

1 **Biogas digestates based on lignin-rich feedstock - Potential as fertilizer and**
2 **soil amendment**

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19 **Biogas digestates based on lignin-rich feedstock – Potential as fertilizer and soil**
20 **amendment**

21

22 **Abstract**

23 With advances in biogas technology, lignocellulosic material may be increasingly
24 included in feedstock due to the abundance of raw materials. The main goal of this
25 study was to evaluate fertilizing and soil amendment effects of digestates based on
26 lignin-rich feedstock. The digestates originated from reactors fed with manure co-
27 digested with *Salix*, wheat straw or sugarcane bagasse, respectively. In pot experiments
28 with three different soils, Italian ryegrass and reed canary grass were grown with 120
29 kg ha⁻¹ total nitrogen or 150 kg ha⁻¹ available nitrogen, respectively, given as either
30 mineral fertilizer or digestate. Soil chemical and physical characteristics were
31 determined after ended experiments. Additionally, an incubation study was carried out
32 to estimate N mineralization from one digestate over time. Digestate addition resulted
33 in similar yields compared to mineral fertilizer, varying from 0.5 (loam) to 1 kg dry
34 matter m⁻² (silt) for Italian ryegrass and 1.2 (loam) to 2.3 kg m⁻² (silt) for reed canary
35 grass. Digestates contributed to a favourable pH for plant growth, reduced bulk density
36 in the loam and improved water retention characteristics in the sand. Biogas digestates
37 based on lignin-rich feedstock appear promising as fertilizers and for soil amelioration
38 but results have to be verified in field experiments.

39

40 **Keywords:** lignocellulosic material; biomass production; Italian ryegrass; reed canary
41 grass; soil physical properties

42

43 **Introduction**

44 Due to the attempt to produce more renewable energy in recent years, the production of
45 biogas from various sources of biomass has increased considerably. Biogas production leaves
46 a digestate that contains organic material but is also rich in plant-available nutrients and
47 should thus be used in new biomass production in order to make the biogas process truly
48 sustainable. In general, the nutrient value of organic fertilizers based on organic waste is
49 highly dependent on the type of organic matter, as well as the processing method (Bungay et
50 al. 2007; Smith et al. 1998). Benefits of organic waste application to soil may include positive
51 effects on, e.g., soil structure and porosity, water retention capacity, trace metal binding,
52 cation exchange capacity, biological activities and thus general soil fertility (Marinari et al.
53 2000; Shiralipour et al. 1992). Recent reviews suggest that digestate additions have a positive
54 effect on soil fertility aspects compared to mineral fertilizer, which is on a similar level as the
55 effect of farmyard manure despite differences in the quality of the organic matter added
56 (Insam et al. 2015; Möller 2015).

57 Anaerobic digestion leaves residues with a lower C/N ratio of the organic material
58 compared to the original feedstock (Arthurson 2009). The residues show good fertilizing
59 properties due to their content of nitrogen (N), phosphorus (P) and potassium (K) in a plant-
60 available form (Tambone et al. 2010). Especially the high content of readily available NH_4^+
61 in biogas digestates is a major advantage for use in plant production is, and digestates could
62 therefore be regarded as mainly a mineral N fertilizer (Svensson et al. 2005). However, the
63 share of NH_4^+ -N of total N may vary widely and even an increased NH_4^+ -N content in
64 digestates does not necessarily imply an improved N uptake (Möller & Müller 2012).

65 So far, the majority of studies on recycling nutrients from biogas digestates in plant
66 production have been conducted using digestates based on feedstock with relatively high N
67 contents and thus low C/N ratios. Examples are food and food industry by-products, crop
68 residues from rape, sunflower or maize or also perennials, often in combination with manure

69 or sewage (Müller-Stöver et al. 2016; Albuquerque et al. 2012b; Odlare et al. 2008;
70 Svensson et al. 2005). However, there is an abundance of more lignin-rich materials such as
71 tree residues or cereal straw that may represent a large potential for biogas production in the
72 future. Lignocellulosic material may be hydrolyzed and further digested anaerobically to
73 methane (Sawatdeenarunat et al. 2015; Hendriks & Zeeman 2009). The challenges connected
74 to exploiting the full biogas potential of these materials have recently been the focus of
75 several studies (Risberg et al. 2013; Vivekanand et al. 2013; Horn et al. 2011; Chandra et al.
76 2012; Estevez et al. 2012). Since it therefore seems likely that more digestates based on
77 lignin-rich feedstock will become available in the future, their value as fertilizer and their
78 effect on soil properties need to be studied. The purpose of the present study was to
79 investigate whether using digestates based on lignin-rich feedstock as fertilizers results in
80 adequate plant growth and/or improves soil quality including physical characteristics.

81

82 **Materials and methods**

83 *Digestate generation*

84 Four different digestates, based partly on lignocellulosic feedstock, were tested as
85 fertilizers in pot experiments. Two digestates were produced from a biogas reactor operating
86 with *Salix viminalis* “Christina” as lignocellulosic substrate. The wood chips were pretreated
87 by steam explosion (210 °C for 10 minutes), and then digested anaerobically with fresh cattle
88 manure in bioreactors with a working volume of 6 L (Dolly - Belach Bioteknik AB, Sweden).
89 Material from a previous experiment with the same feedstock was used as inoculum. After a
90 start-up period, the reactors were fed once a day, 6 days a week, with an organic loading rate
91 (OLR) of 3 g volatile solids (VS) L⁻¹ d⁻¹ and a hydraulic retention time (HRT) of 30 days.
92 Before feeding the reactors with fresh material, an equivalent volume was removed in order
93 to maintain a constant volume in the reactor. The substrate in the first reactor was produced

94 from feeding a feedstock mixture of *Salix* and manure (40/60 % on a VS basis, C/N ratio 39),
95 diluted to the volume with tap water. For the second digestate product, the feedstock fed to
96 the reactor was diluted with the process liquid after filtering the daily removed volume
97 through a 2.5 mm mesh size sieve (40/60 % on a VS basis). The C/N ratio of this second
98 feedstock mixture diluted with the liquid fraction of the digestate was 34. The experiment
99 was run at 37 °C for 3.3 HRT and ended after 100 days. A detailed description of the biogas
100 experiments can be found in Estevez (2013).

