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1 **Characterization and prediction of organic nitrogen**
2 **biodegradability during anaerobic digestion: a bioaccessibility**
3 **approach.**

4
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12

13 **Abstract**

14 Prediction of organic nitrogen mineralization into ammonium during anaerobic digestion is required
15 for optimizing substitution of mineral fertilizer by digestates. The aim of this study was to understand
16 organic nitrogen biodegradability and to investigate how it can be predicted from carbon
17 biodegradability, and nitrogen bioaccessibility, respectively. Bioaccessibility was assessed using
18 fractionation methods based on sequential extractions. Results showed that organic nitrogen was
19 present in fractions whose bioaccessibility levels differed. Organic nitrogen and carbon
20 biodegradability were also determined and compared. Results highlighted two groups of substrates:
21 the first with an initial $\text{NH}_4^+/\text{TKN} < 30\%$, whose carbon and nitrogen biodegradability are similar; the
22 second with an initial $\text{NH}_4^+/\text{TKN} > 30\%$, whose carbon and nitrogen biodegradability differ
23 significantly. To enable prediction on all substrates, partial least square (PLS) regressions were carried
24 out to link organic nitrogen bioaccessibility indicators to biodegradability. The models successfully
25 predicted organic nitrogen biodegradability with a maximum prediction error of 10%.

26
27 **Keywords** : anaerobic digestion, nitrogen, ammonium, biodegradability, bioaccessibility

28 1. INTRODUCTION

29 Anaerobic digestion is currently attracting considerable interest for the management of agricultural
30 waste. The main objectives are reducing greenhouse gas emissions, procuring additional income for
31 farmers and autonomy regarding nutrients. But the expansion of anaerobic digestion plants creates
32 new challenges concerning the management of the digestate. Indeed, residues from anaerobic
33 digestion are rich in nutrients, particularly in nitrogen and are therefore of agronomic interest for their
34 value as fertilizer and amendment (Alburquerque et al., 2012; Tambone et al., 2009). Digestates from
35 agricultural plants are mostly spread on fields after storage or post-processing (phase separation,
36 composting, etc.) with the aim of replacing mineral fertilizers. Indeed, anaerobic digestion results in
37 the mineralization of part of the organic nitrogen into ammonium (Möller and Müller, 2012), enabling
38 short term uptake of nitrogen by crops combined with long term mineralization of slowly
39 biodegradable organic compounds (Fouda et al., 2013) in the soil. However, the digestate management
40 chain is subject to nitrogen leakages in the form of ammonia emissions during storage (Sommer, 1997)
41 and after spreading on the land, or through nitrate leaching (Möller, 2015). In addition to their
42 environmental impacts, nitrogen losses after digestion reduce the fertilizing value of the digestates and
43 negatively impact the goal of replacing mineral fertilizers. Hence, to mitigate environmental impacts
44 and to optimize nitrogen uptake by crops, the anaerobic digestion process has to be designed taking
45 the characteristics of the digestate into account, i.e. ammonia content and organic nitrogen content,
46 together with short or long-term mineralization kinetics in soils. To this end, a detailed understanding
47 of the nitrogen composition of digestates and anaerobic biodegradability of the organic nitrogen in the
48 substrates is required.

49 Nitrogen is present in substrates in two main forms: mineralized (e.g. ammonium, nitrates, etc.) and
50 organic. Anaerobic digestion results in the degradation of part of the organic nitrogen into ammonium,
51 a reduced form (González-Fernández and García-Encina, 2009). In the substrates, the organic nitrogen
52 is mainly contained in proteins and requires hydrolysis to be converted into monomers. In most cases,
53 the amino acids that result from this transformation are then metabolized through acidogenesis to
54 produce volatile fatty acids and ammonium via Stickland or single amino acid fermentation (Ramsay
55 and Pullammanappallil, 2001). However, some authors studied organic nitrogen biodegradation and

56 showed that degradation of the organic nitrogen was not complete during anaerobic digestion, but
57 rather ranged from 18% to 77% depending on the substrate and the hydraulic retention time (HRT), as
58 reported in the different studies (Kobayashi et al., 2012, 2014; Massé et al., 2007; Yang et al., 2015).
59 These results highlight the fact that protein biodegradation is strongly constrained by the
60 bioaccessibility of organic nitrogen.

61 Accessible organic matter refers to the compounds in the substrate that microorganisms can access for
62 intra or extra-cellular degradation. A number of bioaccessibility indicators have been developed using
63 chemical extraction or particle size distribution (Dulekgurgen et al., 2006; Karahan et al., 2008) to
64 fractionate organic matter according to its degree of bioaccessibility. Better characterization of the
65 bioaccessibility of organic nitrogen in substrates will enable a better understanding of the variability of
66 its anaerobic biodegradability. Methods using successive chemical extractions such as the Van Soest
67 method, or more advanced methods can be used for this purpose.

68 The Van Soest fractionation method (Van Soest, 1963) is widely used to fractionate wastes including
69 vegetables, sludges, and plants. It uses different solvents to extract soluble and lignocellulosic
70 compounds. The acidity of the solvents is increased at each step by using hot water, neutral detergent,
71 acid detergent and finally strong acid extraction. The different extraction steps target soluble, protein-
72 like, hemicellulose-like and cellulose-like compounds, respectively. The residual organic matter is
73 considered as lignin-like compounds. Many studies have explored the correlations between the Van
74 Soest composition of organic matter and its biodegradability (Gunaseelan, 2007; Liu et al., 2015;
75 Mottet et al., 2010). Gunaseelan (2007) demonstrated an inverse relationship between the
76 lignin+cellulose-like content and biodegradability of fruit and vegetables in anaerobic conditions.
77 Despite these promising results, Mottet et al., (2010) pointed out that this fractionation method is too
78 aggressive to be representative of biological disintegration that occurs during anaerobic digestion,
79 which limits its use for the prediction of biodegradability. Jimenez et al., (2015) then developed a less
80 aggressive fractionation method based on the protocol of Muller et al., (2014) that targeted
81 extracellular polymeric substances. This protocol, named EPS protocol, uses increasing alkaline
82 solutions, and targets protein-like substances; it ends with strong acid extraction targeting
83 holocellulosic-like products. This fractionation, combined with 3D-fluorescence to assess the

84 complexity of each fraction, successfully predicted the bio-methanogenic potential (BMP) and carbon
85 biodegradability of various substrates using partial least square (PLS) regressions (Jimenez et al.,
86 2017). Hence, EPS fractionation combined with PLS regression enables the fast determination of the
87 BMP value and carbon biodegradability by decreasing the time for analysis from 40-50 days (for a
88 BMP) to 3 days (for EPS fractionation). However, all these studies focused on the biodegradability of
89 volatile solids or carbon compounds. Consequently, no data are available concerning the ability of
90 these accessibility indicators, determined from EPS or Van Soest fractionation, to predict organic
91 nitrogen biodegradability during anaerobic digestion.

92 The aim of this study was thus (i) to investigate the biodegradability of organic nitrogen in
93 mesophilic anaerobic codigestion and (ii) to develop predictive models using carbon biodegradability
94 and organic nitrogen bioaccessibility as variables, respectively. To this end, we first investigated the
95 bioaccessibility of organic nitrogen on various substrates and compared it with carbon bioaccessibility
96 using two fractionation methods. Next we compared the anaerobic biodegradability of nitrogen and
97 carbon on the same panel of substrates. Finally, we performed PLS regressions to identify possible
98 correlations between organic nitrogen biodegradability and bioaccessibility indicators provided by the
99 two fractionation methods to determine which fractionation method better describes organic nitrogen
100 biodegradability.

101

102 2. MATERIAL AND METHODS

103 2.1. Substrates

104 Twenty different substrates were collected in France as representative of the diversity of organic
105 wastes used in agricultural anaerobic digestion plants. They included livestock wastes, wastes from
106 agri-industry and municipalities and agriculture residues. Table 1 lists the substrates sampled and their
107 characteristics. Substrate panel was selected to cover a large range of protein content and C/N ratio.
108 Hence, protein content in this panel ranges from 12 to 67%org-C and C/N ratio from 5.7 to 36.
109 All the samples were ground in a knife mill (R15 V.V., Robotcoupe®) and stored at -20 °C. Samples
110 for elemental analysis were ground in a ball mill (Dangoumill®, PROLABO®).

111

112 2.2. Physico-chemical characterization

113 Total solids (TS), volatile solids (VS), total Kjeldahl nitrogen (TKN) and total ammonia nitrogen were
114 measured using standard methods (APHA, 2005). TKN and ammonia concentrations were determined
115 by distillation (TKN after mineralization). Total carbon and total nitrogen were measured in finely
116 ground raw samples by elemental analysis according to the manufacturer's instructions (ThermoFlash
117 2000, ThermoScientific®). Inorganic carbon in the raw samples was determined by calcination at 480
118 °C and elemental analysis of the ashes. Total organic carbon (TOC) was determined by subtracting
119 inorganic carbon (IC) from total carbon (TC). Protein content in organic carbon was calculated using
120 the following the equation (1):

$$121 \quad (1) \text{ Protein} = ((\text{TKN} - \text{NH}_4^+) * \text{C}/\text{N}_{\text{protein}}) / \text{TOC}$$

122 Where:

- 123 - Protein: protein content (%_{org-C}).
- 124 - C/N_{protein}: carbon/nitrogen ratio equal to 4.348 gC.gN⁻¹ based on Ramsay and
125 Pullammanappallil (2001).

126

127 2.3. Organic matter fractionation

128 The fractionation methods used in this study to characterize organic matter were based on sequential
129 chemical extractions. The successive steps of the two fractionation methods, derived from EPS
130 (Jimenez et al., 2015) and Van Soest (Van Soest, 1963), are described below. Four fractions were
131 extracted with each method and presented similarities in terms of molecules targeted by the extracting
132 solvents as presented in table 2, which made it possible to compare the two methods. Uncertainty on
133 fractionation results was estimated for each extraction method using a Monte Carlo method in order to
134 take error propagation into account.

