measured oven-dried matter (at 105 °C) for loss of volatiles was corrected using coefficients according to Porter and Murray (2001). Furthermore, due to the high solid content of substrates, the total chemical oxygen demand (COD) analytical procedure was modified according to Noguerol-Arias et al. (2012). pH was measured on the leachate after crop: distilled water extraction (1:5 w/v) for 0.5 h. The analyses of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were carried out following the methods of Goering and Van Soest (1970).

Biogas production was daily measured with a volumetric gas counter (Ritter Apparatebau GmbH & Co. KG), and its composition (CH_4, H_2, and CO_2) as well as volatile fatty acids (VFA), acetate, propionate, iso-butyrate, n-butyrate, iso-valerate and n-valerate acids concentrations were determined by gas chromatography as described elsewhere (Campos et al., 2008).

2.7. Statistical analysis

ANOVA analysis and mean separation were applied to the experimental data by using the statistical software Statgraphics Centurion XVIII.

3. Results and discussion

3.1. Catch crop production and nutrient (N, P, Cu and Zn) uptake

The characterisation of the liquid fraction of the digested manure applied to maize fields is summarized as follows (expressed as g kg $^{-1}$ on DM basis): DM = 53.2 ± 9.2; N-NH_4 = 54.5 ± 9.6; P = 23.6 ± 2.8; K = 27.1 ± 1.8; Zn = 0.154 ± 0.087; Cu = 0.604 ± 0.165; Organic nitrogen = 36.8 ± 7.1. The measured pH was 8.60 ± 0.05.

Average ChCp productions (t DM ha^{-1} y^{-1}) and plant nutrient (N, P, Cu, and Zn) uptakes from soil (kg ha^{-1}) for the three years assayed, are shown in Table 1. Biomass yield was significantly different between different years with a minimum value of 3.4 kg DM ha^{-1} and a maximum value of 8.7 kg DM ha^{-1}, since it mainly depends on factors such as the sowing time (linked to harvest time of main crop, maize), the meteorological conditions during the growing period, the available nitrogen in the soil and the time of harvest, among others (Komainda et al., 2016; Alonso-Ayuso et al., 2018; Jeroen et al., 2020).

Despite spontaneous herbage growth (Table 1), N uptake from soil via catch crops was always significantly higher (42–264%) than those cases in which ChCp was not sown (67.7 ± 57.5 kg N ha^{-1}), although very variable among years. Thus, ChCp may reduce the risk of nitrate leaching from soil. In a similar maize-ChCp rotation but in a more arid environment, Gabriel et al. (2016) reported a negligible biomass yield when no catch crops were sown. Thorup-Kristensen (2001) found that much of the nitrate leached to the deeper soil layers when no catch crop was sowed, whereas most of the nitrate generally remained in the topsoil when catch crops were grown.

Biomass yield for the three ChCp species did not show significant differences (p-value = 0.06) considering the three-year average data. Average values ranged from 5.5 (black oat) to 7.1 (forage rape) t_{DM} ha^{-1} y^{-1} (Table 1). In Northern Germany, Komainda et al. (2016) obtained a biomass accumulation of up to 5 t_{DM} ha^{-1} above- and belowground, corresponding to an N amount of up to 83 kg N ha^{-1}, using rye and italian ryegrass sown after maize. Over different years, the highest N extraction was achieved by forage rape (154.1 ± 72.9 kg N ha^{-1}), being 23-57% higher than that achieved by ryegrass (115.5 ± 64.2 kg N ha^{-1}) and 41-82% higher in comparison with black oat (88.9 ± 43.4 kg N ha^{-1}). These results are consistent with those obtained by Thorup-Kristensen (2001), who found N uptakes using ChCps within an organic crop rotation of about 105, 98 and 120 kg N ha^{-1} for ryegrass, winter rape and oat, respectively. He also found that much of the nitrate leached to the deeper soil layers when no catch crop was sowed, whereas most of the nitrate generally remained in the topsoil when catch crops were grown. As for biomass, N uptake variation among years could be explained by ChCp length period, annual meteorological conditions and soil available nitrogen.

Total biomass yield is not the only parameter to be considered. Precocity in the development and soil cover (also affecting nutrient uptake) is desirable in winter catch crops (Justes et al., 2012). In the specific conditions of the trial, black oat was the ChCp specie that more regularly and quickly developed and covered soil surface over the three years, thus, protecting soil against erosion and nutrient losses. Gabriel et al. (2016) found a quicker development of barley than vetch as catch crops, although average biomass yield at the end was higher for vetch, but year dependant. Therefore, specific estimations should be carried out for different geographical zones.

P uptake was also higher using forage rape, with minimum-maximum increases in all rotations of 15-70%, 62-81% and 36-433%, in comparison with ryegrass, black oat and spontaneous herbage, respectively.

It has been reported that anaerobic digestion does not affect the removal of dissolved Cu and Zn in dairy manures. However, Cu and Zn bioavailability is increased during the process, thus making anaerobic digestate less favourable for its application in agriculture (Jin et al., 2015). Nevertheless, this could be solved by introducing catch crops into crop rotation. Zn and Cu uptakes (Table 1) from all catch crops studied were always significantly higher than those obtained by spontaneous herbages (27-197%). The highest Cu and Zn extractions were achieved when using black oat. Zn extractions using black oat (180 g Zn ha^{-1}) were 18-31% and 5-22% higher than when using ryegrass and forage rape, respectively. Besides, Cu uptake (45 g Cu ha^{-1}) was increased by 51-80% in comparison with ryegrass and forage rape.

3.2. Silage process, anaerobic biodegradability and methanogenic potential assessment

Table 2 summarizes the main characteristics of the ChCps used before and after ensiling, during consecutive rotation cycles and under different ensiling periods (3, 6, 9 and 12 months). In view of the mean values and the corresponding standard deviation, it can be assumed that 3 months was enough time for a proper ensiling of the studied ChCps and therefore further ensiling times (6, 9 and 12 months) did not affect the physicochemical properties of these crops. As can be seen, the average DM content in this study ranged between 135-187 g kg^{-1} for all ChCps studied, which is lower than the dry matter content threshold for a proper ensiling process (250-400 g kg^{-1}) stated by Molinuevo-Salces et al. (2015) and Villa et al. (2020). However, the silage process was confirmed by a decrease in pH values to below 4.5 after ensiling whereas dry matter losses remained below 3% for all treatments, as expected (Vervaeren et al. 2010). Nevertheless, studies carried out by Zhao et al. (2017) reported DM losses about 6.6% after ensiling switchgrass without additives for 30 days. The initial DM content in that case was 264 g kg^{-1}.

