

*Full Length Research Paper*

## **Greenhouse gas emissions from an alkaline saline soil amended with urea: A laboratory study**

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**Soil of the former lake Texcoco is nitrogen (N) depleted, so any attempt to vegetate the area will require the application of an N fertilizer. Urea is commonly used as fertilizer, but its application to soil might affect emissions of greenhouse gases (GHG), such as carbon dioxide (CO<sub>2</sub>), nitrous oxide (N<sub>2</sub>O) and methane (CH<sub>4</sub>), and the high pH and electrolytic conductivity (EC) in the Texcoco soil might inhibit the hydrolysis of urea. Four soils of the former lake bed with EC 3.3, 88.3, 96.9, and 121 dS m<sup>-1</sup>, were amended with urea while dynamics of mineral N and emissions of GHG were monitored. Urea increased emission of CO<sub>2</sub> in all soils and emission of N<sub>2</sub>O in soil with EC ≤ 88.3 dS m<sup>-1</sup>, but emission of CH<sub>4</sub> was not affected. Hydrolysis of urea occurred in all soils although it was significantly lower in soil with EC ≥ 88.3 dS m<sup>-1</sup>. Oxidation of NH<sub>4</sub><sup>+</sup> occurred in soil with EC ≤ 96.9 dS m<sup>-1</sup>, but oxidation of NO<sub>2</sub><sup>-</sup> only in soil with EC 3.3 dS m<sup>-1</sup>. It was found that oxidation of NH<sub>4</sub><sup>+</sup> and NO<sub>2</sub><sup>-</sup>, and hydrolysis of urea was inhibited by the high EC in soil of the former lake bed, while emissions of CO<sub>2</sub> and N<sub>2</sub>O, but not CH<sub>4</sub> were affected by application of urea.**

**Key words:** Carbon dioxide (CO<sub>2</sub>), nitrous oxide (N<sub>2</sub>O) and methane (CH<sub>4</sub>), former lake bed, mineral N in soil.

### **INTRODUCTION**

The former lake of Texcoco located in the valley of Mexico City (Mexico) at an altitude of 2240 m above sea level with a mean annual temperature of 16°C and annual precipitation of 705 mm was drained from the 17th century onwards to avoid flooding in Mexico City (O'Hara and Metcalfe, 1997). The drainage of the lake left a soil with a high Ph and salinity and little vegetation

(Dendooven et al., 2010). During the dry season, the wind erosion was high. However, during the rainy season, flooding occurred frequently. The groundwater, which is highly alkaline and saline, was just under the soil surface and after heavy rainfall the area flooded. The national water authority (Commission Nacional de Agua, CNA) installed drainage pipes so that the area could be

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vegetated to stop wind erosion and dust storms in Mexico City (Luna-Guido et al., 2000). However, previous research showed that the soil of the former lake Texcoco is N depleted (Conde et al., 2005). It can be speculated that the high pH and salinity inhibits  $N_2$  fixing microorganisms thereby limiting the amount of mineral N that enters the soil (Barua et al., 2011; Welsh et al., 2007).

Urea is cheap and often applied to fertilize crops. It could easily be applied to grass, shrubs and trees that might be used to vegetate the former lake bed. Hydrolysis of urea can occur in three ways, biotic (Burton and Prosser, 2001), abiotic in soil with a high pH (Ghandi and Paliwal, 1976) and abiotic through extracellular ureases (Conrad, 1996) generating two  $NH_3$  molecules. As such, concentration of  $NH_4^+$  will increase in urea-amended soil (Burton and Prosser, 2001). However, if most of the process is biological then the high pH and salinity might inhibit the release of  $NH_4^+$ . Additionally, application of urea is known to increase emissions of nitrous oxide ( $N_2O$ ) and might affect emissions of carbon dioxide ( $CO_2$ ) and methane ( $CH_4$ ) (Wang et al., 2011). Hydrolysis of urea will generate ammonium ions that can be oxidized by nitrifiers, first to  $NO_2^-$  and subsequently to  $NO_3^-$ . However, high electrolytic conductivity (EC) is known to inhibit the activity of nitrifiers so emissions of  $N_2O$  after application of urea might not increase (Zhu et al., 2011). Therefore, four soils of the former lake Texcoco with different pH and EC were amended with urea, with or without acetylene ( $C_2H_2$ ), known to inhibit nitrification (Bateman and Baggs, 2005), while emissions of  $CO_2$ ,  $CH_4$  and  $N_2O$  were monitored in an aerobic incubation. Acetylene was applied to half of the soil samples so that the importance of the nitrification process in the emissions of  $N_2O$  could be determined. The objective of this study was to investigate the effect of high alkalinity and salinity on hydrolysis of urea, emissions of GHG and dynamics of mineral N (ammonium ( $NH_4^+$ ), nitrite ( $NO_2^-$ ) or nitrate ( $NO_3^-$ )).

