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1 **Physico-chemical characteristics and nitrogen use efficiency of nine human**  
2 **urine-based fertilizers in greenhouse conditions**

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26 like to thank the Swedish University of Agricultural Sciences and TOOPI Organics for the urine-based fertilizer  
27 samples.

28 **Abstract**

29 **Purpose:** Most of the nutrients in wastewater come from human urine and their recycling for agricultural  
30 purposes is very limited. After source separation, urine can be treated to produce various urine-based fertilizers.  
31 This study aims to characterize the nitrogen use efficiency of different urine-based fertilizers.

32 **Methods:** Nine urine-based fertilizers were compared together with ammonium nitrate and cattle slurry in a  
33 greenhouse pot trial with English rye-grass, (*Lolium perenne L.*). The detailed physico-chemical characteristics  
34 of the fertilizers were analyzed. The biomass production and nitrogen uptake of the plants were measured. The  
35 nitrogen use efficiency and the mineral fertilizer equivalent were determined for each fertilizer.

36 **Results:** The urine-based fertilizers were classified in four types based on their nitrogen forms (ammonia,  
37 nitrate, urea or organic). The mineral fertilizer equivalent coefficients of most urine-based fertilizers were above  
38 85% and even higher than 100% for nitrified and alkalinized stored urine. The lowest mineral fertilizer  
39 equivalent were found for fermented urine and the mixture of urine and woodchips but remained between 65 to  
40 75%. In all cases, the nitrogen use efficiencies of urine-based fertilizers were higher than that of cattle slurry.  
41 The differences among the urine-based fertilizers and from the cattle slurry were attributed to the mineral  
42 nitrogen content which was much higher in urine-based fertilizers.

43 **Conclusion:** Urine-based fertilizers contain mainly mineral nitrogen. Their content of trace element  
44 contaminants is low. Their efficiency as nitrogen fertilizers is high and close to that of mineral fertilizer.  
45 However, new valorization pathways from cities to agriculture need to be developed.

46 **Key words**

47 Fertilization - Greenhouse trial - Human urine - Nutrient recycling - Source separation - Urine-based fertilizer

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53 **Conflicts of interest/Competing interests:**

54 The authors have no relevant financial or non-financial interests to disclose.

55 **Availability of data and material:**

56 The datasets generated during and/or analyzed during the current study are available from the corresponding  
57 author on reasonable request.

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59 Not applicable.

60 **Author's contributions:**

61 Tristan Martin, Florent Levavasseur, Kris Dox, Fabien Esculier, Erik Smolders and Sabine Houot contributed to  
62 the study conception and design. Material preparation, data collection and analysis were performed by Tristan  
63 Martin, Kris Dox and Léa Tordera with the help of the other authors. The first draft of the manuscript was  
64 written by Tristan Martin and all authors commented on previous versions of the manuscript. All authors read  
65 and approved the final manuscript.

66 **Ethics approval:**

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## 72 **1. Introduction**

73 Wastewater contains large amounts of nutrients whose release in the environment can have undesirable  
74 environmental impacts (Sutton et al. 2011). The amount of nutrients recycled from wastewater is currently low  
75 (Esculier et al. 2018). Conventional agriculture relies on the use of synthetic nitrogen (N) fertilizers that require  
76 a substantial amount of energy for synthesis and contribute to the disruption of planetary biogeochemical cycles  
77 (Gruber and Galloway 2008). Most nutrients in wastewater have urine as their source (Friedler et al. 2013) and  
78 their recovery could offset a substantial proportion of the mineral fertilizer in agriculture (Trimmer et al. 2019).

79 Urine can be separated from the other constituents of wastewater by source separation (Rossi et al.  
80 2009). Urine is a low concentrated solution compared to mineral fertilizers. The concentration of trace elements  
81 in urine is low (Ronteltap et al. 2007), and while some pathogens can be present in urine, proper storage  
82 inactivates these pathogens to acceptable levels (WHO 2012). In contrast, pharmaceutical residues are of  
83 concern, and the need for specific urine treatments to remove them before application is currently a topic of  
84 debate (Winker 2009; WHO 2012). Collected urines are often treated for: (i) N stabilization (e.g., by  
85 nitrification), to prevent ammonia volatilization and allow volume reduction; (ii) volume reduction, to reduce  
86 transport costs and impacts as well as the work required for application; (iii) nutrient extraction to obtain  
87 concentrated fertilizers; and (iv) treatment of contaminants to produce safer fertilizers (e.g. urine storage as  
88 recommended). All treatments result in different products defined as urine-based fertilizers (UBFs).

89 The fertilization efficiency of stored urine has been studied on different crops (Pandorf et al. 2019), but  
90 other UBFs remain barely studied. As the fertilization efficiency depends on the trial conditions, it is difficult to  
91 directly compare studies. This study aimed to characterize the fertilization efficiency of nine UBFs as N  
92 fertilizers compared to mineral fertilizer and cattle slurry under the same conditions in a greenhouse trial.

## 93 **2. Material and methods**

### 94 **2.1. Urine-based fertilizers**

95 Nine UBFs were used either issued from separate collection followed by treatments intended to stabilize N  
96 (acidification, alkalization and nitrification), or from a frequent collecting practice resulting in a mixture of  
97 urine with woodchips. Detailed information on the treatments can be found in Martin et al. (2020). Some UBFs  
98 were specifically produced for this study.

