



HAL
open science

Fate of nitrogen and phosphorus from source-separated human urine in a calcareous soil

Manon Rumeau, Claire Marsden, Nassim Ait-Mouheb, David Crevoisier,
Chiara Pistocchi

► **To cite this version:**

Manon Rumeau, Claire Marsden, Nassim Ait-Mouheb, David Crevoisier, Chiara Pistocchi. Fate of nitrogen and phosphorus from source-separated human urine in a calcareous soil. *Environmental Science and Pollution Research*, 2023, 10.1007/s11356-023-26895-5 . hal-04081474

HAL Id: hal-04081474

<https://hal.inrae.fr/hal-04081474>

Submitted on 25 Apr 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright



HAL
open science

Fate of nitrogen and phosphorus from source-separated human urine in a calcareous soil

Manon Rumeau, Claire Marsden, Nassim Ait-Mouheb, David Crevoisier,
Chiara Pistocchi

► **To cite this version:**

Manon Rumeau, Claire Marsden, Nassim Ait-Mouheb, David Crevoisier, Chiara Pistocchi. Fate of nitrogen and phosphorus from source-separated human urine in a calcareous soil. *Environmental Science and Pollution Research*, 2023, 10.1007/s11356-023-26895-5 . hal-04081474

HAL Id: hal-04081474

<https://hal.inrae.fr/hal-04081474>

Submitted on 25 Apr 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Metadata of the article that will be visualized in OnlineFirst

ArticleTitle	Fate of nitrogen and phosphorus from source-separated human urine in a calcareous soil	
Article Sub-Title		
Article CopyRight	The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature (This will be the copyright line in the final PDF)	
Journal Name	Environmental Science and Pollution Research	
Corresponding Author	FamilyName	Rumeau
	Particle	
	Given Name	Manon
	Suffix	
	Division	
	Organization	Eco&SolsUniv Montpellier, CIRAD, INRAE, Institut Agro, IRD
	Address	Montpellier, France
	Division	School of Geography, Earth and Environmental Sciences
	Organization	University of Birmingham
	Address	Birmingham, UK
	Phone	
	Fax	
	Email	MLR094@student.bham.ac.uk
	URL	
	ORCID	http://orcid.org/0000-0001-7731-6247
Author	FamilyName	Marsden
	Particle	
	Given Name	Claire
	Suffix	
	Division	
	Organization	Eco&SolsUniv Montpellier, CIRAD, INRAE, Institut Agro, IRD
	Address	Montpellier, France
	Phone	
	Fax	
	Email	
	URL	
	ORCID	
Author	FamilyName	Mouheb
	Particle	
	Given Name	Nassim Ait
	Suffix	
	Division	
	Organization	INRAE, UMR GEAU, Univ Montpellier
	Address	Montpellier, France
	Phone	
	Fax	
	Email	
	URL	
	ORCID	
Author	FamilyName	Crevoisier
	Particle	
	Given Name	David

Suffix
Division
Organization LISAH, Univ Montpellier, INRAE, Institut Agro, IRD
Address Montpellier, France
Phone
Fax
Email
URL
ORCID

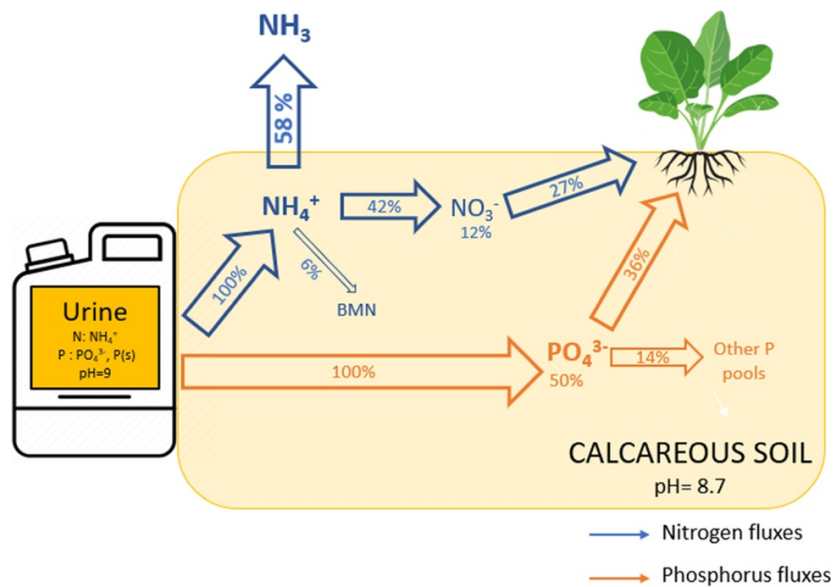
Author	FamilyName	Pistocchi
	Particle	
	Given Name	Chiara
	Suffix	
	Division	
	Organization	Eco&SolsUniv Montpellier, CIRAD, INRAE, Institut Agro, IRD
	Address	Montpellier, France
	Phone	
	Fax	
	Email	
	URL	
	ORCID	

Schedule	Received	19 Dec 2022
	Revised	
	Accepted	4 Apr 2023

Abstract

Human urine concentrates 88% of the nitrogen and 50% of the phosphorus excreted by humans, making it a potential alternative crop fertilizer. However, knowledge gaps remain on the fate of nitrogen in situations favouring NH₃ volatilization and on the availability of P from urine in soils. This study aimed at identifying the fate of nitrogen and phosphorus supplied by human urine from source separation toilets in a calcareous soil. To this end, a spinach crop was fertilized with 2 different doses of human urine (170 kgN ha⁻¹ + 8.5 kgP ha⁻¹ and 510 kgN ha⁻¹ + 25.5 kgP ha⁻¹) and compared with a synthetic fertilizer treatment (170 kgN ha⁻¹ + 8.5 kgP ha⁻¹) and an unfertilized control. The experiment was conducted in 4 soil tanks (50-cm depth) in greenhouse conditions, according to a randomized block scheme. We monitored soil mineral nitrogen over time and simulated nitrogen volatilization using Hydrus-1D and Visual Minteq software. We also monitored soil phosphorus pools, carbon, nitrogen and phosphorus (CNP) in microbial biomass, soil pH and electrical conductivity. Only an excessive input of urine affected soil pH (decreasing it by 0.2 units) and soil conductivity (increasing it by 183%). The phosphorus supplied was either taken up by the crop or remained mostly in the available P pool, as demonstrated by a net increase of the resin and bicarbonate extractable P. Ammonium seemed to be nitrified within about 10 days after application. However, both Visual Minteq and Hydrus models estimated that more than 50% of the nitrogen supplied was lost by ammonia volatilization. Overall, our results indicate that direct application of urine to a calcareous soil provides available nutrients for plant growth, but that heavy losses of volatilized nitrogen are to be expected. Our results also question whether long-term application could affect soil pH and salinity.

Graphical Abstract:



Keywords (separated by '-') Source separation - Fertilization - Hydrus - Ammonia volatilization

Footnote Information Responsible Editor: Kitae Baek • Nitrogen from urine was bioavailable for plants and microbes, but half of the N applied could be lost by volatilization in a calcareous soil. • Phosphorus from urine was either taken up by plants or remained mostly in available pools in a calcareous soil. • Only excessive doses of urine application affected soil pH and conductivity in the short term. The online version contains supplementary material available at <https://doi.org/10.1007/s11356-023-26895-5>.



Fate of nitrogen and phosphorus from source-separated human urine in a calcareous soil

Manon Rumeau^{1,2} · Claire Marsden¹ · Nassim Ait Mouheb³ · David Crevoisier⁴ · Chiara Pistocchi¹

Received: 19 December 2022 / Accepted: 4 April 2023

© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2023

Abstract

Human urine concentrates 88% of the nitrogen and 50% of the phosphorus excreted by humans, making it a potential alternative crop fertilizer. However, knowledge gaps remain on the fate of nitrogen in situations favouring NH_3 volatilization and on the availability of P from urine in soils. This study aimed at identifying the fate of nitrogen and phosphorus supplied by human urine from source separation toilets in a calcareous soil. To this end, a spinach crop was fertilized with 2 different doses of human urine ($170 \text{ kgN ha}^{-1} + 8.5 \text{ kgP ha}^{-1}$ and $510 \text{ kgN ha}^{-1} + 25.5 \text{ kgP ha}^{-1}$) and compared with a synthetic fertilizer treatment ($170 \text{ kgN ha}^{-1} + 8.5 \text{ kgP ha}^{-1}$) and an unfertilized control. The experiment was conducted in 4 soil tanks (50-cm depth) in greenhouse conditions, according to a randomized block scheme. We monitored soil mineral nitrogen over time and simulated nitrogen volatilization using Hydrus-1D and Visual Minteq software. We also monitored soil phosphorus pools, carbon, nitrogen and phosphorus (CNP) in microbial biomass, soil pH and electrical conductivity. Only an excessive input of urine affected soil pH (decreasing it by 0.2 units) and soil conductivity (increasing it by 183%). The phosphorus supplied was either taken up by the crop or remained mostly in the available P pool, as demonstrated by a net increase of the resin and bicarbonate extractable P. Ammonium seemed to be nitrified within about 10 days after application. However, both Visual Minteq and Hydrus models estimated that more than 50% of the nitrogen supplied was lost by ammonia volatilization. Overall, our results indicate that direct application of urine to a calcareous soil provides available nutrients for plant growth, but that heavy losses of volatilized nitrogen are to be expected. Our results also question whether long-term application could affect soil pH and salinity.

Keywords Source separation · Fertilization · Hydrus · Ammonia volatilization

Responsible Editor: Kitae Baek

Highlights

- Nitrogen from urine was bioavailable for plants and microbes, but half of the N applied could be lost by volatilization in a calcareous soil.
- Phosphorus from urine was either taken up by plants or remained mostly in available pools in a calcareous soil.
- Only excessive doses of urine application affected soil pH and conductivity in the short term.

✉ Manon Rumeau
MLR094@student.bham.ac.uk

¹ Eco&SolsUniv Montpellier, CIRAD, INRAE, Institut Agro, IRD, Montpellier, France

² Present Address: School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham, UK

³ INRAE, UMR GEAU, Univ Montpellier, Montpellier, France

⁴ LISAH, Univ Montpellier, INRAE, Institut Agro, IRD, Montpellier, France

Introduction

The global nitrogen (N) cycle has been massively altered for decades, by the synthesis of reactive N from atmospheric N_2 for fertilizer production and by the conversion of reactive N into N_2O or N_2 during wastewater treatment (Gruber and Galloway 2008; Steffen et al. 2015). In these two opposite processes greenhouse gases are emitted and fossil fuel energy is used, while nitrogen pollution is caused as a side effect (Kampschreur et al. 2009). The phosphorus (P) cycle has also been heavily disrupted, with the additional issue that phosphate rock, from which P fertilizers are sourced, is becoming scarce (Desmidt et al. 2015). Wastewater treatment only removes a part of the P, which is however little recycled, and the rest is discharged into surface waters. Therefore, re-looping N and P fluxes appears to be a promising solution to reduce wastewater pollution and synthetic fertilizer dependency. Human urine is of particular interest because it concentrates 88% of the nitrogen and 50% of the phosphorus

excreted by humans (Martin et al. 2022). If recycled, it could account for more than 13% of the global agricultural fertilizer demand (Wald 2022). In addition, urine represents less than 1% of the volume of wastewater but 79% of the nitrogen and 47% of the phosphorus treated by sewage treatment plants (Larsen et al. 2013). Hence, diverting it would reduce by more than half the nutrient pollution from wastewater (Wald 2022). Urine can be considered sterile in most cases and does not present risks of disease transmission if not contaminated with faeces. This is possible in source separation systems (toilets with separated outlets or urinals) (Lienert and Larsen 2010) which allow the safe collection of urine.