101 A third digestate was produced from the same type of reactor (6 L) operating with a
102 mixture of steam-exploded straw and cow manure (78/22 % on VS basis, reactor RTcSS)
103 (Risberg et al. 2013). The reactor had been operating for a total of 350 days, under similar
104 operational conditions, i.e. with a HRT, OLR and temperature of 25 days, 2.8 g (VS) L⁻¹ d⁻¹
105 and 37 °C, respectively. The C/N ratio of the substrate mixture was 30.

106 The last digestate used originated from a reactor operating with milled sugarcane
107 bagasse (*Saccharum officinarum*), supplied from Borregaard (Sarpsborg, Norway) and cattle
108 manure. Sewage from a local wastewater treatment plant (Nordre Follo Wastewater
109 Treatment Plant, Vinterbro, Norway) was used as an inoculum during start-up. The OLR of
110 the substrate mixture (C/N ratio 30) was 3.0 g L⁻¹ d⁻¹ VS, fed 6 days a week and the HRT was
111 25 days. The experiment was run at 37 °C for 3 HRTs and ended after 86 days.

112 ***Digestate and soil analysis***

113 A chemical characterization of the digestates used is shown in Table 1 (pH, macronutrients).
114 Total C was determined in crushed samples by dry combustion (Nelson & Sommers 1982) at
115 1050 °C using a Leco CHN-1000 instrument (St. Joseph, Michigan, USA). Total N was
116 measured on the same instrument according to the Dumas method (Bremner & Mulvaney
117 1982). Ammonium and nitrate (NH₄, NO₃) were measured by flow injection analysis (FIA,
118 Tecator FIAstar 5010 Analyzer, Hillerød, Denmark) after extraction with 2M KCl, with

119 measurements based on the fresh material (wet sample). Other nutrients were analyzed by
120 inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer SCIEX Elan 6000,
121 Waltham, Massachusetts, USA) or inductively coupled plasma optical emission spectrometry
122 (ICP-OES, Perkin Elmer Optima 5300 DV) after ultraclave digestion in concentrated, double-
123 distilled HNO₃ (0.25 g to 0.3 g sample in 5 mL) and subsequent dilution to 50 mL, with a
124 modification for Hg analysis. Determination of Hg was carried out as quickly as possible
125 after first adding 1 mL H₂O₂ to 0.15-0.2 g sample, followed by 5 mL HNO₃. Digestate pH
126 was measured directly in the liquid sample without addition of water.

127 ((Table 1))

128 The soils used in the experiments were collected from the top layer (0-20 cm) of
129 agricultural or forest soils at different locations in south-eastern Norway to represent three
130 different soil textures, i.e. sand, silt and loam. Table 2 shows some chemical characteristics of
131 the soils prior to the experiments. Total C and N in soils were measured using the same
132 methods as described above. Plant-available P and K were estimated by extraction with
133 ammonium acetate lactate solution (0.1 M ammonium lactate and 0.4 M acetic acid, pH 3.75)
134 (Egnér et al. 1960), followed by ICP spectrometry. Particle size distribution was determined
135 by the pipette method (Elonen 1971). Soil pH was measured in H₂O with a soil to solution
136 ratio of 1:2.5.

137 ((Table 2))

138 ***Ryegrass experiment***

139 In order to study the effect of the different biogas digestates as a fertilizer, a pot experiment
140 was conducted under controlled conditions (20 °C, 18-hour day) with three soils differing in
141 texture and Italian ryegrass (*Lolium multiflorum*, var. Macho) as a test crop. The soils used
142 were classified as a sand, a silt and a loam. All soils were air-dried, and the loam and silt
143 were passed through a 5 mm mesh size prior to being filled into pots. Due to its single grain

144 structure, the sand was not sieved prior to use. The pots (diameter 16 cm) were filled with a
145 soil volume of 3 L. Because of its low original pH, the loam was limed with 8 g CaCO₃ per
146 pot, resulting in a pH of approximately 6.

147 The experiment consisted of the following treatments: fertilization with *Salix*
148 digestate, *Salix* digestate where process water was recycled (*Salix* recycled), and wheat straw
149 digestate, as well as a mineral fertilizer control, all in three replicates, respectively. Amounts
150 of fertilizer on a per hectare basis were calculated estimating a soil volume of 2 000 000 L ha⁻¹,
151 which represents a typical Norwegian plough layer of 20 cm. Digestates and mineral
152 fertilizer were mixed into the whole soil volume. The digestate amounts given were
153 calculated based on estimated available N during the experiment, i.e. NH₄-N and
154 approximately half of the organically bound N. All treatments received approximately 0.18 g
155 available N, i.e. 120 kg N ha⁻¹, which represents a normal amount for the first cut in grass
156 production in southern Norway. Amounts of P and K in the digestate treatments depended on
157 the N concentrations in the digestates used, with P applications varying between 35 (wheat
158 straw) and 45 kg ha⁻¹ (*Salix* recycled) and K addition equivalent to approximately 200 kg ha⁻¹.
159 In the mineral fertilizer control, N was given in the form of Ca(NO₃)₂ equivalent to 120 kg
160 N ha⁻¹. Phosphorus was added as Ca(H₂PO₄)₂ equivalent to 20 kg ha⁻¹. Since ryegrass is
161 known for luxury uptake of K (Øgaard et al. 2001), the amount of K (as K₂SO₄) in the
162 mineral control was divided into a rate equivalent to 100 kg ha⁻¹ in the beginning and 50 kg
163 after the first cut to ensure sufficient K later on in the experimental period. Other macro- and
164 micronutrients were added in dissolved form to satisfy plant needs (equivalent to: Mg 1.9, S
165 10, Fe 1.7, Mn 1, Cu 1.3, Zn 0.8, Mo 0.006, B 0.01 kg ha⁻¹). The micronutrients Fe, Mn, Cu
166 and Zn, which were present in relatively low concentrations in the digestates, were also added
167 to the digestate treatments in a mineral form in order to ensure that they were not growth
168 limiting.

