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Cow urine as a source of nutrients for Microbial Induced Calcite Precipitation in sandy soil

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Microbial Induced Calcite Precipitation (MICP) via biostimulation of urea hydrolysis is a biogeochemical process in which soil indigenous ureolytic microorganisms catalyse the decomposition of urea into ammonium and carbonate ions which, in the presence of calcium, precipitate as calcium carbonate minerals. The environmental conditions created by urine in soil resemble those induced by MICP via urea hydrolysis. Thus, this study assesses the suitability of a waste product, cow urine, as a source of nutrients for MICP. Urea stability in fresh and sterilised urine were monitored for a month to cover the length of a potential MICP intervention. An experimental soil column set up was used to compare the soil response to the repeated application of fresh and sterilised cow urine, within pH of 7 and 9, and the chemical-based solution. Urea hydrolysis and the carbonate content in solution were monitored to assess the suitability of the proposed alternative. In addition, the nitrification process was monitored. Key findings indicated i) urea concentration and stability in fresh and sterilised cow urine are suitable for MICP application; ii) the soil response to treatments of cow urine within pH of 7 and 9 are similar to the chemical-based solution; and iii) increasing solution pH results in a faster activation of ureolytic microorganisms and higher carbonate content in solution. These results demonstrate that cow urine is a suitable substitute of the chemical-based MICP application.

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1. Introduction

Microbial Induced Calcite Precipitation (MICP) is a biogeochemical process that induces the precipitation of calcium carbonate minerals ($CaCO_3$). The MICP process is induced in soil through the addition of urea, simple carbon compounds and calcium, provided the ubiquitous presence of urea decomposing, or ureolytic, bacteria (Burbank et al., 2012). Bacteria adsorb calcium ions (Ca^{2+}) present in soil solution onto the cell surfaces, while urea is decomposed by ureolytic bacteria into carbon dioxide (CO_2) and ammonium (NH_4^+) through the enzyme urease secreted from inside the cell (DeJong et al., 2006). The presence of NH_4^+ increases locally the soil environment pH, producing a shift of the CO_2 speciation to carbonates (CO_3^{2-}). Due to the high affinity of Ca^{2+} and CO_3^{2-} ions and the low solubility of calcium carbonate at alkaline pH, calcium carbonate crystals precipitate on and in between soil grains and bacteria, which act as nucleation sites for calcite formation (Gat et al., 2014). At the macroscale, the MICP process induces the cementation of the soil structure thus, MICP is investigated as a stabilisation technique of sandy soils for engineering purposes (for a review see DeJong et al. (2013). The MICP process, however, is not solely restricted to the civil engineering practice. MICP is widely stimulated in agricultural soils with the addition of urea-N fertilisers and liming practices and has applications as an environmental management strategy for the bioremediation of contaminated soils due to replacement of calcium by divalent heavy metals in the calcium carbonate mineral structure (Jalilvand et al., 2020; Kang et al., 2016; Li et al., 2013). Thus, MICP is a relevant process in anthropogenic-related environments.

The principal components of MICP (calcium, urea, and a growth medium for ureolytic microorganisms) are currently based on industry end-products the production of which is considered to be environmentally costly. Urea, for example, is produced by combining NH_3 and CO_2 at high pressure and temperature through the combustion of fossil fuels for which the estimated greenhouse gas (GHG) emissions are 4.02 kg CO_{2e} per kg of urea-N

produced, with specific contribution of CO_2:N_2O:CH_4 gases of 97.5:0.1:2.3 (Wood and Cowie, 2004). The world production of urea-N reached a maximum in 2016 with 46 Mt of urea-N, doubling with respect to 2002 levels, representing total CO_2 emissions of 0.133 Mt of CO_{2e} per year. A change of approach towards a circular economy by considering use of available resources could reduce the environmental cost and increase sustainability of current and developing anthropogenic activities such as MICP.

In this respect, much of the work to date has focused on alternative sources for calcium to the industrially produced calcium chloride used in MICP applications (Cheng et al., 2014; Zhang et al., 2014; Xu et al., 2015; Choi et al., 2016; Liu et al., 2017; Choi et al., 2017; Casas et al., 2019). In particular, crushed basic silicate rocks (dolerite, basalt) <4 mm by-product of the quarrying sector was used effectively to source calcium for MICP, reducing the carbon cost of the technique (Casas et al., 2020). The nutritional requirements of ureolytic microorganisms include simple carbon sources (e.g., glucose), major (i.e., phosphate) and trace elements (Lapierre et al., 2020). Typically, nutrient mediums are composed of laboratory-grade compounds hindering the applicability of MICP (Rajasekar et al., 2017). Accordingly, alternative sources of nutrients based on the by-product of industrial activity have been investigated to reduce the cost of production of the nutrient medium. Lactose mother liquor (Achal, V. et al., 2009), corn steep liquor (Achal, Varenyam et al., 2010; Joshi et al., 2018), effluent of chicken manure (Yoosathaporn et al., 2016) and sugarcane molasses and vinasse (Nikseresht et al., 2020) have been proposed as low-cost nutrient sources, however, they still require of the addition of industrial urea. Recently, Chen et al., (, 2019) successfully used pig urine as a urea-containing nutrient source for laboratory grown ureolytic strains, highlighting the potential of urine by-products.

Urine is a rich nutrient source for soil microorganisms. Along with a typical water content of 95%, urea is the next main component representing in excess of 68% of the nitrogen (N) in the urine produced by cow, sheep and goat (Bristow et al., 1992). Cattle urine in soil stimulates urea hydrolysis by soil indigenous microorganisms, leading to CO_2 emissions derived from urea decomposition (Petersen et al., 2004). Thus, the soil response to cow urine application resembles the urea hydrolysis process of the MICP technique via biostimulation. In the natural environment, the enzyme urease is produced by plants and microorganisms, making urea hydrolysis a common soil trait (Burbank et al., 2012). In MICP, stimulation of native soil ureolytic bacteria (biostimulation) is a preferred

approach to introducing exogenous ureolytic bacteria (bioaugmentation) because in spite of possessing high ureolytic activities, they suffer predation by soil indigenous microorganisms (Burbank et al., 2012).

The World Health Organisation (2012) directive for handling urine indicates six months pre-storage period at 20°C to minimise the potential health risks associated to urine being contaminated with pathogenic bacteria. Although urea is a relatively stable compound, with a half-life of approximately 3.6 years (Zerner, 1991), catalytic activity of the urease enzyme increases urea hydrolysis rates by a factor of 10^4 (Amtul et al., 2002). Methods to prevent or retard urea hydrolysis through the inhibition of urease and urease producing microorganisms include the addition of natural or synthetic organic and inorganic compounds (Hellström et al., 1999; Zhang et al., 2013; Modolo et al., 2015; Ray et al., 2018; Modolo et al., 2018). However, applied to urine, urease inhibition methods would have detrimental effects on soil indigenous ureolytic microorganisms stimulated to induce MICP. A thermal treatment could significantly shorten the pasteurisation period of urine to a week (Zhou et al., 2017), however, the process is energy expensive as it requires sustained elevated temperature.

This study considered cow urine as a single, urea-containing nutrient source for MICP applications. It was hypothesised that cow urine derived from the farming sector could contain sufficient urea and nutrient sources to stimulate the activity of soil indigenous ureolytic bacteria and substitute the laboratory-grade chemical-based solution used in MICP applications. To investigate the potential of the proposed alternative, the first part of the study investigated the stability of urea in fresh and thermally treated urine over a month to cover the length of a possible MICP intervention. The second part of the study used soil column experiments to study the soil response to the application of cow urine providing comparison with the traditional MICP treatment. Urea hydrolysis was induced repeatedly on a naturally occurring sandy soil by biostimulation of soil indigenous ureolytic bacteria with fresh and thermally treated urine, as well as with the traditional, chemical-based, MICP solution treatment. The pH of thermally treated urine was adjusted to investigate the microbial response to a range of alkaline solution pH due to the reported variability of soil ureolytic activity with pH (Tabatabai and Bremner, 1972; Pettit et al., 1976; May and Douglas, 1976; Cabrera et al., 1991; Fisher et al., 2017). The effectiveness of the proposed alternative was assessed through quantification of urea hydrolysis rates and carbonate ion availability for precipitation as carbonate minerals. For this, urea, pH

and dissolved inorganic carbon were monitored. In addition, ammonia and nitrogen oxides in solution were monitored to assess the potential occurrence and magnitude of nitrification.

