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Emission of methane and nitrous oxide from Lake Ellesmere wetlands and their relationship to soil microbial processes

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NIWA Science and Technology Series No. 41

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1.0 INTRODUCTION

Wetlands play an important role in the emission of biogenic gases to the atmosphere. Natural wetlands are the single biggest source of atmospheric methane, an important greenhouse gas, and have been estimated to contribute between 20% and 50% of the total methane flux to the atmosphere (Cicerone and Oremland 1988, Tyler 1991). In addition, they also produce globally significant quantities of the greenhouse gases nitrous oxide and carbon dioxide. The role of wetlands in the global methane and nitrous oxide cycles is not clearly understood due, in part, to an incomplete understanding of the mechanisms controlling the production, emission and consumption of these gases.

In New Zealand wetlands have been estimated to be the forth most important source of methane, emitting between 0.04 and 0.16 Tg y^{-1}, roughly one tenth the rate of the largest methane source, livestock (Lassey *et al.* 1992). Pasture was rated as the largest source of N_2O with organic soils (presumably including wetland soils) contributing much smaller quantities (Sherlock *et al.*, 1992).

A comprehensive study of gas flux from wetlands has not yet been conducted in New Zealand. The objectives of this study were:

- To conduct preliminary investigations of methane and nitrous oxide fluxes from brackish (low to medium sulphate concentrations) and saline (high sulphate concentrations) wetland sites on the margins of Lake Ellesmere, Canterbury.

- Investigate those physical, chemical and microbial processes in the wetland soils which may regulate the emission of these gases.

2.0 SAMPLING SITES

The two sampling sites were situated on lacustrine wetlands on the margin of Lake Ellesmere (Te Waihora), Canterbury (Fig. 1), a shallow brackish coastal lagoon draining a catchment of 22,000 km^2. The area of the lake varies considerably according to its level which is artificially manipulated by the Canterbury Regional Council. The Harts Creek site was in a reserve on a small freshwater wetland on the southern edge of the lake. This site was

surrounded by willows (*Carex* sp.) and contained mainly indigenous vegetation Raupo (*Juncus gregiflora*) and *Agrostis stolinifera*, and some parts of it had experienced agricultural reclamation. The Wolfes Road site was on the eastern edge of the lake and represented the most saline part of the lake margin. This site was dominated by plant communities typical of brackish habitats with the salt tolerant *Juncus maritimus* interspersed with large areas of *Coltula coronopilus*. The hydrological regime of Te Waihora is partly governed by artificial adjustments of the lake level with both sites likely to experience varying degrees of inundation. Lake level over the study period were provided by the Canterbury Regional Council (Fig. 2a).

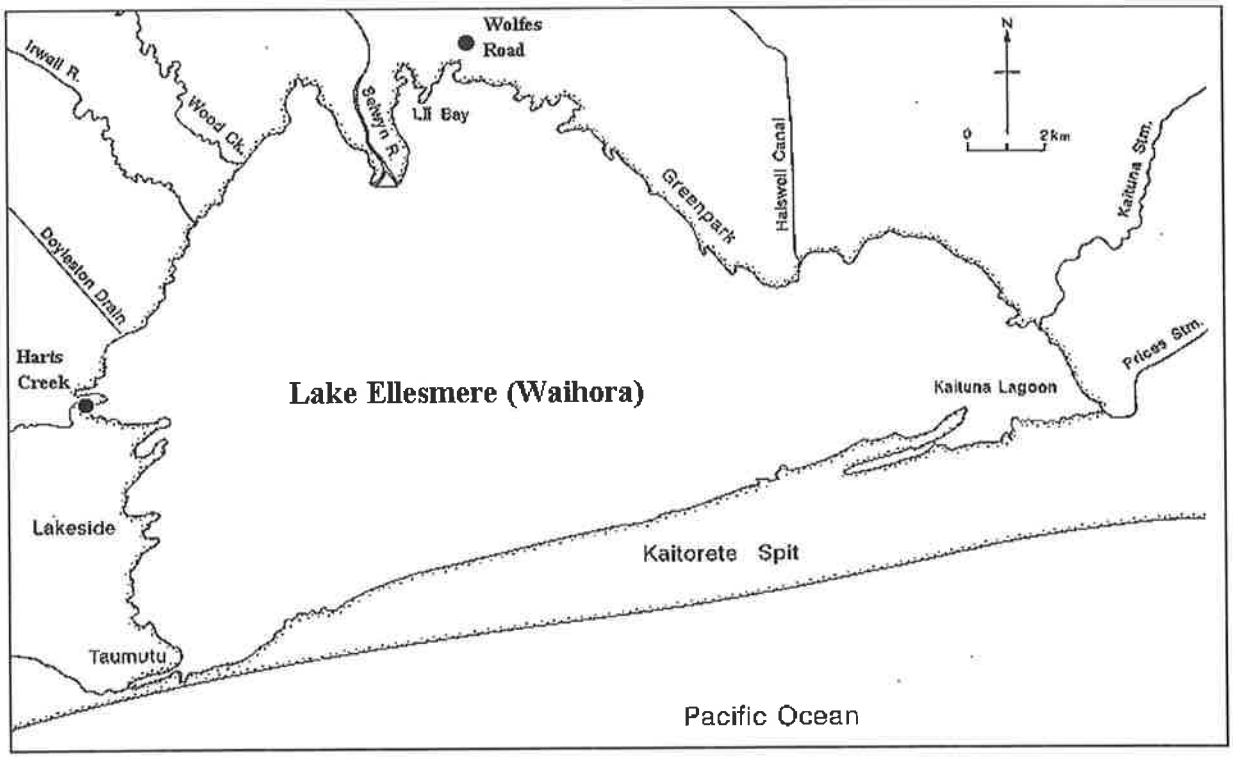


FIGURE 1. Map of Lake Ellesmere shoreline showing the gas flux measuring sites at Harts Creek and Wolfes Road

Preliminary gas flux and soil samplings were carried out from November 1993 to June 1994. From then on six more comprehensive sampling expeditions were carried out between August 1994 and March 1995.

3.0 MATERIALS AND METHODS

3.1 Water Table Position

At each site, PVC pipes (700 mm x 20 mm I.D.), with small (4 mm) holes drilled at 1 cm intervals at each side, were inserted into the ground to a depth of 30 cm and leaving 40 cm protruding. This allowed a constant reference point for measuring the height of the water table relative to the soil surface (Fig. 2b).

3.2 Soil Temperature

Soil temperature was measured at a depth of 10 cm using a thermistor probe which was calibrated against a high sensitivity mercury thermometer (Fig. 2c).

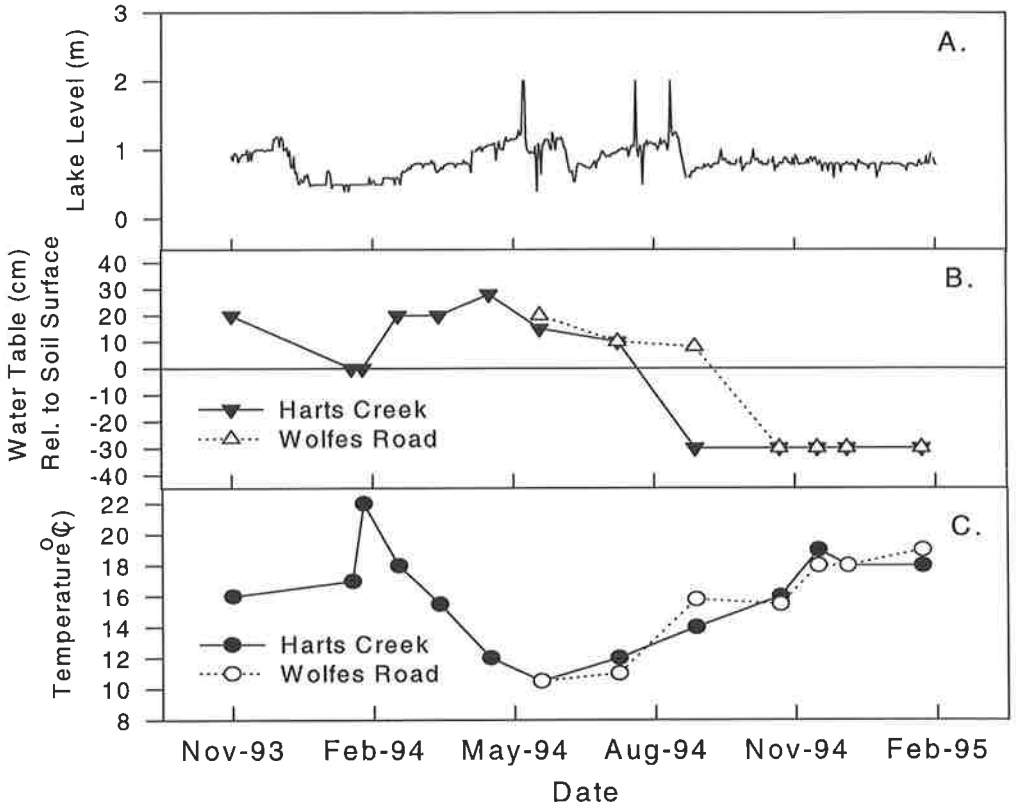


FIGURE 2. (a) Lake Ellesmere lake level, (b) the water table at each site relative to the soil surface and c) soil temperature at 10 cm depth, on each sampling date.

3.3 *In situ* Gas Flux Measurements

Gas fluxes were measured from the accumulation of methane, nitrous oxide and carbon dioxide in static flux chambers. Aluminium collars, enclosing an area of 0.281m^2 and a volume of 56 - 76 litres (Appendix III) , were embedded in the wetland soil to a depth of about 10 cm (Fig. 3). The collars had a trough around their top edge into which a clear acrylic dome could be fitted. An air-tight seal was produced by filling the trough with water. The domes had two, 1 cm diameter, holes drilled through them sealed with tight-fitting Neoprene bungs and through which gas samples could be withdrawn into 50 ml plastic syringes via hypodermic needles. It was found necessary to cover the clear domes with aluminium foil to reduce radiant heating and maintain temperatures close to outside ambient levels inside the enclosures. The collars were put in place at least two weeks before sampling began to allow

any disturbance to the site, caused by the installation of the collars, to subside. Four collars were put in place at each site.

