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	Division Organization	School of Geography, Earth and Environmental Sciences University of Birmingham			
	Address Phone Fax	Birmingham, UK			
	Email URL	MLR094@student.bham.ac.uk			
	ORCID	http://orcid.org/0000-0001-7731-6247			
Author	FamilyName Particle	Marsden			
	Given Name Suffix Division	Claire			
	Organization Address Phone Fax	Eco&SolsUniv Montpellier, CIRAD, INRAE, Institut Agro, IRD Montpellier, France			
	Email URL ORCID				
Author	FamilyName Particle	Mouheb			
	Given Name Suffix Division	Nassim Ait			
	Organization Address Phone	INRAE, UMR GEAU, Univ Montpellier Montpellier, France			
	Fax Email URL ORCID				
Author	FamilyName Particle	Crevoisier			
	Given Name	David			

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Author	FamilyName Particle Given Name Suffix Division	Pistocchi Chiara
	Organization Address Phone Fax Email URL ORCID	Eco&SolsUniv Montpellier, CIRAD, INRAE, Institut Agro, IRD Montpellier, France
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Abstract	Human urine concer it a potential alterna situations favouring at identifying the fa toilets in a calcareou urine (170 kgN ha- fertilizer treatment ( conducted in 4 soil scheme. We monito Hydrus-1D and Viss phosphorus (CNP) i urine affected soil p phosphorus supplied demonstrated by a m nitrified within abou estimated that more results indicate that growth, but that hea whether long-term a <i>Graphical Abstract</i> .	ntrates 88% of the nitrogen and 50% of the phosphorus excreted by humans, making tive crop fertilizer. However, knowledge gaps remain on the fate of nitrogen in NH <sub>3</sub> volatilization and on the availability of P from urine in soils. This study aimed te of nitrogen and phosphorus supplied by human urine from source separation as soil. To this end, a spinach crop was fertilized with 2 different doses of human 1 + 8.5 kgP ha–1 and 510 kgN ha– $1 + 25.5$ kgP ha– $1$ ) and compared with a synthetic (170 kgN ha– $1 + 8.5$ kgP ha– $1$ ) and an unfertilized control. The experiment was tanks (50-cm depth) in greenhouse conditions, according to a randomized block red soil mineral nitrogen over time and simulated nitrogen volatilization using ual Minteq software. We also monitored soil phosphorus pools, carbon, nitrogen and n microbial biomass, soil pH and electrical conductivity. Only an excessive input of H (decreasing it by 0.2 units) and soil conductivity (increasing it by 183%). The d was either taken up by the crop or remained mostly in the available P pool, as the increase of the resin and bicarbonate extractable P. Ammonium seemed to be at 10 days after application. However, both Visual Minteq and Hydrus models than 50% of the nitrogen supplied was lost by ammonia volatilization. Overall, our direct application of urine to a calcareous soil provides available nutrients for plant vy losses of volatilized nitrogen are to be expected. Our results also question upplication could affect soil pH and salinity.



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.

## **RESEARCH ARTICLE**



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

<sup>4</sup> Manon Rumeau<sup>1,2</sup> · Claire Marsden<sup>1</sup> · Nassim Ait Mouheb<sup>3</sup> · David Crevoisier<sup>4</sup> · Chiara Pistocchi<sup>1</sup>

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## 7 Abstract

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<sup>8</sup> Human urine concentrates 88% of the nitrogen and 50% of the phosphorus excreted by humans, making it a potential alternative
 <sup>9</sup> crop fertilizer. However, knowledge gaps remain on the fate of nitrogen in situations favouring NH<sub>3</sub> volatilization and on the

<sup>10</sup> availability of P from urine in soils. This study aimed at identifying the fate of nitrogen and phosphorus supplied by human urine

- <sup>11</sup> from source separation toilets in a calcareous soil. To this end, a spinach crop was fertilized with 2 different doses of human urine <sup>12</sup> (170 kgN ha<sup>-1</sup> + 8.5 kgP ha<sup>-1</sup> and 510 kgN ha<sup>-1</sup> + 25.5 kgP ha<sup>-1</sup>) and compared with a synthetic fertilizer treatment (170 kgN
- $^{13}$  ha<sup>-1</sup>+8.5 kgP ha<sup>-1</sup>) and an unfertilized control. The experiment was conducted in 4 soil tanks (50-cm depth) in greenhouse condi-
- <sup>14</sup> tions, according to a randomized block scheme. We monitored soil mineral nitrogen over time and simulated nitrogen volatiliza-
- <sup>15</sup> tion using Hydrus-1D and Visual Minteg software. We also monitored soil phosphorus pools, carbon, nitrogen and phosphorus
- <sup>16</sup> (CNP) in microbial biomass, soil pH and electrical conductivity. Only an excessive input of urine affected soil pH (decreasing it
- <sup>17</sup> by 0.2 units) and soil conductivity (increasing it by 183%). The phosphorus supplied was either taken up by the crop or remained
- <sup>18</sup> mostly in the available P pool, as demonstrated by a net increase of the resin and bicarbonate extractable P. Ammonium seemed
- <sup>19</sup> to be nitrified within about 10 days after application. However, both Visual Minteq and Hydrus models estimated that more than
- <sup>20</sup> 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
- <sup>22</sup> results also question whether long-term application could affect soil pH and salinity.