169 The pots were sown with 0.3 g seeds of Italian ryegrass. Moisture content in the soil
170 was maintained at 60 % of the water holding capacity of the pot by irrigation with deionized
171 water according to weight loss. The grass was cut after 6 and 10 weeks, respectively. Soil
172 samples were taken after 10 weeks and analysed for different N fractions and other available
173 main nutrients, as well as pH (in H₂O, soil : solution ratio 1:2.5).

174 ***Mineralization study***

175 In order to estimate the amount of N that would be mineralized from the organic fraction of
176 the digestates during the growth experiments, a simple incubation study was carried out. For
177 this study, digestate originating from a digester operating with *Salix* and manure without
178 recycling of process water was used. Since most of the readily available NH₄-N from the
179 fresh sample was lost upon drying (Table 1) and NO₃-N contents were negligible, the N
180 content of the dried digestate consisted almost exclusively of organically bound N. The
181 digestate was ground and the loam soil dried and sieved (2 mm mesh size) before mixing
182 thoroughly with the digestate (average ratio 0.18 g dried digestate: 20.37 g soil) to ensure as
183 little variation between samples as possible. The samples were incubated in the dark for up to
184 11 weeks at 15 °C and a soil moisture of 60 % of water holding capacity. A control with the
185 same soil without digestate was included. Three replicates of both control and soil with
186 digestate were removed for analysis every week for the first seven weeks and after weeks 10
187 and 11. Ammonium and nitrate in the removed replicates were measured by FIA as described
188 above. Nitrogen mineralization from the digestate was calculated as the combined NH₄-N and
189 NO₃-N measured over time in the digestate-amended soil minus the respective values for the
190 control soil. The method is described in more detail in Sogn and Haugen (2011).

191 ***Reed canary grass experiment***

192 In a second pot experiment under controlled conditions (20 °C, 18-hour day), reed canary
193 grass (*Phalaris arundinacea*) was grown with either bagasse digestate or mineral fertilizer.

194 Again, the experiment was conducted with three different soils, i.e. the same loam and silt as
195 in the first experiment, and a sandy soil that differed slightly from the sand used in the first
196 experiment (see Table 2). Reed canary grass was grown in pots (diameter 21 cm) with a soil
197 volume of 6.7 L and three replicates per treatment. In order to increase the pH to
198 approximately 6, 20 g CaCO₃ per pot were added to the loam and 10 g CaCO₃ per pot to the
199 sandy soil.

200 The total amount of available N given to both treatments approximated 150 kg N ha⁻¹
201 or 0.5 g N per pot. The amount of digestate added to each pot was calculated by considering
202 both the NH₄-N content in the fresh sample (23.9 g kg⁻¹ DM) and the amount that was
203 expected to be mineralized over period of 15 weeks (1.3 g kg⁻¹ DM). The latter was estimated
204 based on the results of the incubation study. The P content of the digestate dose was
205 equivalent to approximately 45 kg ha⁻¹ and K equivalent to approximately 125 kg ha⁻¹. The
206 mineral fertilizer control received N equivalent to 150 kg N ha⁻¹ in the form of Ca(NO₃)₂, P
207 equivalent to approximately 20 kg P ha⁻¹, and K equivalent to 200 kg K ha⁻¹. Micronutrients,
208 S and Mg were added to both treatments at the same rate as in the ryegrass experiment to
209 ensure sufficient supply.

210 The pots were sown with approximately 0.07 g seeds. After germination, the amount
211 of plants per pot was reduced to 20 in all pots in order to increase comparability between
212 replicates and treatments. In the loam, however, germination was poorer than in the other
213 soils, resulting in one pot in both the control and the digestate treatment with only 18 plants,
214 respectively. Moisture content in the soil was maintained at 60 % of the water holding
215 capacity of the pot by irrigation with deionized water according to weight loss.

216 *Soil physical studies*

217 In the reed canary grass experiment, selected soil physical properties were studied in order to
218 investigate possible soil amendment effects of digestate addition. Steel cylinders with a

219 volume of 100 cm³ were used to sample undisturbed soil cores for determination of water
220 retention capacity, air porosity and bulk density. One sample per pot was taken
221 approximately three cm below the surface after harvest. Water retention characteristics were
222 determined by exposing the soil cores to 0, -20, -50, -100, -1000 and -15000 hPa matric
223 potential and weighing, using a sand box at -20 and -50 hPa matric potential (Eijkelkamp;
224 <http://pkd.eijkelkamp.com/Portals/2/Eijkelkamp/Files/Manuals/M1-0801e%20Sandbox.pdf>)
225 and ceramic pressure plates at -100, -1000 and -15000 hPa matric potential (Richards 1947;
226 Richards 1948). Air porosity at -100 hPa matric potential was determined with an air
227 pycnometer (Torstensson & Eriksson 1936). Bulk density of the soils was determined after
228 drying the soil cores at 105 °C and weighing.

229 ***Statistical analysis***

230 The effect of the different digestates on yield and soil characteristics in the pot experiments
231 was tested statistically by analysis of variance (General Linear Model). The Student-
232 Newman-Keuls test was performed to identify different means. Results with $p < 0.05$ were
233 considered significant. The statistical analysis was carried out using SAS (SAS Institute Inc.).

234

235 **Results**

236 ***Ryegrass experiment***

237 In all three soils, the combined yield of both cuts was at least similar in the treatments
238 fertilized with digestates compared to the controls fertilized with mineral fertilizer (Figure 1).

239 In the loam, total yields were significantly higher in the digestate treatments compared to the
240 control.

241 (Figure 1))

242 After 10 weeks of growth, pH was significantly higher in both *Salix* digestate
243 treatments in the sand than in the control (Table 3). A similar, though not statistically

244 significant trend was found in the loam, whereas there was no difference in pH between the
245 treatments in the silt.