## MATERIALS AND METHODS

### Soil sampling

Details of the soil of the former lake Texcoco can be found in Dendooven et al. (2010) and mineralogy in Gutiérrez-Castorena et al. (2005). Part of the former lake bed has been drained and irrigated with sewage effluent from a waste water plant to remove excess of salt (Luna-Guido et al., 2000). For instance, the concentration of the sodium ions ( $Na^+$ ) decreased from  $21\text{ g kg}^{-1}$  dry soil to  $3\text{ g kg}^{-1}$  dry soil after 8 years of flooding and the chloride ions ( $Cl^-$ ) from  $21\text{ g kg}^{-1}$  dry soil to undetectable amounts (Luna-Guido et al., 2000). The *Distichlis spicata*, an indigenous grass with a high tolerance to salt and  $Na^+$  and tamarix (*Tamarix* species) have been introduced since the early 1970s to control erosion, and they now cover much of the area. More details on the vegetation and the effluents used to drain the plots can be found in Luna-Guido et al. (2000).

At four locations with different EC and pH due to different periods of drainage, five approximately  $400\text{ m}^2$  plots were defined and

sampled by augering the 0 to 15 cm layer 30-times with a stony soil auger with diameter 7 cm (Eijkelkamp, NI) at random on 7 March 2011. The 30 soil samples taken from each site ( $n = 4$ ) and plot ( $n = 5$ ) were pooled, 5 mm sieved and characterized (Table 1). As such, 20 soil samples were obtained. Details of the sampled soils can be found in Table 1. The soil with an EC  $3.3\text{ dS m}^{-1}$  was denoted soil A, with EC  $88.3\text{ dS m}^{-1}$  soil B, with EC  $96.9\text{ dS m}^{-1}$  soil C and with EC  $121\text{ dS m}^{-1}$  soil D. This field based replication was maintained in the incubation study.

### Treatments and experimental set-up

The experimental design was a completely randomized  $2 \times 4$  factorial with five replications (maintained from the field site replications for each soil). The factors were four soil types and four soil amendments which were: 1)  $200\text{ mg N kg}^{-1}$  applied as urea; 2)  $200\text{ mg N kg}^{-1}$  applied as urea plus acetylene ( $C_2H_2$ ) at 0.1%; 3) unamended soil; and 4) unamended soil plus  $C_2H_2$  at 0.1%.

Sixteen sub-samples of 10 g dry soil from each of the four soils and five sampled plots were added to 120 ml flasks. Eight soil samples were adjusted to 40% water holding capacity (WHC) by adding distilled water and eight by adding an urea solution. The flasks were air-tight sealed with a Suba-seal. Four of the flasks amended with distilled water and four with the urea solution were injected with 0.1 ml acetylene ( $C_2H_2$ ) to inhibit nitrification (Bateman and Baggs, 2005). Additionally, 15 flasks without soil were air-tight sealed and incubated in the same way to determine the concentration of  $CO_2$ ,  $N_2O$  and  $CH_4$  in the atmosphere. The flasks were incubated in the dark at  $25 \pm 2^\circ\text{C}$ . After 0, 1, 3 and 7 days, one flask was selected from each soil and treatment at random and the headspace was analyzed for  $CO_2$ ,  $CH_4$  and  $N_2O$ . Additionally, three flasks without soil were selected at the same time and the headspace analyzed. The flasks were opened, the soil removed and extracted for mineral N (ammonium ( $NH_4^+$ ), nitrate ( $NO_3^-$ ) and nitrite ( $NO_2^-$ )) with 0.5 M  $K_2SO_4$ .