Table 3 summarizes the methane yield for the three ChCp species studied, as a mean value and their standard deviation, for all years and ensiling times assayed for a better comparison. Additionally, the anaerobic biodegradability and methane yield obtained after the BMP assays in each crop rotation, for different ensiling times assayed, are shown in Figs. 1 and 2, respectively.

As can be seen, ensiling improved the biodegradability and methane yield of catch crops under anaerobic conditions. In fact, the anaerobic biodegradability of ryegrass, forage rape and black oat was increased by 10%, 14% and 10%, respectively, which resulted in an increase of the methane yield by 40%, 46% and 50%, in terms of L_{CH4} per tonne of waste (Table 3) and 19%, 25% and 27%, in terms of methane production per volatile solid added (Table 3 and Figure 2A). These results are consistent with those obtained by Amon et al. (2007) who reported increments in methane yield (expressed as L_{CH4} $kg^{-1}_{VSadded}$) by 25% between the anaerobic digestion of non-ensiled and ensiled maize. Values of methane yield reported were also in the same order of magnitude (283-366 L_{CH4} kg^{-1}VS$_{added}$) as those obtained in the present study (Table 3). Zhao et al. (2017) obtained a 35% increase in specific methane yield in BMP trials using ensiled switchgrass, compared to fresh switchgrass. Methane yield did not show significant differences between ChCp species (p-value = 0.51) or between different ensiling times (p-value = 0.42). This analysis showed that ensiling times greater than 3 months do not modify neither the chemical composition nor the energy properties of the different ChCps species. The former could be explained by the fact that during the silage process, lactic acid, acetic acid, methanol, alcohols, and other compounds are formed, which are important precursors for methane formation and reduce the pH of the feedstock causing its preservation against the growth of fungi, bacteria and yeasts (Villa et al., 2020); so, the low pH level (pH < 4) stopped biodegradation thereby preserving its chemical composition (Table 2). During ensiling, the different biochemical processes directly or indirectly affect biogas production by changing the properties of the feedstock. A faster pH decrease produces more water-soluble carbohydrates in the silage mass and therefore more biogas (Villa et al., 2020). . Besides, biochemical processes during ensiling include hydrolysis and acidification. The microbial degradation of crop compounds may lead to a faster conversion or to a better availability of recalcitrant compounds during the anaerobic digestion process so that ensiling can be regarded as a pre-treatment method (Neureiter et al., 2005). Ensiling process can increase the specific methane production of certain substrates by 25–42%, since the produced organic acids can act as a form of chemical pre-treatment solubilizing cellulose and hemicellulose (Janke et al., 2019). Thus, the decomposition of crude fibre during the course of the silage process, which could improve the biodegradability and the availability of nutrients for the methanogenic metabolism, could be another reason for the increase in specific methane yield. However, some studies (Herrmann et al., 2011; Zhao et al., 2017; Chen et al., 2020) have reported no differences in fibre and DM content before and after ensiling crops such as maize, forage ryegrass, switchgrass or oat, which is consistent with

Table 2
Main characteristics of catch crops studied before and after the silage process. Mean value ± standard deviation.

CATCH CROP	pH	DM (g kg^{-1})	VS (g kg^{-1})	VS (% DM)	NDF (% DM)	ADF (% DM)	ADL (% DM)
Ryegrass	5.4 ± 0.5	185 ± 31	162 ± 26	88	42.5 ± n.d	24.1 ± n.d	3.6 ± n.d
Ensiled Ryegrass	3.8 ± 0.3	179 ± 26	156 ± 21	87	47.9 ± n.d	30.5 ± n.d	3.1 ± n.d
Forage rape	5.7 ± 0.9	135 ± 17	117 ± 17	87	40.3 ± n.d	25.0 ± n.d	5.9 ± n.d
Ensiled forage rape	4.0 ± 0.4	136 ± 14	118 ± 15	87	36.8 ± n.d	33.8 ± n.d	8.1 ± n.d
Black oat	5.8 ± 0.6	184 ± 15	164 ± 16	89	45.2 ± n.d	24.7 ± n.d	3.2 ± n.d
Ensiled Black oat	3.9 ± 0.3	187 ± 14	167 ± 14	90	45.9 ± n.d	28.0 ± n.d	4.6 ± n.d

DM: Dry Matter, VS: Volatile Solid, NDF: Neutral Detergent Fiber, ADF: Acid Detergent Fiber, ADL: Acid Detergent Lignin.

Table 3
Mean values of methane yield during consecutive rotations over different ensiling time periods, for the three catch crops studied. Mean value ± standard deviation.

	Ryegrass	Ensiled Ryegrass	Forage rape	Ensiled Forage rape	Black oat	Ensiled Black oat
Methane yield (LCH_4 $kgVS^{-1}$)	287 ± 79	341 ± 72	315 ± 21	394 ± 43	276 ± 37	351 ± 51
Methane yield (LCH_4 $kgCOD^{-1}$)	143 ± 24	180 ± 17	137 ± 51	180 ± 13	115 ± 26	149 ± 29
Methane yield (m^3CH_4 t^{-1})	45 ± 10	63 ± 9	37 ± 8	54 ± 19	48 ± 7	72 ± 11
Methane yield (LCH_4 ha^{-1})	1351 ± 862	1533 ± 903	1287 ± 501	1746 ± 694	1119 ± 653	1496 ± 723

VS: Volatile Solid, COD: Chemical Oxygen Demand.

