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Inorganic N dynamics and N₂O production from tannery effluents irrigated soil under different water regimes and fertilizer application rates: A laboratory study

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ABSTRACT

Effluents from leather processing, a major industry that produces up to 64 kt waste water year⁻¹ in the town of León (Guanajuato, Mexico), are normally discharged to the river Turbio without treatment and used downstream to irrigate agricultural land. Tannery waste water contains valuable nutrients, but also contaminants, such as salts and chromium (Cr) that might affect soil processes and crop production. We investigated how almost 25 years of irrigation of agricultural land with water from the river Turbio affected dynamics of carbon (C) and nitrogen (N) and production of nitrous oxide (N₂O) under different water regimes and fertilizer application rates. Soil sampled from three adjacent fields irrigated with water from the river Turbio (soil A river irrigated) and at a distance of 10 km from the irrigation canals irrigated with non-contaminated well water (soil B well irrigated), was characterized while dynamics of C and N and production of N₂O were measured in an aerobic incubation experiment. Production of CO₂ was 1.33 times larger in soil A than in soil B and addition of 200 mg kg⁻¹ ammonium (NH₄⁺) increased its production 1.16-fold in soil B, but not in soil A. Concentrations of nitrite (NO₂⁻) increased with increasing water content and application of 200 mg NH₄⁺-N kg⁻¹ soil. The soil, the addition of NH₄⁺-N and the water content of the soil all had a significant effect on the N₂O emission. Production of N₂O was >40 times larger in soil incubated at 100% WHC compared to soil incubated at 40% WHC after 1 day. Emission of N₂O was 6.0 times larger from soil A than from soil B. It increased 25.7 times in soil B amended with 200 mg NH₄⁺-N kg⁻¹ soil compared to the unamended soil, but only 1.8 times in soil A after 1 day. It was found that respiration and nitrification was not affected by irrigation with water from the river Turbio, but production of N₂O was larger in soil irrigated with water from the river Turbio than in soil irrigated with well water.

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1. Introduction

Effluents from leather processing, a major industry that produces up to 64 kt waste water year⁻¹ in the town of León (Guanajuato), are normally discharged to the river Turbio without treatment (Robles and Armienta, 2000). The water of the river Turbio is used downstream from León to irrigate up to 5000 ha agricultural land. The water contains large concentrations of nutrients, as tannery effluents are rich in inorganic nitrogen (N) and N-rich organic residues (INE-DGMRAR, 1999). This irrigation water often provides the only nutrients for the cultivated crops. Apart from nutrients and the organic material, that releases valuable nutrients on decomposition, tannery effluent can contain large amounts of chromium (Cr), pathogens and toxic organic components; all of which pose serious threats to the environment (e.g. Sinha et al., 2002). One particular threat is to the microbial functioning of the soil. Our general knowledge of the way the microbial biomass behaves in the presence of pollutants has increased steadily over the last 15 years or so (e.g. Brookes, 1993), but mainly in agricultural soils of the temperate regions. Less is known of its behaviour in arid and semi-arid regions.

Irrigation with water from the river Turbio for over 25 years has significantly increased the electrolytic conductivity from 0.64 to 2.29 dS m⁻¹, organic C and total N content two times, total concentration of Cr four times, copper (Cu) two times and sodium (Na) six times in the clayey soils (Alvarez-Bernal et al., 2006). Microbial biomass was two times larger in soil irrigated with water from the river Turbio than in soil irrigated with well water, while the activity of proteases and hydrolases releasing ninhydrin positive compounds and organic C appeared not to be affected. The concentration of nitrite (NO₂⁻) was 1.71 mg N kg⁻¹ in soil irrigated with water from the river Turbio and approximately twice larger than in soil irrigated with well water. Although there appeared to be no adverse impact on soil microbial biomass, oxidation of NO₂⁻ appeared to be inhibited indicating that the biological functioning of the soil, i.e. nitrification, might be affected. Many substances are known to inhibit nitrification and waste water often contains different kinds of nitrifier inhibitors (e.g. Sverdrup et al., 2002). Chlorate used in tanning is known to inhibit nitrification (Hauck, 1980) and salts and heavy metals all found in tannery effluent might affect nitrification (Cela and Sumner, 2002).

Nitrous oxide (N₂O) has as a greenhouse gas a potential approximately 320 times greater than that of CO₂ (Bouwman, 1990) and is mainly produced through nitrification and denitrification (Wrage et al., 2001). Nitrification appeared to be affected in soil irrigated with tannery waste water for extended periods (Alvarez-Bernal et al., 2006) so the question remained if emission of N₂O was also affected. We investigated how irrigation with water from the river Turbio over an extended period had affected dynamics of carbon (C) and nitrogen (N) and production of N₂O from soil under different water regimes, i.e. 40%, 60%, 80% and 100% of water holding capacity (WHC), and amended with or without 200 mg NH₄⁺-N kg⁻¹ soil. Soil was sampled and characterized from three adjacent fields irrigated with tannery effluent and from three fields irrigated with well water. The dynamics of C and N and production of N₂O in these soils were measured in an aerobic incubation experiment in the laboratory.

2. Materials and methods

2.1. Waste water from tannery industry used to irrigate agricultural fields

Tanning transforms animal skin to leather. The skin is submitted to different processes to eliminate meat, fat and hair in which different chemicals such as sodium hydroxide, sodium hypochlorite, enzymes, lime, chlorides, sulphuric acid, formic acid, NH₄⁺ salts, kerosene, monochlorobenzene and tenso-active reagents, etc. are used. The obtained hide is then treated with Cr³⁺ or tannins, mineral salts and colours to obtain leather. As such, the effluent generated by tannery industry contains large concentrations of sulphates, chlorides and Cr³⁺. The effluents are then discharged into the river Turbio. Water of the river is used downstream of León to irrigate approximately 5000 ha of agricultural land.

2.2. Soil sampling

The experimental sites were located near León (latitude: 21°07'; longitude: 101°41') in the state of Guanajuato (Mexico). Details can be found in Alvarez-Bernal et al. (2006). Its average altitude is 1800 m above sea level and is characterized by a semi-arid and semi-hot climate with a mean annual temperature of 18–20 °C and average annual precipitation of 600–700 mm (mainly from June through August).

