

## Nitrogen requirements for maximizing petroleum bioremediation in a sub-Antarctic soil

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### Abstract

Many contaminated cold region soils are deficient in nitrogen, and addition of the proper amount of this nutrient can increase the biodegradation rate. However, it has been demonstrated that excess nitrogen can depress the rate of microbial activity and petroleum degradation in contaminated soils due to osmotic soil water potential depression. This study was undertaken to optimize nutrient amendments in a sub-Antarctic soil. Soil collected from a petroleum-contaminated site on Macquarie Island, Australia, located in the sub-Antarctic, contained approximately 5250 mg kg<sup>-1</sup> of hydrocarbons and 20.9% H<sub>2</sub>O. Inorganic nitrogen levels prior to fertilization were <1.0 mg kg<sup>-1</sup> of NO<sub>3</sub>-N and 1.3 mg kg<sup>-1</sup> of NH<sub>4</sub>-N. Inorganic nitrogen, in the form of NH<sub>4</sub>Cl, was added at rates of 0, 125, 250, 375, 500, and 625 mg nitrogen kg<sup>-1</sup> of dry soil. On a soil water basis (N<sub>H<sub>2</sub>O</sub>—calculated by dividing inorganic soil nitrogen by the soil water content), applied plus native N levels were 6, 604, 1202, 1800, 2399, and 2997 mg nitrogen kg<sup>-1</sup> of soil water for these treatments. The soil was incubated at 6 °C. O<sub>2</sub> consumption was monitored for approximately 4 months. Maximum O<sub>2</sub> uptake was observed with the 125 and 250 mg nitrogen kg<sup>-1</sup> of soil application rates. Respiration in the 625 mg kg<sup>-1</sup> treatment was slightly lower than that in the untreated soil, although they were not statistically different. Respiration was maximized when N<sub>H<sub>2</sub>O</sub> was 604 mg nitrogen kg<sup>-1</sup> H<sub>2</sub>O, and was depressed when it reached 1800 mg N kg<sup>-1</sup> H<sub>2</sub>O. Residual soil petroleum following incubation was least in soil amended with 125 mg N kg<sup>-1</sup> (N<sub>H<sub>2</sub>O</sub>=604) and was greater in unfertilized soils or in soils receiving 250 mg N kg<sup>-1</sup> or more (N<sub>H<sub>2</sub>O</sub> ≥ 1202). Thus, the rate of bioremediation was maximized when N<sub>H<sub>2</sub>O</sub> was maintained below 1200 mg N kg<sup>-1</sup> soil H<sub>2</sub>O. Whereas previous studies have indicated that bioremediation in polar and sub-polar region soils are inhibited by nitrogen amendments above 2500 mg N kg<sup>-1</sup> H<sub>2</sub>O, results from this study indicated inhibition at a lower level of 1200 mg N kg<sup>-1</sup> H<sub>2</sub>O.

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### 1. Introduction

Nutrients are required to support soil microbial activity and, therefore, bioremediation. Although the

soil microbial community requires numerous nutrients, nitrogen (N) is most often limiting to biological hydrocarbon degradation in cold region soils (Mohn and Stewart, 2000). Treating petroleum-contaminated soil with N can increase cell growth rate (Hoyle et al., 1995), decrease the microbial lag phase (Lewis et al., 1986; Ferguson et al., 2003), help to maintain microbial

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populations at high activity levels (Lindstrom et al., 1991), and increase the rate of hydrocarbon degradation (Braddock et al., 1997, 1999).

Many studies indicate positive effects from supplemental N (Rasiah et al., 1991; Allen-King et al., 1994; Walworth and Reynolds, 1995); however, a surprisingly large number report no benefit, or even deleterious effects when excessive levels of N are applied (Watts et al., 1982; Brown et al., 1983; Huntjens et al., 1986; Morgan and Watkinson, 1990; Genouw et al., 1994; Zhou and Crawford, 1995; Braddock et al., 1997, 1999; Walworth et al., 1997; Mohn et al., 2001; Ferguson et al., 2003).