### Chemical analyses

Details of the techniques used to measure WHC, pH, EC, total N and soil particle size distribution can be found in Ruíz-Valdiviezo et al. (2010). The extracted  $NH_4^+$ ,  $NO_3^-$  and  $NO_2^-$  were measured colourimetrically with a San Plus System-SKALAR automatic analyzer (Breda, The Netherlands).

The headspace of the vials was analyzed for  $CO_2$  and  $N_2O$  on an Agilent Technologies 4890D gas chromatograph fitted with an electron capture detector (ECD) and  $CH_4$  on an Agilent Technologies 4890D gas chromatograph fitted with a flame ionization detector (FID). Details of the columns used, gas flow, and oven, detector and injector temperatures can be found in Ruíz-Valdiviezo et al. (2010). Concentrations of  $CO_2$ ,  $N_2O$  and  $CH_4$  were calculated by comparing peak areas against a standard curve prepared from known concentrations, 10 ppm  $N_2O$  in  $N_2$ , 5 ppm  $CH_4$  in  $N_2$  and 2500, 20000 and 40000 ppm  $CO_2$  in  $N_2$ , every time samples were analysed.

### Statistical analysis

The experimental design was a completely randomized  $2 \times 4$  factorial with five replications. The factors were four soil types with different EC and four soil treatments, that is, unamended soil, urea-amended soil,  $C_2H_2$  applied soil, and urea +  $C_2H_2$ . Emission of  $CH_4$ ,  $N_2O$  and  $CO_2$  was regressed on elapsed time using a linear regression model, which was forced to pass through the origin, but allowed different slopes (production rates) for each treatment. Significant differences for the production of  $CH_4$ ,  $N_2O$  and  $CO_2$

**Table 1.** Some characteristics of the different soils from the former lake Texcoco.

	EC <sup>a</sup> (dS m <sup>-1</sup> )	pH	Organic C	Total N	WHC <sup>b</sup> (g kg <sup>-1</sup> soil)	Clay	Silt	Sand	Textural classification
Soil A	3.3	10.3	21.72	1.22	431	167	47	786	Loamy sand
Soil B	88.3	10.3	9.17	0.88	575	234	60	706	Sandy clay loam
Soil C	96.9	10.3	11.63	0.79	530	174	80	746	Loamy sand
Soil D	121.0	10.5	30.84	1.12	467	147	27	826	Loamy sand

<sup>a</sup>EC : Electrolytic conductivity, <sup>b</sup> WHC : Water holding capacity.

production between treatments, soil and their interactions were determined using PROC MIXED (SAS Institute Inc. 1989).

Concentrations of NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> were subjected to an analysis of variance using PROC GLM (SAS Institute Inc. 1989) to test for significant differences between soils, treatments and their interactions with Tukey's Studentized Range test. All data presented were the mean of five replicates, that is, *n* = 5.

## RESULTS

### Emissions of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O

In the unamended soil, the emission of CO<sub>2</sub> was largest in soil A and lowest in soil D (Figure 1a). Application of C<sub>2</sub>H<sub>2</sub> to the unamended soil had no significant effect on the CO<sub>2</sub> emission rates. The emission of CH<sub>4</sub> was similar in all unamended soils and was not affected by the application of C<sub>2</sub>H<sub>2</sub> (Figure 1b). In the unamended soil, the emission of N<sub>2</sub>O was significantly larger in soil A than in the other soils (*P*<0.05) (Figure 1c). Application of C<sub>2</sub>H<sub>2</sub> had no significant effect on the N<sub>2</sub>O emission rate.

Application of urea increased the emission of CO<sub>2</sub> in soils A, B and D significantly, but not in soil C (*P*<0.05) (Figure 1d). Application of C<sub>2</sub>H<sub>2</sub> to the urea-amended soil decreased the emission of CO<sub>2</sub> significantly in soils A and B, but not in soils C and D (*P*<0.05). The emission of CO<sub>2</sub> was similar in the C<sub>2</sub>H<sub>2</sub>-amended soils applied with or without urea. Application of urea did not affect the emission of CH<sub>4</sub> and was similar in the C<sub>2</sub>H<sub>2</sub>-amended soils applied with or without urea (Figure 1e). Application of urea increased the emission of N<sub>2</sub>O significantly in soil A, but not in the other soils (*P*<0.05) (Figure 1f). Application of C<sub>2</sub>H<sub>2</sub> to the urea-amended soil decreased the emission of N<sub>2</sub>O in soils A and B, but not in soils C and D.