2. Materials and Methods

2.1. Soil sampling and characterisation

Soil was obtained from the William Clark Quarry (N56°29'52'', W2°46'14''; WGS84), located at the Firth of Tay estuary in eastern Scotland discharging into the North Sea. The superficial deposits in this area are detrital raised shallow-marine deposits of Holocene age (https://www.bgs.ac.uk/; January 2018) with sandy layers alternating with layers of finer material. 10 kg of soil were collected from an exposed slope generated by scraping across the top five metres surficial deposits (Figure S1). The sampled soil texture was homogeneous, mainly fine sand with gravels and boulders being scarce, of a reddish-brown colour and with no apparent organic matter. Upon arrival to the laboratory, the soil was air dried for 48 h on trays and sieved through 2 mm and 63 µm sieves. The material remaining on the 2 mm sieve, identified as clay-like hard clumps, and passing the 63 µm sieve were discarded (Figure S1).

The selected soil was characterised by particle size distribution, pH and loss on ignition (LOI) in one, two and three replicates, respectively. The soil's coarse and fine fractions were determined by the dry sieving and hydrometer methods (BS 1377-2, 1990). The soil total organic carbon (TOC) and total nitrogen (TN) were determined by dumas combustion with an elemental analyser (Thermo Finnigan Flash EA, the James Hutton Institute Ltd) on three replicates. The soil total inorganic carbon (TIC) content was determined by dissolution in hydrochloric acid plus back titration (i2 analytical Ltd) on four replicates. The soil inorganic N compounds nitrite (NO_2^-), nitrate (NO_3^-) and ammonium (NH_4^+) were determined colorimetrically with a spectrophotometer (SmartChem 600, the James Hutton Institute Ltd) on three replicates. For nitrite and nitrate a 1:2 soil to water ratio was used, and exchangeable ammonium was extracted in a 0.1 M KCl solution at a 1:5 solution to soil ratio. Soil major extractable elements Ca, Mg, K and elements detrimental to soil ureolytic activity Fe, Mn, Cu, Cd, Zn, Sn, Ni, Co, Al, Mo, Ba (Tabatabai, 1977) were extracted in an 1 M ammonium acetate solution at a 1:5 soil to solution ratio and determined by isotope coupled plasma optical emission spectrometry (ICP-OES, Thermo iCap 6400) on two replicates (i2 analytical Ltd). The

159 moisture content of the soil was determined gravimetrically prior to the beginning of the
160 experiments.

161 **2.2. Cow urine sampling and characterisation**

162 Cow urine was sourced from Auchendreich Farm (Inverbervie, Montrose, DD1 0SY, UK)
163 in November 2018. Urine samples from dairy cows were collected in a bucket at the time
164 of urination, transferred into one litre sealable sampling bottles, stored in a portable fridge
165 during transport to the laboratory and stored frozen at -20°C prior to analysis. Fresh urine
166 collection took place within a period of 40 mins and transported to the laboratory within
167 90 mins. Bottles containing cow urine were allowed to thaw in the fridge at 4°C prior to
168 manipulation, thoroughly mixed inverting the bottles several times, filtered (grade 1
169 Whatman® qualitative filter paper) and redistributed into sealable bottles in preparation
170 for analysis.

171 The initial concentrations of TOC, TIC, TN, urea and pH of urine solutions were
172 determined for each experiment. TOC and TIC were determined by combustion
173 non-dispersive infrared (NDIR) and TN by chemiluminescence elemental analysis with
174 an elemental analyser (TOC-L Analyser with TNM-L unit, Shimazu UK Ltd.; the James
175 Hutton Institute Ltd). Urea was analysed colourimetrically (Knorst et al., 1997)
176 (calibration curve: $n = 5$, $r^2 \geq 0.9995$, $SD_{max} = 0.11$ mM, $CV_{max} = 3.83\%$) and determined
177 using a spectrophotometer (DR5000 Hach Lange, USA) on three replicate samples.
178 Solution pH was determined in triplicate using a FiveEasy Toledo Mettler with LE409
179 probe calibrated at pH of 7 and 10. Urine major elements Ca, K, Mg, Na, and P and trace
180 elements Cu and Zn were determined in cold digested (12.5% HNO_3 nitric acid solution)
181 urine samples and determined by ICP-OES (Perkin Elmer Avio 500; the James Hutton
182 Institute Ltd).

183 **2.3. Urea stability in urine**

184 The stability of urea in fresh and pasteurised urine stored at room temperature was
185 investigated through monitoring urea and pH over a 28-d period. In all cases, urea was
186 measured on three replicate samples and pH in triplicate with the methods described in
187 Section 2.2.

188 Fresh cow urine was allowed to thaw in the fridge for 18 hours prior to storage at room
189 temperature. Storage at room temperature marked the beginning ($t = 0$) of both the urea
190 stability experiment and the soil column experiment running in parallel (see Section 2.4).

Urea stability in fresh urine was studied measuring urea and pH measurements each time the solution was poured into soil columns for a new treatment to determine the initial concentration of urea and pH. Measurements were taken at t = 8, 32, 56, 80, 152, 200, 559, 674, and 703 h from the beginning of the experiment.

Pasteurised urine was obtained using the thermal treatment proposed by Zhou et al. (, 2017). The temperature was controlled using an analogue temperature controller (843/N, Thermosystems S.r.l.) and additionally monitored with a conductivity meter (probe HI 9635, Hanna Instruments Ltd., UK). Urea was determined prior (t = 0) and after the thermal treatment (t = 7 d) plus at t = 14, 21 and 28 d from the beginning of the thermal treatment. The pH was monitored at every urea measurement and every second day after the thermal treatment.

2.4. Soil columns assay

2.4.1. Treatment solutions

The chemical-based, traditional growth and "cementation" solutions to induce MICP were prepared according to common MICP literature (Al Qabany and Soga, 2013; Burbank et al., 2013). For the cementation solution, calcium was excluded because the focus of the study was the urea hydrolysis process, but the term "cementation" is still used for convenience with the MICP literature. The traditional MICP growth solution was prepared adding 1 g L^{-1} of pure cane molasses (Meridian Foods Ltd., UK), 0.1 g L^{-1} yeast (Meridian Foods Ltd., UK) and 100 mM sodium acetate anhydrous (>99%, FCC, FG Sigma-Aldrich Inc., USA) in distilled water. The MICP "cementation" solution was prepared adding 1 g L^{-1} of pure cane molasses (Meridian Foods Ltd, UK), 100 mM of sodium acetate anhydrous and 200 mM of urea (Molecular Biology, Fisher BioReagents™) in distilled water. Fresh solutions were prepared anew each day of treatment.

Fresh and thermally treated urine solutions tested as alternatives to the traditional MICP solution were prepared as follows: fresh urine was withdrawn from refrigerated storage 8 h prior to the beginning of the soil column experiment and subsequently stored at room temperature throughout. An additional batch of fresh urine was thermally treated as in Zhou et al. (2017) one week prior to the beginning of the experiment and subsequently stored at room temperature. Subsamples of thermally-treated urine were brought to initial pH = 7 and pH = 9 adding concentrated hydrochloric acid (HCl~37%; Analytical reagent

grade, Fisher Scientific UK Ltd.) dropwise while stirring and measuring the pH. The solution pH was monitored and adjusted to pH 7 or 9 accordingly when variations were observed prior to addition into soil columns throughout the experiment.