135

136 2.3.1. Modified Van Soest fractionation

137 The modified Van Soest fractionation is based on successive extractions with neutral and acid
138 detergents and ending with a strong acid extraction. Organic matter was separated into four fractions:

- 139 • SOL_VS: This fraction is extracted in water at a temperature of 20 °C for 1 h. The extraction
140 temperature is different from that described in the extraction protocol for the French standard
141 XPU 44-162 (AFNOR) where extraction is performed at 100 °C.
- 142 • PROT_VS : This fraction is extracted with boiling commercial neutral detergent (NDF: 1.86%
143 disodium ethylene diamine tetraacetate, 3% aqueous solution of sodium dodecyl sulfate,
144 0.68% decahydrate sodium tetraborate, 0.45% disodium hydrogen phosphate and 1.0%
145 triethylene glycol; Carlo Erba®) for 1 h from the pellet obtained after the SOL_VS step.
- 146 • HOLO_VS: This fraction results from the two successive extractions of the Van Soest
147 protocol: (1) extraction with boiling commercial acid detergent ADF (2.6% sulfuric acid and
148 2.0% cetrimonium bromide; Carlo Erba®) for 1 h, followed by (2) extraction with 72%
149 sulfuric acid (w/w) at ambient temperature for 3 h. This step used the pellet remaining from
150 the PROT_VS step.
- 151 • LIGN_VS: This fraction contained the remaining materials not extracted during the previous
152 extractions.

153 After the first extraction, the mix was centrifuged at 12100 g for 20 minutes. The supernatant
154 containing the soluble fraction of organic matter was removed and the remaining pellet was then dried
155 at 40°C and then ground to 1 mm. Detergent and strong acid extraction (PROT_VS and HOLO_VS
156 steps) were performed on 1 gram of the dried-ground sample sealed in filter bags (F57 Filter Bags,
157 Ankom Technologies®). Detergent and acid extractions were performed using an automated fiber
158 analyzer (Ankom 2000, Ankom Technologies®). After each extraction step, the content of one bag
159 was used to analyze total organic carbon and total remaining nitrogen using an elemental analyzer
160 (Thermoflash 2000, Thermoscientific®). The organic carbon and nitrogen content in each fraction
161 were determined by the difference between the organic carbon and nitrogen content in the filter bags
162 before and after each extraction.

163

164 2.3.2. Modified “EPS” fractionation

165 EPS fractionation (Jimenez et al., 2015) is based on successive alkaline extractions ending with strong
166 acid extraction. This fractionation method is similar to the Van Soest method including the use of

167 strong acid extraction at the end. Sequential extractions were performed on raw samples (0.5 gram of
168 dry matter) using the method described by Jimenez et al. (2017). This method separates the organic
169 matter into four fractions:

- 170 • SOL_EPS: The first step of the protocol consists of two successive extractions of the sample
171 with 30 mL of a saline solution of CaCl₂ 10mM at 30 °C under stirring (300 rpm) for one
172 hour.
- 173 • PROT_EPS: The pellet remaining from the first step is washed four times with 30 mL of a
174 basic solution of NaOH 10mM + NaCl 10mM at 30 °C and under stirring (300 rpm) for 15
175 minutes. The remaining pellet is washed four times with 30 mL of a strong basic solution of
176 NaOH 0.1M at 30 °C and under stirring (300 rpm), for one hour.
- 177 • HOLO_EPS: The pellet remaining from the previous step is washed three times with 25 ml of
178 a strong acid solution of H₂SO₄ 72% (w/w) at 30 °C under stirring at 300 rpm for three hours.
179 This step is equivalent to the final extractions carried out in Van Soest protocol used to extract
180 holocellulose materials,
- 181 • LIGN_EPS: This fraction contains the most recalcitrant material and is calculated by
182 subtracting the total mass extracted from the original mass of the sample.

183 The organic carbon and total nitrogen content in each fraction (except LIGN_EPS fraction) were
184 measured in the extracts using a TOC-L analyzer (Shimadzu®) and by carbon and nitrogen balance for
185 LIGN_EPS fraction between the raw sample and the sum of the extractible fractions. All extractions
186 and measurements on extracts were performed in triplicate.

187

188 **2.4. Organic carbon and nitrogen biodegradability**

189 Anaerobic biodegradability of organic carbon and nitrogen were analyzed on each substrate studied.
190 The substrate was prepared in the same way as the one used before the extraction protocol (described
191 in section 2.1). The inoculum used for both experiments was obtained from a continuous mesophilic
192 anaerobic digester that is used to process pig slurry and horse feed. Carbon biodegradability was
193 assessed in processing biochemical methanogenic potential (BMP) experiments according to Vedrenne
194 et al., (2008). These batch biodegradability experiments were performed in triplicate with an

195 inoculum:substrate ratio of 1 gVS.gVS⁻¹ and were incubated at 38 °C for 40 days. TC and biogas
 196 production were used to determine carbon mass balance continuity during the monitoring. Whatever
 197 the test, mass balance error is between -8 and 10%.

198 ANMP (Apparent nitrogen mineralized potential) tests were carried out to determine the maximum
 199 apparent organic nitrogen mineralized during anaerobic digestion. This method allows the
 200 determination of the organic nitrogen part of the substrate converted into soluble ammonium which is
 201 the result of organic nitrogen mineralization from substrate, biomass growth and biomass decay. The
 202 experimental conditions were similar to the BMP experiments and the ammonium concentration was
 203 monitored during the test. However, the inoculum used for ANMP tests was previously centrifuged at
 204 12100 g for 20 minutes at ambient temperature and the supernatant was removed in order to reduce the
 205 initial ammonium concentration in the batch reactors. The remaining pellet was suspended in a
 206 KHCO₃ 10 g.L⁻¹ + NaHCO₃ 10 g.L⁻¹ solution to preserve sufficient buffering capacity in the batch and
 207 to prevent inhibitions due to acidification during incubation. A blank experiment was performed
 208 without the addition of substrate to assess the apparent organic nitrogen mineralization due to the
 209 inoculum. The AMNP of each substrate was determined by difference between apparent
 210 mineralization in the batch experiment with a substrate and the control experiment, as described by
 211 equation (2).

$$212 \quad (2) \quad AMNP = \frac{NH_4^+_{-substrate} - NH_4^+_{-blank}}{TS_{substrate}} - NH_4^+_{-Initial \text{ substrate}}$$

213 Where:

214 - AMNP : Apparent Nitrogen Mineralized Potential of the substrate (gN-NH₄⁺.kgTS_{substrate}⁻¹)

215 - NH₄⁺_{-substrate}: total mass of ammonium into the batch experiment with substrate after
 216 incubation (gN-NH₄⁺)

217 - NH₄⁺_{-blank}: total mass of ammonium into the blank batch experiment without substrate after
 218 incubation (gN-NH₄⁺)

219 - MV_{substrate-added}: total mass of the volatile matter of the substrate added to the batch
 220 experiment (kgTS_{substrate})

221 - NH_4^+ -Initial substrate : Ammonium concentration in the substrate before incubation (gN-
222 NH_4^+ .kgTS_{substrate}⁻¹).

223 Ammonium and TKN contents were measured before and after each incubation test using the
224 distillation method to check nitrogen mass balance. The ammonium distillation method is aggressive
225 enough to solubilize ammonium precipitates such as struvite and these ammonium forms are included
226 in total ammonium. Hence, ANMP value considers organic nitrogen mineralization into ammonium
227 which can be soluble or precipitated in the digestate. Whatever the test, difference between initial and
228 final total nitrogen content is between -4% and 5%.

229 ANMP is an apparent mineralization potential which considers organic nitrogen from substrate
230 mineralization and ammonium immobilization for biomass growth. To quantify the impact of
231 ammonium immobilization for biomass growth on ANMP value, a control batch experiment was
232 performed following the ANMP protocol with a volatile fatty acid solution as substrate. This
233 experiment highlights an immobilization rate of $0.006 \text{ gN-NH}_4^+ \text{ immobilized} \cdot \text{gC}_{\text{degraded}}^{-1}$. This value is lower
234 than the references found in the literature that range from 0.017 to $0.009 \text{ gN-NH}_4^+ \text{ immobilized} \cdot \text{gC}_{\text{degraded}}^{-1}$
235 (Batstone et al., 2002; Ramsay and Pullammanappallil, 2001). However, these constants only consider
236 growth, while in this study, the constant is the result of both growth and partial decay during 40 days.
237 This constant was applied to the panel of substrate. Hence, $6 \pm 3\%$ of the TKN brought by the substrate
238 can be immobilized for biomass growth after 40 days of incubation.

239 All the biodegradability tests were performed in triplicate to determine uncertainties due to the
240 operator and the measuring equipment.

241

242 **2.5. Statistical analysis**

243 In order to sort substrates according to their biodegradation rate and investigate the relationship
244 between organic nitrogen biodegradability and organic nitrogen bioaccessibility, hierarchical cluster
245 analysis (HCA) and partial least square (PLS) regressions were performed on the data set using
246 Xlstat® for Excel®. Substrates were sorted depending on their organic carbon and organic nitrogen
247 biodegradability by using HCA.

248 Models based on fractionation methods were developed to describe and predict organic nitrogen
249 biodegradability using PLS regression. PLS regression is an extended multiple linear regression that
250 include principal component analysis principles in the regression structure. This technique build model
251 to link one dependent variable to different explanatory variables by minimizing their covariance. Here,
252 the dependent variable (data to predict) was organic nitrogen biodegradability ($\text{gN-NH}_4^+ \cdot \text{kgTS}^{-1}$) and
253 the explanatory variables (predictor variables) were the organic nitrogen content in each fraction for,
254 in one case modified Van Soest fractionation (expressed in $\text{gN} \cdot \text{kgTS}^{-1}$), and in another case, modified
255 EPS fractionation (expressed in $\text{gN} \cdot \text{kgTS}^{-1}$). In this study, model robustness was investigated using the
256 following quality parameters:

- 257 • The coefficient of determination R^2 , which is the proportion of the dependent variable
258 variance that can be explained by the explanatory variable set and varies between 0 and 1.
259 Based on previous studies using PLS for biodegradability prediction (Jimenez et al., 2017), a
260 coefficient of determination superior to 0.8 is required to ensure a good fitting of the
261 experimental data.
- 262 • The cross validated coefficient of determination Q^2 , which is the variation rate of the
263 dependent variable predicted by the model by cross validation. Q^2 makes it possible to judge
264 the quality of the fit between the predicted and measured output variables and the ability of the
265 model to predict new data. This coefficient varies between $-\infty$ and 1. A Q^2 value above 0.5
266 indicates good predictability of the model (Jimenez et al., 2014). Cross-validation was done
267 using the jackknife method by excluding 1 out of 4 values in the data set chosen at random.
268 External model validation was done by applying the models to another set of data covering a wide
269 range of biodegradability and substrate origin. The predicted organic nitrogen biodegradability was
270 compared with the measured values.