Reports of specific inhibitory effects of excess N include an increased lag phase (Huntjens et al., 1986; Ferguson et al., 2003) and preferential inhibition of aromatic degradation (Fayad and Overton, 1995), although most indicate overall inhibition of microbial respiration and/or hydrocarbon degradation. Genouw et al. (1994) found that addition of 4000 mg N kg<sup>-1</sup> of soil inhibited microbial degradation of an oil sludge. However, microbial inhibition has also been reported at lower application rates. Huntjens et al. (1986) noted that 400 mg N kg<sup>-1</sup> added to a sandy soil inhibited oil degradation. Addition of 100, 200, or 300 mg N kg<sup>-1</sup> of soil to sub-Arctic taiga soils contaminated with Prudhoe Bay crude oil stimulated biodegradation compared to unfertilized controls, although the greatest stimulation was seen at the lowest fertilizer level (100 mg N kg<sup>-1</sup> of soil) (Hunt et al., 1973). Similarly, Braddock et al. (1997) reported that addition of 100 mg N kg<sup>-1</sup> soil resulted in more rapid respiration than did 200 or 300 mg N kg<sup>-1</sup> soil. Addition of 300 mg N kg<sup>-1</sup> soil resulted in respiration rates equivalent to unfertilized soil. In a related study, respiration was greatest when 50 or 100 mg N kg<sup>-1</sup> soil were added to petroleum-contaminated soil, and lesser respiration rates were observed in unfertilized soil or soil amended with 200 mg N kg<sup>-1</sup> soil (Braddock et al., 1999).

Most N fertilizers are composed of highly water soluble nitrate and/or ammonium salts which quickly dissolve into soil pore water. This increases the salt concentration of the soil solution and lowers the soil osmotic potential (the portion of the soil water potential energy attributable to dissolved solutes), which can inhibit microbial activity. An osmotic potential drop of 0.50 MPa can reduce microbial petroleum degradation by roughly 50% (Braddock et al., 1997; Walworth et al., 1997). Populations, as well as activity, of hydrocarbon degraders and heterotrophs in general can be reduced by osmotic stress (Braddock et al., 1997).

The impact of N fertilizer on soil water potential is greater in dry than in moist soils (Walworth et al., 1997).

In a dry soil, there is less pore water for salts to dissolve into than in a moist soil, so the soil solution concentration is greater for a given amount of fertilizer. Therefore, microbial inhibition cannot be predicted simply from the level of fertilizer application.

Direct measurement of soil osmotic potential is problematic. Instead, the contribution of N fertilizer to osmotic potential can be estimated. Dividing the amount of N added (or the soil inorganic N concentration) by the soil moisture content, one can calculate an estimate of the N concentration in the soil solution, which has been termed  $N_{H_2O}$  (Walworth et al., 1997):

$$\frac{\text{mg N}}{\text{kg soil}} \div \frac{\text{kg H}_2\text{O}}{\text{kg soil}} = \frac{\text{mg N}}{\text{kg H}_2\text{O}} = N_{H_2O}$$

Thus, N concentration is calculated as a function of soil water rather than as a function of dry soil weight, which is the conventional notation.

Several studies provide information on the effect of  $N_{H_2O}$  on petroleum biodegradation in contaminated soils. Walworth et al. (1997) added ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>) to a sub-Arctic soil contaminated with diesel fuel and containing between 5% and 10% gravimetric moisture. The rate of microbial respiration was dependent on both the amount of N added to the soil, as well as the soil moisture level. If  $N_{H_2O}$  exceeded approximately 2500 mg N kg<sup>-1</sup> H<sub>2</sub>O, microbial activity was reduced relative to that in soil supplied with lower levels of N. In a study of the effects of nutrients on the hydrocarbon bioremediation potential of Arctic microbes in a hydrocarbon-contaminated coarse Arctic sand, Braddock et al. (1997) showed that amendment with approximately 4000 mg N kg<sup>-1</sup> soil H<sub>2</sub>O stimulated carbon mineralization, whereas 8000 or 12,000 mg N kg<sup>-1</sup> soil H<sub>2</sub>O provided less or no stimulation. In another study on the same soil, Braddock et al. (1999) found that addition of approximately 1500 or 3000 mg N kg<sup>-1</sup> soil H<sub>2</sub>O increased respiration relative to unfertilized soil, but addition of approximately 6000 mg N kg<sup>-1</sup> soil H<sub>2</sub>O resulted in reduced respiration. Mohn and Stewart (2000) also found that amending Arctic soils with small N applications stimulated dodecane mineralization, but that larger amendments of about 8000 mg N kg<sup>-1</sup> soil H<sub>2</sub>O were inhibitory. Ferguson et al. (2003) reported that Special Antarctic Blend fuel degradation in an Antarctic soil was stimulated by addition of 1570 mg N kg<sup>-1</sup> soil H<sub>2</sub>O, but not by addition of approximately 28000 mg N kg<sup>-1</sup> H<sub>2</sub>O.