Each human produces 1 to 1.5L of urine per day (Karak and Bhattacharyya 2011). Urine is composed of 95% water, and the remaining 5% consists of amino compounds (such as urea or creatinine), organic anions and inorganic salts (Maggi and Daly 2006). After urea hydrolysis, nitrogen is mainly in ammonium form and phosphorus is either dissolved in solution or precipitated as struvite (magnesium ammonium phosphate) (Udert et al. 2006). The fertilizing ability of animal urine has been known for a long time (Di and Cameron 2007; Fanjaniaina et al. 2022), while that of human urine has been proven in recent years (Akpan-Idiok et al. 2012; Martin et al. 2022; Pradhan et al. 2009). However, the fate and dynamics of nitrogen and phosphorus from human urine are highly uncertain as soil biotic and abiotic processes involving N and P could be affected by the other compounds and nutrients present in urine. For instance, hippuric acid in urine can inhibit denitrification (Kool et al. 2006), and the formation of ammonium bicarbonate can inhibit nitrification (Clough et al. 2003; Somers et al. 2019).

Calcareous soils are common agricultural land in France. These soils are prone to ammonia (NH_3) volatilization because of their alkaline pH. Therefore, urine application on such soils is likely to result in high NH_3 losses by volatilization. However, published volatilization rates range from 0 to 63% of the ammonium supplied (Mills et al. 1974; Powlson and Dawson 2022). Phosphorus availability is also an important issue in alkaline soils and could be improved by fertilization with urine: firstly because urine is a source of P, and secondly because the application of ammonium can decrease soil pH by enhancing acidifying processes such as ammonia volatilization, nitrification and subsequent nitrate leaching (Bolan et al. 1991; Raza et al. 2021). Hence, a decrease in soil pH could increase the availability of P from urine (i.e. dissolved as phosphate or precipitated as struvite) which largely depends on pH (Frossard et al. 2000; Helfenstein et al. 2020; Meyer et al. 2018).

Aside from the effect on P availability, soil acidification is an important process to monitor as it can generate negative feedbacks on soil fertility through a reduction of the cation exchange capacity (Barak et al. 1997) and an increase in soil CO_2 emissions from the dissolution of carbonate in calcareous soils (Raza et al. 2021).

Moreover, urine is a multi-component solution: as well as N and P, it contains K^+ , S, Ca^{2+} , Mg^{2+} , Na^+ , Cl^- and other micronutrients. Thus, it could represent a complete fertilizing solution even though the bioavailability of urine micronutrients has not been well documented (Olivia et al. 2015). Yet, the high concentrations in Na^+ , Cl^- and SO_4^{2-} could also cause soluble salt accumulation in soils (Boh and Sauborn 2014; Mnkeni et al. 2008; Shingiro et al. 2019).

The objectives of this study were to determine the fates of N and P from urine fertilization on a calcareous soil and the effect on soil pH and salinity. The main hypotheses were: (1) N supplied with urine is readily bioavailable, but a significant amount of N is lost by volatilization potentially causing a decrease in soil pH; (2) urine application and associated decrease in soil pH increase phosphorus availability in soils; (3) urine increases soil salinity because of its soluble salt concentration. To address these hypotheses, we conducted a fertilization trial on a spinach crop (*Spinacia oleracea* L.) where we compared the effect of two different doses of source separated human urine with that of a synthetic fertilizer with equivalent N and P concentrations and an unfertilized control.

Materials and methods

Site and experiment description

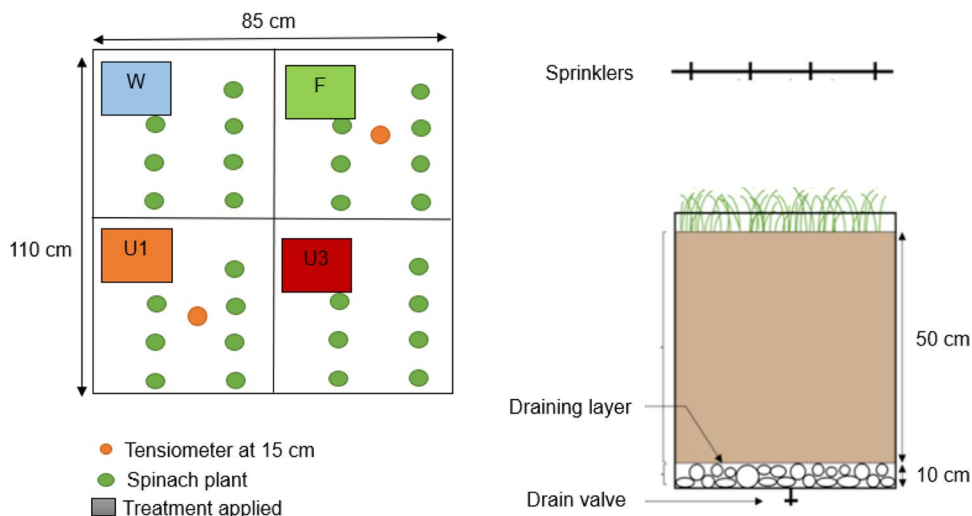
The experiment was carried out in a greenhouse of the UMR G-EAU in Montpellier between May 30th 2020 and July 5th 2020. Meteorological variables were measured by a weather station located at the experimental site. Air temperature and relative humidity in the greenhouse were measured by a temperature and relative humidity probe (model CS215, CAMPBELL SCIENTIFIC), and global radiation was measured by a pyranometer (model SP1110, CAMPBELL SCIENTIFIC) (Table 1).

The experiment was conducted in 4 soil tanks (soil surface equal to 0.935 m² and 50-cm soil depth) (Fig. 1). Each tank was filled with approximately 0.53 tonnes of air-dried loamy clay soil (24% clay, 25.6% silt, 19.5% very fine sand, 16.4% fine sand, 14.4% coarse sand) with 45% of carbonate and a pH of 8.7. A spinach crop (*Spinacia oleracea* L.) was

Table 1 Climatic parameters inside the greenhouse from June 17th to July 2nd, 2020

	Air temperature (°C)	Air relative humidity (%)	Global radiation (kW m ⁻²)
Mean	24.3	63.7	0.203
Minimum	9.5	23.7	0
Maximum	36.0	99.7	1.041

Fig. 1 Scheme of the experimental design (representation of one tank, from above (left) and from the side (right)) with crops and sensor positions. For each tank, the position of the different treatments was randomized



134 sown directly in the tanks on May 30th with a plant density of 17 plant m⁻² and the growing cycle lasted 37 days.
 135
 136 On July 5th, the above-ground biomass was collected and dried for biomass and nutrient content analysis. Spinach was
 137 chosen because of its relatively high N requirements (170 kgN ha⁻¹ under optimal growth conditions) (Frerichs et al.
 138 2022) and short growing cycle. Irrigation was conducted with sprinklers located above the tanks and controlled with
 139 tensiometers placed at 15-cm depth in the soil to maintain soil moisture around field capacity. Approximately 200 mm
 140 of water was supplied over the duration of the experiment.
 141 Each tank was divided into 4 quarters using vertical aluminium sheets driven 30 cm into the soil (below maximum
 142 root depth to avoid transfers of nutrients between quarters).
 143 The experimental treatments were assigned according to a randomized block scheme in which the experimental unit
 144 was a quarter of a tank and the tank was the block. We compared two different doses of human urine with a synthetic
 145 fertilizer and a water control. The four treatments applied were: U1 = Urine dose × 1 (170 kgN ha⁻¹ + 8.5 kgP ha⁻¹
 146 supplied), U3 = Urine dose × 3 (510 kgN ha⁻¹ + 25.5 kgP ha⁻¹ supplied), F = Synthetic fertilizer dose × 1 (170 kgN
 147 ha⁻¹ + 8.5 kgP ha⁻¹ supplied) and W = Water. Treatments U1 and F represent the recommended doses of N for the spin-
 148 ach crop, while treatment U3 represents 3 times this dose and exacerbates the effects of urine as well as approximates
 149
 150
 151
 152
 153
 154
 155
 156
 157
 158
 159

the recommended dose of phosphorus. Nitrogen and phosphorus in the F treatment were added as ammonium nitrate (NH₄NO₃) and potassium phosphate (KH₂PO₄) respectively. Potassium was not limiting in soils (8 mg kg⁻¹ at T0, data not shown) so we assumed that the slight difference in potassium concentration between the urine and the synthetic fertilizer would not affect plant growth.

The treatments were fractionated into 6 applications, and each one was diluted by 11.8 for U1 and F and by 3.8 for U3 (Fig. 2).

The urine used in this experiment was collected by the start-up EcoSec (Montpellier, France, <https://ecosec.fr>), which manufactures and sells source separating toilets. These toilets allow the selective collection of urine and faeces thanks to a gravity system, but do not prevent cross contamination with faeces. Prior to the experiment, the urine was stored for a year in an opaque and airtight container in order to sanitize the effluent according to World Health Organization (WHO) recommendations (Schönning and Stenström 2004). The urine's chemical composition is summarized in Table 2. As expected, P was only in inorganic form and nitrogen was mostly present in ammonium form. However, ammonium concentration in urine decreased by 0.58 gN L⁻¹ between the beginning (T0) and the end of the experiment (TF) due to ammonia volatilization during container openings; this was considered in the N budget. Organic carbon concentration was very low (57 mgC L⁻¹) despite potential cross contamination with faeces. Furthermore, the high concentration

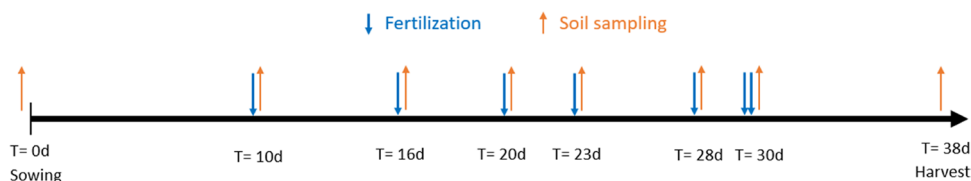


Fig. 2 Timeline of the fertilization treatments. The scale is in day (d) from sowing (day 0) to harvest (day 38). Each fertilization and soil sampling are represented by a blue and an orange arrow respectively. Double dose of fertilization was supplied on day 30

Table 2 Chemical composition at T0 (start of the experiment) of the undiluted urine used in this experiment after 1 year of storage in an air-tight opaque tank

Parameters measured	Values	Method
Electrical conductivity at 25 °C (mS cm ⁻¹)	40.5	NF EN 27,888
pH water	9	NF EN ISO 10523
N-NH ₄ ⁺ (mg L ⁻¹)	4341	Filtered at 0.45 µm, ISO 7150–1
N-NO ₃ ⁻ (mg L ⁻¹)	10.7	Filtered at 0.45 µm, ISO 7890–1-2–1986
Ptotal (mg L ⁻¹)	206	Filtered at 0.45 µm, ICP-AES
Porganic (mg L ⁻¹)	Not detected	
K ⁺ (mg L ⁻¹)	1107.8	Filtered at 0.45 µm, ICP-AES
Mg ²⁺ (mg L ⁻¹)	0.758	Filtered at 0.45 µm, ICP-AES
Ca ²⁺ (mg L ⁻¹)	3.49	Filtered at 0.45 µm, ICP-AES
Na ⁺ (mg L ⁻¹)	1245.6	Filtered at 0.45 µm, ICP-AES
SO ₄ ²⁻ (mg L ⁻¹)	1228.81	ISO 11885
Cl ⁻ (mg L ⁻¹)	3574.85	Water extraction 1/5, NF EN 9297
DBO5 (mg L ⁻¹): 5-day biochemical oxygen demand	3670	NF EN 1899–1
ST-DCO (mg L ⁻¹): chemical oxygen demand	6790	NF T90-101
Labile carbon (mgC L ⁻¹)	11.9	Filtered at 0.45 µm, POXC
Total dissolved carbon/inorganic carbon/organic carbon (mgC L ⁻¹)	2400/2343/57	Filtered at 0.45 µm, TOC-TN analyser
Cu (mg L ⁻¹)	0.264	Filtered at 0.45 µm, ICP-AES
Fe (mg L ⁻¹)	0.126	
Mn (mg L ⁻¹)	<0.008	
Zn (mg L ⁻¹)	0.198	
Bo (mg L ⁻¹)	0.556	
Al (mg L ⁻¹)	0.056	
SAR (sodium absorption ratio)	157.6	
- Solution U1	45.8	
- Solution U3	80.8	

Abbreviations: ICP-AES inductively coupled plasma atomic emission spectroscopy, POXC permanganate-oxidizable carbon, TOC-TN total organic carbon/total nitrogen

of Na⁺ (1289 mg L⁻¹) and Cl⁻ (3574 mg L⁻¹) along with the conductivity (40.6 mS cm⁻¹) showed that the urine had high salinity (Table 2). Additionally, the sodium absorption ratio (SAR, indicating the potential sodium hazard for irrigation or fertirrigation) of pure urine, solution U1 and solution U3 was calculated with the following formula, and SAR values are detailed in Table 2:

$$SAR = \frac{Na^+}{\sqrt{\frac{1}{2}(Ca^{2+} + Mg^{2+})}} \quad (1)$$

where concentrations of cations (Na⁺, Ca²⁺, Mg²⁺) are expressed in meq L⁻¹.