The emission of CO<sub>2</sub> was significantly affected by soil and the interactions between urea × C<sub>2</sub>H<sub>2</sub> and soil × urea × C<sub>2</sub>H<sub>2</sub> (*P*<0.05) (Table 2). The emission of N<sub>2</sub>O was significantly affected by the different interactions between urea, soil and C<sub>2</sub>H<sub>2</sub>, and the emission of CH<sub>4</sub> was affected significantly only by soil (*P*<0.05).

### Dynamics of inorganic N

The concentration of NH<sub>4</sub><sup>+</sup> was similar in the unamended soils and soils applied with C<sub>2</sub>H<sub>2</sub> (Figure 2a). Application

of C<sub>2</sub>H<sub>2</sub> to the unamended soil reduced the concentration of NO<sub>2</sub><sup>-</sup> significantly in soils B and C, but not in soils A and D (*P*<0.05) (Figure 2b). The concentration of NO<sub>3</sub><sup>-</sup> was similar in the unamended soils and soils with applied C<sub>2</sub>H<sub>2</sub> (Figure 2c).

Application of urea increased the concentration of NH<sub>4</sub><sup>+</sup> significantly in all soils and the increase was most accentuated in soil A (*P*<0.05) (Figure 2d). Application of C<sub>2</sub>H<sub>2</sub> to the urea-amended soil decreased the amount of NH<sub>4</sub><sup>+</sup> significantly in soil A, but not in the other soils (*P*<0.05). Application of urea increased the concentration of NO<sub>2</sub><sup>-</sup> significantly in soil A, but not in the other soils (*P*<0.05) (Figure 2e). Application of C<sub>2</sub>H<sub>2</sub> to the urea-amended soil decreased the amount of NO<sub>2</sub><sup>-</sup> significantly in soil A, but not in the other soils (*P*<0.05). Application of urea increased the concentration of NO<sub>3</sub><sup>-</sup> significantly in soil A, but not in the other soils (Figure 2f). Application of C<sub>2</sub>H<sub>2</sub> to the urea-amended soil decreased the amount of NO<sub>3</sub><sup>-</sup> in soil A, but not in the other soils.

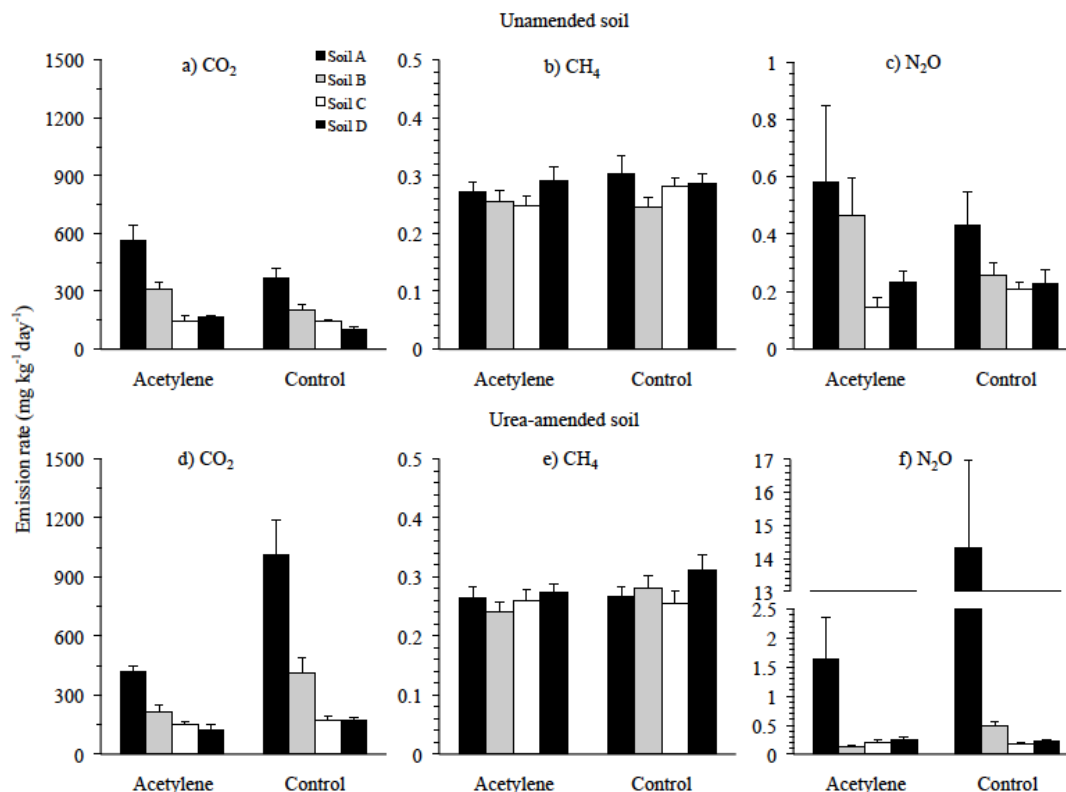
The concentrations of NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> were affected significantly by urea, C<sub>2</sub>H<sub>2</sub>, soil and their interactions, except for the effect of urea and its interaction with C<sub>2</sub>H<sub>2</sub> on the concentration of NO<sub>3</sub><sup>-</sup> (Table 2).

