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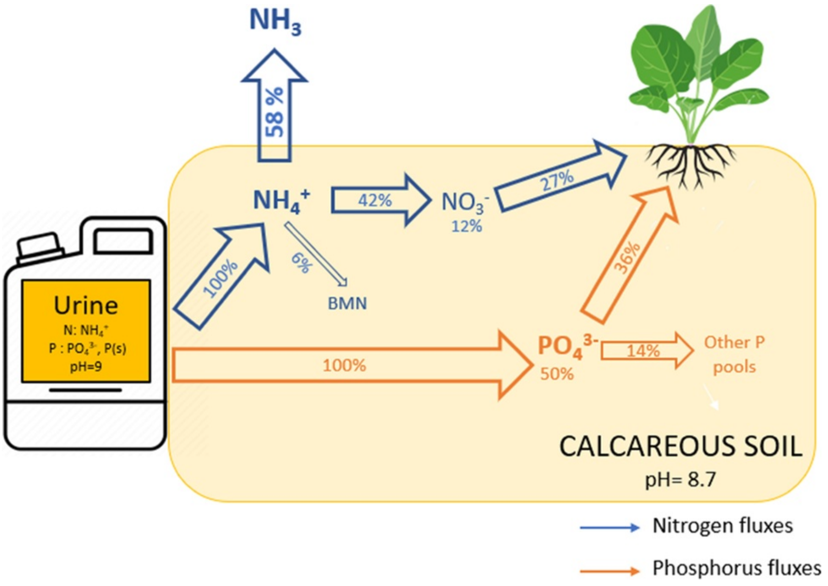
Abstract

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 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.

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RESEARCH ARTICLE

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

Manon Rumeau[1,2] · Claire Marsden[1] · Nassim Ait Mouheb[3] · David Crevoisier[4] · Chiara Pistocchi[1]

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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 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.

Keywords Source separation · Fertilization · Hydrus · Ammonia volatilization

Introduction

AQ1

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

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Responsible Editor: Kitae Baek

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

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

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

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

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

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

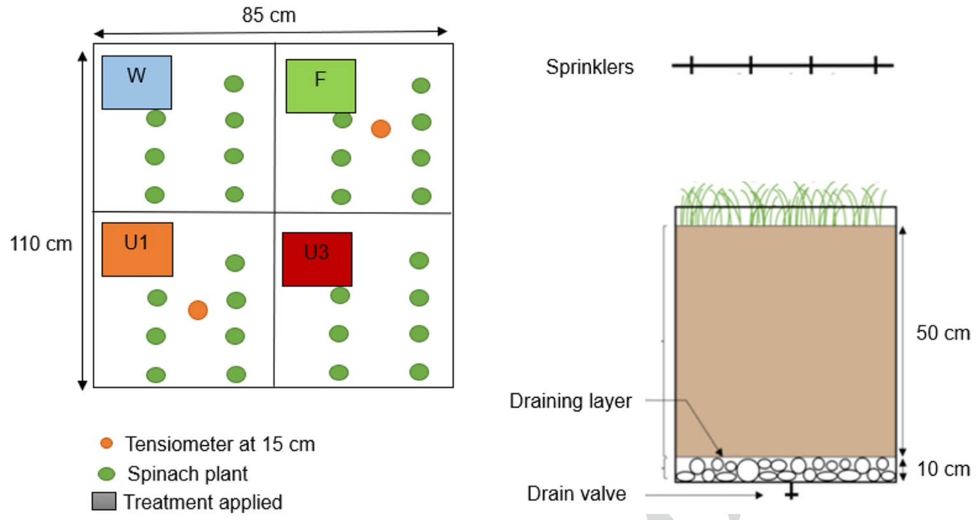

Fig. 1 Scheme of the experimental design (representation of one tank, from above (left) and from the side (right)) with crops and sensor positions. For each tank, the position of the different treatments was randomized