2.4.2. Soil column preparation and treatment sequence

Soil columns were prepared 48 h after soil sampling by placing selected soil into new sterile 60 mL syringes through pluviation up to the 45 mL mark (60 g of wet soil) (Figure S2). To prevent loss of material, sponge cloths (product code: 318012, B&M Retail UK Ltd.) were cut to size and placed at the bottom of the soil columns. Soil columns were prepared in triplicate for each type of treatment (see Section 2.4.1)

Soil columns were treated in two stages designed to follow commonly applied MICP treatments: the growth phase, to enlarge the microbial ureolytic community numbers and; the cementation phase, where calcite precipitation is induced via urea hydrolysis. The treatment sequence was as follows:

i. Soil columns were treated once with the growth solution 3 d after soil collection.
ii. The growth solution was drained 3 d later and soil columns were flushed with 50 mL distilled water.
iii. The first cementation treatment commenced 24 h after drainage, where soil columns were treated with either fresh urine, thermally treated urine at adjusted pH of 7 or 9, or the traditional MICP cementation solution.
iv. The reaction proceeded for 16 h after which solutions were drained and collected in new, sterile centrifuge tubes.
v. The procedure was repeated after rest time intervals of 8 h in between treatments (treatments 4^{th} and 5^{th} were spaced 72 h).

In total, soil columns were treated six times except for soil columns treated with fresh urine, which were treated five times due to runout of fresh urine. To maximise solution replacement within soil columns, addition of growth and precipitation solutions was as follows: initially, 25 mL were pipetted from the top and allowed to gravitationally drain through the soil columns, then the outlet was locked, and additional 15 mL of solution were added on top to maintain the head of the solution above the soil surface.

2.5. Chemical analyses on liquid samples

Urea content and pH of inlet and outlet solutions were determined for each treatment on three replicate samples and in triplicate, respectively, as indicated in Section 2.2). The pH of soil leachates was determined upon sampling. Subsequently, 2 mL of 0.15 mM phenylmercuric acetate (PMA, >97%, Sigma-Aldrich Inc., USA) solution were added to halt microbial activity, such that the final concentration in each centrifuge tube was 0.016±0.001 mM of PMA (Greenan et al., 1995; Fisher et al., 2017). Based on the observed urea decomposition patterns, samples obtained after the first, fourth and sixth treatments were analysed further for TOC, TIC and TN content as in Section 2.2. Additionally, ammonium, nitrite, and total organic nitrogen (TON) were determined colourimetrically (Konelab Discrete Analyser; the James Hutton Institute Ltd.). Nitrate, calculated subtracting nitrite from TON, was reduced to nitrite with hydrazine sulphate, diazotised with sulphanilamide, coupled with N (1 naphthyl)-ethylenediamine dihydrochloride and TON determined at 540 nm with a spectrophotometer.

2.5.1. Carbonate ion in solution

Carbonates in solution were inferred from pH and dissolved inorganic carbon (DIC) measurements as (Gat et al., 2014):

$$CO_3^{2-} = TIC\ \alpha_2 \qquad 1$$

where TIC is the measured inorganic carbon in solution and α_2 is the carbonate mole fraction calculated as:

$$\alpha_2 = \left(\frac{[H^+]^2}{K_1\ K_2} + \frac{[H^+]}{K_2+1}\right)^{-1} \qquad 2$$

where, $K_1 = 5x10^{-7}$ and $K_2 = 5x10^{-11}$ are the solubility constants and $[H^+]$ is the activity of hydrogen cations calculated from the pH.

3. Results

3.1. Urea stability in urine

Urea in fresh urine and thermally treated urine stored at room temperature was stable over the 28-d period and urea in thermally treated urine showed no signs of decomposition three weeks following thermal treatment (Figure 1). Urea in fresh urine remained stable up to Day 28, with an average urea concentration and variability over time of 136±9.5

mM. The pH of fresh urine followed two consistent trends. For a week, pH dropped from the initial pH of 8.8 to just below 8.0 on Day 6. Thereafter, the pH steadily increased to reach a maximum end point of 8.2.

For thermally treated urine, the 7-d thermal treatment induced a consistent pattern of urea concentration decrease and pH increase after which both urea and pH remained stable up to 28 d (Figure 1). Both parameters changed significantly with the thermal treatment: the pH increased from 8.8 to 9.5 and urea decreased up to 103 mM, representing a loss of 36% with respect to the pre-thermal treatment urea content.

3.2. Soil characterisation

The soil used for this study classified as poorly graded, fine-grained silty sand (ASTM, 2017) with 74% sand, 23% silt and <4% clay (Table 1) (Figure S3). The soil chemical properties were typical of a calcareous soil: the soil pH was slightly alkaline (pH = 7.65), the soil TIC (3.64±0.82%) was high and the TOC (0.18%) and TN (<0.03%) contents were low (Table 1). TN was below detectable limits however, taking the sum of ammonium and nitrogen oxides (2.78 mg kg^{-1}) the estimated soil C:N ratio was 647:1. The major extractable elements analysed further confirmed the calcareous nature of the soil, with Ca and Mg being the most abundant extractable elements (Table 1). Extractable elements detrimental to soil ureolytic activity Mn, Al and Fe (<50 mg kg^{-1}), and Cu, Cd, Zn, Sn, Ni, Co, Mo (<1 mg kg^{-1}) (Tabatabai, 1977) were below limits of detection with the exception of Ba.

3.3. Cow urine characterisation

The batches of urine used for the thermal treatment to study urea stability (U1/3) and the soil column experiments (FR, TH7 and TH9) to induce soil urea hydrolysis through biostimulation had variable initial concentrations of organic C (TOC = 541-976 mM) and nitrogen (TN = 297-559 mM) but a constant C:N ratio (1.85) (Table 2). Urea represented between 72 and 83% of TN in urine samples. The variability in urea concentration (123-224 mM) across urine batches was also determined and found to explain the variability determined in TOC and TN. The pH of solutions was similar on the alkaline side (8.4-8.8) and similar TIC (90-107 mM) content were also determined. The elements determined followed common composition of cow urine with K (244 mM) being the most abundant element followed by Na (78 mM), Mg (12 mM) and traces of Ca (<0.3 mM) and P (<0.01 mM). Trace elements Cu and Zn were <0.22 mM.

3.4. Soil urea hydrolysis and pH

Urea hydrolysis through biostimulation of soil indigenous ureolytic microorganisms was found to be successful in all cases. Similar urea hydrolysis patterns with increasing the number of treatments were observed for the solution treatments used: fresh (FR), thermally-treated urine at adjusted pH of 7 and 9 (TH7 and TH9, respectively) and the chemical-based solution (MICP) (Figure 2a).

Initially, the fraction of urea hydrolysed per treatment, $(U_{inlet}-U_{outlet})/U_{inlet}$, did not vary, ranging between 7-18%, with urea hydrolysis rates within 21-48 mmol urea N h^{-1} kg^{-1} of soil. After several treatments, which differed for the different solution treatments, the amount of urea hydrolysed per treatment increased steadily. This initial period of apparent stability is referred herein as "microorganisms' adaptation" or simply "adaptation" phase. Prolongation in time of the microorganisms' adaptation phase was apparently dependent on the initial solution pH, such that the increase in the amount of urea hydrolysed per treatment commenced earlier with higher initial solution pH.

The evolution of soil leachate pH differed with the type of treatment solution but, eventually, pH > 9 were attained in all cases (Figure 2b). The chemical-based solution and fresh urine treatments showed the closest urea hydrolysis and pH trends. In comparison, soil columns treated with pH of 7 urine showed generally a longer adaptation phase and lower pH values, while those treated with pH of 9 urine showed a shorter adaptation phase and 9 and higher pH. At pH <9 and for the same number of treatments, both the fraction of urea hydrolysed, and pH were higher for higher initial solution pH and vice versa. For example, for the pH of 9 urine treatment, the post-adaptation phase commenced from the second treatment onwards with pH >9 while, for the pH of 7 urine treatment, the microorganisms' adaptation phase lasted four treatments and pH of 9 was not reached until the sixth treatment.