A limitation of this approach is that it does not take into account contributions of other soil salts to osmotic

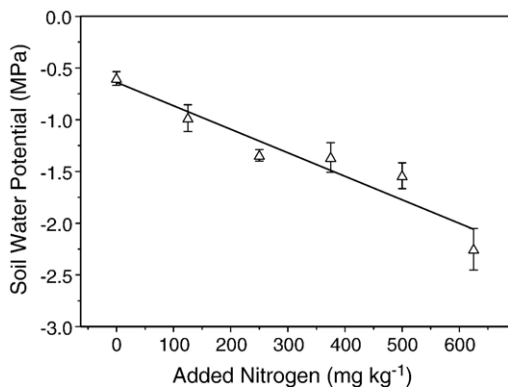


Fig. 1. Soil water potential depression resulting from addition of inorganic ammonium chloride ( $\text{NH}_4\text{Cl}$ ) nitrogen fertilizer. Error bars represent standard deviations.

potential. In soils with saline contaminants, or in saline soils, non-nitrogenous salts can impose a limitation on biodegradation (Haines et al., 1994; Rhykerd et al., 1995). Walworth et al. (1997) demonstrated that microbial activity can be inhibited by osmotic stress whether the osmotic potential is decreased through application of a fertilizer salt such as ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ) or a non-nitrogenous salt such as sodium chloride ( $\text{NaCl}$ ).

Previous data (Braddock et al., 1997, 1999; Ferguson et al., 2003; Walworth et al., 1997) have provided a rough estimate of the level of N application required to inhibit microbial petroleum degradation in Arctic and sub-Arctic soils; resolution was limited by the large increments of N fertilizer used in these studies. We undertook the current study to provide greater resolution to the N versus microbial activity relationship and to clearly define this relationship at a coastal sub-Antarctic site to provide information for field-scale cleanup.

Table 1

Concentration of fertilizer nitrogen added, soil water potential, residual soil nitrogen following incubation, average daily oxygen consumption rate during incubation, and residual petroleum following incubation in petroleum-contaminated soils; numbers in each column followed by different letters are statistically different at  $p < 0.05$

Added N	H <sub>2</sub> O	Residual	O <sub>2</sub>	Petroleum	
mg kg <sup>-1</sup>	potential	soil N	consumption	(mg kg <sup>-1</sup> )	
soil	soil H <sub>2</sub> O	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup>		
	(MPa)		day <sup>-1</sup> )		
0	6	-0.60 a	3 f	67.3 b	2753 a
125	604	-0.98 b	77 e	92.7 a	1520 b
250	1202	-1.36 c	155 d	84.6 a	2160 ab
375	1800	-1.35 c	260 c	69.8 b	2238 ab
500	2399	-1.54 d	527 b	69.8 b	1965 ab
625	2997	-2.25 e	629 a	66.7 b	2086 ab

## 2. Methods and materials

Soil was collected from a petroleum-contaminated site on sub-Antarctic Macquarie Island, Australia. Macquarie Island (54°29'59"S, 158°57'08"E) is located approximately 1500 km south-southeast of Tasmania. Mean daily temperatures range from 1.3 °C in September to 8.6 °C in January (Deprez et al., 1994). Macquarie Island has a moist climate and is ice-free. Annual rainfall is 952.6 mm.