271 3. RESULTS AND DISCUSSION

272 **3.1. Organic carbon and nitrogen distribution in bioaccessibility based fractionation.**

273 Modified Van Soest and modified EPS fractionation were compared on 20 substrates representing the
274 diversity of feedstock in agricultural anaerobic digestion plants. Figure 1 shows the results of Van
275 Soest and EPS fractionations for carbon content (1a) and organic nitrogen content (1b).

276

277 *3.1.1. Fractionation using the modified Van Soest method*

278 Figure 1a shows that the distribution of organic carbon in modified Van Soest fractions differed
279 considerably from one substrate to another, with SOL_VS ranging from 13%_{TOC} to 48%_{TOC},
280 PROT_VS from 5%_{TOC} to 37%_{TOC} and HOLO_VS from 9%_{TOC} to 66%_{TOC}. Only LIGN_VS showed
281 limited distribution, ranging from 1%_{TOC} to 11%_{TOC}. The wide range of values shows that the carbon
282 distribution obtained with this fractionation method is strongly substrate dependent. In figure 1b,
283 organic nitrogen distribution shows that nitrogen was present in all fractions and, like carbon, is
284 strongly substrate dependent. Indeed, the ranges of nitrogen contents were wide in each fraction. On
285 average, only about 30%_{org-N} was contained in the NPROT_VS fraction. The remaining organic
286 nitrogen was mainly split between the NSOL_VS (35%_{org-N} on average) and NHOLO_VS fractions
287 (30%_{org-N} on average). The range of nitrogen contents was wide in all fractions except NLIGN_VS.
288 Only a small proportion of organic nitrogen (between 1 and 15%_{org-N}) was contained in LIGN_VS.
289 Other authors have reported the presence of nitrogen in all the fractions, even in those that are hard to
290 extract like HOLO_VS and LIGN_VS, using the Van Soest method for organic matter characterization
291 before composting (Denes et al., 2015) or soil mineralization of organic waste (Chalhoub et al., 2013;
292 Garnier et al., 2003).

293

294 *3.1.2. Fractionation using the modified EPS method*

295 Figures 1a and 1b show that variability of the carbon and nitrogen content of all EPS fractions was
296 also wide. Extractable fractions SOL_EPS, PROT_EPS and HOLO_EPS ranged from 2 to 31%_{TOC}, 3
297 to 20%_{TOC} and 10 to 45%_{TOC}, respectively, for organic carbon. Non-extractable LIGN_EPS ranged
298 from 29 to 59%_{TOC}. The distribution of organic nitrogen ranged from 2 to 36%_{org-N} for NSOL_EPS, 3

299 to 41%_{org-N} for NPROT_EPS, 3 to 29%_{org-N} for NHOLO_EPS and from 17 to 67%_{org-N} for
300 NLIGN_EPS. The wide range of variability obtained shows that the carbon distribution in the EPS
301 fractions is strongly substrate dependent. In addition, organic carbon and nitrogen are largely present
302 in the non-extractible fraction LIGN_EPS and NLIGN_EPS reflecting that the extraction method uses
303 soft extractants to target organic compounds. These soft extractants are supposed to solubilize specific
304 molecules in organic matter, but organic nitrogen, which is mainly present in protein, was also
305 detected in HOLO_EPS and a large proportion in the LIGN_EPS fraction. According to Jimenez et al.
306 (2015), HOLO_EPS and LIGN_EPS are mainly composed of lignocellulosic materials and other
307 materials entrapped by them. Lignocellulosic materials are hardly biodegradable and biodegradable
308 molecules which are entrapped by them are hardly biodegradable as well (Monlau et al., 2012). Thus,
309 the organic nitrogen contained in HOLO_EPS and LIGN_EPS fractions is the result of the organic
310 nitrogen entrapment by lignocellulosic materials that are not disintegrated by alkali solvents.
311 Consequently, this fractionation method not only takes into account the nature of the extracted
312 molecules, but also their bioaccessibility due to physical structure.

313

314 3.1.3. *Comparison of carbon and nitrogen distribution obtained with the modified Van Soest* 315 *and modified EPS fractionation methods*

316 The distributions of organic carbon and nitrogen obtained with modified EPS and modified Van Soest
317 fractionation methods differed significantly. Indeed, the percentage of organic carbon contained in
318 extractible fractions was higher using modified Van Soest fractionation than the modified EPS
319 fractionation: HOLO_VS was 36%_{TOC} higher than HOLO_EPS and PROT_VS was 66%_{TOC} higher
320 than PROT_EPS. In contrast, the non-accessible fraction was noticeably higher with the modified EPS
321 method than with the modified Van Soest method. The LIGN_EPS fraction reached 41%_{TOC} on
322 average while LIGN_VS represented only 6%_{TOC}. The same kinds of results were obtained for organic
323 nitrogen fractionation even if the trend of distribution between extractible fractions differed
324 significantly. These observations show that a significant part of the organic carbon and nitrogen
325 extracted with the Van Soest method solvents was not extracted by EPS solvents but remained in the
326 non-extractible fraction LIGN_EPS.

327 To explain these results, the composition of solvents used for each fractionation method has to be
328 considered. In particular, as stated by Jimenez et al. (2015) and Muller et al. (2014), EPS fractionation
329 aims to describe the bioaccessibility of organic matter considering the impact of its chemical
330 composition and physical structure. However, Van Soest fractionation only considers the impact of
331 chemical composition on bioaccessibility (the ability of exoenzymes to reach active sites on organic
332 compounds considering its chemical complexity but with no limitation caused by the physical
333 structure of the organic matter). Indeed, as observed by Jimenez et al. (2015), Van Soest solvents are
334 more aggressive than EPS solvents. In particular, the NDF solvent used for the second step of the Van
335 Soest fractionation is a detergent and results in strong disintegration of complex structures composed
336 by lignin and holocellulose. Thus, this extraction step allows the extraction of physically inaccessible
337 organic matter at this and subsequent steps. As a consequence, few carbon and nitrogenous
338 compounds were present in LIGN_VS. On the other hand, the alkaline solvents used for EPS
339 fractionation did not disintegrate the organic matter and extracted only the physically and chemically
340 accessible compounds. These results indicate that, on average, 38%_{org-N} of the organic nitrogen and
341 35%_{TOC} of the organic carbon (estimated by difference between non-extractible fractions obtained for
342 EPS and Van Soest fractionation respectively) are contained inside complex physical structures
343 containing lignocellulose compounds which strongly limits its bioaccessibility.

344

345 **3.2. Organic carbon and nitrogen biodegradability under anaerobic conditions**

346 *3.2.1. Influence of substrate origin on biodegradability*

347 Organic nitrogen biodegradability and carbon biodegradability were determined using the AMNP test
348 and the BMP test. The results are presented in table 3.

349 Organic nitrogen biodegradability is strongly substrate dependent, ranging from 14%_{org-N} of
350 degradation to 82%_{org-N} in the substrate panel, with an average value of 48%_{org-N}. In comparison,
351 organic carbon biodegradability varied less, from 36 to 74%_{TOC}, with a mean value of 54%.

352 Biodegradability of organic nitrogen and carbon was shown to be partial, which is in accordance with
353 the results previously obtained with the EPS fractionation method, which showed that a significant

354 part of the organic nitrogen and carbon is not bioaccessible. Indeed, the EPS method showed that, on
355 average, 41% TOC and 43% org-N cannot be extracted.

356 To further investigate the variability of organic nitrogen and carbon biodegradability, substrate
357 categories can be defined using a statistical method. To this end, three indicators were used that
358 include the biodegradation rate for carbon and nitrogen, respectively, along with the NH_4^+/TKN ratio
359 in the raw substrate. Hierarchical cluster analysis was performed on these variables for all substrates.
360 The dendrogram (fig. 2a) highlights two groups of substrates based on the variables.

361

362 3.2.2. Comparison between organic carbon biodegradability and organic nitrogen 363 biodegradability.

364 As highlighted in figure 3a, the first group G1 is mainly composed of substrates that present a slight
365 difference between organic carbon biodegradation and organic nitrogen biodegradation with high
366 biodegradability (57% on average for organic carbon and nitrogen). Figure 2b shows that G1 is also
367 composed by substrates with a low NH_4^+/TKN ratio ($< 30\%$) and includes all crop residues, non-stored
368 agro-industrial wastes and one silage sample out of three. As the biodegradability values for organic
369 carbon and nitrogen are very close for these kinds of substrates, which contain less than 30% of
370 mineralized organic nitrogen (fig.3a), predicting organic nitrogen biodegradation from organic carbon
371 mineralization is possible with a mean absolute error of 12%. Indeed, some exceptions remain, like
372 supermarket vegetable wastes as well as drain screenings from slaughterhouses with a percentage
373 difference 39% and 37%, respectively between organic carbon and nitrogen biodegradability.

374 As shown in figures 2a, 2b and 3b, the second group, G2, is the opposite and is composed of substrates
375 with a marked difference in the biodegradability of organic carbon and nitrogen, and with a high
376 NH_4^+/TKN ratio ($> 30\%$). Livestock manure is the most representative of this group but silage and
377 stored agro-industrial waste show the same behavior. The biodegradation levels of these substrates
378 containing large amounts of nitrogen in mineralized form are lower than for G1, with average carbon
379 and nitrogen biodegradability of 43% and 30%, respectively. In addition, the biodegradability of
380 organic carbon and nitrogen are not correlated in this type of substrate, making it impossible to
381 accurately predict organic nitrogen biodegradability from the value obtained for organic carbon.