Post-adaptation urea hydrolysis rates, *R,* were computed as the amount of urea-N hydrolysed per hour of reaction time and normalised to the mass of soil (Figure 3). Post-adaptation urea hydrolysis rates increased linearly with coefficients of correlation close to the unit (r^2 >0.91) (Table 3), achieving maximums typically on the last treatment. Urea hydrolysis rates stabilised after repeated treatments in soils treated with pH of 9 urine (Figure 3). This was not observed for the rest of the treatment solutions although signs of flattening were observed for the chemical-based and pH of 7 urine solution treatments.

345 The highest urea hydrolysis rates were determined for soil columns treated with the
346 chemical-based MICP solution (378 mmol urea-N h^{-1} kg^{-1}) and the lowest with fresh
347 urine (243 mmol urea-N h^{-1} kg^{-1}). Despite the different urea hydrolysis and pH trends,
348 soils treated with pH of 9 and 7 urine showed similar maximum urea hydrolysis rates
349 (~264.5 mmol urea-N h^{-1} kg^{-1}) (Table 3). The relation between the initial solution pH and
350 the slope, *m*, of the linear regression of urea hydrolysis rates indicated that following the
351 microorganisms' adaptation phase, urea hydrolysis rates increased faster each treatment
352 with lower initial solution pH (Figure S4). Further, the maximum urea hydrolysis rates
353 were found linearly correlated (r^2 >0.93) to the initial urea-N concentration in solution
354 (Figure S5).

355 **3.5. Carbonate ion availability**

356 The concentration of carbonate ions in solution increased with increasing amounts of urea
357 hydrolysed per treatment (Figure 4a) and increasing pH (Figure 4b), reaching
358 concentrations between 2.5-5.5 mM for the various treatments. The highest carbonate
359 contents in solution were determined with pH of 9 urine (4.1-5.3 mM) and chemical-
360 based solution treatments, followed by the fresh (0.6-2.5 mM) and pH of 7 urine (2.1 mM)
361 treatments.

362 Generally, pH >9 resulted in carbonate content >2 mM, fractions of urea hydrolysed >0.4
363 and pH >8.5 resulted in carbonate ion concentrations >1 mM while lower values resulted
364 in insignificant carbonate content in solution (<0.2 mM). The thermally-treated urine at
365 adjusted pH of 9 resulted in the highest carbonate content in solution (>4 mM) at pH >8.5,
366 as identified by the marker highlighted with "*" in (Figure 4a). Higher carbonate content
367 in solution resulted from urine treatment solutions of higher initial solution pH (e.g., TH9
368 > TH7) for similar fractions of urea hydrolysed. However, this was not always the case,
369 as identified by the marker highlighted with "*" in (Figure 4b). In this case (chemical-
370 based solution, MICP), carbonate <0.5 mM was determined at pH >9, which was
371 associated to a low fraction of urea hydrolysed (<0.3). At low fractions of urea hydrolysed
372 (0.1-0.4), the chemical-based solution (MICP) showed higher soil leachate pH (8.3-9.1)
373 compared to urine treatments (7.3-8.8) but resulted in insignificant carbonate content in
374 solution (≤1 mM).

3.6. N and C mass balance

The sum of urea-N and NH_4-N represented on average 94% of the TN in soil leachates. Nitrite (NO_2-N) was below detectable limits while the maximum concentrations of nitrate (NO_3-N) determined were of 3.3 mg L^{-1}, representing <0.07% of TN in all the analysed samples (data not shown). Figure 5 presents the linear regression between the variation of the urea hydrolysis reaction products, TIC (Figure 5a) and NH4 (Figure 5b), computed from the measured variation urea ($\Delta Urea = U_{inlet} - U_{outlet}$) against the measured variation of the analytes in solution. The figure further includes the molar relation of the urea hydrolysis reaction of 1:1 for TIC in (Figure 5a), and 1:2 for NH_4 in (Figure 5b) which indicate the theoretical variation of the respective products produced by the urea hydrolysis reaction. Variation in solution TIC and NH_4 content were well explained by the determined variation of urea in solution. Values plotting on the negative x-axis suggest that removal of both NH_4 and TIC from solution occurred to some extent during the first treatment. After this initial TIC removal from solution of 40 90 mM (Figure 5a) TIC values plotted much closer to the straight-line indicating TIC content in solution was well explained by variations in urea content. For NH_4 (Figure 5a), values plotting to the left of the expected variation indicated NH4 removal from solution occurred throughout. An average loss of 73 mM of NH_4-N from solution was determined, with the smallest loss observed for the pH of 7 urine treatment.

Similarly, Figure 6 shows the variation in urea-N content with TN determined in solution. The urea hydrolysis reaction should not produce, by itself, a significant change in solution TN as urea-N is converted into NH_4-N. Thus, the variations in solution TN (x-axis) should indicate loss of nitrogen from solution. On the other hand, the straight-line with 1:1 slope in indicates the pattern that would arise from a perfect match between loss of N from solution and conversion of urea-N to NH_4-N via the urea hydrolysis reaction. Thus, values plotting close to the straight-line (highlighted area "A") would indicate that variations in urea-N were also observed in TN, suggesting that the produced NH_4-N through urea hydrolysis was consumed or lost. Instead, values plotting near-zero ΔTN would indicate the produced N through urea hydrolysis remained in solution as NH_4-N (highlighted area "B"). According to this, Figure 6 showed that for urine treatments, most of the NH_4-N produced by urea hydrolysis remained in solution, while markers that plotted close to the straight-line corresponded to the first treatment and are in agreement with the initial NH_4 loss identified in Figure 5. Instead, for the chemical-based solution, most values plotted

close to the straight-line indicating the N produced by urea hydrolysis was partially removed from solution.

4. Discussion

In this study, assessment of the suitability of cow urine for MICP was based on one hand, on the stability of urea in solution and, on the other, on the urea hydrolysis reaction and the environmental conditions favourable to the precipitation of carbonate minerals. Results indicated that both fresh and thermally treated cow urine within the pH range 7 to 9 are a suitable alternative to the traditional chemical-based solution used for MICP.

Urea in cow urine stored at room temperature was found to be stable in fresh urine for a period of at least one month and for thermally-treated urine at least of three weeks following thermal treatment without signs of urea degradation (Figure 1). Urea hydrolysis occurred to some extent during thermal treatment. This was evidenced by the observed pre- and post-treatment decrease in urea-N to TN ratio (0.85 to 0.75) and an increase in NH_4-N to TN ratio (0 to 0.15). The stability of urea in fresh urine indicated urease was not present in the urine sourced for this study. Therefore, the post-thermal treatment presence of NH_4 indicated that urea was chemically hydrolysed, most likely facilitated by relatively high temperature (70ºC) required for the thermal treatment. The stability of urea in fresh urine suggests that fresh urine could remain a reliable source of urea over the duration of an MICP treatment without any prior additional requirement.

The soil response to the application of urine was similar to the traditional, chemical-based solution used for MICP and urea hydrolysis through biostimulation was successfully induced in all cases. This was indicated by the similar urea hydrolysis and pH trends (Figure 2). During the microorganisms' adaptation phase the contribution of ureolytic microorganisms to the total observed soil urease activity (21 to 48 mmol urea-N h^{-1} kg^{-1}) may have been initially small, as suggested by the flat trend and small variation across the various treatments, becoming increasingly relevant up to the point where the linear increase in soil urease activity was observed. In the soil environment, urease is produced by microorganisms and plants (Hoult and McGarity, 1986) and it is estimated that between 37 to 73% of the total soil urease is contained intracellularly (Jahns et al., 1988; Allison and Prosser, 1991; Klose and Tabatabai, 1999). Intracellular urease is released into the soil environment upon cell death (Fisher et al., 2017) where it can continue to function (Krajewska, 2009) adsorbed to organic matter (Hoult and McGarity, 1986) and

secondary minerals (Gianfreda et al., 1992), contributing up to over half of the observed enzymatic activity in some soils (Klose and Tabatabai, 1999; Klose and Tabatabai, 2000).