sown directly in the tanks on May 30th with a plant density of 17 plant m^{-2} and the growing cycle lasted 37 days. On July 5th, the above-ground biomass was collected and dried for biomass and nutrient content analysis. Spinach was chosen because of its relatively high N requirements (170 kgN ha^{-1} under optimal growth conditions) (Frerichs et al. 2022) and short growing cycle. Irrigation was conducted with sprinklers located above the tanks and controlled with tensiometers placed at 15-cm depth in the soil to maintain soil moisture around field capacity. Approximately 200 mm of water was supplied over the duration of the experiment. Each tank was divided into 4 quarters using vertical aluminium sheets driven 30 cm into the soil (below maximum root depth to avoid transfers of nutrients between quarters). The experimental treatments were assigned according to a randomized block scheme in which the experimental unit was a quarter of a tank and the tank was the block. We compared two different doses of human urine with a synthetic fertilizer and a water control. The four treatments applied were: U1 = Urine dose × 1 (170 kgN ha^{-1} + 8.5 kgP ha^{-1} supplied), U3 = Urine dose × 3 (510 kgN ha^{-1} + 25.5 kgP ha^{-1} supplied), F = Synthetic fertilizer dose × 1 (170 kgN ha^{-1} + 8.5 kgP ha^{-1} supplied) and W = Water. Treatments U1 and F represent the recommended doses of N for the spinach crop, while treatment U3 represents 3 times this dose and exacerbates the effects of urine as well as approximates 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^{-1} at T0, data not shown) so we assumed that the slight difference in potassium concentration between the urine and the synthetic fertilizer would not affect plant growth.

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

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

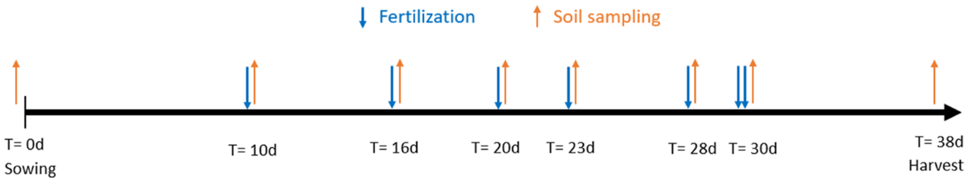

Fig. 2 Timeline of the fertilization treatments. The scale is in day (d) from sowing (day 0) to harvest (day 38). Each fertilization and soil sampling are represented by a blue and an orange arrow respectively. Double dose of fertilization was supplied on day 30

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

Parameters measured	Values	Method
Electrical conductivity at 25 °C (mS cm^{-1})	40.5	NF EN 27,888
pH water	9	NF EN ISO 10523
N-NH$_4^+$ (mg L^{-1})	4341	Filtered at 0.45 µm, ISO 7150–1
N-NO$_3^-$ (mg L^{-1})	10.7	Filtered at 0.45 µm, ISO 7890–1-2–1986
Ptotal (mg L^{-1}) Porganic (mg L^{-1})	206 Not detected	Filtered at 0.45 µm, ICP-AES
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
SO4^{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) *- Solution U1* *- Solution U3*	157.6 *45.8* *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:

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

where concentrations of cations (Na$^+$, Ca^{2+}, Mg^{2+}) are expressed in meq L^{-1}.

Parameter monitoring and measurement

Soil sampling

Bulk soil samples at 0–10-cm depth composed of 2 subsamples 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 (NO$_3^-$ and NH$_4^+$) 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 analysed by continuous flow colorimetry (Skalar SA 3000 analyser). This method measures nitrite and nitrate species together and ammonium separately.

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.

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.

Organic and inorganic P pool

To quantify soil phosphorus pools, we performed a Hedley sequential fractionation as modified by Tiessen and Moir (1993). This method operationally identifies organic and inorganic P pools. Soil samples were sequentially extracted with 4 different reagents in the following order: anionic exchange resin membranes (BDH #55,164, 6 cm × 4 cm, named P resin pool), 0.5 M sodium bicarbonate ($NaHCO_3$, Pi and Po bicarbonate pools), 0.5 M NaOH (Pi and Po NaOH pools) and 1 M HCl (P HCl pool). To quantify organic P, aliquots of bicarbonate and NaOH extracts were mineralized by acid digestion, and the organic P concentration was calculated as the difference between inorganic P in the digested extract (corresponding to the total P in the extract) and inorganic P in the non-digested extract. Inorganic phosphorus concentration in each extract was measured by the malachite green colorimetric method (Ohno and Zibilske 1991). The P resin and the Pi and Po bicarbonate are commonly considered the available P pool (Tiessen and Moir 1993). The exchange times of sequentially extracted P pools with the soil solution increase with the strength of the extractant (Helfenstein et al. 2020). Hence, the P pools extracted with the stronger extractants (i.e. 0.5 M NaOH and 1 M HCl) are most likely less available.