382 The NH_4^+ /TKN ratio indicates if organic nitrogen mineralization occurs before anaerobic digestion or
383 not. As highlighted by the results, these transformations can occur during long storage (supermarket
384 vegetable waste), animal digestion (livestock manures) or silage fermentation process (crop silage
385 samples). During these processes, carbon and nitrogen mineralization can differ. As an example,
386 during crop silaging, fermentation produces organic acids. These organic acids such as lactic acid lead
387 to a pH decrease and an inhibition of methanogenic activity (Palmisano and Barlaz, 1996). Hence,
388 transformations of organic carbon (partial hydrolysis and acidogenesis) occur but without significantly
389 reaching mineralization and most of the BMP is preserved. However, as highlighted by Teixeira
390 Franco et al. (2016), the proteolytic activity can continue and a large amount of ammonia can be
391 produced indicating a significant organic nitrogen mineralization. This process can significantly
392 impact AMNP value. Hence, the impact of silage fermentation process on BMP and ANMP values can
393 significantly differ. Even if processes differ, animal digestion and spontaneous transformations during
394 storage can have a differential impact on BMP and ANMP values. Therefore, the prediction of organic
395 nitrogen biodegradability from the value obtained for organic carbon (derived from BMP values) is
396 not relevant for most of the substrates belonging to the second group G2. Hence, depending on
397 transformations that can occur before anaerobic digestion, a substrate can move from G1 (fresh
398 substrates) to G2. In the results, this phenomenon is observed for oat that belongs to G1 after harvest
399 and to G2 after silage fermentation process.

400 The links between substrate typology and substrate characteristics (protein content, C/N ratio, organic
401 nitrogen fractionation following modified Van Soest and EPS methods) were investigated. C/N ratio,
402 protein content and organic nitrogen fractionation following modified Van Soest method are not
403 significantly different for G1 and G2 (data not shown). However, as presented in figure 4, significant
404 differences are observed on EPS fractionation obtained for G1 and G2. Indeed, carbon fractionation
405 results are close for both groups (fig.4a), but, organic nitrogen fractionation shows significant
406 differences between substrates belonging to G1 and G2, respectively (fig.4b). Hence, NSOL_EPS
407 fraction for G2 (7%_{org-N} in average) is significantly inferior to the average value obtained for G1
408 (27%_{org-N}). In addition, the relative part of NLIGN_EPS for G2 (51%_{org-N} in average) is higher than
409 for G1 (39%_{org-N} in average). Differences between G1 and G2 are less significant for NPROT_EPS and

410 NHOLO_EPS fractions. These results highlight that for substrates belonging to G2, transformations
411 prior to anaerobic digestion (silage fermentation, storage or animal digestion) led to the mineralization
412 of most of the bioaccessible organic nitrogen (NSOL_EPS fraction). Resulting substrates contain more
413 hardly bioaccessible organic nitrogen (NLIGN_EPS fraction). These changes in bioaccessibility of
414 organic nitrogen explain why average ANMP for G2 is significantly inferior to the average value
415 obtained for G1. In addition, results highlight that the impact of transformations taking place before
416 anaerobic digestion (silage fermentation, storage or animal digestion) impact differently organic
417 carbon and nitrogen bioaccessibility, which can explain why prediction of organic nitrogen
418 biodegradability from the value obtained for carbon is impossible for G2. These results highlight that
419 the modified EPS fractionation allowed the determination of accessibility indicators which can explain
420 biodegradability variations for organic nitrogen. Thus, using a fractionation method and especially
421 modified EPS fractionation could help predict organic nitrogen biodegradability.

422

423 **3.3. Prediction of organic nitrogen biodegradability from bioaccessibility indicators**

424 *3.3.1. Partial least squares (PLS) regression for the prediction of organic nitrogen* 425 *biodegradability*

426 PLS analysis was first carried out on the whole set of substrates. Secondly, the same analysis was
427 performed on previously determined substrate groups (G1 and G2, see section 3.2), respectively.

428

429 *3.3.1.1. Development of the statistical model considering the whole substrate set*

430 Two PLS analysis were carried out to investigate correlations between organic nitrogen
431 biodegradability and the bioaccessibility indicators obtained with the modified Van Soest and
432 modified EPS fractionations, respectively, considering the whole set of substrates. Two statistical
433 models were obtained (see Table 4): Model N_{EPS} , which considers modified EPS fractions of organic
434 nitrogen as explanatory variables, and model N_{VS} , which considers modified Van Soest ones as
435 explanatory variables. Both models show good model structure according to the cross validated
436 coefficient of determination Q^2 ($Q^2 > 0.5$) with $Q^2(N_{EPS}) = 0.736$ and $Q^2(N_{VS}) = 0.571$. However, the
437 proportion of the organic nitrogen biodegradability variance that can be explained by the

438 bioaccessibility indicators is higher with the N_{EPS} model ($R^2 = 0.789$) than with the N_{VS} model ($R^2 =$
439 0.640). The N_{EPS} model using modified EPS fractionation thus enables better simulation of the
440 variation in nitrogen biodegradability, but R^2 is inferior of 0.8 so taking into account the typology
441 determined in section 3.2. could improve the predictability of the models.

442

443 *3.3.1.2. Statistical models for the prediction of organic nitrogen biodegradability considering the*
444 *set of substrates and associated quality parameters considering substrate group G1 and*
445 *G2*

446 As mentioned in section 3.2., the substrates can be divided into two groups, G1 (ratio of $NH_4^+/NTK <$
447 30%) and G2 (ratio of $NH_4^+/NTK > 30\%$) whose organic nitrogen mineralization patterns differ. PLS
448 analyses were thus performed to take them into account. Models considering modified EPS fractions
449 as explanatory variables are N_{EPS_G1} for G1 and N_{EPS_G2} for G2 and are detailed in Table 5. Quality
450 parameters shows that the models considering the two typologies are better than for the model N_{EPS}
451 ($Q^2 = 0.736$ and $R^2 = 0.789$). Hence, model N_{EPS_G1} describing the biodegradability of the organic
452 nitrogen for G1 shows $Q^2 = 0.802$ and $R^2 = 0.843$. Model N_{EPS_G2} which considers substrates from G2
453 shows $Q^2 = 0.771$ and $R^2 = 0.943$. Considering models found in literature developed using PLS
454 regression, N_{EPS_G1} and N_{EPS_G2} models have satisfying R^2 values (0.818 for Jimenez et al. (2017), 0.88
455 for Monlau et al. (2012) and 0.925 for Wold et al. (2001)). These results reveal that substrate typology
456 enables better accuracy and predictability with models using EPS fractionation as explanatory
457 variables.

458 Models considering modified Van Soest fractions as explanatory variables (N_{VS_G1} for G1 and N_{VS_G2}
459 for G2, see Table 5) present no real improvement of the R^2 with 0.668 and 0.455 for respectively
460 N_{VS_G1} and N_{VS_G2} . These results showed that the variance of organic nitrogen biodegradability is not
461 well described considering the typology of substrate by models using modified Van Soest variables.
462 Also, the cross-validation parameter Q^2 obtained for N_{VS_G1} (0.551) and for N_{VS_G2} (0.057) were below
463 the value obtained for model N_{VS} (0.571) which took the whole set of substrates into account. These
464 results indicate that the substrate typology does not improve the robustness of statistical models
465 considering Van Soest variables. Consequently, prediction of organic nitrogen biodegradability with

466 models using the modified Van Soest fractionation as explanatory variables is not accurate. This result
467 is due to the lack of information about the physical structure of substrates even though this data
468 strongly impacts bioaccessibility. This statement is mainly due to the use of detergents during
469 extractions that lead to the complete disintegration of the physical structure of the organic matter as
470 stated by Mottet et al. (2010).

471

472 3.3.1.3. External model validation on models considering modified EPS variables

473 For external model validation, the ability of each model developed to predict organic nitrogen
474 biodegradability of four substrates not used for model development was investigated. Predicted
475 organic nitrogen biodegradability and prediction errors were only determined for models that showed
476 satisfying robustness in the previous sections (N_{EPS} , N_{EPS_G1} , N_{EPS_G2}). Results are presented in Table 6.
477 Regarding the prediction of organic nitrogen biodegradability by models using modified EPS fractions
478 as explanatory variables, the prediction errors of the N_{EPS} model that considers the whole set of
479 substrates, range from 5% to 37%. These results are better than those obtained with models using
480 modified Van Soest fractions as explanatory variables but nevertheless underline the poor
481 predictability of the N_{EPS} model. However, models that were specifically designed for each group of
482 substrates, G1 and G2, produced much better results. The N_{EPS_G1} and N_{EPS_G2} models that predict
483 organic nitrogen biodegradability for substrates belonging to the groupe G1 and G2 respectively,
484 produced very accurate predictions with a maximum prediction error of 2% and 10%, respectively.
485 Hence, to accurately estimate organic nitrogen biodegradability, models have to consider modified
486 EPS fractions as explanatory variables and G1 and G2 as validity domains.

487 The EPS fractionation method has been used to develop accurate models for BMP prediction on
488 various substrates (Jimenez et al., 2017). These models were completed with complexity variables
489 acquired with 3D fluorescence spectrometry and reach a R^2 of 0.818 and a $Q^2 = 0.689$. The
490 performance of the models developed in this study were similar without considering complexity,
491 which can be explained by sampling before extraction and the type of substrate considered. Indeed, the
492 preparation of the sample by Jimenez et al. (2017) (freeze-drying + grinding to 1 mm) modifies the
493 physical structure and complexity, but using 3D fluorescence spectrometry makes it possible to track

494 the recalcitrant materials released in the different fractions. The samples in the present study were used
495 raw with only light pretreatment as described in section 2.3.1 which saved the structure of organic
496 matter, and only bioaccessible materials were released during the different extraction steps. These
497 results highlight the fact that EPS fractionation allows the determination of bioaccessibility indicators
498 that take the impact of physical structure of the substrate organic matter into account in its
499 biodegradability.

500

501 *3.3.1.4. Analysis of the impact of bioaccessibility indicators on nitrogen biodegradability*

502 The normalized coefficient of each variable of the model can be determined from the correlation
503 between the explanatory variable and the dependant variable, hence, based on these coefficients, the
504 contribution of each fraction to biodegradability was investigated. As model N_{VS} was not validated in
505 the previous section, only normalized coefficients associated with models N_{EPS_G1} and for N_{EPS_G2} are
506 considered in this section and are presented in Figure 5. Errors bars associated to the normalized
507 coefficients in figure 5 are from uncertainty estimation considering 95 % confidence interval. The
508 impact of each fraction is considered significant if the obtained confidence interval does not contain 0.
509 For both models, the fractions that had the most significant impact on nitrogen biodegradability were
510 NSOL_EPS and NHOLO_EPS. This result is in agreement with the organic fractions targeted by this
511 fractionation method. Indeed, PROT_EPS targets the protein present in extrapolymeric substances
512 (Jimenez et al., 2015) which are recalcitrant to biodegradation. Neither does NLIGN_EPS contribute
513 significantly, due to the strongly limited bioaccessibility of the compounds contained in this fraction.
514 In comparison with a similar study investigating the link between EPS fractionation and carbon
515 biodegradability, our results corroborate the observation made by Jimenez et al. (2017) concerning the
516 contribution of the non-extractible fraction (LIGN_EPS). Indeed, LIGN_EPS on carbon did not
517 contribute to the model like NLIGN_EPS to nitrogen based models. However, the extractible fractions
518 of organic nitrogen (NSOL_EPS, NPROT_EPS and NHOLO_EPS) do not contribute at the same level
519 as organic carbon, which is explained by the difference in the distribution of organic nitrogen and
520 carbon discussed in section 3.1.2.

