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Evaluating the effects of gross nitrogen mineralization, immobilization, and nitrification on nitrogen fertilizer availability in soil experimentally contaminated with diesel

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Abstract

Sandy clay loam soil was contaminated with 5000 mg kg^{-1} diesel, and amended with nitrogen (15.98 atom% ^{15}N) at 0, 250, 500, and 1000 mg kg^{-1} to determine gross rates of nitrogen transformations during diesel biodegradation at varying soil water potentials. The observed water potential values were −0.20, −0.47, −0.85, and −1.50 MPa in the 0, 250, 500, and 1000 mg kg^{-1} nitrogen treatments respectively. Highest microbial respiration occurred in the lowest nitrogen treatment suggesting an inhibitory osmotic effect from higher rates of nitrogen application. Microbial respiration rates of 185, 169, 131, and 116 mg O_2 kg^{-1} soil day^{-1} were observed in the 250, 500, control and 1000 mg kg^{-1} nitrogen treatments, respectively. Gross nitrification was inversely related to water potential with rates of 0.2, 0.04, and 0.004 mg N kg^{-1} soil day^{-1} in the 250, 500, and 1000 mg kg^{-1} nitrogen treatments, respectively. Reduction in water potential did not inhibit gross nitrogen immobilization or mineralization, with respective immobilization rates of 2.2, 1.8, and 1.8 mg N kg^{-1} soil day^{-1}, and mineralization rates of 0.5, 0.3, and 0.3 mg N kg^{-1} soil day^{-1} in the 1000, 500, and 250 mg kg^{-1} nitrogen treatments, respectively. Based on nitrogen transformation rates, the duration of fertilizer contribution to the inorganic nitrogen pool was estimated at 0.9, 1.9, and 3.2 years in the 250, 500, and 1000 mg kg^{-1} nitrogen treatments, respectively. The estimation was conservative as ammonium fixation, gross nitrogen immobilization, and nitrification were considered losses of fertilizer with only gross mineralization of organic nitrogen contributing to the most active portion of the nitrogen pool.

Introduction

Diesel-fuel soil contamination has become a global environmental concern. Within US EPA superfund sites, diesel is the second most frequently treated contaminant after benzene (Buswell 1994). *In situ* bioremediation is considered a favorable approach to the restoration of diesel contaminated soil because it is cost-effective, and under optimal conditions has the potential for complete mineralization of the contaminant, producing only innocuous by-products such as cellular biomass, water, and carbon dioxide.

The influx of carbon at diesel-contaminated sites generally leads to nutritional limitations for the indigenous microbial communities. Although both nitrogen and phosphorous have been reported in short supply in hydrocarbon contaminated ecosystems (Alexander 1999; Walworth & Reynolds 1995), the higher bacterial consumption rate of nitrogen relative to phosphorous during the catabolic breakdown of hydrocarbons often results in nitrogen becoming the primary limiting inorganic nutrient for oil bioremediation (Alexander 1999). Optimizing nitrogen augmentation in bioremediation has been difficult, with several

studies suggesting that too much nitrogen fertilizer may have a negative osmotic effect on hydrocarbon biodegradation resulting from the partitioning of the fertilizer salts into the pore water (Braddock et al. 1997; Walecka-Hutchison & Walworth 2001; Walworth et al. 1997a, b). In their study, Braddock et al., (1997) correlated excessive nitrogen fertilization with decreasing populations of heterotrophs, diesel and gasoline degraders as well as to an overall reduction in hydrocarbon degradation. Other studies evaluating solute effects on hydrocarbon degradation have shown that increasing solute concentrations results in reduced growth rates, demonstrated by a prolonged lag phase, reduction in respiration, and an overall reduction in hydrocarbon biodegradation (Diaz et al. 2000; Haines et al. 1994; Han & New 1994; Holden et al. 1997; Rhykerd et al. 1995; Shapir et al. 1998; Volker et al. 2003). Therefore, depending on soil texture and the resulting water holding capacity, even low levels of fertilization can lead to toxic effects on microbial populations due to osmotic stress (Walworth et al. 1997a).

In most soil environments the majority of nitrogen is present as soil organic material with only a minor fraction present as bioavailable inorganic nitrogen (NO_3^- or NH_4^+). Nitrogen mineralization, or the biological conversion of organically bound nitrogen into inorganic mineral forms, therefore, controls biodegradation efficacy in nitrogen-limited systems. Inorganic nitrogen, whether native or augmented, becomes assimilated by microorganisms (immobilization), and can again be converted to soil mineral nitrogen through re-mineralization. Depending on the form of inorganic nitrogen (NO_3^- or NH_4^+) other processes involved in the dynamics of nitrogen cycling include denitrification (nitrate reduction), nitrification (ammonium oxidation), and ammonium volatilization or fixation to clay. Quantifying rates of the processes involved in nitrogen cycling in contaminated systems allows for a better understanding of how long added inorganic nitrogen fertilizer may contribute to the pool of active nitrogen, thereby allowing for optimal nitrogen augmentation and an overall improved biodegradation efficacy.

A distinction must be made between gross and net rates of nitrogen processes. Net mineralization is the difference between the actual or gross nitrogen mineralization and the concurrent microbial immobilization of the mineralized nitrogen (Hart et al. 1994). In the absence of leaching, denitrification (the reduction of NO_3^- to N_2 or N_2O), and dissimilatory nitrate reduction (the use of NO_3^- as a terminal electron acceptor to oxidize organic compounds producing NH_4^+), net nitrification is equal to gross nitrification minus microbial immobilization of NO_3^-. Although net processes can be calculated from change in soil inorganic nitrogen pool size over time, actual or gross rates can be estimated only by using nitrogen isotope techniques.

The use of ^{15}N in measuring the dynamics of nitrogen cycling can be separated into two techniques: using ^{15}N as a tracer, or the ^{15}N-isotope dilution technique. The ^{15}N tracer technique involves the addition of labeled substrate pool and determining the partitioning of the added ^{15}N, whereas the ^{15}N isotope dilution technique (Kirkham & Bartholomew 1954) involves the addition of a labeled product pool and monitoring the rate at which production alters the isotopic enrichment of that pool.