The length of the microorganisms' adaptation phase was found to be dependent on the initial solution pH with increasing initial pH encouraging a faster microbial response (Figure 2). For example, for soils treated with pH of 9 urine, the amount of urea hydrolysed per treatment increased from the second treatment onwards whilst for soils treated with the pH of 7 urine, an increase in urea hydrolysis was not detected until the fifth treatment. Thus, soil's response to urine treatments was accelerated with increasing initial pH solution, indicating that higher pH had a favourable effect on ureolytic microorganism's development, as observed by Fisher et al. (2017). However, other factors may have also contributed to a slower response of soil ureolytic microbial community treated with pH of 7 urine. Barium, detected in the extractable fraction of the soil used for this study (Table 1), is a non-essential element for soil organisms (Lamb et al., 2013). It has been reported to induce toxicity to ureolytic (Tabatabai, 1977) and non-ureolytic microorganisms (Polonini et al., 2014), as well as to plants and worms, slowing growth rates and reducing body biomass (Lamb et al., 2013). Barium in soil is typically found combined with sulphate or carbonate ions as the minerals barite ($BaSO_4$) and witherite ($BaCO_3$) and adsorbed to clays. Divalent cations are immobile at alkaline pH and become increasingly mobile with increasing acidity. Thus, incorporation of barium into the soil solution may have been favoured by the pH 7 solution treatment, inhibiting urea hydrolysis (Tabatabai, 1977). This should be investigated further, as it may have implications for in-situ MICP regardless of whether MICP is induced through bio-stimulation or bioaugmentation.

The linear increase in the amount of urea hydrolysed following the adaptation phase indicated increasing urea hydrolysis by soil indigenous ureolytic communities (Figure 3 and Table 3). However, results also show that high solution pH led to a slower increase in the amount of urea hydrolysed per treatment, indicating urease activity was generally higher at pH of 7 compared to pH of 9. The rate of reaction urea hydrolysis is reported to follow a two-parameter Michaelis-Menten model (Paulson and Kurtz, 1970; Tabatabai and Bremner, 1972; Pettit et al., 1976). Cabrera et al. (1991) reported that the kinetics in soil are best described by two separate Michaelis-Menten enzymatic reactions for cases of low and high affinity for which the two kinetic parameters v_{max} and K_m are pH dependent. For pH values of 7, the low affinity reaction dominates requiring a large

amount of substrate with high urea hydrolysis rates (v_{max}) whereas for a pH of 9, a high affinity reaction dominates requiring less substrate to reach maximum urease activity. Our results confirm urea hydrolysis rates were higher at pH of 7 than at pH of 9. The maximum urea hydrolysis rates observed across the solution treatments in this study indicated that the urea concentration was not sufficient to saturate the soil's urease enzyme. Differences in maximum urea hydrolysis rates at various pH were therefore not apparent. Our results further suggest that the optimum urease activity at pH of 9 as observed by Tabatabai and Bremner (1972), May and Douglas (1976), and Perez-Mateos and Gonzalez-Carcedo (1988), and could alternatively have been produced from the early response of soil ureolytic communities.

The highest carbonate ion contents in solution product of the decomposition of urea were achieved with higher initial solution pH for soils treated with urine (Figure 4). For similar fractions of urea hydrolysed, soils treated with pH 9 urine showed consistently higher solution pH and carbonate content. Soils treated with the chemical-based solution showed higher solution pH compared to urine treatments for similar amounts of urea hydrolysed, possibly due to higher buffering capacity of urine solutions. However, this was not necessarily related to higher inorganic carbonate content in solution. Our results indicated the combination of pH above 9 and fraction of urea hydrolysed above 0.4 resulted in highest carbonate concentration in solution. For example, combination of a fraction of urea hydrolysed of 0.4 and pH = 9.3 in soils treated with pH of 9 urine led to significant carbonate production (~5 mM) yet either lower urea hydrolysis, determined for soil treated with chemical-based MICP solution (fraction of urea hydrolysed ~ 0.2 and pH = 9.2), or lower solution pH led to carbonate concentrations <1 mM.

Our results show both DIC and ammonia in solution were well explained by variations in urea content following the first treatment. An average loss of 73 mM of NH_4-N produced by urea hydrolysis was determined, which could lead to underestimation of urea hydrolysis. The highest NH_4 removal from solution was determined for the chemical-based MICP solution despite solution pH was generally lower than for pH 9 urine treatment, while for cow urine treatments NH_4 remained mostly in solution. Removal of NH_4 from solution could be a combination of fixation of NH_4 in soil through adsorption or precipitation, as salt or mineral (e.g., struvite) processes; volatilization as NH_3; and microbial consumption for growth and nitrification processes (Nieder et al., 2011). Our data indicated nitrification did not occur in a significant level throughout the experiment,

as more than 80% of NH_4-N produced by urea hydrolysis remained in solution. This was somewhat not surprising as ammonia oxidising microorganisms are naturally slow growing microbes. In MICP applications, nitrification has not been shown significant four weeks following treatment (Gat et al., 2017), indicating that MICP induces ammonia concentrations that exceed toxicity levels of nitrifying bacteria observed in Anthonisen et al. (, 1976).

5. Conclusions

This study of urine derived from the livestock farming sector as source of nutrients for Microbial Induced Calcite Precipitation (MICP) focused on inducing changes in soil environmental conditions compatible with the formation of carbonate minerals such as calcite via urea hydrolysis. Sterilisation and stability of urea in urine are some of the major difficulties that are needed to be overcome for an effective use of urine. Urea was found to be stable in both fresh and sterilised urine for a period of at least one month sufficient to cover the length of a MICP treatment. Results indicate that urine at pH of 9 could be most suitable within the MICP framework as it provided the shortest microorganism adaptation phase and higher production of carbonate ions in solution required for the bio-mediated precipitation of carbonate minerals. However, further studies are required to confirm whether this is the case for longer applications. An excess of 80% of the ammonia produced by urea hydrolysis remained in solution which could pose environmental threats. It is likely oxidation of ammonia by way of nitrification during MICP at the urea levels typically used in MICP inhibit nitrification bacteria therefore, long-term monitoring studies on the fate of accumulated ammonia and nitrogen compounds potentially derived from the MICP technique are necessary, as well as to develop removal strategies of potential pollutants. Overall, our results indicate that urine derived from cow and potentially other mammals was a suitable nutrient solution for MICP. With the adequate infrastructure in place based on a circular economy, this alternative could become an accessible local source which could overcome the need of chemical compounds for the biostimulation of urea hydrolysis for MICP and thus reduce the environmental costs associated with production and transportation of chemicals and source of water. Further studies that include different soils, animal species and animal living conditions should be conducted to assess the wider scope its application.

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712 **Author contributions**

713 EJ, JA and CC, conceptualization; CC, data curation; CC, formal analysis; EJ, CS,
714 funding acquisition; CC investigation; JA, EJ and CC methodology; EJ project

administration; CC resources; EJ, CS and JA supervision; CC, original draft; EJ, CS and JA, review & editing.

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Declaration of interest

None.

Tables

Table 1 Soil physical and chemical properties. Physical properties: particle size distribution (n = 1) and moisture content (n = 3) and; Chemical properties: pH (n = 3), organic (n = 3) and inorganic C (n = 4), total N (n = 3), inorganic N compounds NH_4, NO_2^- and NO_3^- (n = 1), soil major extractable elements and soil trace extractable elements detrimental to urease activity (Tabatabai, 1977) (n = 1). Values "n" in caption indicate number of replicate analysis. In table, values after ± refer to one standard deviation.