246 ((Table 3))

247 Total C in the sand and silt was slightly higher in some of the digestate treatments
248 compared to the control (Table 3). While the amount of total N remained the same in all soils
249 and treatments, there was a trend towards a higher soil NH_4^+ content in treatments with
250 digestate compared to the control. However, the differences were only significant for the
251 straw digestate treatment in the loam and the *Salix* digestate recycled treatment in the silt.
252 The addition of digestates also led to some significantly higher P-AL values of digestate
253 treatments in the loam and K-AL values in the silt compared to the control.

254 ***Mineralization study***

255 Nitrogen release from the digestate treated soil was higher than in the control soil for most of
256 the duration of the incubation (Figure 2A). While mineralization in the control occurred at a
257 similar rate for 11 weeks, mineralization rates in the digestate treated soil showed a first peak
258 in weeks 2 and 3 and a second in weeks 6 and 7. Figure 2B shows the net N release from
259 digestate calculated as the difference in N release between the digestate treated soil and the
260 control soil. Overall, after 11 weeks, mineralization from both control and digestate
261 amounted to approximately the same amount of mineral N released.

262 ((Figure 2))

263 ***Reed canary grass experiment***

264 ((Figure 3))

265 Reed canary grass yields were similar for plants fertilized with either mineral nutrients or
266 biogas digestate, except for in the silt, where fertilization with digestate resulted in a
267 significantly higher yield (Figure 3). From germination and early growth on, plants in the
268 loam were slower to develop, independent of fertilization. This is reflected in a much lower

269 biomass yield after 15 weeks in this soil than in the sand or silt. At the end of the experiment,
270 however, plants in the sandy soil showed clear signs of nutrient deficiencies, especially N, in
271 both treatments and especially in the control. In the silt, plants also started to get lighter-
272 coloured leaves, whereas in the clay no deficiency symptoms were visible. The soil analysis
273 after the experiment confirms that there was still more available N (both NH_4^+ and NO_3^-), P
274 and K in the loam, and to some extent in the silt compared to the sandy soil, which showed
275 the clearest deficiency symptoms (Table 4). In the reed canary grass experiment, the increase
276 in total C in the soils receiving digestate was stronger than in the ryegrass experiment in all
277 three soils. The bagasse/manure digestate did not have any significant effect on soil pH after
278 the growth experiment.

279 ((Table 4))

280 *Soil physical properties*

281 ((Figure 4))

282 Adding biogas digestate had different effects on the soil physical properties in the three soils
283 (Figure 4). The water retention capacity of the loam was little affected by the digestate
284 treatment except for a lower water content at the permanent wilting point (-15000 hPa). In the
285 sandy soil, adding digestate led to a higher overall pore volume, with more water-filled
286 medium-sized pores at field capacity while air porosity was maintained (-100 hPa). In the silt,
287 effects were not significant. Biogas digestate addition significantly increased air porosity at
288 field capacity in the loam but had no significant effects in the other two soils (Table 4). Bulk
289 density was significantly reduced by digestate addition in the loam, with a similar though not
290 significant trend in the silt, whereas no effects were found in the sand.

291

292 **Discussion**

293 An advantage of using biogas digestates in plant production is their high content of
294 readily available NH_4^+ (Albuquerque et al. 2012b; Möller & Müller 2012; Svensson et al.
295 2005). In our experiments, the use of biogas digestates as fertilizer showed good effects on
296 biomass production in both growth experiments. Abubaker et al. (2012) evaluated the effect
297 of four different biogas residues given in three different rates in a pot experiment with spring
298 wheat in a sandy soil. Biomass yields of all digestates were on the same level as equivalent
299 fertilization with mineral fertilizer (NPK). Digestates based on urban wastes resulted in 5 to
300 30 % higher ryegrass yields compared to similar amounts of inorganic N fertilizer in a pot
301 experiment with a sandy soil (Tampio et al. 2016). In a field study with biogas residues and
302 perennial ryegrass (Sieling et al. 2013), however, reduced yields were reported compared to
303 mineral fertilizer.

304 In the reed canary grass experiment, digestate was added mainly based on the amount
305 of $\text{NH}_4\text{-N}$, and so a similar biomass production as in the control treatment was expected. The
306 results of the incubation study suggested that over the experimental period, organically bound
307 N would be released in similar amounts in both control and digestate treatments. In the
308 ryegrass experiment, however, N in the digestate treatments was added based on total N and
309 the digestates would thus have had to contribute with approximately 50 % of the organically
310 bound N in order to supply the same amount of plant-available N as the mineral control. Still,
311 ryegrass biomass production was similar for both mineral fertilizer and digestate treatments,
312 suggesting a more efficient mineralization than found in the incubation study. Furthermore,
313 $\text{NH}_4^+\text{-N}$ at the end of the growing period was significantly increased in some of the digestate
314 treatments in the loam and silt, indicating that not all mineralized N was taken up by the
315 plants. In a field situation, this mineralized N might be considered available for the next
316 growing season unless lost by leaching or N_2O emission during winter. These results suggest
317 that microbial activity and thus mineralization in the limited soil volume (3 L), with intensive

318 rooting and favourable growing conditions was higher than both that measured in the
319 incubation study at 15 °C and what might be expected in a field experiment with more
320 variable conditions.

321 While total C was significantly increased in many of the digestate treatments, total N
322 was not, indicating an effective mineralization as was also found in the mineralization study.
323 There, an initial lag period was seen, most likely due to the necessity to build up microbial
324 biomass in the soil in the beginning. After the most easily degradable organic matter was
325 decomposed, a slight decrease in N mineralization, as seen in week 5, could be accounted for
326 by both changes in substrate and immobilization by microorganisms. However, Albuquerque
327 et al. (2012a) did not find a similar decrease in N mineralization in their incubation study
328 with different digestates. A study including biogas digestate based on pig slurry by Galvez et
329 al. (2012a) showed a fast increase of extractable N over the first week that was sustained over
330 30 days. An explanation for the pattern observed in our study may lie in the nature of the
331 organic material used in the biogas process. Here, manure was co-digested with
332 lignocellulosic plant material (*Salix*) as opposed to manure co-digested with easier
333 decomposable materials such as agro-industrial wastes in the study by Albuquerque et al.
334 (2012) or pig slurry alone as in the study by Galvez et al. (2012). The decrease in N
335 mineralization after five weeks may represent a shift from the more easily decomposable
336 manure-derived organic matter to the *Salix*-derived organic matter.