<sup>23</sup> Keywords Source separation · Fertilization · Hydrus · Ammonia volatilization

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<ul> <li>Hig</li> <li>N</li> <li>but</li> <li>calo</li> <li>P</li> <li>rem</li> <li>C</li> <li>con</li> </ul>	hlights Titrogen from urine was bioavailable for plants and microbes, half of the N applied could be lost by volatilization in a careous soil. hosphorus from urine was either taken up by plants or nained mostly in available pools in a calcareous soil. Only excessive doses of urine application affected soil pH and iductivity 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<sub>2</sub> for fertilizer production and by the conversion of reactive N into N<sub>2</sub>O or N<sub>2</sub> 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

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excreted by humans (Martin et al. 2022). If recycled, it could 42 account for more than 13% of the global agricultural fertilizer 43 demand (Wald 2022). In addition, urine represents less than 44 45 1% of the volume of wastewater but 79% of the nitrogen and 47% of the phosphorus treated by sewage treatment plants 46 (Larsen et al. 2013). Hence, diverting it would reduce by 47 more than half the nutrient pollution from wastewater (Wald 48 2022). Urine can be considered sterile in most cases and does 49 not present risks of disease transmission if not contaminated 50 with faeces. This is possible in source separation systems 51 (toilets with separated outlets or urinals) (Lienert and Larsen 52 2010) which allow the safe collection of urine. 53

Each human produces 1 to 1.5L of urine per day (Karak 54 and Bhattacharyya 2011). Urine is composed of 95% water, 55 and the remaining 5% consists of amino compounds (such 56 as urea or creatinine), organic anions and inorganic salts 57 (Maggi and Daly 2006). After urea hydrolysis, nitrogen is 58 mainly in ammonium form and phosphorus is either dis-59 60 solved in solution or precipitated as struvite (magnesium ammonium phosphate) (Udert et al. 2006). The fertilizing 61 ability of animal urine has been known for a long time (Di 62 63 and Cameron 2007; Fanjaniaina et al. 2022), while that of human urine has been proven in recent years (Akpan-Idiok 64 et al. 2012; Martin et al. 2022; Pradhan et al. 2009). How-65 ever, the fate and dynamics of nitrogen and phosphorus from 66 human urine are highly uncertain as soil biotic and abiotic 67 processes involving N and P could be affected by the other 68 compounds and nutrients present in urine. For instance, hip-69 puric acid in urine can inhibit denitrification (Kool et al. 70 2006), and the formation of ammonium bicarbonate can 71 72 inhibit nitrification (Clough et al. 2003; Somers et al. 2019).

Calcareous soils are common agricultural land in France. 73 These soils are prone to ammonia (NH<sub>3</sub>) volatilization because 74 of their alkaline pH. Therefore, urine application on such soils 75 is likely to result in high NH<sub>2</sub> losses by volatilization. However, 76 published volatilization rates range from 0 to 63% of the ammo-77 nium supplied (Mills et al. 1974; Powlson and Dawson 2022). 78 Phosphorus availability is also an important issue in alkaline 79 soils and could be improved by fertilization with urine: firstly 80 because urine is a source of P, and secondly because the appli-81 cation of ammonium can decrease soil pH by enhancing acidi-82 fying processes such as ammonia volatilization, nitrification 83 84 and subsequent nitrate leaching (Bolan et al. 1991; Raza et al. 2021). Hence, a decrease in soil pH could increase the availabil-85 ity of P from urine (i.e. dissolved as phosphate or precipitated 86 87 as struvite) which largely depends on pH (Frossard et al. 2000; Helfenstein et al. 2020; Meyer et al. 2018). 88

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).

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Moreover, urine is a multi-component solution: as well as 95 N and P, it contains K<sup>+</sup>, S, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, Cl<sup>-</sup> and other 96 micronutrients. Thus, it could represent a complete fertiliz-97 ing solution even though the bioavailability of urine micro-98 nutrients has not been well documented (Olivia et al. 2015). 99 Yet, the high concentrations in Na<sup>+</sup>, Cl<sup>-</sup> and SO<sub>4</sub><sup>2-</sup> could 100 also cause soluble salt accumulation in soils (Boh and Sau-101 erborn 2014; Mnkeni et al. 2008; Shingiro et al. 2019). 102

The objectives of this study were to determine the fates of 103 N and P from urine fertilization on a calcareous soil and the 104 effect on soil pH and salinity. The main hypotheses were: (1) 105 N supplied with urine is readily bioavailable, but a signifi-106 cant amount of N is lost by volatilization potentially causing 107 a decrease in soil pH; (2) urine application and associated 108 decrease in soil pH increase phosphorus availability in soils; 109 (3) urine increases soil salinity because of its soluble salt 110 concentration. To address these hypotheses, we conducted 111 a fertilization trial on a spinach crop (Spinacia oleracea 112 L.) where we compared the effect of two different doses 113 of source separated human urine with that of a synthetic 114 fertilizer with equivalent N and P concentrations and an 115 unfertilized control. 116

## Materials and methods

## Site and experiment description

The experiment was carried out in a greenhouse of the UMR 119 G-EAU in Montpellier between May 30th 2020 and July 5th 120 2020. Meteorological variables were measured by a weather 121 station located at the experimental site. Air temperature and 122 relative humidity in the greenhouse were measured by a tem-123 perature and relative humidity probe (model CS215, CAMP-124 BELL SCIENTIFIC), and global radiation was measured by 125 a pyranometer (model SP1110, CAMPBELL SCIENTIFIC) 126 (Table 1). 127