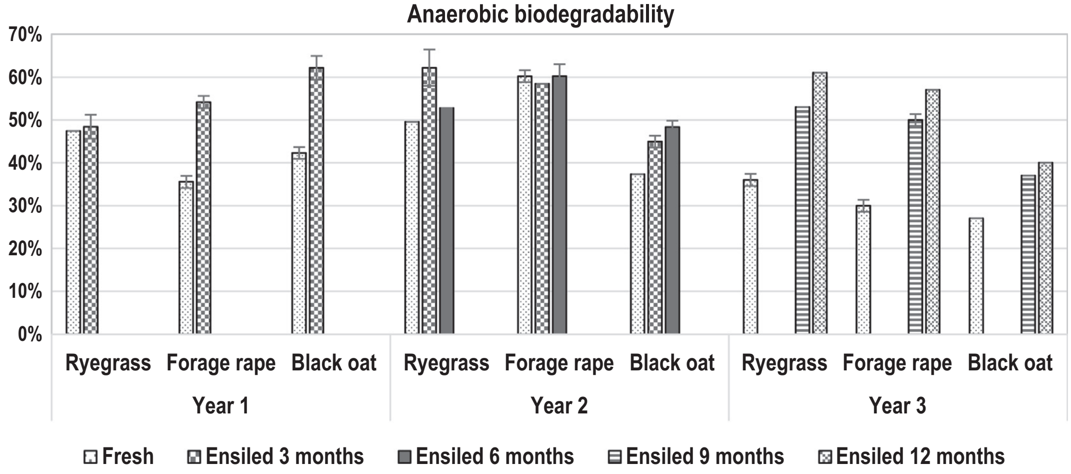

Fig. 1. Anaerobic biodegradability of fresh and ensiled catch crops during consecutive crop rotations, at different ensiling times.

the results obtained in the present study (Table 2). The slight variation in fibre content before and after the silage process in our study could be attributable to sampling and measurement inaccuracies and the biodegradability improvement could only be caused by pre-fermentation during ensiling, since it creates an acidic environment, reducing the risk of feedstock decay and combustion under anaerobic conditions, which can be used as a biochemical pretreatment before AD to improve biomass conversion efficiency (Zhao et al., 2017).

It is important to note that, in terms of economics, the volume of methane per hectare, i.e., the product of the biomass yield as VS per hectare of catch crop (t_{VS} ha^{-1}) and the specific methane yield on VS of the catch crop (m^3 t^{-1}_{VS}) (Villa et al., 2020) (Table 3 and Figure 2B), is one of the key factors determining the feasibility of using catch crops as substrate for biogas production.

In studies on methane potential from ryegrass carried out by Molinuevo-Salces et al. (2013) the results showed that methane yield per hectare should be above 700 m^3_{CH4} ha^{-1} (considering specific methane yields above 350 m^3 t^{-1} of VS and a biomass yield in terms of VS above 2 t ha^{-1}) for an economically sustainable anaerobic co-digestion of ryegrass with animal manure, in biogas plants. In the present study, methane yield was 341 ± 72, 394 ± 43, and 351 ± 51 m^3_{CH4} t^{-1}_{VS} for ensiled ryegrass, forage rape and black oat respectively, and biomass yield ranged between 4.8 and 6.3. Methane yield per hectare was therefore 1533 ± 903, 1746 ± 694 and 1496 ± 653 m^3_{CH4} ha^{-1} for ryegrass, forage rape and black oat, respectively. Besides, the specific methane yield per hectare of ryegrass, forage rape and black oat increased after ensiling by 14%, 36% and 34% respectively, confirming the benefits of this storage method when using crops for biogas production. Methane yield per hectare showed significant differences between fresh and ensiled ChCps (p-value = 0.04) and between different ChCps species (p-value = 0.04). However, in order to assess the technical and economic performance of ChCp as co-substrates in Mediterranean conditions, the co-digestion *Manure + ChCp* performance in continuous or semi-continuous tests should be further analysed in order to determine possible inhibitions or accumulation of recalcitrant compounds during a long-term operation thus optimizing the operational parameters and biogas production.

3.3. Semi-continuous experiments

Table 4 shows the main characteristics of the influent, in terms of organic content, the operational conditions, as well as the results obtained at steady-state conditions for each semi-continuous experiment. Using ChCp as a co-substrate increased the organic content in the influent and therefore, the organic load rate (OLR) rose to between 17 and 46%, while the HRT remained constant at 40 days. Generally, the overall process performance was enhanced; for ryegrass, methane yield increased by 43% (from 8.8 to 12.6 m^3_{CH4} t^{-1}_{waste}) when increasing the organic load rate by 33%, in comparison with the control digester, whereas organic matter degradation was improved by 16% in terms of VS removal. Similarly, co-digestion using forage rape as co-substrate showed an increase of methane yield of 35% and the organic matter degradation increased by 15% in terms of VS added. However, it must be taken into account that the ORL was only increased by 17% in this case. As regards co-digestion when using black oat, methane yield was increased by 48% while VS removal was only 5%. In this case, the OLR increased significantly (46%) in comparison with the other experiments. These results indicate that co-digestion improves methane production, due to the synergism between co-substrates, which increase the efficiency in terms of methane yield per unit of organic matter removed (Aboudi et al., 2020). This synergism was higher when black oat was used as co-substrate which allowed for working at a higher OLR.

The evolution of methane production during the experiments, once the start-up phase was overcome (40 days, until reaching the steady state), is depicted in Figure 3. The digesters were operating for at least 120 days, equivalent to 3 times the established HRT. Co-digestion clearly improved methane yield in terms of m^3