337 The positive effect of digestate addition on biomass production of both ryegrass
338 (loam) and reed canary grass (silt) suggests that mineralization rates in the plant experiments
339 may exceed rates found in the mineralization study without plants, thus rendering more N
340 available for plant growth than calculated. While others have found a certain immobilization
341 of N in soils amended with digestate (de la Fuente et al. 2013), this was not observed in our
342 experiments. Here, mineralization of organic matter did not inhibit plant growth through

343 competition of microorganisms and plant roots for the same nutrients during the
344 mineralization process, possibly because it occurred while enough nutrients were available
345 for both purposes. In general, amounts of organically bound N in the digestates added were
346 too small to detect significant changes in total N in the soils after several months of plant
347 uptake. Repeated additions of digestates over several growing seasons might increase total N
348 content over time by increasing the amount stored as organic N.

349

350 The total amount of added P in the soils treated with digestate was higher than in the
351 mineral fertilizer controls. The significant increase in plant-available P in the recycled *Salix*
352 digestate treatment in the loam (Table 3), as well as in the bagasse digestate treatment in the
353 loam and sand in the reed canary grass experiment (Table 4) reflect the rather high amounts
354 of total P added with these digestates (above 40 kg ha⁻¹), which exceeded fertilizer
355 recommendations. Depending on the initial availability of P in soils and the P concentration
356 in the digestates, it might therefore be necessary to add biogas digestate according to P rather
357 than N content in order to avoid excessive P fertilization with its potential effects on the
358 environment.

359 Soil analysis after the ryegrass experiment showed a clear decrease in available K in
360 the loam and silt in all treatments compared to relatively high values before the growth
361 experiment. This is in accordance with luxury consumption of K in ryegrass as has been
362 found earlier by Øgaard et al. (2001). Also in the reed canary grass experiment, K-AL values
363 decreased in the silt and loam, though to a lesser extent. At the same time, the soil treated
364 with digestate tended to maintain higher amounts of K-AL, despite a similar K fertilization in
365 digestate and control treatments. Since reed canary grass biomass after 15 weeks was
366 considerably higher than ryegrass biomass after 11 weeks, these results suggest that reed
367 canary grass is not taking up excessive K in the same way as ryegrass. Whether K is

368 accumulated in soils amended with digestate will therefore also depend on whether the
369 species growing there is capable of luxury uptake of K. In their field experiment on
370 grassland, Bougnom et al. (2012) observed an increase in K concentrations in plots fertilized
371 with biogas digestate compared with those fertilized with manure.

372 The main N form added differed between the mineral control and the digestate
373 treatments. This should have had an effect on the pH in the soils as measured after the
374 experiments. Nitrogen in the digestates was predominantly in the form of NH_4^+ , which
375 undergoes nitrification in aerated soil, thus releasing H^+ -ions. In addition, NH_4^+ uptake by
376 plant roots occurs in exchange for H^+ -ions. Both processes decrease the pH in the soil
377 compared to the control that received N as NO_3^- , so a lower pH in the digestate treatments
378 could have been expected. However, the original high pH of the digestates seems to have
379 counteracted this effect in all cases. While there were no significant changes in pH in the
380 soils under reed canary grass, in the ryegrass experiment, addition of digestates tended to
381 contribute to maintaining a higher pH than in the controls.

382 Due to a relatively low content of both dry and organic matter in the digestates, their
383 soil amendment potential may be expected to be rather low. The total pore volume and water
384 retention characteristics of the three soils are clearly influenced by the fact that the samples
385 were taken in a pot experiment where the soils used were either sieved through a 5 mm sieve
386 prior to use (silt and loam) or had very weak structure (sandy soil) to start with. In a field
387 experiment on a silty clay loam, Beni et al. (2012) found no improvement in soil surface
388 macroporosity upon digestate addition compared to mineral fertilizer addition, but an
389 increased stability of the soil structure as determined by higher resistance to deformation.

390 Due to more artificial conditions in our experiments with poorly structured soils and
391 regular irrigation, the water retention characteristics cannot be directly related to field
392 conditions. However, bearing in mind these restrictions, they can give some indications of

393 effects in field settings. The sand had the lowest nutrient and organic matter content to start
394 with and was therefore assumed to profit most from the small addition of organic material in
395 the digestate. While no positive effect on yield was observed, soil physical properties were
396 slightly improved in the sand, as shown in the change in water retention capacity. Lack of air
397 is rarely a problem in sandy soils, thus the effect of the digestate on the biggest pores is likely
398 to be of minor importance under field conditions. The amount of plant-available water in the
399 medium-sized pores (-20 to -100 hPa), however, was also significantly increased in the
400 digestate treatment. This could have a positive effect on plant growth on sandy soils if it also
401 occurred under field conditions. Air porosity was significantly increased by digestate addition
402 in the loam but not in the other two soils. In our pot experiments where the soils were sieved
403 prior to the growth experiment, the loam may very likely suffer from a shortage of air-filled
404 pores, and will thus profit from increased air porosity. However, under normal field
405 conditions, the loam with its high organic matter content will show some degree of
406 aggregation and air-filled space in stable macropores.

407

408 **Conclusions**

409 Biogas digestates based on materials relatively rich in lignin, such as *Salix* stems, wheat straw
410 and sugarcane bagasse, have a good potential as fertilizers at least when co-digested with
411 manure as in this study. Depending on the plant type grown, they may also contribute to
412 keeping the pH of arable soils at a beneficial level for plant growth. The digestates tested in
413 this study resulted in similar or even increased amounts of biomass compared to mineral
414 fertilizer treatments that were equivalent with respect to N amounts. Nitrogen seemed to be
415 well available but soil amelioration effects may also have influenced growing conditions in
416 digestate treatments in a positive way. However, applying digestates according to their
417 content of available N may lead to considerable amounts of P added to the soils that may not

418 be entirely used by the plants during the growing season. For digestates with low N contents
419 and high P contents, such as the digestate based on bagasse in this study, it would therefore
420 be more advisable to apply an amount according to P rather than N requirements and add
421 additional N in a mineral form.