Three adjacent agricultural fields were selected at two different sites. Soils were clayey at both sites and classified as Phaeozem (FAO, 1988). The first experimental site (soil A) was located in San Pedro and regularly irrigated with water from the Turbio river (Tables 1 and 2). Irrigation occurs through regular flooding and is dependent on weather conditions and the type of crop. At the time of sampling the crop was alfalfa. The second experimental site (considered soil B) was located in Plan de Ayala (Santa Rosa) at about 10 km from San Pedro. Irrigation of the fields only occurs with well water and when sampled the soil was cultivated with alfalfa. Site B was selected at 10 km from site A, a distance far enough so as to be sure that no water from the river Turbio was used to irrigate fields at site B.

Topsoil (0–20 cm) was sampled by augering 20 times three approximately 400 m² adjacent plots at the two different sites with a stony soil auger (diameter 7 cm, Eijkelkamp, NL) in February 2004. Soil from each plot was pooled, and all stones, visible roots and fauna were removed. As such, six different soil samples were obtained, i.e. from two sites: one site irrigated with water from the river and one site irrigated with well water, and that in triplicate. The soil was then characterized (Table 3).

2.3. Experimental set-up and treatments

Soil samples, approximately 20 kg from each field (three from site A and three from site B), were taken to the laboratory and treated as follows. Soil from each field was sieved separately (5 mm) and 240 sub-samples of 20 g soil were added to 200 ml glass flasks. Soil in 30 glass flasks was adjusted to 40% water holding capacity (WHC) by adding distilled H₂O (3.6 ml to soil A and 2.8 ml to soil B), 30–60% WHC (6.6 ml to soil A and 5.7 ml to soil B), 30–80% WHC (9.6 ml to soil A and 8.7 ml to soil B) and

Table 1 – Characteristics of soils sampled near León (Guanajuato, Mexico)

| Soil ^a | CEC ^b (cmol kg ⁻¹) | EC ^c (dS m ⁻¹) | pH _{H₂O} | WHC ^d | Organic C (g kg ⁻¹ soil) | Inorganic C (g kg ⁻¹ soil) | Total N (g kg ⁻¹ soil) | P (g kg ⁻¹ soil) | Clay (g kg ⁻¹ soil) | Silt (g kg ⁻¹ soil) | Sand (g kg ⁻¹ soil) | Textural classification |
|-----------------------------|--|--|------------------------------|------------------|--|--|--------------------------------------|--------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|----------------------------|
| A | 19.0 | 2.29 | 6.4 | 1030 | 73.5 | 6.1 | 4.6 | 0.043 | 452 | 156 | 392 | Clay |
| B | 18.1 | 0.64 | 6.2 | 1010 | 37.9 | 7.9 | 2.0 | 0.035 | 516 | 196 | 288 | Clay |
| MSD ^e (P < 0.05) | 0.6 | 0.81 | 0.3 | 21 | 30.6 | 8.4 | 0.8 | 0.013 | 52 | 49 | 79 | |

^a Soil A: irrigated with water from river Turbio; soil B: irrigated with well water at 10 km from fields irrigated with water from river Turbio.

^b CEC: cation exchange capacity.

^c EC: electrolytic conductivity.

^d WHC: water holding capacity.

^e MSD: minimum significant difference (P < 0.05).

30–100% WHC (12.6 ml to soil A and 11.5 ml to soil B). Thirty sub-samples of 20 g soil were then amended with the same amounts of distilled H₂O as mentioned before to obtain the same water contents, but also containing 19 mg (NH₄)₂SO₄ so that 200 mg N kg⁻¹ was added to soil. As such, eight treatments were applied to soil from both sites A and B: four different water contents (40%, 60%, 80% or 100% WHC) and two application rates of N (0 or 200 mg N kg⁻¹ soil).

Three flasks were chosen at random and inorganic N was extracted from soil with 100 ml 1 M potassium chloride (KCl), shaken for 60 min and filtered through Whatman[®] No. 42 paper. Concentrations of NH₄⁺, NO₃⁻ and NO₂⁻ were measured. These provided zero time results. The flasks were sealed and stored in the dark for 14 days at 25 ± 1 °C. Although soil temperatures in León might exceed 25 °C, an incubation temperature of 25 ± 1 °C was used so that soils from different regions of Mexico can be compared (e.g. Reyes-Reyes et al., 2003). An additional 12 glass flasks without soil were stoppered and served as controls to account for the CO₂ and N₂O in the atmosphere. After 1, 3, 7 and 14 days, three flasks were selected at random from each field and the headspace was analyzed for N₂O and CO₂. The flasks were opened and soil was extracted for inorganic N with 100 ml 1 M KCl solution as described for zero time samples.

2.4. Chemical analyses

Soil pH was measured in 1:2.5 soil–H₂O suspension using a glass electrode (Thomas, 1996). Total C was determined by oxidation with potassium dichromate (K₂Cr₂O₇) and titration of excess dichromate with ammonium ferrosulphate ((NH₄)₂FeSO₄) (Kalembasa and Jenkinson, 1973), and inorganic C by adding 5 ml 1 M hydrogen chloride (HCl) solution to 1 g air-dried soil and trapping of CO₂ in 1 M NaOH. Total N was measured by the Kjeldhal method using concentrated H₂SO₄, K₂SO₄ and HgO to digest the sample (Bremner, 1996) and soil particle size distribution by the hydrometer method as described by Gee and Bauder (1996). The CO₂ in the 1 M NaOH was determined by titration with 0.1 M HCl (Jenkinson and Powlson, 1976). The NH₄⁺, NO₂⁻ and NO₃⁻ in the 1 M KCl extract were determined colourimetrically on a Skalar automatic analyzer (Bundy and Meisinger, 1994).

The headspace volume of each flask was sampled and analyzed using a Shimadzu gas chromatograph GC-14B fitted with an electron capture detector for the measurement of N₂O and CO₂. A Porapak Q column used to separate N₂O and CO₂ from the other gases with the carrier gas He flowing at a rate of 55 ml min⁻¹ was maintained at 35 °C. The N₂O dissolved in soil water was accounted for as described by Moraghan and Buresh (1977).

One g of soil was digested with concentrated HCl (36.5%) and HNO₃ (70%) in a 1:3 ratio, filtered and analyzed for copper (Cu), chromium (Cr), calcium (Ca), magnesium (Mg), iron (Fe) and sodium (Na). Total concentrations were determined by flame atomic absorption spectrometry (Varian Spectra AA220 fast sequential) while lead (Pb) and cadmium (Cd) were determined by absorption atomic spectrometry fitted with a graphite furnace (Perkin-Elmer, Zeeman 5100, USA). The plastic beakers used for analysis of metals were new and treated with 2% HNO₃ 24 h before use (APHA AWWA WPCF, 1989).