Parameter	Value	Unit
Sand	73.6	%
Silt	22.4	%
Clay	4.0	%
pH	7.65±0.13	-

Moisture (θ_0, t = 48 h)	21	%
TIC	3.64±0.82	%
TOC	0.18±0.01	%
TN	<0.03	%
NH_4^+ (NH_4^+-N)	2.4 (1.86)	mg/kg
NO_3^- (NO_3^--N)	3.7 (0.84)	mg/kg
NO_2^- (NO_2^--N)	0.266 (0.08)	mg/kg
Ca	15.23	mEq/100g
Mg	3.08	mEq/100g
K	0.11	mEq/100g
Ba	0.06	mEq/100g

739

740 ***Table 2 Chemical characterisation of cow urine and MICP solutions used for the***
741 ***different experiments. Initial concentration of total organic and inorganic C, total N***
742 ***(n = 3), urea (n = 3) and pH (n = 3). Values "n" in parenthesis indicate the number of***
743 ***replicate analysis. Value after ± refers to two standard deviations for TOC, TIC and***
744 ***TN measurements and for urea, to the standard deviation of nine (FR) and three (TH7***
745 ***and TH9) replicate samples. Values for TOC-TIC and TN for 'MICP solution' are***
746 ***computed values from known concentration of solution components.***

Parameter	Thermal treatment urine (U1/3)	FR	TH7	TH9	MICP
TOC (mM)	976±27	541±30	772±43	774±43	421**
TIC (mM)	107±5*	90±5	106±5	184±9	-
TN (mM)	559±19	297±18	426±27	403±18	390**
Urea (mM)	224±0.6	123±3	154±2	153±2	195±5
pH	8.37	8.84	7.05	9.05	-

K (mM)	244±19.8	-	-	-	-
Na (mM)	78.17±5.67	-	-	-	-
Mg (mM)	11.59±0.56	-	-	-	-
P (mM)	0.469±0.125	-	-	-	-
Ca (mM)	0.288±0.012	-	-	-	-
Cu (mM)	0.090±0.009	-	-	-	-
Zn (mM)	0.005±0.001	-	-	-	-

747

748 ***Table 3 Urea hydrolysis rates computed from urea concentrations. Parameters***
749 ***describing urea hydrolysis rates in relation to the initial urea concentration (Urea-N0),***
750 ***initial solution pH0 and linear correlation with the number of treatments.***

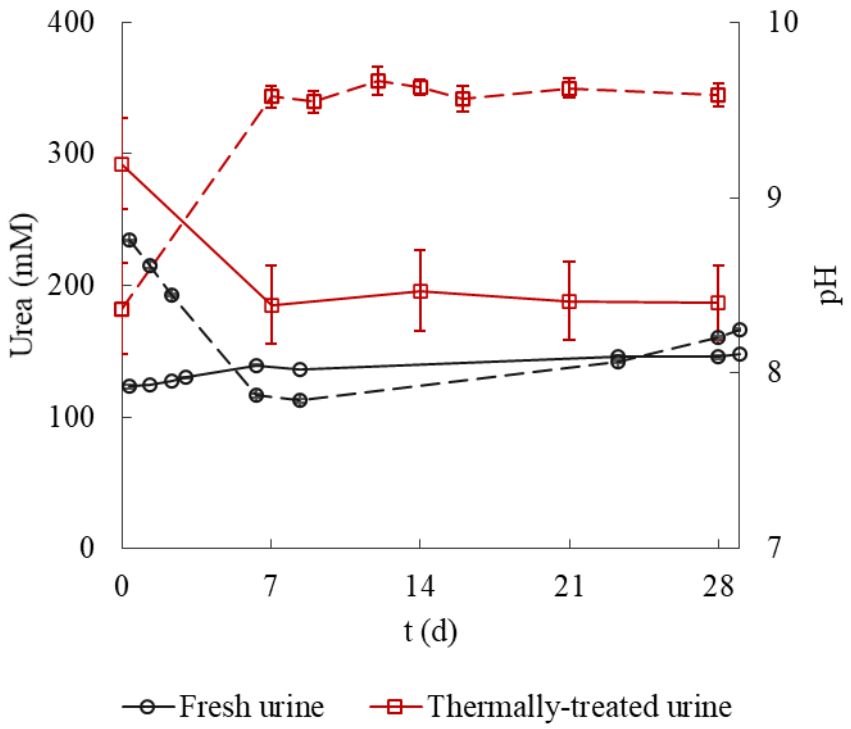
Treatment solution ID	Number of treatments Plateau-linear	m (mM urea-N h^{-1} kg^{-1} n^{-1})	r^2	R_{max} (mM urea-N h^{-1} kg^{-1})
MICP	3-6	124.1	0.924	378.2
TH7	4-6	113.2	0.940	264.1
TH9	2-6	55.1	0.910	264.5
FR	3-5	88.7	0.998	243.1