Each technique has limitations. Three limitations with measuring gross nitrogen process rates using the ^{15}N tracer technique are: (1) addition to the substrate pool may stimulate the processes of interest, (2) influx of ambient nitrogen into the labeled substrate pool will dilute ^{15}N, and (3) consumption of the product pool will result in a lower amount of the isotope in the product pool (Hart et al. 1994). With the ^{15}N isotope dilution technique rates of production should not be stimulated by an increase in substrate, but this method assumes that (1) isotopic fractionation does not occur during microbial transformation of soil nitrogen, (2) rates of processes measured remain constant over the incubation period, and (3) that there is no re-mineralization of assimilated ^{15}N during the course of incubation.

The goal of this research was to evaluate the role of nitrogen dynamics on diesel biodegradation under various water potential conditions in nitrogen fertilized soil systems. More specifically, both ^{15}N tracer and ^{15}N isotope dilution techniques were used to estimate the rates of gross immobilization, nitrification, and mineralization, concurrent with diesel biodegradation. The estimated rates were used to determine how long each level of nitrogen augmentation would contribute to the active nitrogen pool in the diesel-contaminated

soil. Understanding nitrogen dynamics concomitant to hydrocarbon biodegradation is necessary for optimizing nitrogen augmentation and improving biodegradation efficacy.

Materials and methods

Samples of a Gila fine sandy loam soil (coarse loamy, mixed, thermic Typic Torrifluvent) were collected to a depth of 12 inches from the University of Arizona's Campus Agricultural Center in Tucson, Arizona. The soil was alkaline with a pH of 8.4, and contained organic matter at a level of approximately 0.5%. After an initial sieving using a 2-mm sieve, the soil was moistened and re-sieved to achieve uniformity. The final gravimetric moisture content of the re-sieved soil was 16.25% (81% of field capacity). On a dry weight basis, the soil was amended with 150 mg kg^{-1} phosphorous ($Ca(H_2PO_4)_2{\cdot}H_2O$), and 5000 mg kg^{-1} diesel fuel #2. Soil (200 g dry weight) was placed into 500 ml wide mouthed Wheaton glass bottles (respirometric reactors) and supplemented with $^{15}NH_4Cl$ (15.98 atom% ^{15}N, Cambridge Isotope Laboratories) as a solid at 0 (control or background N), 250, 500, and 1000 mg N kg^{-1} soil respectively. Each nitrogen treatment was performed in triplicate. Water potential measurements (ψ, matric plus osmotic) of all nitrogen treatments were determined in triplicate using the Decagon Tru Psi thermocouple psychrometer.

An N-CON Comput-OX System computerized respirometer was used to measure the biological O_2 consumption rates within the sealed respirometer reactors. Each reactor bottle cap assembly contained a 20 ml borosilicate glass vial in which excess potassium hydroxide neutralized CO_2 generated during aerobic diesel degradation. The resulting pressure drop in the reactor was detected by a pressure sensor, which delivered measured pulses of oxygen. The respirometer was maintained at a constant temperature of 25 °C for 821 h (34.2 days) and O_2 uptake was recorded every 0.5 h.

Soil samples of 20 g (dry weight) were aseptically collected from each reactor using a clean disposable teaspoon eight times during the experiment (time 0, 2.5, 4.9, 8.3, 13.3, 17.3, 24.4, and 34.2 days into the study respectively) for determination of nitrate, ammonium, organic nitrogen, and nitrogen isotope ratios in the three nitrogen forms. Soil in each reactor bottle was mixed prior to sampling to ensure sample uniformity and the difference in soil mass re-adjusted in the computerized respirometer to ensure representative oxygen consumption (mg O_2 kg^{-1} soil) throughout the duration of the experiment. Exchangeable NH_4^+ and NO_3^- plus NO_2^- were extracted from the soil samples with 2 M KCl at a 5:1 extractant to soil ratio, filtered using Whatman No. 1 filter paper, and assayed via steam distillation (Keeny & Nelson 1982). Soil extracts were spiked with 5 ml NH_4NO_3 standard (30 μg N ml^{-1}) before distillation to meet the 50–200 μg nitrogen requirement of the mass spectrometer. Distillates were dried at low temperatures (50–60 °C) to a NH_4-salt, residual liquid H_2SO_4 immobilized by adding 2 M KCl to each well in the microplate, and nitrogen isotope ratio analyses were performed using a Nuclide Model 3–60 RMS mass spectrometer equipped with an automated Rittenberg apparatus (ARA-MS, University of Illinois at U-C) (Mulvaney et al. 1990).

Residual KCl extracted soils were ground in a New Brunswick G-10 variable-speed gyrotory shaker (McGee et al. 1999). Organic nitrogen was determined via titration using the semi-microKjeldahl method (Bremner & Mulvaney 1982). After titration with 0.05 N H_2SO_4 to quantify organic nitrogen, 5 ml of 0.02 M H_2SO_4 was added to the distillate and completely dried in an oven at 65 °C. Anhydrous methanol (4 ml) was added to the dried distillate to remove H_3BO_3, and excess methanol was removed by heating to dryness at 65 °C. Two ml of deionized water was added and the sample transferred to a 2 ml polypropylene microcentrifuge tube and evaporated to 0.05–0.3 ml containing 50–200 μg N. The aliquot was transferred to a plastic microplate and dried in an oven at 65 °C to NH_4-salt. Nitrogen isotope ratio analysis was performed using the ARA-MS technique (University of Illinois at U-C).