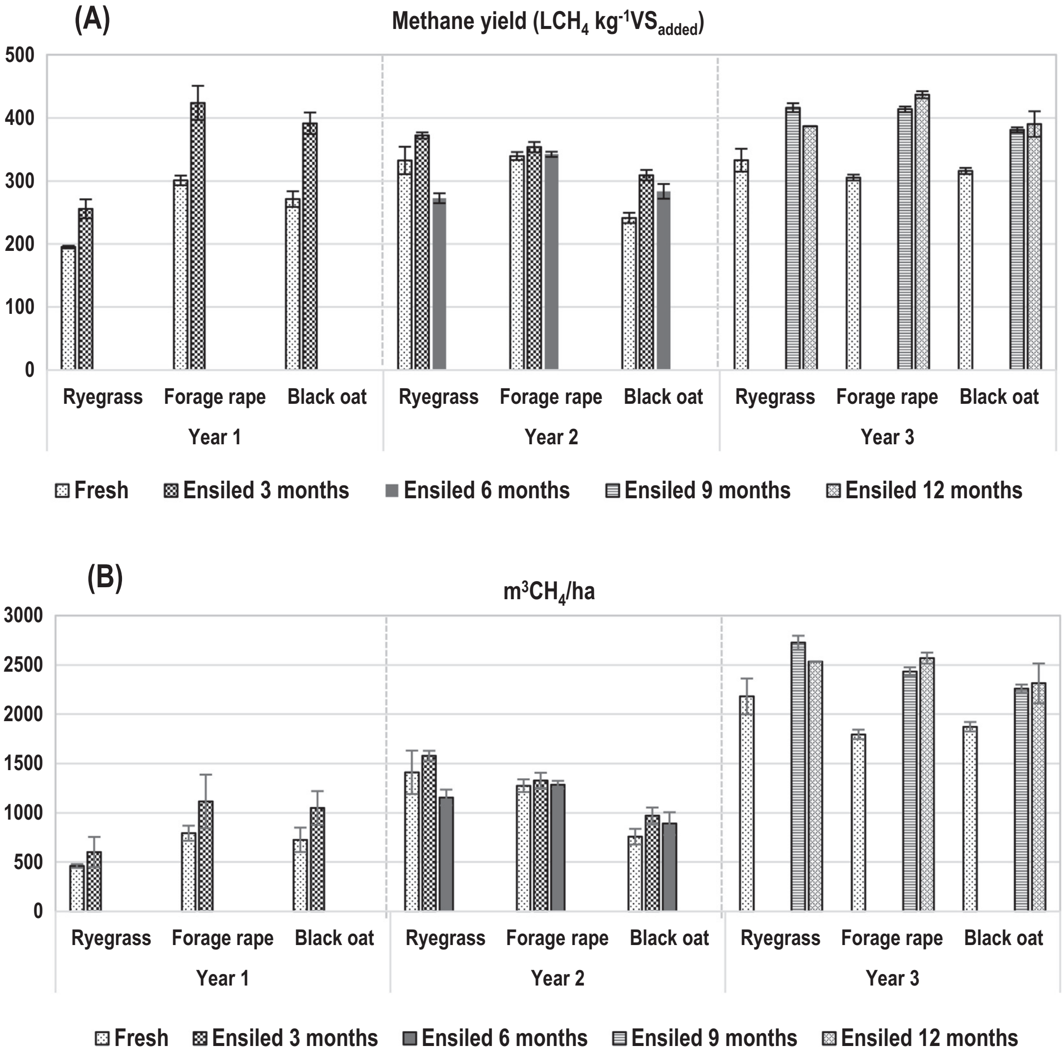

Fig. 2. Methane yield, expressed as (A) litres of methane per kilogram of volatile solid added and (B) cubic meters of methane per hectare, at standard temperature and pressure, for fresh and ensiled catch crops during consecutive crop rotations, at different ensiling times.

Table 4
Organic loading rate, operational conditions and methane yields obtained at steady-state conditions for each semi-continuous experiment. Mean value ± standard deviation.

	Ryegrass		Forage rape		Black oat	
	RC1	RCO1	RC2	RCO2	RC3	RCO3
TS_{INF} (%)	4.5 ± 0.5	6.6 ± 1.2	3.7 ± 0.1	5.0 ± 0.6	4.0 ± 0.4	4.9 ± 0.1
VS_{INF} (%)	3.4 ± 0.4	5.3 ± 1.1	2.8 ± 0.0	4.1 ± 0.5	3.1 ± 0.3	4.4 ± 0.1
COD (g kg^{-1})	62.9 ± 7.2	85.6 ± 12.7	49.9 ± 8.1	56.1 ± 2.5	54.8 ± 9.0	77.2 ± 4.6
OLR (kg COD $m^{-3}·d^{-1}$)	1.8 ± 0.1	2.4 ± 0.4	1.2 ± 0.2	1.4 ± 0.4	1.3 ± 0.2	1.9 ± 0.4
VS removal (%)	40 ± 19	56 ± 7	42 ± 15	57 ± 10	52 ± 10	57 ± 6
Methane content (%)	61 ± 15	63 ± 0	59 ± 5	60 ± 2	65 ± 1	63 ± 1
Methane yield (m^3 CH_4 t^{-1}_{waste})	8.8 ± 2.6	12.6 ± 1.0	8.1 ± 2.0	10.9 ± 3.0	8.7 ± 1.2	12.9 ± 2.8
Average Productivity (m^3 CH_4 $m^{-3}·d^{-1}$)	0.25 ± 0.04	0.33 ± 0.07	0.22 ± 0.04	0.26 ± 0.07	0.25 ± 0.04	0.38 ± 0.07

TS: Total solid. VS: Volatile Solid, COD: Chemical Oxygen Demand, OLR: Organic Load Ratey.

CH_4 t^{-1}_{waste} with respect to control digesters (RC1, RC2 and RC3): 43%, 35% and 48% for RCO1, RCO2 and RCO3, respectively. The highest average volumetric methane production was achieved by the co-digestion reactor using ensiled black oat (0.38 ± 0.07 m^3 CH_4 $m^{-3}·d^{-1}$) as a co-substrate. Preliminary calculations made on the nearby biogas plant, in terms of electric self-sufficiency, foresee an increase in energy production by 42% (from 3.3 to 4.7 mill kWh/a), in comparison to the current plant operation when ChCps are introduced as co-substrates.

