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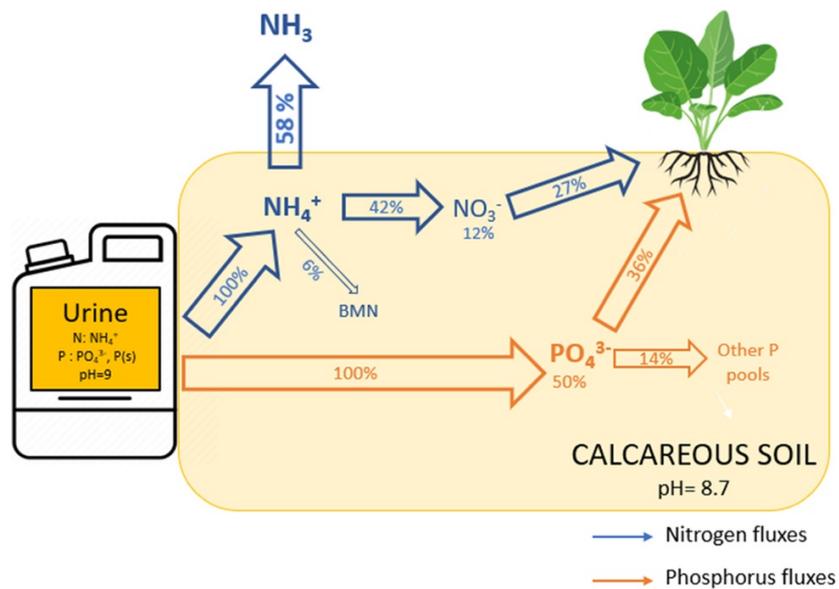
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Abstract

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

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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

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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.

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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 (NH₃) volatilization because of their alkaline pH. Therefore, urine application on such soils is likely to result in high NH₃ 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 CO₂ 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, Ca²⁺, Mg²⁺, 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 SO₄²⁻ 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

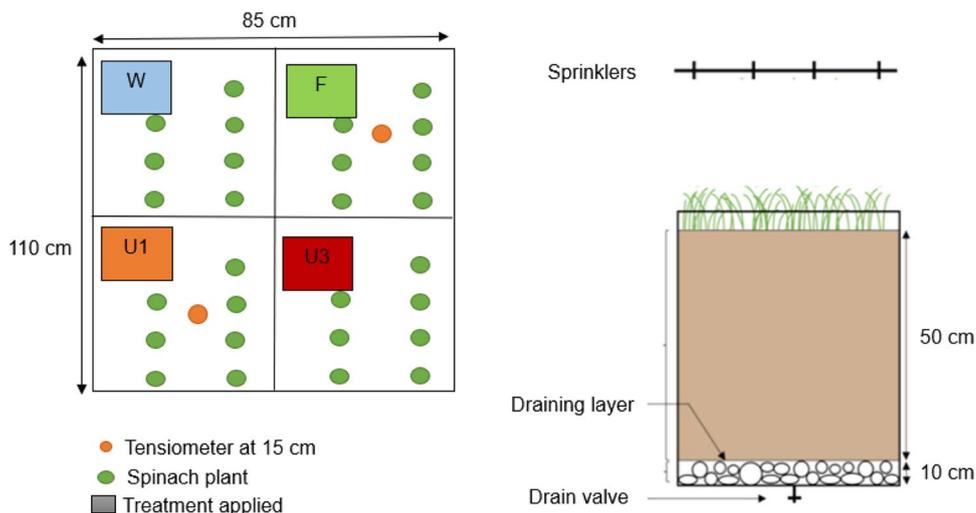
The experiment was carried out in a greenhouse of the UMR G-EAU in Montpellier between May 30th 2020 and July 5th 2020. Meteorological variables were measured by a weather station located at the experimental site. Air temperature and relative humidity in the greenhouse were measured by a temperature 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
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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 startup 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

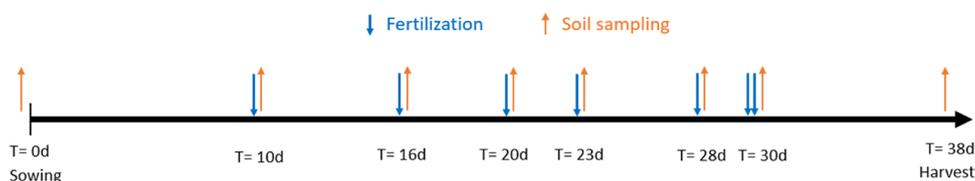


Fig. 2 Timeline of the fertilization treatments. The scale is in day (d) from sowing (day 0) to harvest (day 38). Each fertilization and soil sampling are represented by a blue and an orange arrow respectively. Double dose of fertilization was supplied on day 30