751

752

753 **Figures**

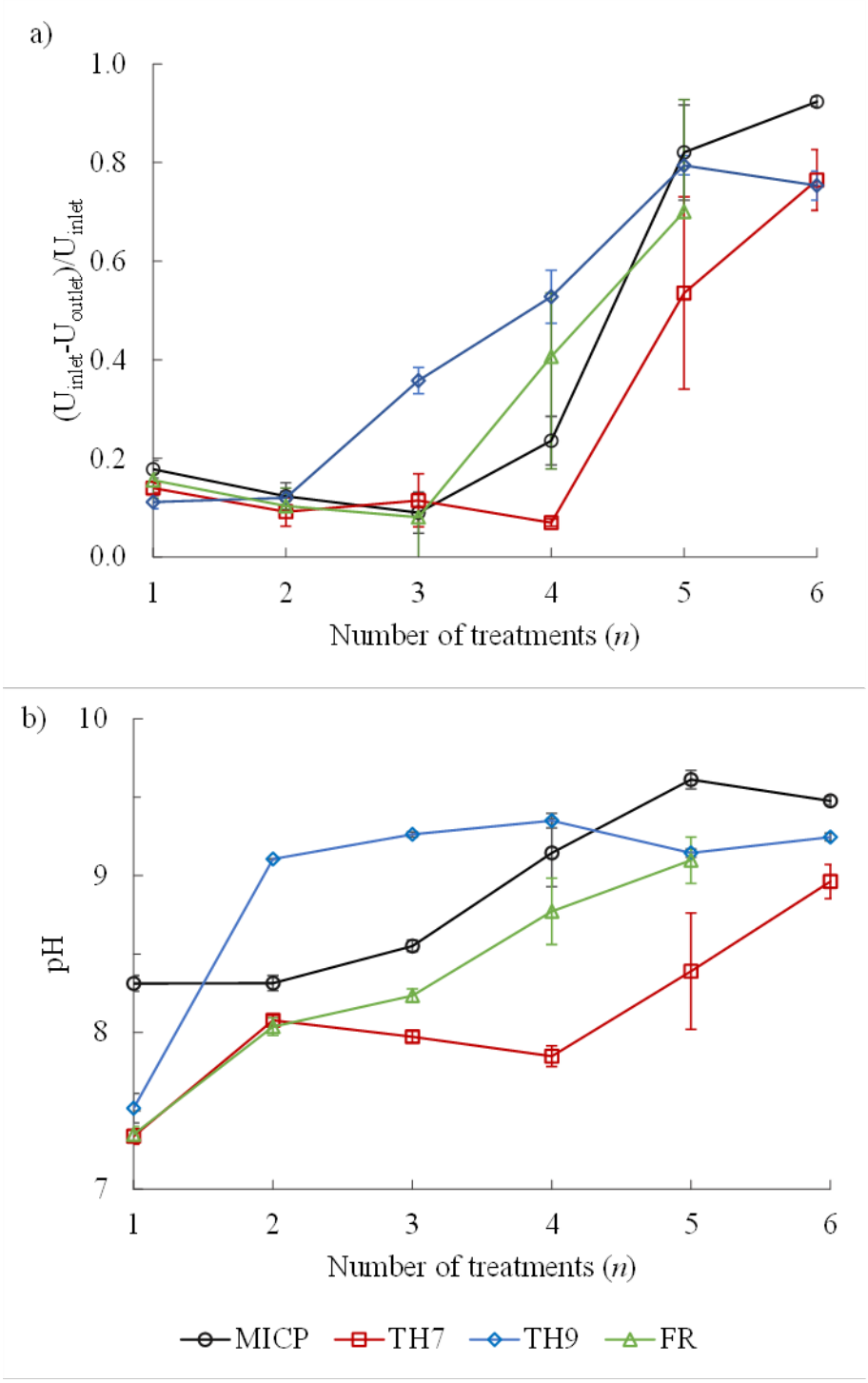
754



755

756 ***Figure 1 Urea stability in fresh urine and thermally-treated urine stored at room***
757 ***temperature over a 28-d period. Urea (solid line) is related to the left y-axis and pH***
758 ***(dashed line) to the right y-axis. For fresh urine, markers and vertical error bars for***
759 ***urea and pH indicate the computed average and standard deviation of three replicate***
760 ***samples and triplicate measurements, respectively. For thermally-treated urine,***
761 ***markers and error bars are computed average and standard deviation values of three***
762 ***replicate samples obtained from three different bottles.***

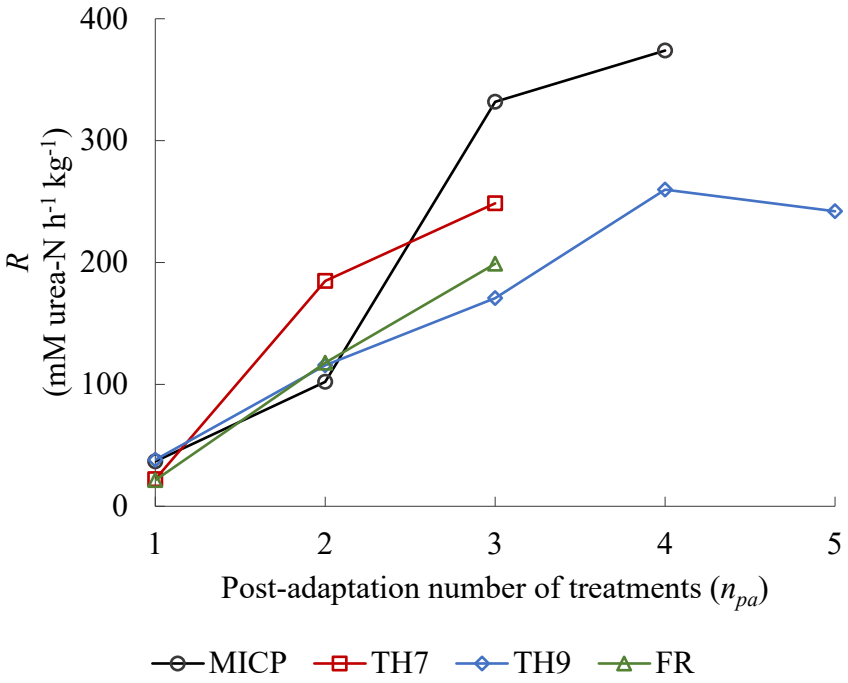
763



764

765 ***Figure 2 Biostimulation of urea hydrolysis in calcareous silty sand of Holocene age.***
766 ***Fraction of urea hydrolysed per treatment (a) and soil leachate pH (b) with number of***
767 ***treatments (n). Treatments comprise the traditional MICP solution (MICP) and***
768 ***variations of cow urine, including thermally treated urine at adjusted initial solution***
769 ***pH of 7 (TH7) and 9 (TH9) and fresh urine (FR). Urea and pH determined in soil***
770 ***leachates. Markers and error bars indicate average and standard deviation results from***
771 ***one composite sample from three replicate soil columns for each solution treatment.***

772



773

774 ***Figure 3 Post-adaptation urea hydrolysis rates with increasing number of treatments***
775 ***(n). Markers indicate average results from one composite sample from three replicate***
776 ***soil columns for each solution treatment. (traditional MICP solution, MICP; thermally***
777 ***treated urine at pH = 7, TH7, and pH = 9, TH9, and; fresh urine, FR)***