Calculations

Measured sample inorganic atom % ^{15}N values were corrected to account for natural abundance nitrogen contamination derived from non-sample sources (reagents or ambient N) using the isotope dilution equation:

$$C = [(B + D)M_1 - B(M_2)]/D$$

where C is the corrected sample atom % ^{15}N value, B the micrograms of non-sample nitrogen (NH_4^+ or NO_3^-) experimentally determined by distillation of standards and controls, D the micrograms of nitrogen (NH_4^+ or NO_3^-) liberated from the sample, M_1 the measured (uncorrected) value of atom % ^{15}N in the sample, and M_2 the measured atom % ^{15}N value of unlabelled standard or control (Khan et al. 1998).

The percent ^{15}N recovered (F_{15N}) at time 0 was determined using the following equation:

$$F_{15N} = {}^{15}\text{N excess}(\text{mg kg}^{-1}) * \text{soil weight}(\text{kg})/{}^{15}\text{N amended}(\text{mg})$$

where ^{15}N excess = [the atom % ^{15}N enrichment of the N pool enriched with ^{15}N minus the atom % ^{15}N enrichment of that pool prior to ^{15}N addition (background enrichment)], multiplied by the N pool size (mg N kg^{-1}) divided by 100% (Hart et al. 1994).

Gross rates of mineralization (m) and NH_4^+ consumption (c_a) were calculated based on isotope dilution principle using the following equations:

$$m = \{([NH_4^+]_0 - [NH_4^+]_t)/t\} * \{\log(APE_0/APE_t)/\log([NH_4^+]_0/[NH_4^+]_t\}$$

$$c_a = m - \{[NH_4^+]_t - [NH_4^+]_0/t\}$$

where m is the gross N mineralization rate (mg of N kg^{-1} soil day^{-1}), c_a is the NH_4^+ consumption rate (mg of N kg^{-1} soil day^{-1}, refers to the sum of all consumptive processes of labeled pool), t the time (days), APE_0 the atom % ^{15}N excess of NH_4^+ pool at time 0, APE_t the atom % ^{15}N excess of NH_4^+ pool at time t (where APE is the atom % ^{15}N enrichment of nitrogen pool enriched with ^{15}N minus the atom % ^{15}N enrichment of that pool prior to ^{15}N addition (assumed 0.3663)), $[NH_4^+]_0$ the total NH_4^+ concentration (mg kg^{-1}) at time 0, and $[NH_4^+]_t$ the total NH_4^+ concentration (mg kg^{-1}) at time t (Hart et al. 1994).

Gross NH_4^+ nitrification and immobilization rates were calculated using ^{15}N tracer principles. The three different nitrogen forms (ammonium-N, nitrate-N, organic-N) derived from the amended fertilizer at varying times throughout the study were quantified using the following equation:

$$F = [T(A_s - A_b)]/A_f]$$

where F is the weight of nitrogen derived from labeled fertilizer in soil sample (mg $^{14+15}N$ kg^{-1} soil), T the total weight of nitrogen in sample mg N kg^{-1} soil), A_s the atom % excess ^{15}N in labeled sample of soil, A_b the atom % excess ^{15}N in control sample of soil that did not receive labeled fertilizer (background enrichment), A_f the atom % excess ^{15}N in labeled fertilizer added (where atom % excess = atom % ^{15}N in material minus 0.3663) (Hauck & Bremner 1976).

Based on the O_2 consumption rates determined respirometrically, the change in the inorganic N pool over time, and assuming $C_{16}H_{34}$ as a representative hydrocarbon, diesel biodegradation was estimated using the following equation:

$$a(C_{16}H_{34}) + b(O_2) + c(NH_3) \rightarrow d(C_5H_7NO_2) + e(CO_2) + f(H_2O)$$

where a, b, c, d, e, and f represent respective mole numbers (Maier et al. 2000).

Results and discussion

Respirometric and Nitrogen pool data

The dissolution of nitrogen fertilizer into the soil solution resulted in a decrease in total soil water potential (matric + osmotic) with highest depression corresponding to highest levels of nitrogen augmentation. The observed water potential values were −0.20, −0.47, −0.85, −1.5 MPa in the 0, 250, 500, and 1000 mg kg^{-1} N treatments respectively. In accordance with our previous research (Walecka-Hutchison & Walworth 2001), highest respiration was observed in the 250 mg kg^{-1} nitrogen treatment, followed by the 500, control and 1000 mg kg^{-1} nitrogen treatments with average rates of 185, 169, 131, and 116 mg O_2 kg^{-1} soil day^{-1} respectively (Figure 1). The addition of nitrogen fertilizer at 1000 mg kg^{-1} inhibited microbial activity as demonstrated by the 37% reduction in total respiration in this treatment relative to that of the 250 mg kg^{-1} nitrogen treatment.

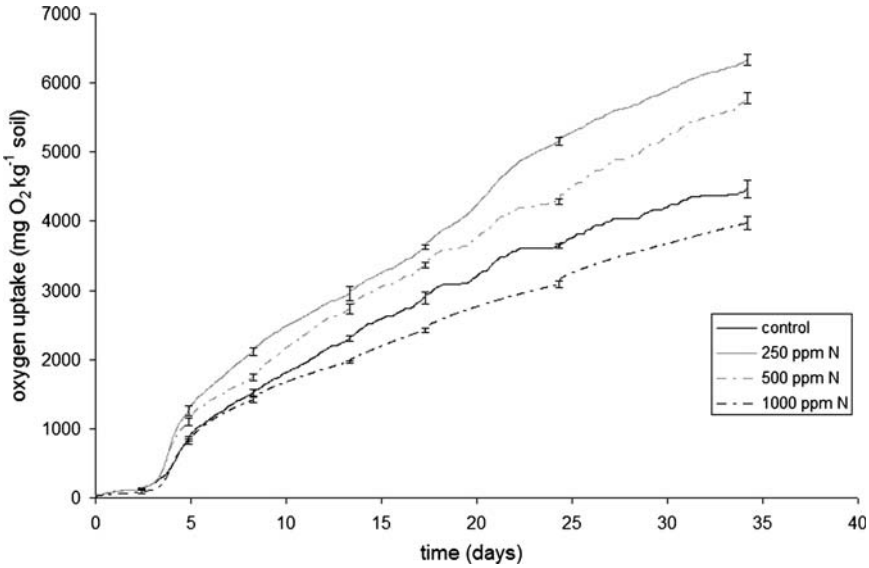

Figure 1. Microbial respiration (mg O_2 kg^{-1} soil) verses incubation time (days). Data represents averages of 3 replicates per nitrogen treatment. Error bars represent ±1.96 standard error (95% confidence interval) at 8 sampling times during the 34.2 days incubation period.