Parameter monitoring and measurement

Soil sampling

Bulk soil samples at 0–10-cm depth composed of 2 sub-samples were taken in every quarter of a tank at the beginning of the experiment before sowing (T0), right after every

fertilization (T1 to T6) and at the end of the experiment (TF). For every sample, the gravimetric water content was measured by drying the soil at 105 °C for 48 h. Mineral nitrogen (NO₃⁻ and NH₄⁺) was measured in all the samples, whereas pH, conductivity, organic and inorganic P concentration and microbial biomass were quantified only in the T0 and TF samples.

Soil bulk density

In each tank, 3 undisturbed soil cores of 5-cm depth were taken. Soil cores were then oven dried at 105 °C, and the bulk density was calculated for each core by dividing the dry mass of soil by the volume of the core.

Mineral nitrogen measurement

Soils were extracted with 1 M KCl (soil to extractant ratio of 1:3) on the day of sampling. The extracts were filtered at 0.45 µm and frozen until analysis. Then, samples were

- 218 analysed by continuous flow colorimetry (Skalar SA 3000
219 analyser). This method measures nitrite and nitrate species
220 together and ammonium separately.
- 221 **pH**
- 222 pH measurement was carried out according to the ISO stand-
223 ard using a pH probe (AFNOR, 2005). Soils were sieved,
224 air dried and extracted with a soil to water ratio of 1:5, and
225 measurements were performed after 1 h of agitation.
- 226 **Electrical conductivity**
- 227 Soils were sieved, air dried and extracted with a soil to water
228 ratio of 1:5. Then, the solution was agitated for 30 min, cen-
229 trifuged and filtered. Electrical conductivity was then meas-
230 ured on the aqueous extract with a conductometer probe.
- 231 **Organic and inorganic P pool**
- 232 To quantify soil phosphorus pools, we performed a Hedley
233 sequential fractionation as modified by Tiessen and Moir
234 (1993). This method operationally identifies organic and
235 inorganic P pools. Soil samples were sequentially extracted
236 with 4 different reagents in the following order: anionic
237 exchange resin membranes (BDH #55,164, 6 cm × 4 cm,
238 named P resin pool), 0.5 M sodium bicarbonate (NaHCO₃,
239 Pi and Po bicarbonate pools), 0.5 M NaOH (Pi and Po NaOH
240 pools) and 1 M HCl (P HCl pool). To quantify organic P,
241 aliquots of bicarbonate and NaOH extracts were mineralized
242 by acid digestion, and the organic P concentration was cal-
243 culated as the difference between inorganic P in the digested
244 extract (corresponding to the total P in the extract) and inor-
245 ganic P in the non-digested extract. Inorganic phosphorus
246 concentration in each extract was measured by the mala-
247 chite green colorimetric method (Ohno and Zibilske 1991).
248 The P resin and the Pi and Po bicarbonate are commonly
249 considered the available P pool (Tiessen and Moir 1993).
250 The exchange times of sequentially extracted P pools with
251 the soil solution increase with the strength of the extractant
252 (Helfenstein et al. 2020). Hence, the P pools extracted with
253 the stronger extractants (i.e. 0.5 M NaOH and 1 M HCl) are
254 most likely less available.
- 255 **C N P in microbial biomass**
- 256 The fumigation extraction method was applied to determine
257 C, N and P in microbial biomass (AFNOR, 1997). For each
258 soil sample, 4 subsamples were weighed, and 2 were fumi-
259 gated with chloroform overnight. Blanks without soil were
260 included. Then, two fumigated/non-fumigated subsamples
underwent potassium sulphate (K₂SO₄, 0.025 M) extrac-
tion for C and N quantification. The two other fumigated/
non-fumigated subsamples underwent sodium bicarbonate
(NaHCO₃ 0.5 M) extraction for P quantification. C and N
were determined in the filtered extracts using a TOC-TN
analyser (VCPH Shimadzu + TN module). Phosphorus in
the fumigated/non-fumigated extracts was measured with
the malachite green method. Microbial C, N and P (MBC,
MBN and MBP) were calculated as the difference in C, N
and P concentration between the fumigated and the non-
fumigated samples and divided by a conversion factor of
0.45 for C and N.
- Soil micronutrient concentrations**
- Soil concentration of K⁺, Na⁺, PO₄²⁻, SO₄²⁻⁻, Mg²⁺, Ca²⁺
were measured at T0 and TF by the laboratory Aurea Agro-
Sciences (<https://www.aurea.eu/>) following the method
ISO 11885 for the determination of selected elements by
inductively coupled plasma optical emission spectrometry
(ICP-OES).
- Crop nutrient uptake**
- The above-ground biomass was dried at 60 °C for 48 h,
weighed and ground. Carbon and nitrogen concentrations were
determined by a CHN elemental analyser (Thermo Fisher Sci-
entific Flash 2000). P, K, Ca, Mg, Na, Cu, Fe, Mn, Zn, Bo and
Al concentrations were measured using ICP-AES spectroscopy.
Nutrient uptake was calculated as the product of tissue
concentration and dry biomass and expressed in kg ha⁻¹.
- Nitrogen fertilizer use efficiency was calculated in all fer-
tilized treatments as the difference between the amount of N
taken up by the crops in the fertilized treatments (NupFT)
and N taken up by the crops in the control treatment (NupW)
divided by the total amount of N supplied by each treatment
(NsupFT).
- $$NUE(\%) = \frac{NupFT - NupW}{NsupFT} \times 100 \quad (2)$$
- Phosphorus fertilizer use efficiency (PUE) was calculated
in the same way as NUE.
- Modelling of ammonia volatilization**
- Two models were used to estimate ammonia volatilization in
our experiment: Hydrus 1D (PC progress, version 4.17) and
Visual Minteq (version 3.1). Each model was able to give
an estimation of the ammonia volatilized using a different
approach. Visual Minteq is an equilibrium speciation model

and simulated the concentration of ammonia in the soil solution by calculating chemical equilibria using the pH, the ionic strength and the concentrations of different chemical species. Hydrus 1D, on the other hand, is a reactive transport model and simulated ammonia volatilization by a first-order reaction process in function of the continuous concentration of ammonium in soils (calibrated with the observed measurements). A comparison of the parameters used for the two models is detailed in Table 5S, Supplementary Data. Finally, the rates of ammonia volatilization simulated by the two different models were compared.

Hydrus 1D model

Model description Hydrus 1D (Šimůnek et al. 2008) is a free software able to simulate water flow and solute transport in one dimension. All the following reactions and processes were considered in the simulation: ammonia volatilization, nitrification, denitrification, NO_3^- leaching, N mineralization and NH_4^+ and NO_3^- root uptake.

Input parameters A homogeneous soil profile of 0–10-cm depth was considered.

Water flow parameters Soil water dynamics were modelled according to the Van Genuchten model (van Genuchten 1980), and the hydraulic parameters were derived from the soil texture (sandy loam). An atmospheric boundary condition (BC) with the surface layer was set for the upper BC, and free drainage was set for the lower BC. The reference evapotranspiration (ET_o) was calculated using the Hargreaves formula using temperature and relative humidity data. Crop evapotranspiration was calculated as $\text{ETP} = \text{Kc} \cdot \text{ET}_o$ (with Kc estimated from the spinach growing stages) accordingly to the FAO 56 (Allan and Smith 1998). Root water uptake was modelled with Feddes parameters (Feddes et al. 1978) using the lettuce parameters embedded in the software. Finally, the average soil moisture measured at T₀ was used to set the initial water content.

Solute transport parameters Ammonium and nitrate were the 2 solutes modelled in Hydrus 1D; their molecular diffusion coefficients in water were 1.52 and 1.64 $\text{cm}^2 \text{day}^{-1}$ respectively (Li et al. 2015). The Henry's law constant for NH_4^+ was 2.95×10^{-4} (Li et al. 2015), and the adsorption coefficient for NH_4^+ (K_d) was set at 3.5 L mg^{-1} (Hanson et al. 2006). The nitrification and denitrification rates were assumed to be 0.2 day^{-1} and 0.04 day^{-1} respectively (Castaldelli et al. 2018; Li et al. 2015). N mineralization was assumed to follow a zero-order reaction process and was calibrated with N content from the control treatment. The rate was set at $1.5 \times 10^{-6} \text{ g cm}^{-3} \text{ day}^{-1}$, falling in the same range as the one used by Tao et al. (2021) for agricultural soils. The boundary

condition at the top was set as “stagnant for volatile solutes” on a 1-cm layer to allow gaseous diffusion of solutes (Jury et al. 1983). A “zero concentration gradient” was used at the bottom of the soil profile to allow N leaching fluxes. Root solute uptake was set as passive for both solutes with the highest soil concentrations as the maximum uptake allowed. Additionally, initial soil concentrations in NO_3^- and NH_4^+ were set using the average concentration measured per treatment at T₀.

Determination of the volatilization rate The volatilization rate was calibrated against soil ammonium and nitrate concentrations with the inverse solution model using the U3 treatment configuration. U3 was chosen because early simulations showed a better fitting between simulated and measured NH_4^+ soil content for this treatment. The inverse solution analysis returned a rate of 1.4 day^{-1} for ammonia volatilization. This value fits the upper range of rates found in the literature (Castaldelli et al. 2018), and we considered that it represented well the optimal conditions for ammonia volatilization in this experiment (i.e. high soil pH and high temperature). This rate was then used to model the nitrogen dynamics in the other treatments.

Model evaluation Simulated ammonium and nitrate soil concentrations in the three fertilized treatments (F, U1 and U3) were compared to measurements to validate the model. The discrepancy between simulated and observed data was evaluated by calculating the coefficient of correlation (r^2) and the root mean square error (RMSE).

$$\text{RMSE (kgN.ha}^{-1}\text{)} = \sqrt{\frac{1}{n} \sum_i^n (S_i - O_i)^2} \quad (3)$$

where S_i (kg ha^{-1}) and O_i (kg ha^{-1}) are respectively simulated and observed nitrogen concentration and n the number of measurements ($n = 8$). The observed nitrogen concentration represents the mean value per treatment ($n = 4$).

