Impact of Sewage Sludge Applications on the Biogeochemistry of Soils


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Abstract: This report describes an investigation into the bioavailability and fate of trace metals and their subsequent impact on important soil microbiological functions such as nitrification, denitrification and methane oxidation in low and high Cu containing soils in the presence and absence of residual organic matter from sewage sludge additions made 10 years earlier. The soils being studied are part of a long term sewage sludge trials and include a low Cu soil (13.3 mg Cu/kg soil, 4.18 LOI%), left un-amended to serve as a control soil, soil amended with a high Cu sewage sludge (278.3 mg Cu/kg soil, 6.52 LOI%) and soil amended with a low Cu sewage sludge (46.3 mg Cu/kg soil, 6.18 LOI%). Soil was also amended with inorganic metal salts (273.4 mg Cu/kg soil, 4.52 LOI%) to further investigate the impact of Cu in the absence of additional organic matter contained in applied sewage sludge. Data from the first two years of a PhD project are presented which has included field-based studies at a long term sewage sludge trials based in Watlington, Oxford, U.K. and laboratory based studies at the Institute of Grassland & Environmental Research, North Wyke, Devon, U.K.

Keywords: Copper; Greenhouse Gases; Methane; Nitrous Oxide; Sewage Sludge

INTRODUCTION

With the ban on dumping sewage at sea (prohibited in the EU since 1998 as a result of the Urban Waste Water Treatment Directive (91/271) and the Bathing Water Directive (76/160)), the land application of sewage sludge to agricultural land in the UK has increased. Land application aims to beneficially recycle the soil enhancing constituents contained within the sewage sludge (Wightman et al., 1996). Sewage sludges contain valuable concentrations of beneficial nutrients, but may also possess elevated concentrations of potentially toxic elements compared with average background concentrations in the soil (Hillman et al., 2003). The current trend of reducing the soil metal loads from sewage sludge applied to agricultural land has resulted in significant reductions in the amounts of trace metals being applied to these soils. Since the metal species most likely to be lost from soils will be the most labile, it is feasible that as levels of bioavailable forms of trace elements decrease, key soil biogeochemical reactions may be affected (Godley, 2003). The hypothesis of this PhD project states that as levels of available Cu fall below a key threshold, the emissions of certain biogenic gases from soils will increase. Nitrous oxide emissions from soil result mainly from the process of denitrification (Robertson and Tiedje, 1987; Firestone and Davidson, 1989) although some is also formed as a result of nitrification (Poth and Focht, 1985; Robertson and Tiedje, 1987). Denitrification involves several sequential intermediate steps that reduce nitrate and nitrite to nitrogen gas. Nitrous oxide is the last intermediate before nitrogen gas, and may be released to the atmosphere alongside N2. Low soil Cu levels may stimulate an increase in the emission of NO to the atmosphere as the conversion of NO to N2 is catalysed by the enzyme nitrous oxide reductase. This enzyme is a Cu-enzyme and its activity is dependent on the availability of Cu (Godley, 2003). Under Cu limiting conditions, the amount of the enzyme produced and its activity may become
reduced, thus the reduction of N\textsubscript{2}O to N\textsubscript{2} may become affected releasing more N\textsubscript{2}O to the atmosphere. The uptake of CH\textsubscript{4} by soils from the atmosphere by methane oxidising bacteria may also be compromised in soils with low available copper. Certain methanotrophic bacteria produce the enzyme methane mono-oxygenase (MMO), allowing them to use methane as an energy source. Stanley \textit{et al.} (1983) have shown that methanotrophic bacteria do not produce pMMO when grown in the absence of Cu. Many studies have documented this relationship including studies by Murrell \textit{et al.} (2000) etc.

**METHODS**

**Site description and treatment**

The experiment is located at Model Farm, Watlington on a sandy loam textured soil (UKWIR 2004). This site has been involved in a long term study of sewage amended soils. To achieve the desired Cu concentrations in the sewage sludge plots, two sewage sludges (with different Cu concentrations) were applied in different amounts to give the same C addition, with the last application occurring in 1997. All plots measured 48 m\textsuperscript{2} except for the inorganic metal amended plots which measured 4.2 m\textsuperscript{2}. An alternate cropping regime operates on the site with grass being followed by wheat. Plots were rotary spaded to 25 cm depth with an Agip spading machine on the 14\textsuperscript{th} February – 6\textsuperscript{th} March 2005. \textit{Paragon} Spring wheat was sown on all plots between 11 and 12\textsuperscript{th} April 2005. Fertilizer (as NPK 21.8.11) was applied to all the plots a rate of 60 kg/ha on the 11\textsuperscript{th} May 05 and 100 kg/ha on the 15\textsuperscript{th} June 05. Fertilizer as MgSO\textsubscript{4} Epsom Salts (30 kg/ha) was also applied during the second application. The control plots also received the same fertilisation regime. In this study, four treatments were investigated with each treatment replicated three times. The following measurements were made monthly except after fertilisation events where biogeneric soil gas sampling frequency was increased. Soil solution collection was not achieved from June onwards due to the dryness of the soils, particularly lower down in the soil profile.

