Physico-chemical Characteristics and Nitrogen Use Efficiency of Nine Human Urine-Based Fertilizers in Greenhouse Conditions

Tristan Martin, Florent Levavasseur, Kris Dox, Léa Tordera, Fabien Esculier, Erik Smolders, Sabine Houot

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

Tristan M.P. Martin[1, 2*], Florent Levavasseur[1], Kris Dox[3], Léa Tordera[1], Fabien Esculier[2, 4], Erik Smolders[3], Sabine Houot[1]

[1]*Université Paris-Saclay, INRAE, AgroParisTech, UMR ECOSYS, Avenue Lucien Bretignières, 78850, Thiverval-Grignon, France*

[2]*École des Ponts ParisTech, UPEC, UMR LEESU, 6-8 Avenue Blaise Pascal, Champs-sur-Marne, 77455 Marne-la-Vallée, France*

[3]*Division of Soil and Water Management, Department of Earth and Environmental Science, KU Leuven, Kasteelpark Arenberg 20, B-3001 Heverlee, Belgium.*

[4] *Sorbonne Universités, CNRS, EPHE, UMR METIS, 4 Place Jussieu, 75005 Paris, France*

***Corresponding author:** Tristan Martin

E-mail address: tristan.martin@inrae.fr

ORCID iD:

Tristan M.P. Martin: https://orcid.org/0000-0002-4008-825X

Florent Levavasseur: https://orcid.org/0000-0002-2164-3334

Kris Dox : https://orcid.org/0000-0003-3035-9164

Fabien Esculier: https://orcid.org/0000-0001-9982-4082

Erik Smolders: https://orcid.org/0000-0003-3054-2444

Sabine Houot: https://orcid.org/0000-0001-7297-7038

Acknowledgments:

This study is part of the AGROCAPI project (www.leesu.fr/ocapi). We would like to thank ADEME (Agence de l'environnement et de la maîtrise de l'énergie), SEDE Environnement, SIAAP (Syndicat interdépartemental pour l'assainissement de l'agglomération parisienne) and the Paris-Saclay University for their financial support. We also wanted to thank Pascal Denoroy to allow us to use the soil from the Folleville long-term trial. We would like to thank the Swedish University of Agricultural Sciences and TOOPI Organics for the urine-based fertilizer samples.

Abstract

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

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

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

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

Key words

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

Declarations

Funding:

This work was supported by ADEME (Agence de l'environnement et de la maîtrise de l'énergie); SEDE Environnement; SIAAP (Syndicat interdépartemental pour l'assainissement de l'agglomération parisienne); and « IDI 2017 » project funded by the IDEX Paris-Saclay, ANR-11-ISEX-0003-02.

Conflicts of interest/Competing interests:

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

Availability of data and material:

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

Code availability:

Not applicable.

Author's contributions:

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

Ethics approval:

Not applicable.

Consent to participate:

Not applicable.

Consent for publication:

Not applicable.

1. Introduction

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

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

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

2. Material and methods

2.1. Urine-based fertilizers

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

The stored urine was collected in a university building using a waterless male urinal and stored for six months in an airtight tank. For the acidified stored urine, 31.3 mL of sulfuric acid (96% pure) was added per liter of stored urine to decrease the pH to 6.5 to reduce ammonia losses (pKa NH_3/NH_4^+ = 9.2).

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

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

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

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

2.2. Greenhouse experiment

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

soil was lightly carbonated (0.8% CaCO3) and had a pH (H_2O) of 8.0. The organic matter content was 13.6 g C kg dry soil^{-1}, and the initial mineral N content was low (11.9 mg N kg^{-1} dry soil). A detailed soil 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).