Temporal fluxes in the ammonium, nitrate, and organic nitrogen pools are depicted in Figure 2a–c. Initial recovered ammonium levels in the 0, 250, 500, and 1000 mg kg^{-1} nitrogen treatments, were 0.9, 233, 441, and 858 mg $N–NH_4^+$ kg^{-1} soil respectively (Figure 2a). The recovered fraction of the added $^{15}NH_4Cl$ at time 0 in the 250, 500, and 1000 mg kg^{-1} nitrogen treatments was 91, 86, and 85% respectively. The un-recovered nitrogen fertilizer was likely attributable to ammonium fixation in the soil due to its considerable mica and clay fractions (D.M. Hendricks, personal communication). Fixation of up to 10% of NH_4^+ added to clay minerals may occur in less then 30 min (Drury & Beauchamp 1991; Trehan 1996). Thus the 9–15% loss of $^{15}NH_4^+$ in this study observed within 15 min of fertilizer application was not unexpected. Ammonium concentrations decreased with time in all treatments, reaching a relatively steady state after 8.3 days. The final ammonium concentration in the 0, 250, 500, and 1000 mg kg^{-1} nitrogen treatments were 0.1, 143, 358, 774 mg $N–NH_4^+$ kg^{-1} soil respectively, representing 90, 38, 19, and 10% losses.

Ambient nitrate found in the soil ranged between 19 and 22 mg kg^{-1} (Figure 2b). Most (18 mg kg^{-1} $N–NO_3^-$) of the nitrate in the control treatment was depleted by 4.9 days into the study. Nitrate concentrations in the 500 and 1000 mg kg^{-1} nitrogen augmented treatments remained relatively steady suggesting microbial preference of NH_4^+ over NO_3^- in the fertilized nitrogen treatments. In our earlier work (Walecka-Hutchison & Walworth 2001), we observed microbial preference for NH_4^+ over NO_3^- with NO_3^- amended soils consuming only 65–70% as much O_2 as those treated with NH_4^+. Chang & Weaver (1997) also noted a preference for ammonium versus nitrate by petroleum degrading soil bacteria.

An increase in nitrate concentrations in the 250 mg kg^{-1} nitrogen treatment was observed beginning at 17.3 days, with final nitrate concentration increasing by 52% to 31 mg kg^{-1} $N–NO_3^-$. Although an increase in nitrate was also observed in both the 500 and 1000 mg kg^{-1} nitrogen treatments after 24.4 days (21 and 20 mg kg^{-1} respectively), it did not exceed the initial nitrate concentrations. Organic nitrogen concentrations increased in all treatments except the control with respect to time (Figure 2c).

Gross rates: isotope dilution technique

Highest gross nitrogen mineralization was observed in the 1000 mg kg^{-1} nitrogen treatment, and was on average 62% higher throughout the experiment than the relatively parallel rates ob-

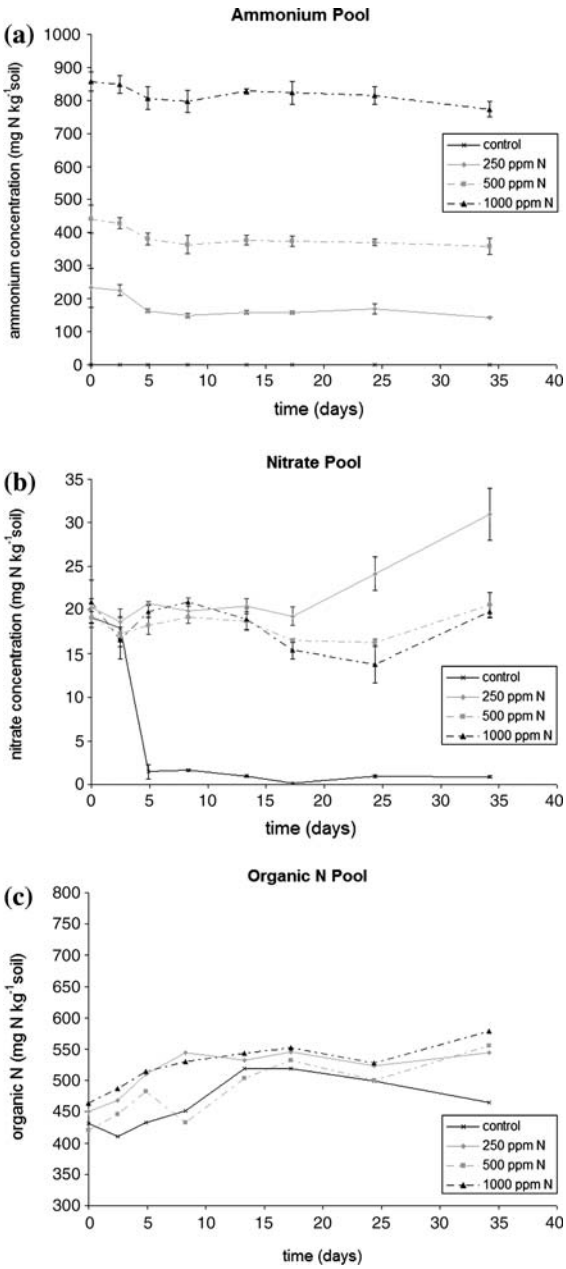

Figure 2. (a) Ammonium pool (mg N–NH_4^+ kg^{-1} soil) verses incubation time (days). (b) Nitrate pool (mg N–NO_3^- kg^{-1} soil) verses incubation time (days). (c) Organic nitrogen pool (mg N-organic kg^{-1} soil) verses incubation time (days). Data represent averages of 3 replicates. Error bars represent ± 1.96 standard error (95% confidence interval). No error bars shown in (c) for clarity.