Table 2 – Total concentrations of metals in soils from León

| Soil ^a | Cu (mg kg ⁻¹ soil) | Cd (mg kg ⁻¹ soil) | Cr (mg kg ⁻¹ soil) | Ca (mg kg ⁻¹ soil) | Mg (mg kg ⁻¹ soil) | Fe (mg kg ⁻¹ soil) | Na (mg kg ⁻¹ soil) |
|---------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| A | 7.2 | 16 | 297 | 35 | 1253 | 4837 | 1014 |
| B | 1.8 | 11 | 44 | 87 | 423 | 5036 | 161 |
| MSD 5% ^b | 3.2 | 7 | 82 | 497 | 664 | 2522 | 248 |

^a Soil A: irrigated with water from river Turbio; soil B: irrigated with well water at 10 km from fields irrigated with water from river Turbio.

^b MSD: minimum significant difference ($P < 0.05$).

2.5. Statistical analysis

Cumulative production of CO₂ 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. This approach is supported by theoretical considerations that no CO₂ is produced at time zero and the control without soil accounted for the CO₂ in the atmosphere.

Soil characteristics, emission of N₂O after 1 day and concentrations of NH₄⁺, NO₂⁻ and NO₃⁻ from three replicates were subjected to one-way analysis of variance using PROC GLM (SAS, 1989) to test for significant differences between soils and treatments with the Tukey's studentized range (HSD) test. Significant differences between the soils and treatments for the cumulative CO₂ production were determined using PROC MIXED (SAS, 1989).

3. Results

3.1. Cumulative production of CO₂

Production of CO₂ was significantly and 1.33 times larger in soil A than in soil B ($P < 0.05$) (Fig. 1a and b). The addition of 200 mg NH₄⁺-N increased production of CO₂ significantly 1.16-fold in soil B (mean of soil incubated at 40%, 60%, 80% and 100% WHC), but had no significant effect in soil A ($P < 0.05$). In soil A, production of CO₂ was significantly larger in soil incubated at 60% and 80% WHC compared to soil incubated at 40% and 100% WHC ($P < 0.05$). In soil A amended with 200 mg NH₄⁺-N, production of CO₂ was significantly larger in soil incubated at 60% and 80% WHC compared to soil incubated at 40% and 100% ($P < 0.05$). In soil B, production of CO₂ was lower in soil incubated at 40% WHC compared to soil

incubated at 60%, 80% and 100% WHC, independent of application of NH₄⁺.

3.2. Dynamics of NH₄⁺, NO₂⁻ and NO₃⁻

Concentration of NH₄⁺ remained <10 mg NH₄⁺-N kg⁻¹ soil in unamended soil A and B (Fig. 2a and b). In soil A amended with 200 mg NH₄⁺-N kg⁻¹ soil, the concentration of NH₄⁺ decreased between time of application and extraction (Fig. 2c). After 1 day, the concentration of NH₄⁺ decreased further with the slowest decrease found in soil incubated at 40% WHC and the fastest in soil incubated at 60% and 80% WHC. In soil B amended with 200 mg NH₄⁺-N kg⁻¹ soil, the concentration of NH₄⁺ decreased sharply at day 1 and further decreases were much smaller especially in soil incubated at 40% WHC (Fig. 2d). Average concentrations of NH₄⁺ were significantly lower in soil A added with 200 mg NH₄⁺-N kg⁻¹ soil compared to soil B (Fig. 2c and d).

Concentrations of NO₂⁻ decreased significantly over time in the unamended soil A and B (Fig. 3a and b) ($P < 0.05$). Average concentrations of NO₂⁻ were similar in the unamended soil A and B ($P < 0.05$). In soil A amended with 200 mg NH₄⁺-N kg⁻¹ soil, concentrations of NO₂⁻ increased the first day and decreased again thereafter (Fig. 3c). In soil B amended with 200 mg NH₄⁺-N kg⁻¹ soil, concentrations of NO₂⁻ increased sharply the first 3 days and decreased again thereafter (Fig. 3d). Maximum concentrations of NO₂⁻ were larger in soil B added with 200 mg NH₄⁺-N kg⁻¹ soil compared to soil A added with 200 mg NH₄⁺-N kg⁻¹ soil (Fig. 3c and d).

In the unamended soil A and B, concentrations of NO₃⁻ did not show large changes over time ($P < 0.05$) (Fig. 4a and b). In soil A amended with 200 mg NH₄⁺-N kg⁻¹ soil, concentrations of NO₃⁻ increased significantly over time independent of water content ($P < 0.05$) (Fig. 4c). In soil B amended with 200 mg NH₄⁺-N kg⁻¹ soil, concentrations of NO₃⁻ increased with the fastest increase found in soil incubated at 60% and 80% WHC (Fig. 4d).

Table 3 – The effect of soil, addition of 200 mg NH₄⁺-N or incubation at 40%, 60%, 80% or 100% water holding capacity (WHC) and its interactions on emissions of N₂O after 1 day

| Treatment and interactions | d.f. | N ₂ O emission [P-value (type II)] |
|--|------|---|
| Soil (SOIL) | 1 | <0.0001 |
| Addition of 200 mg NH ₄ ⁺ -N (AMM) | 1 | 0.0002 |
| Percentage of water holding capacity (40%, 60%, 80% or 100%) (WHC) | 3 | |
| SOIL × WHC | 3 | <0.0001 |
| SOIL × AMM | 1 | 0.2481 |
| AMM × WHC | 3 | 0.0009 |
| SOIL × AMM × WHC | 3 | 0.9188 |

d.f.: degrees of freedom. One-way analysis of variance using PROC GLM (SAS, 1989) to test for significant differences between treatments with Tukey's studentized range (HSD) test (type II SS).