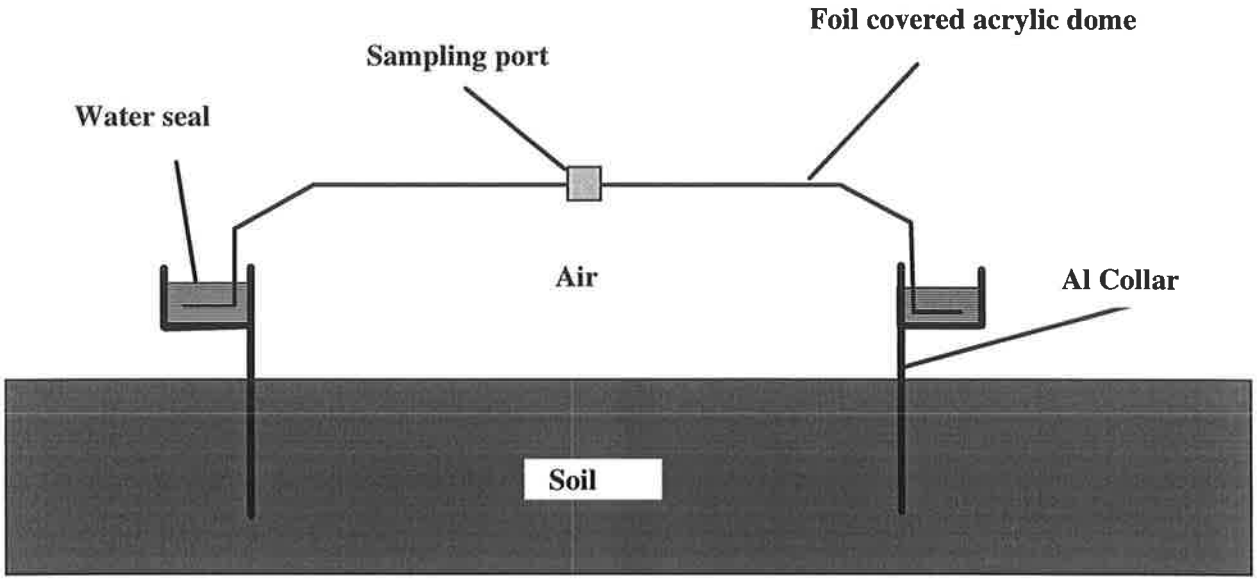


FIGURE 3. Flux box for field measurements of methane and nitrous oxide emissions.

Gas sampling was carried out by first installing the domes with the Neoprene bungs removed, to avoid pressurising the enclosure. The trough was filled with water and the bungs put in place. 50 ml of gas was removed from each enclosure into the 50 ml syringes at 10 minute intervals over a period of one hour. 50 ml of air was injected into each enclosure, prior to the removal of each gas sample, to maintain atmospheric equilibrium.

3.4 Gas Analysis

Gas samples from within the enclosures were analysed within 24 h of collection. Methane was analysed by injecting 1 ml of gas into a Hewlett-Packard 5890 gas chromatograph equipped with a flame-ionisation detector. The methane was separated on a 2 m, 5A molecular sieve column with a helium carrier gas flow of 25 ml min^{-1}. Nitrous oxide was analysed on a Perkin-Elmer Sigma 4 gas chromatograph with an electron capture detector as described by Vincent and Downes (1981). Flux rates were calculated as detailed in Appendix III.

Methane oxidation rates were measured *in situ* on four occasions over the study period by injecting $^{14}CH_4$, of known specific activity, into the hood. The specific activity of the methane was measured as described by Zehnder *et al.* (1979). $^{14}CO_2$ produced by methane oxidation was extracted from a sub-sample of gas into 5 ml of 1M sodium hydroxide. The sodium hydroxide extract was purged with oxygen-free nitrogen to remove $^{14}CH_4$ and 3 ml mixed with Optiphase HiSafe III scintillation cocktail. The samples were then counted in a Wallac Rackbeta scintillation counter with automatic quench correction. The amount of methane oxidised was calculated from the dpm of this sample from the relationship:

$$\text{Methane Oxidised } (\mu M\ m^{-2}) = \frac{CH_4 \times {}^{14}CO_2}{{}^{14}CH_4 \times A}$$

Where:

$^{14}CH_4$ is the activity of methane in the enclosure at the start of the experiment in dpm
CH_4 is the concentration of methane measured in the enclosure at each sampling time in μM.
$^{14}CO_2$ is the dpm activity of the CO_2 in the enclosure at each sampling time in dpm.
A is the area of soil, in m^2, covered by the enclosure.

3.5 Wetland soil analyses

All cores had the same three characteristic soil horizons but the horizons varied in depth and extent. Horizon A was a dark brown/black fine organic layer principally composed of dead and living roots overlying horizon C a grey sandy loam gley soil. Although the gley horizon was sharply demarcated, the organic layer indicated periods of intermittent waterlogging at greater depths. This intermediate layer, horizon B, was characterised by a coarser texture (silty clay) than the A horizon with yellow/grey mottles against a background of a lighter brown, more mineralised soil.

On each sampling occasion four replicate soil cores were taken from each site, using 50 cm lengths of 8 cm diameter plastic water pipe, for analysis in the laboratory. The pipes containing the soil cores were sealed immediately after collection to prevent oxygen intrusion. In the laboratory each soil core was sliced horizontally into four, 6 cm deep, layers and sub-samples of each slice taken for chemical analysis and bacterial assays. For anaerobic assays the soil was handled in a glove box flushed with oxygen free nitrogen to maintain anoxic conditions.

3.5.1 Chemical Analysis

Soil moisture content and dry weights were measured by drying samples at 105 °C for 24 h. Organic matter was determined by the weight loss of oven dry, 0.2 mm sieved soil after combustion at 550 °C for 5 h. and conductivity were measured on air dried samples as described in Blakemore *et al.* (1987).

Soil nitrate and sulphate were measured by extracting 10 ml of 2 mm sieved, field moist soil with 25 ml Milli RQ water in a 50 ml centrifuge tube. The tubes were capped, shaken vigorously by hand and then placed on a rotary shaker at 120 rpm for 30 minutes. The soil extracts were then centrifuged at 5000 rpm for 15 minutes and the supernatent filtered through a Whatman GF/F filter and stored frozen (-20°C) prior to analysis. The anion concentrations were measured on 20 µl of sample by ion chromatography. The anions were separated on a Dionex AS9 anion exchange column using an eluent of CO_3^{2-}/HCO_3^- (1.8/1.7 mM) pumped at 2 ml min^{-1} with a Shimadzu LP6A pump (Fig. 4). Detection was by a Dionex EC10 electrochemical detector in conductivity mode after auto-suppression of the eluent conductivity signal. Peak areas were integrated on a Hewlett-Packard 3990 integrator or by computer using Waters Baseline™ integration and data processing package. The instrument was calibrated using standard anion solutions.

Ammonia was analysed by extraction into 2M potassium chloride solution followed by colorimetric analysis by flow injection.

A summary of some of the physical and chemical soil parameters measured is given in Appendices I and II.

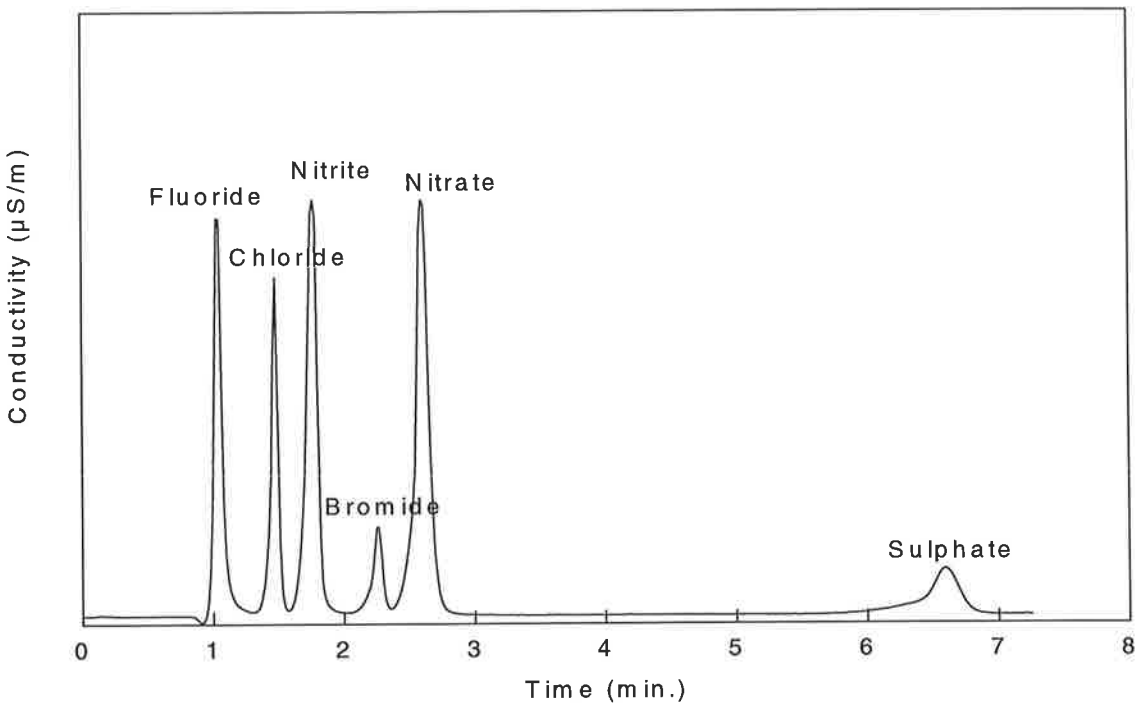


FIGURE 4. Separation of wetland porewater anions by ion chromatography

3.5.2 Bacterial assays

Potential rates of methanogenesis and denitrification enzyme activity (DEA) were conducted by incubation of soil slurries in sealed 150 ml serum vials flushed with oxygen free nitrogen.

Methanogenesis was estimated by measuring the rate of accumulation of methane in vials incubated with constant shaking at 25°C. Methane accumulation in the vial head-space was measured on the same GC system described above. The amount of methane produced was corrected for the methane dissolved in the slurry (<5%) and related to the dry weight of sediment.

DEA was estimated by the acetylene inhibition technique (Baldeston and Payne 1976, Yoshinari and Knowles 1976). The soil slurries were initially incubated under anoxic conditions for six days with 10% by volume of acetylene in the head-space to test for denitrification of "native" nitrate. The head-space gas was analysed for nitrous oxide on a

daily basis. After six days nitrate equivalent to 1 mg N l^{-1} was then added to the slurry and the accumulation of nitrous oxide in the head-space monitored over three days. The total amount of nitrous oxide produced was calculated from the head-space concentration by an empirically derived relationship and was related to the dry weight of sediment (Appendix III).