521 The main difference between $N_{\text{EPS_G1}}$ and $N_{\text{EPS_G2}}$ is the contribution of NPROT_EPS to nitrogen
522 biodegradability. Indeed, for G1 (substrates with $\text{NH}_4^+/\text{NTK} < 30\%$), this explanatory variable
523 contributes moderately to organic nitrogen biodegradability, but has no significant effect for G2
524 (substrates with $\text{NH}_4^+/\text{NTK} > 30\%$). This difference explains the difference in biodegradability of
525 organic nitrogen in the two groups of substrate by showing that for substrates that have a NH_4^+/NTK
526 ratio of less than 30%, part of the organic nitrogen contained in NPROT_EPS fraction is
527 biodegradable. However, for substrates with NH_4^+/NTK ratio greater than 30%, biodegradable organic
528 nitrogen contained in NPROT_EPS fraction was degraded before anaerobic digestion, i.e. during
529 fermentation which occurs during silageing, during animal digestion in the case of livestock manure or
530 during aerobic or anaerobic degradation processes that spontaneously occur during substrate storage.
531 Hence, for that substrate group, the organic nitrogen contained in the PROT_EPS fraction is not
532 significantly biodegradable under anaerobic digestion.

533

534 4. CONCLUSION

535 Our results highlight the fact that the organic nitrogen in substrates is contained in fractions with
536 different bioaccessibility ranging from highly accessible to inaccessible. The distribution of organic
537 nitrogen depends to a great extent on the substrate. Therefore, like for carbon, organic nitrogen
538 biodegradability strongly depends on the substrate and can be predicted using statistical models that
539 use bioaccessibility indicators as variables. These models enable the fast determination of ammonium
540 and organic nitrogen in the digestate and help to optimize anaerobic digestion plants aimed at
541 advancing the substitution of mineral fertilizers by digestates and preventing ammonia inhibition.

542

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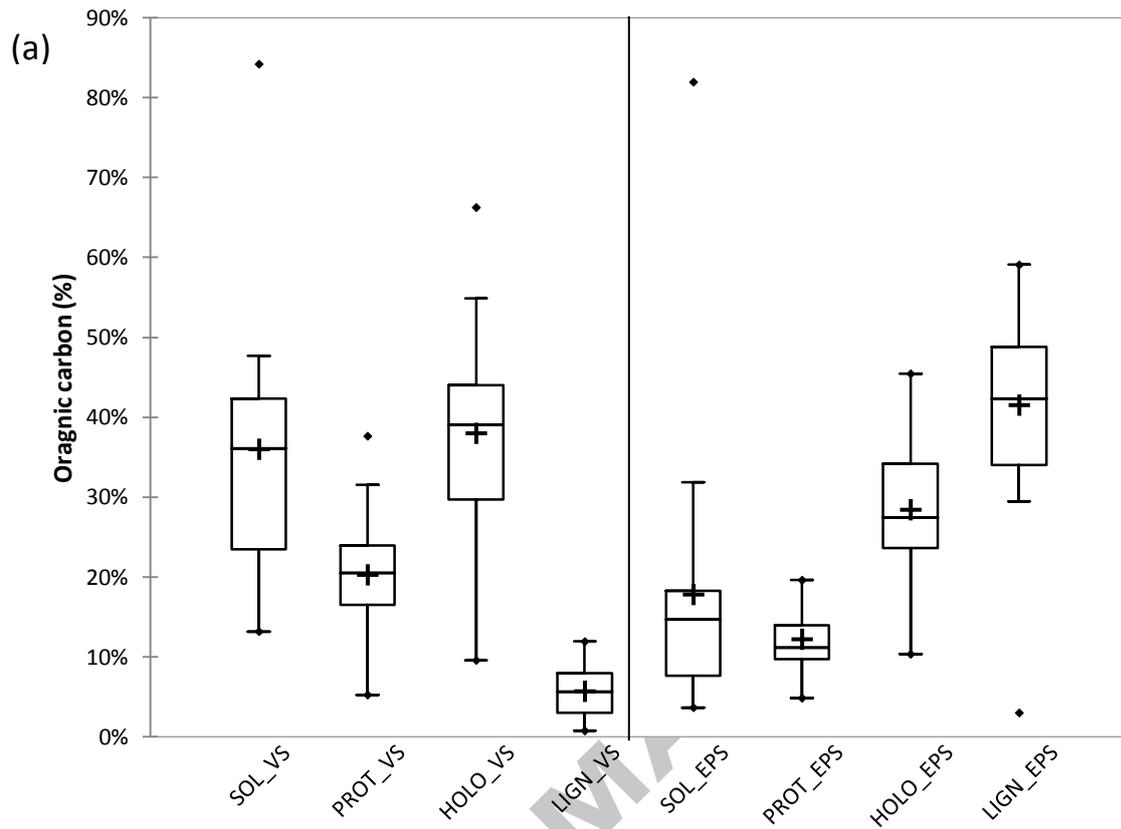
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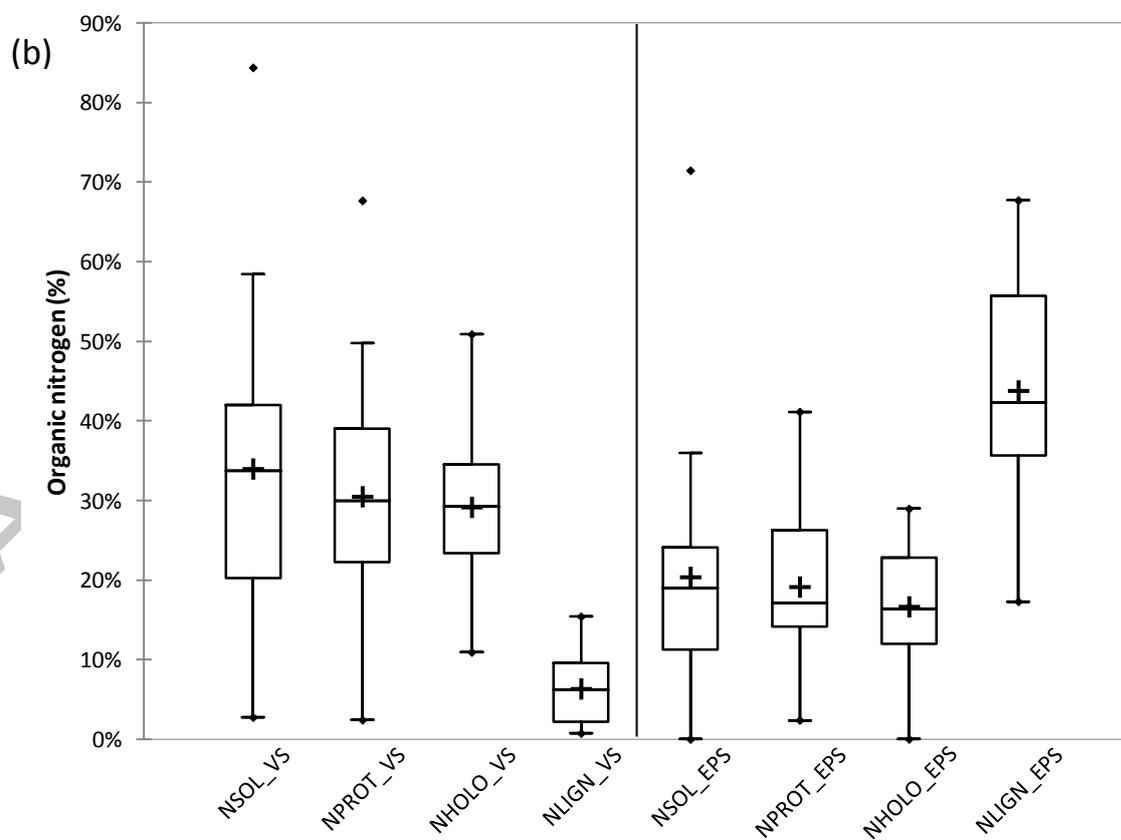
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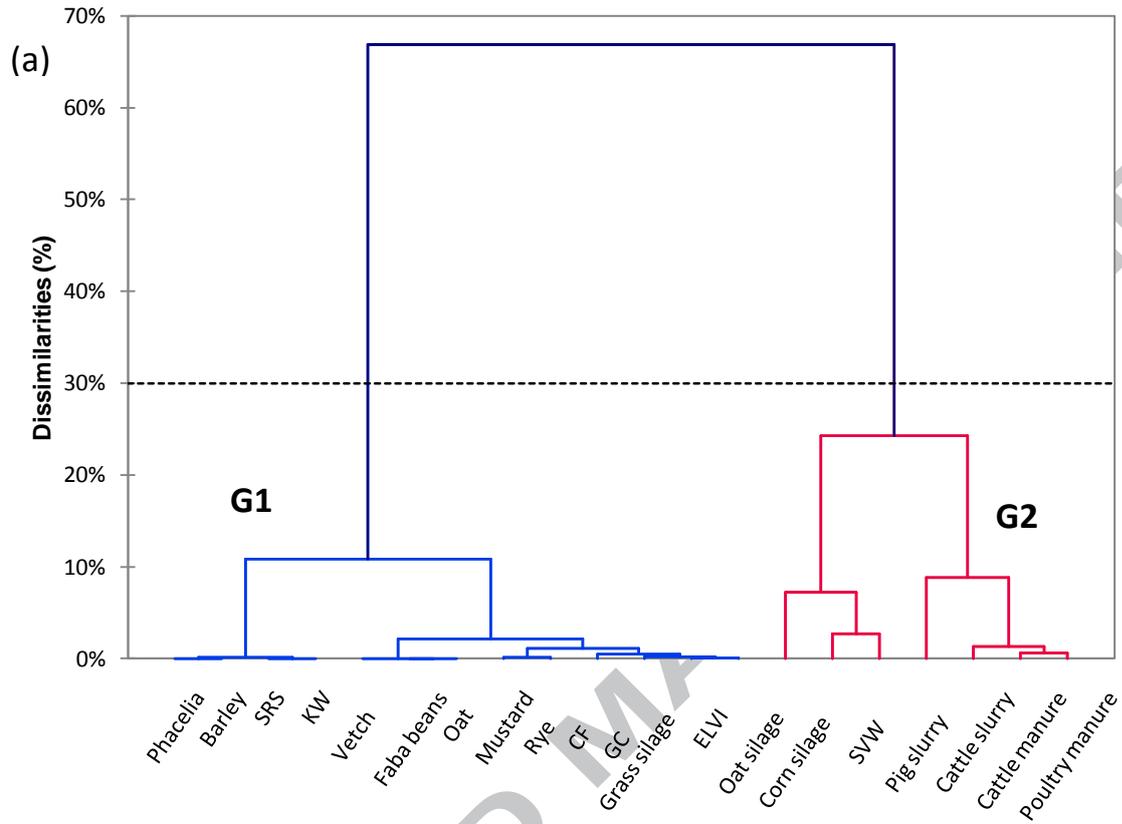
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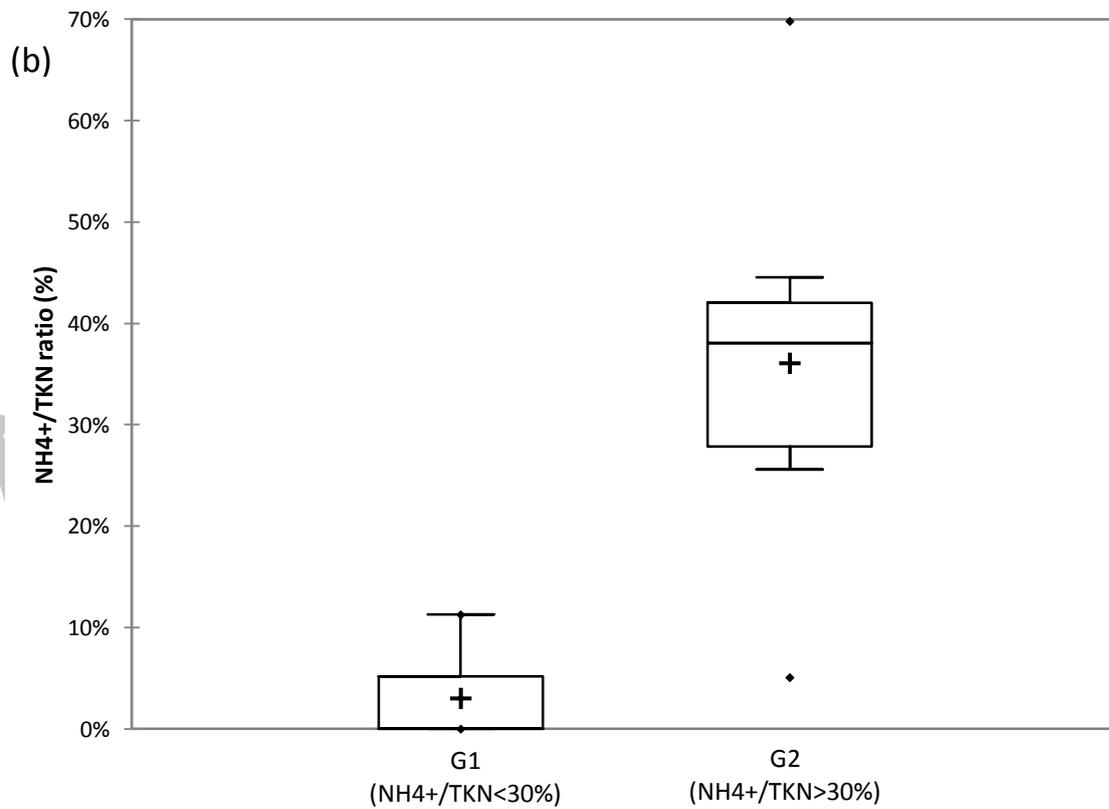
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644 Figure 1: Organic carbon (a) and organic nitrogen (b) distribution according to Van Soest and EPS
 645 fractionation (Fraction abbreviations are explained table 2).