99 The stored urine was collected in a university building using a waterless male urinal and stored for six  
100 months in an airtight tank. For the acidified stored urine, 31.3 mL of sulfuric acid (96% pure) was added per liter  
101 of stored urine to decrease the pH to 6.5 to reduce ammonia losses ( $pK_a \text{ NH}_3/\text{NH}_4^+ = 9.2$ ).

102 For acidified and alkalinized fresh urine as well as the mixture with woodchips, urine was collected  
103 from approximately 20 donors from the university, stored at 4°C and used within three hours. The objective of  
104 acidifying the fresh urine to below pH 4 or alkalinizing the fresh urine to above pH 11 was to prevent urea  
105 hydrolysis and stabilize the N (Hellström et al. 1999; Randall et al. 2016). To produce acidified fresh urine, we  
106 added 60 mmol  $\text{H}^+ \text{ L}^{-1}$  to fresh urine (1.61 mL  $\text{L}^{-1}$  of 96% pure sulfuric acid, Hellström et al. 1999). To produce  
107 alkalinized fresh urine, we added 10 g lime [ $\text{Ca}(\text{OH})_2$ ] per liter of fresh urine (Randall et al. 2016). In dry toilets,  
108 urine is often mixed with absorbent organic substrates. Thus, a mixture of fresh urine and woodchips was  
109 produced one week before the start of the experiment, with 1 kg of woodchips (less than 1 cm pieces) mixed  
110 with 286 g of fresh urine (the maximum amount that the woodchips could absorb).

111 Fermentation and nitrification decrease the risk of ammonia volatilization and make possible further  
112 concentration, respectively. The treatment for fermented urine was similar to the one of Andreev et al. (2017). It  
113 consisted in the acidification of fresh urine, followed by a lactic acid fermentation using lactic acid bacteria. This  
114 pilot batch was produced by the TOOPI Organics company ([www.toopi-organics.com](http://www.toopi-organics.com)). For the nitrified urine,  
115 the biological nitrification of half of the ammonia N in the stored urine was followed by volume reduction by  
116 distillation (Fumasoli et al. 2016). It was produced by the VUNA company ([www.vuna.ch](http://www.vuna.ch)). Alkalinized  
117 dehydrated urine was provided by the Swedish University of Agricultural Sciences. The urine was alkalinized  
118 using two different alkaline media : lime (20.6 g  $\text{L urine}^{-1}$ ) and lime (5.1 g  $\text{L urine}^{-1}$ ) + biochar (15 g  $\text{L urine}^{-1}$ ); and  
119 the mixtures were dehydrated (Simha et al. 2020).

120 The cattle slurry was collected in a conventional dairy farm and used as a reference organic fertilizer. Liquid  
121 ammonium nitrate was used as a reference mineral fertilizer.

122 All fertilizers were analyzed for their contents in water, carbon, nutrients, trace elements and the  
123 different forms of N (Table 1). Information on the used methods is summarized in SI. 1.

## 124 **2.2. Greenhouse experiment**

125 The greenhouse experiment was performed with English ryegrass (*Lolium perenne* L.) sown in a soil sampled  
126 from the surface horizon of a silty luvisol (Fig. 1), sieved at 4 mm and stored at 4°C before the experiment. The

127 soil was lightly carbonated (0.8% CaCO<sub>3</sub>) and had a pH (H<sub>2</sub>O) of 8.0. The organic matter content was  
128 13.6 g C kg dry soil<sup>-1</sup>, and the initial mineral N content was low (11.9 mg N kg<sup>-1</sup> dry soil). A detailed soil  
129 analysis is provided in SI. 2. The pots were filled with 1.30 kg of fresh soil (equivalent to 1.17 kg of dry soil).

130 A control treatment without N addition and 2 ammonium nitrate treatments that received 150 and  
131 250 mg N kg<sup>-1</sup> dry soil were implemented to calculate the response curve of N uptake according to fertilizer  
132 input. The target dose for cattle slurry and the UBFs was 150 mg N kg<sup>-1</sup> dry soil (175.4 mg N pot<sup>-1</sup>). Since the  
133 nutrient concentrations were not available at the start of the experiment, they were estimated; the actual doses are  
134 shown in Table 2. In order to ensure that only N would be a limiting nutrient, phosphorus (P) and potassium (K)  
135 were added as K<sub>2</sub>HPO<sub>4</sub> with 100 mg P kg dry soil<sup>-1</sup> and 250 mg K kg dry soil<sup>-1</sup>. Magnesium (Mg) was added as  
136 MgSO<sub>4</sub> with 40 mg Mg kg dry soil<sup>-1</sup> and iron (Fe) as FeSO<sub>4</sub>.7H<sub>2</sub>O with 1 mg Fe kg dry soil<sup>-1</sup>. The sulfur (S)  
137 input resulting from the Mg and Fe inputs was 53.9 mg S kg dry soil<sup>-1</sup>. All fertilizers were incorporated into the  
138 entire soil mass. One gram of ryegrass seeds was sown in each pot. Three replicates were established for each  
139 treatment. Water losses were measured by weighing, and the soil moisture was readjusted to 90% of the field  
140 capacity (22.5% humidity) three times a week using deionized water. No leachates out of the pot were observed.  
141 The positions of the pots in the greenhouse were randomized and moved twice a week to avoid the potential  
142 effects of heterogeneity in solar radiation. The grass biomass was cut 1 cm above the soil surface in each pot on  
143 days 22, 42, 63 and 75. Then, it was dried at 50°C for 5 days and powdered, after which the N concentrations  
144 were measured. At the end of the trial, the mineral N content in the soils was measured. N uptake was calculated  
145 using the N concentration in tissues and the biomass of each cut. The analytical methods are detailed in SI. 1.