Visual Minteq model

Visual Minteq is a free software modelling chemical equilibria (Gustafsson 2011). In our study, it was used to calculate the theoretical amount of NH_3 produced in solution after urine application. The input parameters were: soil pH, ionic strength and soil solution concentration of major ions, i.e. NH_4^+ , NO_3^- , K^+ , Na^+ , PO_4^{3-} , SO_4^{2-} , Mg^{2+} , Ca^{2+} after each application. Except for NH_4^+ and NO_3^- , the other concentrations were measured only at the beginning and the end of the experiment. Therefore, they were considered equal to their concentration at T₀ until mid-experiment and then equal to their concentration at TF until the end. Regarding phosphorus, the amount of P contained in urine was added to the PO_4^{3-} concentrations.

The other inputs of major ions added with urine additions were considered negligible for this specific model application. The model outputs give all chemical species in solution likely to precipitate at thermodynamic equilibrium and the distribution of each element among its different species. The percentage of NH_3 after each urine application calculated by the model was multiplied by the initial NH_4^+ concentration to estimate the total amount of NH_3 produced in kg ha^{-1} for each treatment. To compare Visual Minteq and Hydrus 1D outputs, we assumed that all the NH_3 produced in solution was volatilized.

N and P budget

Nitrogen

A nitrogen budget approach was used to compare the main nitrogen fluxes in our experiment and give an estimation of the unaccounted losses (i.e. denitrification and leaching). The N budget was calculated for the 0–10-cm depth soil layer using the N applied by fertilization (Ferti_N), the measured soil concentration of NH_4^+ and NO_3^- at T0 and TF to calculate ΔN_{soil} , the measured crop N uptake between 0 and 10 cm (N_{upt}), the measured microbial N pool (ΔMBN) and the measured soil bulk density to convert values from mgN kg^{-1} to kgN ha^{-1} . Ammonia volatilization (Vol) was fitted according to the Hydrus model outputs. Nitrogen mineralization (Min) was calculated as the only input of N in the water treatment. The nitrogen budget error was then calculated (as described below) for each treatment; it can be interpreted as a measure of the unaccounted losses and of the experimental error. We assumed that a budget error below 10% of N supplied means that unaccounted losses were minimal.

$$\text{Nitrogen budget error } (\delta N)(\text{kg} \cdot \text{ha}^{-1}) = \text{Ferti}_N - \Delta N_{\text{soil}} - \Delta \text{MBN} - N_{\text{upt}} - \text{Vol} + \text{Min} \quad (4)$$

where

$$\Delta N_{\text{soil}} = (\text{NH}_4^+ + \text{NO}_3^-) T_F - (\text{NH}_4^+ + \text{NO}_3^-) T_0 \quad (5)$$

$$\Delta \text{MBN} = \text{MBN}_F - \text{MBN}_0 \quad (6)$$

$$N_{\text{upt}} = N_{\text{uptake}} \times \% \text{root biomass}_{0-10\text{cm}} \text{ (estimated at 44\% according to And Heinrich et al. (2013))} \quad (7)$$

Phosphorus

The P budget was calculated for the 0–10-cm depth soil layer excluding the pool of P extractable with HCl. As the HCl-extractable P pool is very large in our calcareous soil, variations occurring during the experiment were not detectable against the analytical error. Hence, the P budget error can be interpreted as a net variation of the HCl-extractable

P pool size. Similarly, a net change in a P pool size gives an indication of the net flux involving the pool; however, this variation also includes the experimental error. The P budget error is calculated with the amount of P supplied by fertilization (Ferti_P), the P taken up by the crops between 0 and 10 cm (P_{upt}) and the concentration of P in the different soil pools ($P_{i_{\text{resin}}}$, $P_{i_{\text{HCO}_3}}$, $P_{i_{\text{NaOH}}}$, $P_{o_{\text{HCO}_3}}$, $P_{o_{\text{NaOH}}}$). Microbial P is already included in the organic P pools due to the extraction method; therefore, it does not appear in the budget calculation.

$$P \text{ budget error } (\delta P)(\text{kg} \cdot \text{ha}^{-1}) = \text{Ferti}_P - \Delta P_{\text{soil}} - P_{\text{upt}} \quad (8)$$

where

$$\Delta P_{\text{soil}} = (P_{i_{\text{resin}}} + P_{i_{\text{HCO}_3}} + P_{i_{\text{NaOH}}} + P_{o_{\text{HCO}_3}} + P_{o_{\text{NaOH}}}) T_F - (P_{i_{\text{resin}}} + P_{i_{\text{HCO}_3}} + P_{i_{\text{NaOH}}} + P_{o_{\text{HCO}_3}} + P_{o_{\text{NaOH}}}) T_0 \quad (9)$$

P crop uptake between 0 and 10 cm is calculated the same way as the N crop uptake on 0–10 cm (see above).

Statistical analysis

Statistical analyses were carried out with Rstudio software (version 3.6.1) (R Core Team 2017). To test the homogeneity of initial variable values at T0 across blocks (tanks) and treatments, we used a linear model with 2 factors: block ($n=4$) and treatment ($n=4$) (16 samples in total), and significance was determined with the ANOVA (analysis of variance) function. Significant differences were only found for the variables Pi resin, and MBN with differences of up to 41% and 52% respectively in the mean pool size between the richest and the poorest tank. On T1 to TF values, ANOVAs were performed to assess the effect of treatments on each variable and date separately. For each variable measured, a linear mixed model (“lmer” function, lme4 package, (Bates et al. 2015, p. 4)) was produced with “treatment” ($n=4$) as fixed factor and “block” ($n=4$) as random factor to account for the initial differences between tanks observed for certain variables. A Tukey’s multi-comparison test was performed when the treatments had a significant effect on the variable (significance level: p value < 0.05). Correlations between variables were assessed using Pearson correlation tests.

Results

Global result analysis

Soil analysis

At the end of the experiment, soil pH was lower in the U3 treatment (urine at 510 kgN ha^{-1}) than in all other treatments, reaching 8.5, while it was over 8.8 in all other

484 treatments. At T3, shortly after a fertilizer application, soil
485 conductivity was higher in all the fertilized treatments than
486 in the water treatment, but at the end of the experiment,
487 only the U3 treatment still had higher conductivity (+ 187%
488 compared to the water treatment, $p=0.002$) (Table 3). In
489 the U3 treatment, soil concentrations in Na^+ and Cl^- were
490 also twice as high at TF as in the other treatments (Table 2S,
491 Supplementary data). MBC and MBP showed no significant
492 response, but MBN showed a significant difference between
493 U3 and the control W at TF (+ 370% in U3 compared to W)
494 (Table 3). Soil nitrate and ammonium concentrations and
495 soil P pool concentrations are commented in Sect. 2.1 and
496 3 respectively.

497 Biomass and plant nutrient uptake

498 Aboveground biomass at the end of the experiment was
499 significantly different only between the control treatment
500 (W) and the synthetic fertilizer treatment (F). N and P
501 uptake by plants were significantly higher ($p < 0.05$) in the
502 F and U3 treatments than in the W treatment, and inter-
503 mediate in the U1 treatment, i.e. not significantly differ-
504 ent from both W and U3 and F treatments. In addition,
505 the spinach crops in the tanks 1 and 8 took up more N
506 and P than the ones in the tanks 4 and 5 (up to 138% and
507 210% more for N and P respectively, data not shown). N
508 and P uptake were highly correlated (Pearson coefficient
509 $r = 0.97$, $p < 0.0001$). N uptake was correlated with soil
510 nitrate content from day 23 to day 28 ($p = 0.03$, $p = 0.04$,
511 $p = 0.01$ for day 23, 26 and 28 respectively) but was not
512 correlated with soil ammonium content. P uptake was cor-
513 related with none of the P pools at the end of the experi-
AQ5 ment ($p > 0.05$) (Table 4).

515 Nitrogen stock evolution and losses

516 N- NH_4^+ , N- NO_3^- soil concentrations

517 In the water treatment, soil ammonium and nitrate contents
518 were very close to 0 kgN ha^{-1} throughout the experiment
519 (Fig. 3). In the synthetic fertilizer treatment (F), both nitrate
520 and ammonium increased after the first application (day 10),
521 but, while ammonium content stayed relatively stable under
522 100 kgN ha^{-1} , nitrate content increased up to 150 kgN ha^{-1}
523 at day 30. In U3 and U1 treatments, ammonium reached a
524 peak during the experiment (at day 30 and day 20 respec-
525 tively) and then decreased to 0 kgN ha^{-1} at the end (Fig. 3,
526 left panel). Nitrate concentration in U1 and U3 showed a
527 slow increase between day 10 and 20 and a sharp increase
528 between day 20 and 30 (Fig. 2). Overall, the nitrate and
529 ammonium curves of U1 and F were very similar, although
530 in the U1 treatment twice more ammonium was added than
531 in the F treatment.

Estimation of ammonia volatilization

532 In order to validate the N fluxes simulated by Hydrus 1D,
533 simulated and observed ammonium and nitrate soil concen-
534 trations were compared (Fig. 4).
535

536 Modelled NH_4^+ soil concentrations agreed relatively well
537 with measurements in the 3 treatments ($\text{RMSE} < 69 \text{ kg ha}^{-1}$),
538 although they were slightly underestimated for U3. However,
539 NO_3^- dynamics were poorly simulated by Hydrus 1D espe-
540 cially in the U3 treatment ($\text{RMSE} = 142 \text{ kg ha}^{-1}$). As soil
541 NO_3^- is not used in the calculation of ammonia volatilization,
542 this poor fitting should not affect the estimation of NH_3 volatil-
543 ization. It prevents, however, a good estimation of NO_3^- leach-
544 ing and denitrification. Therefore, we did not use the Hydrus
545 1D model outputs to estimate these losses in the N budget.

546 As expected, ammonia volatilization simulated by
547 Hydrus 1D was especially high in the U3 treatment reach-
548 ing 260 kg ha^{-1} , and it was almost two times higher in the
549 U1 treatment than in the F treatment (Table 5).

550 Visual Minteq estimated that 21% of NH_4^+ in soils would
551 be in NH_3 form for a soil at $\text{pH} = 8.7$; however, this pro-
552 portion decreases to 13.9% when the pH is at 8.5 as in the
553 treatment U3 at the end of the experiment (Table 1S, Sup-
554 plementary data).

555 Despite the models' dissimilarities, their output for the flux
556 of NH_3 is similar (Table 5). Both models agree that more than
557 half of the nitrogen applied with the urine fertilizer was lost
558 by ammonia volatilization, whereas only approximately 30%
559 was lost with the NH_4NO_3 synthetic fertilizer.

Fate of phosphorus from urine

560 P was similarly distributed in the different P pools in F,
561 U1 and W treatments, whereas in U3 treatment, the three
562 inorganic phosphorus pools were larger than in all others
563 at the end of the experiment, in particular Pi resin (+ 179%
564 compared to the W treatment) ($p = 0.00005$) and Pi NaOH
565 (+ 38%) ($p = 0.0015$). (Fig. 5).