## DISCUSSION

### Emissions of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O

The emission of CO<sub>2</sub> decreased with increased EC in the soils. It is well known that increased salinity reduces the soil microbial biomass and inhibits microbial activity (Setia et al., 2011a, b). However, it has to be remembered that other characteristics, such as soil organic matter content, clay content and pH, are also known to affect microbial activity and thus emissions of CO<sub>2</sub> (Setia et al., 2011a, b).

The application of urea increased the CO<sub>2</sub> emission rate significantly in soils A, B and D compared to the unamended soil. It is well known that application of an N fertilizer to an N depleted soil can increase emission of CO<sub>2</sub> as microbial activity is stimulated (Wang et al., 2011). The high salt content in the Texcoco soils will inhibit N<sub>2</sub> fixation, which will limit the N content of the soil (Barua et al., 2011; Welsh et al., 2007). Additionally,



**Figure 1.** (a) Emission of CO<sub>2</sub>, (b) CH<sub>4</sub> and (c) N<sub>2</sub>O (mg kg<sup>-1</sup> soil day<sup>-1</sup>) from the unamended Texcoco soil and soil amended with urea (d), (e) and (f). Bars are ± one standard deviation.

hydrolysis of urea will release CO<sub>2</sub> (Snyder et al., 2009). The emission of CO<sub>2</sub> will thus increase with 86 mg CO<sub>2</sub> after the application of 200 mg N kg<sup>-1</sup> soil if all urea was hydrolyzed.

The application of C<sub>2</sub>H<sub>2</sub> had no effect on emission of CO<sub>2</sub>. Acetylene can be used by certain organisms, e.g. *Rhodococcus opacus*, *Rhodococcus ruber* and *Gordona* species, as C substrate thereby increasing emission of CO<sub>2</sub> (Rosner et al., 1997). Soil characteristics are known to affect C<sub>2</sub>H<sub>2</sub> degradation (Brzezinska et al., 2011), although the limited time that the soil microorganisms were exposed to C<sub>2</sub>H<sub>2</sub> (7 days) might have reduced the possibility that they use C<sub>2</sub>H<sub>2</sub> as C source, that is, they were not yet adapted.

Agricultural soils can be a source or a sink for CH<sub>4</sub>, but they are normally a sink and fluxes are normally small (Wang et al., 2011). Large amounts of CH<sub>4</sub> are only emitted from paddy soils or wetlands (Wright et al., 2011). Production of CH<sub>4</sub> occurs under anaerobic and oxidation under aerobic conditions. Although, the soils were incubated aerobically, emission of CH<sub>4</sub> occurred in all soils. Anaerobic micro-sites exist even in a soil at 40% WHC that will stimulate production of CH<sub>4</sub>, and oxidation of CH<sub>4</sub> did not match production. The high salt content might have inhibited methanotrophic activity.