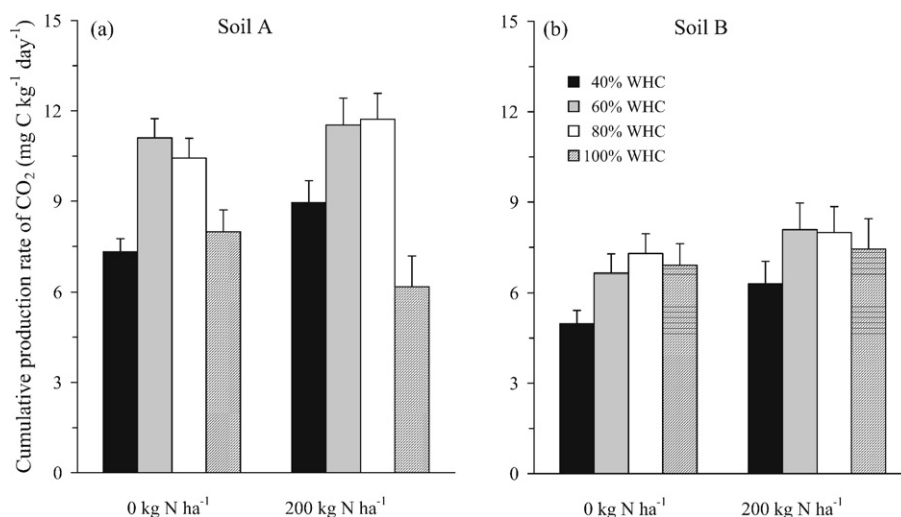


Fig. 1 – (a) Cumulative CO₂ production rate (mg C kg⁻¹ soil) from soil irrigated with water from river Turbio (soil A); (b) irrigated with well water (soil B). Soil adjusted to 40% (■), 60% (▒), 80% (□) or 100% water holding capacity (▨). Bars are standard errors of the estimates ($P < 0.05$).

3.3. Cumulative production of N₂O

In the unamended soil A, production of N₂O remained <0.1 mg N₂O-N kg⁻¹ soil for soil incubated at 40% and 60% WHC (Fig. 5a). Production of N₂O increased over time when unamended soil was incubated 80% WHC, but after an initial increase decreased again in soil incubated at 100% WHC. In the unamended soil B, production of N₂O remained <1 mg N₂O-N kg⁻¹ soil in all treatments (Fig. 5b). In soil A amended with 200 mg NH₄⁺-N, production of N₂O increased over time when soil was incubated ≤60% WHC, but after an initial increase, decreased again in soil incubated at 80% and 100% WHC

(Fig. 5c). In soil B added with 200 mg NH₄⁺-N, production of N₂O increased over time, but no decreases were found (Fig. 5d).

The soil, the addition of 200 mg NH₄⁺-N and the water content of the soil all had a significant effect on the N₂O emission within the first day (Table 3). Production of N₂O increased significantly 25.7 times in soil B amended with 200 mg NH₄⁺-N kg⁻¹ soil compared to the unamended soil (mean of soil incubated at 40%, 60%, 80% and 100% WHC), but only 1.8 times in soil A ($P < 0.05$) after 1 day. Production of N₂O increased significantly with increased water content and was >40 times larger in soil incubated at 100% WHC compared to soil incubated at 40% WHC after 1 day ($P < 0.05$).

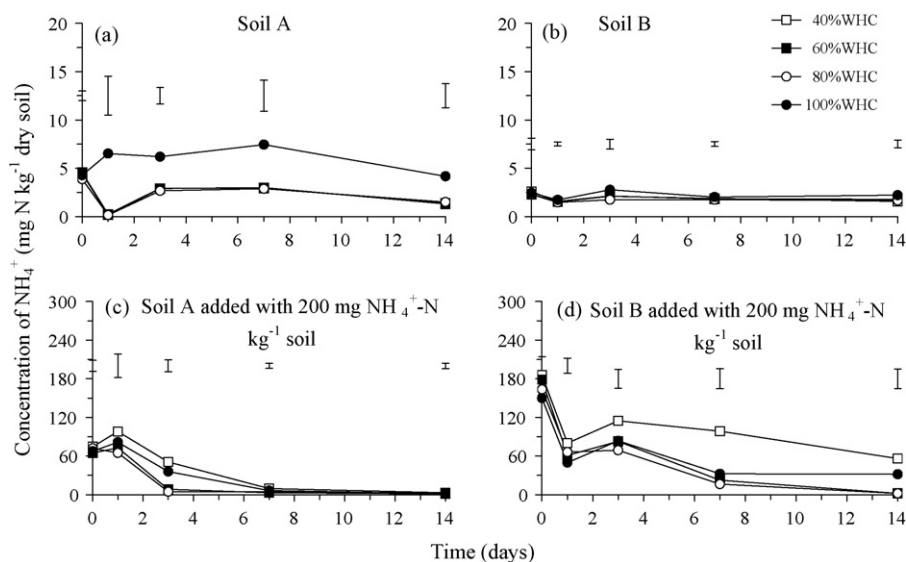


Fig. 2 – (a) Concentration of NH₄⁺ (mg N kg⁻¹ soil) from soil irrigated with water from river Turbio (soil A); (b) irrigated with well water (soil B); (c) soil A amended with 200 mg NH₄⁺-N kg⁻¹ soil; (d) soil B amended with 200 mg NH₄⁺-N kg⁻¹ soil. Soil adjusted to 40% (□), 60% (■), 80% (○) or 100% water holding capacity (●). Bars are standard errors of the estimates ($P < 0.05$) and each point in the graph is mean of $n = 9$.

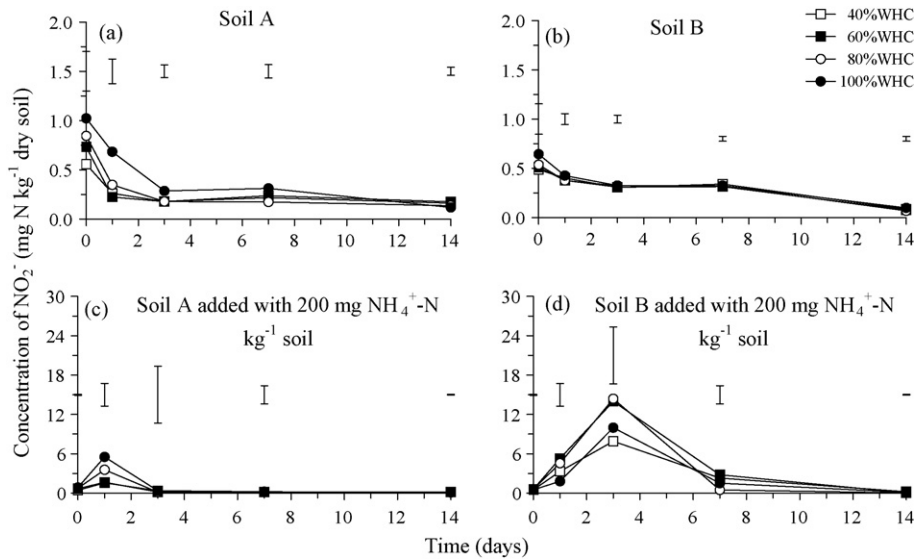


Fig. 3 – (a) Concentration of NO_2^- (mg N kg⁻¹ soil) from soil irrigated with water from river Turbio (soil A); (b) irrigated with well water (soil B); (c) soil A amended with 200 mg NH_4^+ -N kg⁻¹ soil; (d) soil B amended with 200 mg NH_4^+ -N kg⁻¹ soil. Soil adjusted to 40% (□), 60% (■), 80% (○) or 100% water holding capacity (●). Bars are standard errors of the estimates ($P < 0.05$) and each point in the graph is mean of $n = 9$.