served in the 250 and 500 mg kg^{-1} nitrogen treatments respectively (Figure 3). By 4.9 days 13.8, 6.8, and 4.9 mg of ambient organic nitrogen

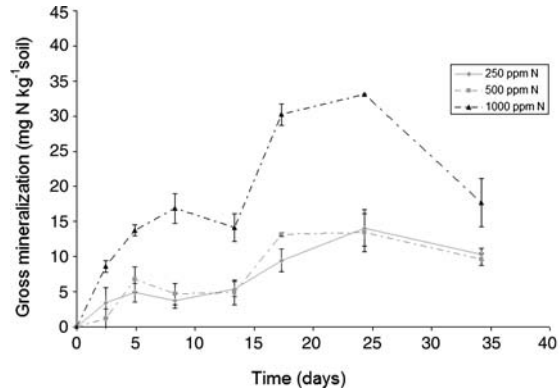

Figure 3. Cumulative gross mineralization (mg N kg^{-1} soil) verses incubation time (days). Error bars represent ± 1.96 standard error (95% confidence interval).

was mineralized per kg soil in the 1000, 500, and 250 mg kg^{-1} nitrogen treatments respectively, resulting in gross mineralization rates of 2.8, 1.4, and 1.0 mg N kg^{-1} soil day^{-1} in the treatments. At 13.3 days an unexplained increase in gross mineralization occurred in all treatments. Although soil samples were collected aseptically throughout the study, it is possible that the increase in mineralization may have resulted from a microbial population shift related to sample perturbation.

At 24.4 days the cumulative gross mineralization in the 1000, 500, and 250 mg kg^{-1} nitrogen treatments was approximately 33.2, 13.4, and 14.1 mg N kg^{-1} soil, resulting in mineralization rates of 1.4, 0.6, 0.6 mg N kg^{-1} soil day^{-1} respectively. At the end of the experiment the average mineralization decreased to 17.7, 9.6, and 10.3 mg N kg^{-1} soil in the 1000, 500, and 250 mg kg^{-1} nitrogen treatments respectively. It is possible that re-mineralization of immobilized biomass nitrogen occurred as suggested by a trend toward increasing ^{15}N-ammonium enrichment after 24.4 days (Figure 4; significant in the 1000 mg kg^{-1} N treatment only). This is in accordance with the work of Davidson et al. (1992) who estimated mean turnover time of the microbial biomass nitrogen at 1–2 months. Therefore, the final mineralization rates of 0.5, 0.3, and 0.3 mg N kg^{-1} soil day^{-1} in the 1000, 500, and 250 mg kg^{-1} nitrogen treatments, respectively, likely underestimate actual mineralization. An explanation as to why highest mineralization was observed in the 1000 mg kg^{-1} N treatment which demonstrated the greatest

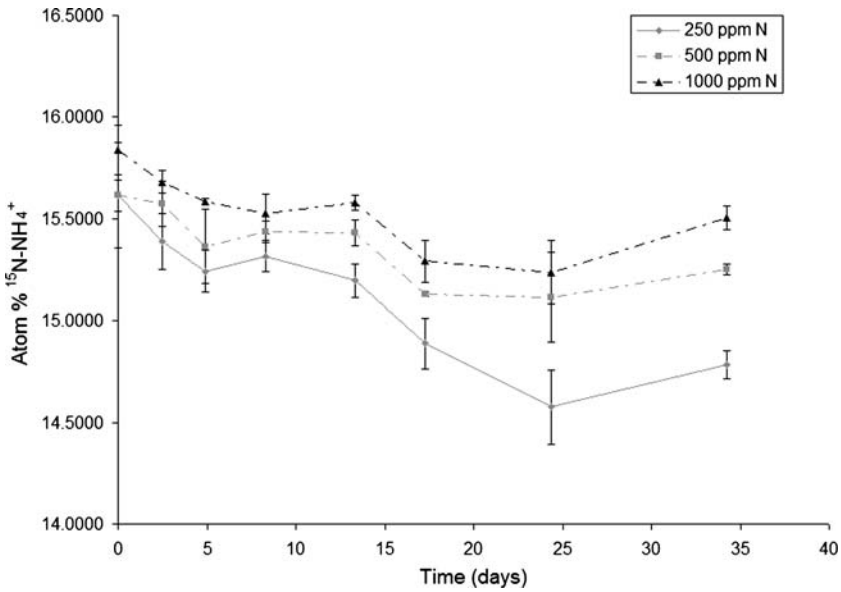

Figure 4. Atom % $^{15}N–NH_4^+$ verses time (days). Error bars represent ± 1.96 standard error (95% confidence interval).

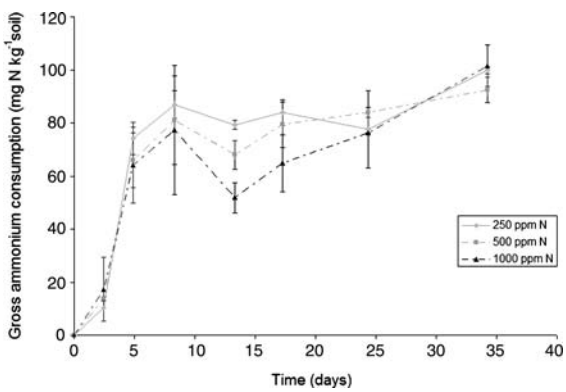

Figure 5. Cumulative gross ammonium consumption (mg N–NH_4^+ kg^{-1} soil) verses incubation time (days). Error bars represent ± 1.96 standard error (95% confidence interval).

depression in soil water potential (−1.5 MPa), may be that ammonifiers are fairly tolerant of osmotic stress, and capable of growth in environments with water potentials reaching −25 MPa (Paul & Clark 1989).