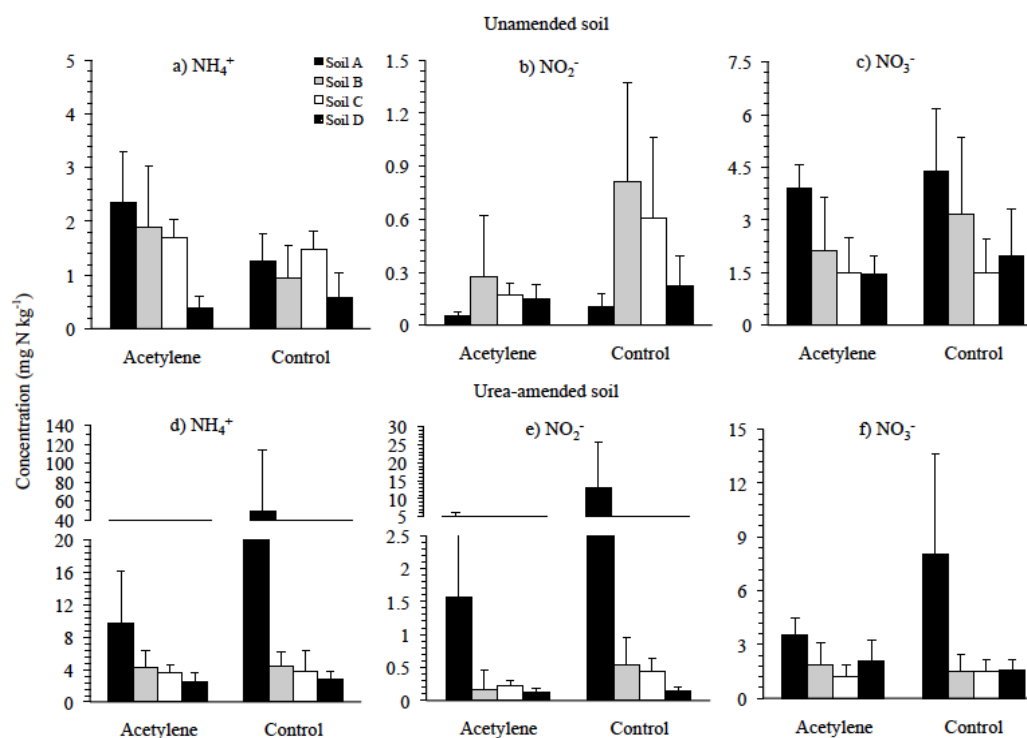
Application of urea or C<sub>2</sub>H<sub>2</sub> did not affect emissions of

CH<sub>4</sub>. Application of NH<sub>4</sub><sup>+</sup>, released after the hydrolysis of urea, is known to inhibit oxidation of CH<sub>4</sub>, but not in the Texcoco soils (Stiehl-Braun et al., 2011; Xu et al., 2011). Aronson and Helliker (2010) reported after a meta-analysis that not only the amount of N applied, but also the history of the soil affected the inhibitory effect. They reported that managed soil and soil with a longer duration of fertilizer application showed greater inhibition of CH<sub>4</sub> uptake with added N. The Texcoco soil was not fertilized and is N depleted so it can be assumed that N fertilizer would not inhibit CH<sub>4</sub> oxidation. Bronson and Mosier (1994) reported a strong inhibitory effect of C<sub>2</sub>H<sub>4</sub> on oxidation of CH<sub>4</sub> (76 to 100% inhibition) in two soils. No such inhibitory effect was found in the Texcoco soil, so it can be speculated that little or no CH<sub>4</sub> oxidation occurred as stated before.

Application of urea increased emission of N<sub>2</sub>O in soil A compared to the unamended soil, but not in the other soils. It is well known that application of urea to soil increases emission of N<sub>2</sub>O (Wang et al., 2011). Emission of N<sub>2</sub>O from soil is mainly due to nitrification, that is, the oxidation of NH<sub>4</sub><sup>+</sup> to NO<sub>2</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> to NO<sub>3</sub><sup>-</sup> under aerobic conditions and denitrification, that is, the reduction of NO<sub>3</sub><sup>-</sup> to NO<sub>2</sub><sup>-</sup>, N<sub>2</sub>O and N<sub>2</sub> under anaerobic conditions (Wrage et al., 2001). As the soil was incubated under aerobic conditions and the concentration of NH<sub>4</sub><sup>+</sup> sharply

**Table 2.** Effect of urea, acetylene, soil and their interaction on the emissions of CO<sub>2</sub>, CH<sub>4</sub> (mg C kg<sup>-1</sup> day<sup>-1</sup>), and N<sub>2</sub>O (mg N kg<sup>-1</sup> day<sup>-1</sup>), and concentrations of mineral N (NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup> or NO<sub>3</sub><sup>-</sup>) (mg N kg<sup>-1</sup> dry soil).