The Australian Antarctic Division has maintained a permanent station on Macquarie Island since 1948. Large volumes of fuel and lubricants are needed to supply, maintain, and operate this station. The soil used in this study was collected from the Main Power House, where an overflow of Special Antarctic Blend fuel was reported in 1975 (Deprez et al., 1994); additional spills at this site may have been unrecorded. This site is a small wetland with some standing water, and covered with thick clumps of tussock grass. The upper 15 cm of soil has an elevated organic matter content (5.4%) compared to the deeper soil profile horizons which are medium- to coarse-grained sand with very little finer textured material. A homogenized bulk composite sample from the top 75 cm of the soil profile was used for the current study. The entire area is underlain by impervious bedrock.

Soil was placed in glass bottles, stored, and transported to the University of Arizona in Tucson, Arizona, at 4 °C until analysis. Soil analyses for electrical conductivity (EC) and pH were conducted in 1:1 (soil:water) extracts. Soil EC was measured via electrical conductance. Organic carbon was measured colorimetrically by the Walkley–Black method (Nelson and Sommers, 1996). Phosphorus and potassium were measured by ICP-AES on a 1:100 soil to 0.5 M bicarbonate extract with the pH adjusted to 8.5 with NaOH (Olsen and Dean, 1965). Inorganic N was extracted using 2.0 M KCl and analyzed on a continuous flow

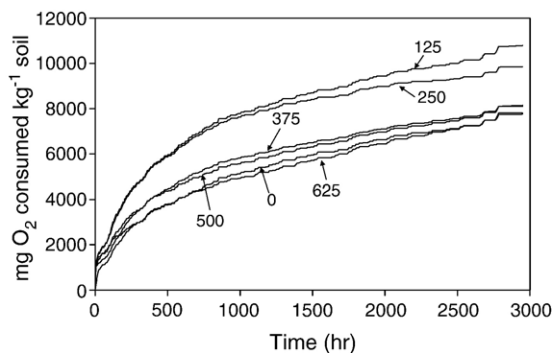


Fig. 2. Oxygen consumption during incubation of petroleum-contaminated soil. Numbers in figure indicate added nitrogen. Each line represents the average of four replicates.

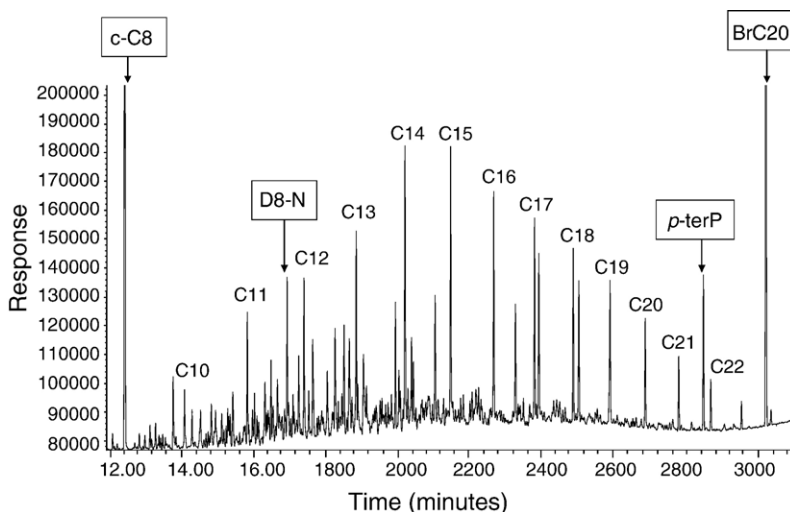


Fig. 3. Chromatogram of unweathered diesel fuel. Cyclo-octane (c-C8), D8-naphthalene (D8-N), *p*-terphenyl (*p*-terP), and bromoeicosane (BrC20) are internal standards.

analyzer. Nitrate was analyzed using the cadmium reduction method (Maynard and Kalra, 1993) and  $\text{NH}_4^+$  with the salicylate–hypochlorite method (Mulvaney, 1996).