566 In contrast, the HCl-extractable Pi (Pi HCl) did not vary
567 significantly among treatments (Table 3). The P budget
568 calculation (δ_p) was negative in the U1 and U3 treatments
569 suggesting a potential decrease of approximately 10 kgP
570 ha^{-1} of the Pi HCl pool, which was not detectable against
571 replicate variability (Table 1S, Supplementary data).
572

Discussion

Effect of urine fertilization on soil pH and salinity

573 In our study, only the excessive dose of urine (U3) lowered
574 the pH of the calcareous soil. Previous studies on acidic soils
575 found a decrease in soil pH even with the appropriate urine
576
577

Table 3 Results of ANOVA (*p* value) and post hoc Tukey tests on the experiment variables. Values in the table are the means of the 4 replicates in each treatment. Treatments are noted with letters, W=control, F=synthetic fertilizer, U1=urine dosed at 170 kgN ha⁻¹, U3=urine in excess at 510 kgN ha⁻¹. Different letters (a, b, c) indi-

cate significant differences between treatments (*p*<0.05) for a same date (T0 at the beginning of the experiment before sowing, T1 to T6 right after every fertilization event and TF at the end of the experiment)

Variables	Time	W	F	U1	U3	<i>p</i> value treatment effect	<i>p</i> value tank effect	
pH	T0	8.73 a	8.76 a	8.73 a	8.74 a	0.3	0.4	
	TF	9.01 a	8.84 b	8.88 ab	8.52 c	<0.0001 ***	Nd	
Conductivity (μS cm ⁻¹)	T0	160 a	135 a	155 a	149 a	0.23	0.33	
	T3	150 c	248 bc	341 b	528 a	<0.0001	Nd	
NO ₃ ⁻ +NO ₂ ⁻ (mgN kg ⁻¹)	TF	147 b	154 b	177 b	423 a	0.002*	Nd	
	T0	7.6 a	5.0 a	8.2 a	6.3 a	0.21	0.6	
NH ₄ ⁺ (mgN kg ⁻¹)	T1	2.4 a	19.1 a	4.8 a	3.9 a	0.06	Nd	
	T2	8.8 b	47.0 a	23.1 ab	42.5 a	0.002 *	Nd	
	T3	6.6 b	44.3 a	26.5 ab	39.7 a	0.01 *	Nd	
	T4	10.4 c	88.8 b	80.1 bc	226.1 a	0.00004 ***	Nd	
	T5	13 c	135.9 b	104.8 b	221.3 a	0.00001 ***	Nd	
	T6	6.7 c	102.7 b	57.1 bc	214.3 a	<0.00001 ***	Nd	
	TF	4.1 b	22 b	22 b	121 a	0.003 **	Nd	
	T0	0 a	0 a	0 a	0 a	Nd	Nd	
	T1	0 b	25 ab	22 ab	58 a	0.01	Nd	
	T2	2.9 c	35 bc	66 b	145 a	0.0001	Nd	
Pi Resin (mgP kg ⁻¹)	T3	1.1 c	38 c	86 b	187 a	<0.00001 ***	Nd	
	T4	0 b	35 b	76 b	206 a	0.0001	Nd	
	T5	0.3 b	35 b	51 b	260 a	<0.00001 ***	Nd	
	T6	0.8 b	51 b	52 b	315 a	0.0001	Nd	
	TF	0.6 a	0.9 a	0.8 a	1 a	0.5	Nd	
	T0	10.5 a	9.7 a	9.7 a	10.8 a	0.4	0.009 **	
	TF	9.4 b	11.7 b	11.7 b	26.2 a	0.00005 **	Nd	
	Pi Bicarbonate (mgP kg ⁻¹)	T0	7.8 a	11.6 a	9.3 a	8.9 a	0.65	0.28
		TF	11.5 a	11.5 a	11.1 a	17.0 a	0.05	Nd
	Pi NaOH (mgP kg ⁻¹)	T0	11.5 a	9.9 a	9.5 a	9.58 a	0.13	0.02 *
TF		9.8 b	10.9 b	9.9 b	13.5 a	0.0015 **	Nd	
Pi HCl (mgP kg ⁻¹)	T0	224 a	209 a	206 a	221 a	0.45	0.10	
	TF	225 a	217 a	219 a	230 a	0.42	Nd	
Po Bicarbonate (mgP kg ⁻¹)	T0	10.1 a	5.7 a	7.5 a	7.7 a	0.33	0.07	
	TF	6.9 a	12.8 a	12.1 a	8.1 a	0.43	Nd	
Po NaOH (mgP kg ⁻¹)	T0	24.5 a	22.9 a	20.5 a	22.5 a	0.81	0.09	
	TF	23.9 a	21.3 a	23.8 a	20.9 a	0.6	Nd	
P total (mgP kg ⁻¹)	T0	281.3 a	269.9 a	254.3 a	289.3 a	0.21	0.07	
	TF	286.9 a	285.8 a	288.5 a	316.3 a	0.03 *	Nd	
MBC (mgC kg ⁻¹)	T0	127 a	134 a	140 a	140 a	0.67	0.03	
	TF	96 a	122 a	126 a	134 a	0.10	Nd	
MBN (mgN kg ⁻¹)	T0	9.8 a	11.3 a	11.1 a	10.5 a	0.17	0.001 **	
	TF	8.4 b	20.1 ab	21.3 ab	39.5 a	0.05 *	Nd	
MBP (mgP kg ⁻¹)	T0	7.1 a	7.1 a	8.8 a	7.6 a	0.9	0.8	
	TF	6.3 a	5.1 a	7.8 a	10.5 a	0.08	Nd	

578 dosage (Mnkeni et al. 2008; Sangare et al. 2015). However,
579 calcareous soils have a stronger pH buffering capacity (Mag-
580 doff and Bartlett 1985; Raza et al. 2021); they are probably

more resistant to the acidifying effect of urine in the short 581
term. The effect of long-term urine application on soil pH is 582
uncertain and is potentially much greater for non-calcareous 583

Table 4 Results of ANOVA (p value) and post hoc Tukey tests on plant biomass and plant nutrient uptake variables assessed at the end of the experiment. NUE and PUE (respectively nitrogen and phosphorus use efficiency) were assessed as the efficiency of N and P fertilizer, i.e. the difference in nutrient uptake between a fertilized and non-fertilized treatment, divided by the amount of applied nutrient. Values in the table are the means of the 4 replicates. Different letters (a, b, c) indicate significant differences between treatments ($p < 0.05$) for a same date

Variables	W	F	U1	U3	p value treatment effect
Aboveground biomass (g)	15.2 b	27.8 a	22.3 ab	23.2 ab	0.02 *
Nuptake (kg/ha)	23.6 b	52.9 a	41.8 ab	49.4 a	0.02 *
Puptake (kg/ha)	2.2 b	4.3 a	3.1 ab	4.1 a	0.03 *
C/N	10.19 a	8.03 b	8.21 b	7.57 b	0.005 **
C/P	130 a	106 a	119 a	101 a	0.17
NUE	/	17 a	10 a	5 a	0.06
PUE	/	24 a	10 a	7 a	0.05

584 than for calcareous soils. Soil acidification can nevertheless
585 be reduced by applying correct amounts and most importantly
586 by reducing urine-derived ammonia volatilization,
587 which exacerbates soil acidification.

588 Similarly, urine caused a buildup of soluble salt concentration
589 and specifically NaCl only in the U3 treatment. However, crops
590 under the U3 treatment did not take up more sodium than under
591 the other treatments (Table 3S, Supplementary data), and the soil
592 salinity was still below the spinach salt tolerance threshold
593 established at 9.4 dS m^{-1}

594 by Ferreira et al. (2018); so, it is unlikely that the spinach
595 crops underwent a saline stress. Other studies on urine ferti-
596 lization confirm that critical values of soil conductivity
597 are only observed when urine is applied in excess (up to
598 13 dS m^{-1} with 800 kgN ha^{-1}) (Boh and Sauerborn 2014;
599 Mnkeni et al. 2008; Neina 2013) and that the crop response
600 depends on its salt tolerance threshold (Mnkeni et al. 2008).
601 Nevertheless, the effects of urine fertilization on soil salinity
602 have not been investigated so far for longer than one or
603 two cropping seasons. The sodium adsorption ratio (SAR)
604 of diluted urine being high (Table 2), the potential effect
605 of urine on the buildup of harmful concentrations of soil
606 exchangeable sodium should be assessed in the long term,
607 and suitable solutions can be envisaged to avoid such a risk,
608 such as the addition of Ca and Mg amendments (Ayers and
609 Westcot 1985).

610 Considerable losses of nitrogen by volatilization

611 More than half of the nitrogen applied was estimated to be
612 lost by volatilization with urine application. Similar rates of
613 volatilization were obtained with liquid ammonium fertilizer
614 on calcareous soils (Hargrove et al. 1977; Powlson and Dawson
615 2022; Whitehead and Raistrick 1990). In addition, volatili-
616 zation is almost double with urine than with ammonium
617 nitrate fertilizer; similar values were found when comparing
618 urea and ammonium nitrate (Eckard et al. 2003).

619 According to Visual Minteq simulations, a small shift
620 in soil pH can considerably affect the NH_4/NH_3 chemical
621 equilibrium and so the potential of our soil for ammonia
622 volatilization. This highlights the interaction between

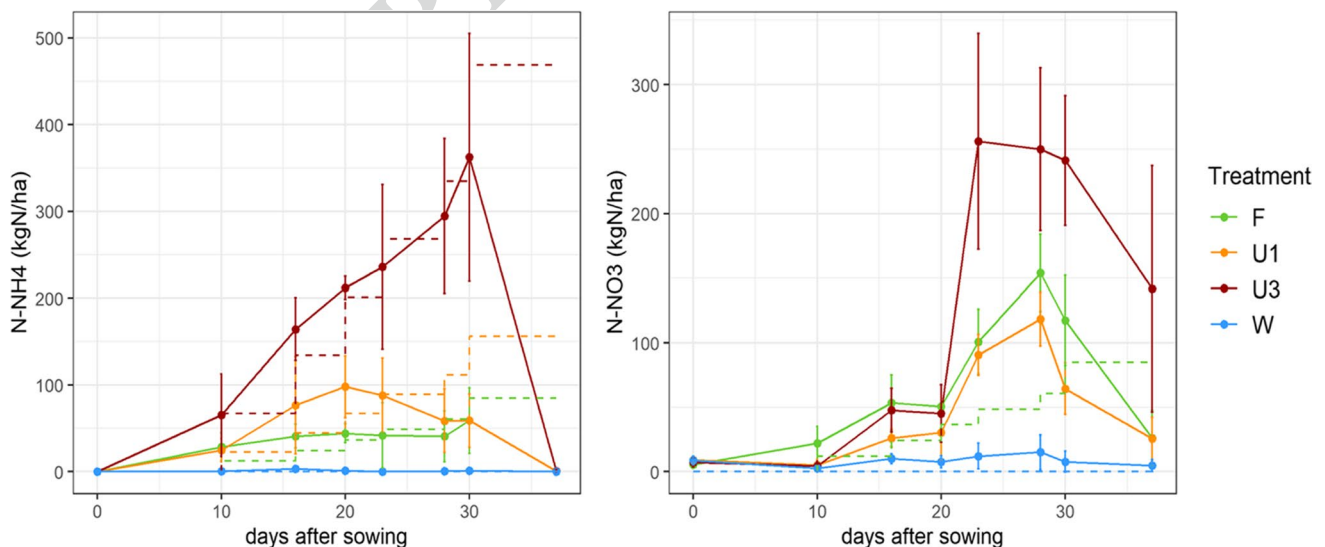


Fig. 3 Evolution of soil mineral nitrogen stocks during the experiment as a function of treatment: F: synthetic fertilizer dose $\times 1$, U1: urine dose $\times 1$, U3: urine dose $\times 3$, W: unfertilized control (ammo-

nium on the left panel and nitrate on the right panel). The dashed lines represent the cumulative fertilization inputs and the errors bars represent the standard error between the four replicates