422 In the artificial conditions of a pot experiment with sieved soils, the latter digestate
423 showed a positive effect on soil physical properties in the sand and the loam tested. In both
424 soils, digestate addition increased soil porosity, leading to higher air porosity in the loam and
425 plant-available water in the sand at field capacity. This effect may be expected to be more
426 pronounced if digestates with a higher dry matter and thus a higher organic matter content
427 could be applied. Whether similar effects can be detected under field conditions, still needs to
428 be investigated.

429

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435

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535 **Tables**536 Table 1. Dry matter, pH, and main nutrient content (g kg^{-1} DM) of the biogas digestates used.

537

Treatment	pH	Dry matter	Total C	Total N ^a	NH ₄ -N ^a	P	K
<i>Salix</i> + manure	7.3	4.42	442	29.0	9.7	6.6	32
<i>Salix</i> + manure, recycled	7.4	6.47	437	26.8	9.3	6.3	31
Straw + manure	7.7	5.37	377	36.3	17.6	7.8	49
Bagasse + manure	7.3	5.19	380	36.5	23.9	8.1	22

538

539 Analyses were conducted in one representative sample per treatment.

540 ^aTotal N and NH₄-N given in Table 1 refer to contents in fresh digestate samples. Because541 most NH₄-N was volatilized upon drying (NH₄ measured in dried samples equalled542 approximately 0.07 g kg^{-1}) and NO₃ content in digestates was negligible ($<0.1 \text{ mg l}^{-1}$), total N543 was calculated as the sum of total N determined in a dried sample and NH₄-N in fresh544 digestate- NH₄-N in dried digestate.

545 Table 2. Soil texture, pH, organic matter and nitrogen phosphorus and potassium content of
 546 the soils used in the ryegrass, reed canary grass and incubation experiments.

547

Soil texture class	Sand	Silt	Clay	Organic matter	Total N	P-AL ^a	K-AL ^a	pH
Loam	45	38	17	6.1	2.1	58	195	5.2 ^d
Sand ^b	96	4	0	0.4	0.0	19	6	6.0
Silt	2	93	5	3.7	1.0	49	200	6.5
Sandy soil ^c	94	3	3	1.3	0.1	16	10	5.1 ^d

548

549 Analyses were conducted in one representative sample per soil.

550 ^a AL: Plant-available P and K was estimated as the ammonium-lactate extractable fraction.

551 ^b Used in the ryegrass experiment

552 ^c Used in the reed canary grass experiment

553 ^d Original pH; the soil was limed to approximately pH 6 for the growth experiments.

554

555

556 Table 3. Carbon, nitrogen, phosphorus and potassium content, and pH in the test soils after
 557 the second cut in the ryegrass experiment (after 10 weeks of growth).

558

Soil	Treatment	pH	Total C		Total N		NH ₄ -N	P-AL*	K-AL*
			g kg ⁻¹		mg kg ⁻¹				
Loam	Control	5.9 a	21.1 a	1.7 a	5.0 a	57 a	66 a		
	<i>Salix</i> /Manure	6.1 a	22.2 a	1.9 a	5.4 a	61 ab	62 a		
	<i>Salix</i> /Manure rec.	6.2 a	22.3 a	1.7 a	5.3 a	65 b	67 a		
	Straw/Manure	6.0 a	22.2 a	1.6 a	5.6 a	58 a	67 a		
Sand	Control	5.9 a	0.1 a	0 a	1.2 a	18 a	9 a		
	<i>Salix</i> /Manure	6.2 b	0.3 a	0 a	1.5 a	18 a	12 a		
	<i>Salix</i> /Manure rec.	6.3 b	0.3 a	0 a	1.4 a	16 a	14 a		
	Straw/Manure	6.1 ab	0.5 b	0 a	1.4 a	16 a	10 a		
Silt	Control	6.7 a	14.9 a	0.9 a	2.8 a	51 a	25 a		
	<i>Salix</i> /Manure	6.8 a	16.0 b	1.0 a	3.0 a	52 a	27 a		
	<i>Salix</i> /Manure rec.	6.6 a	15.9 b	1.0 a	3.4 b	52 a	34 b		
	Straw/Manure	6.6 a	15.3 ab	1.0 a	3.1 a	52 a	10 c		

559

560 All treatments were carried out in triplicates. Figures followed by different letters indicate
 561 significantly different results within a soil texture class (p<0.05).

562 * AL: Plant-available P and K was estimated as the ammonium-lactate extractable fraction.

563

564 Table 4. Carbon, nitrogen, phosphorus and potassium content, pH, bulk density and air
 565 porosity in the test soils after 15 weeks of reed canary grass growth.

566

Soil	Treatment	pH	Total	Total	NH ₄ -	NO ₃ -N	P-AL	K-AL	Bulk	Air
			C	N	N					
			g kg ⁻¹		mg kg ⁻¹			g cm ⁻³		%
Loam	Control	6.3 a	23.0 a	1.9 a	7.1 a	21.3 a	51 a	114 a	1.05 a	38.4 a
	Digestate	6.0 a	26.0 b	2.1 a	6.6 a	25.3 a	67 b	164 a	0.88 b	45.9 b
Sandy soil	Control	6.8 a	2.9 a	0.2 a	2.0 a	<0.2 a	19 a	15 a	1.21 a	49.3 a
	Digestate	6.8 a	4.8 b	0.2 a	2.2 a	<0.2 a	24 b	15 a	1.21 a	45.3 a
Silt	Control	6.7 a	15.5 a	1.0 a	3.4 a	0.9 a	54 a	66 a	0.91 a	24.2 a
	Digestate	6.6 a	18.4 b	3.1 a	3.9 a	<0.2 a	66 a	91 a	0.77 a	34.1 a

567

568 All treatments were carried out in triplicates. Figures followed by different letters indicate
 569 significantly different results within a soil (p<0.05).