Petroleum hydrocarbons were extracted from 10 g samples of soil with 10 mL of hexane, 10 mL of water, and 0.5 mL of an internal standard solution containing  $1000 \text{ mg L}^{-1}$  cyclo-octane,  $100 \text{ mg L}^{-1}$  D<sub>8</sub>-naphthalene,  $100 \text{ mg L}^{-1}$  *p*-terphenyl, and  $1000 \text{ mg L}^{-1}$  1-bromoeicosane dissolved in hexane. Vials containing soil and extractant were tumbled end-over-end at 50 rpm overnight and allowed to settle. The clear hexane layer was analyzed via GC-FID with a 30 m, 0.32 mm i.d., 0.25  $\mu\text{m}$  film thickness BP-1 capillary column (SGE) and a split/splitless FocusLiner (SGE). Reported soil petroleum concentrations represent compounds ranging from C<sub>9</sub> to C<sub>28</sub>.

For the incubation study, six aliquots of field-moist (20.9%) soil were amended with dry ammonium chloride ( $\text{NH}_4\text{Cl}$ ) to supply 0, 125, 250, 375, 500, and 625  $\text{mg N kg}^{-1}$  soil (on a dry soil weight basis) and thoroughly physically mixed with the soil. Four 30 g sub-samples of soil treated with each level of N were placed into 500 mL respirometer jars. Each jar contained an alkali trap filled with 10 mL of 6 M KOH. Jars were capped, placed inside a 6 °C incubator, and attached to a COMPUT-OX Respirometer (N-CON Systems, Crawford Ga). The respirometer recorded O<sub>2</sub> consumption every 30 min.

The experiment was ended after 2950 h (123 days). Jars were then opened and the alkali traps removed and capped. Unreacted KOH was titrated with a standard acid at the completion of the incubation to verify CO<sub>2</sub> production and O<sub>2</sub> consumption (CO<sub>2</sub> production verified O<sub>2</sub> consumption and data are not shown). The soil was removed and placed

into screw top glass jars. Soil water potential was measured in a SC-10 psychrometer using a Peltier thermocouple (Decagon Devices, Inc. Pullman, WA).

Statistical analyses were conducted with SAS PROC ANOVA and PROC REG functions (SAS Institute, 2004). Mean separations were performed by least significant difference (LSD) analysis.

### 3. Results and analyses

The soil used in this study was a sand that contained 3.1% organic carbon. At the initiation of the study, the soil had a pH of 6.1, contained  $250 \text{ mg PO}_4\text{-P kg}^{-1}$ ,  $91 \text{ mg K kg}^{-1}$ ,  $<1.0 \text{ mg NO}_3\text{-N kg}^{-1}$ ,  $1.3 \text{ mg NH}_4\text{-N kg}^{-1}$ , and  $5250 \text{ mg petroleum kg}^{-1}$ . EC in the 1:1 soil:water slurry was  $2.06 \text{ dS m}^{-1}$ . The soil had a gravimetric moisture content of 20.9%.

Based on the soil moisture content of 20.9%, and the sum of added N plus pre-treatment soil inorganic N, fertilizer treatments resulted in the following soil water N ( $\text{N}_{\text{H}_2\text{O}}$ ) concentrations: 6, 604, 1202, 1800, 2399, and 2997  $\text{mg N kg}^{-1}$  soil water. Soil water potential was significantly ( $P < 0.0001$ ) related to the level of N added to the soil (Fig. 1; Table 1). Regression analysis indicated that water potential decreased approximately 0.23 MPa upon addition of each  $100 \text{ mg N kg}^{-1}$  soil. The linear regression equation was

$$\Psi = -0.64 - 0.002265 * \text{nitrogen}, \quad r^2 = 0.86$$

where N is in units of  $\text{mg N kg}^{-1}$  soil and water potential ( $\Psi$ ) is in MPa.