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_2SO_4, 0.025 M) extraction for C and N quantification. The two other fumigated/non-fumigated subsamples underwent sodium bicarbonate ($NaHCO_3$ 0.5 M) extraction for P quantification. C and N were determined in the filtered extracts using a TOC-TN analyser (VCPH Shimadzu + TN module). Phosphorus in the fumigated/non-fumigated extracts was measured with the malachite green method. Microbial C, N and P (MBC, MBN and MBP) were calculated as the difference in C, N and P concentration between the fumigated and the non-fumigated samples and divided by a conversion factor of 0.45 for C and N.

Soil micronutrient concentrations

Soil concentration of K^+, Na^+, 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).

Crop nutrient uptake

The above-ground biomass was dried at 60 °C for 48 h, weighed and ground. Carbon and nitrogen concentrations were determined by a CHN elemental analyser (Thermo Fisher Scientific Flash 2000). P, K, Ca, Mg, Na, Cu, Fe, Mn, Zn, Bo and Al concentrations were measured using ICP-AES spectroscopy. Nutrient uptake was calculated as the product of tissue concentration and dry biomass and expressed in kg ha^{-1}.

Nitrogen fertilizer use efficiency was calculated in all fertilized treatments as the difference between the amount of N taken up by the crops in the fertilized treatments (NupFT) and N taken up by the crops in the control treatment (NupW) divided by the total amount of N supplied by each treatment (NsupFT).

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

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

Modelling of ammonia volatilization

Two models were used to estimate ammonia volatilization in our experiment: Hydrus 1D (PC progress, version 4.17) and Visual Minteq (version 3.1). Each model was able to give an estimation of the ammonia volatilized using a different approach. Visual Minteq is an equilibrium speciation model and simulated the concentration of ammonia in the soil solution by calculating chemical equilibria using the pH, the ionic strength and the concentrations of different chemical species. Hydrus 1D, on the other hand, is a reactive transport model and simulated ammonia volatilization by a first-order reaction process in function of the continuous concentration of ammonium in soils (calibrated with the observed measurements). A comparison of the parameters used for the two models is detailed in Table 5S, Supplementary Data. Finally, the rates of ammonia volatilization simulated by the two different models were compared.

Hydrus 1D model

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

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

Water flow parameters Soil water dynamics were modelled according to the Van Genuchten model (van Genuchten 1980), and the hydraulic parameters were derived from the soil texture (sandy loam). An atmospheric boundary condition (BC) with the surface layer was set for the upper BC, and free drainage was set for the lower BC. The reference evapotranspiration (ETo) was calculated using the Heargreaves formula using temperature and relative humidity data. Crop evapotranspiration was calculated as ETP = Kc*ETo (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 $cm^2\ day^{-1}$ 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^+ (Kd) 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 1.5×10^{-6} g cm^{-3} 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). AQ2

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

where Si (kg ha^{-1}) and Oi (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 ΔNsoil, the measured crop N uptake between 0 and 10 cm (Nupt), 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.

$$Nitrogen\ budget\ error\ (\delta N)\left(kg.ha^{-1}\right) = Ferti_N - \Delta Nsoil - \Delta MBN - Nupt - Vol + Min \tag{4}$$

where

$$\Delta Nsoil = \left(NH_4^+ + NO_3^-\right)T_F - \left(NH_4^+ + NO_3^-\right)T_0 \tag{5}$$

$$\Delta MBN = MBNT_F - MBNT_0 \tag{6}$$

$$Nupt = N_{uptake} \times \%root\ biomass_{0-10cm}\ (estimated\ at\ 44\%\ according\ to) \tag{7}$$

And Heinrich et al. (2013)

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 (Pupt) and the concentration of P in the different soil pools (Pi_{resin}, Pi_{HCO3}, Pi_{NaOH}, Po_{HCO3}, Po_{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.

$$P\ budget\ error\ (\delta P)(kg.ha^{-1}) = Ferti_P - \Delta Psoil - Pupt \tag{8}$$

where

$$\Delta Psoil = \left(Pi_{resin} + Pi_{HCO3} + Pi_{NaOH} + Po_{HCO3} + Po_{NaOH}\right)T_F - \left(Pi_{resin} + Pi_{HCO3} + Pi_{NaOH} + Po_{HCO3} + Po_{NaOH}\right)T_0 \tag{9}$$