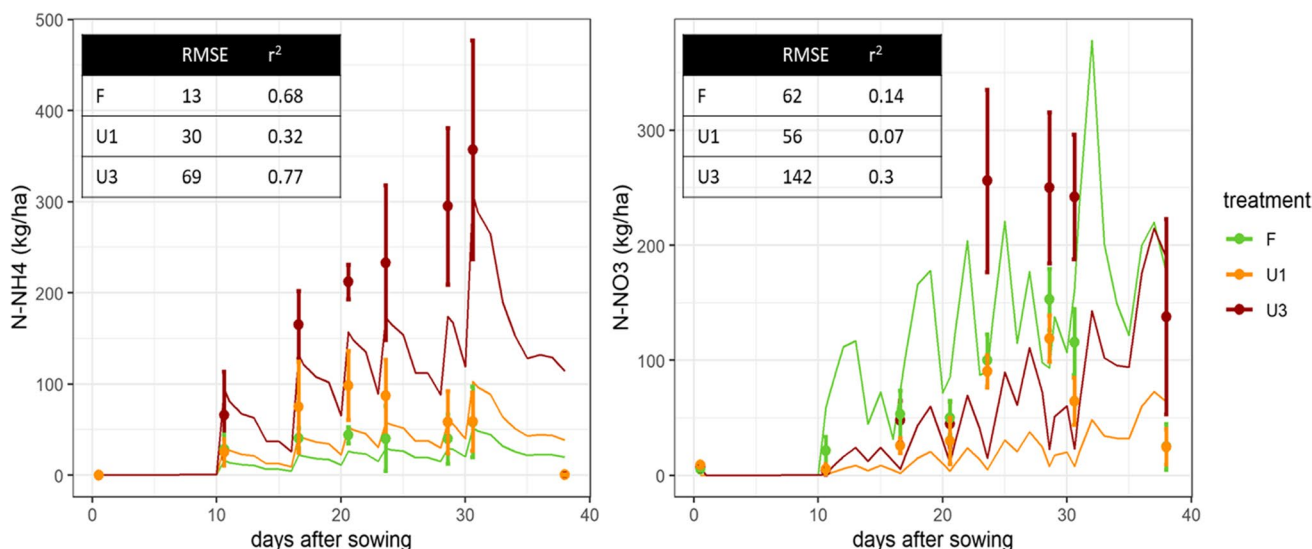


Fig. 4 Comparison between concentration of ammonium and nitrate in soils, as simulated by Hydrus 1D (lines) and observed (points) for which the errors bars represent the standard error between the four

replicates. Tables in the figures report the RMSE (in kg ha⁻¹) and r² calculated for each treatment

Table 5 Nitrogen lost by ammonia volatilization, as modelled by Visual Minteq and Hydrus 1D models

Treatment	Hydrus	Visual Minteq
U1	57% (90 kg ha ⁻¹)	67% (105 kg ha ⁻¹)
U3	55% (260 kg ha ⁻¹)	58% (270 kg ha ⁻¹)
F	28% (48 kg ha ⁻¹)	38% (66 kg ha ⁻¹)

fertilization, soil pH and ammonia volatilization that needs to be considered when studying nitrogen fluxes. It is likely that a high load of urine or long-term urine fertilization by causing high losses through ammonia volatilization and high nitrification rates decreases the pH of calcareous soils, thus decreasing the potential of the soil for subsequent ammonia volatilization.

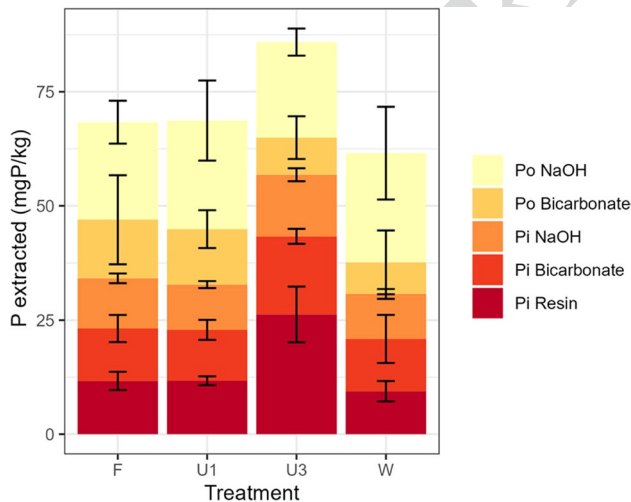
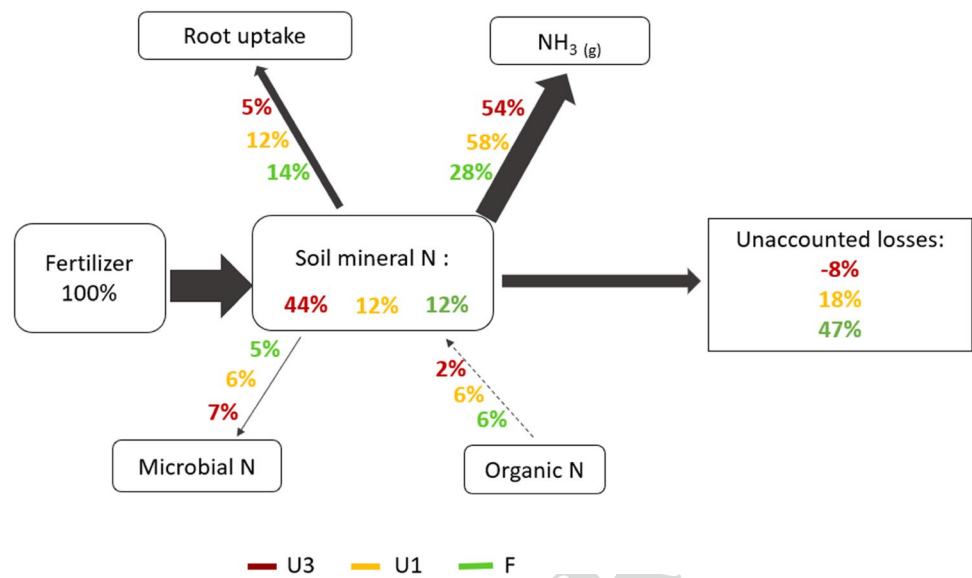


Fig. 5 Distribution of phosphorus in different soil pools at the end of experiment. The errors bars represent the standard error of four replicates. Pi Resin=resin extractable inorganic P; Pi Bicarbonate=sodium bicarbonate extractable inorganic P; Po Bicarbonate=sodium bicarbonate extractable organic P; Pi NaOH=sodium hydroxide extractable inorganic P; Po NaOH=sodium hydroxide extractable organic P

The nitrogen budget error was especially high in the F treatment (Fig. 6 and Table 4S, Supplementary data), suggesting that unaccounted losses, most probably leaching and denitrification, had a higher contribution in the N budget of this treatment. Hence, nitrate leaching and denitrification were probably higher in the fertilizer treatment than in the urine treatments. Most likely, the NH₄NO₃ fertilizer induced a smaller volatilization rate but higher leaching and denitrification rates because of its partition between nitrate and ammonium (Eckard et al. 2003; Fernández-Escobar et al. 2004). In addition, such high levels of excess N were not expected as the U1 and F fertilization rate had supposedly been adjusted to meet crop requirements, but crop growth was limited in our experiment possibly because of excessive heat during that summer. In conditions of adequate crop uptake, lower total N losses are to be expected.

In this experimental setup, soil conditions were optimal for ammonia volatilization (pH at 8.7 and air temperature ranging between 20 and 30 °C), so the high rates of volatilization simulated in the urine treatments can be considered an upper limit for urine fertilization. Although these high ammonia emissions raise concern, it is possible to reduce them with appropriate application techniques, timing and dosage (Mencaroni et al. 2021; Rodhe et al. 2004).

Fig. 6 Nitrogen fate in each fertilized treatment on a 0–10-cm soil profile. Percentages represent the estimated percentage of the N supplied by fertilizer that had followed each pathway at the end of the experiment. Unaccounted losses are calculated by the nitrogen budget error. A small budget error (< 10% of N supplied) suggests that unaccounted losses are close to 0



654 Bioavailability of N from urine fertilizer for plants 655 and microbes

656 In our experiment, most of the ammonium supplied by urine
657 was readily nitrified in the soil. This result is consistent with
658 other studies with urine fertilization showing that the nitro-
659 gen applied was in nitrate form at the end of their experiment
660 (Cuttle et al. 2001). Ledgard and Saunders (1982) observed
661 a nitrate peak 10 days after fertilization, which is consist-
662 ent with our peak 13 days after the first urine application.
663 Ammonium oxidation into nitrite is faster than nitrite oxi-
664 dation into nitrate (Monaghan and Barraclough 1992). As
665 with our analytical method we could not distinguish nitrate
666 and nitrite, we cannot exclude that nitrite might have been
667 present along with nitrate. This could have caused nitro-
668 gen stress in the urine treatments, especially at the start of
669 vegetative growth, given that nitrite is not bioavailable and
670 that spinach prefers nitrate over ammonium (Okazaki et al.
671 2009). The lower crop uptake in the U1 treatment (Table 4)
672 could support the hypothesis of a nitrogen limitation due to
673 incomplete nitrification at the beginning of the experiment.
674 Another explanation for the lower N uptake in the U1 treat-
675 ment could lie in the high rate of ammonia volatilization,
676 reducing the amount of N available in the soil. In this case,
677 the nitrogen stress should have occurred at the end of the
678 growth cycle where the N demand was higher. However, the
679 plant growth data are not sufficiently detailed to determine
680 whether or when nitrogen stress occurred in the U1 treat-
681 ment (Fig. 2S, Supplementary data).

682 The microbial N pool appears to have increased in the
683 fertilized treatments between the beginning and end of
684 the experiment, with a stronger response in the U3 treat-
685 ment. Microbes were, therefore, able to immobilize N from
686 urine, making it unlikely that urine had a negative effect on

687 microbial activity. However, this increase was never accom-
688 panied by an increase in microbial C, causing a shift in the
689 microbial C to N ratio. Mason-Jones et al. (2022) recently
690 highlighted that soil microbes have the capacity to store
691 surplus nutrients to reduce their loss and release them later
692 upon microbial death. Thus, under urine application, part
693 of the added ammonium was probably stored in the micro-
694 bial biomass forming a readily available N pool. Similarly,
695 Zaman et al. (2006) found an increase of only microbial N
696 with NH_4Cl fertilizer, while both N and C microbial pools
697 increased with C-rich dairy shed effluent. Urine alone is
698 relatively poor in dissolved organic carbon (Table 2) and,
699 therefore, does not stimulate microbial growth.

700 Bioavailability of P from urine fertilizer

701 N and P uptake were highly correlated (Pearson coefficient
702 $r=0.97$). Therefore, the lower P uptake observed for the U1
703 treatment might be a consequence of the nitrogen stress that
704 likely occurred under U1.

705 At low doses, the fate of phosphorus from urine and
706 synthetic fertilizer seemed relatively similar as there was
707 no difference in concentrations of the different P pools or
708 total P between U1 and F. The fate of P from urine was
709 clearly detectable in the U3 treatment, where the high dose
710 of urine supplied significantly increased the available P and
711 secondarily the less available P pools (Fig. 5). Therefore,
712 phosphorus from urine distributed mostly into available and
713 slightly available inorganic P (Pi Bicarbonate) with a resid-
714 ual effect that was still detectable after 5 weeks of cropping.
715 This result is in line with a study by Pandorf et al. (2018)
716 finding that P from urine can be taken up by snap beans and
717 turnips. In another experiment, Bonvin et al. (2015) applied

718 synthetic and nitrified urine and found a similar P recovery
719 rate in the crops as under mineral fertilizer.

720 In contrast, the application of urine did not affect the size
721 of organic P pools, including microbial P. Again, this might
722 be explained by the low concentration of dissolved organic C
723 in urine, which does not foster microbial activity or growth.
724 However, unlike nitrogen, there was no storage of P in the
725 microbial pool. According to Chen et al. (2019), the more
726 the element is limiting the more it is stored once it becomes
727 available. At T0, the soil concentration in mineral nitrogen
728 was around 8 mgN kg⁻¹, while the available phosphorus (Pi
729 resin + Pi Bicarbonate) was around 19 mgP kg⁻¹. Hence,
730 at the start of the experiment N was more limiting than P,
731 explaining the stronger N than P storage in the microbial
732 biomass.