Cumulative gross ammonium consumption rates are depicted in Figure 5. Except at 13.3 days, no difference was observed between gross ammonium consumption rates of the 3 nitrogen treatments. By 4.9 days into the study 74.2, 66.0, and 64.2 mg N–NH_4^+ kg^{-1} soil was consumed in the 250, 500, and 1000 mg kg^{-1} nitrogen treatments resulting in consumption rates of 15.1, 13.5, and 13.1 mg N–NH_4^+ kg^{-1} soil day^{-1} respectively. At the end of the experiment 99.8, 92.4, and 101.6 mg N–NH_4^+ kg^{-1} soil was consumed in the 250, 500, and 1000 mg kg^{-1} nitrogen treatments resulting in final cumulative ammonium consumption rates of 2.9, 2.7, and 3.0 mg N–NH_4^+ kg^{-1} soil day^{-1} respectively. These rates, however, represent the flux in the tracer derived ammonium concentrations and are not limited to microbial immobilization alone, but reflect all losses of ammonium including immobilization, nitrification, volatilization, as well as fixation to clay.

Research suggests that re-mineralization of immobilized nitrogen can occur within 1 week of adding the ^{15}N label (Bjarnason 1988; Bristow et al. 1987). Accordingly therefore, the gross mineralization and ammonium consumption rates determined via isotope dilution technique in this study are most accurate for up to 1 week of the experiment (gross mineralization rates of 0.5, 0.6, and 2.0 mg N kg^{-1} soil day^{-1} and gross ammonium consumption of 10.5, 9.8, 9.3 mg N–NH_4^+ kg^{-1} soil day^{-1} in the 250, 500, and 1000 mg kg^{-1} N treatments respectively at 8.3 days), after which re-mineralization of the previously immobilized ^{15}N may begin to result in error. However, the work of Davidson et al. (1992) concluded that the mean residence time of microbial biomass

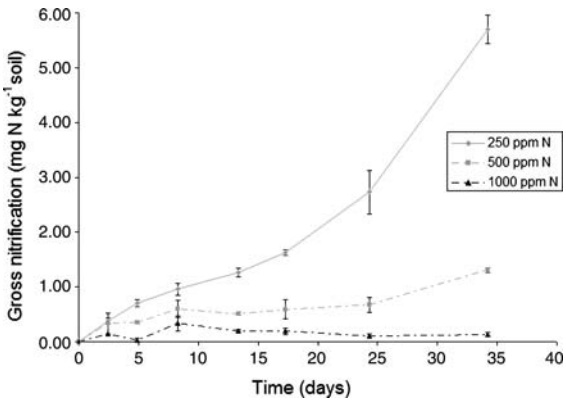

Figure 6. Cumulative gross nitrification (mg $N–NO_3^-$ kg^{-1} soil) verses incubation time (days). Error bars represent ± 1.96 standard error (95% confidence interval).

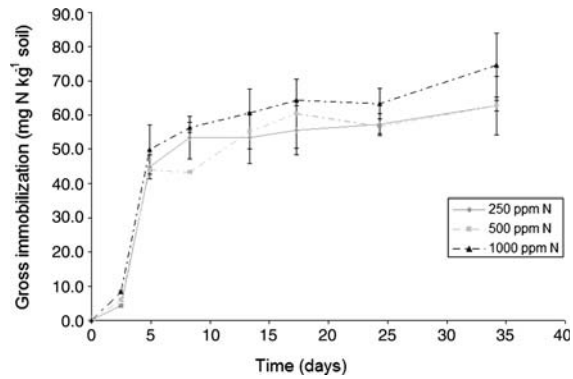

Figure 7. Cumulative gross immobilization (mg N kg^{-1} soil) verses incubation time (days). Error bars represent ± 1.96 standard error (95% confidence interval).

nitrogen was 1–2 months. Similarly, we observed a trend toward increasing $^{15}NH_4^+$ enrichment after 24.4 days suggestive of re-mineralization of immobilized biomass nitrogen after that time (Figure 4; significant in the 1000 mg kg^{-1} N treatment only). Furthermore, the sampling design in this experiment was intended for extrapolation to determine the duration of nitrogen fertilizer contribution to the active inorganic nitrogen pool during hydrocarbon bioremediation. Cleanup of contaminated sites generally necessitates prolonged timeframes in order to meet regulatory requirements. Therefore, the final rates estimated via this method were considered appropriate.

Gross rates: tracer technique

Figure 6 depicts the cumulative gross nitrification rates calculated using ^{15}N tracer principles. Highest gross nitrification was observed in the lowest fertilized treatment (250 mg kg^{-1} N, soil water potential = −0.47 MPa) suggesting that higher nitrogen application was inhibitory to this process, possibly through osmotic effects. The added ammonium in the 250 mg kg^{-1} nitrogen treatment began nitrifying almost immediately after fertilizer addition, with 0.7 mg $N–NO_3^-$ kg^{-1} soil being produced by 4.9 days into the study. By 17.3 days, 1.6 mg $N–NO_3^-$ kg^{-1} soil was generated in this treatment, reaching a final 5.7 mg $N–NO_3^-$ kg^{-1} soil at 34.2 days (final cumulative nitrification rate of 0.2 mg $N–NO_3^-$ kg^{-1} soil day^{-1}). Nitrification in the 500 mg kg^{-1} nitrogen treatment (soil water potential = −0.85 MPa) appeared to have a longer lag period, with the final nitrification rate being only 23% of that in the 250 mg kg^{-1} nitrogen treatment (0.04 mg $N–NO_3^-$ kg^{-1} soil day^{-1}, or 1.3 mg $N–NO_3^-$ generated per kg soil by 34.2 days). Nitrification was most inhibited in the highest nitrogen treatment (1000 mg kg^{-1} N, soil water potential = −1.50 MPa), where little change in the nitrate concentration took place throughout the experiment (final nitrification rate of 0.004 mg $N–NO_3^-$ kg^{-1} soil day^{-1}). Other research has also found nitrification to be sensitive to salinity or depression in soil water potential, with optimum rates occurring at moisture contents near or at field capacity (Malhi & McGill 1982; Stark & Firestone 1995; Zaman et al. 1999).