A control treatment without N addition and 2 ammonium nitrate treatments that received 150 and 250 mg N kg^{-1} dry soil were implemented to calculate the response curve of N uptake according to fertilizer input. The target dose for cattle slurry and the UBFs was 150 mg N kg^{-1} dry soil (175.4 mg N pot^{-1}). Since the nutrient concentrations were not available at the start of the experiment, they were estimated; the actual doses are shown in Table 2. In order to ensure that only N would be a limiting nutrient, phosphorus (P) and potassium (K) were added as K_2HPO_4 with 100 mg P kg dry soil^{-1} and 250 mg K kg dry soil^{-1}. Magnesium (Mg) was added as $MgSO_4$ with 40 mg Mg kg dry soil^{-1} and iron (Fe) as $FeSO_4.7H_2O$ with 1 mg Fe kg dry soil^{-1}. The sulfur (S) input resulting from the Mg and Fe inputs was 53.9 mg S kg dry soil^{-1}. All fertilizers were incorporated into the entire soil mass. One gram of ryegrass seeds was sown in each pot. Three replicates were established for each treatment. Water losses were measured by weighing, and the soil moisture was readjusted to 90% of the field capacity (22.5% humidity) three times a week using deionized water. No leachates out of the pot were observed. The positions of the pots in the greenhouse were randomized and moved twice a week to avoid the potential effects of heterogeneity in solar radiation. The grass biomass was cut 1 cm above the soil surface in each pot on days 22, 42, 63 and 75. Then, it was dried at 50°C for 5 days and powdered, after which the N concentrations were measured. At the end of the trial, the mineral N content in the soils was measured. N uptake was calculated using the N concentration in tissues and the biomass of each cut. The analytical methods are detailed in SI. 1.

2.3. Nitrogen use efficiency and mineral fertilizer equivalent

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

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

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

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

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^{-1}) and the
162 concentrated UBFs (up to 107 g N L^{-1}, 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^{-1}, respectively). The organic N may have been mineralized during
167 acidification (Antonini et al. 2012). In contrast, in fresh urine stabilized by acidification or alkalinization, 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^{-1} instead of the 3.7 g N kg^{-1} 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^{-1} would be below the limit of the French
182 standard for the use of sewage sludge compost in agriculture (NF U 44-095).

3.2. Biomass production and nitrogen uptake

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

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

3.3. Mineral fertilizer equivalent

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

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

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

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

4. Conclusion

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

References

Antonini S, Nguyen PT, Arnold U, Eichert T, Clemens J (2012) Solar thermal evaporation of human urine for nitrogen and phosphorus recovery in Vietnam. Sci Total Environ 414:592–599. https://doi.org/10.1016/j.scitotenv.2011.11.055

Benoît P, Brugère H, Casellas M, Dabert P, Fuchs J, Giamberini L, Patureau D, Pons M-N, Pourcher A-M (2014) Physico-chemical and biological characteristics of fertilizing materials of residual origin [Caractéristiques physico-chimiques et biologiques des matières fertilisantes d'origine résiduelle]. In: Houot, S., Pons, M.-N., Pradel, M. (Eds.) Valorization of fertilizers of residual origin on soils for agricultural or forestry use. Final report of the collective scientific expertise [Valorisation des matières fertilisantes d'origine résiduaire sur les sols à usage agricole ou forestier. Rapport final de l'expertise scientifique collective]. INRA, CNRS, Irstea, France, pp 115–363

Bonvin C, Etter B, Udert KM, Frossard E, Nanzer S, Tamburini F, Oberson A (2015) Plant uptake of phosphorus and nitrogen recycled from synthetic source-separated urine. AMBIO 44:217–227. https://doi.org/10.1007/s13280-014-0616-6

Dox K (2020) Phosphorus recycling from urine using layered double hydroxides. PhD Thesis, KU LEUVEN

Esculier F, Le Noë J, Barles S, Billen G, Créno B, Garnier J, Lesavre J, Petit L, Tabuchi J-P (2018) The biogeochemical imprint of human metabolism in Paris Megacity: A regionalized analysis of a water-agro-food system. J Hydrol. https://doi.org/10.1016/j.jhydrol.2018.02.043

Friedler E, Butler D, Alfiya Y (2013) Wastewater composition. In: Larsen, T. A., Udert, K. M. & Lienert, J. (Eds.) Source Separation and Decentralization for Wastewater Management. IWA Publishing, London, pp 241–257

Fumasoli A, Etter B, Sterkele B, Morgenroth E, Udert KM (2016) Operating a pilot-scale nitrification/distillation plant for complete nutrient recovery from urine. Water Sci Technol 73:215–222. https://doi.org/10.2166/wst.2015.485

Gómez-Muñoz B, Magid J, Jensen LS (2017) Nitrogen turnover, crop use efficiency and soil fertility in a long-term field experiment amended with different qualities of urban and agricultural waste. Agric Ecosyst Environ 240:300–313. https://doi.org/10.1016/j.agee.2017.01.030

Gruber N, Galloway JN (2008) An Earth-system perspective of the global nitrogen cycle. Nature 451:293–296. https://doi.org/10.1038/nature06592