The specific methane production ranged between 229-300 L_{CH4} kg^{-1}_{VS} for RC1, RC2 and RC3 and between 239-341 L_{CH4} kg^{-1}_{VS} for RCO1, RCO2 and RCO3. These results are in concordance with several studies in which energy crops have been used as co-substrates. Energy crops, their controversial use apart, are being utilized more and more as co-substrates in certain countries due to the higher methane yield relative to animal manure (174–300 L_{CH4} kg^{-1}_{VS}) (Lansing et al., 2010; Kalamaras and Kotsopoulos, 2014). Some of the most widely used energy crops are maize (225–450 L_{CH4} kg^{-1}_{VS}) (Amon et al., 2007;

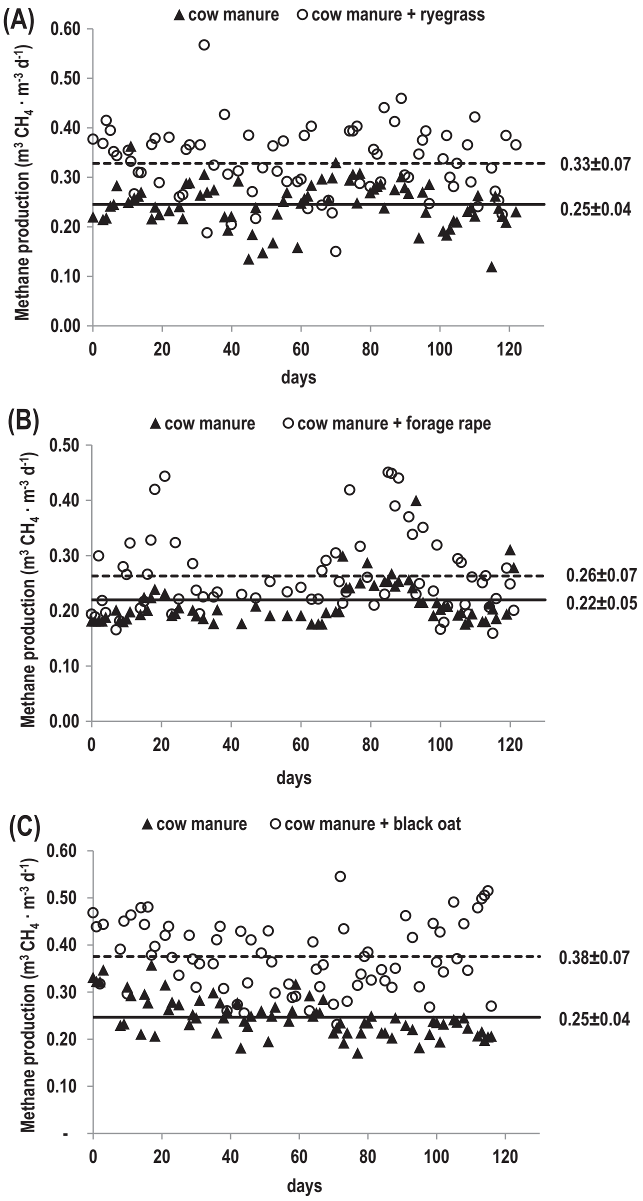

Fig. 3. Evolution of the volumetric methane production during the semi-continuous experiments, expressed as m^3 CH_4 $m^{-3}d^{-1}$ at standard temperature and pressure. (A) RC1 vs RCO1; (B) RC2 vs RCO2; and (C) RC3 vs RCO3. Numbers and lines represent mean values ± standard deviations.

Bruni et al., 2010), switchgrass (191–467 L_{CH_4} kg_{VS}^{-1}) (Massé et al., 2010; Zheng et al., 2015) and sugar beets (243–314 L_{CH_4} kg_{VS}^{-1}) (Aboudi et al., 2016), among others. Thus, in view of these results, it is possible to obtain comparable methane yields using catch crops with lower cultivation costs, as opposed to energy crops, especially in Southern Europe where high-frequency irrigation systems are crucial for increasing the biomass yield from maize and other energy crops (Kalamaras and Kotsopoulos, 2014).

As can be seen in Table 2, forage rape presented higher lignin content than the other tested ChCps, and therefore its methanogenic potential was affected when semi-continuous conditions were established, since cellulose and hemicellulose polymers of lignocellulosic materials are protected by lignin fraction, which acts as a shield during enzymatic hydrolysis (Usman Khan, 2021). In fact, the lowest volumetric production (Figure 3) and the lowest methane production per ton of waste (Table 4) in the co-digestion semi-continuous experiments were achieved in RCO2 (forage rape as co-substrate), whereas the productivity of control digesters was almost similar for all the assays carried out (Figure 3).

With respect to operational parameters such as pH, ammonia nitrogen, total volatile fatty acids (TVFA) and total alkalinity, reactors did not show significant inhibition symptoms over the operational period. VFA ranged between 0-540 and 0-266 mg acetic acid L^{-1} for co-digestion and control digesters, respectively. It has to be noted that, the maximum values (Figure 4) corresponded to slight and punctual imbalances in the operational performance such as the start-up, obstructions or temperature fluctuations; those were solved without significant effects on the overall performance of the reactors. In fact, total alkalinity (9.44 ± 1.56 g L^{-1} and 9.65 ± 1.32 g L^{-1} on average for control and co-digestion reactors, respectively) counteracts the peaks of VFA, and therefore pH was almost constant at around 7.6-7.7 throughout the experiment period (Figure 4), which indicates that the buffer capacity of the system is well-balanced that favors the fermentative bacteria metabolism and the development of methanogenic archaea (Micolucci et al., 2016). Besides, average TVFA concentrations were far lower than those stated as inhibitory concentrations (e.g. acetic acid exceeding 1.5 g/L lead to AD failure according to Caliskan et al. (2014), and the propionic to acetic acid ratio (maximum value of 0.7 and 0.4 for co-digestion and control digesters, respectively)), and was always below the limit of 1.4, indicative of the anaerobic digester stability (Zhang et al., 2014). Moreover, it has been reported that isoforms of butyrate and valerate are considered the best indicators of anaerobic process imbalance (Hill and Holmberg, 1988). In the present study, isobutyrate and isovalerate (data not shown) were almost totally consumed during the anaerobic process with concentrations under 0.6 mg L^{-1} in all reactors. The average ammonia concentrations in the effluent at the steady state were 1.6 ± 0.1 g L^{-1} for RCO1, 1.3 ± 0.1 g L^{-1} for RCO2 and 1.5 ± 0.1 g L^{-1} for RCO3,