Nitrification potentials were measured by monitoring nitrate production in soil slurries incubated under aerobic conditions over six days. The slurries were amended with ammonium sulphate equivalent to 1 mg NH_4-N l^{-1} to ensure an adequate supply of substrate. Nitrate concentrations in the slurries was measured by ion chromatography as described above.

4.0 RESULTS AND DISCUSSION

4.1 Emissions of methane and nitrous oxide

4.1.1 Methane

Methane and nitrous oxide were emissions measured on 13 occasions from spring 1993 to late summer 1995. The seasonal pattern of methane emission at both sites is shown in Figure 5. The highest emission rates were in October 1993 and methane flux decreased over the two subsequent sampling occasions. Emission rates from individual flux boxes at each sampling date over this period were quite variable ranging from 1.94 to 15.8 mM m^{-2} day^{-1}. From late February to June emission rates were detectable but remained below 0.5 mM m^{-2} day^{-1}. A second emission peak was recorded for both Wolfes Road and Harts Creek in September 1994 but methane emissions were undetectable after this period. A slight negative flux was observed at Harts Creek in November 1994, indicating net methane consumption on this date.

Methane emission was generally higher at Harts Creek than at Wolfes Road with the highest individual flux of methane (8.56 mM m^{-2} day^{-1}) measured at Harts Creek in October, 1993. The overall mean of methane emission across all sampling periods at Harts Creek was 1.27 mM m^{-2} day^{-1}. In contrast, the overall mean emission rate of methane at Wolfes Road from May, 1994 to March, 1995 was only 0.15 mM m^{-2} day^{-1} . Over the same period, the mean emission from Harts Creek was 0.81 mM m^{-2} day^{-1}.

The within-site and between-site variability was greater at higher emission rates. Interestingly, methane emission at Wolfes Road was not much lower than at Harts Creek considering the higher pore water sulphate concentrations at Wolfes Road (Appendix I). No obvious

relationship existed between methane production and soil temperature or water level, although high rates of methane emission appeared to follow periods of high lake levels.

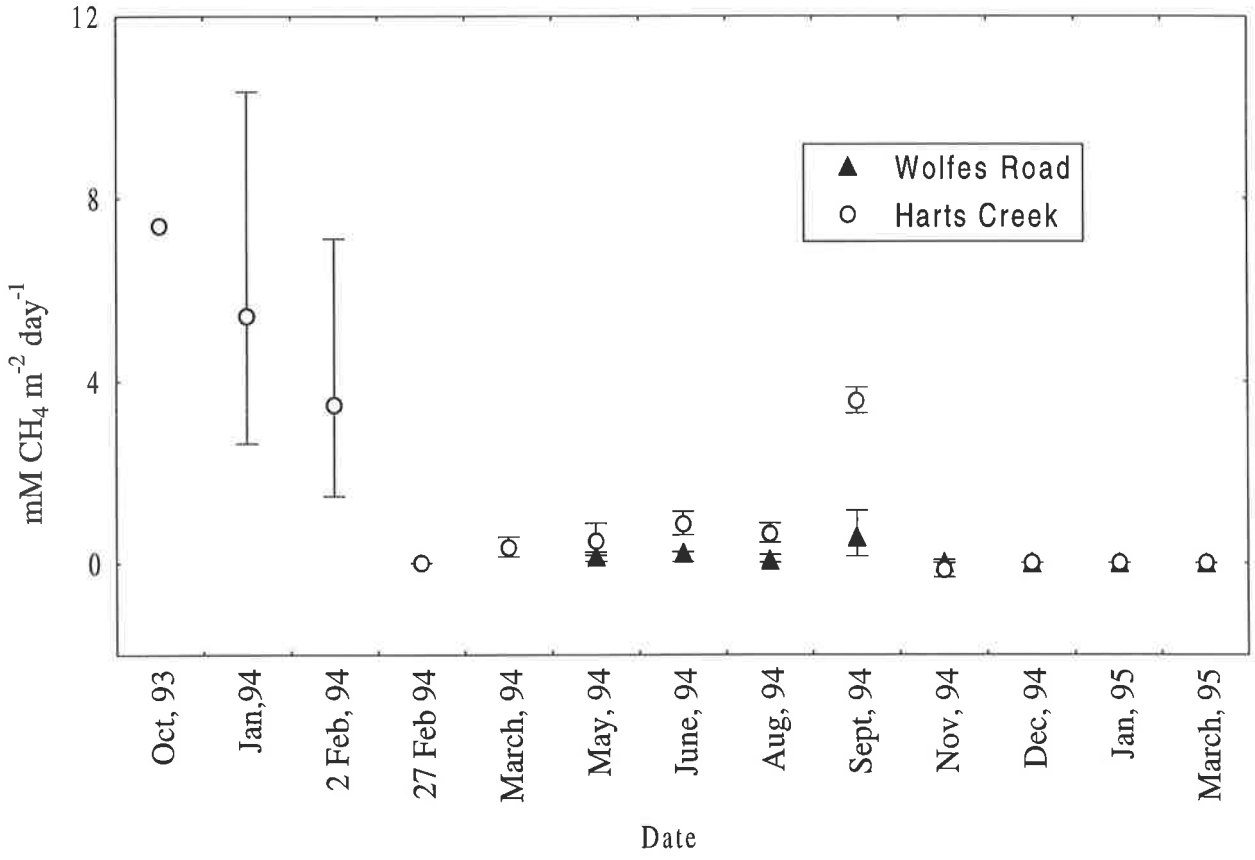


FIGURE 5. Seasonal variability in methane emission from Harts Creek and Wolfes Road sites (geometric mean ± one standard deviation).

4.1.2 Methane Oxidation

A preliminary field experiment in a single enclosure at Harts Creek on 4 February 1994, showed that methane oxidation could have a significant effect on methane flux (Fig. 6.). Total potential methane production (methane flux rate + methane oxidised) was 0.34 mM m^{-2} day^{-1} with methane oxidation accounting for 26% of the potential methane flux. Additional field measurements at Wolfes Road in May gave methane flux rates slightly lower than those measured at Harts Creek however the methane oxidation rate varied from 0.5% to 28% of the potential methane flux between enclosures (Table 1.). Workers using different techniques to

those described here have reported that methane oxidation can account for up to 91% of total methanogenesis (King *et al.* 1990). Net methane fluxes to the atmosphere were undetectable at both sites in January 1995 and methane oxidation rates were 0.0005 mM m^{-2} day^{-1} or lower (Table 1), suggesting that methane oxidation may be higher at times of higher methane emission. This suggestion conflicts with the general view that methane oxidation is controlled mainly by changes in water table position (Harris *et al.* 1982; Conrad 1989).

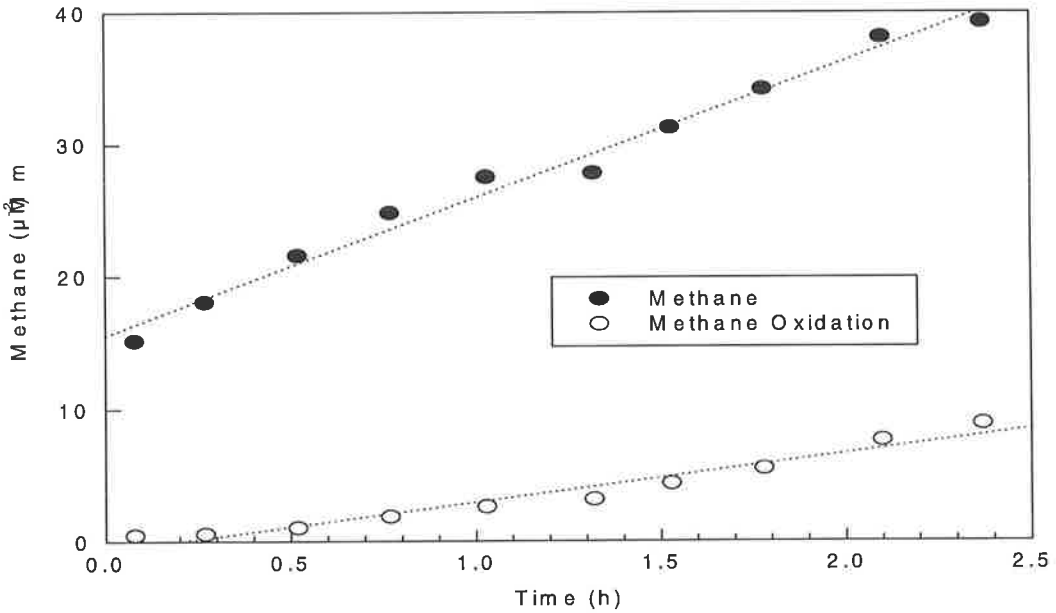


FIGURE 6 Field data from a single enclosure showing accumulation of methane and the amount of methane oxidised to carbon dioxide

These experiments were intended to provide a gross measurement of methane oxidation for inter-site comparisons and between different sampling periods. Since the rates of exchange of methane and carbon dioxide between the soil atmosphere and the external atmosphere are not known it is difficult to derive an accurate estimate of how much of the methane produced in the soil undergoes biological oxidation. However, they do demonstrate that these wetlands have the potential to provide a significant sink for methane, through oxidation to carbon dioxide, at methane concentrations close to the atmospheric mixing ratio, even under waterlogged conditions.

Table 1. Field measurements of methane flux and rates of methane oxidation on three sampling occasions. Measurements were taken over a one hour period

Date	Site[a]	Hood	Total potential CH_4 flux (mM m^{-2} day^{-1})	Measured methane flux (mM m^{-2} day^{-1})	Oxidised methane (mM m^{-2} day^{-1})	% Methane oxidised[b]
4 Feb. 1994	HC	C	0.335	0.250	0.089	26.1
3 May 1994	WR	A	0.108	0.094	0.014	12.9
		B	0.082	0.059	0.023	27.7
		C	0.092	0.092	0.0005	0.50
		D	0.109	0.108	0.0008	0.75
12 Jan. 1995	HC	A	0	0	0.0002	na
		C	0	0	0.0002	na
	WR	A	0	0	0.0005	na
		C	0	0	0.0002	na

[a] Hc = Harts Creek
WR = Wolfes Road
[b] = Methane oxidised as a percentage of the total potential methane flux.
na = not applicable as there was no measurable methane flux.