### 146 **2.3. Nitrogen use efficiency and mineral fertilizer equivalent**

147 The fertilization efficiency was estimated using two calculations: the nitrogen use efficiency (NUE) and the  
148 mineral fertilizer equivalent (MFE). The NUE of ammonium nitrate corresponded to the slope of the response  
149 curve of N uptake by plants according to the amount of N added. In the UBF treatments, the NUE was directly  
150 calculated using the following equation (1):

$$151 \quad \text{NUE (\%)} = \frac{\text{Nitrogen uptake by fertilized crop} - \text{Nitrogen uptake by unfertilized control crop}}{\text{Nitrogen added by fertilizer}} \times 100 \quad (1)$$

152 The NUE of UBFs can be compared with that of mineral fertilizer by calculating the MFE as the ratio  
153 of the NUE of the UBF and the NUE of ammonium nitrate (2):

$$154 \quad \text{MFE (\%)} = \frac{\text{NUE Urine-based fertilizer}}{\text{NUE Ammonium nitrate}} \times 100 \quad (2)$$

155 The results are expressed as the mean of the 3 replicates with the standard error. Significant differences  
156 between treatments were tested using an ANOVA followed by a Tukey HSD post hoc test. Significant  
157 differences between UBF and ammonium nitrate were tested using Student's t-test, or a sign test if the  
158 distribution was not normal. All tests were performed using R, version 3.3.2 (R Core Team 2016).

### 159 **3. Results and discussion**

#### 160 **3.1. Urine-based fertilizers characteristics**

161 There was a strong difference in the N concentrations of the non-concentrated UBFs (below 7 g N L<sup>-1</sup>) and the  
162 concentrated UBFs (up to 107 g N L<sup>-1</sup>, Fig. 2). Most UBFs had N forms similar to those in the typical mineral N  
163 fertilizer: urea and ammonia or nitrate N (Fig. 2). N is excreted in urine mainly as urea (Udert et al. 2006);  
164 however, in stored urine and acidified stored urine, most of the urea is hydrolyzed during storage, and ammonia  
165 N is the main form of N. Interestingly, the content of organic N was much lower in the acidified stored urine  
166 than in the stored urine (0.04 and 1.4 g N kg<sup>-1</sup>, respectively). The organic N may have been mineralized during  
167 acidification (Antonini et al. 2012). In contrast, in fresh urine stabilized by acidification or alkalization, urea  
168 was the main form of N. We did not observe mineralization of organic N in the acidified fresh urine, but 20  
169 times less acid was added to the acidified fresh urine than was added to the acidified stored urine. The N forms  
170 in nitrified urine were half nitrate and half ammonia N with a very low content of organic N because the organic  
171 N had been mineralized during nitrification (Fumasoli et al. 2016). In the mixture of woodchips and fresh urine,  
172 most of the N was under organic form. This may be explained by N immobilization by microorganisms during  
173 storage due to the high carbon input from the woodchips (Reichel et al. 2018). The concentration of N in the  
174 woodchips was not determined, but, assuming an N content of 0.06%, as was measured in sawdust in Reichel et  
175 al. (2018), the expected concentration of the mixture would be 4.3 g N kg<sup>-1</sup> instead of the 3.7 g N kg<sup>-1</sup> measured.  
176 This suggests that at least 15% of the urine N may have been volatilized during storage. In the cattle slurry,  
177 approximately 60% of the N was under organic form, which is typical for cattle slurry (Benoît et al. 2014).

178 In addition to the nutrient concentrations, the contents of contaminants (trace elements, pathogens,  
179 pharmaceutical residues) must also be considered. Trace element concentrations were low in each UBF and in  
180 the cattle slurry (Table 1), which was also observed for stored urine by Ronteltap et al. (2007). The fluxes of  
181 trace elements in the amounts of fertilizer required to apply 200 kg N ha<sup>-1</sup> would be below the limit of the French  
182 standard for the use of sewage sludge compost in agriculture (NF U 44-095).

### 183 **3.2. Biomass production and nitrogen uptake**

184 The N uptake by the above-ground biomass according to the N input is presented in Fig. 3. The response curve  
185 for ammonium nitrate was linear ( $r^2 = 0.99$ ). Fertilizers above the response curve had higher NUE than  
186 ammonium nitrate and those below the response curve had lower NUE than ammonium nitrate.

187 A strong increase in biomass production, biomass N content and N uptake were observed in the fertilized  
188 treatments (Table 2, Fig. 3). The NUE of ammonium nitrate was 83% and was similar to those in previous pot  
189 trials (Mnkeni et al. 2008). The soil mineral N content was low at the end of the experiment, indicating that most  
190 available mineral N was taken up by the ryegrass (SI. 3).