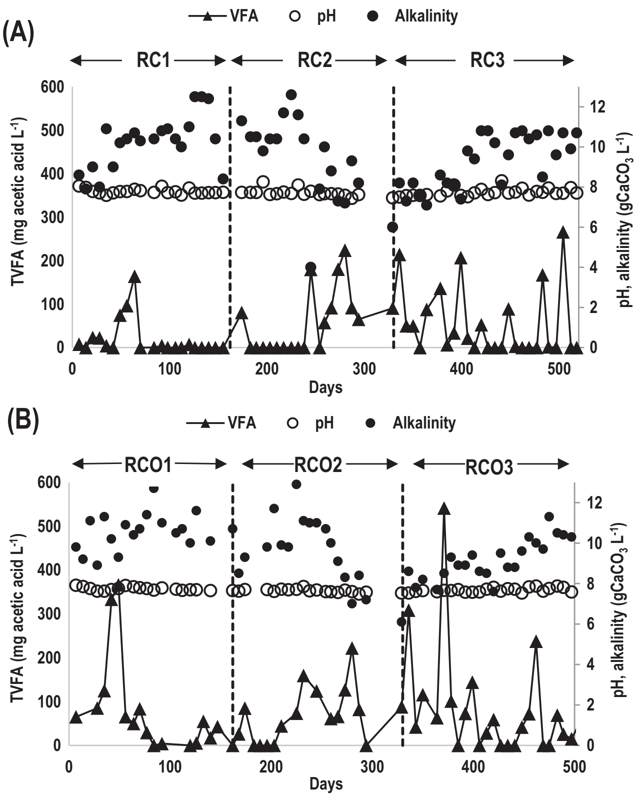

Fig. 4. Evolution of total volatile fatty acid (TVFA) concentration, total alkalinity and pH during the semi-continuous experiments. (A) Control reactors and (B) Co-digestion reactors.

whereas ammonia in the control reactors was 1.8 ± 0.3 g L^{-1} on average. There is a broad range of NH_4-N levels reported that cause inhibitory effects on the anaerobic bacteria with thresholds ranging from 1.5 up to 5 g L^{-1} if the acclimatization of microorganisms is properly carried out (Jahn et al., 2020). Besides, not all methanogens are affected to ammonia exposure in the same way; the less sensitive hydrogenotrophic methanogenic archaea are able to remain active at ammonia concentrations above 5 g L^{-1} (Ruiz-Sánchez et al., 2019)

4. Conclusions

Catch crops biomass yield is strongly dependent on the geographical and meteorological conditions. In this sense, black oat appeared to be the specie that more regularly and quickly developed and covered soil surface. Besides, the use of catch crops is shown to be a good strategy for reducing nutrients (N and P) and other elements (Cu and Zn), leaching and runoff. N extraction from soil via catch crops was always significantly higher (42-264%), than those cases in which ChCp was not sown. Moreover, ensiling was demonstrated to be an economically viable storing method, that can act as a form of chemical pre-treatment for biogas production, as potential methane production per hectare is increased by 14%, 36% and 34% for ryegrass, forage rape and black oat, respectively. Semi-continuous experiments confirmed the viability of the implementation of anaerobic co-digestion of dairy manure and ensiled catch crops since methane production was increased by 43%, 35% and 48% for ryegrass, forage rape and black oat, respectively, in comparison to the single anaerobic digestion of dairy manure. It is therefore possible to establish a closed system between dairy and crop production by applying the Circular Economy principles in terms of waste valorisation and nutrient recycling.

Declaration of Competing Interest

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

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References

Aboudi, K., Álvarez-Gallego, C.J., Romero-García, L.I., 2016. Biomethanization of sugar beet byproduct by semi-continuous single digestion and co-digestion with cow manure. Bioresource Technol. 200, 311–319.

Aboudi, K., Gómez-Quiroga, X., Álvarez-Gallego, C.J., Romero-García, L.I., 2020. Insights into anaerobic co-digestion of lignocellulosic biomass (sugar beet by-products) and animal manure in long-term semi-continuous assays. Appl. Sci. (Switzerland) 10 (15), 5126. https://doi.org/10.3390/app10155126.

Alonso-Ayuso, M., Quemada, M., Vanclooster, M., Ruiz-Ramos, M., Rodriguez, M., Gabriel, J.L., 2018. Assessing cover crop management under actual and climate change conditions. Sci. Total Environ. 621, 1330–1341.

Amon, T., Amon, B., Kryvoruchko, V., Zollitsch, W., Mayer, K., Gruber, L., 2007. Biogas production from maize and dairy cattle manure-Influence of biomass composition on the methane yield. Agr. Ecosyst. Environ. 118 (1-4), 173–182.

Aronsson, H., Torstensson, G., 1998. Measured and simulated availability and leaching of nitrogen associated with frequent use of catch crops. Soil Use Manag. 14 (1), 6–13.

Askegaard, M., Askegaard, M., Olesen, J.E., Kristensen, K., 2005. Nitrate leaching from organic arable crop rotations: effects of location manure and catch crop. Soil Use Manag. 21 (2), 181–188.

AWWA, APHA, WEF, 2005. Standard methods for the examination of water and waste water, 21th ed. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC,USA.

Bonmatí, A., Flotats, X., 2003. Pig slurry concentration by vacuum evaporation: influence of previous mesophilic anaerobic process. J. Air Waste Manag. Assoc. 53, 21–31.

Bonmatí, A., Campos, E., Flotats, X., 2003. Concentration of pig slurry by evaporation: Anaerobic digestion as the key process. Water Sci. Technol. 48 (4), 189–194.

Bruni, Emiliano, Jensen, Anders Peter, Pedersen, Erik Silkjær, Angelidaki, Irini, 2010. Anaerobic digestion of maize focusing on variety, harvest time and pretreatment. Appl. Energ. 87 (7), 2212–2217.

Caliskan, G., Giray, G., Gundogdu, T.K., Azbar, N., 2014. Anaerobic biodegradation of beer production wastewater at a field scale and exploitation of bioenergy potential of other solid wastes from beer production. Int. J. Renew. Energy Biofuels. 10. https://doi.org/10.5171/2014.664594.

Campos, E., Almirall, M., Mtnez-Almela, J., Palatsi, J., Flotats, X., 2008. Feasibility study of the anaerobic digestion of dewatered pig slurry by means of polyacrylamide. Bioresource Technol. 99 (2), 387–395.