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The experiment was conducted in 4 soil tanks (soil surface equal to  $0.935 \text{ m}^2$  and 50-cm soil depth) (Fig. 1). Each tank was filled with approximatively 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  $17^{\text{th}}$  to July  $2^{\text{nd}}$ , 2020

	Air tempera- ture (°C)	Air relative humidity (%)	Global radiation (kW m <sup>-2</sup> )
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



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

the recommended dose of phosphorus. Nitrogen and phosphorus in the F treatment were added as ammonium nitrate  $(NH_4NO_3)$  and potassium phosphate  $(KH_2PO_4)$  respectively. Potassium was not limiting in soils (8 mg kg<sup>-1</sup> 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-169 up EcoSec (Montpellier, France, https://ecosec.fr/), which manu-170 factures and sells source separating toilets. These toilets allow 171 the selective collection of urine and faeces thanks to a gravity 172 system, but do not prevent cross contamination with faeces. Prior 173 to the experiment, the urine was stored for a year in an opaque 174 and airtight container in order to sanitize the effluent accord-175 ing to World Health Organization (WHO) recommendations 176 (Schönning and Stenström 2004). The urine's chemical com-177 position is summarized in Table 2. As expected, P was only in 178 inorganic form and nitrogen was mostly present in ammonium 179 form. However, ammonium concentration in urine decreased 180 by 0.58 gN  $L^{-1}$  between the beginning (T0) and the end of the 181 experiment (TF) due to ammonia volatilization during container 182 openings; this was considered in the N budget. Organic carbon 183 concentration was very low (57 mgC  $L^{-1}$ ) despite potential cross 184 contamination with faeces. Furthermore, the high concentration 185



**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

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

Parameters measured	Values	Method
Electrical conductivity at 25 °C (mS cm <sup>-1</sup> )	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 <sup>-1</sup> )	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 <sup>-1</sup> )	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
$SO4^{2-}$ (mg L <sup>-1</sup> )	1228.81	ISO 11885
$Cl^{-}$ (mg $L^{-1}$ )	3574.85	Water extraction 1/5, NF EN 9297
DBO5 (mg L <sup>-1</sup> ): 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	
$\operatorname{Zn}(\operatorname{mg} L^{-1})$	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

(1)

of Na<sup>+</sup> (1289 mg L<sup>-1</sup>) and Cl<sup>-1</sup> (3574 mg L<sup>-1</sup>) along with the 186 conductivity (40.6 mS  $cm^{-1}$ ) showed that the urine had high 187 salinity (Table 2). Additionally, the sodium absorption ratio 188 (SAR, indicating the potential sodium hazard for irrigation or 189 fertirrigation) of pure urine, solution U1 and solution U3 was 190 calculated with the following formula, and SAR values are 191 detailed in Table 2: 192

<sup>193</sup> 
$$SAR = \frac{Na^+}{\sqrt{\frac{1}{2}(Ca^{2+} + Mg^{2+})}}$$

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where concentrations of cations (Na<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>) are 195 expressed in meg  $L^{-1}$ . 196

#### Parameter monitoring and measurement 197

#### Soil sampling 198

Bulk soil samples at 0-10-cm depth composed of 2 sub-199 samples were taken in every quarter of a tank at the begin-200 ning of the experiment before sowing (T0), right after every 201

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fertilization (T1 to T6) and at the end of the experiment 202 (TF). For every sample, the gravimetric water content was 203 measured by drying the soil at 105 °C for 48 h. Mineral 204 nitrogen (NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) was measured in all the samples, 205 whereas pH, conductivity, organic and inorganic P concen-206 tration and microbial biomass were quantified only in the 207 T0 and TF samples. 208

## Soil bulk density

#### In each tank, 3 undisturbed soil cores of 5-cm depth were 210 taken. Soil cores were then oven dried at 105 °C, and the 211 bulk density was calculated for each core by dividing the 212 dry mass of soil by the volume of the core. 213

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## Mineral nitrogen measurement

Soils were extracted with 1 M KCl (soil to extractant ratio 215 of 1:3) on the day of sampling. The extracts were filtered 216 at 0.45 µm and frozen until analysis. Then, samples were 217

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analysed by continuous flow colorimetry (Skalar SA 3000

analyser). This method measures nitrite and nitrate speciestogether and ammonium separately.

### 221 pH

pH measurement was carried out according to the ISO standard using a pH probe (AFNOR, 2005). Soils were sieved,
air dried and extracted with a soil to water ratio of 1:5, and
measurements were performed after 1 h of agitation.

## 226 Electrical conductivity

Soils were sieved, air dried and extracted with a soil to water
ratio of 1:5. Then, the solution was agitated for 30 min, centrifuged and filtered. Electrical conductivity was then measured on the aqueous extract with a conductometer probe.