4.1.3 Nitrous Oxide

Over the total period of sampling, positive fluxes of nitrous oxide were recorded on only five occasions (Fig. 7). The highest fluxes were recorded in October 1993 and March 1995. Smaller fluxes were observed from January to March 1994. Nitrous oxide was emitted on only one occasion (6 March 1995) during the measurements where the two sites were compared. Here, both sites emitted over 10 μM m^{-2} day^{-1} but there was no statistical difference between the sites. Due to the high number of zero flux measurements, it was difficult to relate nitrous oxide emissions to temporal and spatial variation in environmental parameters. The highest flux measurement was 40.4 μM m^{-2} day^{-1} from a single chamber in March 1995. Smaller fluxes (4.1 - 5.2 μM m^{-2} day^{-1}) were observed between January and March 1994.

4.2 Soil Assays

4.2.1 Methanogenesis

Soil methanogenic activity varied both seasonally and with soil depth over the period of measurement especially at the Harts Creek site (Fig. 8a). Methanogenic activity in the top two

soil layers increased to a maximum in December and November for Harts Creek and Wolfes Road, respectively. The lower soil layers showed little variation either seasonally or with substrate

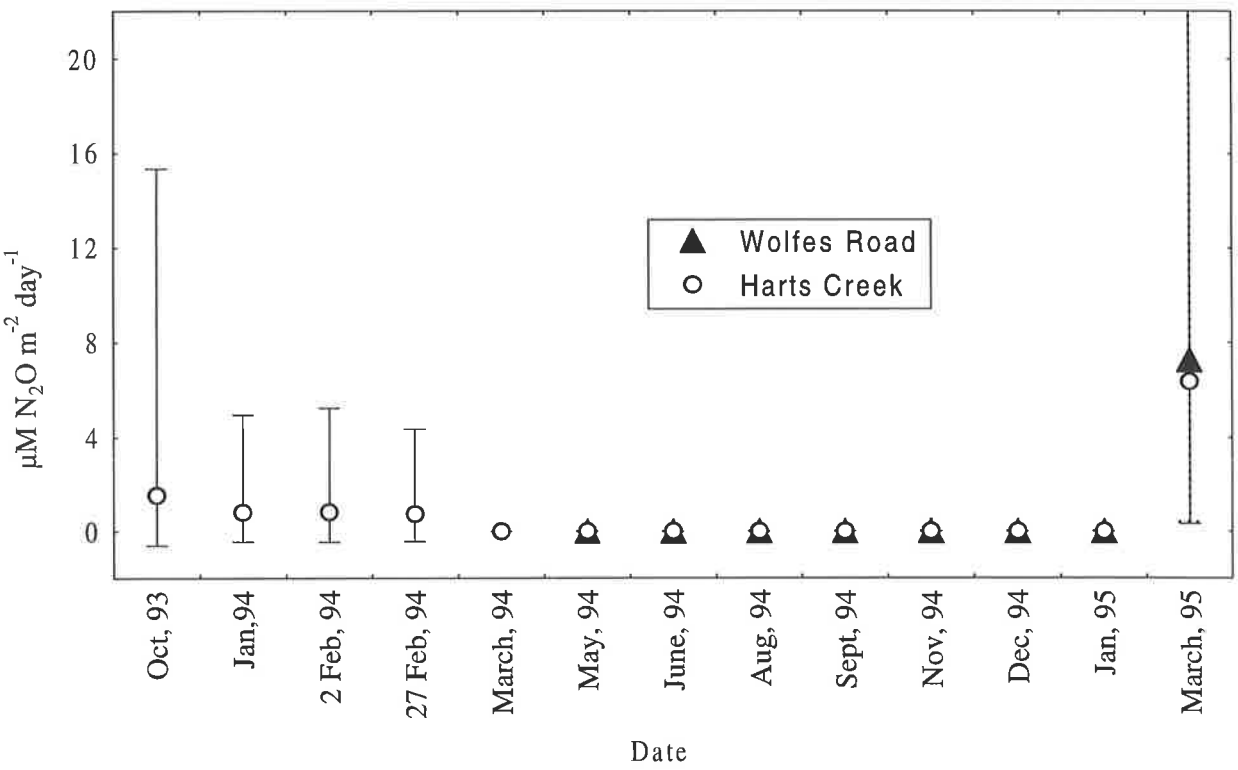
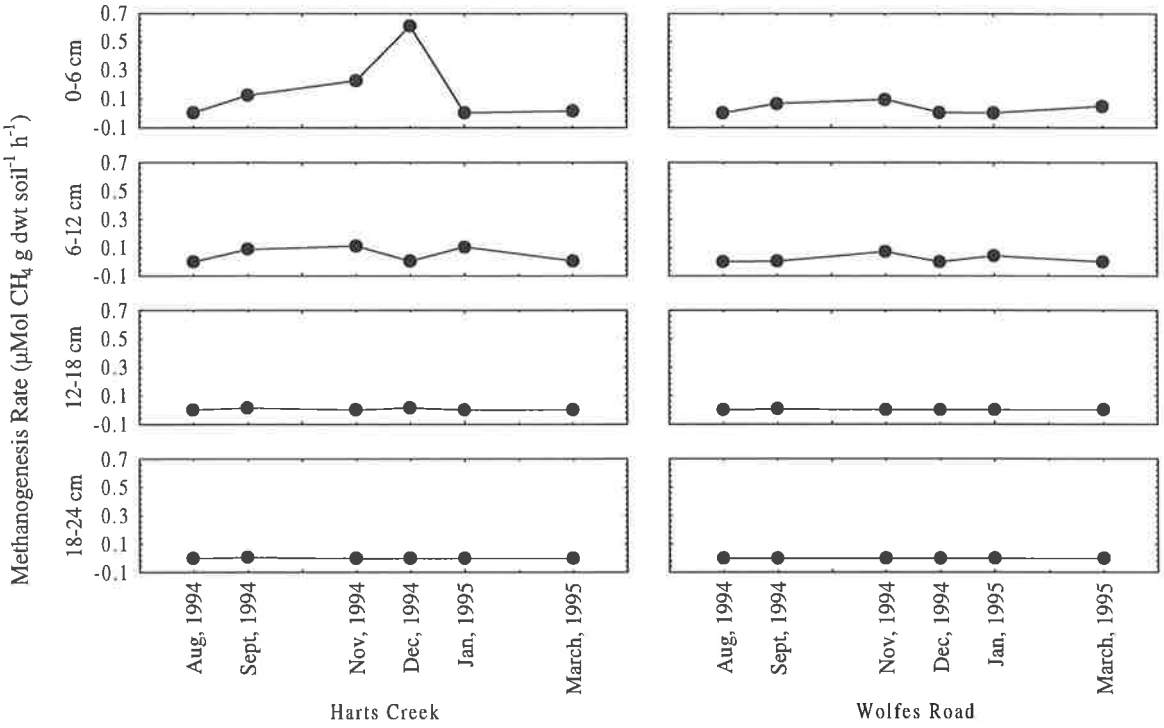


Figure 7. Seasonal variability in nitrous oxide emissions from Harts Creek and Wolfes Road (geometric mean ± one standard deviation)

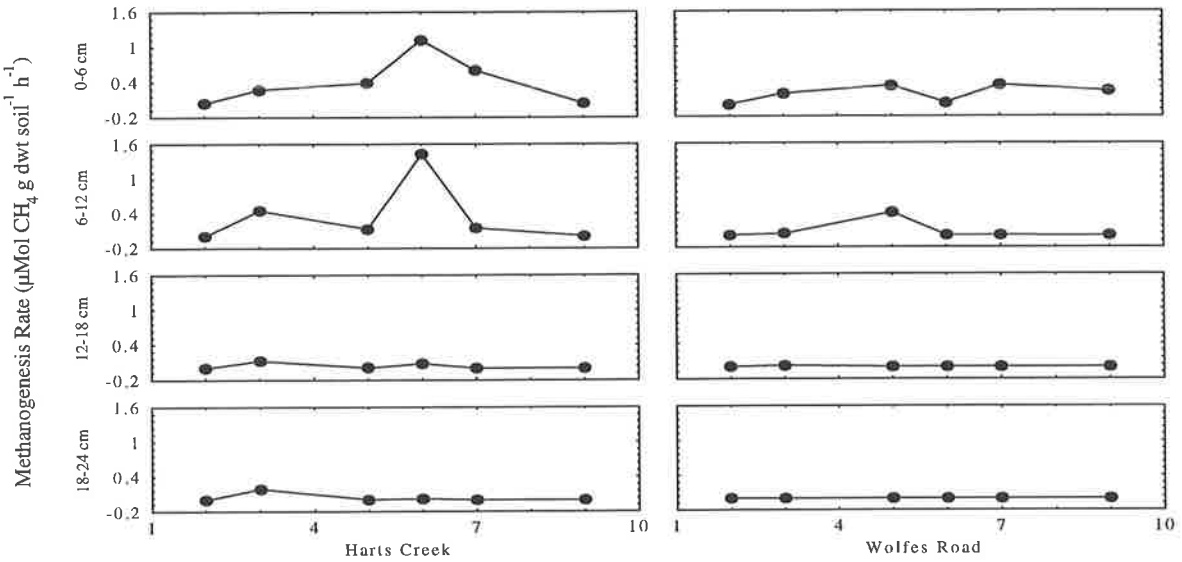
amendment. In general the substrate amended and non-amended methanogenic activity in the soil layers showed a similar seasonal pattern although acetate amended rates were about twice as high as the unamended rates at Harts Creek and four times higher at Wolfes Road (Fig. 8b). Average rates of unamended methanogenic activity varied between 0 and 7.91 µg CH_4 g dwt^{-1} h^{-1} and the overall mean methanogenic activity across all assay measurements was 0.44 µg CH_4 g dwt^{-1} h^{-1}.

The only occasion when methanogenic activity at Wolfes Road was significantly higher than Harts Creek was from soil collected at the March sampling period. At the same time sulphate levels in the soil were elevated at Harts Creek and lowered at Wolfes Road.

There were large differences between the substrate-amended and non-amended rates of methanogenesis suggesting that the supply of the methanogenic precursor, acetate, was limiting. There was a weak positive relationship between the rate of unamended methanogenesis for a particular soil sample and the corresponding rate when acetate was added (Figure 9). This indicates that factors other than acetate may be limiting the potential rate of methanogenesis. However, there



(a)



(b)

FIGURE 8 Seasonal pattern of methanogenic activity (a) without addition of acetate. (b) with 1 mg l^{-1} carbon as acetate.