733 Additionally, in the U3 treatment, P availability was
734 likely increased by the decrease in soil pH (0.2 units) caus-
735 ing calcium phosphate dissolution. In our experiment, it is
736 likely that the subsequent mobilized phosphate was partially
737 adsorbed on the soil exchange complex (Frossard et al. 1995)
738 explaining the increase of iron and aluminium bonded P
739 (Pi NaOH pool) (Adhami et al. 2006; Prietzel et al. 2016)
740 and partially remained in solution increasing the P available
741 pool.

742 Conclusion

743 In this experiment, urine supplied in appropriate doses
744 had a very similar behaviour to that of a synthetic fertil-
745 izer in a calcareous soil suggesting that other compounds
746 present in urine did not affect N and P uptake by the crop
747 and their fate in the soil in the short term. Appropriate
748 doses of urine altered neither soil pH nor soil conductiv-
749 ity, and provided nitrogen and phosphorus in bioavailable
750 forms. Supplied phosphorus was either taken up by the
751 crop or dissolved in the soil, and the ammonium was nitrif-
752 ied within about 10 days after application. However, this
753 study raises awareness on the amount of ammonia that
754 can be lost by volatilization in a calcareous soil. Indeed,
755 chemical equilibrium and solute transfer models agreed on
756 the estimation that about half of the N applied with urine
757 could be lost by volatilization. Therefore, it is imperative
758 to find more suitable fertilization techniques to mitigate
759 ammonia volatilization on alkaline soils. Overall, our
760 results demonstrate that although direct usage of human
761 urine for fertilization is possible, timing, dosage and soil
762 type need to be carefully considered for this practice to be
763 environmentally sustainable. Future studies are encour-
764 aged to focus on the long-term effect of urine fertilization
765 especially on soil pH and salinity which are key factors
766 of soil quality and disentangle the various indirect effects
767 that human urine could have on soil and plant health.

Supplementary Information The online version contains supplement- 768
ary material available at <https://doi.org/10.1007/s11356-023-26895-5>. 769

Acknowledgements We thank EcoSec for providing the urine used in 770
this experiment and Jean-Louis Aznar for helpful assistance. 771

Author contribution MR: writing—original draft, investigation, data 772
curation, formal analysis, visualization. CP: conceptualization, meth- 773
odology, resources, funding acquisition, writing—review and editing. 774
CM: conceptualization, methodology, resources, funding acquisition, 775
writing—review and editing. NAM: resources, funding acquisition, 776
writing—review and editing. DC: formal analysis, writing—review 777
and editing. 778

Funding This work was supported by Institut Agro Montpellier, Mont- 779
pellier, France. 780

Data availability The datasets are available from the corresponding 781
author on reasonable request. 782

Declarations 783

Ethical approval Not applicable. 784

Consent to participate All authors gave their consent to participate in 785
the conception of this paper. 786

Consent for publication All authors read and approved the final manu- 787
script. 788

Competing interests The authors declare no competing interests. 789

References

- Adhami E, Maftoun M, Ronaghi A, Karimian N, Yasrebi J, Assad MT 791
(2006) Inorganic phosphorus fractionation of highly calcareous 792
soils of Iran. *Commun Soil Sci Plant Anal* 37:1877–1888. <https://doi.org/10.1080/00103620600767116> 793
Akpan-Idiok AU, Udo IA, Braide EI (2012) The use of human urine 794
as an organic fertilizer in the production of okra (*Abelmoschus* 795
esculentus) in South Eastern Nigeria Resour. Conserv Recycl 796
62:14–20. <https://doi.org/10.1016/j.resconrec.2012.02.003> 797
Allan R, Smith M (1998) Crop evapotranspiration—guidelines for com- 798
puting crop water requirements-FAO Irrigation and drainage paper 799
Ayers RS, Westcot D (1985) Water quality for agriculture. FAO Irri- 800
gation and Drainage Paper 29, Food and Agriculture Organiza- 801
tion of the United Nations, Rome 802
Barak P, Jobe BO, Krueger AR, Peterson LA, Laird DA (1997) 803
Effects of long-term soil acidification due to nitrogen fertilizer 804
inputs in Wisconsin. *Plant Soil* 197:61–69. <https://doi.org/10.1023/A:1004297607070> 805
Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed- 806
effects models using lme4. *J Stat Softw* 67. <https://doi.org/10.18637/jss.v067.i01> 807
Boh MY, Sauerborn J (2014) Effect of NaCl-induced salinity and 808
human urine fertilization on substrate chemical properties. *Open* 809
J Soil Sci 04:16–25. <https://doi.org/10.4236/ojss.2014.41003> 810
Bolan NS, Hedley MJ, White RE (1991) Processes of soil acidification 811
during nitrogen cycling with emphasis on legume based pastures. 812
Plant Soil 134:53–63. <https://doi.org/10.1007/BF00010717> 813
Bonvin C, Etter B, Udert KM, Frossard E, Nanzer S, Tamburini F, 814
Oberson A (2015) Plant uptake of phosphorus and nitrogen 815
816
817
818

- 819 recycled from synthetic source-separated urine. *Ambio* 44:217–
820 227. <https://doi.org/10.1007/s13280-014-0616-6>
- 821 Castaldelli G, Colombani N, Tamburini E, Vincenzi F, Mastrocicco M
822 (2018) Soil type and microclimatic conditions as drivers of urea
823 transformation kinetics in maize plots. *CATENA* 166:200–208.
824 <https://doi.org/10.1016/j.catena.2018.04.009>
- 825 Chen J, Seven J, Zilla T, Dippold MA, Blagodatkaya E, Kuzyakov Y
826 (2019) Microbial C:N: P stoichiometry and turnover depend on
827 nutrients availability in soil: A 14C, 15N and 33P triple labelling
828 study. *Soil Biol Biochem* 131:206–216. [https://doi.org/10.1016/j.
AQ7 soilbio.2019.01.017](https://doi.org/10.1016/j.soilbio.2019.01.017)
- 830 Clough TJ, Sherlock RR, Mautner MN, Milligan DB, Wilson PF, Free-
831 man CG, McEwan MJ (2003) Emission of nitrogen oxides and
832 ammonia from varying rates of applied synthetic urine and cor-
833 relations with soil chemistry. *Soil Res* 41:421. [https://doi.org/10.
834 1071/SR02105](https://doi.org/10.1071/SR02105)
- 835 Cuttle SP, Scurlock RV, Davies BMS (2001) Comparison of fertilizer
836 strategies for reducing nitrate leaching from grazed grassland, with
837 particular reference to the contribution from urine patches. *J Agric
838 Sci* 136:221–230. <https://doi.org/10.1017/S0021859601008516>
- 839 Desmidt E, Ghyselbrecht K, Zhang Y, Pinoy L, Van der Bruggen B,
840 Verstraete W, Rabaey K, Meesschaert B (2015) Global phospho-
841 rus scarcity and full-scale P-recovery techniques: a review. *Crit
842 Rev Environ Sci Technol* 45:336–384. [https://doi.org/10.1080/
843 10643389.2013.866531](https://doi.org/10.1080/10643389.2013.866531)
- 844 Di HJ, Cameron KC (2007) Nitrate leaching losses and pasture yields
845 as affected by different rates of animal urine nitrogen returns
846 and application of a nitrification inhibitor—a lysimeter study.
847 *Nutr Cycl Agroecosyst* 79:281–290. [https://doi.org/10.1007/
848 s10705-007-9115-5](https://doi.org/10.1007/s10705-007-9115-5)
- 849 Eckard RJ, Chen D, White RE, Chapman DF (2003) Gaseous nitrogen
850 loss from temperate perennial grass and clover dairy pastures in
851 south-eastern Australia. *Aust J Agric Res* 54:561. [https://doi.org/
852 10.1071/AR02100](https://doi.org/10.1071/AR02100)
- 853 Fanjaniaina ML, Stark F, Ramarovaoka NP, Rakotoharinaivo JF,
854 Rafolisy T, Salgado P, Becquer T (2022) Nutrient flows and bal-
855 ances in mixed farming systems in Madagascar. *Sustainability*
856 14:984. <https://doi.org/10.3390/su14020984>
- 857 Feddes R, Kowalik PJ, Zaradny H (1978) Simulation of field water use
858 and crop yield. [WWW Document]. URL [https://library.wur.nl/
859 WebQuery/wurpubs/fulltext/172222](https://library.wur.nl/WebQuery/wurpubs/fulltext/172222). Accessed 4.19.22
- 860 Fernández-Escobar R, Benlloch M, Herrera E, García-Novelo JM
861 (2004) Effect of traditional and slow-release N fertilizers on
862 growth of olive nursery plants and N losses by leaching. *Sci Hortic*
863 101:39–49. <https://doi.org/10.1016/j.scienta.2003.09.008>
- 864 Ferreira J, Sandhu D, Liu X, Halvorson J (2018) Spinach (*Spinacea
865 oleracea* L.) response to salinity: nutritional value, physiological
866 parameters, antioxidant capacity, and gene expression. *Agriculture*
867 8:163. <https://doi.org/10.3390/agriculture8100163>
- 868 Frerichs C, Key G, Broll G, Daum D (2022) Nitrogen fertilization
869 strategies to reduce the risk of nitrate leaching in open field cul-
870 tivation of spinach (*Spinaciaoleracea* L.)#. *J Plant Nutr Soil Sci*
871 185:264–281. <https://doi.org/10.1002/jpln.202100275>
- 872 Frossard E, Brossard M, Hedley MJ, Metherell A (1995) Reactions
873 controlling the cycling of P in soils 16
- 874 Frossard E, Condron LM, Oberson A, Sinaj S, Fardeau JC (2000)
875 Processes governing phosphorus availability in temperate soils. *J
876 Environ Qual* 29:15–23. [https://doi.org/10.2134/jeq2000.00472
877 425002900010003x](https://doi.org/10.2134/jeq2000.00472425002900010003x)
- 878 Gruber N, Galloway JN (2008) An Earth-system perspective of the
879 global nitrogen cycle. *Nature* 451:293–296. [https://doi.org/10.
880 1038/nature06592](https://doi.org/10.1038/nature06592)
- 881 Gustafsson JP (2011) Visual MINTEQ 3.0 user guide. KTH, Depart-
882 ment of Land and Water Resources, Stockholm, Sweden
- 883 Hanson BR, Šimůnek J, Hopmans JW (2006) Evaluation of urea-
884 ammonium–nitrate fertigation with drip irrigation using numerical
885 modeling. *Agric Water Manag* 86:102–113. [https://doi.org/10.
886 1016/j.agwat.2006.06.013](https://doi.org/10.1016/j.agwat.2006.06.013)
- 887 Hargrove WL, Kissel DE, Fenn LB (1977) Field measurements of
888 ammonia volatilization from surface applications of ammonium
889 salts to a calcareous soil¹. *Agron J* 69:473–476. [https://doi.org/
890 10.2134/agronj1977.00021962006900030035x](https://doi.org/10.2134/agronj1977.00021962006900030035x)
- 891 Heinrich A, Smith R, Cahn M (2013) Nutrient and water use of fresh
892 market spinach. *HortTechnol* 23:325–333. [https://doi.org/10.
893 21273/HORTTECH.23.3.325](https://doi.org/10.21273/HORTTECH.23.3.325)
- 894 Helfenstein J, Pistocchi C, Oberson A, Tamburini F, Goll DS, Frossard
895 E (2020) Estimates of mean residence times of phosphorus in
896 commonly considered inorganic soil phosphorus pools. *Biogeo-
897 sciences* 17:441–454. <https://doi.org/10.5194/bg-17-441-2020>
- 898 Jury WA, Spencer WF, Farmer WJ (1983) Behavior assessment model for
899 trace organics in soil: I Model Description. *J Environ Qual* 12:558–
900 564. <https://doi.org/10.2134/jeq1983.00472425001200040025x>
- 901 Kampschreur MJ, Temmink H, Kleerebezem R, Jetten MSM, van
902 Loosdrecht MCM (2009) Nitrous oxide emission during wastewa-
903 ter treatment. *Water Res* 43:4093–4103. [https://doi.org/10.1016/j.
904 watres.2009.03.001](https://doi.org/10.1016/j.watres.2009.03.001)
- 905 Karak T, Bhattacharyya P (2011) Human urine as a source of alterna-
906 tive natural fertilizer in agriculture: a flight of fancy or an achiev-
907 able reality. *Resour Conserv Recycl* 55:400–408. [https://doi.org/
908 10.1016/j.resconrec.2010.12.008](https://doi.org/10.1016/j.resconrec.2010.12.008)
- 909 Kool D, Hoffland E, Hummelink E, Vangroenigen J (2006) Increased
910 hippuric acid content of urine can reduce soil N₂O fluxes. *Soil
911 Biol Biochem* 38:1021–1027. [https://doi.org/10.1016/j.soilbio.
912 2005.08.017](https://doi.org/10.1016/j.soilbio.2005.08.017)
- 913 Larsen TA, Udert KM, Lienert J (2013) Source separation and decen-
914 tralization for wastewater management. IWA Publishing. [https://
915 doi.org/10.2166/9781780401072](https://doi.org/10.2166/9781780401072)
- 916 Ledgard SF, Saunders WMH (1982) Effects of nitrogen fertiliser and
917 urine on pasture performance and the influence of soil phosphorus
918 and potassium status. *N Z J Agric Res* 25:541–547. [https://doi.
919 org/10.1080/00288233.1982.10425219](https://doi.org/10.1080/00288233.1982.10425219)
- 920 Li Y, Šimůnek J, Zhang Z, Jing L, Ni L (2015) Evaluation of nitrogen
921 balance in a direct-seeded-rice field experiment using Hydrus-
922 1D. *Agric Water Manag* 148:213–222. [https://doi.org/10.1016/j.
923 agwat.2014.10.010](https://doi.org/10.1016/j.agwat.2014.10.010)
- 924 Lienert J, Larsen TA (2010) High acceptance of urine source separa-
925 tion in seven European countries: a review. *Environ Sci Technol*
926 44:556–566. <https://doi.org/10.1021/es9028765>
- 927 Magdoff FR, Bartlett RJ (1985) Soil pH buffering revisited. *Soil Sci
928 Soc Am J* 49:145–148. [https://doi.org/10.2136/sssaj1985.03615
929 995004900010029x](https://doi.org/10.2136/sssaj1985.03615995004900010029x)
- 930 Maggi F, Daly E (2006) Decomposition pathways and rates of human
931 urine in soils. *Agric Food Chem*
- 932 Martin TMP, Esculier F, Levavasseeur F, Houot S (2022) Human
933 urine-based fertilizers: a review. *Crit Rev Environ Sci Technol*
934 52:890–936. <https://doi.org/10.1080/10643389.2020.1838214>
- 935 Mason-Jones K, Robinson SL, Veen GF, Manzoni S, van der Putten
936 WH (2022) Microbial storage and its implications for soil ecology.
937 *ISME J* 16:617–629. <https://doi.org/10.1038/s41396-021-01110-w>
- 938 Mencaroni M, Dal Ferro N, Furlanetto J, Longo M, Lazzaro B, Sar-
939 tori L, Grant BB, Smith WN, Morari F (2021) Identifying N fer-
940 tilizer management strategies to reduce ammonia volatilization:
941 towards a site-specific approach. *J Environ Manage* 277:111445.
942 <https://doi.org/10.1016/j.jenvman.2020.111445>
- 943 Meyer G, Frossard E, Mäder P, Nanzer S, Randall DG, Udert KM,
944 Oberson A (2018) Water soluble phosphate fertilizers for crops
945 grown in calcareous soils—an outdated paradigm for recycled
946 phosphorus fertilizers? *Plant Soil* 424:367–388. [https://doi.org/
947 10.1007/s11104-017-3545-x](https://doi.org/10.1007/s11104-017-3545-x)
- 948 Mills HA, Barker AV, Maynard DN (1974) Ammonia volatilization
949 from soils¹. *Agron J* 66:355–358. [https://doi.org/10.2134/agron
950 j1974.00021962006600030005x](https://doi.org/10.2134/agronj1974.00021962006600030005x)