Variable	Concentration of						Emission of					
	NH <sub>4</sub> <sup>+</sup>		NO <sub>2</sub> <sup>-</sup>		NO <sub>3</sub> <sup>-</sup>		CO <sub>2</sub>		N <sub>2</sub> O		CH <sub>4</sub>	
	F value	P value	F value	P value	F value	P value	F value	P value	F value	P value	F value	P value
Urea	14.97	0.0001	14.87	0.0002	1.19	0.2769	0.01	0.9091	1.83	0.1779	0.01	0.9258
Acetylene (C <sub>2</sub> H <sub>2</sub> )	4.77	0.0302	13.36	0.0003	13.13	0.0004	0.32	0.5715	1.92	0.1669	0.06	0.8085
Soil	9.49	<0.0001	12.96	<0.0001	42.27	<0.0001	16.13	<0.0001	1.96	0.1217	5.12	0.0020
Urea C <sub>2</sub> H <sub>2</sub>	4.88	0.0283	10.77	0.0012	1.81	0.1807	21.26	<0.0001	5.46	0.0205	0.35	0.5526
Urea Soil	7.82	<0.0001	15.83	<0.0001	4.79	0.0031	2.02	0.1138	6.39	0.0004	0.18	0.9096
Soil C <sub>2</sub> H <sub>2</sub>	4.43	0.0049	8.01	<0.0001	5.14	0.0019	1.35	0.2616	3.87	0.0101	0.07	0.9780
Urea Soil C <sub>2</sub> H <sub>2</sub>	4.51	0.0044	9.12	<0.0001	5.78	0.0008	4.91	0.0029	4.51	0.0044	0.22	0.8816



**Figure 2.** (a) Concentration of NH<sub>4</sub><sup>+</sup>, (b) NO<sub>2</sub><sup>-</sup> and (c) NO<sub>3</sub><sup>-</sup> (mg N kg<sup>-1</sup> soil) in the unamended Texcoco soil and soil amended with urea (d), (e) and (f). Bars are ± one standard deviation.

increased after application of urea and its subsequent hydrolysis, emission of  $N_2O$  was most likely due to oxidation of  $NH_4^+$ . Application of  $C_2H_2$  (as an inhibitor of the oxidation of  $NH_4^+$ ) to soil A sharply reduced the emission of  $N_2O$  confirming that oxidation of  $NH_4^+$  was the main source of  $N_2O$  emission. The emission of  $N_2O$  in soil A amended with urea plus  $C_2H_2$ , however, was still higher than in the unamended control soil. As such, although the soil was incubated aerobically, it is likely that some anaerobic microsites were formed in soil stimulating denitrification and thus emission of  $N_2O$ .

Emission of  $N_2O$  also increased when urea was added to soil B, and  $C_2H_2$  decreased it. As such, nitrification contributed to the emission of  $N_2O$  in soil B. No increase in emission of  $N_2O$  occurred in soils C and D amended with urea. As such, the high salt content inhibited the nitrification process in soils C and D. Application of urea might be used as N fertilizer for a pioneering vegetation to minimise  $N_2O$  emission in the alkaline saline Texcoco soil. However, it would have to be injected into the soil as the high pH will favour  $NH_3$  volatilization.

The soils were incubated at a constant water content in this experiment. In the field, water content will fluctuate continuously thereby changing soil conditions constantly. These constantly changing conditions will put a further strain on the microbial population. The soil microorganisms will have to adapt strategies to survive a dried out or flooded environment, to altering anaerobic and aerobic conditions, and salt concentrations that decrease in the rainy season but increase in the dry season when evaporation will concentrate the salt ions mostly in the upper soil layer.

