Effect of sulfadiazine on soil nitrogen mineralization

Yan Wang\textsuperscript{A,B}, Fangbai Li\textsuperscript{A} and Juan Boo Liang\textsuperscript{B}

\textsuperscript{A} Guangdong Institute of Eco-Environmental and Soil Sciences, Guangzhou 510650, PR China , Email cefbli@soil.gd.cn
\textsuperscript{B} Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia, Email jbliang@ibs.upm.edu.my

Abstract
Soil nitrogen (N) mineralization is important for soil fertility, and has been suggested to provide 20 to 80\% of the N required by plants. Effects of antibiotic sulfadiazine (SD), a widely used antibiotic in livestock production, on the amount of net N mineralized in the soil were studied. The results showed that SD significantly inhibited the mineralization of N, and the inhibition increased with increasing SD concentration and reaction time. This suggested that soil fertility may be reduced with the presence of SD, with higher reduction rates when the concentration of SD increased from 0 to 2 mg/kg and during the first 21 reaction days.

Key Words
Nitrogen mineralization, sulfadiazine, soil

Introduction
Antibiotics are widely used in intensive livestock farming as growth promoters and/or to treat infectious diseases (Boxall \textit{et al.}, 2003), particularly for pig and poultry (Boxall \textit{et al.}, 2004). Tetracyclines and sulphonamides are the most widely used antibiotics due to their effectiveness and price (Boxall \textit{et al.}, 2004). It was estimated that annual consumption of antibiotics for livestock farming accounted for 95 tonnes in the European countries, of which 78 tonnes were for pig production (Diaz-Cruz \textit{et al.}, 2003). Since 2006, the EU countries have prohibited the use of antibiotics as growth promoters, but the use of such drugs for prevention and treatment of diseases is still allowed. And it has been reported that over ten thousand tons of antibiotic were used for prevention and treatment of animal diseases (Diaz-Cruz \textit{et al.}, 2003). The actual consumption of the veterinary drugs in most developing countries including China and many south-east Asian countries, such as Malaysia, is not known.

Most of the antibiotics are poorly assimilated by the animals. About 25 to 75\% of the antibiotics are excreted via feces or urine as the parent compounds or their metabolites and enter the environment either directly by spreading as manure or after collection and storage in form of sludge (Diaz-Cruz, \textit{et al.}, 2003). The presence of traces of these antibiotic residues in the environment can induce the development of antibiotic-resistant pathogens, causing serious problems to human health (Hirsch \textit{et al.}, 1999). Currently, the environmental hazards of veterinary antibiotics have received greater attentions (Boxall \textit{et al.}, 2004; O’Neil \textit{et al.}, 2001). Residual concentrations of antibiotics have been observed in soils, surface and ground water (Hamscher \textit{et al.}, 2002; Kolpin \textit{et al.}, 2002; Simon, 2005). They may affect the beneficial bacteria communities in the environment. The fate of antibiotics in the soil and aquatic environments such as sorption and fixation, mobility and transport, eco-toxicity, resistance and degradation are well documented (Tolls, 2001; Figueroa, \textit{et al.}, 2004; Kulshreshtha \textit{et al.}, 2004; Thiele-Bruhn, 2003; Kong \textit{et al.}, 2006; Wang and Yates, 2008), but information on the effect of antibiotics on soil nitrogen mineralization is scanty.

Nitrogen is important for efficient crop production. The main sources of N used by crops are from (i) mineralization of soil organic N, (ii) decomposition of plant residues or organic amendments such as manure, and (iii) addition of N as inorganic fertilizer. Inefficient use of the applied N to the soil is likely to cause undesirable environmental impacts from NO\textsubscript{3} leaching or gaseous N losses by denitrification and/or volatilization. Accurate estimates of the contributions of soil N to crop production are needed to minimize environmental impacts and production costs from overuse of N fertilizer, (Rice and Havlin, 1994). Soil N mineralization has been shown to provide 20 to 80\% of the N required by plants (Broadbent, 1984). Manure, as readily decomposable organic materials, is an important source of plant nutrients (Zaman \textit{et al.}, 2004), and has been shown to increase soil total N (Mikha and Rice, 2004) and to improve the nutrient status of the soil (Zaman \textit{et al.}, 2004). Currently, the use of manure as fertilizer for crop production has been widely practised. However, mineralization of soil organic matter and crop residue is a complex process, and is mainly via the activity of microorganisms. Sulfadiazine (SD) is broad spectrum bacteriostatic antibiotics, which inhibits dihydropteroate synthesis in the folic acid pathway (O’Neil \textit{et al.}, 2001), and reduces the
reproductive functions of bacteria. Therefore, antibiotic residue in the livestock manure, when applied to the soil as fertilizer, may affect the activity of microorganism and thus soil N mineralization. As mentioned earlier, that information on the effect of antibiotics on soil N mineralization is scanty, this study was conducted to provide such information.