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	underwent potassium sulphate (K_2SO_4 , 0.025 M) extrac-	261
219	analyser). This method measures nitrite and nitrate species	tion for C and N quantification. The two other fumigated/	262
220	together and ammonium separately.	non-fumigated subsamples underwent sodium bicarbonate	263
		($NaHCO_3$ 0.5 M) extraction for P quantification. C and N	264
221	pH	were determined in the filtered extracts using a TOC-TN	265
		analyser (VCPH Shimadzu + TN module). Phosphorus in	266
222	pH measurement was carried out according to the ISO stand-	the fumigated/non-fumigated extracts was measured with	267
223	ard using a pH probe (AFNOR, 2005). Soils were sieved,	the malachite green method. Microbial C, N and P (MBC,	268
224	air dried and extracted with a soil to water ratio of 1:5, and	MBN and MBP) were calculated as the difference in C, N	269
225	measurements were performed after 1 h of agitation.	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
226	Electrical conductivity		
227	Soils were sieved, air dried and extracted with a soil to water	Soil micronutrient concentrations	273
228	ratio of 1:5. Then, the solution was agitated for 30 min, cen-		
229	trifuged and filtered. Electrical conductivity was then meas-	Soil concentration of K^+ , Na^+ , PO_4^{2-} , SO_4^{2--} , Mg^{2+} , Ca^{2+}	274
230	ured on the aqueous extract with a conductometer probe.	were measured at T0 and TF by the laboratory Aurea Agro-	275
		Sciences (https://www.aurea.eu/) following the method	276
231	Organic and inorganic P pool	ISO 11885 for the determination of selected elements by	277
		inductively coupled plasma optical emission spectrometry	278
232	To quantify soil phosphorus pools, we performed a Hedley	(ICP-OES).	279
233	sequential fractionation as modified by Tiessen and Moir		
234	(1993). This method operationally identifies organic and	Crop nutrient uptake	280
235	inorganic P pools. Soil samples were sequentially extracted		
236	with 4 different reagents in the following order: anionic	The above-ground biomass was dried at 60 °C for 48 h,	281
237	exchange resin membranes (BDH #55,164, 6 cm × 4 cm,	weighed and ground. Carbon and nitrogen concentrations were	282
238	named P resin pool), 0.5 M sodium bicarbonate ($NaHCO_3$,	determined by a CHN elemental analyser (Thermo Fisher Sci-	283
239	Pi and Po bicarbonate pools), 0.5 M NaOH (Pi and Po NaOH	entific Flash 2000). P, K, Ca, Mg, Na, Cu, Fe, Mn, Zn, Bo and	284
240	pools) and 1 M HCl (P HCl pool). To quantify organic P,	Al concentrations were measured using ICP-AES spectro-	285
241	aliquots of bicarbonate and NaOH extracts were mineralized	scopy. Nutrient uptake was calculated as the product of tissue	286
242	by acid digestion, and the organic P concentration was cal-	concentration and dry biomass and expressed in $kg\ ha^{-1}$.	287
243	culated as the difference between inorganic P in the digested	Nitrogen fertilizer use efficiency was calculated in all fer-	288
244	extract (corresponding to the total P in the extract) and inor-	tilized treatments as the difference between the amount of N	289
245	ganic P in the non-digested extract. Inorganic phosphorus	taken up by the crops in the fertilized treatments (NupFT)	290
246	concentration in each extract was measured by the mala-	and N taken up by the crops in the control treatment (NupW)	291
247	chite green colorimetric method (Ohno and Zibilske 1991).	divided by the total amount of N supplied by each treatment	292
248	The P resin and the Pi and Po bicarbonate are commonly	(NsupFT).	293
249	considered the available P pool (Tiessen and Moir 1993).		
250	The exchange times of sequentially extracted P pools with	$NUE(\%) = \frac{NupFT - NupW}{NsupFT} \times 100 \quad (2)$	294
251	the soil solution increase with the strength of the extractant		295
252	(Helfenstein et al. 2020). Hence, the P pools extracted with	Phosphorus fertilizer use efficiency (PUE) was calculated	296
253	the stronger extractants (i.e. 0.5 M NaOH and 1 M HCl) are	in the same way as NUE.	297
254	most likely less available.		
255	C N P in microbial biomass	Modelling of ammonia volatilization	298
256	The fumigation extraction method was applied to determine	Two models were used to estimate ammonia volatilization in	299
257	C, N and P in microbial biomass (AFNOR, 1997). For each	our experiment: Hydrus 1D (PC progress, version 4.17) and	300
258	soil sample, 4 subsamples were weighed, and 2 were fumi-	Visual Minteq (version 3.1). Each model was able to give	301
259	gated with chloroform overnight. Blanks without soil were	an estimation of the ammonia volatilized using a different	302
260	included. Then, two fumigated/non-fumigated subsamples	approach. Visual Minteq is an equilibrium speciation model	303

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).