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

Biomass and plant nutrient uptake

Aboveground biomass at the end of the experiment was significantly different only between the control treatment (W) and the synthetic fertilizer treatment (F). N and P uptake by plants were significantly higher ($p < 0.05$) in the F and U3 treatments than in the W treatment, and intermediate in the U1 treatment, i.e. not significantly different from both W and U3 and F treatments. In addition, the spinach crops in the tanks 1 and 8 took up more N and P than the ones in the tanks 4 and 5 (up to 138% and 210% more for N and P respectively, data not shown). N and P uptake were highly correlated (Pearson coefficient $r = 0.97$, $p < 0.0001$). N uptake was correlated with soil nitrate content from day 23 to day 28 ($p = 0.03$, $p = 0.04$, $p = 0.01$ for day 23, 26 and 28 respectively) but was not correlated with soil ammonium content. P uptake was correlated with none of the P pools at the end of the experiment ($p > 0.05$) (Table 4).

Nitrogen stock evolution and losses

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

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

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 with measurements in the 3 treatments (RMSE < 69 kg ha^{-1}), although they were slightly underestimated for U3. However, NO_3^- dynamics were poorly simulated by Hydrus 1D especially in the U3 treatment (RMSE = 142 kg ha^{-1}). As soil NO_3^- is not used in the calculation of ammonia volatilization, this poor fitting should not affect the estimation of NH_3 volatilization. It prevents, however, a good estimation of NO_3^- leaching and denitrification. Therefore, we did not use the Hydrus 1D model outputs to estimate these losses in the N budget.

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

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.

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 (δ_P) was negative in the U1 and U3 treatments suggesting a potential decrease of approximatively 10 kgP ha^{-1} of the Pi HCl pool, which was not detectable against replicate variability (Table 1S, Supplementary data).

Discussion

Effect of urine fertilization on soil pH and salinity

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

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) indicate 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
	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_4^+ (mgN kg^{-1})	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
	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 (mgP kg^{-1})	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

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 term. The effect of long-term urine application on soil pH is uncertain and is potentially much greater for non-calcareous

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

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

Considerable losses of nitrogen by volatilization

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

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

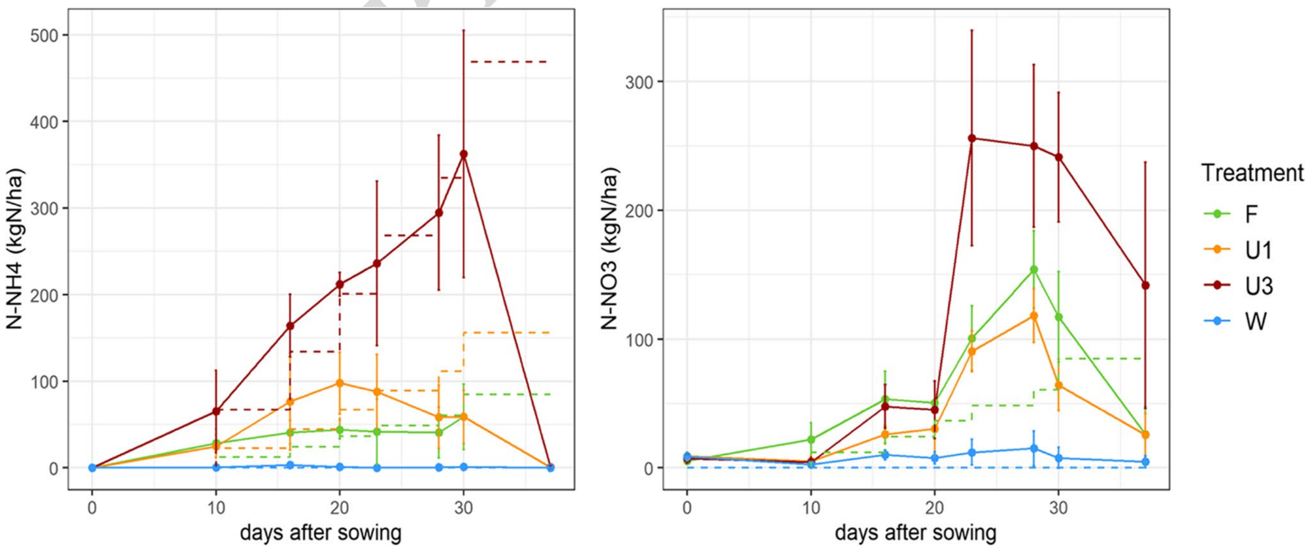

Fig. 3 Evolution of soil mineral nitrogen stocks during the experiment as a function of treatment: F: synthetic fertilizer dose × 1, U1: urine dose × 1, U3: urine dose × 3, W: unfertilized control (ammonium on the left panel and nitrate on the right panel). The dashed lines represent the cumulative fertilization inputs and the errors bars represent the standard error between the four replicates