Gutser R, Ebertseder Th, Weber A, Schraml M, Schmidhalter U (2005) Short-term and residual availability of nitrogen after long-term application of organic fertilizers on arable land. J Plant Nutr Soil Sci 168:439–446. https://doi.org/10.1002/jpln.200520510

Hellström D, Johansson E, Grennberg K (1999) Storage of human urine: acidification as a method to inhibit decomposition of urea. Ecol Eng 12:253–269. https://doi.org/10.1016/S0925-8574(98)00074-3

Kirchmann H, Pettersson S (1995) Human urine-chemical composition and fertilizer use efficiency. Fertil Res 40:149–154. https://doi.org/10.1007/BF00750100

Martin TMP (2018) Valorization of human urine as a source of nitrogen for plants: a greenhouse experiment [Valorisation des urines humaines comme source d'azote pour les plantes : une expérimentation en serre]. Master Thesis, Université Paris Diderot

Martin TMP, Esculier F, Levavasseur F, Houot S (2020) Human urine-based fertilizers: A review. Crit Rev Environ Sci Technol 0:1–47. https://doi.org/10.1080/10643389.2020.1838214

Mnkeni PNS, Kutu FR, Muchaonyerwa P, Austin LM (2008) Evaluation of human urine as a source of nutrients for selected vegetables and maize under tunnel house conditions in the Eastern Cape, South Africa. Waste Manag Res 26:132–139. https://doi.org/10.1177/0734242X07079179

Pandorf M, Hochmuth G, Boyer TH (2019) Human Urine as a Fertilizer in the Cultivation of Snap Beans (*Phaseolus vulgaris*) and Turnips (*Brassica rapa*). J Agric Food Chem 67:50–62. https://doi.org/10.1021/acs.jafc.8b06011

R Core Team (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria

Randall DG, Krähenbühl M, Köpping I, Larsen TA, Udert KM (2016) A novel approach for stabilizing fresh urine by calcium hydroxide addition. Water Res 95:361–369. https://doi.org/10.1016/j.watres.2016.03.007

Reichel R, Wei J, Islam MS, Schmid C, Wissel H, Schröder P, Schloter M, Brüggemann N (2018) Potential of Wheat Straw, Spruce Sawdust, and Lignin as High Organic Carbon Soil Amendments to Improve Agricultural Nitrogen Retention Capacity: An Incubation Study. Front Plant Sci 9. https://doi.org/10.3389/fpls.2018.00900

Ronteltap M, Maurer M, Gujer W (2007) The behaviour of pharmaceuticals and heavy metals during struvite precipitation in urine. Water Res 41:1859–1868. https://doi.org/10.1016/j.watres.2007.01.026

Rossi L, Lienert J, Larsen TA (2009) Real-life efficiency of urine source separation. J Environ Manage 90:1909–1917. https://doi.org/10.1016/j.jenvman.2009.01.006

Simha P, Lalander C, Nordin A, Vinnerås B (2020) Alkaline dehydration of source-separated fresh human urine: Preliminary insights into using different dehydration temperature and media. Sci Total Environ 733:139313. https://doi.org/10.1016/j.scitotenv.2020.139313

Simons J (2008) Suitability of nutrient-rich substrates from central & decentralized wastewater treatment as fertilizer [Eignung nährstoffreicher Substrate aus zentraler & dezentraler Abwasserbehandlung als Düngemittel]. PhD Thesis, Rheinischen Friedrich-Wilhelms-Universität Bonn

Sutton EMA, Howard CM, Erisman JW, Billen G, Bleeker A, Bleeker A, Grennfelt P, van Grinsven H, Grizzetti B (2011) The European Nitrogen Assessment: Sources, Effects and Policy Perspectives. Cambridge University Press, Cambridge

Trimmer JT, Margenot AJ, Cusick RD, Guest JS (2019) Aligning Product Chemistry and Soil Context for Agronomic Reuse of Human-Derived Resources. Environ Sci Technol 53:6501–6510. https://doi.org/10.1021/acs.est.9b00504

Udert KM, Larsen TA, Gujer W (2006) Fate of major compounds in source-separated urine. Water Sci Technol 54:413–420. https://doi.org/10.2166/wst.2006.921

Viskari E-L, Grobler G, Karimäki K, Gorbatova A, Vilpas R, Lehtoranta S (2018) Nitrogen Recovery With Source Separation of Human Urine—Preliminary Results of Its Fertiliser Potential and Use in Agriculture. Front Sustain Food Syst 2. https://doi.org/10.3389/fsufs.2018.00032