646

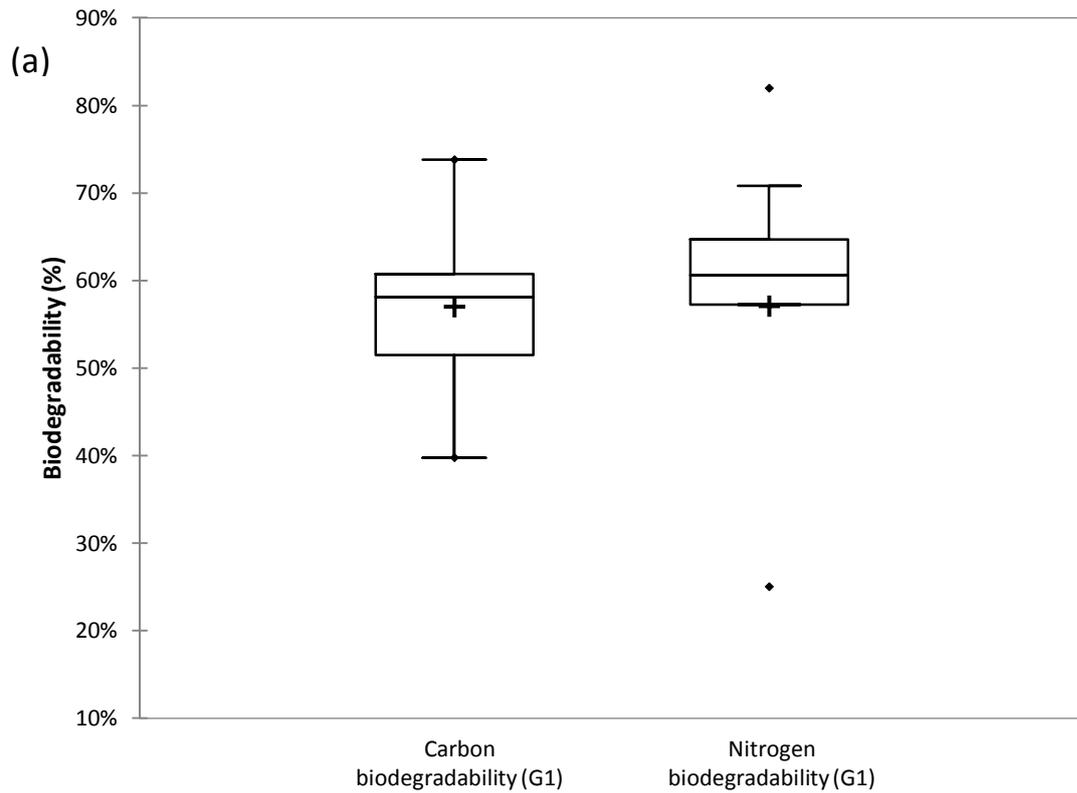


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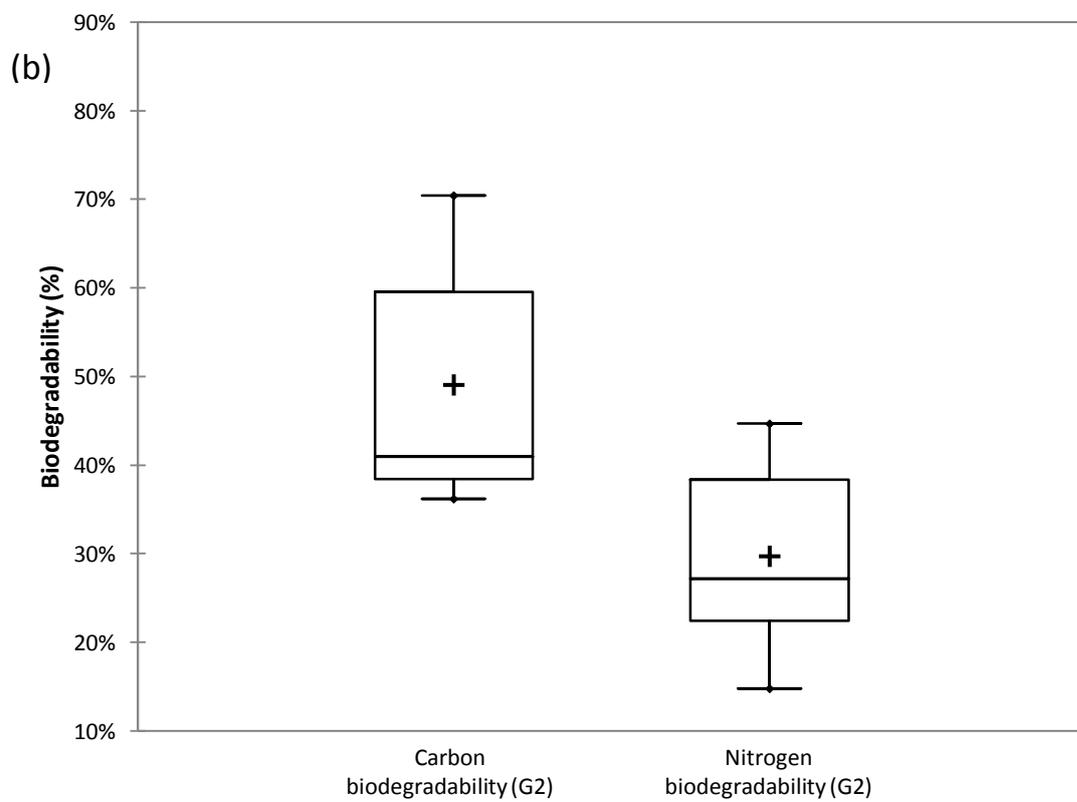


648

649 Figure 2: Hierarchical Cluster Analysis of the panel of substrates (a) and (b) the distribution of the
650 ratio NH_4^+/TKN according to the substrate typology (Substrate abbreviations are explained in table
651 1).