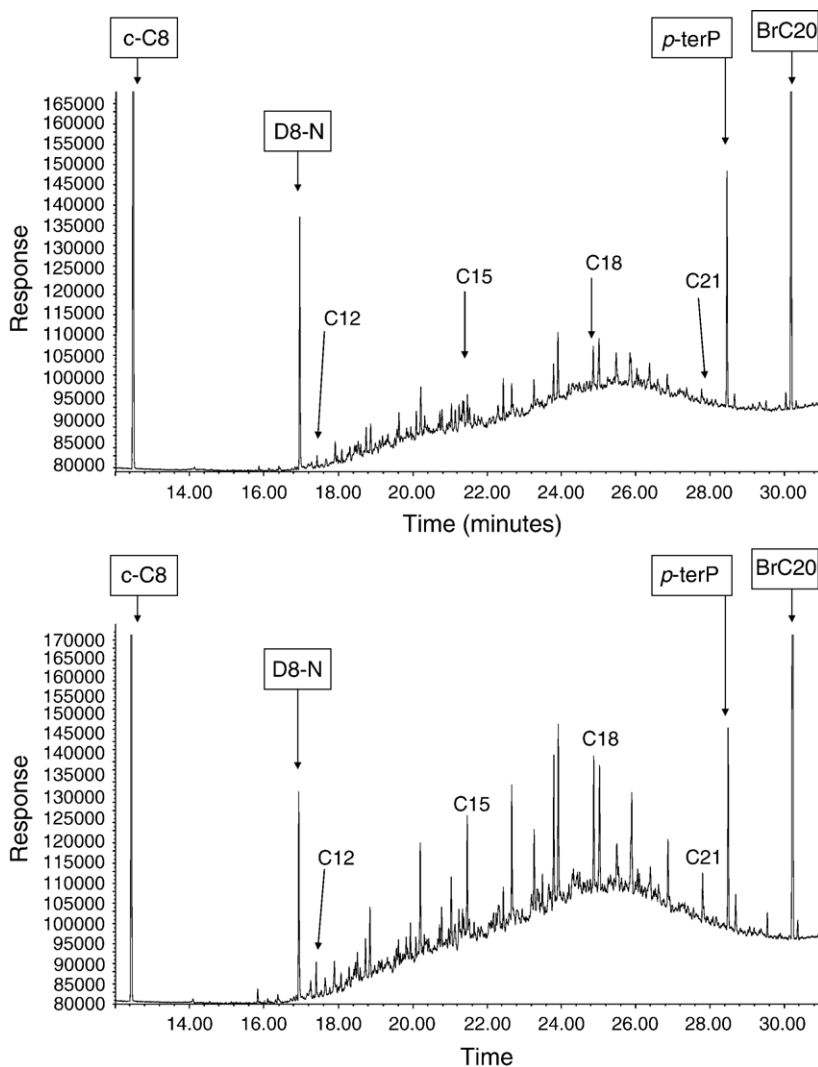


Fig. 4. Chromatogram of residual soil petroleum in soil with 125 mg N kg<sup>-1</sup> following incubation (top) and of residual soil petroleum in soil with 375 mg N kg<sup>-1</sup> following incubation (bottom). Cyclo-octane (c-C8), D8-naphthalene (D8-N), *p*-terphenyl (*p*-terP), and bromoicosane (BrC20) are internal standards.

Oxygen consumption, measured over a period of 123 days, is shown in Fig. 2. No noticeable effect of N addition on the microbial lag phase was expressed in the oxygen consumption data. Oxygen consumed during the incubation was divided by the number of days of incubation to give the average oxygen consumption (mg kg<sup>-1</sup> day<sup>-1</sup>) over the entire incubation period (Table 1). Without additional N, oxygen consumption averaged 67.3 mg oxygen kg<sup>-1</sup> day<sup>-1</sup>. Addition of 125 mg N kg<sup>-1</sup> soil increased the oxygen consumption to 92.7 mg kg<sup>-1</sup> day<sup>-1</sup>. Oxygen consumption appeared to slightly decrease upon addition of 250 mg N kg<sup>-1</sup> soil; however, the decrease was not statistically significant. Addition of greater levels of N (375, 500, or 675 mg N kg<sup>-1</sup> soil)

significantly depressed oxygen consumption to levels equivalent to that of the untreated (no N) control.