### Dynamics of mineral N

Concentration of  $NH_4^+$  increased in all urea-amended soils. Hydrolysis of urea can occur in three ways, biotic (Burton and Prosser, 2001), abiotic in soil with a high pH (Ghandi and Paliwal, 1976) and abiotic through extracellular ureases (Conrad, 1996) generating two  $NH_3$  molecules. As such, concentration of  $NH_4^+$  will increase in urea-amended soil (Burton and Prosser, 2001) as found in this study. The increase in the concentration of  $NH_4^+$  was lower in soil with  $EC \geq 88.3 \text{ dS m}^{-1}$  than in soil with  $EC 3.3 \text{ dS m}^{-1}$ , so the high EC had an inhibitory effect on the hydrolysis of urea (Wilson et al., 1999).

Application of  $C_2H_2$  reduced the concentration of  $NO_2^-$  in the unamended or urea-amended soils A, B and C, but not in soil D. Consequently, oxidation of  $NH_4^+$  occurred in soil with  $EC \leq 96.9 \text{ dS m}^{-1}$ . Ammonium oxidizing organisms have been found in extreme environments (Sorokin and Kuenen, 2005) so it would come as no surprise that oxidation of  $NH_4^+$  occurred in soil with  $EC \leq 96.9 \text{ dS m}^{-1}$ . Oxidation of  $NO_2^-$  only occurred in the soil with the lowest EC. Although  $NO_2^-$  oxidizing bacteria have been isolated from alkaline environments (Sorokin

et al., 1998), it might well be that the extreme high EC inhibited  $NO_2^-$  oxidation as the energetic gain from this process is low (Oren, 2011).

Under the experimental conditions,  $NO_2^-$  oxidation seems to be absent in soils with  $EC \geq 88.3 \text{ dS m}^{-1}$ . However,  $NH_4^+$  oxidation occurred even at  $96.9 \text{ dS m}^{-1}$  suggesting that  $NH_4^+$  oxidation is less sensitive to salinity than  $NO_2^-$  oxidation. From a biological point of view, there are several possible explanations for this phenomenon. First, aerobic ammonium oxidation yields more energy for growth than nitrite oxidation (Bock and Wagner, 2006). Second, aerobic  $NH_4^+$  oxidation is done not only by a restricted group of Bacteria, but also by Archaea belonging to the phylum Thaumarchaeota (Leininger et al., 2006). These Archaea have a different physiology than  $NH_4^+$  oxidizing Bacteria. In some environments, these Archaea can even be the major  $NH_4^+$  oxidizers (Prosser and Nicol, 2008). Furthermore, even when aerobic  $NH_4^+$  and  $NO_2^-$  oxidizers form tight associations, that is, the  $NO_2^-$  produced by  $NH_4^+$  oxidizers is consumed by  $NO_2^-$  oxidizers, there seems to be ecophysiological differences between both groups that might be in part the consequence of the evolution of their metabolic life styles. Nitrite oxidizers can be heterotrophic/mixotrophic or strict chemotrophs (Bock and Wagner, 2006), and even between them there are differences since *Nitrospira* species can be K-strategists with high substrate affinity and low growth rate, while *Nitrobacter* species might be r-strategists (Schramm et al., 1999). However, more studies need to be done as new nitrite oxidizers groups are emerging and their physiological must still be studied.

In this study, the microbial population was not investigated. It would be interesting to study the microbial population in each of the treatments and investigate which organisms were involved in each of the processes discussed, e.g. nitrifiers, methanogens and methanotrophs. A transcriptomics analysis would surely reveal genes that are relevant in these extreme environments, but absent in more normal soil conditions.

It was found that urea increased emission of  $CO_2$  in all soils and emission of  $N_2O$  in soil with  $EC \leq 88.3 \text{ dS m}^{-1}$ , but emission of  $CH_4$  was not affected. Hydrolysis of urea occurred in all soils although it was significantly slower in soil with  $EC \geq 88.3 \text{ dS m}^{-1}$ . Oxidation of  $NH_4^+$  occurred in soil with  $EC \leq 96.9 \text{ dS m}^{-1}$ , but oxidation of  $NO_2^-$  only in soil with  $EC 3.3 \text{ dS m}^{-1}$ .

### CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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