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

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

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

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	p value treatment effect	p 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 impor-
586 tantly by reducing urine-derived ammonia volatilization,
587 which exacerbates soil acidification.

588 Similarly, urine caused a buildup of soluble salt con-
589 centration and specifically NaCl only in the U3 treatment.
590 However, crops under the U3 treatment did not take up more
591 sodium than under the other treatments (Table 3S, Sup-
592 plementary data), and the soil salinity was still below the
593 spinach salt tolerance threshold 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 fer-
596 tilization 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 salin-
602 ity 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 Daw-
615 son 2022; Whitehead and Raistrick 1990). In addition, vola-
616 tilization 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 chemi-
621 cal equilibrium and so the potential of our soil for ammo-
622 nia volatilization. This highlights the interaction between

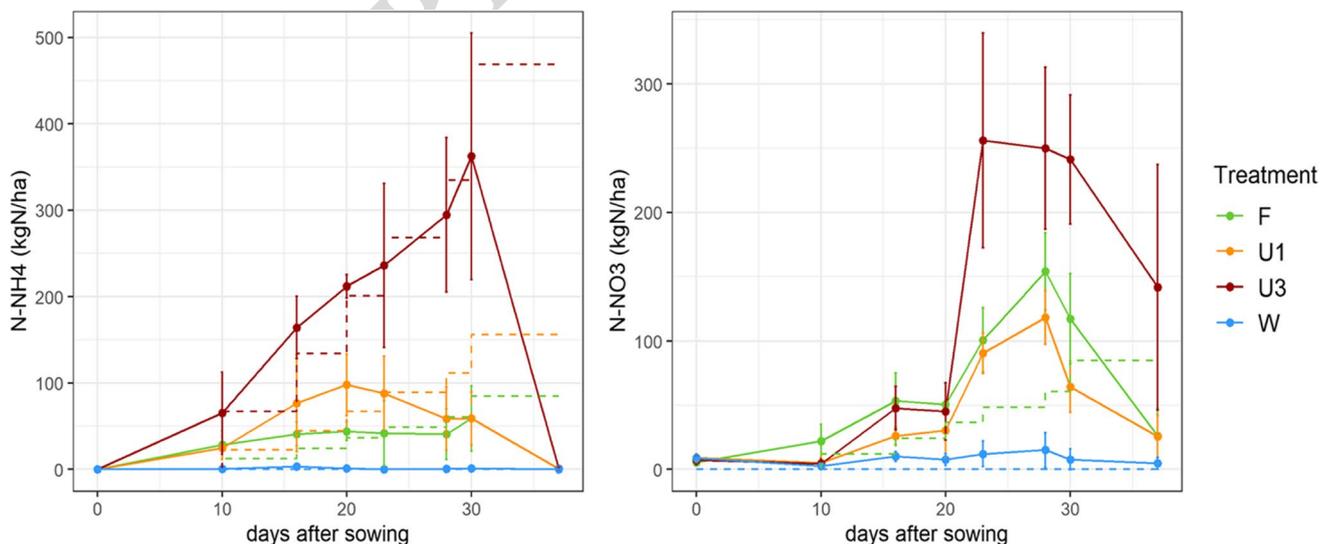


Fig. 3 Evolution of soil mineral nitrogen stocks during the experiment as a function of treatment: F: synthetic fertilizer dose $\times 1$, U1: urine dose $\times 1$, U3: urine dose $\times 3$, W: unfertilized control (ammo-