Materials and Methods

Chemicals
Sulfadiazine (SD) (Sigma-Aldrich, USA) stock solutions were prepared at a concentration of 50 mg/L in deionised water, and further diluted to the experimental concentration standards in mobile phase to construct a standard calibration curve. Other chemicals of analytical grade were from Guangzhou Chemical Co., China.

Preparation of the soil samples
Red soils, widely used for the growing of vegetable, were collected from Dazhen village, Nanhai district, Foshan city of South China (23.08656°N, and 113.10952°E) for this study. The sampling depth was 0-20 cm. Prior to the experiment, the soils were dried under open shade, ground to pass through 200 mesh screen.

Experimental procedure
Sulfadiazine (SD) was used as the model antibiotic for this study, to investigate the effect of SD on soil net N mineralization. The SD stock solutions were added into the soil samples to achieve the respective experimental concentrations of 0, 0.2, 0.5, 1, 2, 6, 10, 15 and 20 mg/kg. On day 7, the soils were sampled and stored for the study of net N mineralization. For the experiment on SD reaction times in the soil N mineralization, the concentrations of SD in the soil were 0, 0.5, 2, 20 mg/kg, respectively. On day 0, 7, 14, 21, 28 and 35, net N mineralizations of the respective treatments were determined.

Laboratory analysis of soil N mineralization
Soil samples from the treatments were shaken and extracted in 2 M KCl solution three times (15 min each in a serial extraction) to extract all available NH$_4^+$-N and NO$_3^-$-N. NH$_4^+$-N were analysed by Nessler's colorimetric method and NO$_3^-$-N were by Ion Chromatography Analyzer (Dionex ICS-90, USA) equipped with a Polysulfonate Ion Exclusion Column. The eluent of the ion analytical column contained the following: 8 mM of Na$_2$CO$_3$, 1 mM of NaHCO$_3$, and 45 mM vitriol. The amount of N mineralized in the soil at each sampling time was determined by subtracting the concentration of NH$_4^+$-N and NO$_3^-$-N in the soil at the beginning of the study from that at each sampling time and multiplying the dry weight of the total soil in each tube. Potentially mineralizable N ($N_0$) and the rate constant ($k$) were determined by laboratory incubation and applying a first-order exponential model, based on the leaching method proposed by Cabrera and Kissel (1988) as modified by Garcia (1992).

Results

Effect of SD concentrations on the soil N mineralization
Figure 1 presents the effect of different SD concentrations on soil net N mineralized. The result indicated that net N mineralized reduced with increased SD concentration. The inhibition of soil net N mineralized was more sensitive in the lower concentrations of SD than in the higher concentrations. The turning point of the inhibition effect (i.e. the point at which rapid inhibition ended and a more gradual inhibition began) was at 2 mg/kg SD. When the concentration of SD increased from 0 to 2 mg/kg, net N mineralized decreased from 0.170 to 0.148 mg/g, but when the concentration of SD increased from 2 to 20 mg/kg, net N mineralized decreased at a slower rate, from 0.148 to 0.142 mg/g. The above results suggest that soil fertility may be reduced with increasing SD concentrations, at a higher rate initially (0-2 mg/kg) than the subsequent higher SD rates (2-20 mg/kg).
SD reaction times on soil N mineralization

Net N mineralized remained almost the same with time in control and initially rapid (0-21 day) and then slowed down (21-35 day) in the SD treatment. There were significant differences in the net N mineralized rates (r value) between the control and SD treatments. This indicated that the soil fertility was influenced by both, the SD concentration and the reaction time. And the fertility decreased with the time, especially during the first 21 days.

Conclusion

This study presented the effects of SD concentration and reaction time on the soil net N mineralized. Result showed the net N mineralized significantly affected by SD concentration and reaction time in the red soils used in this study. Higher SD concentrations resulted in lower net N mineralized. The amount of net N mineralized reduced with the time, initially rapid and later slowed down. This indicated that red soil fertility reduced with increased SD concentration and reaction time.
References