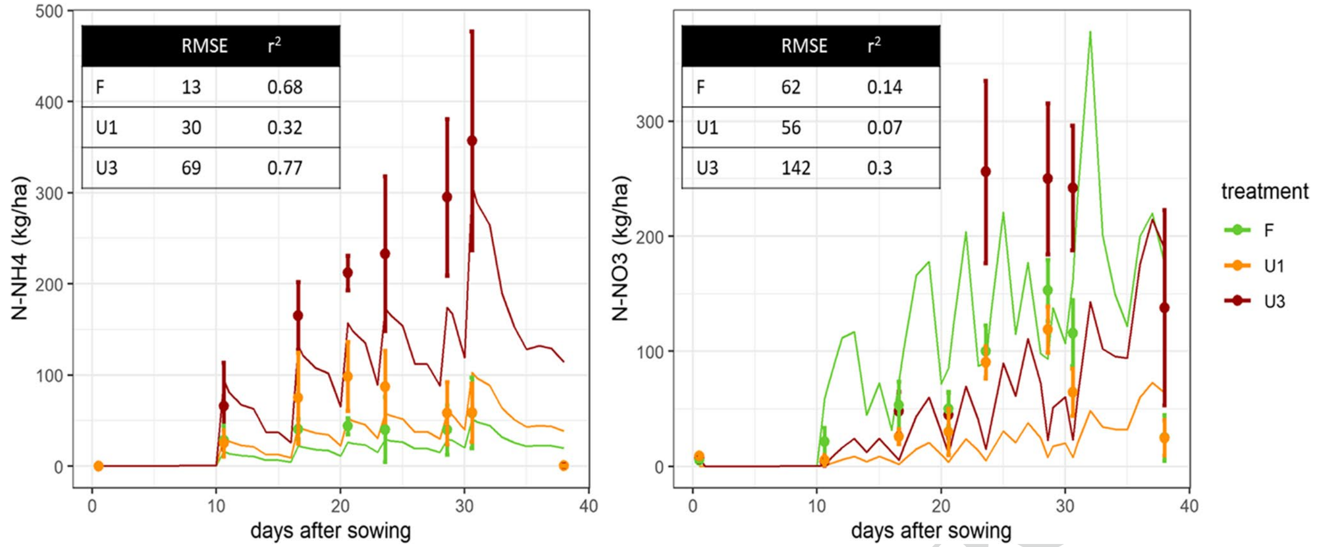

Fig. 4 Comparison between concentration of ammonium and nitrate in soils, as simulated by Hydrus 1D (lines) and observed (points) for which the errors bars represent the standard error between the four replicates. Tables in the figures report the RMSE (in kg ha^{-1}) and r^2 calculated for each treatment

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})

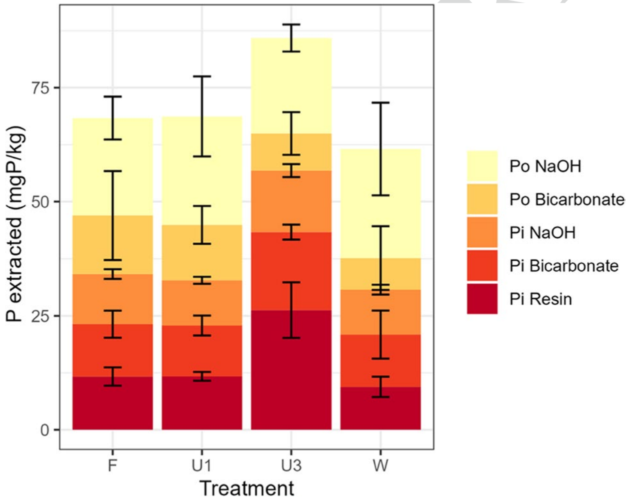

Fig. 5 Distribution of phosphorus in different soil pools at the end of experiment. The errors bars represent the standard error of four replicates. Pi Resin = resin extractable inorganic P; Pi Bicarbonate = sodium bicarbonate extractable inorganic P; Po Bicarbonate = sodium bicarbonate extractable organic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

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.