### 191 **3.3. Mineral fertilizer equivalent**

192 Only acidified stored urine and nitrified urine presented MFE values higher than 100% (Fig. 4), although not  
193 significantly different from 100%. The MFE of the nitrified urine was significantly higher than that of all other  
194 UBFs, and the MFE of acidified stored urine was significantly higher than that of stored urine. In previous trials,  
195 the efficiency of nitrified urine and acidified stored urine was also high (Bonvin et al. 2015). The stored urine,  
196 acidified fresh urine, and all three alkalized urine samples had MFEs between 85% and 95%. Stored urine has  
197 been tested on different crops and generally showed similar or slightly lower efficiency values than mineral  
198 fertilizer (Kirchmann and Pettersson 1995; Viskari et al. 2018). The other UBFs have been little studied. A lower  
199 MFE for stored urine (91%) than for acidified stored urine (102%) has been previously observed in ryegrass  
200 (Simons 2008). The fermented urine has not been tested previously and showed a lower MFE than the other  
201 treatments. The mixture of fresh urine and woodchips had a significantly lower MFE than most UBFs, as  
202 observed for compost impregnated with urine (Martin 2018). The cattle slurry MFE (51%) was significantly  
203 lower than those of all UBFs except the mixture with woodchips; this result is consistent with that in a previous  
204 experiment (Gómez-Muñoz et al. 2017). The efficiencies observed in this trial are similar to those observed by  
205 Gutser et al. (2005) for animal urine and cattle slurry.

206 The variation in MFE values could be related to the N form. Both acidified stored urine and nitrified  
207 urine presented the highest MFEs; these UBFs contained only mineral N and no organic N. For the other UBFs  
208 (except the mixture with woodchips), the percentage of organic N ranged from 4% to 19% of the total N; this  
209 may explain the MFE values lower than 100%, because this organic fraction must be mineralized before  
210 becoming available to plants. The lower efficiencies observed for the mixture with woodchips and the cattle

211 slurry may be explained by the even higher proportion of organic N (more than 50% of the total N) in these  
212 treatments. To a lesser extent, the mineral N forms in fertilizers may impact yield and MFE (Watson 1986,  
213 1987). Even though the soil was supplemented with a mixture of other nutrients, the MFE values higher than  
214 100% may be partly explained by the micronutrient inputs from the UBFs; this is particularly true for the  
215 acidified stored urine, which had much higher sulfate content than the other treatments. In parallel with this trial,  
216 the phosphorus availability of some of the UBFs was characterized by Dox (2020); the phosphorus availability  
217 of some of these UBFs was not different from that of the mineral fertilizer, which confirmed that the UBFs can  
218 supply multiple nutrients. The lower MFE of the fermented urine may be due to the bacteria in the UBF which  
219 may have increased N immobilization in the soil. Most UBFs have a fertilizing efficiency similar to that of  
220 mineral fertilizer, i.e., most of their N is immediately available to the crops after application, contrary to organic  
221 fertilizers. Thus they could be used under similar conditions than mineral fertilizer. However the large  
222 differences in N concentration among UBFs raise the question of the technical constraints regarding the  
223 application of UBFs with very different rates of application for similar amounts of N (from 1 t ha<sup>-1</sup> for  
224 dehydrated alkalized urine to more than 30 t ha<sup>-1</sup> for fermented fresh urine to bring 100 kg N ha<sup>-1</sup>).

225 In the experimental conditions of this study, fertilizers short-term efficiencies were maximized and  
226 ammonia volatilization was greatly limited because the fertilizers were incorporated into the whole soil mass.  
227 However, under field conditions, substantial differences in ammonia volatilization can be expected due to the  
228 various pH values and N forms of the UBFs.

#### 229 **4. Conclusion**

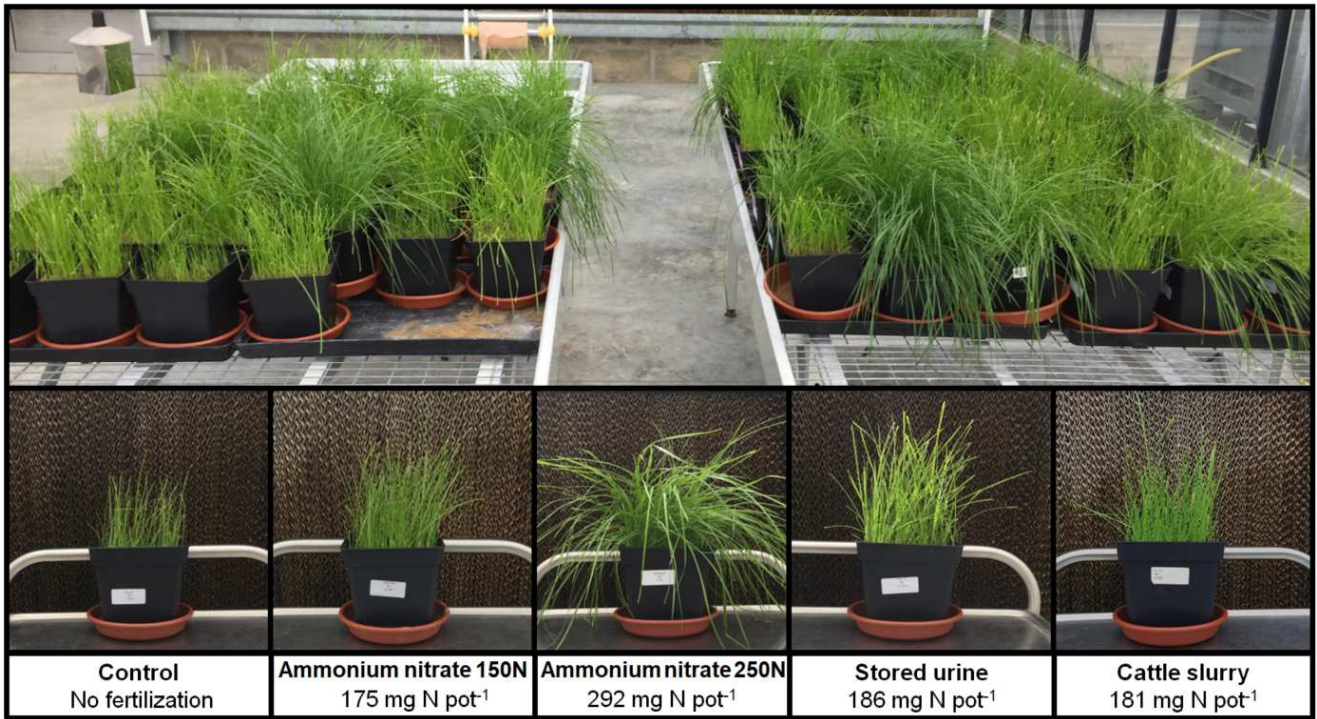
230 The mineral fertilizer equivalents (MFE) of seven out of the nine urine-based fertilizers (UBF) were similar and  
231 higher than 85%. The main factor explaining the differences in MFE was the proportion of organic N. Mixing  
232 urine with organic matter like woodchips strongly reduced the MFE. To a lesser extent, fermentation also  
233 reduced the MFE. It would be necessary to perform further trials under field conditions to confirm the  
234 tendencies. Furthermore, the fertilization efficiency of UBFs may be balanced by other aspects, such as ammonia  
235 volatilization that must be studied in real conditions of application. However, urine source separation should be  
236 developed in new neighborhoods or existing buildings and new valorization pathways adapted to the  
237 geographical context (e.g., urban characteristics, transport distance to farm) need to be implemented. The  
238 constraints associated with field application of large volume of UBFs, their insertion in fertilization strategies  
239 and the fate of contaminants potentially present in the UBFs also call for further investigation.