570

571 **Figure captions**

572 Figure 1. Italian ryegrass yield (kg DM m⁻²) in pots fertilized with biogas digestates based on
573 *Salix* or straw co-digested with manure.

574 All treatments were carried out in triplicates. Statistically significant differences in the first
575 cut are indicated by different letters in the respective bars (per soil), statistically significant
576 differences in total biomass between treatments (only in the loam) are indicated by different
577 letters on top of the figure (p<0.05).

578

579 Figure 2. N mineralization from biogas digestate produced from a mixed feedstock of *Salix*
580 and manure during 11 weeks of incubation with a loam soil. Part A shows N mineralization
581 over time in control and digestate treatments, Part B the net mineralization due to digestate
582 addition calculated as the difference between N mineralization in control and digestate
583 treatments.

584

585 Figure 3. Reed canary grass biomass (kg DM m⁻²) after 15 weeks of growth.

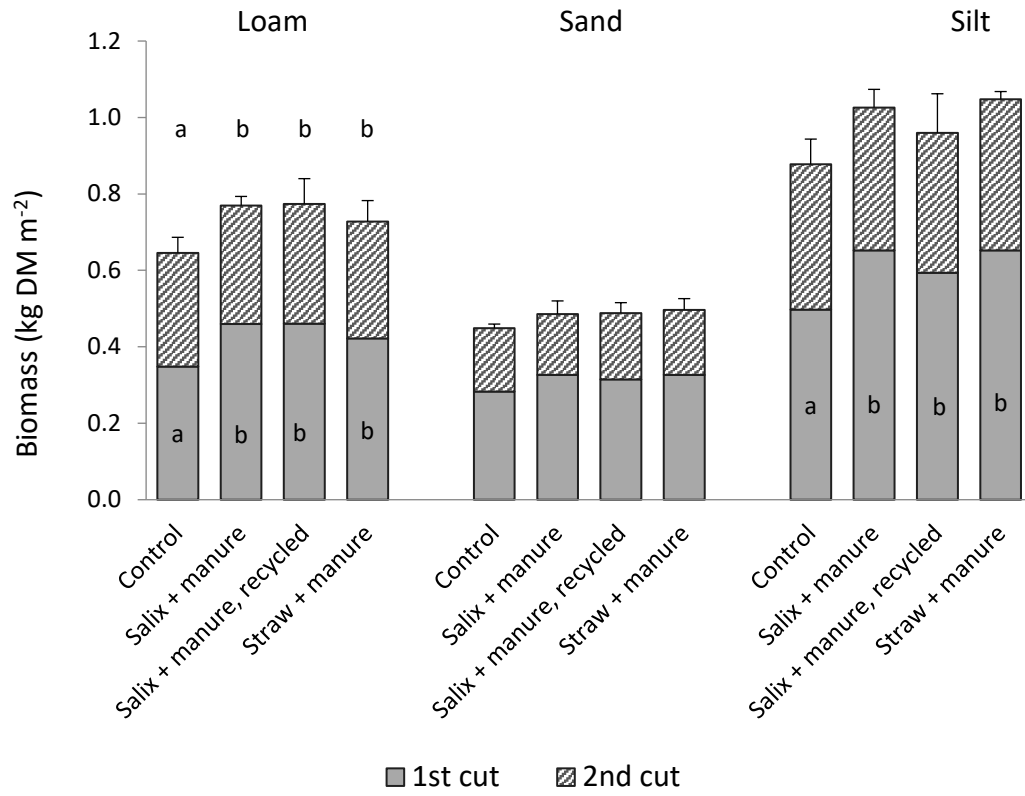
586 All treatments were carried out in triplicates. Statistically significant differences in total
587 biomass between treatments in the loam are indicated by different letters (p<0.05).

588

589 Figure 4. Water retention curve for the three soils fertilized with either mineral N (min N) or
590 bagasse and manure-based digestate after 15 weeks of reed canary grass growth.

591 All treatments were carried out in triplicates. Differences in water content at -15000 hPa in
592 the loam, and at -20, -50 and -100 hPa in the sand are statistically significant (p<0.05).