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 NH_4NO_3 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).

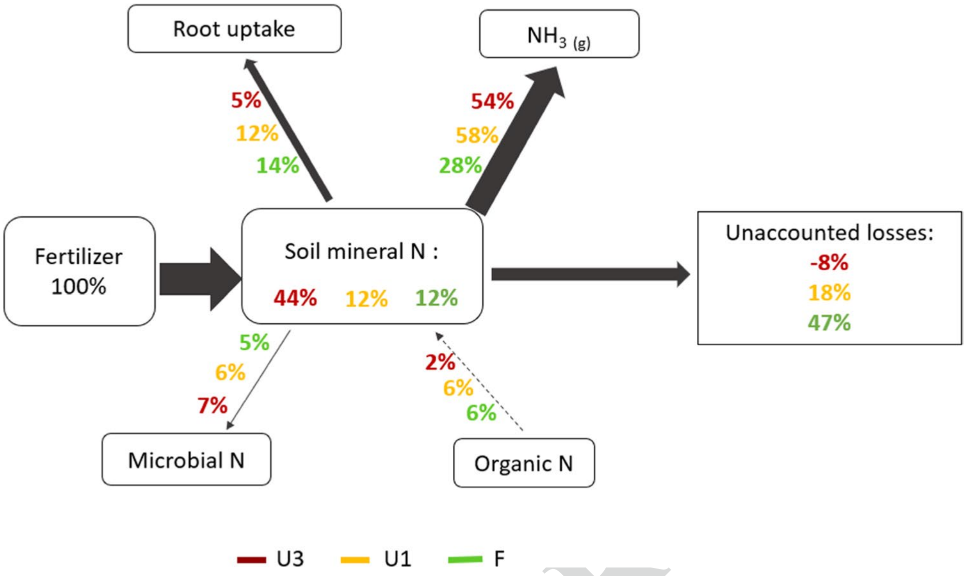

Fig. 6 Nitrogen fate in each fertilized treatment on a 0–10-cm soil profile. Percentages represent the estimated percentage of the N supplied by fertilizer that had followed each pathway at the end of the experiment. Unaccounted losses are calculated by the nitrogen budget error. A small budget error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

Bioavailability of N from urine fertilizer for plants and microbes

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

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

Bioavailability of P from urine fertilizer

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

At low doses, the fate of phosphorus from urine and synthetic fertilizer seemed relatively similar as there was no difference in concentrations of the different P pools or total P between U1 and F. The fate of P from urine was clearly detectable in the U3 treatment, where the high dose of urine supplied significantly increased the available P and secondarily the less available P pools (Fig. 5). Therefore, phosphorus from urine distributed mostly into available and slightly available inorganic P (Pi Bicarbonate) with a residual effect that was still detectable after 5 weeks of cropping. This result is in line with a study by Pandorf et al. (2018) finding that P from urine can be taken up by snap beans and turnips. In another experiment, Bonvin et al. (2015) applied synthetic and nitrified urine and found a similar P recovery rate in the crops as under mineral fertilizer.

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

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

Conclusion

In this experiment, urine supplied in appropriate doses had a very similar behaviour to that of a synthetic fertilizer in a calcareous soil suggesting that other compounds present in urine did not affect N and P uptake by the crop and their fate in the soil in the short term. Appropriate doses of urine altered neither soil pH nor soil conductivity, and provided nitrogen and phosphorus in bioavailable forms. Supplied phosphorus was either taken up by the crop or dissolved in the soil, and the ammonium was nitrified within about 10 days after application. However, this study raises awareness on the amount of ammonia that can be lost by volatilization in a calcareous soil. Indeed, chemical equilibrium and solute transfer models agreed on the estimation that about half of the N applied with urine could be lost by volatilization. Therefore, it is imperative to find more suitable fertilization techniques to mitigate ammonia volatilization on alkaline soils. Overall, our results demonstrate that although direct usage of human urine for fertilization is possible, timing, dosage and soil type need to be carefully considered for this practice to be environmentally sustainable. Future studies are encouraged to focus on the long-term effect of urine fertilization especially on soil pH and salinity which are key factors of soil quality and disentangle the various indirect effects that human urine could have on soil and plant health.

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

Declarations

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