4. Discussion

4.1. C mineralization

Both soils contained easily decomposable organic matter as a flush in production of CO_2 was observed at the onset of the incubation (Azam et al., 1995). Application of water to air-dried soil stimulates and increases the microbial activity, which will

decompose the microorganisms killed upon drying (Parton et al., 1996). It can also change the amount and quality of dissolved organic matter (Marschner and Noble, 2000), which is an easily available substrate for soil microorganisms. The larger organic C content in soil A compared to soil B resulted in a larger production of CO_2 in soil A than in soil B.

Production of CO_2 in soil B was 4.98 and 7.32 mg CO_2 -C kg⁻¹ day⁻¹ for soil A. Similar values have been reported for other agricultural soils of the Central Highlands of Mexico.

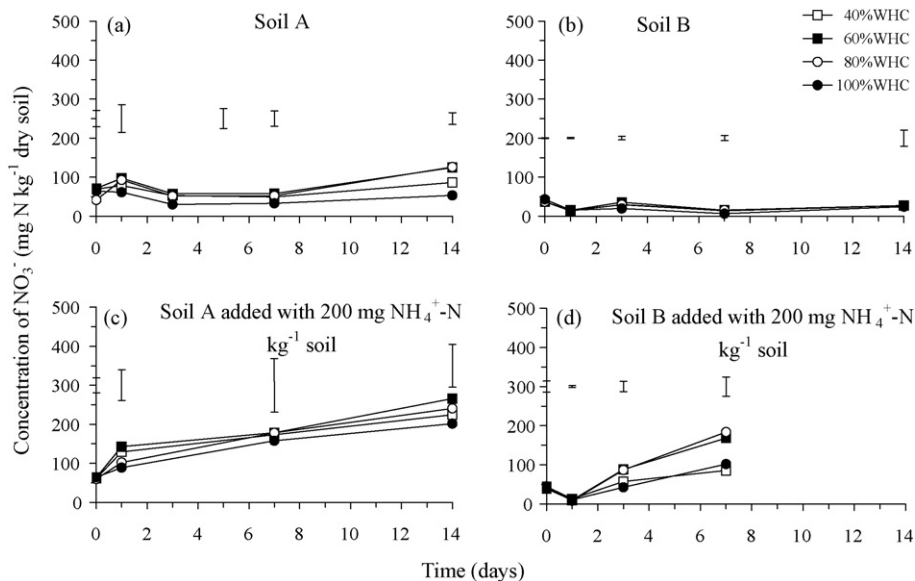


Fig. 4 – (a) Concentration of NO_3^- (mg N kg⁻¹ soil) from soil irrigated with water from river Turbio (soil A); (b) irrigated with well water (soil B); (c) soil A amended with 200 mg NH_4^+ -N kg⁻¹ soil; (d) soil B amended with 200 mg NH_4^+ -N kg⁻¹ soil. Soil adjusted to 40% (□), 60% (■), 80% (○) or 100% water holding capacity (●). Bars are standard errors of the estimates ($P < 0.05$) and each point in the graph is mean of $n = 9$.

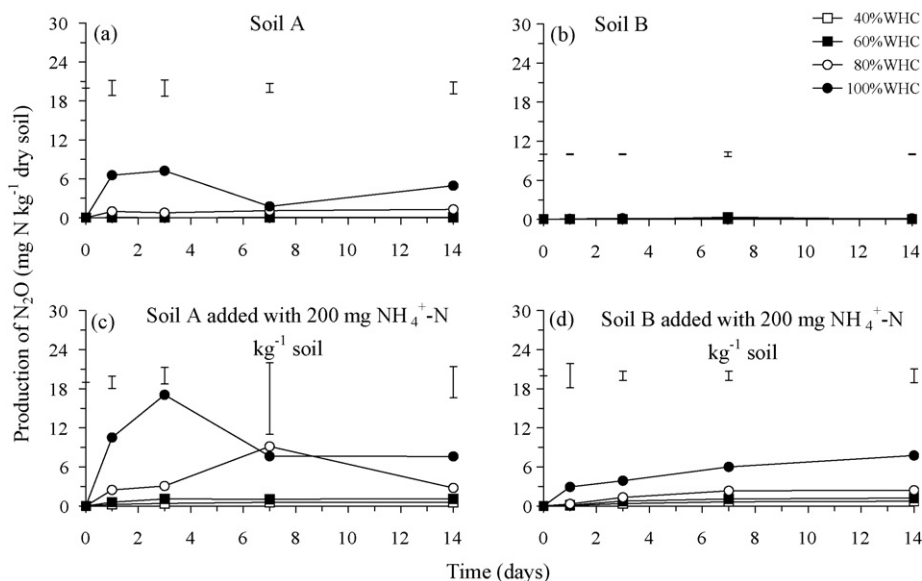


Fig. 5 – (a) Production of N₂O (mg N kg⁻¹ soil) from soil irrigated with water from river Turbio (soil A); (b) irrigated with well water (soil B); (c) soil A amended with 200 mg NH₄⁺-N kg⁻¹ soil; (d) soil B amended with 200 mg NH₄⁺-N kg⁻¹ soil. Soil adjusted to 40% (□), 60% (■), 80% (○) or 100% water holding capacity (●). Bars are standard errors of the estimates (P < 0.05) and each point in the graph is mean of n = 9.

Angoa Pérez et al. (2004) reported values of 9.88 mg CO₂-C kg⁻¹ day⁻¹ for an agricultural soil of Dolores Hidalgo (Guanajuato, Mexico) while Reyes-Reyes et al. (2002) 3.66 mg CO₂-C kg⁻¹ day⁻¹.