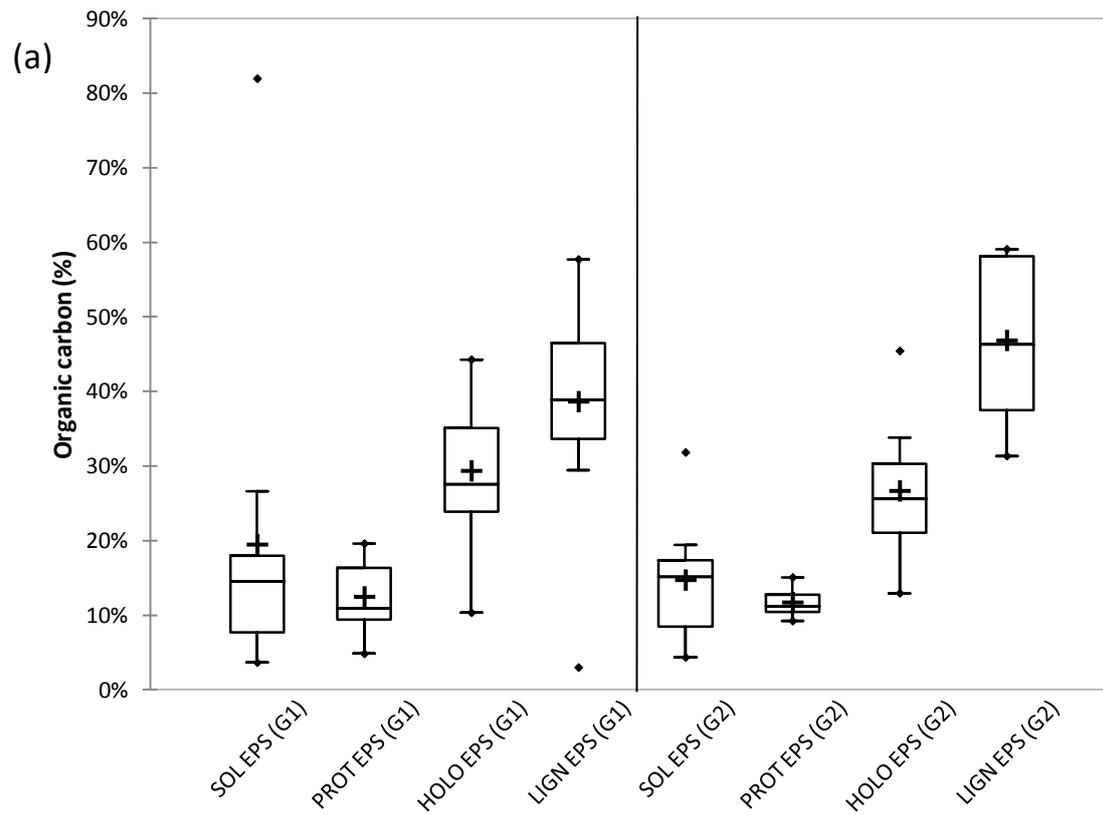
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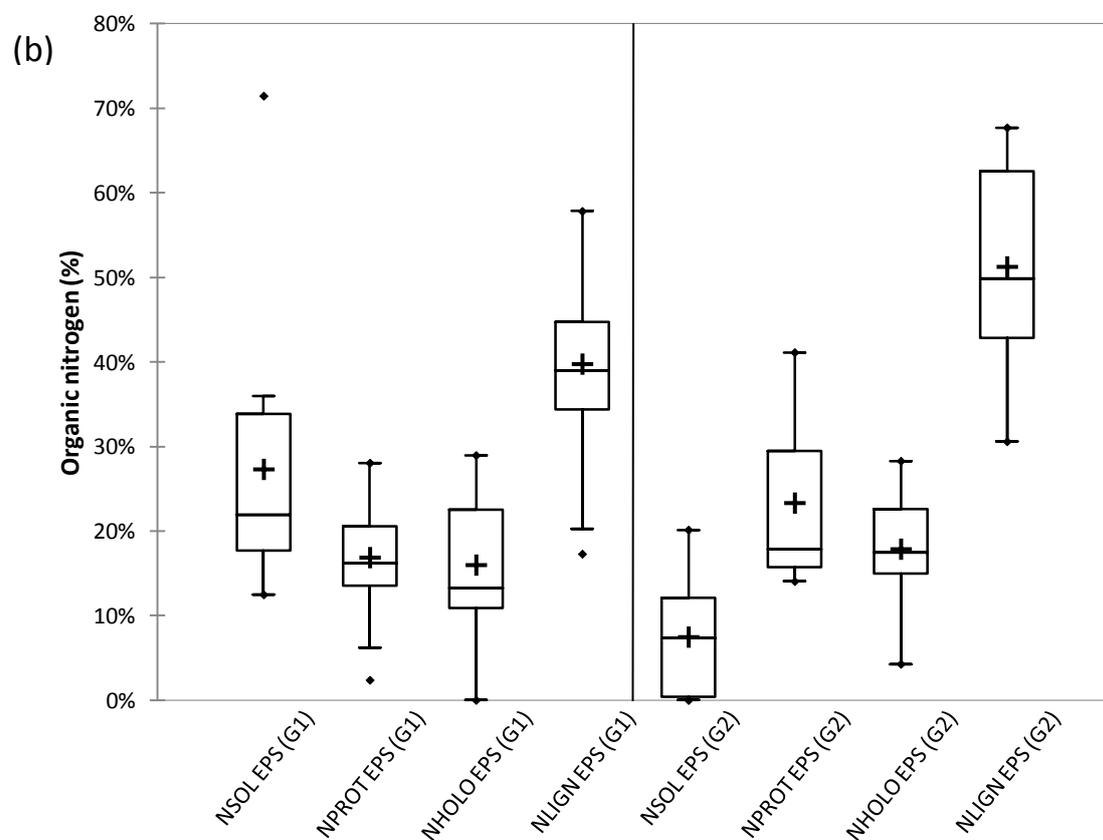
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654 Figure 3: Box plots of the distribution of organic carbon and nitrogen biodegradability for G1 (a) and
655 G2 (b).