## 231 Organic and inorganic P pool

To quantify soil phosphorus pools, we performed a Hedley 232 sequential fractionation as modified by Tiessen and Moir 233 (1993). This method operationally identifies organic and 234 inorganic P pools. Soil samples were sequentially extracted 235 with 4 different reagents in the following order: anionic 236 exchange resin membranes (BDH #55,164, 6 cm×4 cm, 237 named P resin pool), 0.5 M sodium bicarbonate (NaHCO<sub>3</sub>, 238 Pi and Po bicarbonate pools), 0.5 M NaOH (Pi and Po NaOH 239 pools) and 1 M HCl (P HCl pool). To quantify organic P, 240 aliquots of bicarbonate and NaOH extracts were mineralized 241 by acid digestion, and the organic P concentration was cal-242 culated as the difference between inorganic P in the digested 243 extract (corresponding to the total P in the extract) and inor-244 ganic P in the non-digested extract. Inorganic phosphorus 245 concentration in each extract was measured by the mala-246 chite green colorimetric method (Ohno and Zibilske 1991). 247 The P resin and the Pi and Po bicarbonate are commonly 248 considered the available P pool (Tiessen and Moir 1993). 249 The exchange times of sequentially extracted P pools with 250 the soil solution increase with the strength of the extractant 251 (Helfenstein et al. 2020). Hence, the P pools extracted with 252 the stronger extractants (i.e. 0.5 M NaOH and 1 M HCl) are 253 most likely less available. 254

## 255 C N P in microbial biomass

The fumigation extraction method was applied to determine C, N and P in microbial biomass (AFNOR, 1997). For each soil sample, 4 subsamples were weighed, and 2 were fumigated with chloroform overnight. Blanks without soil were included. Then, two fumigated/non-fumigated subsamples underwent potassium sulphate (K<sub>2</sub>SO<sub>4</sub>, 0.025 M) extrac-261 tion for C and N quantification. The two other fumigated/ 262 non-fumigated subsamples underwent sodium bicarbonate 263 (NaHCO<sub>3</sub> 0.5 M) extraction for P quantification. C and N 264 were determined in the filtered extracts using a TOC-TN 265 analyser (VCPH Shimadzu + TN module). Phosphorus in 266 the fumigated/non-fumigated extracts was measured with 267 the malachite green method. Microbial C, N and P (MBC, 268 MBN and MBP) were calculated as the difference in C, N 269 and P concentration between the fumigated and the non-270 fumigated samples and divided by a conversion factor of 271 0.45 for C and N. 272

### Soil micronutrient concentrations

Soil concentration of  $K^+$ ,  $Na^+$ ,  $PO_4^{2-}$ ,  $SO_4^{2--}$ ,  $Mg^{2+}$ ,  $Ca^{2+}$ 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).

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## **Crop nutrient uptake**

The above-ground biomass was dried at 60 °C for 48 h,281weighed and ground. Carbon and nitrogen concentrations were282determined by a CHN elemental analyser (Thermo Fisher Sci-283entific Flash 2000). P, K, Ca, Mg, Na, Cu, Fe, Mn, Zn, Bo and284Al concentrations were measured using ICP-AES spectros-285copy. Nutrient uptake was calculated as the product of tissue286concentration and dry biomass and expressed in kg ha<sup>-1</sup>.287

Nitrogen fertilizer use efficiency was calculated in all fer-<br/>tilized treatments as the difference between the amount of N<br/>taken up by the crops in the fertilized treatments (NupFT)288<br/>290<br/>291and N taken up by the crops in the control treatment (NupW)<br/>divided by the total amount of N supplied by each treatment<br/>(NsupFT).291<br/>292

$$NUE(\%) = \frac{\text{NupFT} - \text{NupW}}{NsupFT} \times 100$$
(2)

Phosphorus fertilizer use efficiency (PUE) was calculated 296 in the same way as NUE. 297

## Modelling of ammonia volatilization

Two models were used to estimate ammonia volatilization in<br/>our experiment: Hydrus 1D (PC progress, version 4.17) and<br/>Visual Minteq (version 3.1). Each model was able to give<br/>an estimation of the ammonia volatilized using a different<br/>approach. Visual Minteq is an equilibrium speciation model299<br/>300300<br/>301<br/>302301<br/>302

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and simulated the concentration of ammonia in the soil solu-304 tion by calculating chemical equilibria using the pH, the 305 ionic strength and the concentrations of different chemical 306 species. Hydrus 1D, on the other hand, is a reactive transport 307 model and simulated ammonia volatilization by a first-order 308 reaction process in function of the continuous concentration 300 of ammonium in soils (calibrated with the observed meas-310 urements). A comparison of the parameters used for the two 311 models is detailed in Table 5S, Supplementary Data. Finally, 312 the rates of ammonia volatilization simulated by the two dif-313 ferent models were compared. 314

## 315 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 324 according to the Van Genuchten model (van Genuchten 325 1980), and the hydraulic parameters were derived from 326 the soil texture (sandy loam). An atmospheric bound-327 ary condition (BC) with the surface layer was set for the 328 upper BC, and free drainage was set for the lower BC. The 329 reference evapotranspiration (ETo) was calculated using 330 the Heargreaves formula using temperature and relative 331 humidity data. Crop evapotranspiration was calculated as 332 ETP = Kc\*ETo (with Kc estimated from the spinach growing 333 stages) accordingly to the FAO 56 (Allan and Smith 1998). 334 Root water uptake was modelled with Feddes parameters 335 (Feddes et al. 1978) using the lettuce parameters embedded 336 in the software. Finally, the average soil moisture measured 337 at T0 was used to set the initial water content. 338