Cerrillo, M., Palatsi, J., Comas, J., Vicens, J., Bonmatí, A., 2015. Struvite precipitation as a technology to be integrated in a manure anaerobic digestion treatment plant – removal efficiency, crystal characterization and agricultural assessment. J. Chem. Technol. Biotechnol. 90 (6), 1135–1143. 10.1002/jctb.4465.

Chen, Liangyin, Bai, Shiqie, You, Minghong, Xiao, Bingxue, Li, Ping, Cai, Yimin, 2020. Effect of a low temperature tolerant lactic acid bacteria inoculant on the fermentation quality and bacterial community of oat round bale silage. Anim. Feed Sci. Tech. 269, 114669. https://doi.org/10.1016/j.anifeedsci.2020.114669.

De Waele, Jeroen, Vandecasteele, Bart, Elsen, Annemie, Haesaert, Geert, Wittouck, Daniël, Horemans, Dorien, Zerssa, Gebeyanesh Worku, De Neve, Stefaan, 2020. Risk assessment of additional nitrate leaching under catch crops fertilized with pig slurry after harvest of winter cereals. Agr. Ecosyst. Environ. 304, 107113. https://doi.org/10.1016/j.agee.2020.107113.

Ferrer, Ivet, Palatsi, Jordi, Campos, Elena, Flotats, Xavier, 2010. Mesophilic and thermophilic anaerobic biodegradability of water hyacinth pre-treated at 80°C. Waste Manage. 30 (10), 1763–1767.

Ferrer, Pablo, Cambra-López, María, Cerisuelo, Alba, Peñaranda, David S., Moset, Verónica, 2014. The use of agricultural substrates to improve methane yield in anaerobic co-digestion with pig slurry: Effect of substrate type and inclusion level. Waste Manage. 34 (1), 196–203.

Gabriel, J.L., Alonso-Ayuso, M., García-González, I., Hontoria, C., Quemada, M., 2016. Nitrogen use efficiency and fertiliser fate in a long-term experiment with winter cover crops. Eur. J. Agron. 79, 14–22.

Goering, H.K., Van Soest, P.J., 1970. Forage Fiber Analysis. Agric. Handbook No. 379. ARS, USDA, Washington, DC.

Herrmann, Christiane, Heiermann, Monika, Idler, Christine, 2011. Effects of ensiling, silage additives and storage period on methane formation of biogas crops. Bioresource Technol. 102 (8), 5153–5161.

Hill, D.T., Holmberg, R.D., 1988. Long chain volatile fatty acid relationships in anaerobic digestion of swine waste. Biol. Wastes 23 (3), 195–214.

Holm-Nielsen, J.B., Al Seadi, T., Oleskowicz-Popiel, P., 2009. The future of anaerobic digestion and biogas utilization. Bioresource Technol. 100 (22), 5478–5484.

Jahn, Lydia, Baumgartner, Thomas, Krampe, Jörg, Svardal, Karl, 2020. Effect of NH_3 and organic loading on the inhibition of mesophilic high-solid digestion. J. Chem. Technol. Biotechnol. 95 (3), 702–709.

Janke, Leandro, McCabe, Bernadette Kathleen, Harris, Peter, Hill, Andrew, Lee, Seonmi, Weinrich, Sören, Marchuk, Serhiy, Baillie, Craig, 2019. Ensiling fermentation reveals pre-treatment effects for anaerobic digestion of sugarcane biomass: An assessment of ensiling additives on methane potential. Bioresource Technol. 279, 398–403.

Jin, H., Fu, G., Chang, Z., 2015. Effects of mesophilic anaerobic digestion of pig and dairy manures on Cu and Zn. Res. Environ. Sci. 28 (3), 474–480.

Justes, E., Beaudoin, N., Bertuzzi, P., Charles, R., Constantin, J., Dürr, C., Hermon, C., Joannon, A., Le Bas, C., Mary, B., Mignolet, C., Montfort, F., Ruiz, L., Sarthou, J.P., Souchère, V., Tournebize, J., Savini, I., Réchauchère, O., 2012. Réduire les fuites de nitrate au moyen de cultures intermédiaires : conséquences sur les bilans d'eau et d'azote, autres services écosystémiques. Synthèse du rapport d'étude, INRA (France), p. 60.

Kafle, Gopi Krishna, Kim, Sang Hun, 2013. Effects of chemical compositions and ensiling on the biogas productivity and degradation rates of agricultural and food processing by-products. Bioresource Technol. 142, 553–561.

Kalamaras, S.D., Kotsopoulos, T.A., 2014. Anaerobic co-digestion of cattle manure and alternative crops for the substitution of maize in South Europe. Bioresource Technol. 172, 68–75.

Komainda, Martin, Taube, Friedhelm, Kluß, Christof, Herrmann, Antje, 2016. Above- and belowground nitrogen uptake of winter catch crops sown after silage maize as affected by sowing date. Eur. J. Agron. 79, 31–42.

Lansing, Stephanie, Martin, Jay F., Botero, Raúl Botero, da Silva, Tatiana Nogueira, da Silva, Ederson Dias, 2010. Methane production in low-cost, unheated, plug-flow digesters treating swine manure and used cooking grease. Bioresource Technol. 101 (12), 4362–4370.

Laureni, M., Palatsi, J., Llovera, M., Bonmatí, A., 2013. Influence of pig slurry characteristics on ammonia stripping efficiencies and on the quality of the recovered ammonium-sulfate solution. J Chem Tech Biotech. 88, 1654–1662.

Liu, Jian, Bergkvist, Göran, Ulén, Barbro, 2015. Biomass production and phosphorus retention by catch crops on clayey soils in southern and central Sweden. Field Crop. Res. 171, 130–137.

Mantovi, P., Fabbri, C., Soldano, M., Piccinini, S., 2010. Effect of solid/liquid separation on raw and digested slurries. Proceedings of the 14th International Ramiran Conference.

Massé, Daniel, Gilbert, Yan, Savoie, Philippe, Bélanger, Gilles, Parent, Gaétan, Babineau, Daniel, 2010. Methane yield from switchgrass harvested at different stages of development in Eastern Canada. Bioresource Technolog. 101 (24), 9536–9541.