**Flux measurements and analysis**

Gas fluxes into and out of the soil were measured using a closed chamber system. The chambers were made from cylindrical plastic piping, measuring 19.5 cm diameter. Each chamber lid was made from perspex and fitted with a rubber sampling port. Chambers were inserted into the soil to a depth of 5.5 cm to leave an enclosed headspace of approx. 3.4 l. The extracted gas samples were analysed by a Hewlett Packard GC equipped with a flame ionisation detector (FID) for CH\textsubscript{4} and an electron capture detector (ECD) for N\textsubscript{2}O analysis. Carbon dioxide was reduced by a methanizer and then analyzed by the FID.

**Soil sampling and analysis**

Aqua-Regia digestion of soil samples was used to determine the total element content (Vercoutere \textit{et al.} 1995). BCR sequential extraction (Rauaret, 1999) was used to fractionate trace metals into groups of decreasing solubility. Potential plant available metal concentrations were determined by DTPA extraction (Lindsay and Norvell, 1978). Metal digests and extractions were analysed using a Perkin Elmer Optima 3000 inductively coupled plasma optical emission spectrometry (ICP-OES). Soil available ammonium (NH\textsubscript{4}\textsuperscript{+}), nitrate (NO\textsubscript{3}\textsuperscript{-}) and nitrite (NO\textsubscript{2}\textsuperscript{-}) were determined by extracting 10 g soil with 50 ml 1M KCl and analysis with a ChemLab England Autoanalyser. Soil moisture content was measured gravimetrically by the determination of weight loss of soil samples on drying at 105\textdegreeC overnight. Loss on ignition (LOI) was measured as a proxy for organic matter contents by drying 10 g soil samples (already air-dried) at 105\textdegreeC for 16 h, and determining the mass loss after 16 h at 550\textdegreeC. Soil pH was measured in a 1:2.5 soil to water suspensions. Soil total nitrogen was determined by combustion and analysis by a Europa Roboprep/VG 622 mass spectrometer. Available P was determined by Olsen's procedure as simplified by Rowell (1994) following extraction with 0.5 M NaHCO\textsubscript{3}. 

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Soil solution and rainfall sampling and analysis

Two rhizon soil moisture samplers (RSMS) were installed at two different depths (30 & 45 cm) in each plot to collect soil solution. A vacuum was placed on each of the moisture samplers prior to sampling using 50ml polypropylene syringes. Rainfall was collected from two areas on opposite ends of the site. Rainfall and soil solutions were analysed using a Perkin Elmer Optima 3000 inductively coupled plasma optical emission spectrometry (ICP-OES). Soil solution ammonium (NH$_4^+$), nitrate (NO$_3^-$) and nitrite (NO$_2^-$) were determined using a ChemLab England Autoanalyser.

Laboratory experiments

An automated laboratory soil incubation system at IGER, North Wyke enabled the quantification of nitrogen and nitrous oxide gases from Watlington soils of varying copper concentrations (see abstract). Soil cores were collected from the long term sewage sludge and metal salt trials at Watlington. The soil was wetted to 75 – 80% water filled pore space (WFPS) before being placed into a cylindrical incubation vessel. The system and methodology is described in Cardenas et al. (2003). The incubation lasted 27 days. Watlington soil cores each from the sewage sludge I and II treatments were used as well as six cores from control plots, three of which would be amended whilst the remaining three would be used as pure controls. Three periods of amendment occurred where a CuSO$_4$.5H$_2$O solution with nutrients were added to the chambers. The cores were allowed to stabilise for 5 days before a CuSO$_4$.5H$_2$O solution with KNO$_3$ and glucose was added to three control cores.

RESULTS

For the purpose of this report, only the gaseous emissions analysis from the year long monitoring programme at Watlington and the first 10 days of using the automated denitrification system at IGER are discussed. During the monthly monitoring period from January to December, all four treatments showed similar low N$_2$O fluxes (Figure 1) from January to June. Fluxes for sewage sludge II (high Cu) were significantly higher than both the control and metal salt (high Cu) treatments in January. No significant differences in N$_2$O fluxes existed between treatments for February, March, April, May and June. N$_2$O fluxes were very low for this period with N$_2$O emissions not exceeding 9 g N ha$^{-1}$ d$^{-1}$ from any treatment on any observation date. Sampling was intensified around fertilisation events as it was hypothesised that any potential differences in biogenic soil gas fluxes between treatments would be most evident after these events when the soils had been enriched with N. N$_2$O fluxes remained at low levels for 10 days after fertilisation, at which time all treatments exhibited an increase in N$_2$O fluxes. The highest N$_2$O fluxes, on this day were exhibited by those treatments which had received sewage sludge applications whilst the smallest increase in N$_2$O flux was experienced by the inorganic metal salt amended plots. No significant differences in N$_2$O fluxes between treatments were observed for all sampling days with the exception of 21.05.05 where fluxes from the metal salt treatment were significantly different from the sewage sludge II treatment. Sampling frequency was increased again to cover this second fertilisation event. N$_2$O fluxes increased 3 days after fertilisation with a second high flux exhibited by the sewage sludge treatment 11 days after fertilisation. Fluxes from all treatments displayed some minor N$_2$O emissions for approximately 3 weeks after fertilisation after which fluxes tailed off to < 5 g N ha$^{-1}$ d$^{-1}$.