Solute transport parameters Ammonium and nitrate were 339 the 2 solutes modelled in Hydrus 1D; their molecular dif-340 fusion coefficients in water were 1.52 and 1.64  $\text{cm}^2 \text{ day}^{-1}$ 341 respectively (Li et al. 2015). The Henry's law constant for 342  $NH_4^+$  was  $2.95 \times 10^{-4}$  (Li et al. 2015), and the adsorption 343 coefficient for  $NH_4^+$  (Kd) was set at 3.5 L mg<sup>-1</sup> (Hanson 344 et al. 2006). The nitrification and denitrification rates were 345 assumed to be 0.2 day<sup>-1</sup> and 0.04 day<sup>-1</sup> respectively (Castal-346 delli et al. 2018; Li et al. 2015). N mineralization was assumed 347 to follow a zero-order reaction process and was calibrated 348 with N content from the control treatment. The rate was set at 349  $1.5 \times 10^{-6}$  g cm<sup>-3</sup> day<sup>-1</sup>, falling in the same range as the one 350 used by Tao et al. (2021) for agricultural soils. The boundary 351

condition at the top was set as "stagnant for volatile solutes" 352 on a 1-cm layer to allow gaseous diffusion of solutes (Jury 353 et al. 1983). A "zero concentration gradient" was used at the 354 bottom of the soil profile to allow N leaching fluxes. Root sol-355 ute uptake was set as passive for both solutes with the highest 356 soil concentrations as the maximum uptake allowed. Addi-357 tionally, initial soil concentrations in  $NO_3^-$  and  $NH_4^+$  were set 358 using the average concentration measured per treatment at TO. 350

Determination of the volatilization rate The volatilization rate 360 was calibrated against soil ammonium and nitrate concentrations 361 with the inverse solution model using the U3 treatment configu-362 ration. U3 was chosen because early simulations showed a better 363 fitting between simulated and measured  $NH_4^+$  soil content for 364 this treatment. The inverse solution analysis returned a rate of 365 1.4 day<sup>-1</sup> for ammonia volatilization. This value fits the upper 366 range of rates found in the literature (Castaldelli et al. 2018), and 367 we considered that it represented well the optimal conditions for 368 ammonia volatilization in this experiment (i.e. high soil pH and 369 high temperature). This rate was then used to model the nitrogen 370 dynamics in the other treatments. 371

**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).

RMSE 
$$(kgN.ha^{-1}) = \sqrt{\frac{1}{n} \sum_{i}^{n} (Si - Oi)^{2}}$$
 (3)

where Si (kg ha<sup>-1</sup>) and Oi (kg ha<sup>-1</sup>) are respectively simulated and observed nitrogen concentration and *n* the number of measurements (n=8). The observed nitrogen concentration and *n* the number of measurements the mean value per treatment (n=4).

384

### **Visual Minteq model**

Visual Minteq is a free software modelling chemical equilib-385 ria (Gustafsson 2011). In our study, it was used to calculate 386 the theoretical amount of NH<sub>3</sub> produced in solution after urine 387 application. The input parameters were: soil pH, ionic strength 388 and soil solution concentration of major ions, i.e. NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, 389 K<sup>+</sup>, Na<sup>+</sup>,  $PO_4^{3-}$ ,  $SO_4^{2-}$ ,  $Mg^{2+}$ ,  $Ca^{2+}$  after each application. 390 Except for  $NH_4^+$  and  $NO_3^-$ , the other concentrations were 391 measured only at the beginning and the end of the experiment. 392 Therefore, they were considered equal to their concentration 393 at T0 until mid-experiment and then equal to their concentra-394 tion at TF until the end. Regarding phosphorus, the amount of 395 P contained in urine was added to the  $PO_4^{3-}$  concentrations. 396

The other inputs of major ions added with urine additions were 397 considered negligible for this specific model application. The 398 model outputs give all chemical species in solution likely to 399 precipitate at thermodynamic equilibrium and the distribution 400 of each element among its different species. The percentage of 401 NH<sub>3</sub> after each urine application calculated by the model was 402 multiplied by the initial  $NH_4^+$  concentration to estimate the 403 total amount of NH<sub>3</sub> produced in kg ha<sup>-1</sup> for each treatment. To 404 compare Visual Minteq and Hydrus 1D outputs, we assumed 405 that all the NH<sub>3</sub> produced in solution was volatilized. 406

#### N and P budget 407

#### Nitrogen 408

A nitrogen budget approach was used to compare the main 409 nitrogen fluxes in our experiment and give an estimation of 410 the unaccounted losses (i.e. denitrification and leaching). The 411 N budget was calculated for the 0-10-cm depth soil layer 412 using the N applied by fertilization (Ferti<sub>N</sub>), the measured soil 413 concentration of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> at T0 and TF to calculate 414  $\Delta$ Nsoil, the measured crop N uptake between 0 and 10 cm 415 (Nupt), the measured microbial N pool ( $\Delta$ MBN) and the meas-416 ured soil bulk density to convert values from mgN kg<sup>-1</sup> to 417 kgN ha<sup>-1</sup>. Ammonia volatilization (Vol) was fitted according 418 to the Hydrus model outputs. Nitrogen mineralization (Min) 419 was calculated as the only input of N in the water treatment. 420 The nitrogen budget error was then calculated (as described 421 below) for each treatment; it can be interpreted as a measure 422 of the unaccounted losses and of the experimental error. We 423 assumed that a budget error below 10% of N supplied means 424 that unaccounted losses were minimal. AQ3