The water content of the soil had an effect on C mineralization in soil A. At 40% and 100% WHC, cumulative production of CO₂ was lower than for soil incubated at 60% and 80% WHC. Similar results were reported by Ponce-Mendoza et al. (2006). They found a CO₂ production of 33.6 mg C kg⁻¹ for soil incubated at 60% WHC which was similar for soil incubated at 80% WHC, but decreased 1.13 times for soil incubated at 40% and 1.16 times for soil incubated at 100% WHC. For soil incubated at 40% WHC, H₂O was limited reducing microbial activity (Parton et al., 1996). For soil incubated at 100% WHC, diffusion of O₂ was sometimes restricted and efficiency for C or the amount of C used for metabolic activity is known to be lower under anaerobic conditions so that production of CO₂ is lower (Dendooven and Anderson, 1994). The effect of high water content on production of CO₂ in soil B, i.e. soil incubated at 100% WHC, was not that clear as in soil A, but C mineralization at 40% WHC was lower than for soil incubated at higher water contents.

4.2. Dynamics of NH₄⁺, NO₂⁻ and NO₃⁻

Of the 200 mg NH₄⁺ added to soil A and B, approximately 140 mg was not recovered in soil A between time of application and extraction with KCl, but not in soil B. There are mainly four processes that might have affected concentration of NH₄⁺ between time of application and extraction, i.e. NH₃ volatilization, NH₄⁺ fixation, nitrification and microbial immobilization of NH₄⁺. High pH stimulates NH₃ volatilization (Kirchmann and Witter, 1989). However, the pH of both soils was low and

similar so volatilization could not explain the difference in concentrations of NH₄⁺ between both soils. Part of NH₄⁺ could have been fixed on the soil matrix, but values reported in literature are far less than the 140 mg that could not be accounted for. Drury et al. (1991) found that the maximum amount of ¹⁵N-labelled fixed NH₄⁺ in soil was 18.7 mg kg⁻¹. Nitrification could not explain the drop in concentration of NH₄⁺ as the concentration of NO₃⁻ was similar for the unamended soil A and soil A added with 200 mg NH₄⁺ at the onset of the incubation. This indicated that most of the NH₄⁺ appeared to be immobilized by the soil microbial biomass between the time of application and extraction with KCl. However, microbial biomass C values were 547 mg C kg⁻¹ in soil A and 367 mg C kg⁻¹ in soil B (Alvarez-Bernal et al., 2006) and it might be difficult to explain an immobilization of 140 mg NH₄⁺-N when microbial biomass C-to-N generally ranges between 4 and 8. A similar fast immobilization of NH₄⁺ was found when NH₄⁺ was added to an alkaline saline soil of the former lake Texcoco (Vega-Jarquin et al., 2003) and the dynamics of NH₄⁺ and NO₃⁻ in the NH₄⁺-amended soil could not be simulated unless an immobilization of NH₄⁺ into the microbial biomass occurred in the first day of the incubation without an immediate incorporation of it into microbial organic material (Luna-Guido and Dendooven, 2001). Most of the NH₄⁺ immobilized into the microbial biomass at the onset of the incubation was released again and then oxidized to NO₃⁻.

Oxidation of NO₂⁻ is the time limiting step in nitrification and more sensitive than the oxidation of NH₄⁺ to adverse conditions, such as low pH (De Boer and Kowalchuk, 2001) or large salt concentrations (Oren, 1999). In a previous experiment with the same soils, larger concentrations of NO₂⁻ were found in soil A than B, and we stated that this might indicate an inhibition of nitrification due to the use of water from the

river Turbio. In this study, small amounts of NO_2^- ($\leq 1 \text{ mg NO}_2^- \text{-N kg}^{-1}$ soil) were detected in the unamended soil A and B, but concentrations were similar in both soils so no inhibition of the nitrification process occurred. In soil added with 200 mg NH_4^+ concentrations of NO_2^- in soil A $< 6 \text{ mg N kg}^{-1}$ soil and peaked after 1 day while they were $> 6 \text{ mg N kg}^{-1}$ in soil B and peaked after 3 days, i.e. oxidation of NO_2^- was faster in soil A than in soil B. It can be postulated that the continuous application of NH_4^+ to soil A through irrigation with water from the river Turbio increased populations of nitrifiers and/or their activity so nitrification was faster in soil A than in soil B (Williams et al., 1998). Consequently, concentrations of NO_2^- were lower in soil A than in soil B. The larger concentration of NO_2^- in the previous experiment was presumably due to more NH_4^+ being oxidized in soil A than in soil B, resulting in larger concentrations of NO_2^- , i.e. N cycling was larger in soil A than in soil B.

4.3. Production of N_2O

Production of N_2O is primarily related to nitrification, nitrifier denitrification and denitrification (Wrage et al., 2001). Some other processes such as aerobic denitrification, heterotrophic nitrification and chemodenitrification might contribute to production of N_2O , but their overall impact is considered small (Robertson and Tiedje, 1987). Water is the most important factor to control nitrification and denitrification and thus production of N_2O (e.g. Bandibas et al., 1994). At 40% WHC, nitrifier denitrification and denitrification will not contribute substantially to production of N_2O so nitrification will be the main source. The N_2O production rate was low in the soil not amended with $200 \text{ mg NH}_4^+\text{-N}$ ($< 0.07 \text{ mg N}_2\text{O-N kg}^{-1} \text{ day}^{-1}$; mean of all treatments), but increased 4.6 times when $200 \text{ mg NH}_4^+\text{-N}$ was added. Large concentrations of NH_4^+ will induce nitrification and thus production of N_2O (Beauchamp et al., 1989). However, the amount of N_2O produced after 14 days as a percentage of NO_3^- produced after 14 days was low and $< 0.5\%$.

Emission of N_2O from soil B was $1.80 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ day}^{-1}$ and $3.38 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ day}^{-1}$ for soil A. Similar values have been reported for other agricultural soils of the Central Highlands of Mexico. For instance, Angoa Pérez et al. (2004) reported values of $1.66 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ day}^{-1}$ for an agricultural soil of Dolores Hidalgo (Guanajuato, Mexico).