Expressing the quantity of soil N (native inorganic N plus added N fertilizer) as N<sub>H<sub>2</sub>O</sub>, calculated by dividing N concentration by the soil moisture content, initial N<sub>H<sub>2</sub>O</sub> levels ranged from 6 to 2997 mg N kg<sup>-1</sup> soil H<sub>2</sub>O (Table 1). Among the N<sub>H<sub>2</sub>O</sub> levels in this experiment, maximum oxygen consumption occurred in soils with levels ranging from 604 to 1202 mg N kg<sup>-1</sup> soil H<sub>2</sub>O. Microbial inhibition occurred with 1800 mg N kg<sup>-1</sup> soil H<sub>2</sub>O and higher levels of N addition.

Residual inorganic N (NH<sub>4</sub><sup>+</sup> + NO<sub>3</sub><sup>-</sup>) levels at the end of the incubation period are shown in Table 1. In all soils less than 2 mg kg<sup>-1</sup> of the inorganic N were present in the

$\text{NO}_3^-$  form, suggesting that little nitrification took place (data not shown), although no measurements of  $\text{NO}_3^-$  production were made. The remainder of the measured inorganic N was in the  $\text{NH}_4^+$  form. Net consumption of inorganic N can be estimated by comparing the added N levels with levels of residual N following incubation, but gross rates of biological N consumption cannot be determined from the current experiment. Net inorganic N consumption was not closely related to oxygen consumption; however, little or no net consumption was observed in the soils that were fertilized with N rates of 500 or 625  $\text{mg kg}^{-1}$ . In soils fertilized with 125, 250, or 375  $\text{mg kg}^{-1}$ , 38%, 38%, and 31%, respectively, of the added N was not recovered in the inorganic form at the end of incubation, suggesting the greatest microbial consumption in those treatments.

Residual petroleum hydrocarbon remaining in the soil at the end of the incubation period is shown in Table 1 and roughly corresponds to oxygen consumption data ( $r^2=0.46$ ). Residual soil petroleum was greatest in the untreated control, in which 2086  $\text{mg kg}^{-1}$  of the original 5250  $\text{mg kg}^{-1}$  remained following incubation. Losses may be a combination of sequestration into the soil matrix, biodegradation, and volatilization; however, abiotic controls were not included, so specific mechanisms of petroleum loss cannot be evaluated. Residual petroleum in soil with an  $\text{N}_{\text{H}_2\text{O}}$  level of 604  $\text{mg N kg}^{-1}$  soil  $\text{H}_2\text{O}$  was 1520  $\text{mg kg}^{-1}$ , which was significantly less than the unfertilized control. All soils receiving higher rates of N had residual petroleum levels ranging from 1965 to 2238  $\text{mg kg}^{-1}$ , levels which were not significantly different from either the untreated control or the soil with 604  $\text{mg N kg}^{-1}$  soil  $\text{H}_2\text{O}$  according to least significant difference analysis. However, an orthogonal contrast comparing the lowest level of N application to all higher application rates indicated a significant difference ( $p < 0.05$ ), indicating that the 604  $\text{mg N kg}^{-1}$  soil  $\text{H}_2\text{O}$  was superior to other treatments.

A chromatogram of unweathered diesel fuel is shown in Fig. 3, with typical *n*-alkane peaks from C10 to C22. In contrast, chromatograms from residual petroleum extracted from soil at the conclusion of the experimental incubation period are noticeably weathered, lacking components more volatile than *n*-C12, and exhibiting highly degraded *n*-alkanes (Fig. 4). The upper chromatogram in Fig. 4 is from the soil with a supplemental N treatment rate of 125  $\text{mg kg}^{-1}$ , whereas the lower chromatogram is from soil that received 375  $\text{mg kg}^{-1}$ . The degree of weathering following the 123-day incubation period is reflective of the average oxygen consumption and of the residual soil petroleum concentration (Table 1). Compared to the soil with 375  $\text{mg kg}^{-1}$ ,

the soil with 125  $\text{mg kg}^{-1}$  contains less *n*-alkanes and has a reduced C18:phytane ratio, indicative of microbial hydrocarbon degradation.