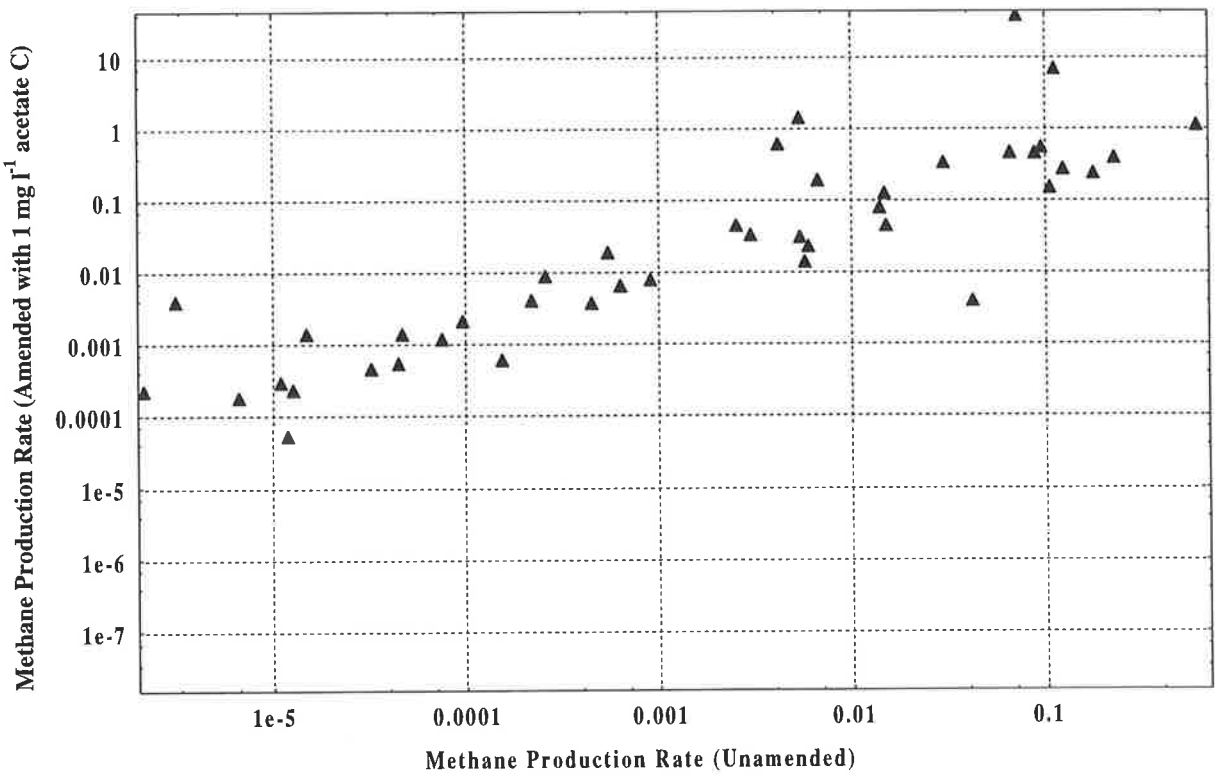


Figure 9. The relationship between amended and unamended methanogenesis in soil assays. Units are mM methane g dwt soil^{-1} day^{-1}.

was no association between the degree of response which each individual soil sample showed to amendment with acetate and the level of sulphate in the soil.

The top 12 cm of the soil exhibited the greatest response to the addition of acetate. There did not appear to be any seasonal pattern associated with the degree of response to acetate.

Figure 10 shows the variation in unamended methanogenic activity, integrated over the whole 24 cm soil core, from August 1994 to March 1995 for both sites. The integrated methanogenic activity under both substrate-amended and unamended treatments was generally lower at Wolfes Road than at Harts Creek and differences between sites were generally less than an order of magnitude with the exception of the November assays. On this occasion Harts Creek soil exhibited methanogenic activity about ten times higher than that of Wolfes Road.

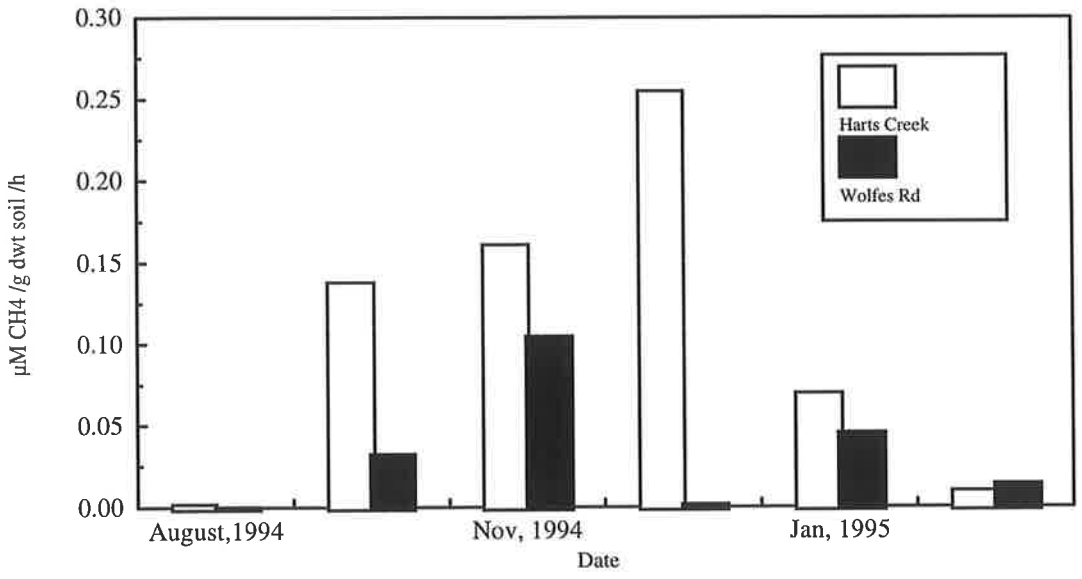


FIGURE 10 Trends in unamended methanogenic activity. Rates are calculated by integrating the methanogenic activity over the whole 24 cm of soil core.

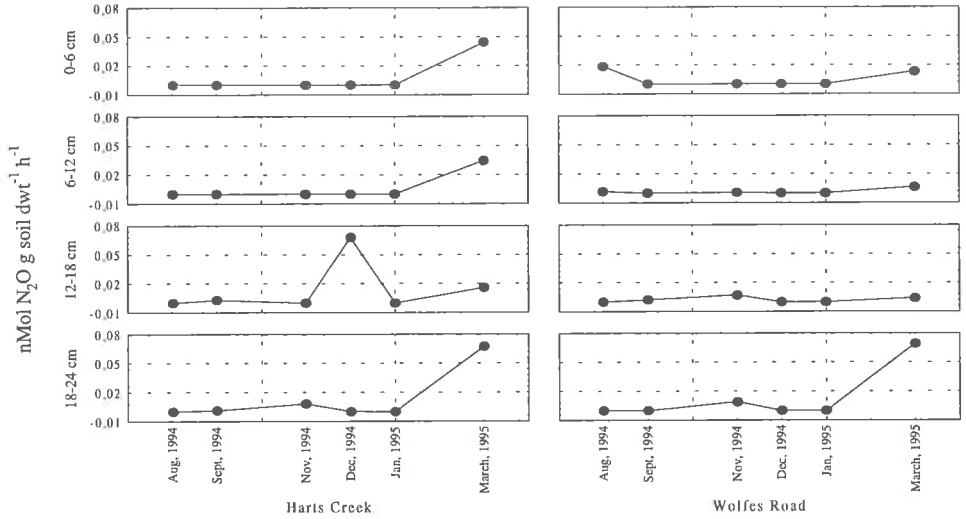
4.2.2 Denitrification enzyme activity (DEA)

Levels of DEA were highly variable among soil replicates and the standard deviations were correlated with the means suggesting a high degree of heterogeneity in the soil. Accordingly, most of the statistical tests were conducted on log-transformed rates.

DEA in unamended soil incubations did not differ significantly between sites or layers if the results from each sampling period were pooled. Very low rates (up to 3 nMol N_2O g dwt^{-1} h^{-1}) were detected in some of the incubation vials under the non-amended treatment. After the addition of 1 mg l^{-1} of nitrate higher rates of N_2O production (up to 13 nM N_2O g dwt^{-1} h^{-1}) resulted indicating that the DEA was limited by nitrate.

The non-amended DEA were highest in March while the amended DEA was highest in August (Fig. 11a). There was no clear relationship between the levels of soil nitrate and DEA. However, the lowest non-amended rates of DEA corresponded with periods of low nitrate in the soil.

The vertical distribution of DEA was not as clearly defined as it was for methanogenic activity. During the preliminary studies, the vertical distribution of DEA was investigated at a 3 cm



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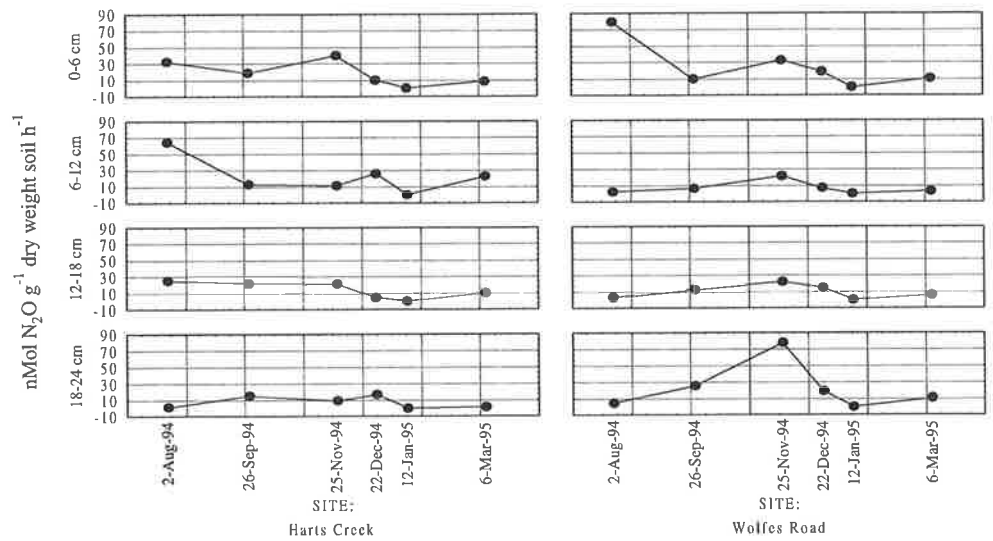


(b)

FIGURE 11 Seasonal changes in DEA in soil assays as measured by N_2O production rates in acetylene inhibited soil samples a.) with no amendments and b.) amended with 1 mg l^{-1} NO_3-N in the soil slurry

resolution in soil cores from Harts Creek. This study revealed that DEA was greatest in the 6 to 18 cm depths of the soil (Fig. 12). DEA appeared to be suppressed in the top 6 cm and the bottom 6 cm of the soil profile. Competition for nitrate from wetland plants may have been responsible for the inhibition of DEA in the top 6 cm since this was in the main root-zone.