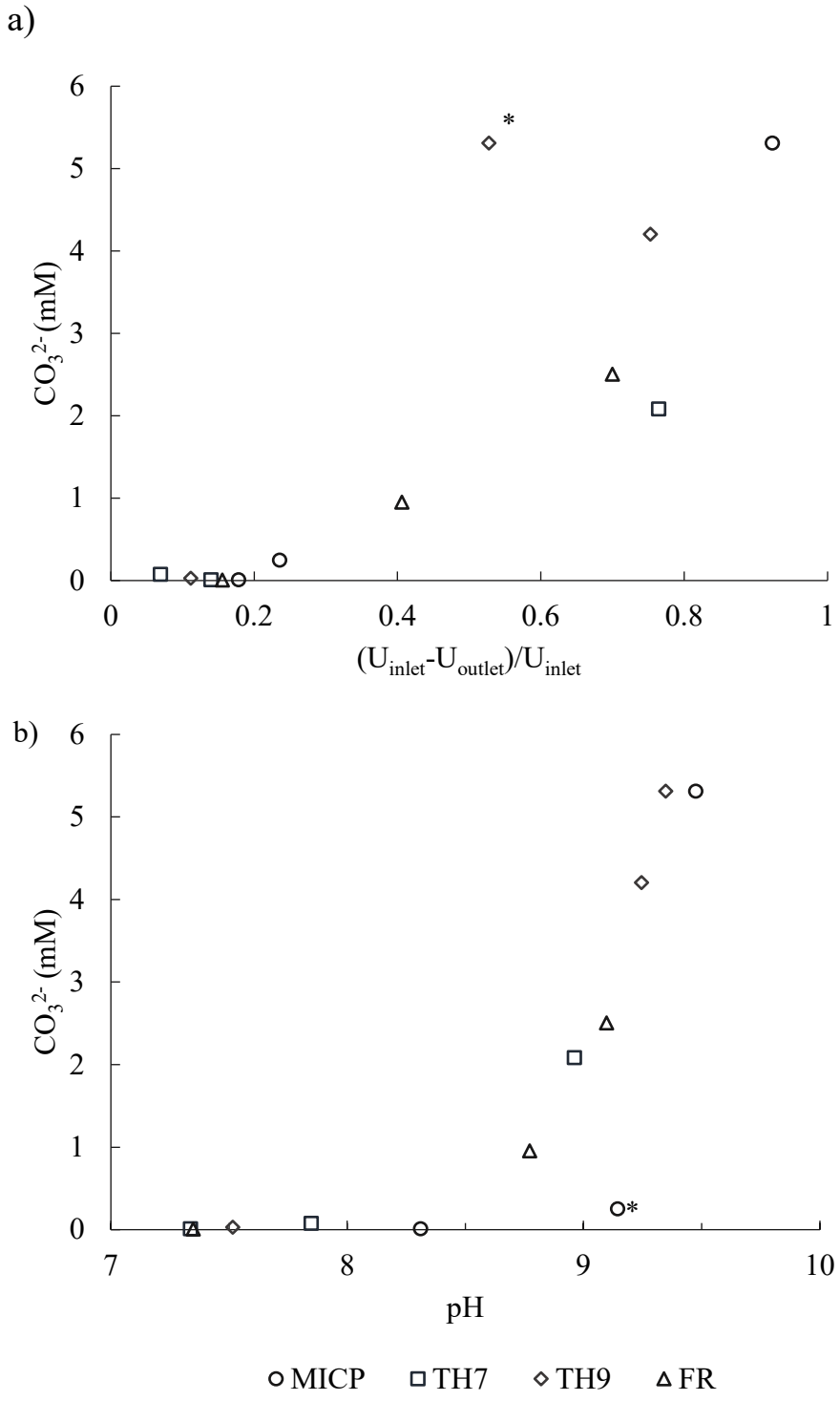
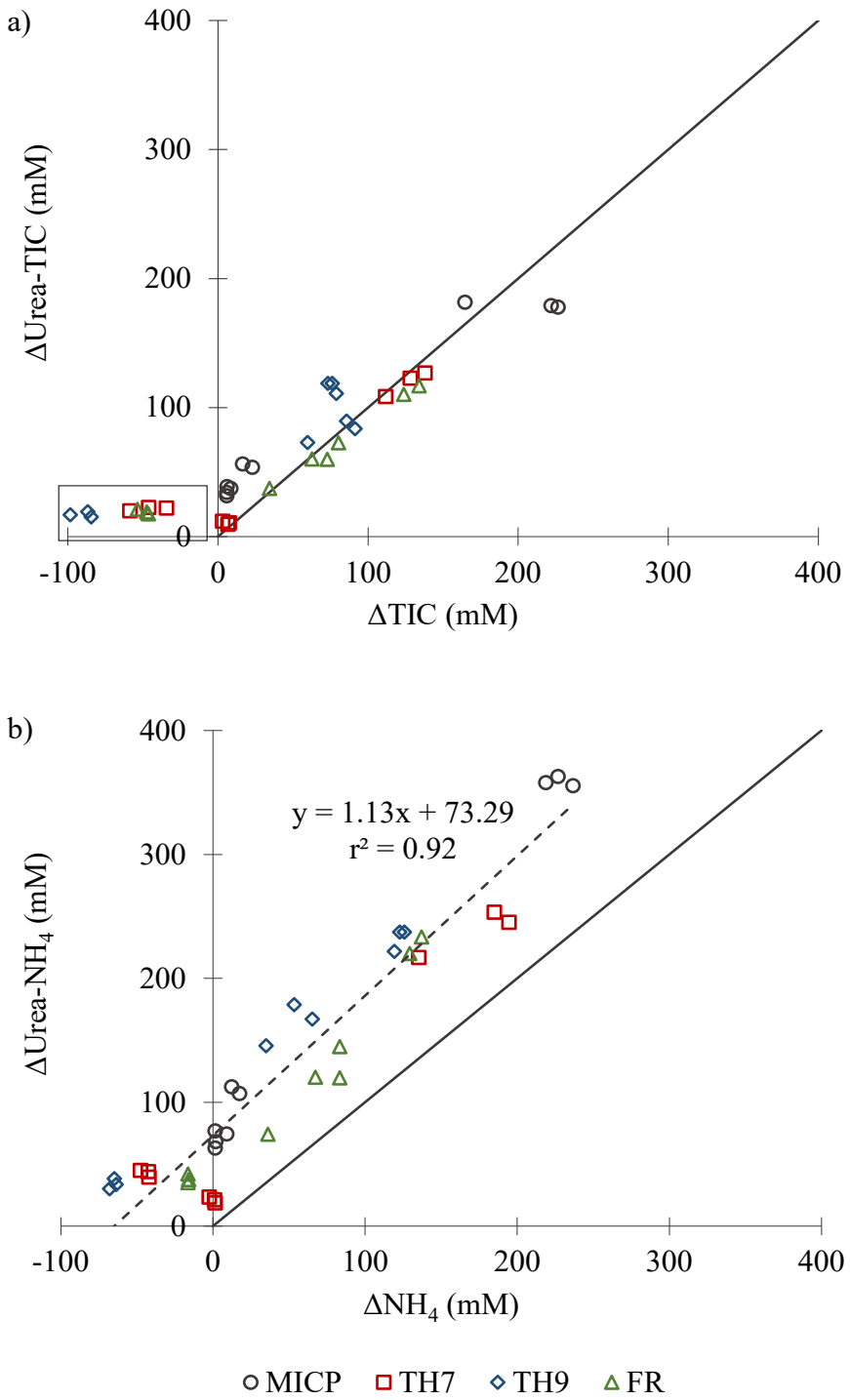


Figure 4 Estimated carbonate ion concentration in solution (mM) as a function of (a) the fraction of urea hydrolysed per treatment and (b) pH determined in soil leachates for soils treated with the traditional MICP solution (MICP), thermally-treated urine at adjusted pH of 9 (TH9) and 7 (TH7) and fresh urine (FR). The analysed soil leachates were obtained after the first, fourth and last cementation treatments. Carbonate content

784 *in solution was evaluated from soil leachate DIC and pH measurements using Eq. 1*
785 *and 2.*

786



787

788 ***Figure 5 Variation in soil leachate TIC (a) and NH_4 (b) (mM) computed from the***
789 ***measured variation in urea content in solution plotted against the determined variation***
790 ***in solution of TIC and NH_4. The variation of the respective analyte was computed as***

the initial minus the final concentration. Straight lines with a 1:1 molar relation indicate the theoretical variation of the analyte produced by the urea hydrolysis reaction. In (b), the linear regression includes all the dataset. Markers indicate average results from one composite sample from three replicate soil columns for each solution treatment (traditional MICP solution, MICP; thermally treated urine at pH = 7, TH7, and pH = 9, TH9, and; fresh urine, FR).

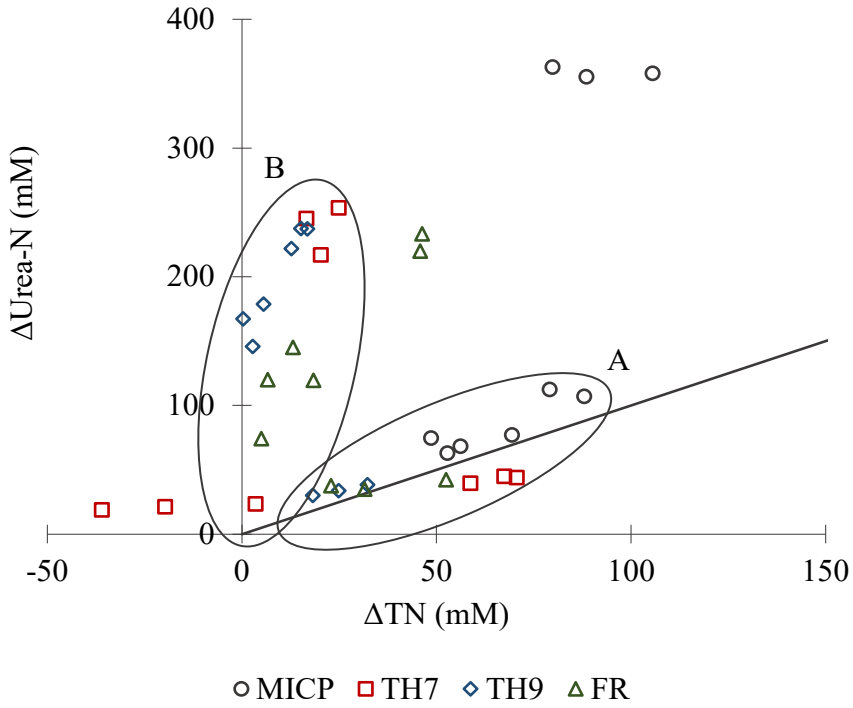


Figure 6 Urea-N hydrolysed plotted against the variation of total nitrogen (TN) in soil leachates. Markers indicate average results from one composite sample from three replicate soil columns for each solution treatment (traditional MICP solution, MICP; thermally treated urine at pH = 7, TH7, and pH = 9, TH9; and fresh urine, FR).