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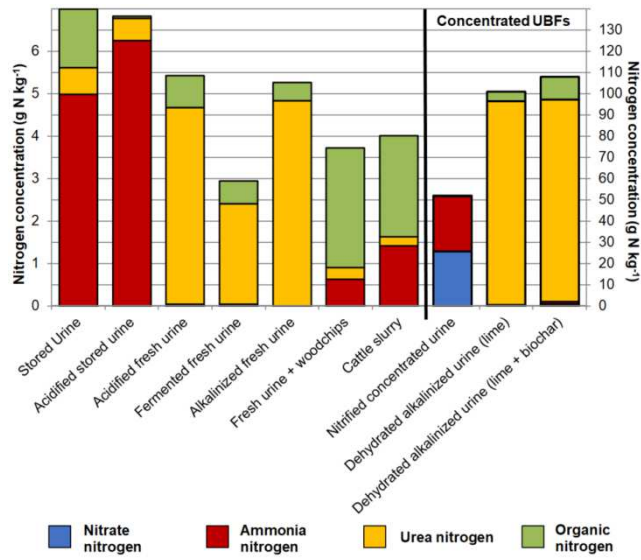
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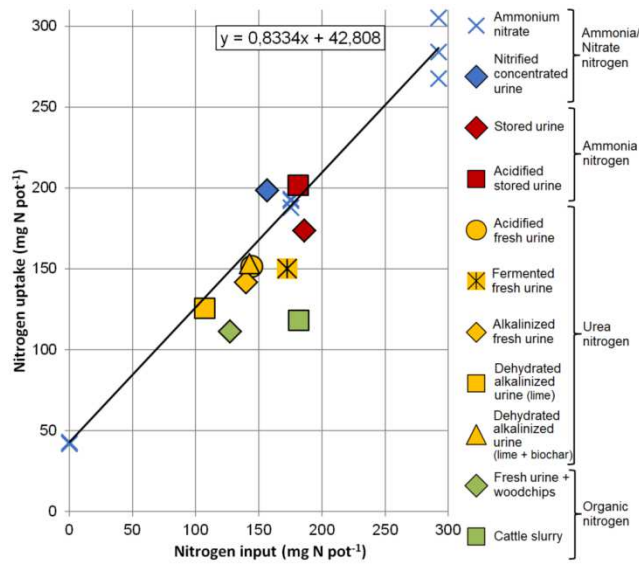


328 **Fig. 1** Greenhouse trial and biomass in some treatments before the third cut (day 63).



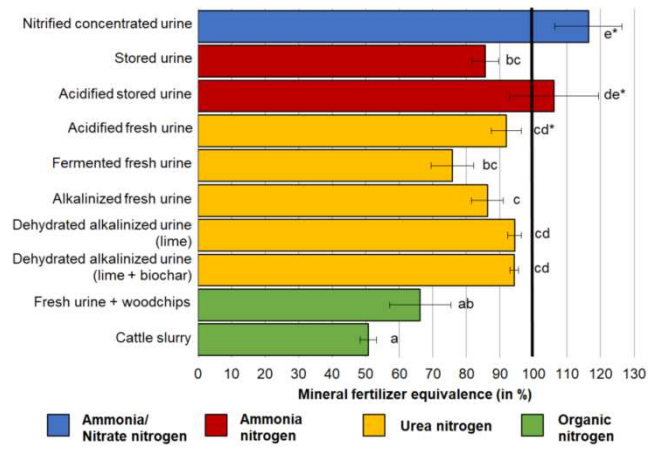
329

330 **Fig. 2** Nitrogen concentration ( $\text{g N kg}^{-1}$ ) and nitrogen forms in the different UBFs and cattle slurry. Concentrated  
 331 UBFs are plotted on another axis. Colors correspond to the nitrogen form.