Nitrogen budget error 
$$(\delta N)(kg.ha^{-1}) = Ferti_N - \Delta Nsoil - \Delta MBN$$

– Nupt – Vol + Min

where 428

 $\Delta Nsoil = \left(NH_4^+ + NO_3^-\right)T_F - \left(NH_4^+ + NO_3^-\right)T_0$ 429 (5)430

$$\begin{array}{l} 431\\ 432 \end{array} \quad \Delta MBN = MBNT_F - MBNT_0 \tag{6}$$

433 Nupt = 
$$N_{uptake} \times \%$$
 root biomass<sub>0-10cm</sub> (estimated at 44% according to) (7)  
A04 And Heinrich et al. (2013)

#### Phosphorus 435

The P budget was calculated for the 0-10-cm depth soil 436 layer excluding the pool of P extractable with HCl. As the 437 HCl-extractable P pool is very large in our calcareous soil, 438 variations occurring during the experiment were not detect-439 able against the analytical error. Hence, the P budget error 440 can be interpreted as a net variation of the HCl-extractable 441

P pool size. Similarly, a net change in a P pool size gives an 442 indication of the net flux involving the pool: however, this 443 variation also includes the experimental error. The P budget 444 error is calculated with the amount of P supplied by fertili-445 zation (Ferti<sub>P</sub>), the P taken up by the crops between 0 and 446 10 cm (Pupt) and the concentration of P in the different soil 447 pools (Piresin, PiHCO3, PiNaOH, POHCO3, PONAOH). Microbial P is 448 already included in the organic P pools due to the extraction 449 method; therefore, it does not appear in the budget calculation. 450

$$P \text{ budget error } (\delta P)(kg.ha^{-1}) = Ferti_P - \Delta Psoil - Pupt$$
<sup>451</sup>

(8)

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where

$$\Delta Psoil = (Pi_{resin} + Pi_{HCO3} + Pi_{NaOH} + Po_{HCO3} + Po_{NaOH})T_{F}$$

$$- (Pi_{resin} + Pi_{HCO3} + Pi_{NaOH} + Po_{HCO3} + Po_{NaOH})T_{0}$$

$$(9)$$

$$455$$

P crop uptake between 0 and 10 cm is calculated the same 456 way as the N crop uptake on 0-10 cm (see above). 457

## **Statistical analysis**

Statistical analyses were carried out with Rstudio software (ver-459 sion 3.6.1) (R Core Team 2017). To test the homogeneity of ini-460 tial variable values at T0 across blocks (tanks) and treatments, 461 we used a linear model with 2 factors: block (n=4) and treat-462 ment (n=4) (16 samples in total), and significance was deter-463 mined with the ANOVA (analysis of variance) function. Signif-464 icant differences were only found for the variables Pi resin, and 465 MBN with differences of up to 41% and 52% respectively in the 466 mean pool size between the richest and the poorest tank. On T1 467 to TF values, ANOVAs were performed to assess the effect of 468 treatments on each variable and date separately. For each vari-469 able measured, a linear mixed model ("Imer" function, Ime4 470 package, (Bates et al. 2015, p. 4)) was produced with "treat-471 ment" (n=4) as fixed factor and "block" (n=4) as random fac-472 tor to account for the initial differences between tanks observed 473 for certain variables. A Tukey's multi-comparison test was per-474 formed when the treatments had a significant effect on the vari-475 able (significance level: p value < 0.05). Correlations between 476 variables were assessed using Pearson correlation tests. 477

### **Results** 478

## **Global result analysis**

## Soil analysis

At the end of the experiment, soil pH was lower in the U3 481 treatment (urine at 510 kgN ha<sup>-1</sup>) than in all other treat-482 ments, reaching 8.5, while it was over 8.8 in all other 483

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(4)

treatments. At T3, shortly after a fertilizer application, soil 484 conductivity was higher in all the fertilized treatments than 485 in the water treatment, but at the end of the experiment, 486 only the U3 treatment still had higher conductivity (+187% 487 compared to the water treatment, p = 0.002) (Table 3). In 488 the U3 treatment, soil concentrations in Na<sup>+</sup> and Cl<sup>-</sup> were 480 also twice as high at TF as in the other treatments (Table 2S, 490 Supplementary data). MBC and MBP showed no significant 491 response, but MBN showed a significant difference between 492 U3 and the control W at TF (+370% in U3 compared to W)493 (Table 3). Soil nitrate and ammonium concentrations and 494 soil P pool concentrations are commented in Sect. 2.1 and 495 3 respectively. 496

## 497 Biomass and plant nutrient uptake

Aboveground biomass at the end of the experiment was 498 significantly different only between the control treatment 499 (W) and the synthetic fertilizer treatment (F). N and P 500 uptake by plants were significantly higher (p < 0.05) in the 501 F and U3 treatments than in the W treatment, and inter-502 mediate in the U1 treatment, i.e. not significantly differ-503 ent from both W and U3 and F treatments. In addition, 504 the spinach crops in the tanks 1 and 8 took up more N 505 and P than the ones in the tanks 4 and 5 (up to 138% and 506 210% more for N and P respectively, data not shown). N 507 and P uptake were highly correlated (Pearson coefficient 508 r = 0.97, p < 0.0001). N uptake was correlated with soil 509 nitrate content from day 23 to day 28 (p = 0.03, p = 0.04, 510 p = 0.01 for day 23, 26 and 28 respectively) but was not 511 correlated with soil ammonium content. P uptake was cor-512 related with none of the P pools at the end of the experi-513 MQ5 ment (p > 0.05) (Table 4).