It is well known that production of N_2O increases with increased water content. For instance, Ponce-Mendoza et al. (2006) reported that the emission of N_2O increased significantly 32 times when soil was incubated at 100% WHC compared to soil at 40%. When water content increases in soil, nitrifier denitrification and denitrification is induced (Wrage et al., 2001), and already in both soils incubated at 60% WHC and independent of addition of $200 \text{ mg NH}_4^+\text{-N}$, production of N_2O increased threefold compared to soil incubated at 40% WHC. Losses of N through denitrification were presumably larger as reduction of N_2O to N_2 was not taken into account. The effect of further increases in water content (80% and 100% WHC) on production of N_2O was different between soil A and B. In soil A production of N_2O was much larger than in soil B. There are different possible explanations for this. First, microbial activity in soil A was larger than in soil B, so emissions of N_2O through nitrification and/or denitrification

will be larger. The increase in production of N_2O (25 times at 100% WHC), however, was larger than the increase in CO_2 (on average 1.33 times). Second, concentrations of NO_3^- were larger in soil A (mean $67 \text{ mg NO}_3^- \text{-N kg}^{-1}$ in unamended soil) than in soil B ($24 \text{ mg NO}_3^- \text{-N kg}^{-1}$ in unamended soil). Denitrification follows a competitive Michaelis–Menten type kinetics so larger concentrations of NO_3^- limit reduction of N_2O to N_2 (Betlach and Tiedje, 1981). As a result more N_2O is released in the environment. Third, it might be that irrigation with water from the river Turbio changed some characteristics of the soil, which affected production–reduction of N_2O . It might have been, for instance, that *de novo* synthesis of N_2O -reductase took longer in soil A and as such production of N_2O was larger because it was not reduced to N_2 (Dendooven and Anderson, 1994).

It has often been reported that an increase in soil organic matter, such as in soil A, increases N_2O emissions (e.g. Li et al., 2005b). Larger amounts of soil organic matter means that more C is sequestered reducing atmospheric CO_2 . However, increased N_2O emissions might offset reductions in climate radiative forcing (Li et al., 2005a).

It is well known that addition of fertilizer to soil increases production of N_2O . For instance, Williams et al. (1998) reported a 9.5-fold increase when NH_4NO_3 was added to soil at field capacity. Addition of $(\text{NH}_4)_2\text{SO}_4$ in this experiment increased production of N_2O significantly (mean of all treatments), 25.7 times in soil B and 1.7 times in soil A after 1 day.

5. Conclusion

It was found that the characteristics of soil irrigated with water from the river Turbio appeared not to have deteriorated, although concentrations of salt had increased. Biological functioning of the soil, such as nitrification, was not affected by irrigation with water containing tannery effluent. The production of N_2O was much larger in soil irrigated with water containing tannery effluent water than in soil irrigated with well water. The larger production of N_2O in soil irrigated with tannery effluents might be related to the larger microbial activity as more easily decomposable material was available as C substrate or an effect of irrigation with tannery effluent. The large residual concentrations of NO_3^- found in soil irrigated with water from the river Turbio indicate that substantial losses of NO_3^- might occur after irrigation through leaching and denitrification.