ACCEPTED MANUSCRIPT

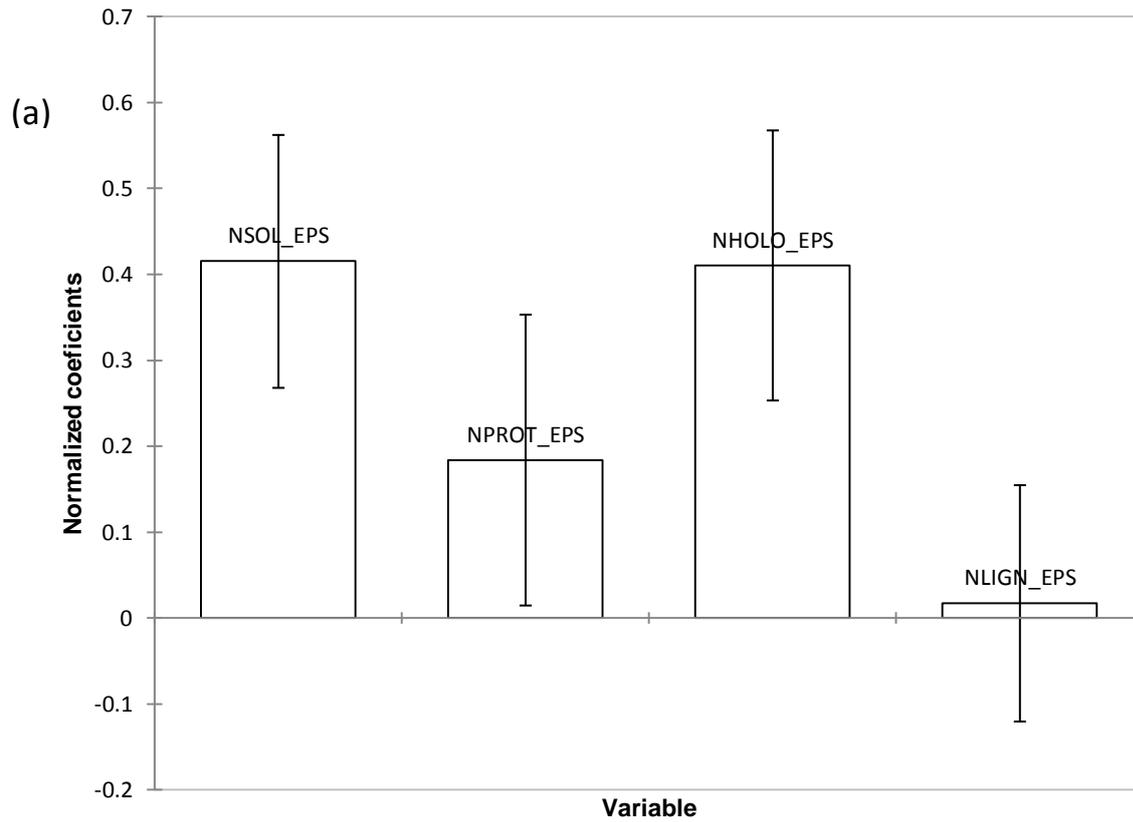


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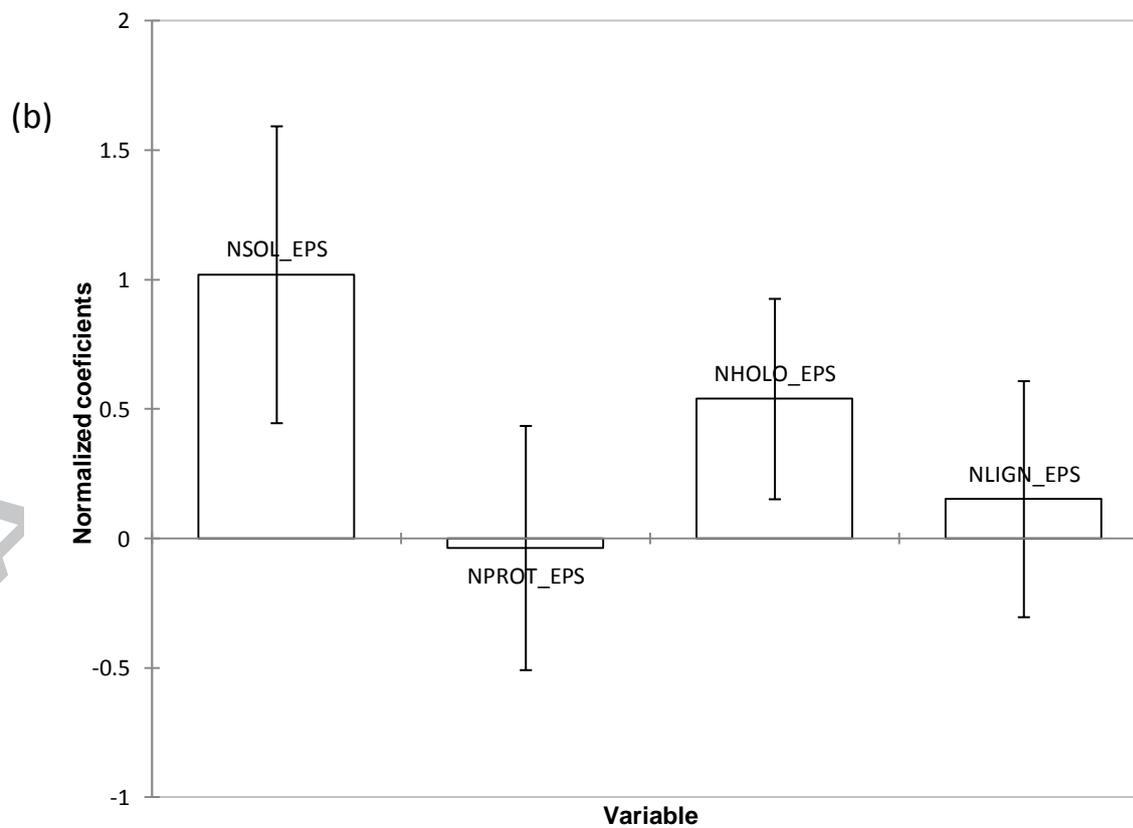


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659 Figure 4: Organic carbon (a) and organic nitrogen (b) content for G1 and G2 substrates according to
 660 modified EPS fractionation (Fraction abbreviations are explained table 2).



661



662

663 Figure 5: Normalized coefficient for model N_{EPS_G1} (a) and N_{EPS_G2} (b) explaining organic nitrogen
 664 biodegradation on G1 and on G2 (Fraction abbreviations are explained table 2).

665

666 **Table 1:** Characterization of the panel of substrates.

Class	Sample	TS (g _{TS} .kg ⁻¹)	VS (g _{VS} .kg ⁻¹)	NH ₄ ⁺ (gN.kg ⁻¹)	TKN (gN.kg ⁻¹)	TOC (gC.kg ⁻¹)	Protein (% _{org-C})
Crop residues	Faba beans	140.2	125.6	0	6.4	72.4	38%
	Vetch	174.5	158.6	0	8.4	86.5	42%
	Phacelia	216.6	197.7	0	2.8	49.9	24%
	Mustard	178.1	158.7	0	5.2	89.2	25%
	Oat	157.1	141.1	0	4.8	106.7	20%
	Rye	207.6	195.6	0	3.6	128.9	30%
	Barley	246.5	221.3	0	4.0	149.1	12%
	Corn silage	298.2	275.6	0.3	6.2	133.4	19%
	Grass silage	267.4	234.0	0.7	7.7	138.8	22%
	Oat silage	161.9	134.0	1.9	4.2	85.6	12%
Stock manure	Cattle manure	133.7	103.8	1.3	4.3	71.0	18%
	Pig slurry	50.3	37.1	2.3	3.2	25.1	17%
	Cattle slurry	92.4	73.4	0.9	2.4	48.3	14%
	Poultry manure	328.1	252.2	5.5	13.8	148.4	24%
Agroindustry and municipalities	Screening refusal from slaughterhouse (SRS)	294.1	282.7	0.2	7.8	165.4	20%
	Grass clippings (GC)	261.4	213.3	0.4	8.1	112.0	30%
	Cattle feed waste (CF)	698.1	628.1	3.4	34.0	321.9	41%
	Kitchen Waste (KW)	259.1	206.2	0.1	11.5	139.7	36%
	Waste from yeast production (ELVI)	587.7	465.0	4.9	43.9	251.3	67%
	Vegetable waste from supermarkets (SVW)	107.4	95.7	0.7	2.7	62.6	14%

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669 **Table 2:** Fractions and extractions steps in the modified Van Soest and EPS methods.

Modified Van Soest method		Modified EPS method	
Fraction name**	Extraction step	Fraction name**	Extraction step
SOL_VS	Water extraction	SOL_EPS	CaCl ₂ extraction
PROT_VS	Neutral detergent extraction (NDF)	PROT_EPS	NaOH, NaCl 10mM + Na 0.1M extractions
HOLO_VS	Acid detergent (ADF) + H ₂ SO ₄ 72% extraction	HOLO_EPS	H ₂ SO ₄ 72% extraction
LIGN_VS	Non-extractible	LIGN_EPS	Non-extractible

670 * In addition to targeted molecules, other compounds are also liberated by modifying accessibility.

671 ** If N prefix is added prior to the fraction name, organic nitrogen content in the fraction is considered,

672 otherwise, organic carbon content is considered.

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674 **Table 3:** Results of carbon and nitrogen biodegradability

Sample	BMP (Nm ³ .kgTS ⁻¹)	%CH ₄	Organic carbon biodegradability			AM
			gC.kgTS ⁻¹	% _{org-C}	gN-NH ₄ ⁺ .kgTS ⁻¹	
Faba beans	267.9	53%	291.6	54%	21.3	
Vetch	297.8	56%	289.1	58%	36.3	
Phacelia	167.8	54%	171.0	74%	11.7	
Mustard	241.7	45%	321.8	61%	19.7	
Oat	325.2	52%	360.0	51%	19.1	
Rye	301.0	44%	271.8	45%	5.4	
Barley	272.5	44%	262.1	43%	4.4	
Corn silage	220.9	45%	225.9	56%	3.1	
Grass silage	320.5	46%	310.3	58%	13.3	
Oat silage	365.9	60%	323.0	63%	2.2	
Cattle manure	212.0	56%	196.4	36%	6.8	
Pig slurry	235.3	70%	182.8	36%	3.4	
Cattle slurry	251.9	64%	242.7	41%	3.7	
Poultry manure	221.2	65%	182.8	41%	13.1	
Screenings refusal from slaughterhouse (SRS)	299.2	60%	249.8	40%	6.0	
Grass clippings (GC)	270.6	54%	230.4	54%	17.1	
Cattle feed waste (CF)	396.6	65%	329.8	72%	35.7	
Kitchen waste (KW)	220.9	54%	391.0	72%	20.4	
Waste from yeast production (ELVI)	320.5	55%	248.1	58%	31.0	
Supermarket vegetable waste (SVW)	365.9	41%	384.9	70%	7.6	

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677 **Table 4:** Statistical models for the prediction of organic nitrogen biodegradability considering the
 678 whole set of substrates and associated quality parameters.

Fractionation method	Explanatory variables	Biodegradability (BD) equation	Model quality
			Q ²
Modified EPS	NSOL_EPS, NPROT_EPS, NHOLO_EPS, NLIGN_EPS	$BD(orgN_{EPS}) = -5.14 + 1.48*NSOL_EPS + 0.07*NPROT_EPS + 1.76*NHOLO_EPS + 0.12*NLIGN_EPS$	0.736
Modified Van Soest	NSOL_VS, NPROT_VS, NHOLO_VS, NLIGN_VS	$BD(orgN_{VS}) = -1.91 + 0.80*NSOL_VS + 0.72*NPROT_VS + 0.44*NHOLO_VS - 0.178*NLIGN_VS$	0.571

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681 **Table 5:** Statistical models for the prediction of organic nitrogen biodegradability considering
 682 substrate group G1 and G2 and associated quality parameters.

Fractionation method	Group considered	Biodegradability (BD) equation	Model quality
			Q ²
Modified EPS	G1	$BD(orgN_{N_{EPS_G1}}) = -4,22 + 1,43*NSOL_EPS + 0,84*NPROT_EPS + 1,22*NHOLO_EPS + 0,03*NLIGN_EPS$	0.802
Modified Van Soest		$BD(orgN_{N_{VS_G1}}) = -1,94 + 0,89*NSOL_VS + 0,81*NPROT_VS + 0,24*NHOLO_VS - 0,59*NLIGN_VS$	0.551
Modified EPS	G2	$BD(orgN_{N_{EPS_G2}}) = -2,78 + 1,29*NSOL_EPS - 0,07*NPROT_EPS + 1,14*NHOLO_EPS + 0,19*NLIGN_EPS$	0.771
Modified Van Soest		$BD(orgN_{N_{VS_G2}}) = -3,32 + 0,56*NSOL_VS + 0,28*NPROT_VS + 0,63*NHOLO_VS + 0,27*NLIGN_VS$	0.057

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685 **Table 6:** Comparison of organic nitrogen biodegradability values obtained from experiments and
 686 models.

Model used	Livestock feed waste									
			Oat (G1)		Oat (G1)		Cattle slurry (G2)		Oat silage (G2)	
	Q ²	R ²	Prediction (g/kg TS ⁻¹)	Prediction error (%)	Prediction (g/kg TS ⁻¹)	Prediction error (%)	Prediction (g/kg TS ⁻¹)	Prediction error (%)	Prediction (g/kg TS ⁻¹)	Prediction error (%)
N _{EPS}	0.736	0.789	34.1	5%	15.2	20%	2.4	37%	1.8	19%
N _{EPS_G1}	0.802	0.843	35.7	2%	18.7	2%	n.t.	n.t.	n.t.	n.t.
N _{EPS_G2}	0.771	0.943	n.t.	n.t.	n.t.	n.t.	3.9	5%	2.0	10%

687 n.t.: not tested

688

689 **Highlights:**

- 690 • Organic nitrogen is contained in materials with various bioaccessibility levels.
- 691 • Organic nitrogen bioaccessibility differs from organic carbon bioaccessibility.
- 692 • Organic nitrogen biodegradability cannot be estimated from carbon biodegradability.
- 693 • Van Soest fractionation does not predict organic nitrogen biodegradability.
- 694 • Models using bioaccessibility data from EPS fractionation show good predictability.

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