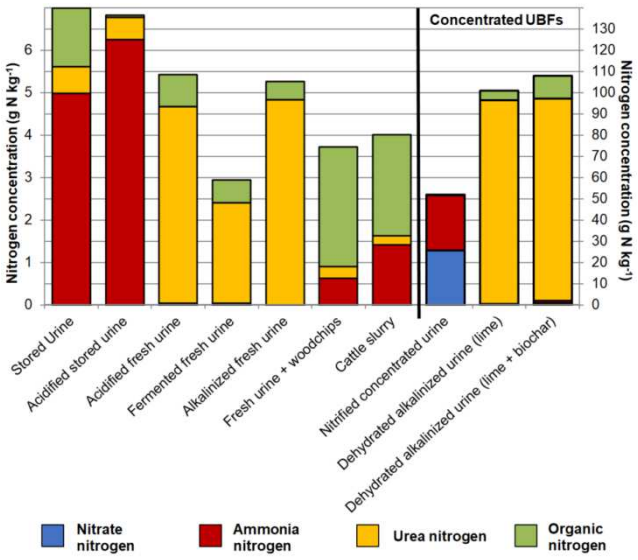
Watson CJ (1986) Preferential uptake of ammonium nitrogen from soil by ryegrass under simulated spring conditions. J Agric Sci 107:171–177. https://doi.org/10.1017/S0021859600066922

321 Watson CJ (1987) The comparative effects of ammonium nitrate, urea or a combined ammonium
322 nitrate/urea granular fertilizer on the efficiency of nitrogen recovery by perennial ryegrass. Fertil Res
323 11:69–78. https://doi.org/10.1007/BF01049565

324 WHO (2012) WHO guifelines for the safe use of wastewater, excreta and greywater. Volume 4
325 Excreta and greywater use in agriculture. World Health Organization, Geneva

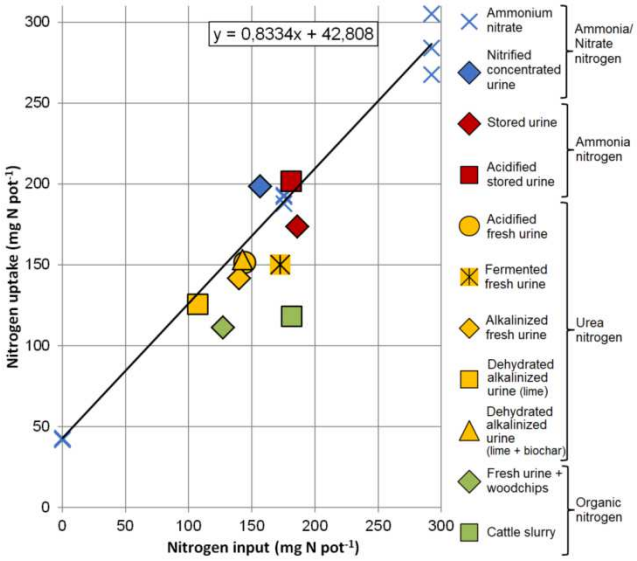
326 Winker M (2009) Pharmaceuticals residues in urine and potential risks related to usage as fertiliser in
327 agriculture. PhD Thesis, Technischen Universität Hamburg-Harburg

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



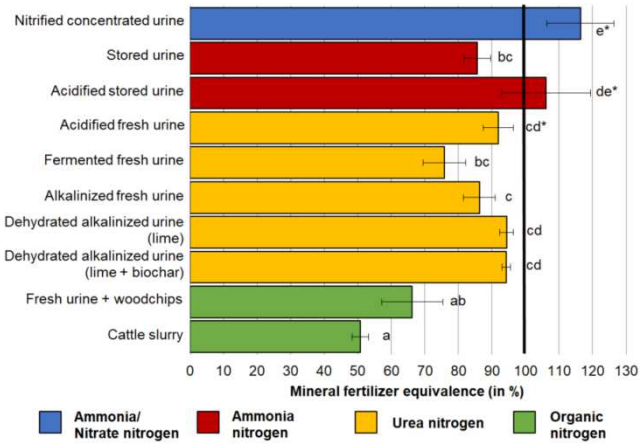
329

330 **Fig. 2** Nitrogen concentration (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