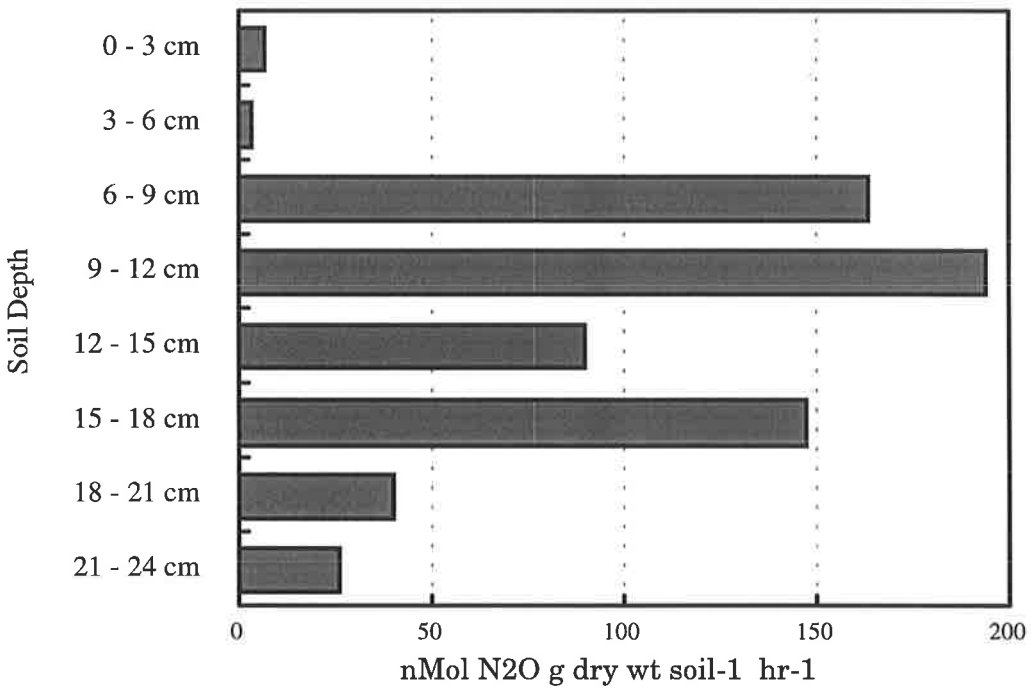
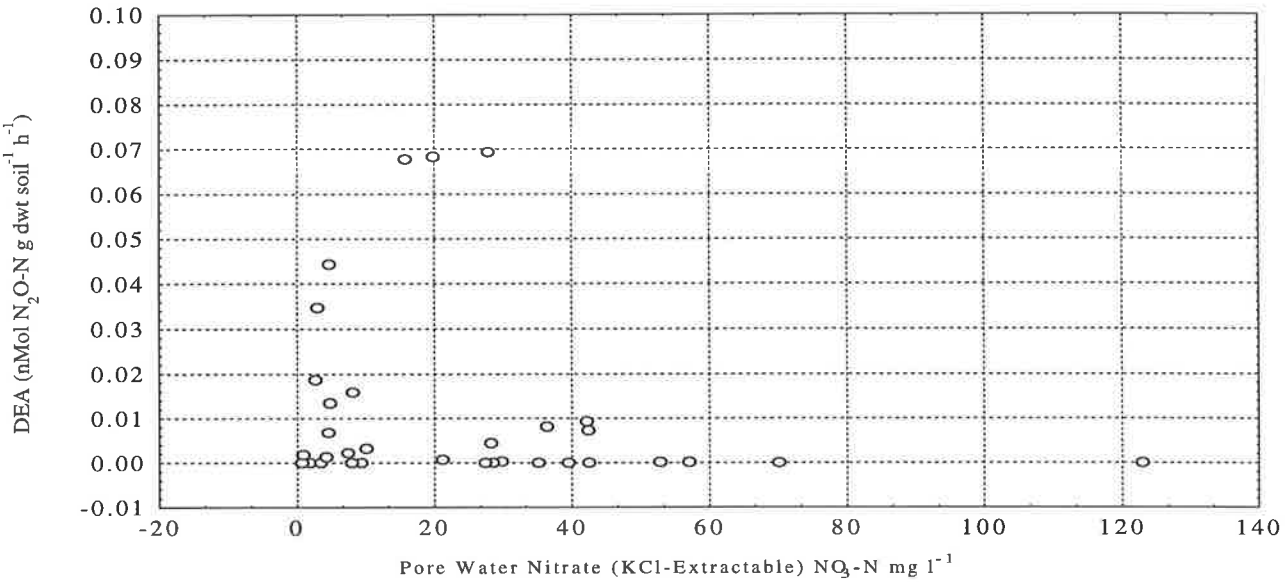


FIGURE 12 Distribution of DEA in a wetland soil core from Harts Creek.

The relationship between nitrate and the DEA suggests a depletion of soil nitrate in samples with high denitrifying activity. However, because the rates are relatively low and variable for the unamended treatment, it is difficult to detect the presence or absence of a nitrate dependence for DEA (Fig. 13a). However, a greater linear dependence was apparent after nitrate was added to the incubation vials (Fig. 13b).

Similar patterns of nitrate-amended DEA were observed from November, 1994 to March, 1995. Rates fell consistently from November to a minimum in January, 1995. In March, DEA increased for all sites and all layers. The lack of denitrifying activity that was observed in January coincided with the seasonal minimum dissolved organic carbon values (Appendix II), although DOC was not a good predictor of DEA. In general, relationships between *in situ* environmental variables and DEA activity were weak.



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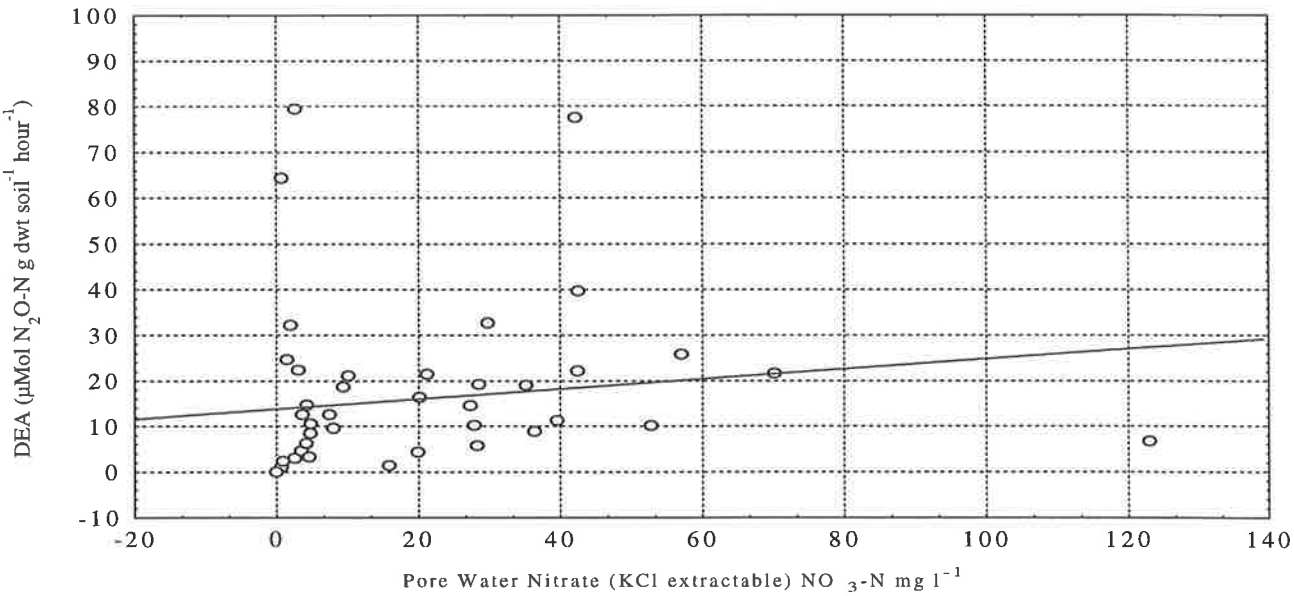

(b)

FIGURE 13. DEA activity in wetland soils vs. *in situ* nitrate concentrations. (a) No added nitrate. (b) With the addition of 1 mg l^{-1} NO_3-N.

Factors thought to regulate denitrification vary in importance with different environments. Cooke and White (1987) found a strong relationship between *in situ* denitrification and readily mineralisable carbon in an agricultural stream, whereas, Dodds and Jones (1987) found a relationship between DEA and redox potential but not with nitrate, ammonium or organic carbon content. However, Lockaby *et al.* (1994) stated that environmental variables often lack the predictive capacity to explain variations in denitrification rates.

4.2.3 Nitrification

The rates of nitrification were in the range of 0 to 3 mM N g^{-1} h^{-1} although nitrate occasionally decreased in the incubation jars suggesting active anaerobic denitrifying micro-sites were present in the soil slurries despite continuous shaking.

Generally rates of nitrification were low at *in situ* ammonium concentrations and were in the range 0 to 0.03 µg NO_3^--N g^{-1} dwt soil h^{-1}. With the exception of the August sampling period, rates of nitrification were at, or close to zero over the whole course of the study. The nitrification rates observed in August was also at a time of lower than average *in situ* ammonium concentrations.

The rate of nitrification increased by over two orders of magnitude when slurries were amended with 1 mg l^{-1} NH_4-N. This was despite the high background levels of KCl-extractable ammonium. This may indicate that either (a) KCl-extractable ammonium was not available to the nitrifiers or (b) competition for ammonium was occurring (e.g. by methane oxidising bacteria). Figure 14 shows the relationship between the rate of nitrifying activity and pore-water ammonium concentrations. A weak linear relationship existed between *in situ* ammonium concentrations and nitrifying activity.

Under the NH_4-N amended phase of the incubation, Harts Creek soil samples showed the maximal potential nitrifying activity from November to January while Wolfes Road was the most productive in the lower layer (12-18 cm) in December. Generally the potential rate of nitrification was below 2 µg NO_3^--N g dwt $soil^{-1}$ h^{-1} and overall the maximum potential rates at both sites were observed during the November to January period. In addition a pronounced dip in potential nitrification activity was observed for both sites during the January period

No trend was apparent between potential (NH_4-N amended) nitrifying activity and nitrate concentrations in the soil. In addition, the few data for non-amended nitrification rates also

showed no relationship with nitrate. This suggests that the supply of nitrate during the dry periods is limited by the rate of nitrification and that nitrous oxide fluxes from nitrification are unlikely to be large because of the low rates. Ammonium appears to be limiting for the nitrifiers but even under non-limiting conditions maximum rates are comparatively low.