593



595

596

597

598

Figure 2

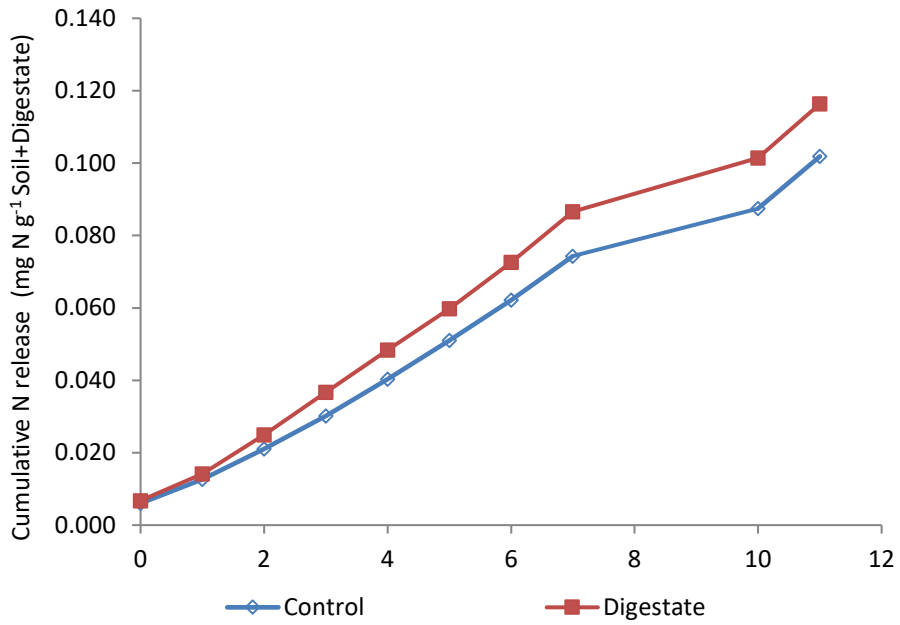
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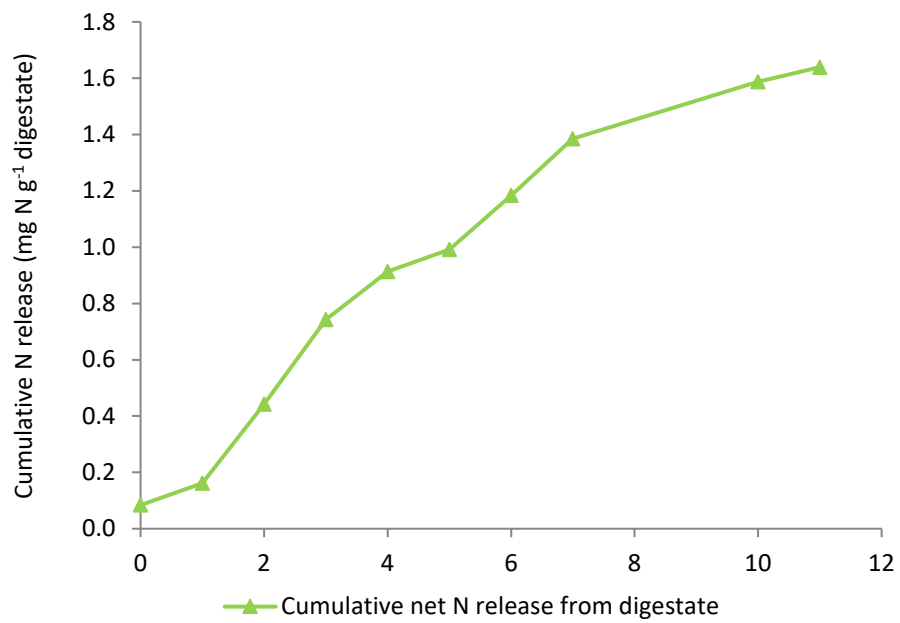
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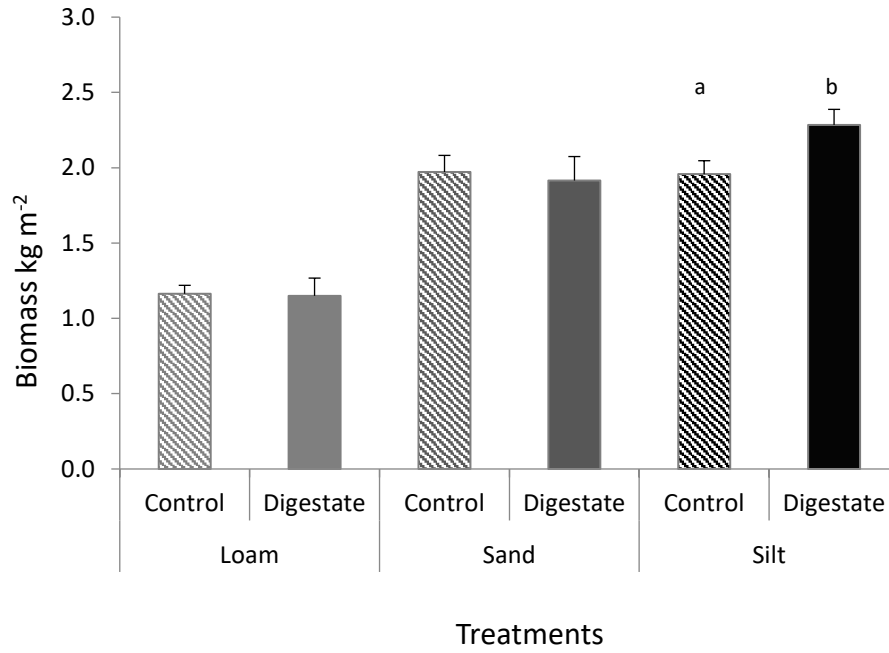
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Figure 3

604



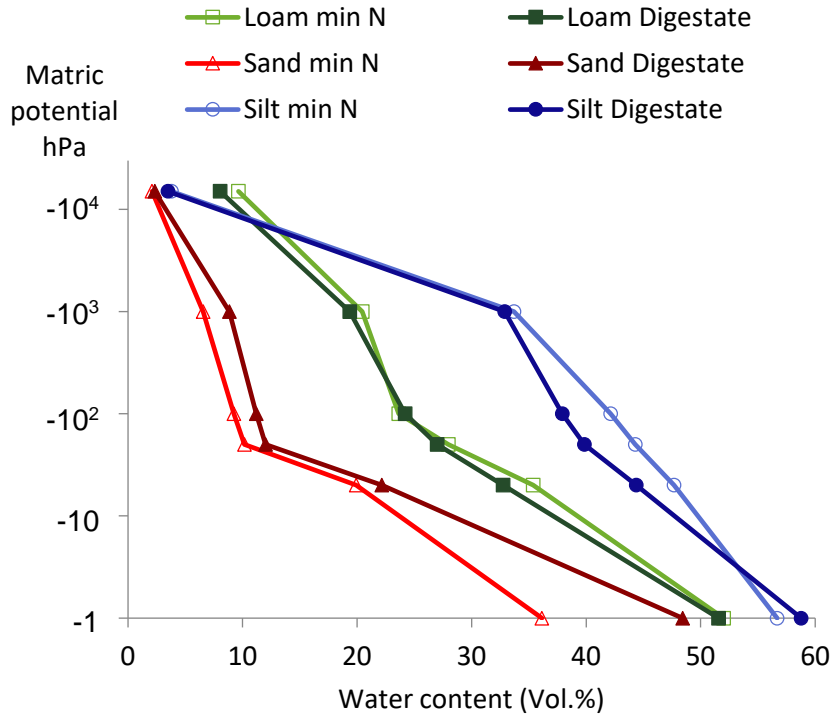
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Figure 4

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609

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