## 515 Nitrogen stock evolution and losses

## 516 N-NH<sub>4</sub><sup>+</sup>, N-NO<sub>3</sub><sup>-</sup> soil concentrations

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

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## Estimation of ammonia volatilization

In order to validate the N fluxes simulated by Hydrus 1D, simulated and observed ammonium and nitrate soil concentrations were compared (Fig. 4).

Modelled  $NH_4^+$  soil concentrations agreed relatively well 536 with measurements in the 3 treatments (RMSE < 69 kg ha<sup>-1</sup>), 537 although they were slightly underestimated for U3. However, 538 NO<sub>3</sub><sup>-</sup> dynamics were poorly simulated by Hydrus 1D espe-539 cially in the U3 treatment (RMSE =  $142 \text{ kg ha}^{-1}$ ). As soil 540  $NO_3^{-1}$  is not used in the calculation of ammonia volatilization, 541 this poor fitting should not affect the estimation of NH<sub>3</sub> volatil-542 ization. It prevents, however, a good estimation of NO<sub>3</sub><sup>-</sup> leach-543 ing and denitrification. Therefore, we did not use the Hydrus 544 1D model outputs to estimate these losses in the N budget. 545

As expected, ammonia volatilization simulated by Hydrus 1D was especially high in the U3 treatment reaching 260 kg ha<sup>-1</sup>, and it was almost two times higher in the U1 treatment than in the F treatment (Table 5). 549

Visual Minteq estimated that 21% of  $NH_4^+$  in soils would be in  $NH_3$  form for a soil at pH = 8.7; however, this proportion decreases to 13.9% when the pH is at 8.5 as in the treatment U3 at the end of the experiment (Table 1S, Supplementary data).

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

## Fate of phosphorus from urine

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

In contrast, the HCl-extractable Pi (Pi HCl) did not vary significantly among treatments (Table 3). The P budget calculation ( $\delta_p$ ) was negative in the U1 and U3 treatments suggesting a potential decrease of approximatively 10 kgP ha<sup>-1</sup> of the Pi HCl pool, which was not detectable against replicate variability (Table 1S, Supplementary data). 569 570 571 572 573

## Discussion

## Effect of urine fertilization on soil pH and salinity 574

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

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**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<sup>-1</sup>, U3 = urine in excess at 510 kgN ha<sup>-1</sup>. 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	p value treatment effect	<i>p</i> value tank effect
рН	TO	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 <sup>-1</sup> )	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
	TF	147 b	154 b	177 b	423 a	0.002*	Nd
$NO_3^- + NO_2^- (mgN kg^{-1})$	T0	7.6 a	5.0 a	8.2 a	6.3 a	0.21	0.6
	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
NH <sub>4</sub> <sup>+</sup>	T0	0 a	0 a	0 a	0 a	Nd	Nd
(mgN kg <sup>-1</sup> )	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
	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
Pi Resin	T0	10.5 a	9.7 a	9.7 a	10.8 a	0.4	0.009 **
$(mgP kg^{-1})$	TF	9.4 b	11.7 b	11.7 b	26.2 a	0.00005 **	Nd
Pi Bicarbonate	T0	7.8 a	11.6 a	9.3 a	8.9 a	0.65	0.28
$(mgP kg^{-1})$	TF	11.5 a	11.5 a	11.1 a	17.0 a	0.05	Nd
Pi NaOH	T0	11.5 a	9.9 a	9.5 a	9.58 a	0.13	0.02 *
$(mgP kg^{-1})$	TF	9.8 b	10.9 b	9.9 b	13.5 a	0.0015 **	Nd
Pi HCl	T0	224 a	209 a	206 a	221 a	0.45	0.10
$(mgP kg^{-1})$	TF	225 a	217 a	219 a	230 a	0.42	Nd
Po Bicarbonate	Т0	10.1 a	5.7 a	7.5 a	7.7 a	0.33	0.07
$(mgP kg^{-1})$	TF	6.9 a	12.8 a	12.1 a	8.1 a	0.43	Nd
Po NaOH	Т0	24.5 a	22.9 a	20.5 a	22.5 a	0.81	0.09
$(mgP kg^{-1})$	TF	23.9 a	21.3 a	23.8 a	20.9 a	0.6	Nd
P total	Т0	281.3 a	269.9 a	254.3 a	289.3 a	0.21	0.07
$(mgP kg^{-1})$	TF	286.9 a	285.8 a	288.5 a	316.3 a	0.03 *	Nd
MBC	T0	127 a	134 a	140 a	140 a	0.67	0.03
$(mgC kg^{-1})$	TF	96 a	122 a	126 a	134 a	0.10	Nd
MBN	T0	9.8 a	11.3 a	11.1 a	10.5 a	0.17	0.001 **
$(mgN kg^{-1})$	TF	8.4 b	20.1 ab	21.3 ab	39.5 a	0.05 *	Nd
MBP	T0	7.1 a	7.1 a	8.8 a	7.6 a	0.9	0.8
$(mgP kg^{-1})$	TF	6.3 a	5.1 a	7.8 a	10.5 a	0.08	Nd

dosage (Mnkeni et al. 2008; Sangare et al. 2015). However,
calcareous soils have a stronger pH buffering capacity (Magdoff 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