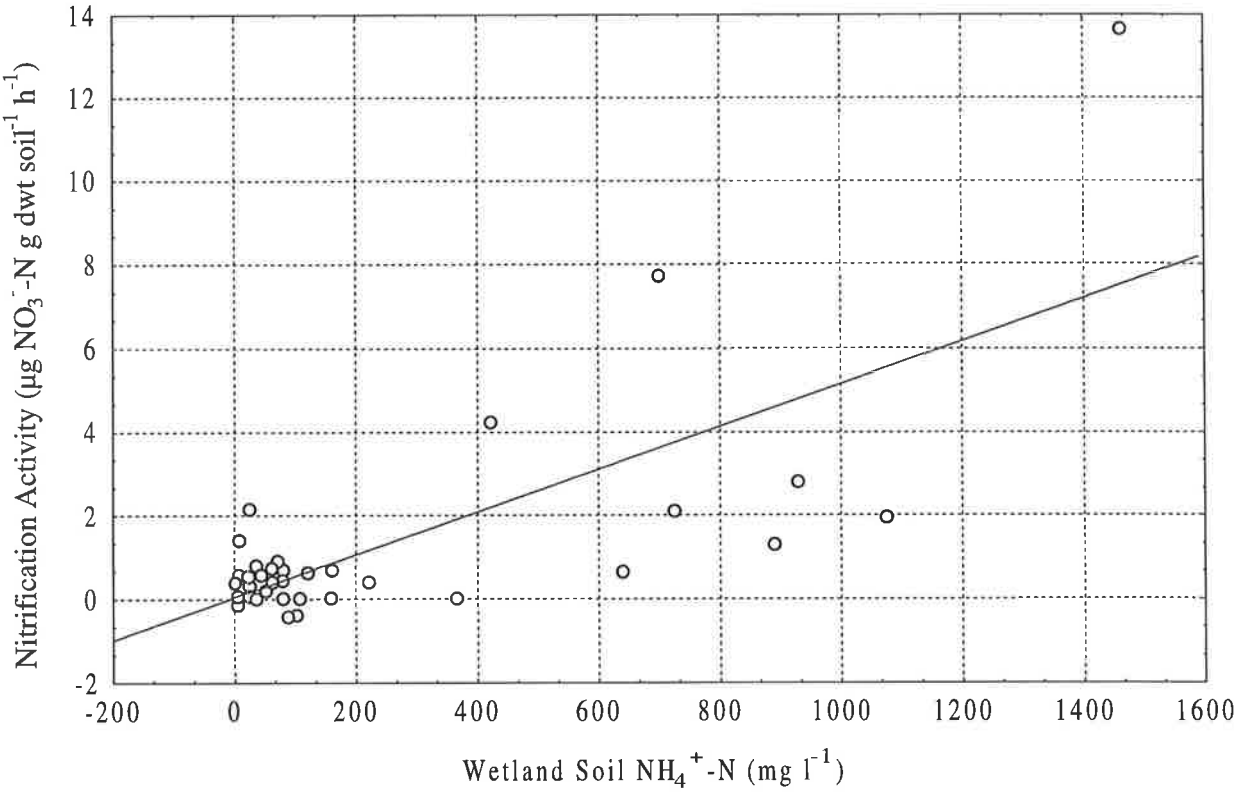

FIGURE 14. The relationship between wetland soil pore-water ammonia concentrations andnitrification activity. Nitrification Activity = 0.014 + 0.005 (NH_4^+-N)

5.0 SUMMARY

1. In general there was a high spatial and temporal variability of methane flux rates at Harts Creek and very low flux rates of all gases at Wolfes Road. Methane flux rates ranged from slightly negative, indicating net methane consumption, to a positive flux of 16 mM m^{-2} day^{-1}.

2. Field estimates of methane oxidation indicate that as much as 27% of the total methanogenesis may be oxidised to carbon dioxide. Low levels of methane oxidation

were also detectable when there was no measurable methane flux and methane concentrations were at normal atmospheric concentrations.

3. Nitrous oxide flux rates were frequently undetectable at both sites and were only detectable on two occasions at Harts Creek. The highest rate in a single enclosure was 40 μM m^{-2} day^{-1} in March 1995 with smaller fluxes (4.1 - 5.2 μM m^{-2} day^{-1}) observed between January and March 1994. Because of the large number of zero nitrous oxide flux measurements it was not possible to derive reliable relationships between nitrous oxide flux rates and *in situ* environmental variables.

4. Relationships between *in situ* environmental variables (temperature, soil nitrate, pore-water DOC and soil organic carbon) and DEA were weak.

5. Nitrification rates in the wetland soils were consistently low and appeared to be limited by ammonium despite the high levels of extractable ammonium measured. Since the nitrification process produces nitrous oxide as well as substrate for denitrifying bacteria this may partly explain the low nitrous oxide fluxes at these sites. There was a weak relationship between soil pore-water ammonia concentrations and nitrification activity.

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Appendix I: Wetland Soil; Physical and Chemical Characteristics.

Date	Site	Soil Depth	Organic Matter	Bulk Density g dwt/cm3	µg NH4-N/ g dwt Soil	µg NO3-N/ml Porewater	ug NO3-N/g dwt Soil	µg SO4/ml Porewater	µg SO4/g dwt Soil	DOC µg /ml Porewater
02/08/94	Harts	0-6 cm	62.98%	0.17	111.2	1.89	8.57	18.5	84.1	239.55
02/08/94	Harts	6-12 cm	27.86%	0.29	14.7	0.71	1.35	14.2	27.1	303.57
02/08/94	Harts	12-18 cm	10.40%	0.48	5.4	1.45	1.30	352.9	316.7	252.46
02/08/94	Harts	18-24 cm	8.31%	1.13	3.3	0.63	0.37	253.1	146.3	97.93
02/08/94	Wolfes	0-6 cm	70.47%	0.23	103.1	2.69	11.10	882.4	3641.4	100.95
02/08/94	Wolfes	6-12 cm	31.77%	0.86	17.0	0.88	1.19	2000.1	2706.9	106.87
02/08/94	Wolfes	12-18 cm	5.06%	1.33	2.3	2.58	0.82	2852.7	909.9	113.77
02/08/94	Wolfes	18-24 cm	2.93%	1.33	1.6	3.37	0.90	1455.9	387.5	98.52
26/09/94	Harts	0-6 cm	65.40%	0.17	1473.1	9.39	32.73	72.0	250.8	121.26
26/09/94	Harts	6-12 cm	26.24%	0.29	154.0	3.63	6.97	121.0	232.8	80.72
26/09/94	Harts	12-18 cm	8.88%	0.48	28.3	10.11	12.64	52.0	65.1	65.71
26/09/94	Harts	18-24 cm	9.62%	1.13	12.8	4.21	2.20	216.0	112.8	39.6
26/09/94	Wolfes	0-6 cm	59.20%	0.23	1910.8	7.99	23.85	1268.97	3786.7	93.6
26/09/94	Wolfes	6-12 cm	21.67%	0.86	33.1	4.19	5.45	2114.0	2745.7	70.01
26/09/94	Wolfes	12-18 cm	4.61%	1.33	5.4	7.42	1.67	2523.9	569.1	22.45
26/09/94	Wolfes	18-24 cm	8.12%	1.33	2.3	2.03	0.48	2211.9	525.6	19.67
25/11/94	Harts	0-6 cm	69.40%	0.17	2267.5	42.49	137.45	89.0	288.1	151.37
25/11/94	Harts	6-12 cm	25.12%	0.29	105.7	39.56	81.34	98.0	201.7	40.65
25/11/94	Harts	12-18 cm	6.24%	0.48	36.6	70.16	73.30	152.0	158.8	31.18
25/11/94	Harts	18-24 cm	7.21%	1.13	17.6	36.35	21.53	143.0	84.7	16.72
25/11/94	Wolfes	0-6 cm	66.70%	0.23	1534.4	29.77	62.98	1151.0	2434.6	82.60
25/11/94	Wolfes	6-12 cm	12.36%	0.86	82.7	21.19	22.31	1795.0	1890.8	14.91
25/11/94	Wolfes	12-18 cm	1.47%	1.33	15.6	42.30	10.61	1969.0	495.3	10.60
25/11/94	Wolfes	18-24 cm	3.45%	1.33	2.2	42.20	93.32	1818.9	402.0	11.11

Continued on next page

Appendix I: Wetland Soil; Physical and Chemical Characteristics contd.

Date	Site	Soil Depth	Organic Matter	Bulk Density g dwt/cm3	µg NH4-N/ g dwt Soil	µg NO3-N/ml Porewater	ug NO3-N/g dwt Soil	µg SO4/ml Porewater	µg SO4/g dwt Soil	DOC µg /ml Porewater
22/12/94	Harts	0-6 cm	68.98%	0.17	2688.8	52.79	152.60	105.4	304.6	275.32
22/12/94	Harts	6-12 cm	31.88%	0.29	197.2	57.05	110.78	121.4	235.7	27.74
22/12/94	Harts	12-18 cm	5.98%	0.48	113.0	19.89	31.63	245.4	390.4	19.67
22/12/94	Harts	18-24 cm	9.99%	1.13	9.3	20.18	11.83	161.0	94.6	16.53
22/12/94	Wolfes	0-6 cm	58.90%	0.23	1784.6	28.52	47.32	1335.1	2215.6	198.81
22/12/94	Wolfes	6-12 cm	9.93%	0.86	136.5	122.98	75.69	1994.1	1227.4	21.06
22/12/94	Wolfes	12-18 cm	2.16%	1.33	20.9	27.29	6.46	3218.3	764.7	10.97
22/12/94	Wolfes	18-24 cm	9.43%	1.33	0.7	35.14	8.25	1896.1	444.8	9.56
12/01/95	Harts	0-6 cm	56.93%	0.17	4297.0	0.00	0.00	197.8	580.9	92.25
12/01/95	Harts	6-12 cm	17.29%	0.29	277.2	0.00	0.00	199.1	346.3	37.12
12/01/95	Harts	12-18 cm	5.60%	0.48	105.5	0.00	0.00	112.0	109.7	63.09
12/01/95	Harts	18-24 cm	3.00%	1.13	10.8	0.00	0.00	48.9	14.7	10.00
12/01/95	Wolfes	0-6 cm	54.32%	0.23	2894.4	0.00	0.00	1661.8	2654.5	66.22
12/01/95	Wolfes	6-12 cm	7.35%	0.86	240.4	0.00	0.00	1774.3	1163.2	59.03
12/01/95	Wolfes	12-18 cm	1.90%	1.33	17.6	0.00	0.00	14801.7	3271.2	20.00
12/01/95	Wolfes	18-24 cm	2.11%	1.33	0.4	0.00	0.00	1684.0	387.34	8.00
06/03/95	Harts	0-6 cm	63.39%	0.17	2046.6	4.75	10.91	953.2	2190.3	108.25
06/03/95	Harts	6-12 cm	12.51%	0.29	170.5	3.02	4.25	269.46	379.4	36.70
06/03/95	Harts	12-18 cm	2.42%	0.48	38.1	8.07	7.07	379.1	332.2	30.81
06/03/95	Harts	18-24 cm	2.21%	1.13	6.3	15.81	4.05	193.7	49.7	43.29
06/03/95	Wolfes	0-6 cm	66.88%	0.23	11.4	4.81	6.51	929.6	1258.6	96.25
06/03/95	Wolfes	6-12 cm	31.34%	0.86	78.0	4.54	2.18	834.1	402.2	26.911
06/03/95	Wolfes	12-18 cm	6.86%	1.33	6.3	28.21	2.86	255.2	26.0	60.21
06/03/95	Wolfes	18-24 cm	2.93%	1.33	0.3	27.69	3.26	1063.4	125.1	28.08