Figure 7 depicts cumulative gross nitrogen immobilization. No difference in microbial immobilization of the enriched ammonium was observed among the nitrogen treatments. Steady state was approached by the 3rd sampling interval (4.9 days) with 44.8, 43.9, and 49.9 mg N kg^{-1} soil was immobilized in the 250, 500, and 1000 mg kg^{-1} nitrogen treatments resulting in rates of 9.1, 9.0, and 10.2 mg N kg^{-1} soil day^{-1} respectively. Final cumulative immobilization was 62.7, 62.8, and 74.6 mg N kg^{-1} soil resulting in final cumulative immobilization rates of 1.8, 1.8, and 2.2 mg N kg^{-1} soil day^{-1} in the 250, 500, and 1000 mg kg^{-1} nitrogen treatments respectively.

Abiotic Nitrogen losses

The initial abiotic losses of the nitrogen fertilizer (9–15% of the added $^{15}NH_4^+$ likely lost to NH_4^+

Table 1. N-total ($N–NH_4^+$ + $N–NO_3^-$ + N-organic) derived from tracer (mg N kg^{-1} soil)

Time (days)	250 mg kg^{-1} N		500 mg kg^{-1} N		1000 mg kg^{-1} N	
	mg^{14+15} N kg^{-1}	Total ^{15}N (%)	mg^{14+15} N kg^{-1}	Total ^{15}N (%)	mg^{14+15} N kg^{-1}	Total ^{15}N (%)
0	227	100.0	430	100.0	849	100.0
2.5	222	97.6	423	98.3	840	98.9
4.9	201	88.5	410	95.4	836	98.5
8.3	197	86.9	395	91.9	830	97.7
13.3	205	90.3	419	97.5	865	101.9
17.3	204	89.9	414	96.4	850	100.1
24.4	214	94.1	406	94.6	838	98.7
34.2	201	88.3	405	94.1	824	97.1

fixation within 15 min of application) were accounted for in calculating all gross rates of nitrogen processes occurring throughout the experiment. However, additional abiotic losses of nitrogen were observed during the study. In comparing rates determined via both techniques, gross immobilization and nitrification estimated via the ^{15}N tracer technique collectively accounted for only 69–74% of the overall ammonium consumption. By the end of the study gross immobilization and nitrification combined accounted for 68.4, 64.1, and 74.8 mg $N–NH_4^+$ kg^{-1} soil in the 250, 500, and 1000 mg kg^{-1} nitrogen treatments respectively, whereas ammonium consumption calculated via isotope dilution was 99.8, 92.4, and 101.6 mg $N–NH_4^+$ kg^{-1} soil respectively, suggesting that gross immobilization and nitrification accounted for only 68.5, 69.4, and 73.6% of the ammonium consumed. Due to the conducive experimental conditions (alkaline desert soil under relatively high constant temperature of 25 °C), the remaining 26–31% of ammonium (32, 28, and 27 mg $N–NH_4^+$ kg^{-1} soil in the 250, 500, and 1000 mg kg^{-1} N treatments respectively) may have been lost to NH_3 volatilization, although no quantitive analyses were performed. However, ammonium fixation may have also played a role in the abiotic losses of the nitrogen fertilizer.

Table 1 shows the total nitrogen ($N–NH_4^+$ + $N–NO_3^-$ + N-organic) derived from the tracer throughout the study after the initial abiotic losses of the fertilizer have been accounted for. By 4.9 days the ^{15}N balance in the 250, 500, and 1000 mg kg^{-1} nitrogen treatments decreased to 88.5, 95.4, and 98.5% and remained close to these values throughout the remainder of the experiment with final values of 88.3, 94.1, and 97.1% respectively. Highest percent of tracer lost was observed in the 250 mg kg^{-1} nitrogen treatment in which 11.7% of the fertilizer was not accounted for by the end of the experiment relative to 5.9 and 2.9% in the 500 and 1000 mg kg^{-1} nitrogen treatments respectively. Quantitatively, however, the abiotic loss of $^{14+15}NH_4^+$ in the treatments was very similar (27, 25, and 25 mg N kg^{-1} soil in the 250, 500, and 1000 mg kg^{-1} nitrogen treatments respectively, Table 1) and as mentioned above, likely attributed to ammonia volatilization due to the conducive experimental conditions.

Petroleum degradation

An aerobic biodegradation mass balance equation was used to estimate hydrocarbon biodegradation based on the final quantitative O_2 and inorganic nitrogen consumption in each treatment, assuming $C_{16}H_{34}$ as a representative hydrocarbon. The losses of oxygen to nitrification in the 250 and 500 mg kg^{-1} nitrogen treatments (0.31 and 0.08% of the total O_2 utilized in each treatment respectively) were accounted for in the calculations. Degradation decreased with increasing level of nitrogen fertilization, with the highest removal occurring in the 250 mg kg^{-1} nitrogen treatment (42% or 2098 mg/kg diesel degraded). Degradation of 1939, 1425, and 1351 mg kg^{-1} diesel (39, 29, and 27%) was estimated for the 500, 1000, and 0 mg kg^{-1} nitrogen treatments respectively. The estimated cell yield (g cell mass/g hydrocarbon consumed, data not shown) was lower than expected (approximately 0.3) and might be attributable to the microbial production of compatible solutes in response to solute water stress (Welsh et al. 1996; Shapir et al. 1998). Research of

Table 2. Estimated steady state (34.2–4.9 days) net nitrogen fertilizer consumption (mg N kg^{-1} soil). *i*, *n*, and *m* represent cumulative gross immobilization, nitrification, and mineralization rates for the three amended ^{15}N treatments at 4.9 and 34.2 days

Nitrogen treatment (mg kg^{-1})	^{15}N–NH_4^+ recovered at time 0 (mg kg^{-1})	4.9 days		34.2 days	Steady state cumulative nitrogen fertilizer consumption (mg kg^{-1})
		$(i+n) - m$ (mg kg^{-1})	Nitrogen remaining (mg kg^{-1})	$(i+n) - m$ (mg kg^{-1})	
250	227	40.6	187	58.0	17.4
500	430	37.5	392	54.4	16.9
1000	849	36.2	813	57.1	20.9

Geerdink et al. (1996) found cell yields of 0.1 and 0.3 Cmol/Cmol during the biodegradation of diesel and hexadecane respectively under batch experimental conditions. The authors hypothesized that the low cell yields may have resulted from the microbial production of emulsifiers to increase solubilization of the hydrocarbons in water.