332

333 **Fig. 3** Nitrogen uptake by plants according to nitrogen inputs for the different fertilizers. The linear regression  
 334 used to compute the nitrogen use efficiency of the reference fertilizer (ammonium nitrate) is represented with a  
 335 black solid line (with the corresponding equation). Colors correspond to the nitrogen form.



336

337 **Fig. 4** Mineral fertilizer equivalent (nitrogen) of the different UBFs and cattle slurry. Statistically significant  
 338 differences among treatments are represented by letters. Treatments not significantly different from 100%  
 339 (mineral fertilizer) are marked with \*. Colors correspond to the nitrogen form.  
 340

341 **Table 1.** Physico-chemical characteristics of fertilizers tested. Three samples of each product were analyzed but as the replicates were homogenous, only the mean value is  
342 presented. The density of liquids UBFs was taken as 1 except for nitrified urine for which is 1.14. Initial urine was the same between stored urine and acidified stored urine  
343 and between acidified fresh urine and liquid alkalized urine. Dry residue includes crystalized salt. \*Not measured because of crystallization. \*\*Value given by the producer  
344 (VUNA). \*\*\*As dry matter was performed at 105°C, urea chemical hydrolysis occurs at that temperature. Only trace elements were measured based on dry matter. Then, dry  
345 matter and trace elements may have been slightly underestimated.

Parameters	Unit	Nitrified concentrated urine	Stored urine	Acidified stored urine	Acidified fresh urine	Fermented fresh urine	Alkalized urine	Dehydrated alkalized urine (lime)	Dehydrated alkalized urine (lime + biochar)	Fresh urine + woodchips	Cattle slurry
pH	-	<b>4.0</b>	<b>9.2</b>	<b>6.5</b>	<b>2.1</b>	<b>3.5</b>	<b>12.3</b>	<b>11.2</b>	<b>8.9</b>	<b>8.2</b>	<b>7.3</b>
Conductivity	mS cm <sup>-1</sup>	43.3	39.0	49.5	16.3	6.2	18.2	16.2	18.9	0.8	3.1
Dry residue (liquid) or dry matter (solid)	g 100g raw material <sup>-1</sup>	-*	1.4	3.7	1.8	0.7	2.7	84.0***	82.0***	30.7	5.3
Carbon	Tot-C	-	-	-	-	-	-	129.0	331.3	153.3	22.7
	Organic-C	2.4	3.3	2.7	5.4	8.3	5.0	82.9	321.0	153.0	22.4
	Inorganic-C	-	-	-	-	-	-	46.2	10.5	0.5	0.5
Nitrogen	Total-N	<b>51.8</b>	<b>7.0</b>	<b>6.8</b>	<b>5.4</b>	<b>2.9</b>	<b>5.3</b>	<b>100.6</b>	<b>107.0</b>	<b>3.7</b>	<b>4.0</b>
	NH <sub>4</sub> -N	26.1	5.0	6.2	0.1	0.04	0.01	0.3	1.3	0.6	1.4
	NO <sub>3</sub> -N	25.6	< 0.0002	< 0.0002	0.002	< 0.0002	0.002	0.3	0.7	<0.01	0.002
	Urea-N	0.1	0.6	0.5	4.6	2.4	4.8	95.8	95.2	0.27	0.21
	Organic-N	0.1	1.4	0.04	0.8	0.5	0.4	4.5	10.5	2.8	2.4
Other nutrients	P <sub>2</sub> O <sub>5</sub>	<b>8.2</b>	<b>0.6</b>	-	<b>0.6</b>	<b>0.5</b>	-	<b>14.6</b>	<b>16.4</b>	<b>0.7</b>	<b>1.4</b>
	K <sub>2</sub> O	<b>32.2</b>	<b>2.4</b>	-	<b>1.7</b>	<b>0.6</b>	-	<b>51.6</b>	<b>50.3</b>	<b>1.4</b>	<b>3.4</b>
	MgO	0.1	< 0.1	-	< 0.1	< 0.1	-	6.5	3.3	0.2	1.0
	CaO	0.7	< 0.1	-	< 0.1	< 0.1	-	273.7	76.8	0.5	2.7
	SO <sub>3</sub>	10.9	0.6	-	1.5	0.2	-	14.6	14.8	0.7	0.8
	Na <sub>2</sub> O	24.5	3.1	-	2.3	1.0	-	49.4	50.3	1.7	1.2
	Cl-	54.8	3.9	-	2.8	1.3	-	24.8	24.2	0.6	1.2
	B	9.5	1.1	-	1.4	0.3	-	23.9	21.5	2.4	0.1
	Fe	< 18	< 20	-	< 20	< 20	-	712.0	728.2	31.9	95.5
	Cu	0.34**	< 0.4	-	< 0.4	< 0.4	-	< 2	< 2	0.4	0.5
Trace elements	Mn	0.4	< 0.2	-	< 0.2	0.3	-	44.7	46.4	14.2	36.4
	Mo	0.6	< 0.2	-	0.9	0.3	-	< 2	< 2	< 0.6	0.01
	Zn	6.5	0.1	-	1.1	1.5	-	12.6	15.6	4.5	1.8
	Se	0.4	< 0.2	-	< 0.2	< 0.2	-	< 1.3	< 1.2	< 0.5	0.01
	As	< 0.2	< 0.2	-	< 0.2	< 0.2	-	1.0	1.0	< 0.3	< 0.05
	Cd	0.03	< 0.02	-	0.05	0.06	-	< 0.1	0.07	0.11	0.04
	Co	< 0.2	< 0.2	-	< 0.2	< 0.2	-	< 0.8	< 0.8	< 0.3	0.02
	Cr	< 0.2	< 0.2	-	< 0.2	< 0.2	-	6.2	2.8	0.3	0.01
	Hg	< 0.0004	< 0.0004	-	< 0.0004	< 0.0004	-	< 0.2	< 0.2	< 0.1	< 0.01
	Ni	0.6	< 0.2	-	< 0.2	< 0.2	-	1.4	< 0.8	< 0.3	0.02
Pb	< 0.2	< 0.2	-	< 0.2	< 0.2	-	< 2	3.7	< 0.6	0.01	