- 951 Mkeni 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>
- 952
953
954
955 Monaghan RM, Barraclough D (1992) Some chemical and physical factors affecting the rate and dynamics of nitrification in urine-affected soil. *Plant Soil* 143:11–18. <https://doi.org/10.1007/BF00009124>
- 956
957
958
959
960 Neina D (2013) Short-term effects of human urine fertiliser and wood ash on soil pH and electrical conductivity 12
- 961
962 Ohno T, Zibilske LM (1991) Determination of low concentrations of phosphorus in soil extracts using malachite green. *Soil Sci Soc Am J* 55:892–895. <https://doi.org/10.2136/sssaj1991.03615995005500030046x>
- 963
964
965
966 Okazaki K, Oka N, Shinano T, Osaki M, Takebe M (2009) Metabolite profiling of spinach (*Spinaciaoleracea* L.) leaves by altering the ratio of NH_4^+ / NO_3^- in the culture solution. *Soil Sci Plant Nutr* 55:496–504. <https://doi.org/10.1111/j.1747-0765.2009.00383.x>
- 967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000
1001
1002
1003
1004
1005
1006
1007
- Olivia SNS, Marcelo BT, Hans RG, Hans RG, Luciano MQ, da Silva Paz P, da Vital C, Linge Silva, Asher K (2015) Bermudagrass fertilization with human urine as a tool to close nutrient cycles: the use of micronutrients. *Afr J Agric Res* 10:3189–3199. <https://doi.org/10.5897/AJAR2015.9942>
- Pandorf M, Hochmuth G, Boyer T (2018) Human urine as a fertilizer in the cultivation of snap beans (*Phaseolus vulgaris*) and turnips (*Brassica rapa*) [WWW document]. ResearchGate. URL https://www.researchgate.net/publication/329407271_Human_Urine_as_a_Fertilizer_in_the_Cultivation_of_Snap_Beans_Phaseolus_vulgaris_and_Turnips_Brassica_rapa. Accessed 3.27.20
- Powelson DS, Dawson CJ (2022) Use of ammonium sulphate as a sulphur fertilizer: implications for ammonia volatilization. *Soil Use Manag* 38:622–634. <https://doi.org/10.1111/sum.12733>
- Pradhan SK, Holopainen JK, Heinonen-Tanski H (2009) Stored human urine supplemented with wood ash as fertilizer in tomato (*Solanum lycopersicum*) cultivation and its impacts on fruit yield and quality. *J Agric Food Chem* 57:7612–7617. <https://doi.org/10.1021/jf9018917>
- Prietzl J, Klysubun W, Werner F (2016) Speciation of phosphorus in temperate zone forest soils as assessed by combined wet-chemical fractionation and XANES spectroscopy. *J Plant Nutr Soil Sci* 179:168–185. <https://doi.org/10.1002/jpln.201500472>
- R Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. [WWW Document]. URL <https://www.R-project.org/>. Accessed 8.1.22
- Raza S, Zamanian K, Ullah S, Kuzyakov Y, Virto I, Zhou J (2021) Inorganic carbon losses by soil acidification jeopardize global efforts on carbon sequestration and climate change mitigation. *J Clean Prod* 315:128036. <https://doi.org/10.1016/j.jclepro.2021.128036>
- Rodhe L, RichertStintzing A, Steineck S (2004) Ammonia emissions after application of human urine to a clay soil for barley growth. *Nutr Cycl Agroecosystems* 68:191–198. <https://doi.org/10.1023/B:FRES.0000019046.10885.ee>
- Sangare D, Sou/Dakoure M, Hijikata N, Lahmar R, Yacouba H, Coulibaly L, Funamizu N (2015) Toilet compost and human urine used in agriculture: fertilizer value assessment and effect on cultivated soil properties. *Environ Technol*. 36:1291–1298 <https://doi.org/10.1080/09593330.2014.984774>
- Schönning, Stenström (2004) Guidelines for the safe use of urine and faeces in ecological sanitation systems
- Shingiro C, Nyagatare G, Hirwa H, Solange U (2019) Effect of human urine and compost tea on soil physical and chemical properties case study Rubirizi Marshland 7
- Šimůnek J, Genuchten MTh, Šejna M (2008) Development and applications of the HYDRUS and STANMOD software packages and related codes. *Vadose Zone J* 7:587–600. <https://doi.org/10.2136/vzj2007.0077>
- Somers C, Girkin NT, Rippey B, Lanigan GJ, Richards KG (2019) The effects of urine nitrogen application rate on nitrogen transformations in grassland soils. *J Agric Sci* 157:515–522. <https://doi.org/10.1017/S0021859619000832>
- Steffen W, Richardson K, Rockstrom J, Cornell SE, Fetzer I, Bennett EM, Biggs R, Carpenter SR, de Vries W, de Wit CA, Folke C, Gerten D, Heinke J, Mace GM, Persson LM, Ramanathan V, Rayers B, Sorlin S (2015) Planetary boundaries: guiding human development on a changing planet. *Science* 347:1259855–1259855. <https://doi.org/10.1126/science.1259855>
- Tao Y, Li N, Wang S, Chen H, Guan X, Ji M (2021) Simulation study on performance of nitrogen loss of an improved subsurface drainage system for one-time drainage using HYDRUS-2D. *Agric Water Manag* 246:106698. <https://doi.org/10.1016/j.agwat.2020.106698>
- Tiessen H, Moir J (1993) Characterisation of available P by sequential extraction
- 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>
- van Genuchten MTh (1980) A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. *Soil Sci Soc Am J* 44:892–898. <https://doi.org/10.2136/sssaj1980.03615995004400050002x>
- Wald C (2022) The urine revolution: how recycling pee could help to save the world. *Nature* 602:202–206. <https://doi.org/10.1038/d41586-022-00338-6>
- Whitehead DC, Raistrick N (1990) Ammonia volatilization from five nitrogen compounds used as fertilizers following surface application to soils. *J Soil Sci* 41:387–394. <https://doi.org/10.1111/j.1365-2389.1990.tb00074.x>
- Zaman M, Di HJ, Cameron KC (2006) A field study of gross rates of N mineralization and nitrification and their relationships to microbial biomass and enzyme activities in soils treated with dairy effluent and ammonium fertilizer. *Soil Use Manag* 15:188–194. <https://doi.org/10.1111/j.1475-2743.1999.tb00087.x>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.

Journal:	11356
Article:	26895

Author Query Form

Please ensure you fill out your response to the queries raised below and return this form along with your corrections

Dear Author

During the process of typesetting your article, the following queries have arisen. Please check your typeset proof carefully against the queries listed below and mark the necessary changes either directly on the proof/online grid or in the 'Author's response' area provided below

Query	Details Required	Author's Response
AQ1	The city Birmingham has been inserted for the present address of Manon Rumeau. Please check.	
AQ2	Equations has been renumbered. Please check if correct.	
AQ3	Please check all equations are captured/presented correctly.	
AQ4	Reference list Heinrich et al. (2013) was transferred here. Please check if correct.	
AQ5	Table 4 has been inserted here for sequencing. Please check.	
AQ6	Please provide complete bibliographic details of this reference (Allan and Smith 1998, Ayers and Westcot 1985, Frossard et al. 1995, Gustafsson 2011, Maggi and Daly 2006, Schönning and Stenström 2004, Shingiro et al. 2019, Tiessen and Moir 1993).	
AQ7	Please check article title in reference Chen et al. 2019 if correct.	