Duration of Nitrogen fertilizer availability

A better understanding of the nitrogen processes occurring concurrent with diesel degradation is necessary for estimating how long the added nitrogen fertilizer will contribute to the active nitrogen pool at contaminated sites. In this study, the estimated duration of fertilizer contribution to the inorganic nitrogen pool was based on three assumptions: (1) ammonium immobilization was considered a loss of fertilizer as biomass ^{15}N is not immediately available, and re-mineralization of immobilized ^{15}N biomass could not be calculated, (2) nitrification was also considered a loss of nitrogen because heterotrophic preference for ammonium over nitrate has been noted (Walecka-Hutchison & Walworth 2001; Chang & Weaver 1997), and because the process is primarily performed by chemoautotrophic organisms in alkaline soils which compete for ammonium with the hydrocarbon degrading heterotrophic organisms, and (3) the ammonium initially lost to fixation was considered a loss of fertilizer as non-exchangeable ammonium was assumed not bioavailable. The work of Breitenbeck & Paramasivam (1995) found that although heterotrophic soil microorganisms assimilated non-exchangeable $^{15}NH_4^+$ when the availability of an organic carbon substrate created demand, the fixed NH_4^+ was only 23–46% as available as NH_4^+ added directly to soil.

Gross ammonium immobilization was the overall net process occurring in this experiment, with gross mineralization of native organic nitrogen assumed the only process re-supplying the diminishing inorganic nitrogen pool. Net fertilizer consumption was therefore determined as follows: [(gross immobilization + gross nitrification) − gross mineralization]. Gross immobilization rates were much higher during the first 4.9 days (Figure 7) reaching steady state thereafter. Therefore, nitrogen losses during the initial 4.9 days were accounted for in calculating the steady state net fertilizer consumption (Table 2). It was estimated that at the consumptive rates calculated in this experiment the fertilizer would contribute to the active nitrogen pool for 0.9, 1.9, and 3.2 years in the 250, 500, and 1000 mg kg^{-1} N treatments respectively (Table 3). The estimated duration of fertilizer contribution to the active nitrogen pool is

Table 3. Estimated duration of nitrogen fertilizer availability

Nitrogen treatment (mg kg^{-1})	Nitrogen remaining after 4.9 days (mg N kg^{-1} soil)	Steady state cumulative fertilizer consumption (mg N kg^{-1}soil)	Steady state fertilizer consumption rate (mg $kg^{-1}day^{-1}$)	Nitrogen fertilizer availability (days)	Nitrogen fertilizer availability (years)
250	187	17.4	0.59	314	0.9
500	392	16.9	0.57	678	1.9
1000	813	20.9	0.71	1140	3.2

conservative as nitrification, re-mineralization, and utilization of fixed NH_4^+ all play a role in re-supplying the active inorganic nitrogen pool.

Conclusions

This study found that increasing nitrogen augmentation resulted in greater depression in soil water potential, reduced microbial respiration, and estimated diesel degradation. Highest respiration (185 mg O_2 kg^{-1} soil day^{-1}) and estimated diesel degradation (42%) were observed with the lowest nitrogen application of 250 mg N kg^{-1} soil (soil water potential = −0.47 MPa). Fertilizer addition at a concentration of 1000 mg kg^{-1} nitrogen (soil water potential = −1.50 MPa) inhibited microbial activity as demonstrated by a 37% reduction in maximum respiration.

Nitrification was also inversely related to soil water potential, and decreased with increasing nitrogen fertilizer concentrations. The highest cumulative gross nitrification was observed in the lowest nitrogen amended soil treatment (250 mg kg^{-1} N) resulting in 5.7 mg of N kg^{-1} soil nitrified by the end of the experiment (cumulative gross rate of 0.2 mg N kg^{-1} soil day^{-1}). In the 500 mg kg^{-1} nitrogen treatment, only 1.3 mg N kg^{-1} soil was nitrified throughout the study (0.04 mg N kg^{-1} soil day^{-1}). Nitrification was most inhibited in the highest nitrogen treatment (1000 mg kg^{-1} N) with final nitrification rate of 0.004 mg N kg^{-1} soil day^{-1}.

Reduction in soil water potential did not inhibit gross nitrogen immobilization or mineralization rates. No difference in gross immobilization rates was established between the treatments. However, gross nitrogen mineralization was on average 62% higher in the 1000 mg kg^{-1} N treatment, remaining nearly the same in the other two treatments: 17.7, 9.6, and 10.3 mg of the ambient nitrogen was mineralized per kg soil in the 1000, 500, and 250 mg kg^{-1} N treatments respectively at the end of the study (gross mineralization rates 0.5, 0.3, and 0.3 mg N kg^{-1} soil day^{-1} respectively).

The nitrogen transformation rates calculated in this experiment were used to determine the duration of fertilizer contribution to the inorganic nitrogen pool at each level of nitrogen fertilization. The assessment was conservative as ammonium fixation, gross ammonium immobilization, and nitrification were assumed losses of nitrogen fertilizer with only gross mineralization of native organic nitrogen contributing to the active nitrogen pool. It was estimated that the augmented ammonium fertilizer would contribute to the active inorganic nitrogen pool for 0.9, 1.9, and 3.2 years in the 250, 500, and 1000 mg kg^{-1} nitrogen treatments respectively, assuming nitrogen processes remained at steady state.

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