#### 4. Discussion and conclusions

Previous research indicated that inhibition of microbial respiration occurred at a  $\text{N}_{\text{H}_2\text{O}}$  level of approximately 2500  $\text{mg N kg}^{-1}$  soil  $\text{H}_2\text{O}$ , and that a 50% reduction occurred at approximately 5000  $\text{mg N kg}^{-1}$  soil  $\text{H}_2\text{O}$  (Walworth et al., 1997). In that study, 50% reduction of microbial respiration corresponded to a soil water potential of approximately  $-0.8$  MPa. In the current study, we observed inhibition of microbial oxygen consumption at a  $\text{N}_{\text{H}_2\text{O}}$  level of 1800  $\text{mg N kg}^{-1}$  soil  $\text{H}_2\text{O}$ . This  $\text{N}_{\text{H}_2\text{O}}$  level corresponded to a water potential of  $-1.36$  MPa. When  $\text{N}_{\text{H}_2\text{O}}$  was increased to 2997  $\text{mg N kg}^{-1}$  soil  $\text{H}_2\text{O}$ , with a corresponding soil water potential of  $-2.25$  MPa, microbial oxygen consumption was still 72% of the maximum observed.

The difference in the relationship between  $\text{N}_{\text{H}_2\text{O}}$  and soil water potential in these two studies may have two sources. Soil in the earlier study was a silt loam versus sand in the current study. At a given soil water content, the soil water matric potential (the potential energy attributable to attraction of water to soil particle surface) is lower (more negative) in finer textured soils. However, at the soil moisture levels used in both studies, the matric potential should have been between 0 and  $-0.05$  MPa, and this magnitude does not explain the observed differences. The differences are more likely due to the presence of non-nitrogenous salts. The relatively high EC ( $2.06 \text{ dS m}^{-1}$ ) and low soil water potential ( $-0.6$  MPa) in the untreated Macquarie Island soil are probably due to the close proximity of the site to the ocean (approximately 50 m). Conversely, the soil used in the Walworth et al. (1997) study was not subject to salt deposition, and would be expected to have very low salinity levels, although salinity measurements were not taken. Maximum microbial respiration rates in the current study were slightly higher than those observed by Walworth et al. (1997) even though native salinity levels were higher, suggesting microbial adaptation to elevated salinity in the Macquarie Island soil.

The N application rates related to inhibition of microbial oxygen consumption and/or petroleum loss in the current study are somewhat lower than in previous studies, although we are not aware of studies with N application rates that would allow direct comparisons to be made (i.e., with small enough N addition increments). The oxygen consumption data from the current study suggest that a reasonable cutoff level for added plus native

inorganic soil N should be approximately 1800 mg N kg<sup>-1</sup> soil H<sub>2</sub>O, a slightly lower value than previously recommended. On the other hand, petroleum loss was maximized with an N<sub>H<sub>2</sub>O</sub> level of 604 mg N kg<sup>-1</sup> soil H<sub>2</sub>O, and less petroleum loss was observed at levels at or above 1202 mg N kg<sup>-1</sup> soil H<sub>2</sub>O, suggesting that N<sub>H<sub>2</sub>O</sub> levels should be maintained below 1200 mg N kg<sup>-1</sup> soil H<sub>2</sub>O. The design of the current study does not permit more precise interpretation; however, studies with smaller increments of N fertilization could help to more accurately identify levels of application deleterious to microbial petroleum degradation. However, a precise number is probably not attainable because of differing responses of various soil microbial communities, differences in levels of non-nitrogenous soil salts, and because biologically available soil N levels change temporally as inorganic N is used as a substrate for or is a product of biological and chemical reactions.

Maintaining N<sub>H<sub>2</sub>O</sub> below 1200 to 1800 mg N kg<sup>-1</sup> H<sub>2</sub>O limits the dose of inorganic N fertilizers that can be added to a soil. Use of sparingly soluble N sources may permit addition of higher N doses by minimizing osmotic stress. For example, Mohn and Stewart (2000) found that addition of 5265 mg N kg<sup>-1</sup> soil H<sub>2</sub>O as non-water soluble Inipol EAP22 to an Arctic soil stimulated mineralization, whereas 7897 mg N kg<sup>-1</sup> soil H<sub>2</sub>O as urea and diammonium phosphate did not. Alternatively, N applications can be split into multiple smaller applications to keep N concentrations below inhibitory levels.

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