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REFERENCES

- Alvarez-Bernal, D., Contreras-Ramos, S.M., Trujillo-Tapia, N., Olalde-Portugal, V., Frías-Hernández, J.T., Dendooven, L., 2006. Effects of tanneries wastewater on chemical and biological soil characteristics. *Appl. Soil Ecol.* 33, 269–277.
- Angoa Pérez, M.V., González Castañeda, J., Franco-Hernández, O., Van Cleemput, O., Dendooven, L., Olalde, V., 2004. Trace gas emissions from soil of the central highlands of México as affected by natural vegetation: a laboratory study. *Biol. Fertil. Soils* 40, 252–259.
- APHA AWWA WPCF, 1989. Standard Methods for the Examination of Water and Wastewater, 17th edition. Published jointly by the American Public Health Association, American Water Works Association and Water Environment Federation, American Public Health Association, 2015 Fifteenth Street, NW Washington, DC 20005, USA.
- Azam, F., Simmons, F.W., Mulvaney, R.L., 1995. Effect of ammonium and nitrate on mineralization of N from leguminous residues. *Biol. Fertil. Soils* 20, 49–52.
- Bandibas, J., Vermoesen, A., De Groot, C.J., Van Cleemput, O., 1994. The effect of different moisture regimes and soil characteristics on nitrous oxide emission and consumption by different soils. *Soil Sci.* 158, 106–114.
- Beauchamp, E.G., Trevors, J.T., Paul, J.W., 1989. Carbon sources for bacterial denitrification. *Adv. Soil Sci.* 10, 113–142.
- Betlach, M.R., Tiedje, J.M., 1981. Kinetic explanation for accumulation of nitrite, nitric oxide, and nitrous oxide during bacterial denitrification. *Appl. Environ. Microbiol.* 42, 1074–1084.
- Bouwman, A.F., 1990. Exchange of greenhouse gases between terrestrial ecosystems and the atmosphere. In: Bouwman, A.F. (Ed.), *Soils and the Greenhouse Effect*. Wiley, Chichester, pp. 100–120.
- Bremner, J.M., 1996. Nitrogen-Total. In: Sparks, D.L. (Ed.), *Methods of Soil Analysis*. Part 3. Soil Science Society of America Inc., American Society of Agronomy Inc., Madison, WI, USA, pp. 1085–1122.
- Brookes, P.C., 1993. In: Schulin, K., Desaulles, A., Webster, R., von Steigen, B. (Eds.), *The Potential of Microbiological Properties as Indicators in Soil Pollution Monitoring*. Soil Monitoring Birhauser, Basel, pp. 229–254.
- Bundy, L.G., Meisinger, J.J., 1994. Nitrogen availability indices. In: Weaver, R.W., Angle, J.S., Bottomley, P.S. (Eds.), *Methods of Soil Analysis*. Part 2. Microbiological and Biochemical Properties. Soil Science Society of America Inc., American Society of Agronomy Inc., Madison, WI, USA, pp. 951–984.
- Cela, S., Sumner, M.E., 2002. Critical concentrations of copper, nickel, lead, and cadmium in soils based on nitrification. *Commun. Soil Sci. Plan.* 33, 19–30.
- De Boer, W., Kowalchuk, G.A., 2001. Nitrification in acid soils: micro-organisms and mechanisms. *Soil Biol. Biochem.* 33, 853–866.
- Dendooven, L., Anderson, J.M., 1994. Dynamics of reduction enzymes involved in the denitrification process in pasture soil. *Soil Biol. Biochem.* 26, 1501–1506.
- Drury, C.F., Voroney, R.P., Beauchamp, E.G., 1991. Availability of NH_4^+ -N to microorganisms and the soil internal N cycle. *Soil Biol. Biochem.* 23, 165–169.
- FAO, 1988. www.fao/waicent/faoinfo/agricult/agl/agll/key2soil.stm.
- Gee, G.W., Bauder, J.W., 1996. Particle size analysis. In: Klute, A. (Ed.), *Methods of Soil Analysis*. Part 1. Physical and Mineralogical Methods. 2nd edition. Soil Science Society of America Inc., American Society of Agronomy Inc., Madison, WI, USA, pp. 383–411.
- Hauck, R.D., 1980. Mode of action of nitrification inhibitors. In: Meisinger, J.J., Randall, G.W., Vitosh, M.L. (Eds.), *Nitrification Inhibitors—Potentials and Limitations* ASA Special Publication Number 38. American Society of Agronomy Soil Science Society of America, Madison, WI, pp. 19–32.
- INE-DGMRAR, 1999. Manual de procedimientos para el manejo adecuado de los residuos de la curtiduría. Dirección General de Materiales, Residuos y Actividades riesgosas. Instituto Nacional de Ecología, México, D.F.
- Jenkinson, D.S., Powlson, D.S., 1976. The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. *Soil Biol. Biochem.* 8, 209–213.
- Kalembasa, S.J., Jenkinson, D.S., 1973. A comparative study of titrimetric and gravimetric methods for the determination of organic carbon in soil. *J. Sci. Food Agric.* 24, 1085–1090.
- Kirchmann, H., Witter, E., 1989. Ammonia volatilization during aerobic and anaerobic manure decomposition. *Plant Soil* 115, 35–41.
- Li, C.S., Frolking, S., Butterbach-Bahl, K., 2005a. Carbon sequestration in arable soils is likely to increase nitrous oxide emissions, offsetting reductions in climate radiative forcing. *Climate Change* 72, 321–338.
- Li, C.S., Frolking, S., Xiao, X.M., Moore, B., Boles, S., Qiu, J.J., Huang, Y., Salas, W., Sass, R., 2005b. Modelling impacts of farming management alternatives on CO_2 , CH_4 , and N_2O emissions: a case study for water management of rice agriculture of China. *Global Biogeochem. Cycles* 19 (art. no. GB3010).
- Luna-Guido, M.L., Dendooven, L., 2001. Simulating the dynamics of glucose and NH_4^+ in alkaline soils of the former lake Texcoco with the Detran model. *Eur. J. Soil Sci.* 52, 269–277.
- Marschner, B., Noble, A., 2000. Chemical and biological processes leading to the neutralisation of soil acidity after incubation with different litter materials. *Soil Biol. Biochem.* 32, 805–813.
- Moraghan, J.T., Buresh, R., 1977. Correction for dissolved nitrous oxide in nitrogen studies. *Soil Sci. Soc. Am. J.* 41, 1201–1203.
- Oren, A., 1999. Bioenergetic aspects of halophilism. *Microbiol. Mol. Biol. R.* 63, 334–348.
- Parton, W.J., Mosier, A.R., Ojima, D.S., Valentine, D.W., Schimel, D.S., Weier, K., Kulmala, A.E., 1996. Generalized model for N_2 and N_2O production from nitrification and denitrification. *Global Biogeochem. Cycles* 10, 401–412.
- Ponce-Mendoza, A., Boeckx, P., Van Cleemput, O., Dendooven, L., 2006. Influence of water regime and N fertilization on emission of nitrous oxide and carbon dioxide from tropical semi-arid soils of Chiapas, México. *J. Arid Environ.* 64, 137–151.
- Reyes-Reyes, G., Baron-Ocampo, L., Cuali-Alvarez, I., Frías-Hernández, J.T., Olalde-Portugal, V., Varela-Fregoso, L., Dendooven, L., 2002. C and N dynamics in soil from the central highlands of México as affected by mesquite (*Prosopis* spp.) and huizache (*Acacia tortuosa*): a laboratory investigation. *Appl. Soil Ecol.* 19, 27–34.
- Reyes-Reyes, B.G., Zamora-Villafranco, E., Reyes-Reyes, M.L., Frías-Hernández, J.T., Olalde-Portugal, V., Dendooven, L., 2003. Decomposition of leaves of huizache (*Acacia tortuosa*) and mesquite (*Prosopis laevigata*) in soil of the central highlands of México. *Plant Soil* 256, 359–370.
- Robertson, G.P., Tiedje, J.M., 1987. Nitrous oxide sources in aerobic soils: nitrification, denitrification and other biological processes. *Soil Biol. Biochem.* 19, 187–193.
- Robles, J., Armienta, M.A., 2000. Natural chromium contamination of groundwater at León Valley, México. *J. Geochem. Explor.* 68, 167–181.
- SAS Institute Inc., 1989. SAS/STAT[®]. *Statistic Guide for Personal Computers*. Version 6.04. SAS Institute Inc., Cary, NC.
- Sinha, S., Saxena, R., Singh, S., 2002. Comparative studies on accumulation of Cr from metal solution and tannery

- effluent under repeated metal exposure by aquatic plants: its toxic effects. *Environ. Monit. Assess.* 80, 17–31.
- Sverdrup, L.E., Ekelund, F., Krogh, P.H., Nielsen, T., Johnsen, K., 2002. Soil microbial toxicity of eight polycyclic aromatic compounds: effects on nitrification, the genetic diversity of bacteria, and the total number of protozoans. *Environ. Toxicol. Chem.* 21, 1644–1650.
- Thomas, G.W., 1996. Soil pH and soil acidity. In: Sparks, D.L. (Ed.), *Methods of Soil Analysis: Chemical Methods. Part 3.* Soil Science Society of America Inc., American Society of Agronomy Inc., Madison, WI, USA, pp. 475–490.
- Vega-Jarquin, J.C., Garcia-Mendoza, M., Jablonowski, A.N., Luna, G.M., Dendooven, L., 2003. Rapid immobilization of applied nitrogen in alkaline saline soils. *Plant Soil* 256, 379–388.
- Williams, P.H., Jarvis, S.C., Dixon, E., 1998. Emission of nitric oxide and nitrous oxide from soil under field and laboratory conditions. *Soil Biol. Biochem.* 30, 1885–1893.
- Wrage, N., Velthof, G.L., van Beusichem, M.L., Oenema, O., 2001. Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biol. Biochem.* 33, 1723–1732.