Appendix II: Soil Redox Measurements. (mV - Standard Calomel mV)

Date	Soil Depth	Harts Creek mV	Wolfes Road mV
02-Aug-94	1.5 cm	-56	-100
02-Aug-94	4.5 cm	-56	-175
02-Aug-94	7.5 cm	-56	-100
02-Aug-94	10.5 cm	-56	-175
02-Aug-94	13.5 cm	-6	-225
02-Aug-94	16.5 cm	34	-175
02-Aug-94	19.5 cm	44	100
02-Aug-94	22.5 cm	94	350
26-Sep-94	1.5 cm	-156	-91
26-Sep-94	4.5 cm	-146	-76
26-Sep-94	7.5 cm	-142	-61
26-Sep-94	10.5 cm	-126	-49
26-Sep-94	13.5 cm	-80	-41
26-Sep-94	16.5 cm	-30	4
26-Sep-94	19.5 cm	20	48
26-Sep-94	22.5 cm	42	248
25-Nov-94	1.5 cm	-275	162
25-Nov-94	4.5 cm	-210	150
25-Nov-94	7.5 cm	-135	183
25-Nov-94	10.5 cm	-30	170
25-Nov-94	13.5 cm	15	241
25-Nov-94	16.5 cm	180	171
25-Nov-94	19.5 cm	300	162
25-Nov-94	22.5 cm	380	352
22-Dec-94	1.5 cm	40	238
22-Dec-94	4.5 cm	44	238
22-Dec-94	7.5 cm	44	263
22-Dec-94	10.5 cm	93	275
22-Dec-94	13.5 cm	180	263
22-Dec-94	16.5 cm	210	232
22-Dec-94	19.5 cm	224	282
22-Dec-94	22.5 cm	381	338
12-Jan-95	1.5 cm	7	238
12-Jan-95	4.5 cm	-44	338
12-Jan-95	7.5 cm	-11	307
12-Jan-95	10.5 cm	24	263
12-Jan-95	13.5 cm	137	275
12-Jan-95	16.5 cm	239	263
12-Jan-95	19.5 cm	347	232
12-Jan-95	22.5 cm	362	282
06-Mar-95	1.5 cm	289	277
06-Mar-95	4.5 cm	334	255
06-Mar-95	7.5 cm	323	218
06-Mar-95	10.5 cm	339	244
06-Mar-95	13.5 cm	341	196
06-Mar-95	16.5 cm	360	242
06-Mar-95	19.5 cm	377	265
06-Mar-95	22.5 cm	408	277

Appendix III: Gas Flux Calculations.

Methane.

The rate of **methane emission in mg m^{-2} day^{-1} (E)** was calculated from:

$E = C \times (1/RT) \times (273/298) \times (H_v/A) \times 1440 \times 0.001 \times M_w$

Where C = Rate of change in methane concentration in the flux box (ppmv/min.)

RT = 22.414

273/298 =correction for room temperature of 25°C

H_v = Volume of the flux box above the soil (L)

A = Area enclosed by the flux box (m^2)

1440 = minutes per day

0.001 = mg/µg

M_w = Molecular weight of methane (16)

Nitrous oxide.

The rate of **nitrous oxide emission** in µg N m^{-2} day^{-1} **(N),** was calculated from:

$N = C \times (1/RT) \times (273/298) \times (H_v/A) \times 1440 \times M_w$

Where C = Rate of change in nitrous oxide concentration in the flux box (ppmv/min.)

RT = 22.414

273/298 =correction for room temperature of 25°C

H_v = Volume of the flux box above the soil (L)

A = Area enclosed by the flux box (m^2)

1440 = minutes per day

M_w = Molecular weight of nitrogen in N_2O (28)

Flux box volumes (L)

Harts Creek Site

Box 1 (B)	Box 2 (D)	Box 3 (C)	Box 4 (A)
76.4	74.3	75.1	76.5

Wolfes Road Site

Box 1 (A)	Box 2 (B)	Box 3 (C)	Box 4 (D)
61.2	56.4	57.0	55.8

Appendix IV: Microbial Assay Calculations

DEA Assays

The solubility of nitrous oxide in the soil slurry is corrected for using the Henry's Law Constant for N2O of 0.0279 mol L^{-1} atm^{-1}. This is calculated from the distibution coefficient, H=1.6, (Liss and Slater, 1974; cited in Stumm and Morgan, 1981) where:

$$K_H = 1/HRT$$
$$= 1/(1.6)(22.414)$$
$$= 0.0279$$

The Bunsen Absorption Coefficient (α_B) is:

$$\alpha_B = K_H \times RT = (0.0279 \times 22.414) = 0.625$$

At each sampling occasion the amount of nitrous oxide produced from the soil, C, was the sum of A+B where:

$A = N_2O$ in soil solution ($\mu M\ l^{-1}$) = $[N_2O(aq.)]=K_H \times pN_2O \times H_v$
$B = N_2O$ in the headspace (μAtm) = $[N_2O(g)] = pN_2O \times (1/22.414) \times (273/298) \times H_v$

$C = (A+B) = K_H \times pN_2O \times H_v + pN_2O \times (1/22.414) \times (273/298) \times H_v$

Where:

K_H = Henry's Law Coefficient for N_2O ($\mu Mol\ L^{-1}\ atm^{-1}$) = 0.0279

pN_2O = the partial pressure of N_2O in the headspace of the vials in ppmv.

H_v = the headspace volume of the vials = 0.081 L

22.414 = the molar volume of an ideal gas at 0°C and 1 atmosphere pressure = RT = L mol^{-1}

273/298 =correction for room temperature of 25°C

Note: This assumes equal headspace and soil solution volumes.

The final calculation to get from ppmv N_2O to µM per vial at a room temperature of 25° C was:

µM per vial = ppmv in headspace x headspace vol. x (K_H + 1/22.414 x 273/298)

= ppmv x (0.081 L) x (0.0279 + (1/22.414 x 273/298))

= ppmv x 0.00557

Continued on the next page

Appendix IV: Microbial Assay Calculations Contd.

Methanogenic rate (M).

$$M = \Delta U/\Delta t$$

Where: M = Methane production rate (μM g dwt soil^{-1} h^{-1})

U = μM methane in the headspace

t = Time (h)

U is calculated by

$$U = (P \times 2^R/Cal.) \times (1/22.414) \times (273/298) \times H_v$$

Where:

P = Peak area unit on the selected GC detector range

R = Selected GC detector range

22.414 = Gas constant at STP (L Mol^{-1} atm^{-1})

273/298 = correction for room temperature of 25°C

H_v = Volume of vial headspace

Nitrification Rate (N_f).

$$N_f = (\Delta N_s/\Delta t)/(S_w \times D_m)$$

Where:

N_f = μg NO_3-N produced per g dry weight of soil per hour (μg NO_3-N g dwt^{-1} h^{-1})

N_s = Amount of nitrate in the soil slurry (μg NO_3-N)

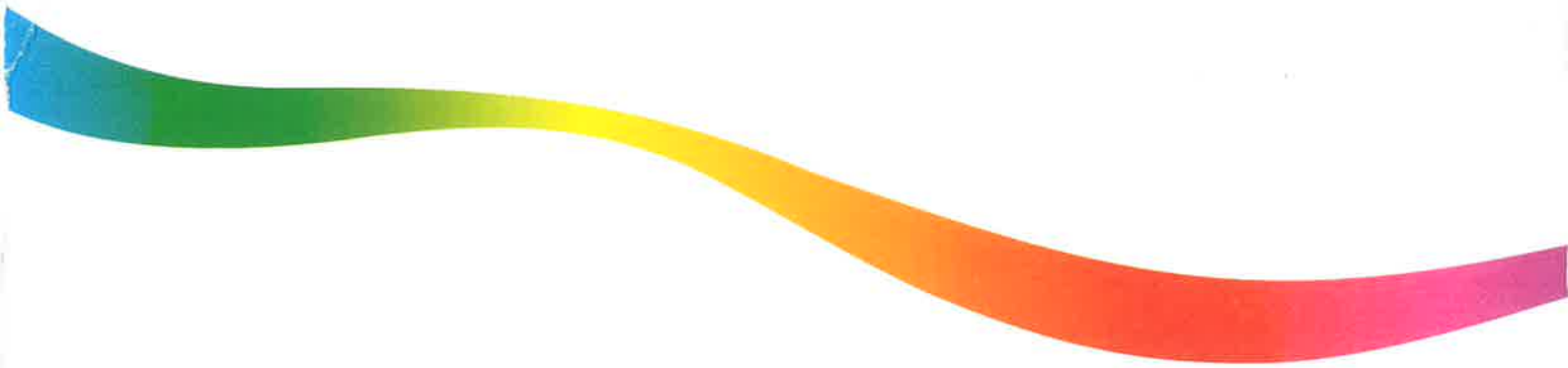
t = Time (h)

S_w = Fresh soil weight (g).

D_m = Dry weight of soil (g) N_s is calculated from $N_c \times S_v$

Where S_v = Soil solution volume

N_c = NO_3-N concentration in the whole slurry.



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