346 **Table 2.** Pot experiment: experimental conditions (doses of fertilizers and nitrogen input) and results (plant biomass and nitrogen concentration, nitrogen uptake and nitrogen  
 347 use efficiency). Statistically significant (p-value  $\leq 0.05$ ) differences among treatment are represented by letters. Treatments not significantly different from ammonium nitrate  
 348 are marked with \* (p-value  $>0.05$ ).

Treatment	Fertilizer doses (g pot <sup>-1</sup> )	Nitrogen input (mg N pot <sup>-1</sup> )	Dry biomass (g DM pot <sup>-1</sup> )		Dry biomass nitrogen tissue concentration (mg N g DM <sup>-1</sup> )		Nitrogen uptake (mg N pot <sup>-1</sup> )		Nitrogen use efficiency (%)		Mineral fertilizer equivalent (%)	
			Mean	±	Mean	±	Mean	±	Mean	±	Mean	±
Control	0	0.0	1.63	0.02	25.9	0.5	42	1	-	-	-	-
Ammonium nitrate	150N	3.51	4.48	0.03	42.6	0.4	191	3	83	3	100	-
	250N	5.85	6.60	0.11	43.2	2.2	285	19				
Nitrified concentrated urine	3.02	157	4.99	0.04	39.8	3.0	198	13	100 <sup>e*</sup>	8	120 <sup>e*</sup>	10
Stored urine	26.57	186	4.29	0.16	40.5	0.4	174	6	71 <sup>c</sup>	3	86 <sup>c</sup>	4
Acidified stored urine	26.57	181	4.94	0.34	40.8	2.0	202	20	88 <sup>de*</sup>	11	106 <sup>de*</sup>	13
Acidified fresh urine	26.57	144	4.14	0.20	36.7	0.8	152	5	77 <sup>cd*</sup>	4	92 <sup>cd*</sup>	4
Fermented fresh urine	58.46	172	4.33	0.25	34.7	2.0	150	9	63 <sup>bc</sup>	5	76 <sup>bc</sup>	6
Alkalinized urine	26.57	140	3.78	0.13	37.5	0.5	142	6	72 <sup>c</sup>	4	86 <sup>c</sup>	5
Dehydrated alkalinized urine (lime)	1.06	107	3.62	0.09	34.7	1.2	126	2	79 <sup>cd</sup>	2	94 <sup>cd</sup>	2
Dehydrated alkalinized urine (lime + biochar)	1.33	142	4.08	0.26	37.6	2.1	153	2	79 <sup>cd</sup>	1	94 <sup>cd</sup>	1
Fresh urine + woodchips	34.17	127	3.35	0.46	33.6	4.3	111	10	55 <sup>ab</sup>	8	66 <sup>ab</sup>	9
Cattle slurry	44.97	181	3.30	0.09	35.7	0.6	118	4	42 <sup>a</sup>	2	51 <sup>a</sup>	2

349

# Physico-chemical characteristics and nitrogen use efficiency of nine human urine-based fertilizers in greenhouse conditions

## Journal of Soil Science and Plant Nutrition

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## Supplementary Information

### SI. 1 Analysis method for plant and fertilizers

#### Fertilizers analysis:

Parameters	Methods liquid product	Methods for solid product and cattle slurry	
<b>pH</b>	ISO 10523	NF EN 13037	
<b>Conductivity</b>	ISO 7888	NF EN 13038	
<b>Dry matter</b>	Determination of dry residue at 105 ° CNF EN 13040		
<b>Carbon</b>	<b>Tot-C</b>	- ISO 10694	
	<b>Organic-C</b>	NF EN 1484 ISO 10694	
	<b>Inorganic-C</b>	- NF U44-001	
<b>Nitrogen</b>	<b>Total-N</b>	Kjeldahl method NF EN 25663 + NO <sub>3</sub> Kjeldahl Method + NO <sub>3</sub> (NF EN 13654.-2 for dehydrated alkalinized urine)	
	<b>NH<sub>4</sub>-N</b>	Berthelot and Griess method	NF EN 13652
	<b>NO<sub>3</sub>-N</b>		NF EN 13652
	<b>Urea-N</b>		PDAB colorimetry. N total – N mineral – N organic for the mixture with woodchips
	<b>Organic-N</b>		N total – N mineral
	<b>Other nutrients</b>	<b>P<sub>2</sub>O<sub>5</sub></b>	
<b>K<sub>2</sub>O</b>		ICP MS dosage	NF EN 13650
<b>MgO</b>			
<b>CaO</b>			
<b>Na<sub>2</sub>O</b>			
<b>SO<sub>3</sub></b>		ISO 11885	NF EN 13650 ISO 11885
<b>Cl-</b>	ISO 10304-1	ISO 10304-1	
<b>Trace metals</b>	<b>B</b>		
	<b>Fe</b>		
	<b>Cu</b>		
	<b>Mn</b>		
	<b>Mo</b>		
	<b>Zn</b>	ICP MS dosage	NF EN 13650
	<b>Se</b>		
	<b>As</b>		
	<b>Cd</b>		
	<b>Co</b>		
	<b>Cr</b>		
	<b>Ni</b>		
<b>Pb</b>			
<b>Hg</b>	NF EN 1484	Dry combustion and cold vapor AAS dosage	