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

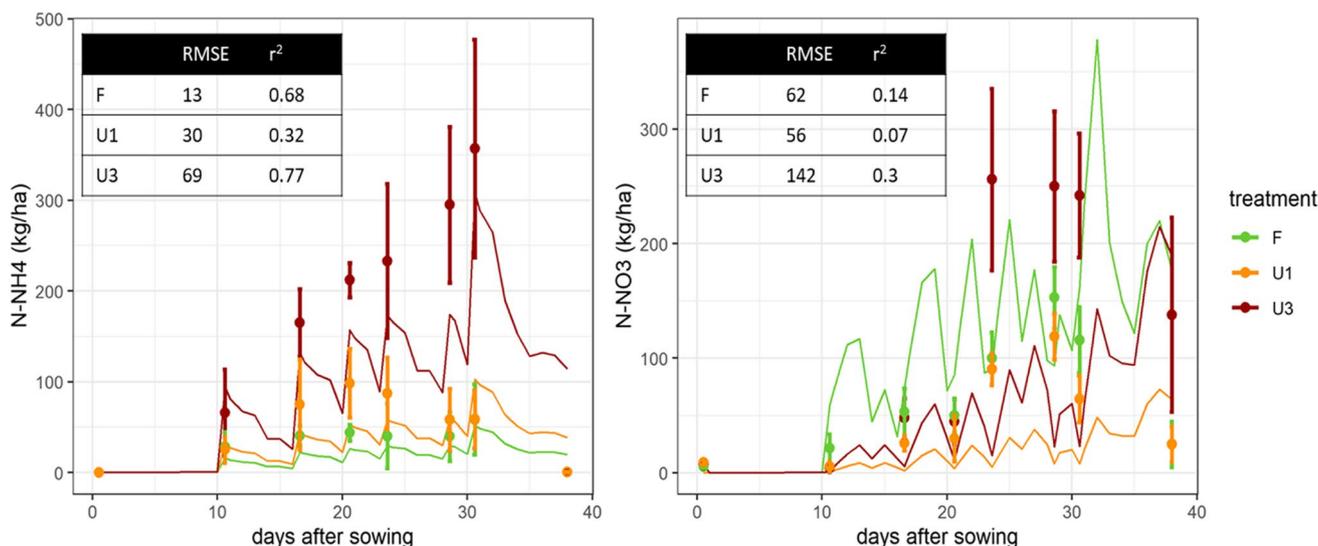


Fig. 4 Comparison between concentration of ammonium and nitrate in soils, as simulated by Hydrus 1D (lines) and observed (points) for which the errors bars represent the standard error between the four

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.

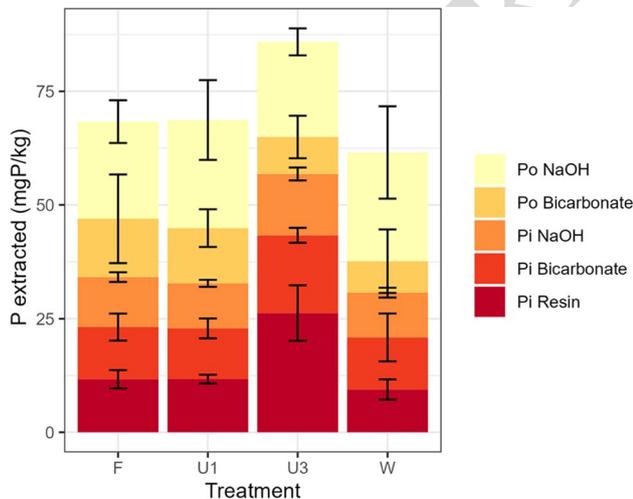
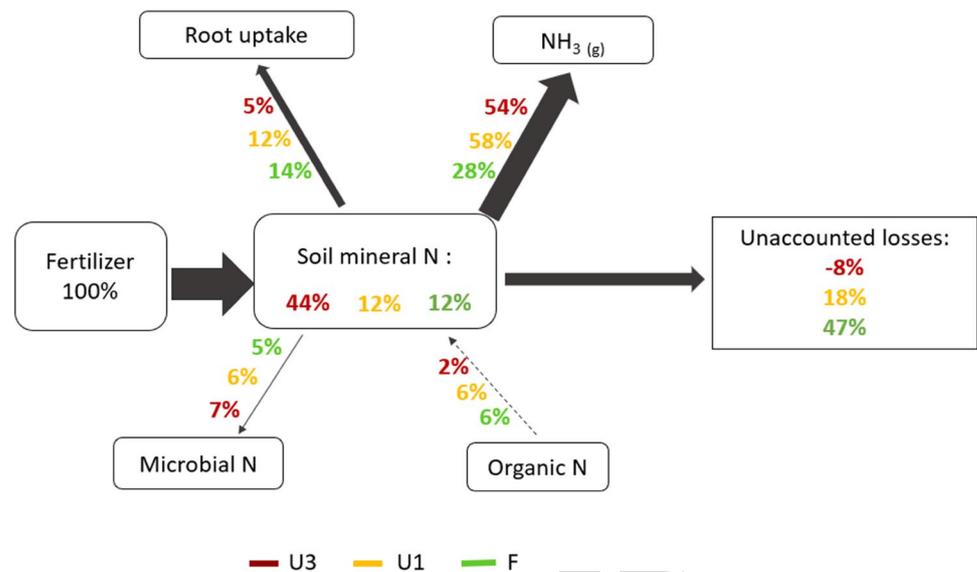


Fig. 5 Distribution of phosphorus in different soil pools at the end of experiment. The errors bars represent the standard error of four replicates. Pi Resin=resin extractable inorganic P; Pi Bicarbonate=sodium bicarbonate extractable inorganic P; Po Bicarbonate=sodium bicarbonate extractable organic 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 NH₄Cl 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.

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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
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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.	