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 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 and between acidified fresh urine and liquid alkalinized urine. Dry residue includes crystalized salt. *Not measured because of crystallization. **Value given by the producer (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 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	Alkalinized urine	Dehydrated alkalinized urine (lime)	Dehydrated alkalinized urine (lime + biochar)	Fresh urine + woodchips	Cattle slurry
pH		-	**4.0**	**9.2**	**6.5**	**2.1**	**3.5**	**12.3**	**11.2**	**8.9**	**8.2**	**7.3**
Conductivity		mS cm^{-1}	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 $material^{-1}$	-*	1.4	3.7	1.8	0.7	2.7	84.0***	82.0***	30.7	5.3
Carbon	**Tot-C**	g kg raw $material^{-1}$	-	-	-	-	-	-	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**		**51.8**	**7.0**	**6.8**	**5.4**	**2.9**	**5.3**	**100.6**	**107.0**	**3.7**	**4.0**
	NH_4-N		26.1	5.0	6.2	0.1	0.04	0.01	0.3	1.3	0.6	1.4
	NO_3-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_2O_5**		**8.2**	**0.6**	**-**	**0.6**	**0.5**	**-**	**14.6**	**16.4**	**0.7**	**1.4**
	K_2O		**32.2**	**2.4**	**-**	**1.7**	**0.6**	**-**	**51.6**	**50.3**	**1.4**	**3.4**
	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_3		10.9	0.6	-	1.5	0.2	-	14.6	14.8	0.7	0.8
	Na_2O		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
Trace elements	**B**	mg kg raw $material^{-1}$	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
	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

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

Treatment		Fertilizer doses (g pot^{-1})	Nitrogen input (mg N pot^{-1})	Dry biomass (g DM pot^{-1})		Dry biomass nitrogen tissue concentration (mg N g DM^{-1})		Nitrogen uptake (mg N pot^{-1})		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	175	4.48	0.03	42.6	0.4	191	3	83	3	100	-
	250N	5.85	292	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^{e}*	8	120^{e}*	10
Stored urine		26.57	186	4.29	0.16	40.5	0.4	174	6	71^{c}	3	86^{c}	4
Acidified stored urine		26.57	181	4.94	0.34	40.8	2.0	202	20	88^{de}*	11	106^{de}*	13
Acidified fresh urine		26.57	144	4.14	0.20	36.7	0.8	152	5	77^{cd}*	4	92^{cd}*	4
Fermented fresh urine		58.46	172	4.33	0.25	34.7	2.0	150	9	63^{bc}	5	76^{bc}	6
Alkalinized urine		26.57	140	3.78	0.13	37.5	0.5	142	6	72^{c}	4	86^{c}	5
Dehydrated alkalinized urine (lime)		1.06	107	3.62	0.09	34.7	1.2	126	2	79^{cd}	2	94^{cd}	2
Dehydrated alkalinized urine (lime + biochar)		1.33	142	4.08	0.26	37.6	2.1	153	2	79^{cd}	1	94^{cd}	1
Fresh urine + woodchips		34.17	127	3.35	0.46	33.6	4.3	111	10	55^{ab}	8	66^{ab}	9
Cattle slurry		44.97	181	3.30	0.09	35.7	0.6	118	4	42^{a}	2	51^{a}	2

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

Journal of Soil Science and Plant Nutrition

Tristan M.P. Martin[1, 2*], Florent Levavasseur[1], Kris Dox[3], Léa Tordera[1], Fabien Esculier[2, 4], Erik Smolders[3], Sabine Houot[1]

[1]*Université Paris-Saclay, INRAE, AgroParisTech, UMR ECOSYS, Avenue Lucien Bretignières, 78850, Thiverval-Grignon, France*

[2]*École des Ponts ParisTech, UPEC, UMR LEESU, 6-8 Avenue Blaise Pascal, Champs-sur-Marne, 77455 Marne-la-Vallée, France*

[3]*Division of Soil and Water Management, Department of Earth and Environmental Science, KU Leuven, Kasteelpark Arenberg 20, B-3001 Heverlee, Belgium.*

[4] *Sorbonne Universités, CNRS, EPHE, UMR METIS, 4 Place Jussieu, 75005 Paris, France*

***Corresponding author:** Tristan Martin

E-mail address: tristan.martin@inrae.fr

Supplementary Information

SI. 1 Analysis method for plant and fertilizers

Fertilizers analysis:

Parameters		Methods liquid product	Methods for solid product and cattle slurry
pH		ISO 10523	NF EN 13037
Conductivity		ISO 7888	NF EN 13038
Dry matter		Determination of dry residue at 105 ° C	NF EN 13040
Carbon	**Tot-C**	-	ISO 10694
	Organic-C	NF EN 1484	ISO 10694
	Inorganic-C	-	NF U44-001
Nitrogen	**Total-N**	Kjeldahl method NF EN 25663 + NO_3	Kjeldahl Method + NO_3 (NF EN 13654.-2 for dehydrated alkalinized urine)
	NH_4-N	Berthelot and Griess method	NF EN 13652
	NO_3-N		NF EN 13652
	Urea-N		PDAB colorimetry. N total – N mineral – N organic for the mixture with woodchips
	Organic-N		N total – N mineral
Other nutrients	**P_2O_5**	ICP MS dosage ISO 17294-2	NF EN 13650 ISO 17294
	K_2O		
	MgO		
	CaO		
	Na_2O		
	SO_3	ISO 11885	NF EN 13650 ISO 11885
	Cl-	ISO 10304-1	ISO 10304-1
Trace metals	**B**	ICP MS dosage ISO 17294-2	NF EN 13650 ISO 17294
	Fe		
	Cu		
	Mn		
	Mo		
	Zn		
	Se		
	As		
	Cd		
	Co		
	Cr		
	Ni		
	Pb		
	Hg	NF EN 1484	Dry combustion and cold vapor AAS dosage

Plant analysis:

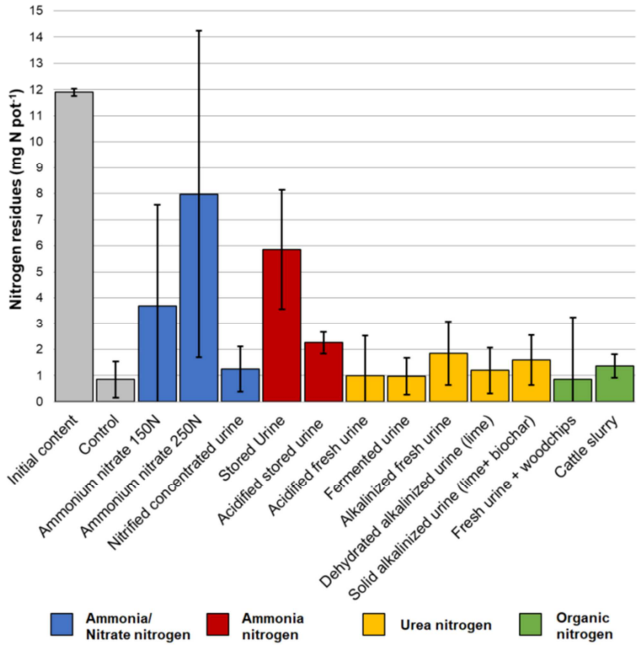
The dried samples of each replicate pot and harvest were powdered. Subsamples of 50 mg were digested on 10 mL tubes with 1 mL of 67-70% HNO_3 and allowed to rest overnight prior to boiling for about 4h to almost dryness, then diluted to 10 mL and analyzed for elements by ICP-OES. Internal reference material 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_2 and N_2 with gas chromatography.

SI. 2 Detailed soil analysis

Parameter		Unit	Mean	Standard deviation	Method
Soil texture	**Clay**	g kg^{-1}	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 $CaCL_2$	-	6.7	-	ISO 10390
	CEC	mEq kg^{-1}	179	1	Cobaltihexamine extraction / ISO 23470
	C/N ratio	-	9.6	0.2	-
	Total organic carbon	g kg^{-1}	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_4		0.0003	0.00	KCl extraction (1 M), Berthelot method for NH_4. Cd reduction and Griess method for NO_3. NH_4 + NO_3 for mineral nitrogen.
	NO_3		0.012	0.00	
	Mineral nitrogen		0.012	0.00	
Other nutrients	**P_2O_5 Olsen**		0.042	0	
	Sulfur		0.26	0.02	ISO 11466 / ICP AES dosage
	K_2O exchangeable		0.41	0	Cobaltihexamine extraction / ISO 23470
	MgO exchangeable		0.20	0	
	CaO exchangeable		4.65	0.04	
	Na_2O exchangeable		0.013	0.002	
	Boron	mg kg^{-1}	0.50	0.01	Boiling water extraction
	Copper		2.7	0.0	DTPA extraction / ICP AES dosage
	Iron		15.3	2.4	
	Manganese		8.4	0.9	
	Zinc		2.7	0.1	
	Chloride		9.80	0.31	Water extraction & potentiometric dosage
	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^{-1}). 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.