**Plant analysis:**

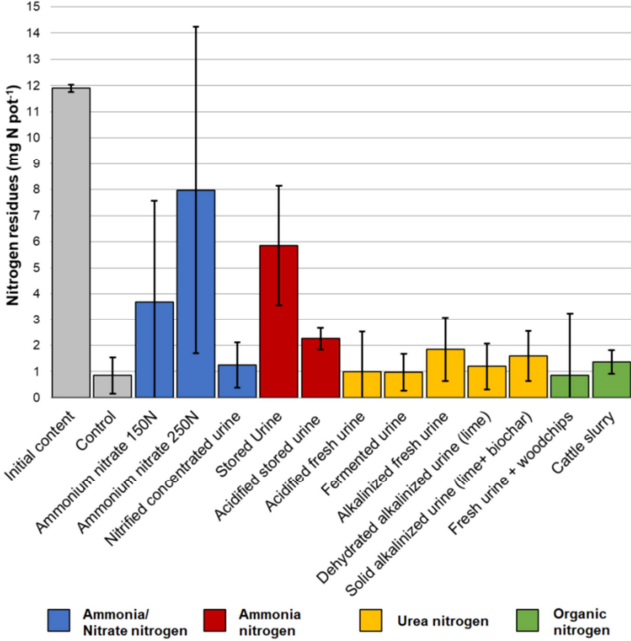
The dried samples of each replicate pot and harvest were powdered. Subsamples of 50 mg were digested on 10 mL tubes with 1 mL of 67-70% HNO<sub>3</sub> and allowed to rest overnight prior to boiling for about 4h to almost dryness, then diluted to 10 mL and analyzed for elements by ICP-OES. Internal reference material was used to verify analytical accuracy. Total N was measured by dry combustion with an elemental analyzer (Thermo Scientific, EA1108) via combustion at 900 °C and subsequent analysis of CO<sub>2</sub> and N<sub>2</sub> with gas chromatography.

## SI. 2 Detailed soil analysis

Parameter	Unit	Mean	Standard deviation	Method	
Soil texture	Clay	259	3	Pipette method with decarbonation	
	Fine silt	240	6		
	Coarse silt	407	5		
	Fine sand	65.1	1.1		
	Coarse sand	25.7	1.0		
General characteristics	pH (water)	-	8.0	0	ISO 10390
	pH CaCl <sub>2</sub>	-	6.7	-	ISO 10390
	CEC	mEq kg <sup>-1</sup>	179	1	Cobaltihexamine extraction / ISO 23470
	C/N ratio	-	9.6	0.2	-
	Total organic carbon		13.6	0.1	ISO 14235
	Organic matter		23.5	0.2	ISO 14235
	Total carbonate		8	1	ISO 10693
Nitrogen	Total nitrogen		1.41	0.03	Dumas method / ISO 13878
	NH <sub>4</sub>		0.0003	0.00	KCl extraction (1 M), Berthelot method for NH <sub>4</sub> . Cd reduction and Griess method for NO <sub>3</sub> . NH <sub>4</sub> + NO <sub>3</sub> for mineral nitrogen.
	NO <sub>3</sub>		0.012	0.00	
	Mineral nitrogen	g kg <sup>-1</sup>	0.012	0.00	
	Other nutrients	P <sub>2</sub> O <sub>5</sub> Olsen		0.042	0
Sulfur			0.26	0.02	
K <sub>2</sub> O exchangeable			0.41	0	
MgO exchangeable			0.20	0	
CaO exchangeable			4.65	0.04	Cobaltihexamine extraction / ISO 23470
Na <sub>2</sub> O exchangeable			0.013	0.002	Boiling water extraction
Boron			0.50	0.01	
Copper			2.7	0.0	
Iron		mg kg <sup>-1</sup>	15.3	2.4	
Manganese			8.4	0.9	
Zinc			2.7	0.1	
Chloride	9.80		0.31		
Molybdenum		<2	0	ISO 11466 / ICP-MS dosage (ISO 17294)	

**SI. 3 Nitrogen residues at the end of the experimentation**

An extraction was performed with KCl (1 mol L<sup>-1</sup>). Ammonia nitrogen was measured using Berthelot colorimetric method. Nitrite and nitrate were measured using a reduction on a cadmium column and the Griess colorimetric method. Colors correspond to the nitrogen form.



Nitrogen residues were low for each treatment at the end of the experiment indicating a good uptake by crops. They are all below the initial nitrogen content before the launching of the experiment.