Micolucci, Federico, Gottardo, Marco, Cavinato, Cristina, Pavan, Paolo, Bolzonella, David, 2016. Mesophilic and thermophilic anaerobic digestion of the liquid fraction of pressed biowaste for high energy yields recovery. Waste Manage. 48, 227–235.

Molinuevo-Salces, Beatriz, Larsen, Søren U., Ahring, Birgitte K., Uellendahl, Hinrich, 2013. Biogas production from catch crops: Evaluation of biomass yield and methane potential of catch crops in organic crop rotations. Biomass Bioenerg. 59, 285–292.

Molinuevo-Salces, Beatriz, Larsen, Søren U., Ahring, Birgitte K., Uellendahl, Hinrich, 2015. Biogas production from catch crops: Increased yield by combined harvest of catch crops and straw and preservation by ensiling. Biomass and Bioenerg. 79, 3–11.

Neureiter M, dos Santos JTP, Lopez CP, Pichler H, Kirchmayr R, Braun R. Effect of silage preparation on methane yields from whole crop maize silages. In: Proceedings of the 4th international symposium on anaerobic digestion of solid waste; 2005. p. 109–15. Copenhagen, Denmark.

Noguerol-Arias, Joan, Rodríguez-Abalde, Angela, Romero-Merino, Eva, Flotats, Xavier, 2012. Determination of Chemical Oxygen Demand in Heterogeneous Solid or Semisolid Samples Using a Novel Method Combining Solid Dilutions as a Preparation Step Followed by Optimized Closed Reflux and Colorimetric Measurement. Anal. Chem. 84 (13), 5548–5555.

Pakarinen, Outi, Lehtomäki, Annimari, Rissanen, Sanna, Rintala, Jukka, 2008. Storing energy crops for methane production: Effects of solids content and biological additive. Bioresource Technol. 99 (15), 7074–7082.

Pitk, Peep, Palatsi, Jordi, Kaparaju, Prasad, Fernández, Belén, Vilu, Raivo, 2014. Mesophilic co-digestion of dairy manure and lipid rich solid slaughterhouse wastes: Process efficiency, limitations and floating granules formation. Bioresource Technol. 166, 168–177.

Porter, M.G., Murray, R.S., 2001. The volatility of components of grass silage on oven drying and the inter-relationship between dry-matter content estimated by different analytical methods. Grass Forage Sci. 56 (4), 405–411.

Rico, Carlos, Rico, José Luis, Tejero, Iñaki, Muñoz, Noelia, Gómez, Beatriz, 2011. Anaerobic digestion of the liquid fraction of dairy manure in pilot plant for biogas production: Residual methane yield of digestate. Waste Manage. 31 (9-10), 2167–2173.

Ruiz-Sánchez, J., Guivernau, M., Fernández, B., Vila, J., Viñas, M., Riau, V., Prenafeta-Boldú, F.X., 2019. Functional biodiversity and plasticity of methanogenic biomass from a full-scale mesophilic anaerobic digester treating nitrogen-rich agricultural wastes. Sci. Total Environ. 649, 760–769.

Taxonomy, Soil, Edition, Second, 1999. A Basic System of Soil Classification for Making and Interpreting Soil Surveys. U.S.D.A, Agricultural Handbook, p. 436.

Søndergaard, Marie M., Fotidis, Ioannis A., Kovalovszki, Adam, Angelidaki, Irini, 2015. Anaerobic Co-digestion of Agricultural Byproducts with Manure for Enhanced Biogas Production. Energ. Fuel 29 (12), 8088–8094.

Soto, M., Méndez, R., Lema, J.M., 1993. Methanogenic and non-methanogenic activity tests. Theoretical basis and experimental set up. Water Res. 27 (8), 1361–1376.

Thorup-Kristensen, K., 2001. Are differences in root growth of nitrogen catch crops important for their ability to reduce soil nitrate-N content, and how can this be measured?. Plant Soil 230 (2), 185–195.

Torrellas, Marta, Burgos, Laura, Tey, Laura, Noguerol, Joan, Riau, Victor, Palatsi, Jordi, Antón, Assumpció, Flotats, Xavier, Bonmatí, August, 2018. Different approaches to assess the environmental performance of a cow manure biogas plant. Atmos. Environ. 177, 203–213.

Usman Khan, Muhammad, Kiaer Ahring, Birgitte, 2021. Improving the biogas yield of manure: Effect of pretreatment on anaerobic digestion of the recalcitrant fraction of manure. Bioresource Technol. 321, 124427. https://doi.org/10.1016/j.biortech.2020.124427.

Vervaeren, H., Hostyn, K., Ghekiere, G., Willems, B., 2010. Biological ensilage additives as pretreatment for maize to increase the biogas production. Renew. Energ. 35 (9), 2089–2093.

Villa, Raffaella, Ortega Rodriguez, Lelia, Fenech, Cecilia, Anika, Ogemdi Chinwendu, 2020. Ensiling for anaerobic digestion: A review of key considerations to maximise methane yields. Renew. Sust. Energ. Rev. 134, 110401. https://doi.org/10.1016/j.rser.2020.110401.

Zhang, Cunsheng, Su, Haijia, Baeyens, Jan, Tan, Tianwei, 2014. Reviewing the anaerobic digestion of food waste for biogas production. Renew. Sust. Energ. Rev. 38, 383–392.

Zhao, Xiaoling, Liu, Jinhuan, Liu, Jingjing, Yang, Fuyu, Zhu, Wanbin, Yuan, Xufeng, Hu, Yuegao, Cui, Zongjun, Wang, Xiaofen, 2017. Effect of ensiling and silage additives on biogas production and microbial community dynamics during anaerobic digestion of switchgrass. Bioresource Technol. 241, 349–359.

Zheng, Zehui, Liu, Jinhuan, Yuan, Xufeng, Wang, Xiaofen, Zhu, Wanbin, Yang, Fuyu, Cui, Zongjun, 2015. Effect of dairy manure to switchgrass co-digestion ratio on methane production and the bacterial community in batch anaerobic digestion. Appl. Energ. 151, 249–257.