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**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	<i>p</i> value treatment effect
Aboveground bio- mass (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 <i>b</i>	8.21 <i>b</i>	7.57 b	0.005 **
C/P	130 a	106 a	119 a	101 a	0.17
NUE	1	17 a	10 a	5 a	0.06
PUE	/	24 a	10 a	7 a	0.05

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

Similarly, urine caused a buildup of soluble salt concentration and specifically NaCl only in the U3 treatment. However, crops under the U3 treatment did not take up more sodium than under the other treatments (Table 3S, Supplementary data), and the soil salinity was still below the spinach salt tolerance threshold established at 9.4 dS m<sup>-1</sup> 610

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

## Considerable losses of nitrogen by volatilization

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

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



**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

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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

 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 <sup>-1</sup> )	67% (105 kg ha <sup>-1</sup> )
U3	55% (260 kg ha <sup>-1</sup> )	$58\% (270 \text{ kg ha}^{-1})$
F	28% (48 kg ha <sup>-1</sup> )	38% (66 kg ha <sup>-1</sup> )



**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

replicates. Tables in the figures report the RMSE (in kg  $ha^{-1}$ ) and  $r^2$  calculated for each treatment

fertilization, soil pH and ammonia volatilization that needs623to be considered when studying nitrogen fluxes. It is likely624that a high load of urine or long-term urine fertilization by625causing high losses through ammonia volatilization and high626nitrification rates decreases the pH of calcareous soils, thus627decreasing the potential of the soil for subsequent ammonia628volatilization.629

The nitrogen budget error was especially high in the F 630 treatment (Fig. 6 and Table 4S, Supplementary data), sug-631 gesting that unaccounted losses, most probably leaching and 632 denitrification, had a higher contribution in the N budget of 633 this treatment. Hence, nitrate leaching and denitrification 634 were probably higher in the fertilizer treatment than in the 635 urine treatments. Most likely, the NH<sub>4</sub>NO<sub>3</sub> fertilizer induced 636 a smaller volatilization rate but higher leaching and deni-637 trification rates because of its partition between nitrate and 638 ammonium (Eckard et al. 2003; Fernández-Escobar et al. 639 2004). In addition, such high levels of excess N were not 640 expected as the U1 and F fertilization rate had supposedly 641 been adjusted to meet crop requirements, but crop growth 642 was limited in our experiment possibly because of exces-643 sive heat during that summer. In conditions of adequate crop 644 uptake, lower total N losses are to be expected. 645

In this experimental setup, soil conditions were optimal 646 for ammonia volatilization (pH at 8.7 and air temperature 647 ranging between 20 and 30 °C), so the high rates of volatili-648 zation simulated in the urine treatments can be considered 649 an upper limit for urine fertilization. Although these high 650 ammonia emissions raise concern, it is possible to reduce 651 them with appropriate application techniques, timing and 652 dosage (Mencaroni et al. 2021; Rodhe et al. 2004). 653

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# Bioavailability of N from urine fertilizer for plantsand microbes

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

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

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microbial activity. However, this increase was never accom-687 panied by an increase in microbial C, causing a shift in the 688 microbial C to N ratio. Mason-Jones et al. (2022) recently 689 highlighted that soil microbes have the capacity to store 690 surplus nutrients to reduce their loss and release them later 691 upon microbial death. Thus, under urine application, part 692 of the added ammonium was probably stored in the micro-693 bial biomass forming a readily available N pool. Similarly, 694 Zaman et al. (2006) found an increase of only microbial N 695 with NH<sub>4</sub>Cl fertilizer, while both N and C microbial pools 696 increased with C-rich dairy shed effluent. Urine alone is 697 relatively poor in dissolved organic carbon (Table 2) and, 698 therefore, does not stimulate microbial growth. 699

## **Bioavailability of P from urine fertilizer**

N and P uptake were highly correlated (Pearson coefficient 701 r=0.97). Therefore, the lower P uptake observed for the U1 702 treatment might be a consequence of the nitrogen stress that 1 likely occurred under U1. 704

700

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

synthetic and nitrified urine and found a similar P recoveryrate in the crops as under mineral fertilizer.

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

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

## 742 Conclusion

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

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Author contribution MR: writing—original draft, investigation, data curation, formal analysis, visualization. CP: conceptualization, meth- odology, resources, funding acquisition, writing—review and editing. CM: conceptualization, methodology, resources, funding acquisition, writing—review and editing. NAM: resources, funding acquisition, writing—review and editing. DC: formal analysis, writing—review and editing.	772 773 774 775 776 777 778
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Declarations	783
Ethical approval Not applicable.	784
<b>Consent to participate</b> All authors gave their consent to participate in the conception of this paper.	785 786
<b>Consent for publication</b> All authors read and approved the final manuscript.	787 788
Competing interests The authors declare no competing interests.	789

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