Very low or negative CH$_4$ fluxes (indicating a sink function of soil for CH$_4$) were evident throughout most of the monthly sampling programme (Figure 2). An exception to this occurred in March as shown by the sewage sludge II treatment where the CH$_4$ flux for this treatment was quite high when compared to the control and sewage sludge I treatments. The metal salt treatment displayed an opposite trend with a high CH$_4$ removal from the soil atmosphere. No significant differences in CH$_4$ fluxes existed between treatments for all the monthly measurements with the exception of February. Here the CH$_4$ fluxes from the metal salt treatment were significantly higher than all other treatments. No significant differences in CH$_4$ fluxes between treatments were observed for all sampling days during the first fertilisation period. CH$_4$ fluxes were again very low for the increased sampling programme surrounding the second fertilisation period. No significant differences in CH$_4$ fluxes between treatments were observed for the duration of this intensive sampling phase.
CO$_2$ fluxes for January, February and March were similar for all treatments (Figure 3). CO$_2$ fluxes increased for all treatments until July, with the exception of May where CO$_2$ fluxes fell for the metal salt, control and sewage sludge I treatments. After July CO$_2$ fluxes continued to fall for all treatments until November. CO$_2$ fluxes increased slightly in December for the control and sewage sludge I treatments. CO$_2$ fluxes were very similar for all treatments for the duration of this monitoring period. Statistical analysis revealed no significant differences in CO$_2$ fluxes within the control and sewage sludge II treatments for the duration of this intensive sampling phase. CO$_2$ fluxes for this sampling period showed a similar pattern to N$_2$O fluxes from the same period. Two increased periods of CO$_2$ fluxes occurred 3 and 14 days after fertiliser application, after which CO$_2$ fluxes generally decreased for all treatments until 29th July where CO$_2$ fluxes increased for all treatments. The highest CO$_2$ fluxes observed were exhibited again by the sewage sludge treatment II.

Results from the first condition controlled experiment using the denitrification system is shown in Figure 4. A sharp increase in the N$_2$O emissions from all treatments is observed approximately 24 hours after KNO$_3$ application. Increased N$_2$O emissions were highest from the control treatment i.e. the lowest copper treatment. Conversely the lowest N$_2$O emissions were from the high copper sludge treatment. The amended control (which had its copper concentration raised to the same concentration as that of the low copper sludge treatment) showed a very similar flux pattern to that of the low copper sludge treatment. The increased N$_2$O levels subsided 3 days after fertiliser application to low emission levels.

![Figure 1](image1.png)

**Figure 1.** Mean fluxes of N$_2$O from soils of all treatments for January to December 2005 (N=3, vertical bars denote standard errors). Soil copper concentration of the metal salt, control, sewage sludge I and sewage sludge II treatments are 273.4, 13.3, 46.3 and 278.3 mg Cu/kg soil respectively.

![Figure 2](image2.png)

**Figure 2.** Mean fluxes of CH$_4$ from soils of all treatments for January to December 2005 (N=3, vertical bars denote standard errors). Soil copper concentration of the metal salt, control, sewage sludge I and sewage sludge II treatments are 273.4, 13.3, 46.3 and 278.3 mg Cu/kg soil respectively.
Figure 3. Mean fluxes of CO$_2$ from soils of all treatments for January to December 2005 (N=3, vertical bars denote standard errors). Soil copper concentration of the metal salt, control, sewage sludge I and sewage sludge II treatments are 273.4, 13.3, 46.3 and 278.3 mg Cu/kg soil respectively.

Figure 4. Mean fluxes of N$_2$O from soils of all treatments from 8th – 17th June 2006. Soil copper concentration of the sewage sludge I and sewage sludge II, amended control and control treatments are 46.3, 278.3, 46.3 and 13.3 mg Cu/kg soil respectively.

CONCLUSIONS

In the field monitoring programme, those treatments with low soil copper levels did not demonstrate any significant increase in the emissions of N$_2$O and CH$_4$ from soils with low copper concentrations as compared to treatments containing high soil copper concentrations. However from the laboratory study, emissions from treatments with low soil copper levels are elevated with respect to emissions from high copper treatments. The increased N$_2$O emissions from the soil cores from the laboratory experiments lasted for only 2 days under ideal conditions. This increase could have easily been missed from the field monitoring scheme even when been intensively sampled. The hypothesis has not been proven false to date. Soil chemical analyses (data not shown here) confirm some of the beneficial aspects of using sewage sludge e.g. the significantly higher levels of soil Total N, LOI and moisture contents in the sewage amended soils. Molecular biology techniques are being employed in the final year of the PhD project to investigate differences in the microbial populations between treatments and aim to elucidate whether increased trace metal concentrations have an effect on microbial communities and more specifically on their role in key biogeochemical reactions in soil.
REFERENCES


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